AN EPIDEMIOLOGICAL STUDY OF NOSOCOMIAL INFECTIONS AT MAYO HOSPITAL, LAHORE

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Microbiology

University of Veterinary and Animal Sciences, Lahore

2005
Acknowledgements

The author feels actuated from within to offer her humblest and sincerest thanks to Almighty ALLAH The Merciful and Beneficent, Who bestowed her the ability to perceive and pursue higher ideals of life.

Heart is warm with love and thoughts have turned to Holy Prophet Muhammad (PBUH), the very special entity God has brought into our lives, whose saying learn from cradle to grave awakened the strong desire in myself to undertake this course of studies.

Special thanks to Prof. Dr. Manzoor Ahmad, Vice Chancellor, University of Veterinary and Animal Sciences, Lahore for his moral support, encouragement and ever helping behaviour and valuable guidance.

I wish to thank Dr. Mansur-ud-Din Ahmad, Associate Professor/Chairman, Department of Microbiology, University of Veterinary and Animal Sciences, Lahore for his constructive and valuable guidance during the write up of this dissertation.

Special thanks and appreciations are due to Prof. Dr. Muhammad Athar Khan, Chairman, Department of Preventive Medicine and Public Health, for his remarkable contributions during the study period and guidance.

I feel immense pleasure in recording my heartfelt and sincerest thanks to Prof. Dr. Rashid Ahmed Chaudhry, Ex Principal of CVS, Lahore for his moral support, encouragement and ever helping behaviour.

I have the honour to express my deep sense of gratitude and indebtedness to Dr. Ata-ur-Rehman Rizvi, Professor (Retd.) Department of Microbiology, CVS, Lahore for his skillful guidance, learned patronage and inspiring attitude during the course of my study at C.V.S., Lahore.

I will be failing in my duties if I do not extend my special thanks to Prof. Dr. H.A. Hashmi, Director, Advanced Studies & Research, UVAS, Lahore, for his moral support and technical guidance for preparation of this manuscript.
I will never forget the guidance, kindness and supportive attitude of Dr. Syed Zafar Haider, Professor (Retd.) K.E.M.C., Dr. Ejaz ul Hassan (Late) Ex-Medical Superintendent, and Dr. Ehsan Ullah Khan Tareen, Ex-DMS, Mayo Hospital, to upraise my career. I am also thankful to Prof. Dr. Ajmal Niazi for his valuable support and appreciation.

This chapter will incomplete if I do not extend my heartiest thanks to Mr. Badar Munir, P.A. to the Dean, Faculty of Veterinary Science, UVAS, Lahore, for his everlasting support and academic guidance during postgraduate study programmes and finally composing this manuscript with full enthusiasm.

I express my special thanks to my class fellows namely; Dr. Rashid Ahmed, Dr. Mehboob Alam Qureshi and Dr. Waheeda Raana for their constant moral support.

I have Special regards for Mr. Waqar Gilani, who always helped me as a guide. I will never forget his efforts, constant support, motivation and encouragement throughout my career.

I wish to extend my zealous thanks to my respected family members and brothers Zafar Iqbal, Aftab Iqbal, Riaz Iqbal, Atta Ullah Khan Niazi, Nadeem Ghauri and Irfan, for their valuable support and encouraging behaviour in my studies. I also wish my thanks to dear sisters Samina, Sadia and Shaista for their support and encouragement.

I would appreciate the role of my dear brothers Masood Ahmad Khan, Farid Ahmad Khan, who always sacrificed a lot of time and make their efforts in completion of this great task. I am also thankful to my lovely sisters Zakira and Fakhra for their ever lasting moral support and sincerity throughout my career.

Lastly a word of great love and affection for my sweet daughters Saadia (Sonia) and Nayab (Mohnia) for their loving attitude. In fact they suffered a lot in my absence during the period of my studies. It was impossible to achieve this goal without a full support of my loving husband Mian Ijaz Iqbal, who really sacrificed a lot of his precious time for me. I will never forget his care and concern throughout my life.

Tayyaba
DEDICATED

To

My Loving MOM
and
my great FATHER (Late)
who always prayed for
my brilliant career
# CONTENTS

**DEDICATION** (i)

**ACKNOWLEDGEMENT** (ii)

**LIST OF TABLES** (v)

**LIST OF FIGURES** (vii)

<table>
<thead>
<tr>
<th>CHAPTER #</th>
<th>TITLE</th>
<th>PAGE #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>REVIEW OF LITERATURE</td>
<td>8</td>
</tr>
<tr>
<td>o</td>
<td>Sources of Nosocomial Infections</td>
<td>8</td>
</tr>
<tr>
<td>o</td>
<td>Hospital Staff</td>
<td>8</td>
</tr>
<tr>
<td>o</td>
<td>Hospital Environment</td>
<td>11</td>
</tr>
<tr>
<td>o</td>
<td>Hospital Inmates</td>
<td>22</td>
</tr>
<tr>
<td>o</td>
<td>Inanimate Objects</td>
<td>28</td>
</tr>
<tr>
<td>o</td>
<td>Nosocomial Pathogens</td>
<td>33</td>
</tr>
<tr>
<td>i.</td>
<td>Genus Staphylococcus</td>
<td>36</td>
</tr>
<tr>
<td>ii.</td>
<td>Genus Streptococcus</td>
<td>47</td>
</tr>
<tr>
<td>iii.</td>
<td>Genus Enterococcus</td>
<td>48</td>
</tr>
<tr>
<td>iv.</td>
<td>Genus Pseudomonas</td>
<td>52</td>
</tr>
<tr>
<td>v.</td>
<td>Genus Enterobacter</td>
<td>57</td>
</tr>
<tr>
<td>vi.</td>
<td>Genus Salmonella</td>
<td>59</td>
</tr>
<tr>
<td>vii.</td>
<td>Genus Escherichia</td>
<td>59</td>
</tr>
<tr>
<td>viii.</td>
<td>Genus Klebsiella</td>
<td>60</td>
</tr>
<tr>
<td>ix.</td>
<td>Genus Serratia</td>
<td>61</td>
</tr>
<tr>
<td>x.</td>
<td>Genus Acinetobacter</td>
<td>62</td>
</tr>
<tr>
<td>xi.</td>
<td>Genera Aerobacter and Aeromonas</td>
<td>65</td>
</tr>
<tr>
<td>xii.</td>
<td>Genus Flavobacterium</td>
<td>65</td>
</tr>
<tr>
<td>xiii.</td>
<td>Genus Clostridium</td>
<td>66</td>
</tr>
<tr>
<td>xiv.</td>
<td>Genus Haemophilus</td>
<td>67</td>
</tr>
<tr>
<td>xv.</td>
<td>Genus Mycobacterium</td>
<td>67</td>
</tr>
<tr>
<td>xvi.</td>
<td>Genus Corynebacterium</td>
<td>71</td>
</tr>
<tr>
<td>xvii.</td>
<td>Yeast and Fungi</td>
<td>71</td>
</tr>
<tr>
<td>xviii.</td>
<td>Uncommon Organisms</td>
<td>73</td>
</tr>
<tr>
<td>o</td>
<td>Drug Resistance</td>
<td>74</td>
</tr>
<tr>
<td>o</td>
<td>Epidemiology of Nosocomial Infections</td>
<td>108</td>
</tr>
<tr>
<td>3.</td>
<td>MATERIALS AND METHODS</td>
<td>126</td>
</tr>
<tr>
<td>o</td>
<td>Glassware, culture tubes, chemicals</td>
<td>126</td>
</tr>
<tr>
<td>o</td>
<td>Specimen</td>
<td>132</td>
</tr>
<tr>
<td>o</td>
<td>Collection of samples</td>
<td>135</td>
</tr>
<tr>
<td>o</td>
<td>Sampling of health care workers</td>
<td>135</td>
</tr>
<tr>
<td>o</td>
<td>Sampling of hospital environment</td>
<td>136</td>
</tr>
<tr>
<td>o</td>
<td>Sampling of hospital Inmates</td>
<td>136</td>
</tr>
<tr>
<td>o</td>
<td>Processing of samples</td>
<td>137</td>
</tr>
<tr>
<td>o</td>
<td>Examination of body fluids</td>
<td>138</td>
</tr>
<tr>
<td>o</td>
<td>Examination of cerebrospinal fluid</td>
<td>139</td>
</tr>
<tr>
<td>o</td>
<td>Examination of Urine,blood specimens</td>
<td>140</td>
</tr>
<tr>
<td>o</td>
<td>Examination of fecal specimens</td>
<td>141</td>
</tr>
<tr>
<td>o</td>
<td>Examination of pus, ulcers &amp; skin specimens</td>
<td>142</td>
</tr>
<tr>
<td>o</td>
<td>Examination of sputum &amp; pulmonary exudates</td>
<td>143</td>
</tr>
<tr>
<td>o</td>
<td>Air samples from operation theatre &amp; hospital environment</td>
<td>143</td>
</tr>
<tr>
<td>o</td>
<td>Examination of smears</td>
<td>144</td>
</tr>
<tr>
<td>o</td>
<td>Identification of bacteria</td>
<td>145</td>
</tr>
</tbody>
</table>
4. RESULTS
   • Sources of Materials
     o Patients 163
     o Hospital staff 163
     o Hospital’s environment 164
     o Non Human inmates 167
   • Bacteriological Studies
     o Staphylococcus 172
     o Streptococcus 180
     o Enterococcus 186
     o Pseudomonas 192
     o Enterobacter 197
     o Acinetobacter 202
     o Klebsiella 205
     o Proteus 210
     o Escherichia 214
     o Serratia 220
     o Haemophilus 223
     o Uncommon Gram positive bacteria 228
     o Uncommon Gram negative organisms 234
     o Yeast & Fungi 236

5. DISCUSSION
   o Source of materials 241
   o Bacteriology 244
     i. Staphylococcus 245
     ii. Streptococcus 254
     iii. Enterococcus 256
     iv. Pseudomonas 260
     v. Enterobacter 264
     vi. Acinetobacter 267
     vii. Klebsiella 268
     viii. Proteus 270
     ix. Escherichia 272
     x. Serratia 275
     xi. Haemophilus 277
     xii. Uncommon Gram positive bacteria 278
     xiii. Uncommon Gram negative organisms 279
     xiv. Yeast & fungi 279

CONCLUSION 281
RECOMMENDATIONS 283
FUTURE QUEST 290
6. SUMMARY 292
LITERATURE CITED 295
APPENDICES 327
<table>
<thead>
<tr>
<th>TABLE #</th>
<th>TITLE</th>
<th>PAGE #</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Types of samples collected from patients</td>
<td>128</td>
</tr>
<tr>
<td>3.2</td>
<td>Details of specimens collected from various wards of the Mayo hospital, Lahore</td>
<td>129</td>
</tr>
<tr>
<td>3.3</td>
<td>Details of specimens collected from hospital Staff</td>
<td>130</td>
</tr>
<tr>
<td>3.4</td>
<td>Details of samples from hospital environment</td>
<td>131</td>
</tr>
<tr>
<td>3.5</td>
<td>Details of samples collected from non human animates</td>
<td>131</td>
</tr>
<tr>
<td>4.1</td>
<td>Detailed bacteriology of samples collected from the patients.</td>
<td>165</td>
</tr>
<tr>
<td>4.2</td>
<td>Detailed bacteriology of hospital staff samples</td>
<td>166</td>
</tr>
<tr>
<td>4.3</td>
<td>Bacteriology of samples collected from hospital environment</td>
<td>169</td>
</tr>
<tr>
<td>4.4(a)</td>
<td>Bacteriology of samples collected from non human inmates</td>
<td>170</td>
</tr>
<tr>
<td>4.4(b)</td>
<td>Bacteriology of samples collected from non human inmates</td>
<td>171</td>
</tr>
<tr>
<td>4.5</td>
<td>Isolation of various Staphylococcus species from patient samples</td>
<td>174</td>
</tr>
<tr>
<td>4.6</td>
<td>Isolation of Staphylococcus species from hospital staff samples</td>
<td>174</td>
</tr>
<tr>
<td>4.7</td>
<td>Isolation of Staphylococcus species from hospital environment</td>
<td>175</td>
</tr>
<tr>
<td>4.8</td>
<td>Isolation of Staphylococcus species from non human inmates</td>
<td>175</td>
</tr>
<tr>
<td>4.9</td>
<td>Isolation of Staphylococcus species from various sources</td>
<td>175</td>
</tr>
<tr>
<td>4.10</td>
<td>Colonization of Staphylococcus isolates in various wards in Mayo Hospital, Lahore</td>
<td>177</td>
</tr>
<tr>
<td>4.11</td>
<td>Isolation of Streptococcus isolates from the patient samples</td>
<td>183</td>
</tr>
<tr>
<td>4.12</td>
<td>Isolation of Streptococcus isolates from hospital staff samples</td>
<td>183</td>
</tr>
<tr>
<td>4.13</td>
<td>Isolation of Streptococcus isolates from hospital environment</td>
<td>184</td>
</tr>
<tr>
<td>4.14</td>
<td>Isolation of Streptococcus isolates from non human inmates</td>
<td>184</td>
</tr>
<tr>
<td>4.15</td>
<td>Isolation of Streptococcus isolates from various sources</td>
<td>184</td>
</tr>
<tr>
<td>4.16</td>
<td>Isolation of Enterococcus isolates from the patient samples</td>
<td>188</td>
</tr>
<tr>
<td>4.17</td>
<td>Isolation of Enterococcus isolates from hospital staff samples</td>
<td>189</td>
</tr>
<tr>
<td>4.18</td>
<td>Isolation of Enterococcus isolates from hospital environment</td>
<td>189</td>
</tr>
<tr>
<td>4.19</td>
<td>Isolation of Enterococcus isolates from non human inmates</td>
<td>190</td>
</tr>
<tr>
<td>4.20</td>
<td>Isolation of Enterococcus isolates from various hospital sources</td>
<td>190</td>
</tr>
<tr>
<td>4.21</td>
<td>Isolation of Psudomonas isolates from the patient samples</td>
<td>194</td>
</tr>
<tr>
<td>4.22</td>
<td>Isolation of Psudomonas isolates from hospital staff samples</td>
<td>194</td>
</tr>
<tr>
<td>4.23</td>
<td>Isolation of Psudomonas isolates from hospital environment</td>
<td>195</td>
</tr>
<tr>
<td>4.24</td>
<td>Isolation of Psudomonas isolates from non human inmates</td>
<td>195</td>
</tr>
<tr>
<td>4.25</td>
<td>Isolation of Psudomonas isolates from various sources</td>
<td>195</td>
</tr>
<tr>
<td>4.26</td>
<td>Isolation of Enterobacter isolates from the patient samples</td>
<td>199</td>
</tr>
<tr>
<td>4.27</td>
<td>Isolation of Enterobacter isolates from hospital staff samples</td>
<td>199</td>
</tr>
<tr>
<td>4.28</td>
<td>Isolation of Enterobacter isolates from hospital environment</td>
<td>200</td>
</tr>
<tr>
<td>4.29</td>
<td>Isolation of Enterobacter isolates from non human inmates</td>
<td>200</td>
</tr>
<tr>
<td>4.30</td>
<td>Isolation of Enterobacter isolates from various sources</td>
<td>200</td>
</tr>
<tr>
<td>4.31</td>
<td>Isolation of Acinetobacter isolates from patient samples</td>
<td>203</td>
</tr>
<tr>
<td>4.32</td>
<td>Isolation of Acinetobacter isolates from various sources</td>
<td>203</td>
</tr>
<tr>
<td>4.33</td>
<td>Isolation of Klebsiella isolates from patient samples</td>
<td>207</td>
</tr>
<tr>
<td>4.34</td>
<td>Isolation of Klebsiella isolates from hospital staff</td>
<td>207</td>
</tr>
<tr>
<td>4.35</td>
<td>Isolation of Klebsiella isolates from hospital environment</td>
<td>208</td>
</tr>
<tr>
<td>4.36</td>
<td>Isolation of Klebsiella isolates from various sources</td>
<td>208</td>
</tr>
<tr>
<td>4.37</td>
<td>Isolation of Proteus isolates from the patient samples</td>
<td>211</td>
</tr>
<tr>
<td>4.38</td>
<td>Isolation of Proteus isolates from hospital environment</td>
<td>211</td>
</tr>
<tr>
<td>4.39</td>
<td>Isolation of Proteus isolates from non human inmates</td>
<td>211</td>
</tr>
<tr>
<td>4.40</td>
<td>Isolation of Proteus isolates from various hospital sources</td>
<td>212</td>
</tr>
<tr>
<td>4.41</td>
<td>Isolation of Escherichia isolates from the patient samples</td>
<td>216</td>
</tr>
<tr>
<td>4.42</td>
<td>Isolation of Escherichia isolates from hospital staff</td>
<td>217</td>
</tr>
<tr>
<td>4.43</td>
<td>Isolation of Escherichia isolates from hospital environment</td>
<td>217</td>
</tr>
<tr>
<td>4.44</td>
<td>Isolation of Escherichia isolates from non human inmates</td>
<td>218</td>
</tr>
<tr>
<td>4.45</td>
<td>Isolation of Escherichia isolates from various sources</td>
<td>218</td>
</tr>
<tr>
<td>4.46</td>
<td>Isolation of Serratia isolates from the patient samples</td>
<td>221</td>
</tr>
<tr>
<td>4.47</td>
<td>Isolation of Serratia isolates from various sources</td>
<td>221</td>
</tr>
<tr>
<td>4.48</td>
<td>Isolation of Haemophilus isolates from patient samples</td>
<td>225</td>
</tr>
<tr>
<td>4.49</td>
<td>Isolation of Haemophilus isolates from hospital staff</td>
<td>225</td>
</tr>
<tr>
<td>4.50</td>
<td>Isolation of Haemophilus isolates from various sources</td>
<td>226</td>
</tr>
<tr>
<td>4.51</td>
<td>Isolation of Gram positive organisms from patient samples</td>
<td>231</td>
</tr>
<tr>
<td>4.52</td>
<td>Isolation of Gram positive organisms from hospital staff</td>
<td>231</td>
</tr>
<tr>
<td>4.53</td>
<td>Isolation of Gram positive organisms from hospital environment</td>
<td>232</td>
</tr>
<tr>
<td>4.54</td>
<td>Isolation of Gram positive organisms from non human inmates</td>
<td>232</td>
</tr>
<tr>
<td>4.55</td>
<td>Isolation of Gram positive organisms from various sources</td>
<td>232</td>
</tr>
<tr>
<td>4.56</td>
<td>Isolation of Gram negative organisms from the patient samples</td>
<td>235</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIG. #</th>
<th>TITLE</th>
<th>PAGE #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colonization of Staphylococci in wards</td>
<td>179</td>
</tr>
<tr>
<td>2.</td>
<td>Colonization of Streptococci in wards</td>
<td>185</td>
</tr>
<tr>
<td>3.</td>
<td>Colonization of Enterococci in wards</td>
<td>191</td>
</tr>
<tr>
<td>4.</td>
<td>Colonization of Pseudomonas in wards</td>
<td>196</td>
</tr>
<tr>
<td>5.</td>
<td>Colonization of Enterobacter in wards</td>
<td>201</td>
</tr>
<tr>
<td>6.</td>
<td>Colonization of Acinetobacter in wards</td>
<td>204</td>
</tr>
<tr>
<td>7.</td>
<td>Colonization of Klebsiella in wards</td>
<td>209</td>
</tr>
<tr>
<td>8.</td>
<td>Colonization of Proteus in wards</td>
<td>213</td>
</tr>
<tr>
<td>9.</td>
<td>Colonization of Escherichia in wards</td>
<td>219</td>
</tr>
<tr>
<td>10.</td>
<td>Colonization of Serratia in wards</td>
<td>222</td>
</tr>
<tr>
<td>11.</td>
<td>Colonization of Haemophilus in wards</td>
<td>227</td>
</tr>
<tr>
<td>12.</td>
<td>Colonization of Gram positive (others) in wards</td>
<td>233</td>
</tr>
<tr>
<td>13.</td>
<td>Colonization of yeast and fungi in wards</td>
<td>237</td>
</tr>
<tr>
<td>14.</td>
<td>The most prevalent organisms in various wards of Mayo</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>Hospital, Lahore</td>
<td></td>
</tr>
</tbody>
</table>
 Various types of nosocomial infections are acquired by hospitalized patients after 48-72 hours of their admission, in addition to their prior ailments. The immuno suppressed, immuno compromised and even the patients with normal immune system are vulnerable to nosocomial infections under certain circumstances. The potential impact of nosocomial infections is considerable in terms of incidence, morbidity, mortality, and financial burden (Wallace and Doebbeling, 1998).

Nosocomial infections have been a serious problem ever since sick patients first congregated in the hospitals (Semmelweiss, 1861; Nightingale, 1863; Simpson and Spensor, 1869). Interest in nosocomial infection grew at a very rapid rate from earlier twentieth century, when new basis of hospital infections was reported, and alarming increase in the number of serious cases of Streptococcus pyogenes infections in hospitals were noted (Cruickshank, 1935).

Urinary tract infections (UTI) are considered to be the most common infections which are acquired from the hospitals. The causal pathogens of those infections have been reported as Escherichia coli, Klebsiella spp, Proteus spp, Enterococcus spp, and Enterobacter spp. which are part of the patient’s endogenous bowel flora. The isolation of pathogens such as Serratia marcescens and Pseudomonas cepacia from cases of UTI is also considered to be quite important (Wagenletner and Naber, 2000).

Nosocomial pneumonia is the leading cause of hospital acquired respiratory tract infections (RTI) in both the industrialized and developing countries. Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis are the most common pathogens which cause early onset of nosocomial pneumonia. Late-onset of nosocomial pneumonia is usually caused by Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae, Serratia marcescens, and Acinetobacter baumannii (Craven et al., 1997).
Surgical wound infections (SWI) are common nosocomial infections which may develop following surgery in a contaminated environment. The most common causative agent of SWI is *Staphylococcus aureus*, followed by *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Burn wound patients and burn wound units are potential sources of entry for nosocomial infection with Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. *Staphylococcus aureus* alone has been reported responsible for 25% of all burn wound infections. However, *Pseudomonas aeruginosa* has also been incriminated in burn wound infections (Trilla and Miro, 1995).

Consumption of contaminated food has been regarded as an important cause of nosocomial diarrhea, while administration of certain antibiotics (Clindamycin etc.) against infectious diseases may also cause diarrhea in some patients. Micro-organisms responsible for diarrhea in the community are also capable to cause a nosocomial outbreak in hospitalized patients. Diarrhea caused by *Bacillus cereus*, *Clostridium botulinum* and *Staphylococcus aureus*, has been found least common but the micro-organisms causing nosocomial gastro-enteritis include *E. coli.*, *Salmonella* and *Shigella* spp., *Yersinia enterocultica*, *Vibrio cholerae* and *Clostridium difficile* (Cooksan et al., 1997; Dupont and Ribner, 1998).

Various nosocomial blood stream infections (BSIs) are related to the use of intravascular devices. BSIs are quite high among patients using intravascular devices than those without such devices. The species of micro-organisms causing BSI are coagulase negative *Staphylococci*, *Enterococci*, *Staphlococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* and the *Candida* spp. (Pittet, 1997).

Discovery of penicillin in the later years of World War II provided considerable benefits (Fraser, 1984) and banished chronic cases of sepsis from hospitals (Fletcher, 1984). Use of antibiotics replaced the Penicillin sensitive *Staphylococcus* with Penicillin resistant *Staphylococcus* (Williams, 1956). In later years Penicillin resistant and later multiple drug resistant organisms especially the
Staphylococcus aureus appeared on the scene (Clarke et al., 1952). Use of combination of antibiotics, however, has changed the pattern of nosocomial infections (Ayliffe et al., 1979). In many hospitals outbreaks of diseases caused by multi drug resistant organisms are now commonly encountered. It is important to note that these infections cause serious disease problems and place the patients at serious risk. The stubborn infections make recovery difficult and slow, prolong convalescence and add in difficulties of the patient, both in terms of health and economics (Hart, 1982; Holmberg et al., 1987). The hospital is a threatening environment because a variable number of virulent pathogens are brought to the hospital from the community through admitted patients and their attendants. These patients are not only exposed to the indigenous hospital flora but also to the flora of other sick individuals and hospital personnels under treatment (Wenzel, 1987; McGowan, 1989).

Nosocomial infections are quite a common feature in the hospitals throughout the world. Bannett and Brachman (1989) estimated that 5% of all the hospitalized patients develop nosocomial infection and the problem of getting such infections varies in severity from hospital to hospital. Haley and Schaberg (1981) reported that in randomly selected patients of general medical and surgical services during 1975-1976, the nosocomial infection rate was 5.2%. They identified that the intrinsic factors like age, sex, underlying disease and immuno-suppressive therapy increased the risk of infection while the extrinsic factors like indwelling catheters, duration of catherization and invasive procedures also contributed to the risk of hospital acquired infections. Brewer et al. (2002) reported that in developed countries 5-10% of infections are acquired in hospitals. This figure can exceed to 25% in developing countries.

The nosocomial infections not only contribute, significantly, to morbidity and mortality of patients but also increase cost of hospitalization. In USA alone nearly 5% of patients admitted to acute care centers acquired nosocomial infections and their treatment cost the Federal Government an additional 2 billion US$ each year (Dixon, 1978; Holmberg et al., 1987; Stone et al., 2002).
Nosocomial infection may be endogenous or exogenous in origin. Endogenous infections are caused by the organisms that are present on or in the body of a patient as a part of the normal flora and under circumstances of physical or pathological stress, when the resistance of the host is suppressed some apparently non-pathogenic strains may become more active and could invade the deeper tissues setting up productive infection. The organisms entering the body which are not normally present on or in the body of the host and are acquired from the hospital environment, hospital personnel, medical devices, infected surgical instruments, infected food and water and insect vectors living in the hospital environment. Some bacteria such as \textit{Staphylococcus aureus}, \textit{Streptococcus} spp., \textit{Mycobacterium tuberculosis}, and most viral and fungal pathogens are usually acquired from the animate environment (Khan \textit{et al.}, 1983), and sometimes from the inanimate hospital environment (Zai \textit{et al.}, 1980).

Before the introduction of antimicrobial agents, the organisms commonly associated with hospital acquired infections have been claimed to be \textit{Streptococcus} spp. belonging to the Genus \textit{Streptococcus} groups A and D. With the introduction of antimicrobial agents the susceptible strains of most of the bacteria have been replaced with antibiotic resistant strains of the same species complicating the nosocomial problems. Evolutions of new wide spectrum antibiotics greatly improved the prognosis of nosocomial infections, and prophylactic antibiotic therapy successfully covers short periods of risk of infections. Failure to complete antibiotic coverage and under dosage of antibiotic administration is a common practice in many hospitals, which leads to the survival and rapid multiplication of drug resistant strains of bacteria in the body of the host. These resistant strains not only replace the drug sensitive pathogens but in some cases it may eliminate the normal commensals intestinal flora. New antibiotics are being developed and are more affective against various resistant bacterial strains, a big proportion of the nosocomial infections may be successfully treated with newly discovered antibiotics, however there are still several bacterial species that may be resistant to such newly developed antibiotics.
Medical practitioners usually ignore the commensal flora and pathogens, present on the skin and mucous membrane of an individual being admitted in the hospital (Mayer and Opal, 1989; Zhang, 1991). It has been observed that patients recovering from typhoid fever and allied infections may be passing Salmonellae in their secretions and excretions for over a year after recovery (Hammami et al., 1991; Karim et al., 1991). During active infection and just near recovery a patient may be passing out large quantities of pathogens in the environment (Soomro, 1988; Chou et al., 1991). Group A Streptococci have been recovered from the throat secretions of individuals recovering from Septic Sour Throat and Scarlet Fever (Rose et al., 1981). Tuberculosis suffering patients may pass large number of *Mycobacterium tuberculosis* in their sputum and other excretions endangering the health of hospital staff and other patients (Franzetti et al., 1999). Alternatively the apparently healthy visitors and individuals suffering from innocuous problems may acquire pathogenic organisms from indoor patients, their attendants and the hospital staff treating such patients.

The cockroaches are one of the most common insects found in the hospitals throughout the world and these are considered as important mechanical vector of pathogens. These insects are generally present abundantly in ward pantries, stores, bathrooms, kitchens, dining rooms, false ceiling, hollow partitioning such as in bed frames and side tables. These insects freely come in contact with the patient’s utensils and fomites, spread the pathogens and infections via their hair, bristles, their legs, fecal material and salivary secretions (Okafor, 1981 and Service, 1986). The environment in the hospitals is ideal for their uninhibited multiplication (Frishman and Alcamo, 1977). In pathogenic species of Salmonella, Shigella, Escherichia, Klebsiella, Enterobacter, Serratia, Proteus, Morganella, Providencia, Citrobacter, Edwardsiella, Alcaligens, Acinetobacter, Pseudomonas and Bacillus were isolated from the external surface of cockroaches (Burgess and Chetwyn, 1981; Soomro, 1988).

The principal factors that contribute to the risk of acquiring a nosocomial infection include; prolonged hospital stay (Larson et al., 1986; Lodise et al. (2003),

Though it is not possible to draw a sharp line between the endogenous and exogenous sources of infection yet the other factors which may play a major role in the cause of nosocomial infections are: (i) airborne transmission (Riley et al., 1962; Lowbury et al., 1971; Lidwell, 1974 and Gould et al., 1987), (ii) Food and water (Tobin et al., 1981; and Taylor et al., 1982), (iii) hospital staff (Burnie, 1986 and Larson, 1988), (iv) hospital environment (Mulligan and George, 1980; Sanderson and Rawal, 1987; Weinstein, 1991), (v) equipment (Curie and Speller, 1978; Im et al., 1981; Chastre and Fagon, 2002), (vi) accidental inoculation (Hawkey et al., 1980; Mollisson et al., 1987), (vii) exposed mucus membranes and peritoneum (Forster and Zachary, 1976), (viii) intravascular cannulae (Maki et al., 1973), (ix) indwelling catheters (Kunin, 1987), (x) prolonged catheterization (Clayton et al., 1982; O’Donnell and Hofmann, 2002), (xi) Tracheal intubation etc. (Nair et al., 1986).

In view of the above explained facts, the present study had therefore been designed to investigate the sources and causes of nosocomial infections in patients admitted to the Mayo Hospital Lahore. The major objectives of the study were:

1. To investigate the prevalence of nosocomial bacterial infections in Mayo hospital, Lahore.
2. To identify the etiology of nosocomial infections.
3. To investigate prevalence of drug resistant bacterial strains in Mayo hospital, Lahore.
4. To identify the reservoirs of bacterial infections in the hospital environment in:
i) patients
ii) cockroaches (insects)
iii) cats (pets)

5. To recommend the measures for prevention of nosocomial infections in Mayo hospital.
CHAPTER – 2
REVIEW OF LITERATURE

SOURCES OF NOSOCOMIAL INFECTIONS:

All living and non-living objects contribute towards the bacterial load of hospitals. The bacteria present on the body of an inanimate and within the body of a living subject are transferred to its environments; thus everything that happens to pass through the hospital may become source of nosocomial pathogens (Gordon and Lavoipierre, 1978). In nosocomial infections the most commonly reported organisms are those belonging to Genera *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Mycobacterium*, *Meningococcus*, *Clostridium*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Aeromonas*, *Serratia* (Aoun and Klastersky, 1991; Begue, 1991; Kadoya *et al*., 1991; Schaberg *et al*., 1991; Zhang, 1991; Ismail, *et al*., 1997; Richard *et al*., 1998; Valero and Saenz, 1998). The viral and fungal pathogens are also acquired from the animate environment. Some individuals and patients may be the carriers of pathogenic bacteria at the time of their admission or visit to hospital and spread them in the hospital environment and to other patients. The inanimate environment of the hospital including ward dust, linen, insects, contaminated instruments and other fomites, also provide an important potential reservoir of bacteria and source of nosocomial infections. Gram-negative bacilli, in particular, establish their reservoir in hospitals especially in moist or wet areas (Berk and Verghese, 1989; Dryden and Munro, 1989; McGowan *et al*., 1989; Meyer *et al*., 1989).

The era of chemotherapy is over fifty years old and represents continuous development and introduction of new and potent antimicrobial agents (Wise *et al*., 1989). During this period significant changes have taken place in the character of nosocomial infections (Gentry, 1991). Antimicrobial therapy has greatly improved the prognosis of infective disease and it can be given as prophylactic measure to cover short periods of risks of infections.
Hospital authorities usually ignore the commensal flora and pathogens, present on the skin and mucous membrane of individuals as no measures could be effective against such transient sources of infection. It has been observed that patients recovering from typhoid fever and allied infections may be passing Salmonellae in their secretions and excretions almost 12-14 months after recovery from this disease (Hammami et al., 1991). Just near recovery a patient may be passing out large quantities of pathogen in his stools in the environment. Streptococci belonging to group A have been isolated from the throat secretions of individuals recovering from the Septic Sour Throat and Scarlet Fever. An individual suffering from tuberculosis may be passing large numbers of *Mycobacterium tuberculosis* in sputum and other excretions (Macfie, 1922; Coronado et al., 1993). These individuals are potential danger for other patients and if the individual happens to be a health care worker of unhygienic habits, he could play havoc with the health of other patients. Such infections are difficult to control because their source is not immediately known. The visitors and individuals suffering from innocuous infection may acquire pathogenic organisms from already admitted patients, their attendants and even from the staff of wards while attending a hospital (Ismail et al., 1997; Valero and Saenz, 1998 and Brett et al., 1999).

The inanimate environment of the hospital including ward dust, linen, insects, contaminated instruments and other fomites are important sources and potential reservoirs of bacteria and nosocomial infections. Gram-negative bacilli, establish reservoirs in the dark, moist and wet areas of hospitals especially in the washbasins and sinks (Fernandez and Zaror 1972; Zuberi et al., 1972; Curie and Speller, 1978)). Contaminated food, water medication and medical devices, are quite common sources of nosocomial pathogens (Okafor, 1981). These objects serve as medium of growth and transmission of infectious agents that may cause sporadic diseases or even outbreaks (Macfie, 1922). Many of the common nosocomial gram-negative pathogens can persist or even proliferate in the moist environments (Burgess, 1979; Okafor, 1981; Lyon et al., 1984; Cotton et al., 1989; Bennett and Brachman, 1986).
Under-staffing of nurses and other health-care workers and overcrowding with patients increase the risk of nosocomial infection by making it difficult for the health care workers and nurses to adhere to hygienic and aseptic procedures and by enhancing contamination of the hospital environment with infected dust. Goldmann et al. (1981) studied the host rate and therapeutic risk factors for nosocomial infections and impact of staffing and environment on the rate of nosocomial infection in a neonatal intensive care units in which infants occupied a crowded NICU that lacked basic infection control features. A 5.2% of the infants had at least 1 major nosocomial infection. The risk of nosocomial infection was associated with low birth weight, patent ductus arteriosus, surgery and multiple supportive measures. They further reported that after a new NICU was opened in February 1977, only 0.9% of patients had suffered from major nosocomial infections. Host and therapeutic risk factors for nosocomial infections were comparable in old and new nurseries. The decrease in rate of nosocomial infections therefore appeared to be due to improved staffing and environment. Improvement included 50% more nurses, increased space per infant and isolation facilities for the seriously ill patients.

Hospital Staff

It is generally accepted that the hands of staff are an important vehicle for pathogenic transfer and that hand washing makes a significant contribution to the control of hospital acquired infection (Doebbeling et al., 1992; Wenzel et al., 2002). The relevant micro-organisms can readily be demonstrated on the hands of staff and may easily be transferred to the skin of others by brief contact. The importance of such contact in colonization and infection has been demonstrated (Wenzel et al., 1991). The most important micro-organisms spread by hands are; Staph. aureus, Strep. pyogenes and other gram positive and gram negative bacilli (Zuliani et al., 2002).

The clothing of personnel can be shown to become contaminated with potential pathogens, such as Staph. aureus and, less frequently, gram negative bacilli, particularly after the handling of heavily colonized patients.
Khan et al. (1983) isolated strains of *Staphylococcus aureus* from the nasal swabs of hospital staff including doctors, nurses, bearers, orderlies, attendants and sweepers working at Services Hospital Lahore. Specimens were taken from the nose and wound of patients admitted in the surgical wards. All the *Staphylococcus aureus* strains were coagulase positive and harboured phage type 29. The same phage type was isolated from the blankets used by patients. Obviously the patients acquired infection from the contaminated surroundings and hospital workers.

Cafferky et al. (1983) isolated antibiotic resistant *Staphylococcus aureus strains* from burns, surgical wounds and traumatic skin lesions. The same strains were isolated from the patients and hospital staff. The isolate harboured phage type 29.

Zuliani et al. (2002) observed that stethoscope is in direct contact with many patients and can therefore be a vehicle in the dissemination of bacterial infections. They experimented 300 stethoscope diaphragms and found that 87% of the analyzed stethoscopes were contaminated with Gram-positive cocci, yeasts, fungi and Gram-positive and negative bacilli. They concluded that Stethoscopes presented a high rate of contamination and their use without precautions could spread nosocomial infections.

**Hospital Environment**

The environment of the hospital is equally important in transmission and perpetuation of nosocomial infections. Nosocomial infections of exogenous origin spread from person to person by direct contact, through hands of nurses, physicians’ aides, and other personnel (Crossley et al., 1979; Cross et al., 1983). Persons with an active infection and asymptomatic carriers may transmit infection to other patients and hospital inmates (Khan et al., 1983; Larson, 1988). Staphylococcus species usually spread by contact (Etienne et al., 1989; Mayer and Opal, 1989). Contaminated hands are an important source of dissemination of infections with gram-negative bacteria (Weinstein, 1991). Body secretions and excretions, in some diseases, are highly contaminated with causal agents and any direct or indirect
contact with them may lead nosocomial of infection. AIDS, Tuberculosis, Influenza, Infectious Diseases of respiratory tract and uro-genital tract, many viral infections, meningococcal infections and purulent infections spread through direct or indirect contact (Crossley et al., 1983).

Cross et al. (1983) reported a hospital-wide outbreak of septicemia due to some strains of *Staphylococcus aureus* at Walter Reed Hospital. They stated that 38% of *Staphylococcus aureus*, isolated from the hospital personnel, harbored the same phage type, which was causing septicemia in the hospital patients.

McGowan et al. (1989) observed that the gram negative aerobic bacilli, isolated from the blood of patients suffering from nosocomial infections, were more resistant as compared to those isolated from community acquired cases. They compared the susceptibility of organisms of a given species that caused community-acquired bacteremia with the susceptibilities of isolates from nosocomial cases. Antibiograms of 1077 isolates were tested against 9 antimicrobial agents during non-epidemic period, the nosocomial isolates exhibited a higher resistance rate compared to community isolates. The major factor leading to the greater prevalence of antimicrobial resistance in hospital organisms was the markedly different distribution of organisms in the nosocomial and community acquired groups. Alpuche et al. (1989) determined the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in community acquired and nosocomial strains of *Staphylococcus aureus* isolated between January 1986 and March 1989. They detected 24.2% MRSA in nosocomial strains and 5% MRSA in community acquired strains.

Uttley et al. (1989) isolated Vancomycin resistant strains of *Enterococci* (VRE) from 41 patients which were suffering from the renal disease. These vancomycin resistant enterococci were cultured from many sources including blood. The emergence of transferable high level vancomycin resistance in enterococci causing significant clinical infections was of particular importance since vancomycin was widely used as a reserve antibiotic for the management of infections with multi drug resistant Gram positive bacteria.
Wise et al. (1989) reported the occurrence of pandemic of Staphylococcal infection in the mid twentieth century. *Staphylococcus aureus* strains, originally sensitive to penicillin, took nearly 20 years from 1946 to 1966, to become this antibiotic resistant. Hospital personnel became carriers of antibiotic resistant staphylococci. These carriers further contaminated the newborn infants and hospitalized children and adults, who themselves became carriers of antibiotic resistant epidemic strains and spread them into the communities resulting in infections to others. They further reported the development of local, national and international programs for the epidemiological research, hospital surveillance and education in methods of prevention and control. Due to these control programs, however, the carrier rates of *S. aureus* among hospital personnel’s remained 33%. However, the incidence of nosocomial staphylococcal infection declined.

Meyers et al. (1989) studied 100 episodes of nosocomial bacteremia, in patients over 65 years of age, at the Mount Sinai Hospital during a period from October, 1984 to October, 1986. They isolated gram-positive organisms from 30% of the cases including *Staphylococcus aureus* from 13%, and *Streptococcus faecalis* from 10% cases. Sixty percent of the isolates were gram-negative organisms including *Escherichia coli* from 22% cases and *Klebsiella* species from 10% of patients. All the positive blood cultures were analysed for the site of acquisition of infection, source of bloodstream infection, causative microorganisms and the drug susceptibility pattern. Other factors like age, sex, underlying illness, mental status and leukocyte counts were also analyzed. Majority of the patients were female (63%) and had altered mental status (52%). Fifty percent of the infections were nosocomial and 44% were community acquired (home and nursing home).

Etienne et al. (1989) studied the incidence of pefloxacin (a fluoroquinolone) resistant *Staphylococcus epidermidis* from 1986 to 1988 at the Cardiology and Neurology Hospital in Lyon. They investigated the source of this nosocomial pathogen and traced back 22% of infections to infected fingers of staff members and 2% infections to the environment. The rate of pefloxacin resistance increased from 31% to 57% during this period. An association between the carriage rate and work
place was found.

Berk and Verghese (1989) reported the isolation of beta hemolytic group B Streptococci and gram-positive organisms like enterococci from cases of nosocomial respiratory infections. McGowan et al. (1989) isolated gram-negative aerobic bacilli from cases of nosocomial infections. Dryden and Munro (1989) reported the isolation of *Aeromonas sorbia* from 10 patients (77%) and *Aeromonas hydrophila* from 3 (23%) cases of septicemia in thirteen patients that were brought at Westmead hospital during 1983 to 1987.

Griffith et al. (1989) studied the colonization of *Pseudomonas aeruginosa* in a 36 bed mixed general medical oncology unit. They selected 283 patients, the environment and the hospital personnel; 12 of the patients were colonized at the time of admission and 10% acquired infection of *Pseudomonas aeruginosa* from the hospital. They identified 63 genetically distinct strains of Pseudomonas and concluded that only 5 out of 3 nosocomial acquisitions were due to horizontal transmission and nine such acquisitions were linked to the contaminated sink.

Brown et al. (1989) isolated *Flavobacterium meningosepticum* and *Pseudomonas aeruginosa* from the patients suffering from a respiratory problem in the medical and surgical intensive care units.

Levy and Katz (1989) isolated a virulent strain of *Pseudomonas cepacia* from a nosocomial patient receiving topical steroid therapy for eye infection due to use of contact lenses; the isolates were resistant to conventional anti-pseudomonal therapy. They developed an experimental model of *Pseudomonas cepacia* keratitis in the rabbit and this organism was considered as a cause of infectious keratitis especially in nosocomial infections.

Zaidi et al. (1989) reported an epidemic of bacteremia and meningitis caused by *Serratia mercescens* in the neonatal intensive care unit and special care nursery of general hospital in Mexico City, Mexico. The cause of epidemic was attributed to
Review of Literature

catheters and fluids used for intravenous infusion. Hand cultures were found to be positive in 16.7% of personnel working in hospitals.

Khalifa *et al.* (1989) isolated 93 strains of *Staphylococcus aureus* from in-patient wards of Ismailia General Hospital out of which 48 strains (51%) were methicillin resistant. Of these 48 strains 44 were isolated from patients and 4 were isolated from healthy individuals working in the same ward.

Cotton *et al.* (1989) studied nosocomial infections in black African infants and children in general ward of a hospital in South Africa. Among 1350 individuals admitted during 5 months period, they observed that 193 (14.3%) patients developed nosocomial infections. The major risk factors were malnutrition, less than 2 years of age and prolonged hospitalization. The most common sites of infection were lower respiratory and gastrointestinal tracts; most frequently isolated organisms were *Staphylococcus aureus* and Klebsiella species. Among the isolates 70% were found to be resistant *in vitro* against conventional antibiotics.

Patterson and Zervos (1990) observed the epidemiology of nosocomial enterococci infection remarkably similar to that of nosocomial infection caused by methicillin resistant *Staphylococci* and by multi drug resistant Gram-negative bacilli. They further reported that the most probable way these resistant bacteria are spread among hospital patients is via transient carriage on the hands of personnel, patient-to-patient and inter-hospital transmission. They further reported that antibiotic resistance is an ever-increasing problem in enterococci as these bacteria acquire and disseminate antibiotic resistance genes. A high level resistance to gentamycin has spread worldwide through various routes including plasmid mediated and aminoglycoside modifying enzymes.

Archer (1991) isolated antibiotic resistant strains of *Staphylococcus epidermidis* from patients of cardiac surgery. The skin flora of the patients, before and after cardiac surgery, was examined and it was observed that post-operative isolated strains of *Staphylococcus epidermidis* were more resistant to antimicrobial
agents. He further observed that prophylactic administration of antimicrobial agents has profound effect on the microbial flora of the skin.

Begue (1991) described that in tropical areas high mortality in neonates is due to bacterial infections and severe sepsis caused by mother-linked multi-resistant hospital strains of *Streptococcus* group B and *Escherichia coli*.

Gentry (1991) suggested that the infection control measures must take into consideration the contribution of the hospital worker as reservoir and mediator of antibiotic resistance strains. He observed that pathogenic bacteria remain adaptable to an increasingly hostile environment and a wider variety of more potent antibiotics.

Hammami *et al.* (1991) isolated *Salmonella wien* from stools of all 27 infants and from blood of 4 babies suffering from acute gastroenteritis in a single intensive care unit during a period from January to May 1988 in a Tunisian hospital. The same strain was isolated from the stools of one nurse working in the same unit.

Hekker *et al.* (1991) reported nosocomial outbreak of amoxycillin resistant non-typable *Haemophilus influenzae* causing acute bronchitis in a 23-bed unit. They observed 13 patients and two previously healthy staff members affected within a period of one month. The isolated strains were studied by various typing techniques and it was found that 13 of the isolates belonged to the same biotype indicating a cross infection.

Karim *et al.* (1991) isolated species of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella*, *Serratia* and *Acinetobacter* from cases of immune-compromised patients of bacteremia at Agha Khan University Hospital in Karachi. The isolated strains were either of nosocomial nature or were community acquired.

Lin and Huang (1991) from January 1981 to December 1988 observed 11 cases of neonatal meningitis caused by *Flavobacterium meningosepticum*. They concluded that neonate meningitis due to this organism was more frequent in
premature babies and usually appeared in nosocomial infections.

Menzies et al. (1991) isolated the strains of *Staphylococcus epidermidis* from the skin of 13 patients before and after surgery and compared them by antimicrobial susceptibility testing, plasmid profile and slime production. This organism appeared to be responsible for few cases of prosthetic value endocarditis. Three strains isolated from patients of endocarditis resembled the antibiotic resistant nosocomial strains recovered from the skin of eight patients and the environment of the operation theaters.

Nishi et al. (1991) while studying methicillin resistance of *Staphylococcus aureus* isolated this organism from the respiratory tract of patients in surgical wards, ICU and pediatric ward. They observed that 43.6% of the isolates were methicillin resistant and that frequency of isolation of MRSA gradually increased from January to August.

Ortega et al. (1991) isolated *Enterococcus faecalis* from two patients of nosocomial bacteremia in a Spanish hospital.

Schaberg et al. (1991) determined trends in the microbial etiology of nosocomial infections in 1980’s based on documented nosocomial infection reported to the National Nosocomial Infections Surveillance System (NNISS) and from the University of Michigan Hospital. Antimicrobial susceptibility testing data from both sources were also analyzed. It was observed that *Escherichia coli* infections were 23% in 1980 and decreased to 16% in 1981-1989, *Klebsiella* dropped from 7% to 5% while coagulase-negative *Staphylococci* increased from 4% to 9% and *Candida albicans* increased from 2% to 5%. The frequency of isolation of organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter* and *Enterococci* species were shown to be mildly increased.

Wong et al. (1991) reported an increase in the incidence of *Serratia mercescens* in hospital acquired nosocomial infections. They reviewed 23 episodes of *Serratia mercescens* bacteremia in 1985 and among them 17 (74%) were hospital
acquired and 6 (26%) were community acquired. Eleven patients (48%) had no clinically apparent source of infection, 5 (22%) patients had urinary tract infection, 3 (13%) had pneumonia, 2 (9%) biliary tract infection, 1 (4%) patient had intra-abdominal infection and 1 (4%) had skin and soft tissue infections.

Boyce et al. (1994) observed that chances of environmental contamination by the epidemic ampicillin-resistant strains of Enterococcus faecium were higher when case patients had diarrhea. Risk factors for acquiring the epidemic strains included proximity to other case patients and exposure to a nurse who cared other case patients. Use of gloves and gowns by hospital staff reduced the risk of cross spread of the contaminants. Savitz et al. (1994) found that in 3 patients of cervical disk surgery the skin of patients was the source of wound infection with coagulase-positive Staphylococcus aureus strains.

Sanders et al. (1994) studied, during two years period, the microbiological effects of selective decontamination of digestive tract (SDD) as prophylaxis against nosocomial respiratory tract infections. Surveillance specimens from the alimentary tract and trachea were obtained from each patient on admission and then twice weekly until 48 hours after the discharge from the unit. The isolates were identified on basis of colonial morphology. The antibiotic susceptibility of each isolate was studied using disc diffusion method. From 239 patients 5960 specimens were thus obtained and comparisons were made with placebo group. Studies revealed the evidence of nosocomial infections with aerobic gram-negative bacilli, Candida species, Enterococci, coagulase positive Staphylococci, methicillin-resistant Staphylococci, Acinetobacter species, Pseudomonas aeruginosa and cefotaxime-and/ or tobramycin-resistant Enterobacteriaceae, tobramycin-resistant Proteus, Morganella and Providencia spp.

Brett et al. (1998) assessed the prevalence of nosocomial infections at a rural government hospital during 1992 to 1995 period. Retrospective review of data regarding rates of nosocomial infections, cost to government, and infection control practices were studied. The hospital was a 653-bed rural hospital providing primary
and tertiary care. During 1992-1995 period 7,158 nosocomial infections were identified from 72,532 patients (10.0/100 admissions). Nosocomial infection rates assessed in the intensive-care unit were (67/100 of admissions; urology 30/100 of admissions; microsurgery was 29.5/100 of admissions, and newborn nursery was 28.4/100 of admissions). Urinary tract infections (4.1/100 admissions) accounted for most nosocomial infections (42%), followed by postoperative wound infections (26.8%) with a rate of 2.6/100 admissions. Nosocomial pneumonias and bloodstream infections also were common with their incidence at 13.2% and 8.0%, respectively. The highest rates were recorded on the intensive-care unit for both pneumonia (26.4/100 admissions) and blood stream infection (7.0/100 admissions). The cost to the government for nosocomial infections was estimated at US $697,000 annually (US $1=$6 Trinidad and Tobago). Poor infection control practice, inadequate hand washing facilities, lack of supplies, and non-existence of garbage cans in most of the wards.

Valero and Saenz (1998) studied the etiologic variations of nosocomial infections (NI) in the surgery departments of a university hospital. Active surveillance of NI in the departments of general, vascular and urologic surgery was undertaken in 1988 and 1996. The frequency of the presentation of different microorganisms was globally calculated and based on the localization of the infection. Microorganisms isolated were \textit{E. coli} (20.6%), \textit{Enterococcus} spp. (15.6%), \textit{S. epidermidis} (8.8%), \textit{Streptococcus} spp. (8.5%), other coagulase negative staphylococci (CNS) (5.7%), \textit{Pseudomonas} spp. (5.5%), \textit{S. aureus} (5.2%), and \textit{Candida} spp. (4.3%). On analysis of the temporal evolution an increase was observed in prevalence of gram positives (27.4% in 1988 and 46.4% in 1996). \textit{Enterococcus} sp. increased in surgical infections (5.8% in 1988 and 15.8% in 1996) and in the urinary tract (8.5% in 1988 and 25.6% in 1996). Contrary to the \textit{S. epidermidis}, the CNS increased in importance mainly in infections at the site of surgery (0% in 1988 to 5.1% in 1996). The incidence of diseases due to species of \textit{Klebsiella}, \textit{Enterobacter}, and \textit{Proteus} however, decreased.

Revathi et al. (1999) isolated a strain of \textit{Salmonella senftenberg} from burn
wounds of eight patients on a burns ward of a hospital in Delhi, India that was resistant to ceftazidime, gentamicin, chloramphenicol and ciprofloxacin. The organism, which had probably been spread from patient to patient on staff hands, produced the extended-spectrum beta-lactamase SHV-5 and the aminoglycoside-modifying enzymes AAC (3) II + AAC (6). The strain could not be isolated from stool cultures of any of the patients or staff, apart from the index patient who had a history of diarrhea and fever before admission. The outbreak ended in three weeks, after the implementation of strict hand washing.

Harbarth et al. (1999) conducted a one-week period-prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Standard definitions by the Centers for Disease Control and Prevention were used except that asymptomatic bacteriuria was not classified as a nosocomial infection. A total of 176 nosocomial infections were found among 156 of the 1349 surveyed patients (prevalence 11.6%; inter-hospital range 9.8-13.5%). Surgical site infections were most prevalent (30% of all the nosocomial infections), followed by urinary tract (22%), lower respiratory tract (15%), and bloodstream infections (13%). The most frequently isolated microorganisms were *Enterobacteria* (n = 44; 28%), *S. aureus* (n = 20; 13%), *Pseudomonas* spp (n = 17; 11%), and Candida spp (n = 16; 10%). One third of all episodes of nosocomial infections were not microbiologically documented. The overall prevalence of nosocomial infections in surgical patients (n = 562) was 16.2% compared to 8.6% for non-surgical patients (prevalence ratio, 1.9; 95% confidence interval [CI95], 1.4-2.5). In one center, the in-hospital mortality of patients with nosocomial infections was 9.2% (10/109) compared to 3.9% (25/637) for patients without nosocomial infections.

Wiener et al. (1999) identified, between November 1990 and October 1992, 55 hospital patients infected or colonized with ceftazidime-resistant *Escherichia coli*, *Klebsiella pneumoniae* or both. The infection possibly spread from patient to patient. Of the 35 admitted from 8 nursing homes, 31 harboured the resistant strains on admission. All the strains were resistant to ceftazidime,
gentamicin, and tobramycin, 96% of the isolates were resistant to trimethoprim-sulfamethoxazole and 41% to ciprofloxacin hydrochloride. Plasmid studies on isolates from 20 hospital and nursing home patients revealed that 17 had a common plasmid, which conferred ceftazidime resistance and mediated resistance to trimethoprim-sulfamethoxazole, gentamicin, and tobramycin. Molecular fingerprinting showed 7 different strain / types of resistant Kl. pneumoniae and E. coli distributed among the nursing homes.

Chastre and Fagon (2002) studied ventilator-associated pneumonia (VAP) in complicated patients on mechanical ventilation. They observed that the predominant organisms responsible for infection were Staphylococcus aureus, Pseudomonas aeruginosa, and Enterobacteria.

Stone et al. (2002) reported that Nosocomial infections (NIs) were a serious patient safety issue. Infection control personnel were responsible for implementing interventions to reduce this risk. The purpose of this systematic review was to audit the published economic evidence of the attributable cost of NIs and interventions conducted by infection control professionals and to evaluate the methods used. "Hospital acquired infections" cross-referenced with "costs," "cost analysis," "economics," or "cost-effectiveness analysis" were conducted and this included articles published between 1990 and 2000. Results were standardized into a common currency. Fifty-five studies were eligible. Approximately one quarter examined NIs in intensive care patients (n = 13). Most studies were conducted from the hospital perspective (n = 48). The costs attributable to bloodstream (mean = $38,703) and methicillin-resistant Staphylococcus aureus infections (mean = $35,367) were the largest. Increased standardization and a liaison between clinicians, economists and policy analysts to improve the economic evidence available to reduce hospital acquired infections.
Hospital Inmates

Cockroaches:

Cockroach is one of the most abundant insects present in the hospital environment. During daytime it usually hides in the drainage system, washbasin pipes, dark and moist places and gutters and during night, in search of food, it visits almost all places inside the hospitals including the operation theaters (Jessen and Wedberg, 1952). By itself the insect is harmless but it is capable of acting as a biological carrier and mechanical transmitter of a number of bacterial, viral, fungal and parasitic pathogens (Steinhaus, 1941; Okafor, 1981; Steinhaus, 1994). The germs present in the environment may stick to the bristles present on its legs and be deposited at the places it visits in search of food (Okafor, 1981). It is voracious eater and scavenges on anything it comes across; thus picking up a number of pathogens, these organisms usually remain undigested and passed out with its excreta (Macfie, 1922) contaminating the environment. In this way it disseminates a number of infectious agents in the hospital (Burgess, 1979). Jessen and Wedberg (1952) reported that filthy habits and crusorial nature of the cockroaches make them ideally suitable for the mechanical spread of certain pathogenic organisms by contaminating foodstuffs and fomites thorough contact with their infected appendages.

Macfie (1922) observed that organisms like *Mycobacterium tuberculosis*, *Mycobacterium laprae*, cyst of *Entamoeba histolytica*, *Entamoeba coli* and cyst of *Giardia intestinalis* and eggs of certain helminths including *Ascaris lumbricoides*, *Taenia saginata* and *Schistosoma haematobium* pass unharmed through the intestines of the cockroach.

Steinhaus (1941) studied the bacterial flora of cockroach, captured from campus building of Ohio State University, and isolated organisms like *Escherichia coli*, *Aerobacter cloacae*, *Streptococcus faecalis* and unidentified yeast from its body harboring infectious organisms.

Herms (1953) reported that cockroaches pick up organisms by crawling over cultures and filthy material and then during feeding deposit the bacteria on food.
Using simple bacteriological techniques he showed that these cockroaches carried on average 13370 bacteria / insect. More bacteria were found on hind pair of legs due to the fact that these legs are more constantly in contact with surfaces harbouring infectious organisms.

Roth and Willis (1957) studied the pathogenic organisms that were isolated from domestic pest cockroaches in their natural environment. They listed four strains of Poliomyelitis virus, bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, several species of *Salmonella* and *Shigella*, *Klebsiella pneumonia* and *Serratia marcescens*. The fungus isolates included species of *Aspergillus fumigtus*. The protozoan isolated from intestine of cockroach was *Entamoeba histolytica*. A number of helminthes, that infest human, were also isolated from these cockroaches.

Hiro and Fuji (1966) isolated organisms like *Pseudomonas aeruginosa*, *Staphylococcus*, *Moraxella*, *Neisseria*, *Shigella*, *Serratia*, *Actinomyces* and yeast from the surface of cockroaches in Japan. Burgess et al (1969) while studying the gut flora of wild cockroaches isolated *Bacillus* sp., *Streptococci*, *Enterobacter* and few strains of *Corynaebacterium*, *Pseudomonas*, *Micrococci* and *Acinetobacter*.

Fernandez and Zarror (1972) isolated species of *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Serratia merscens*, *Proteus vulgaris*, *Proteus mirabilis* and *Klebsiella pneumoniae* from the German cockroaches which were captured in maternity and gynecology ward of John F. Kennedy Hospital of Valdivia, Chile during a study of four months. Zuberi et al. (1972) isolated *Pseudomonas aeruginosa* and *Streptococcus citrus* strains from the surface washings of German cockroaches at PCSIR Karachi. During another experiment 13 species of those bacteria were isolated from the surface, body fat and alimentary tract of these insects (Zuberi et al. (1972).

Bhaumik and Raychaudhri (1975) isolated 22 types of bacteria from haemocoelic fluid of female American cockroaches. The isolates included *Bacillus*
spp., *Klebsiella rhinoscleromatis*, *Listeria*, *Serratia mercescens*, *Shigella dispar*, and *Staphylococcus albus*.

Cochran *et al.* (1975) reported that cockroaches are the most primitive of all the winged insects, they are medically important as they harbour pathogenic bacteria and also they serve as intermediate host for pathogenic helminths and carry the helminth’s eggs, viruses, protozoa and fungi pathogenic for man and other vertebrate animals. Cockroaches have the habits of regurgitating some of their partially digested food and dropping faces, often at the same time they are eating and as such they play an important role in pathogen transmission.

Svec *et al.* (1975) studied the microflora of 502 individual cockroaches (*Blatta germanica*) obtained from various sanitary institutions. They isolated species of *Proteus* 10%, *Pseudomonas aeruginosa* 8.5%, *Staphylococcus aureus* 5%, *Bacillus cereus* and *Streptococcus faecalis* from greater number of cases.

Frishman and Alcamo (1977) studied the role of cockroaches as mechanical vector of pathogens. They collected three species of cockroaches from different areas including hospitals, nursery schools, dairy and candy stores etc. These cockroaches were held with tweezers and walked across the surface of seven different culture media. The infected culture media yielded species of *Escherichia coli*, *Salmonella*, *Staphylococci* and *Streptococci*.

Gorden and Lavoipierre (1978) reported the injurious role of insects as destroyers of human’s crops both growing and stored. They discussed that they have been found responsible for vast famines and long sustained malnutrition over wide areas. While as vectors of diseases they have caused great sufferings and mortality to both man and domestic stocks. Further they have been incriminated as playing vital role in the world’s important epidemics and epizootic diseases.

Burgess (1979) investigated five London hospitals and proved that bacteria found on the external surface and gut of cockroach were same which were isolated from the environment from which these insects were trapped. The bacteria were
isolated both from the cockroaches and from the swabs taken from the same environment. Isolation comparison showed exact correlation between the organisms found in cockroach’s guts and those found in their environment.

Dolmierski and ldzkowski (1979) conducted bacteriological examination of 716 German cockroaches collected from the 14 ships of different ocean lines. The cultures were made from body washings and from intestines of cockroaches. A total of 391 cultures were made from which 312 various bacterial strains were isolated, among them 301 (96%) of the strains belonged to family *Enterobacteriaceae* and 4 strains belonged to genus *Salmonella*.

Burgess and Chetwyn (1981) investigated large number of cockroaches from sewers, hospitals and hotels in London. They isolated organisms like *Escherichia coli*, *Klebsiella pneumonia*, *Serratia mercescens*, *Pseudomonas aeruginosa* and *Proteus*. They also isolated *Salmonella typhimurium* from cockroaches (*Blatta germanica*) infesting a children ward in a Belgian hospital in which there was an epidemic of gastroenteritis due to the same organism.

Okafor (1981) studied bacteriology of 120 American cockroaches collected form various places and isolated 310 bacterial and fungal species from the intestine of these insects. The isolates included species of *Bacillus*, *Proteus*, *Escherichia*, *Staphylococci*, *Streptococci*, *Salmonella*, *Shigella* and five fungal genera including *Mucor*, *Aspergillus*, *Geotrichum*, *Candida* and *Trichosporon*. He further reported that the cockroaches have hair and bristle on their legs and their feeding habits involve considerable use of saliva. In this way the transmission of pathogenic bacteria takes place.

Panthora *et al.* (1981) recovered three sero-types of Salmonellae viz. *S. barilely*, *S. newport* and *S. senftenber* from the gut of cockroaches that were collected from the kitchens of Nehru Hospital, Chandigarh, India.

Razia and Jafri (1986) isolated six types of pathogenic bacteria from American
cockroaches captured from various localities of Lahore and Gujranwala. The isolated organisms included *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp.

Umunnabuike (1986) conducted a study in which 690 adult cockroaches were captured alive from domestic kitchens and near poultry house. Using selective media the external surface and gut contents were examined for the presence of Campylobacter. Four *Campylobacter jejuni* isolates were recovered; three form the gut contents and one from the external surface.

Fotedar *et al.* (1991) isolated and identified the microorganisms of medical importance from cockroaches trapped from hospitals and residential area of the same hospitals. They studied their role in the epidemiology of nosocomial infections. They reported that 158 out of 159 cockroaches collected from hospitals and 113 out of 120 cockroaches collected from residential areas (control) were carrying microorganisms of medical importance. The test cockroaches carried higher bacterial load as compared to the controls. Multiple-drug resistant bacteria were isolated from the test cockroaches. The diversity of drug-resistant bacterial species isolated from the test cockroaches suggests their involvement in the transmission of drug-resistant bacteria. Various fungi and parasitic cysts of medical importance were also isolated from the test as well as control cockroaches.

Fotedar and Banerjee (1991) isolated a variety of fungi of medical importance from cockroaches collected from hospital wards (159) and residential areas (128) in different seasons. The fungi isolated were of various species of *Candida*, *Rhizopus*, *Mucor*, *Alternaria* and *Aspergillus*. Candida species were isolated in the highest percentage from the hospital and residential area surveyed.

Fotedar *et al.* (1991) reported the role of cockroaches as vectors of drug-resistant strains of Klebsiella species at the all India Institute of Medical Sciences hospital. Klebsiella species (majority *Klebsiella pneumoniae*) were isolated from
28.3% of the hospital cockroaches and 29.1% of the infected wounds of patients. Most of these isolates, 96.3% from patients and 859% from cockroaches, showed multiple drug-resistances. The strains isolated from patients and cockroaches were similar indicating the possible role of cockroaches as vector for the nosocomial infections.

Sarmova et al. (1992) isolated 116 strains of microorganisms of medical importance from the arthropods (Cockroaches, flies, chironomus and tenebrio), collected from the premises of health institutions. They observed that 88% of the isolates were gram-negative rods and 12% of the isolates were gram-positive cocci. The bacterial isolates included Enterobacter, Acinetobacter, Klebsiella, Citrobacter and Pseudomonas spp. Of the isolated bacteria 33% of the strains showed multiple antibiotic resistances. The investigation was supplemented by the isolation of similar strains from hospital environment and nosocomial patients.

**Cats:**

The hospitals in most of the scientifically advanced countries are free from cats and other such animals that may lead to spread of nosocomial infections.

Scott et al. (1988) recorded an outbreak of epidemic methicillin-resistant Staphylococcus aureus (MRSA) on a rehabilitation geriatric ward. Intensive screening of patients and staff revealed an unusually high carriage rate in the nursing staff (38%), thought to be related to a ward cat which was heavily colonized from the environment. Adoption of various infection control measures and removal of the cat from the ward led to rapid resolution of the outbreak.

Rodriguez et al. (1993) isolated Salmonella choleraesuis from a cat suffering from pneumonia. The disease was presumed to be of nosocomial origin.

Fulton and Walker (1992) isolated Candida albicans from a cat suffering from urocystitis secondary to urethral stricture and administration of antibiotics.
Review of Literature

Muller (1997) reported that the cat-scratch disease (CSD) is known as a nosological entity since 1950. It was diagnosed by the clinical symptoms, epidemiologic data, and the intracutaneous test of Hanger and Rose. The etiologic agent is *Bartonella* (formerly *Rochalimaea*) *henselae* occurring in thirty to fifty percent of healthy cats. The gram-negative alpha-2-proteobacteria cause the CSD but also fever in healthy humans. Patients suffering from AIDS show bacillary angiomatosis, bacillary peliosis hepatis, endocarditis, and septicemia. There is an open question for other etiologic agents causing CSD as cofactors. For example, *Afipia felis* is found to a certain extent from patients suffering from CSD. Furthermore, *Rothia dentocariosa* was isolated in lymph nodes of CSD patients, and also other gram positive rods may play an important role together with *B. henselae* in CSD.

Drusin *et al.* (2000) reported an outbreak of nosocomial ringworm involved five infants in a neonatal intensive care unit. The index case was a nurse infected with *Microsporum canis* by her cat. Upon initiation of standard infection control measures, the outbreak was resolved successfully by an interdisciplinary professional collaboration of Medical Physician and Veterinary Dermatologists and infection control personnels.

Inanimate Objects:


Bennett and Brachman (1986) reported that plastic bandages have caused *Clostridium perfringes* skin infections, and contaminated blood collection tubes have caused pseudobacteremias and true bacteremias.
Zai Salar et al. (1980) were successful in isolating a number of pathogenic gram-positive cocci and gram-negative aerobic rods, from different objects in the Khyber Hospital Peshawar, mostly from the hospital wards.

Khan et al. (1983) at Services Hospital Lahore isolated strains of *Staphylococcus aureus* from the blankets of patients in the surgical ward. Ladeas et al. (1983) studied rectal swabs from 122 patients and 497 environmental swabs from several wards in order to assess the role of environmental spread of *Clostridium difficile*. The study indicated that environmental contamination is important in the spread of *Cl. difficile* in hospital patients, and the implementation of patient isolation procedure may limit this spread.

Ladeas et al. (1983) attributed the dissemination of *Clostridium difficile* in hospital to environmental contamination. Levin et al (1984) studied the sink drains in a medical and surgical intensive care unit during 6 consecutive weeks as a part of 7 months prospective study of acquisition of *Pseudomonas aeruginosa* by intensive care patients. They confirmed that sinks may be reservoir for large number of highly resistant *P. aeruginosa* but were rarely the source of organisms colonizing patients in their intensive care unit.

Mayer and Opal (1989) discussed the factors responsible for the emergence of constant changes in the nosocomial microflora. These factors include the widespread use of antibiotics, the spread of bacterial resistance plasmids, transposons and the increased use of invasive procedures. They observed that the introduction of new technologies, including enhanced life support techniques ranging from blood products to parental nutrient supplementation, also provide opportunities for non-pathogenic organisms to become true pathogens. In addition, the changes in the inanimate environment, such as ventilator system and changes in personnel and infection control protocols also alter the resident nosocomial flora. They suggested that awareness of changes in the prevalence of different organisms and their susceptibility pattern, in local and community hospitals, and to get the latest
information about the new antimicrobial agents that have the activity against multi-drug resistant strains were important to note. They further proposed to study the molecular epidemiological information to anticipate the emergence and spread of new distinctive nosocomial pathogens.

Khalifa et al. (1989) isolated 93 strains of MR *Staphylococcus aureus* from cases of nosocomial infections from in-patient wards of Ismailia General Hospital, 48 (51%) isolates were proven to be methicillin resistant (MR). Of these MR *S. aureus* strains, 44 were isolated from patients and 4 were isolated from healthy carriers, who were newly arrived interns working in the same wards. Takesue et al. (1989) isolated 214 strains of *Staphylococcus aureus* form a surgical ward. They also collected 62 airborne isolates from the operation room during 1983 to 1988.

Nakagomi et al. (1989) observed a considerable increase in the methicillin resistant *S. aureus* hospital-acquired infection. They suggested measures for control over the routes of transmission and foci of infection of multiple drug resistance strains of MRSA that circulate within the hospital wards.

Klastersky (1989) reported that compromised patients are predisposed to acquire drug resistant bacteria from hospital environment. In compromised hosts prolonged hospitalization leads to infection with gram-negative bacteria and *Staphylococcus* spp. He advised use of effective antibiotics, careful hand washing by the hospital personnel and low microbial diets to the patients for controlling these infections. Careful use of intravenous devices will help to minimize the infections of *Staphylococcus epidermidis*.

Begue (1991) stated that in tropical areas the neonatal infections are due to the multi-resistant hospital acquired species of *Enterobacteriaceae, Pseudomonads* and *Staphylococci*. The babies acquire infection during delivery or from the environment which lack hygiene. Chou et al (1991) found that *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most important nosocomial
pathogens observed during a five year study (1985-89) in a hospital causing 56.2% of all the nosocomial infections particularly the skin infections in new-born nursery.

Hammami et al. (1991) studied 27 babies in a single intensive care unit suffering from acute gastroenterities in a period from January to May 1988 in a Tunisian hospital. They isolated *Salmonella wien* from one mattress.

Wang et al. (1991) studied two outbreaks of nosocomial infection, due to multiple resistant *Enterobacter cloacae* occurring in September 1987 and then from December 1988 to January 1989, in a pediatric intensive care unit. The organisms were isolated from eight neonates who were receiving ventilator support. Plasmid analysis established that the strains from the first outbreak were different from the strains obtained in the second outbreak. Environmental survey showed that the distilled water containers were contaminated with same strains of *Enterobacter cloacae*. Change of water containers and use of aseptic techniques terminated the infection.

Callaghan (1988) examined the hypothesis that the wearing of plastic aprons during direct patient contact would reduce significantly the number of bacteria carried on nurses' uniforms, and therefore reduce the probability of the transmission of nosocomial infections. Current nursing practices and overall bacterial uniform contamination levels were investigated. The study demonstrated that these contaminations significantly contribute to the spread of nosocomial infections.

Savitz et al. (1994) isolated coagulase-positive *Staphylococcus aureus* from the gloves in one case and from the operation room in 2 cases as the source of wound infection in cervical disk surgery. The isolated nosocomial strains were resistant to various antibiotics.

Blanc (1997) isolated two genetically distinct clones of *Pseudomonas aeruginosa* from bronchial specimens of 65% patients, during or after bronchoscopy.
This epidemic was traced back to contamination of bronchoscopes during in washing machines. Reintroduction of manual disinfection of endoscopes significantly decreased the incidence of *P. aeruginosa*. This procedure reduced the incidence due to epidemic clones but not the cases due to nonepidemic *P. aeruginosa*. The distinction between sporadic and epidemic cases was possible only with the use of a molecular typing method (ribotyping).

Bert *et al.* (1998) from July 1995 to November 1996, isolated multi-resistant *Pseudomonas aeruginosa* 011, from 36 patients which were admitted to a neurosurgery intensive care unit. The strain was resistant to ticarcillin, ceftazidime, imipenem, gentamicin and ciprofloxacin, and susceptible to amikacin. Nine patients were colonized only; the remaining 27 patients had at least one infected site (17 urinary infections, 10 pneumonias and four with sinusitis). Strains of *P. aeruginosa* 011 with the same resistance pattern were isolated from tap water. The strain was also cultured from enteral nutrition solutions given to two infected patients. Changing the mode of enteral nutrition and replacement of all sinks in the unit resulted in decreased incidence. The sinks were found to be the main source of *P. aeruginosa* during this outbreak. The sink contamination had occurred via the hands of the nursing staff or nutrition solutions contaminated with tap water.

Richards *et al.* (1998) described the epidemiology of nosocomial infections in Coronary Care Units (CCU) in the United States, using the data collected between 1992 and 1997, and the standard protocols of the National Nosocomial Infections Surveillance (NNIS) Intensive Care Unit (ICU) surveillance component. Data on 227,451 patients with 6,698 nosocomial infections were analyzed. Urinary tract infections (35%), pneumonia (24%), and primary bloodstream infections (17%) were almost always associated with the use of an invasive device (93% with a urinary catheter, 82% with a ventilator, 82% with a central line, respectively). The distribution of pathogens differed from that reported from other types of ICUs. *Staphylococcus aureus* (21%) was the most common isolate from pneumonia and *Escherichia coli* (27%) from the urine. Only 10% of reported urine isolates were
Review of Literature

*Candida albicans. Staphylococcus aureus* (24%) was the more common bloodstream isolate than the enterococci (10%). The mean overall patient infection rate was 2.7%. Device-associated infection rates for bloodstream infections, pneumonia, and urinary tract infections did not correlate with length of stay, number of hospital beds, number of CCU beds, or the hospital teaching affiliation. Use of invasive devices was lower than that in other types of ICUs. Overall patient infection rates were lower than in other types of ICUs, which was largely explained by lower the rates of invasive procedures.

Bernard *et al.* (1999) conducted a study in a 450-bed general hospital to evaluate the role of stethoscopes in transmission of nosocomial infections; bacterial contamination and bacterial survival on stethoscope membranes; the kinetics of the bacterial load on stethoscope membranes during clinical use; and the efficacy of 70% alcohol or liquid soap for membrane disinfection. Among the 355 stethoscopes tested, 234 carried ≥ different bacterial species; 31 carried potentially pathogenic bacteria. Although some bacteria deposited onto membranes could survive 6 to 18 hours, none survived after the application of above disinfectants.

**NOSOCOMIAL PATHOGENS**

The organisms isolated from nosocomial infections are derived from the human body and human environment. All the living and non-living objects contribute towards the bacterial load of hospitals. The bacteria present on the body of an inanimate and within and outside the body of a living subject are by and large transferred to its environments; thus everything that happens to pass through the hospital may become source of nosocomial pathogens. Microorganisms such as *Staphylococcus aureus, Streptococcus spp., Mycobacterium tuberculosis* and many viral and fungal pathogens, are usually acquired from the animate environment. Some individuals through inadequate antibiotic therapy, during some recent infection may become immune carriers of virulent pathogenic bacteria at the time of admission or visit to hospital and may vertically transmit those pathogens to other nearby patients.
The inanimate environment of the hospital including ward dust, linen, insects, contaminated instruments and other fomites, also provide important reservoirs of bacteria and source of nosocomial infections. Gram-negative bacilli, in particular, establish reservoirs in the hospitals especially in the moist or wet areas.

Most of the nosocomial infections of exogenous origin spread from person to person by direct contact from hands of nurses, physicians’ aides, and other staff. Persons with an active infections disease, as well, asymptomatic carriers may transmit infectious pathogens. Gram-positive bacteria such as Staphylococci spread by this route. Hand contamination is an important source for spread of endemic and epidemic Gram-negative infections. Some diseases such as viral respiratory infections (meningococcal, Staphylococcal and Streptococcal infections) occur by indirect contact of contaminated secretions of patients through ventilators and endotracheal tubes.

Under-staffing with nurses and overcrowding with patients greatly increase the risk of nosocomial infections by making it difficult for the nurses to adhere to strict hygienic and aseptic procedures and by enhancing contamination of the air with infected dust.

Contaminated vehicles are the second most common mode of spread of nosocomial pathogens. In this, a contaminated inanimate vehicle such as food, water medication or medical devices, serves as medium for transmission of infectious agents, which may cause sporadic infections or outbreaks of disease. Many of the common nosocomial Gram-negative pathogens can persist or even proliferate in the moist environments.

The era of chemo and antibiotic therapies is marked with continuous development and introduction of new antimicrobial agents. These drugs are effective against a variety of microbial agents and as such are commonly known as Broad Spectrum Antibiotics and life saving drugs. As a living entity the bacteria has also
responded reciprocally against these medicines and new multi drug resistant strains of many common pathogens have emerged. During this period significant changes have occurred in the character of nosocomial infections. Antimicrobial therapy has greatly improved the prognosis of infectious diseases and it can be prescribed prophylactically to cover short periods of infection risks. Since antibiotics are widely used, sensitive bacteria causing infections continue to disappear and are being replaced by the drug resistant ones. Although a proportion of these resistant strains can be dealt with the newer antibiotics yet there are many common species that resist almost all types of antibiotics. Berk and Verghese (1989) reported that the organisms responsible for nosocomial pneumonia are continuously evolving, and that Gram-negative bacilli have become the most common nosocomial agents over the last 20 years. They further reported that Gram-positive organisms like enterococci, beta hemolytic group B Streptococci and methicillin resistant Staphylococcus aureus have taken new significance in nosocomial respiratory infections. They observed that the behaviour of etiologic agents also change with the introduction of new antibiotics.

Schaberg et al. (1991) examined trends in the etiology of nosocomial infection in the 1990s. Surveillance data on the microbiology of documented nosocomial infections reported to the National Nosocomial Infections Surveillance System and from the University of Michigan were analyzed. Anti microbial susceptibility data on selected pathogens from both sources were reviewed. Overall, Escherichia organism decreased from 23% of infection in 1980 to 16% in 1986-1989, Klebsiella pneumoniae infection rate dropped from 7% to 5%, whereas coagulase- negative Staphylococci increased from 4% to 9% and infection with Candida albicans increased from 2% to 5%. Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter species and Enterococci registered minor increase, but antimicrobial resistant strains for these pathogens as well as coagulase-negative Staphylococci were seen more frequently. In contrast to the 1970s, major shifts in the etiology of nosocomial infections have occurred in the decade of the 1980s. Taken as a whole, the shifts are away from more easily treated pathogens towards more resistant pathogens with fewer options for therapy. These shifts underscore the continued need for prevention and control to
accompany the newer developments in therapy.

In nosocomial infections the most commonly encountered organisms are the species belonging to genera *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Mycobacterium*, *Meningococcus*, *Clostridium*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Aeromonas*, *Serratia* etc.

**Genus Staphylococcus:**

The bacterial strains belonging to the genus Staphylococcus are the most common cause of nosocomial infections in man. Murtaugh and Mason (1989) and Wise et al. (1989) reported pandemic of nosocomial infections due to *Staphylococcus aureus* in mid twentieth century; the outbreaks were caused by penicillin resistant strains of this organisms.

Crossley et al. (1979) isolated strains of *Staphylococcus aureus* from 108 inpatients who had suffered from nosocomial infections during their longer hospital stay. They concluded that antibiotic resistant stains of *Staphylococcus aureus* might cause serious infections and significant mortality. Crossley et al. (1979a) isolated antibiotic resistant strains of *Staphylococcus aureus* from 201 patients over a period of 2.5 years. Of those 201 patients, 56 strains were isolated from burn patients while 145 strains were recovered from hairs, hands of personnel and from the air and inanimate objects.

Zai Salar et al. (1980) were successful in isolating a number of different types of pathogenic gram-positive cocci and gram-negative aerobic rods, from different objects in the Khyber Hospital Peshawar. Weinstein et al. (1982) isolated 22 antibiotic resistant strains of *Staphylococcus aureus* from Michael Reese Hospital, Chicago, in 1979 form 22 otherwise unrelated hospital patients.

Cafferky et al. (1983) reported that the antibiotic resistant strains of *Staphylococcus aureus* were first recorded in the Dublin hospitals in 1976. The
incidence of these strains rapidly increased especially in 1979 and by 1980 these drug resistant strains became widely disseminated. Most of the isolates were recovered from burns, surgical wounds and traumatic skin lesions. Carrol et al. (1983) isolated two new strains of methicillin and gentamycin resistant *Staphylococcus aureus* (MGRSA), from a Dublin hospital, which had affected a total of 65 patients and subsequently the same type of infection spread to a second Dublin hospital. Detailed laboratory investigations demonstrated that the “new” MGRSA organisms termed as Phenotype Ill Dublin isolates were completely different and unrelated to those isolated before 1985. These Phenotype Ill isolates were found very much similar to MGRSA organisms isolated from a Baghdad hospital during 1984. Lyon et al. (1984) studied outbreaks of nosocomial infections due to *Staphylococcus aureus* in hospitals in Australia. The strains isolated from geographically related hospitals had a uniform plasmid pattern indicating the same source of origin. Cross et al. (1983) at Walter Reed Hospital during a six month period reported a hospital-wide outbreak of septicemia due to some strains of *Staphylococcus aureus* during a six-month period. They observed that 38% of the organisms isolated from hospital personnel were of the same phage type which was causing septicemia in the hospital patients.

Khan et al. (1983) in a study at Services Hospital Lahore isolated strains of *Staphylococcus aureus* from the nasal swabs of hospital staff including doctors, nurses, bearers, orderlies, female attendants and sweepers. Specimens were also taken from the nose, wounds and blankets of patients admitted in the surgical wards. All the isolates were coagulase positive and harbored phage type 29. The prevalent Staphylococcal phage type isolated was phage type 29. It was carried in the nose of one of the nurse and one sweeper. Obviously the patients had acquired infection from the contaminated surroundings and the hospital workers.

Alpuche et al. (1989) determined the prevalence of MRSA of community acquired and nosocomial strains of *Staphylococcus aureus* isolated between January 1986 and March 1989. They observed that out of their isolated strains 24.2% MRSA were of nosocomial in origin and 5% MRSA strains were of community acquired in origin.
Berk and Verghese (1989) reported that the organisms responsible for nosocomial pneumonia are continuously evolving and methicillin resistant *Staphylococcus aureus* has taken new significance in nosocomial respiratory infections. Cotton *et al.* (1989) studied nosocomial infections in infants and children in a general ward of a hospital among black South African children and reported most frequent isolation of *Staphylococcus aureus*. Khalifa *et al.* (1989) isolated 93 strains of MR *Staphylococcus aureus* from cases of nosocomial infections from in-patient wards of Ismailia General Hospital.


Etienne *et al.* (1989) reported an increased incidence of *Staphylococcus epidermidis*, from 1986 to 1988 at the Cardiology and Neurology Hospitals in Lyon due to intensive use of pefloxacin (a new fluoroquinolone). Archer (1991) isolated antibiotic resistant strains of *Staphylococcus epidermidis* from patients who had undergone cardiac surgery.

Klastersky (1989) reported that the immune compromised patients, on prolonged hospitalization, are predisposed to nosocomial infections due to resistant strains of *Staphylococcus epidermidis* derived from the hospital environment. Immune compromised patients are usually at a higher risk of getting infected with antibiotic resistant strains of bacteria especially methicillin resistant strains of *Staphylococcus aureus*. Patterson and Zervos (1990) and Begue (1991) while studying the epidemiology of nosocomial infections reported the isolation of methicillin resistant *Staphylococcus* spp. along with other bacteria. Karim *et al.* (1991) isolated
Staphylococcus aureus from 15% cases of immuno-compromised patients suffering from bacteremia treated at Agha Khan University Hospital, Karachi.

Aoun and Klastersky (1991) stated that nosocomial pneumonia due to Staphylococcus aureus infections resulted in high mortality and morbidity. Successful treatment of pulmonary infections depends on several factors such as type of infection, offending pathogen, status of the host defense, and accurate choice of antibiotic therapy.

Kadoya et al. (1991) isolated methicillin resistant strain of Staphylococcus aureus from the cases of nosocomial bacteremia, from April 1983 to March 1990, in Nagoya University Hospital. Lee et al. (1991) during a study on nosocomial infections, in 1988 at University Hospital in Koalalumpur Malaysia, observed that patients over 50 years of age, who underwent microsurgery, cardio thoracic surgery or were treated for major burns and those who stayed in the hospital for more than 14 days, were prone to get infected with MR Staphylococcus aureus. They observed that of 148 patients, 78 were clinically ill while 70 were colonized during their hospital stay. Nettleman et al. (1991) reported that out of each 1000 patients admitted to a hospital 1.025 were prone to get nosocomial infection with methicillin resistant Staphylococcus aureus (MRSA). Chou et al. (1991) reported that Staphylococcus aureus and Staphylococcus epidermidis were the most important nosocomial pathogens isolated during a five year study (1985-89) in a hospital causing 56.2% of all the nosocomial infections particularly the skin infection in the new-born nursery.

Ichiyama et al. (1991) studied the genomic DNA fingerprinting of MR Staphylococcus aureus isolated from the nosocomial infection. Using this technique they performed an epidemiological investigation and reported 10 chromosomal types of this bacteria causing nosocomial outbreaks.

Nishi et al. (1991) and Begue (1991) studied the incidence of methicillin resistant S. aureus (MRSA) and methicillin sensitive S. aureus (MSSA) isolated in
1989. They found that 43.6% of the isolates were MRSA type and were isolated mostly from the respiratory tract specimens. They also observed that frequency of isolation of MRSA gradually increased from January to August and most of the isolates were recovered from surgical wards, ICU and pediatric ward but on the other hand MSSA did not show a similar tendency. Isolation of *Staphylococcus aureus* from cases of nosocomial infections has also been reported by Begue (1991).

Karchmer (1991) reported that the risk of nosocomial prosthetic value endocarditis (PVE) is between 1.4% to 3% with cases occurring throughout the year. Methicillin-resistant coagulase negative Staphylococci are predominant cause of nosocomial PVE and account for 60% of the cases. Intra-operative contamination with *Staphylococcus epidermidis* has led to epidemics of PVE. Prophylactic antibiotics have become useful in preventing PVE. Menzis *et al.* (1991) reported the occurrence of a reservoir of antibiotic resistant *Staphylococcus epidermidis* strains in cardiac surgery unit producing three cases of early prosthetic valve endocarditis. Based on plasmid profile, antimicrobial susceptibility and slime production, the three strains reported above resembled antibiotic-resistant nosocomial strains of *Staphylococcus epidermidis* isolated from the skin of 8 out of 13 patients of general surgery, pre- and post-operation, and from the environment of the operation theatre.

Schaberg *et al.* (1991) reported that in accordance with the observations of National Nosocomial Infections Surveillance System (NNISS) from the University of Michigan Hospital that coagulase negative Staphylococci were common cause of nosocomial infections in 1980’s and incidence of infections due to this group increased from 4% to 9% by 1989.

Tenover (1991) observed that nosocomial pathogens are frequently resistant to antimicrobial agents and an increase in the incidence of nosocomial infections due to methicillin-resistant strains of *Staphylococcus aureus* is emerging. He observed the occurrence of several new types of resistance determinants among the organisms causing hospital-acquired infections is limiting the choice of effective drugs available to the clinicians.
Ben-Hassen et al. (1992) isolated 221 strains of oxacillin-resistant *Staphylococcus aureus* from nosocomial infection in patients, in an intensive care unit, from 1985 to 1989, in Tunisia. The strains showing similar resistance pattern were also isolated from the medical staff and the hospital environments.

Tanaka et al. (1992) isolated 282 strains of drug-resistant strains of *Staphylococcus aureus* from the ward environment of a University hospital, Tunisia. The analysis revealed that 84 isolated strains were *Staphylococcus epidermidis* (30%), 65 strains were *Staphylococcus aureus* (23%) and 58 strains were *Staphylococcus haemolyticus* (21%). These results indicated that the routine testing, along with testing of the internal nares of the medical staff are necessary to monitor nosocomial transmission of infectious agents.

Parras et al. (1991) experienced a nosocomial outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in a Spanish hospital that had started in a surgical ward and spread to the other wards affecting 245 patients in one month period. The isolated MRSA strains belonged to phage type III and showed multiple-antibiotic resistance. Vindel et al. (1994) reported a dramatic increase in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Spain; in 1986 there were only 1.2% MRSA amongst all nosocomial *Staphylococcus aureus* (SA) isolates, by 1989 this percentage had risen to 44% in some hospitals causing a very serious epidemic situation in the country. The isolates were characterized by direct reverse and phage typing and for an additional local set of phages to help in differentiating these strains. The authors were able to differentiate an epidemic strain from other MRSA strains that cause sporadic hospital outbreaks.

Venditti (1994) reported that the incidence of oxacilline-resistance nosocomial *Staphylococcus aureus* infections was increasing during the period of January 1991 to March 1993. The isolation of *S. aureus* increased during the study period: of the 265 clinically significant isolates, 45 were isolated in 1990, 50 in 1991, 130 in 1992 and 40 in the first trimester of 1993. The annual rates of oxacillin-
resistant *S. aureus* (ORSA) among *S. aureus* isolates varied from 62% to 68% through these years. Most of these strains were obtained from surgery patients and/or from the surgical wounds. Based on these findings, the need for a stringent application of infection control measures was outlined.

Takesue et al. (1994) compared nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* with those caused by *Pseudomonas aeruginosa*. Institution of strict control measures reduced the incidence of nosocomial infections due to enterotoxin type B and C MRSA; this change lead to the emergence of nosocomial infections due to multi-antibiotic-resistant strains of *Pseudomonas aeruginosa*.

Pujol et al. (1994) in surveillance of a 1000 bed hospital, on nosocomial infection caused by methicillin-resistant strains of *Staphylococcus aureus* reported that 309 (33%) patients were colonized or infected and 67% of the affected patients carried MRSA. The patients infected with MRSA developed more severe underlying diseases as compared to the patients infected with methicillin-susceptible *Staphylococcus aureus* (MSSA) and the risk factors included severe disease (intensive care unit P<0.01), prior antibiotic treatment (P<0.01), and MRSA infection (P<0.01). The patients receiving intravascular catheterization for more than 48 hours were at a greater risk of being infected.

Coello et al. (1994) investigated outbreaks of nosocomial infection in 990 patients, over a period of 3 years, due to methicillin-resistant strains of *Staphylococcus aureus*. The distributions of patients with carriage, colonization or infection were investigated prospectively. It was observed that 928 patients had acquired MRSA nosocomial infection. Carrier status in 98% of the patients could be detected by screening of nose, throat and perineum. The most common sites of isolation were surgical wounds, urinary tract and skin. Auto-infection from nasal carriage or cross infection, probably by staff hands, seemed to be the most likely mode of acquisition of MRSA infections.
Huebner et al. (1994) assessed the long-term nosocomial transmission, trends in antibiotic resistance, and expression of potential virulence factors in 86 randomly selected *Staphylococcus epidermidis* blood stream isolates obtained from 80 patients over a 10 years period from a neonatal intensive care unit. The finding suggested that the distinct clones of *Staphylococcus epidermidis* could become endemic in NICU over long periods of time; as long as a decade and that nosocomial transmission plays an important role in the neonatal *Staphylococcus epidermidis* bacteremia.

Uehara (1994) reported that the nosocomial infection due to methicillin-resistant *Staphylococcus aureus* is an important and serious problem in Japanese hospitals. About 70% of the Staphylococcal isolates are MRSA. Coagulase type of methicillin-resistant *Staphylococcus aureus* were isolated from patient nasal cavities, fingertips of medical staff, swabbed material in the ward floor and patient’s lavatory. Control measures suggested improvement of hospital environmental conditions and personal hygiene of patients and hospital staff.

Nonoyama et al. (1994) surveyed 387 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) obtained from 26 hospitals in 1993 to determine whether they became resistant to arbekacin (ABK). A total of 25 ABK resistant MRSA (6.5 %) were isolated from 9 hospitals. Analysis of genomic DNA fingerprinting by pulsed field gel electrophoresis was used to confirm the classification by resistance patterns, phage typing and other biological characters. After digestion with endonuclease Sma-I, two or three types of restriction patterns were found in ABK-resistant MRSA isolated from the hospitals. They concluded that ABK- resistant MRSA might spread through nosocomial MRSA infection.

Witte et al. (1994) reported two outbreaks of nosocomial infections with MRSA, one in a urological unit in connection with Transurethral prostatectomy (TURP), and the other in an orthopedic clinic with infections after implantation of prosthetic hips. The MR Staphylococcal isolates were analyzed on the basis of typing by phage-patterns; plasmid profiles and genomic DNA fragment patterns. Main
reasons for these outbreaks were obviously mistakes in hospital hygiene and an inappropriate antibiotic prophylaxis (in the first outbreak a quinolone over about 7 days, in the second, a third generation cephalosporin). Both outbreaks could be stopped by measures of hospital hygiene including isolated or cohort nursing of affected patients, and change in the antibiotic prophylaxis.

Emmerson (1994) reported that *Staphylococcus aureus* is the most frequently isolated single bacterial species from the surgical wounds. During the years 1954-59 *Staphylococcus aureus* phage type 80/81 was responsible for 20% of the epidemics in British Hospitals. With introduction of methicillin, strains resistant to this antibiotic rapidly emerged.

Sakumoto *et al.* (1996) detected the presence of methicillin-resistance mec-A gene in the methicillin resistant strains of *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus epidermidis* (MRSE) isolated from complicated cases of urinary tract infections. All of these strains showed a low susceptibility to other broad-spectrum antibiotics. This gene mec-A was not seen in any of the methicillin susceptible strains of *Staphylococcus aureus* (MSSA). It was further observed that while controlling MRSA nosocomial infections it must be kept in mind that MRSE acted as reservoir for the gene mec-A.

Brucker (1996) described that in France the nosocomial infection prevalence rate was approximately 6 to 16% in hospitals. The epidemiological situation in Europe showed that nosocomial infections due to methicillin resistant *Staphylococcus aureus*, and other multiple antibiotic-resistant bacterial strains were over 40% of infections.

Ressnde and Figuiredo (1997) observed that methicillin resistant strains of *Staphylococcus aureus* (MRSA) frequently cause serious nosocomial infections. MR resistance is a variable characteristic and often heterogeneous within a strain. Highly resistant strains can be detected in a medium containing 25 mg methicillin /Lit and
medium with 10 mg methicillin or 6 mg oxacillin. Lit can be employed to detect heterogeneously resistant strains. The borderline-resistant and susceptible *Staphylococcus aureus* failed to grow on these media.

Sloos *et al.* (1998) determined the diversity of types of *Staphylococcus epidermidis* in a neonatal care unit of a secondary care hospital in the Netherlands. In a prospective study, specimens from nose, ear, axilla, umbilicus, and groin were taken from patients, twice a week, up to two weeks. All the isolates were typed by both pulsed field gel electrophoresis (PFGE) and antibiogram analysis. Fifty-three *S. epidermidis* isolates were obtained from 15 of 24 patients in one to four surveys. Fourteen isolates from six patients had a common PFGE pattern and were of one multi-resistant antibiogram type. The remaining 39 isolates were allocated to 24 sporadic PFGE types and were more susceptible to antibiotics. Colonization with the multi-resistant strain correlated with a long period of stay and with the use of specific antibiotics. The multi-resistant isolates were related closely to isolates of *S. epidermidis* found in a recent study in a teaching hospital in the vicinity of the secondary care hospital. Raad *et al.* (1998) observed that *Staphylococcus epidermidis* and other coagulase negative Staphylococci had emerged among the leading causes of nosocomial blood infections and that these isolates showed multidrug-resistance.

Richards *et al.* (1998) described the epidemiology of nosocomial infections from data collected between 1992 and 1997 in Coronary Care Units (CCUs) in the United States, using the standard protocols of the National Nosocomial Infections Surveillance (NNIS) Intensive Care Unit (ICU) surveillance component. Data on 227,451 patients with 6,698 nosocomial infections were analyzed. The distribution of pathogens differed from that reported from other types of ICUs. *Staphylococcus aureus* was the most common species isolated from 21% of cases of nosocomial pneumonia and from the blood of 24% of patients suffering from bacteremia. Valero and Saenz (1998) reported the etiologic variations of nosocomial infections (NI). Active surveillance of NI in the departments of general, vascular and urologic surgery was undertaken in 1988 and 1996. The frequency of the presentation of different
microorganisms was globally calculated and based on the localization of the infection. *Staphylococcus epidermidis* was isolated from 8.8% of the cases and coagulase negative Staphylococci from 5.7% of the cases. Raad *et al.* (1998) observed that *Staphylococcus epidermidis* and other coagulase negative Staphylococci have emerged among the leading causes of nosocomial blood infections and pathogenic strains show multidrug-resistance.

Tabe *et al.* (1998) tested 55 *Staphylococcus haemolyticus* isolates obtained from patients and neonatal intensive care unit staff in order to determine their source. They examined all isolates by pulsed-field gel electrophoresis (PFGE) after digestion with restriction enzymes and detected an endemic PFGE pattern in multi-resistant isolates. Their findings suggested that local spread of multi-resistant *S. haemolyticus* was hospital acquired, and that the hospital staffs functioned as a reservoir of infection with *S. haemolyticus*.

Huebner and Goldmann (1999) recognized the increasing pathogenic role of multi-antibiotic-resistant coagulase-negative Staphylococci in recent years. Bacterial polysaccharide components are involved in attachment and/or persistence of bacteria on foreign materials. Coagulase-negative staphylococci are by far the most common cause of bacteremia related to indwelling devices. These organisms are associated with central nervous system shunt infections, native or prosthetic valve endocarditis, urinary tract infections, and endophthalmitis.

Harbarth *et al.* (1999) conducted a one-week period-prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Standard definitions by the Centers for Disease Control and Prevention were used. The cases of asymptomatic bacteriuria were not considered nosocomial infections. *Staphylococcus aureus* was isolated from 13% of the cases.

Wu *et al.* (2000) observed a very high case mortality due to nosocomial pneumonia caused by MRSA at 3 Chinese hospitals. In addition to common causes,
underlying diseases, leukocytopenia, malnutrition and complex infections are also risk factors of MRSA infection. Case mortality of 64 patients with nosocomial MRSA pulmonary infections were 44%. Secondary invaders like fungi, *Klebsiella pneumoniae*, *Pseudomonas maltophilia* and *Enterococcus spp.* worsened the condition and caused high mortality.

Lodise *et al.* (2003) determined the effect of delayed therapy on morbidity and mortality associated with nosocomial *Staphylococcus aureus* bacteremia. Their study included all episodes of S. aureus bacteremia that developed 2 days after hospital admission during 1999 to 2001. Classification and regression tree analysis (CART) was used to select the mortality breakpoint between early and delayed treatment. During the 25 months study, 167 patients met the inclusion criteria. The breakpoint between delayed and early treatment derived using CART was 44.75 hours. On multivariate analysis, delayed treatment was found to be an independent predictor of infection-related mortality (odds ratio, 3.8; 95% confidence interval, 1.3-11.0; P=.01) and was associated with a longer hospital stay.

**Genus Streptococcus:**


De Galan *et al.* (1999) isolated multidrug-resistant strains of *Streptococcus pneumoniae*, between July 1995 to August 1997, from the sputum of 36 patients who were hospitalized at a Dutch medical center. Nosocomial transmission was confirmed by typing of the bacterial isolates: as all the 36
multidrug-resistant isolates shared the same genotype, serotype, and displayed overlapping drug resistance profiles. Thirty-two of the 36 (89%) patients had chronic obstructive pulmonary disease (COPD). The source of outbreak was a 76-year old patient, who had been colonized with same strain of *Streptococcus pneumoniae*. Because staff screening of the hospital and pulmonary function department was negative, patient-to-patient spread was the most likely cause of this outbreak. Brett *et al.* (1999) isolated 386 clinical strains of *Streptococcus pneumoniae*, from general practice or inpatients from 4 laboratories (Auckland, Wellington, Hamilton and Christchurch) and determined their antibiotic susceptibility patterns.

Ismail *et al.* (1997) studied, retrospectively, nosocomial and community acquired infections in a consecutive series of 191 patients aged over 60 years admitted in 1995 in Singapore. In 57.5% of patients the infection was acquired from the community, in 33% cases it was of nosocomial origin and in 9.5% cases it was result of long-term hospitalization. Common organisms cultured in community-acquired infections were *Streptococcus* species.

**Genus Enterococcus:**

Gram-positive enterococci have emerged as the most common cause of nosocomial diseases and on global bases Enterococci were isolated from 15.6% of cases of nosocomial bacteremia (Venditti *et al.* 1994; Valero and Saenz (1998). Christie *et al.* (1994) concluded that Enterococci were the most important cause of serious morbidity and mortality among critically ill children. Nicoletti and Stefani (1995) observed a dramatic increase in the nosocomial isolates of ampicillin-resistant strains of Enterococcus, these strains also showed remarkable resistance to all synergistic combinations of beta-lactam antibiotics and aminoglycosides, making it increasingly difficult to control them.

Low *et al.* (1995) observed that Enterococcus had emerged as an important nosocomial pathogen. It is the most common pathogen associated with
blood stream infections and the second most commonly isolated pathogen overall. It is now frequently recognized as a cause of super-infection in the surgical patients as the possible result of the frequent use of infective antimicrobials for prophylaxis and treatment. Both of these findings are due in part, to the intrinsic antimicrobial resistance of the enterococci. An important fact about enterococci is their ability to acquire antibiotic-resistance traits. The risk factors for nosocomial acquisition of antibiotic-resistant Enterococci (ARE) infection included use of more than three antibiotics, empiric use of antibiotics, use of third generation cephalosporins, and enteral tube feeding.

Ortega et al. (1991) reported two cases of nosocomial bacteremia due to vancomycin resistant Enterococcus faecalis, from 2 different hospitals in Spanish hospital. None of the patients had previous exposure to vancomycin. Kadoya et al. (1991) in their study, from April 1983 to March 1990, in Nagoya University Hospital, reported that of 34 cases of nosocomial bacteremia due to gram-positive Enterococci, 24 were due to Enterococcus faecalis and 10 were caused by Enterococcus faecium. Zhang (1991) reported an incidence of 13.1% nosocomial infections in a total of 1826 hospitalized patients at Hua, Shan Hospital from 1985 to 1987. The incidence of nosocomial infections, due to gram-positive cocci was 22.9%.


Watanakunakorn and Jura (1991) reviewed 196 episodes of bacteremia during a 10-year period (1980-1989) in a community teaching hospital in the USA and isolated Enterococci from nosocomial infections from 194 patients between the age groups of 70-84 years. Venditti et al. (1994) in a study identified 17 (20%) high-
level ampicillin-resistant enterococci from 83 hospitalized patients. Of these, 16 isolates were identified as Enterococcus faecium and 1 isolate as Enterococcus raffinosus.

Spera and Farber (1994) reported an increase in the prevalence of Enterococci as nosocomial pathogen over the past 15 years. They observed that nosocomial Enterococci had become increasingly resistant to antimicrobial agents, traditionally useful, in the treatment of invasive diseases due to enterococci. Vancomycin resistance, first described in clinical isolates in 1988, has disseminated worldwide. Vancomycin resistance is usually associated with high-level resistance to penicillins and aminoglycosides rendering the treatment of patients very difficult. Several investigators have reported mortality rates greater than 50% for vancomycin-resistant enterococcal bacteremia. Risk factors associated with vancomycin-resistant enterococcal bacteremia include prolonged hospital stay, neutropenia, prior oral or parenteral vancomycin use, and broad-spectrum antibiotics. Since there is no uniformly effective antimicrobial therapy for patients infected with vancomycin-resistant enterococci, prevention of infection with the rigorous application of barrier precaution and other infection control techniques was recommended.

Christie et al. (1994) reported an apparent increase in the incidence of Enterococcal bacteremia from 7 to 48 cases / 1000 bacteremias during 1986 to 1991. Eighty-three episodes of Enterococcal bacteremias occurred in 80 children between 1986 and 1992. Most community-acquired cases were in the infants in comparison with nosocomial episodes (24 /26 and 34 / 57; P < 0.01) many of them were neonates (10 / 26 and 6 / 57; P < 0.01). Nosocomial cases were associated with underlying conditions such as 56% to major surgery, 49% to immunosuppression, 30% to organ and tissue transplants, and 32% to cardiac, 25% to pulmonary, 21% to renal and 21% to hepatic disorders. Nosocomial episodes developed after a period of 32 days. There were 58 primary and 25 cases of secondary bacteremias. Thirty-two episodes were polymicrobial and 44 organisms were involved. Twenty-six percent
of the patients died. Of the 75 isolates, 82% were *Enterococcus faecalis* and 14% were *Enterococcus faecium*. It was concluded that the enterococci were the most important cause of high morbidity and mortality among critically ill children.

Boyce *et al.* (1994) recorded that ampicillin-resistant strains of *Enterococcus faecium* also showed high levels of resistance to gentamicin, and vancomycin but were susceptible to tioceplanin (vanB class vancomycin-resistance). These strains had been isolated from 37 patients during an outbreak involving a 250-bed university-affiliated hospital. Contamination of the environment, by the epidemic strain, occurred significantly more often when case patients had diarrhea. *E. faecium* strains have emerged as important nosocomial pathogens. Patients with diarrhea may cause extensive environmental contamination therefore barrier precautions, including the use of both gowns and gloves were recommended as soon as these pathogens were encountered.

Weinstein *et al.* (1996) studied the prevalence of nosocomial acquisition of antibiotic resistant Enterococci in 350 patients admitted to general medical ward and the medical intensive care unit. Rectal culture were obtained within 24 hours of admission or transfer on to the study ward and repeated at weekly intervals at the time of discharge. Antibiotic resistant Enterococci (ARE) were isolated from 52 patients; 19 were obtained at admission to study and 33 patients later acquired resistant strains. At the time of admission 5.4% were colonized with ampicillin-resistant enterococci including 1.1% that were colonized with vancomycin-resistant strains of Enterococci. Independent risk factors for nosocomial acquisition of ARE infection included use of more than three antibiotics, empiric use of antibiotics, use of third generation cephalosporins, and use of enteral tube for feeding.

Singer *et al.* (1997) observed an increase in the incidence of nosocomial infections caused by vancomycin resistant strains of Enterococci in USA since 1989. Species like *E. faecalis, E. faecium, E. raffinosus* and *E. caselialflavus* and *E. durans* have been implicated with clusters of infection. The Center for Disease Control and
Genus Pseudomonas:

*Pseudomonas aeruginosa* has emerged as the common nosocomial pathogen during the recent years. Hsueh *et al.* (1998) describing an outbreak due to this organism documents the fact that a single clone of multidrug-resistant *P. aeruginosa* can cause long-term persistence in different body sites of burn patients and that the colonization can subsequently result in various severe infections.

Most of the strains isolated from such burn patients are highly resistant to antibiotics. Valero and Saenz (1998) calculated that on global basis Pseudomonas species were isolated from 5.5% cases of nosocomial infections.

Levin *et al.* (1984) isolated during 6 consecutive weeks, large number of highly resistant strains of *Pseudomonas aeruginosa* from the sink drains from medical and surgical intensive care units. Bawn *et al.* (1989) isolated highly resistant strain of *Pseudomonas aeruginosa* from medical and surgical intensive care unit patients facing respiratory problem due to this organism. Griffith *et al.* (1989) isolated *Pseudomonas aeruginosa* from 12 cases of nosocomial infections which were admitted to a general medical oncology unit.

Berk and Verghese (1989) elucidated the role of gram negative bacilli in cases of nosocomial pneumonia and that these bacteria have become the most common causative agents over the last 20 years. Klastersky (1989) reported that the immune compromised patients are at a greater risk of developing nosocomial
infections due to gram-negative bacilli after prolonged hospitalization. McGowan et al. (1989) isolated gram-negative aerobic bacilli from the cases of nosocomial infections.

Voutsinas et al. (1989) isolated 173 multi-drug resistant strains of *Pseudomonas aeruginosa* from a case of nosocomial infection. Levy and Katz (1989) isolated virulent *Pseudomonas cepacia* from the cases of nosocomial infection in patients who had developed infectious keratitis due to wearing of contact lenses and were receiving topical steroid therapy.

Begue (1991) isolated *Pseudomonas aeruginosa* from the cases of nosocomial infections. Aoun and Klastersky (1991) isolated these organisms from severe cases of nosocomial pneumonia. Karim et al. (1991) isolated *Pseudomonas aeruginosa* from 31% cases of immune compromised patients suffering from bacteremia at Agha Khan University Hospital in Karachi.

Kadoya et al. (1991) isolated *Pseudomonas aeruginosa* from the cases of nosocomial bacteremia, from April 1983 to March 1990, in Nagoya University Hospital. Zhang (1991) in his work on nosocomial infections in hospitalized patients of Hua, Shan Hospital from 1985 to 1987 observed an incidence of 13.10% due to infection with *Pseudomonas aeruginosa* among 1826 patients. The incidence in dermatology ward was 19.8%, Medical ward 16.5%, Surgical ward 14.8%, Neurology ward 1.7% and Microsurgery ward was 12.75. Lower respiratory tract infection was the most frequent in 45.2% cases; urinary tract infection in 17% cases, wound infection in 10%, biliary tract infection in 7% and bacteremia in 5% cases. Of 271 nosocomial cases, Gram-negative organisms were isolated from 66.4% and *Pseudomonas aeruginosa* from 13.3% cases.

Armstrong et al. (1992) in a study at the selective decontamination of digestive tract (SDD) to reduce the risk of nosocomial infections in critical care patients isolated Pseudomonas from 27% of the 161 SDD cases and 30% of the 170-
placebo control group. A total of 108 Pseudomonas isolates were recovered from the environment. SDD partially suppressed the incidence of colonization of the gastro-respiratory tract but not in the rectum region.

Takesue *et al.* (1994) investigated nosocomial infections by the comparison of MRSA and *Pseudomonas aeruginosa*. They serologically classified 262 strains of *P. aeruginosa* between 1983-1991. Group E strains of *Pseudomonas aeruginosa* prevalent in 1987 were replaced by carbapenem-resistant strains *Pseudomonas aeruginosa* after 1990.

Blanc (1997) reported that during a 6-month period, two genetically distinct clones were isolated from 65% of patients with *Pseudomonas aeruginosa* being isolated from bronchial specimens obtained during or after bronchoscopy. This epidemic was due to contamination of bronchoscopes by washing machines. After reintroduction of manual disinfections of endoscopes, a significant decrease in the incidence of the epidemic clones was observed, but the incidence of non-epidemic *Pseudomonas aeruginosa* did not change. The distinction between sporadic and epidemic cases was possible only with the use of a molecular typing method.

Hsueh *et al.* (1998) observed that long-term colonization of various body sites with a multidrug-resistant *Pseudomonas aeruginosa* clone (resistant to piperacillin, cefoperazone, ceftazidime, azteonam, imipenem, cefepime, cefpirome, cofloxacin, ciprofloxacin, minocycline, and aminoglycosides) with subsequent severe infections in burn patients, has not been reported previously. The outbreak was attributed to a multidrug-resistant *P. aeruginosa* clone belonging to serogroup O:F (serogroup 0:4) by means of antimicrobial susceptibility testing, O serogrouping, and analysis of the randomly amplified polymorphic DNA patterns generated by arbitrarily primed PCR of the isolates.

Valero and Saenz (1998) studied the etiologic variations of nosocomial infections (NI) in the surgery departments of a university hospital. Active
surveillance of NI in the departments of general, vascular and urologic surgery was undertaken in 1988 and 1996. The frequency of the presentation of different microorganisms was globally calculated and based on the localization of the infection. Pseudomonas species were isolated from 5.5% of the total cases.

Traub et al. (1998) employed serogrouping (determination of O antigen) and bacteriocin typing (based on susceptibility to one or more of 18 bacteriocins) to survey 210 isolates of Pseudomonas aeruginosa from 201 patients in 8 intensive care units (ICU) during an observation period of 18 months. The most common serogroups were O1, O9, O11, and O3y and that 88 isolates (41.9%) were nonserogroupable (NT). All except 5 isolates (97.6%) were bacteriocin-typable. Bacteriocin susceptibility profiles were not predictive of serogrouping and vice versa. Workup of 19 isolates from 9 patients disclosed phenotypic variation of antibiotic susceptibility in 3 patients super infection by different strains in 4 patients and persistence (3 months) of the same strain in 2 patients, respectively. Serotyping and bacteriocin susceptibility data revealed 15 clusters of putative cross infection of 2 patients each, 8 clusters involving 3 patients each, one outbreak. Pulsed-field gel electrophoresis (PFGE) macrorestriction analysis confirmed the pediatric and surgical ICU strains as singular strains. However, the two putative outbreaks in the pneumonology ICU were due to one particular strain which had infected 13 of the 15 patients as determined with the PFGE genotypic method. Isolates of P. aeruginosa were found susceptible to aztreonam and ceftazidime.

Bert et al. (1998) isolated multi-resistant Pseudomonas aeruginosa 011, from July 1995 to November 1996 from 36 patients admitted to a neurosurgery intensive care unit. The isolate was resistant to ticarcillin, ceftazidime, imipenem, gentamicin and ciprofloxacin, and susceptible to amikacin. Nine patients were colonized only; the remaining 27 patients had at least one infected site (17 urinary infections, 10 pneumonias and 4 with sinusitis) and P. aeruginosa 011 with the same resistance pattern was isolated from tap water and the same strain was also isolated from nutritional solution given to infected patients. The sinks were presumably the
main source of *P. aeruginosa* during this outbreak, which were contaminated via the hands of the nursing staff or nutrition solutions contaminated with tap water.

Harbarth *et al.* (1999) conducted a one-week period-prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Standard definitions by the Centers for Disease Control and Prevention were used except that asymptomatic bacteriuria was not classified as a nosocomial infection. Pseudomonas species were isolated from 11% of the cases.

Buttery *et al.* (1999) in a nosocomial out break of *Pseudomonas aeruginosa* in pediatric hospitals conducted a case-control study involving 8 cases and 24 disease-matched controls. It was demonstrated that there was a significant association between *P. aeruginosa* infection and use of infected bath toys (*P*=0.004), use of bubble bath (*P*=0.014), duration of stay (*P*=0.007) and previous antibiotic exposure (*P*=0.026). Cultures from the bubble bath liquid were negative. This is the first description of a nosocomial outbreak associated with toys. These workers were cautioned against the use of water-retaining bath toys in wards treating immunocompromised children.

Arruda *et al.* (1999) isolated multi-resistant *Pseudomonas aeruginosa* strains from the patients receiving immunosuppressive and antimicrobial therapy. Mokaddas and Sanyal (1999) reported that *Pseudomonas aeruginosa* as a multi-resistant nosocomial pathogen is a major problem. The common infection sites were wounds, respiratory tract, urine, blood and intravascular lines. The patients with burns, cardiac surgery, neurosurgery, pediatric surgery, cancer, transplantation and immunocompromised status were more susceptible to acquiring infection due to multi-resistant *P. aeruginosa*.

Sartor *et al.* (2002) observed leeches as a factor contributing to nosocomial infections. A 5-year retrospective survey of *Aeromonas hydrophila* nosocomial infections at a hospital in Marseille, France, revealed infections in 5 (4.1%) of an
estimated 122 patients treated with leeches in the Hand Surgery Unit and 2 (2.4%) of an estimated 85 patients treated with leeches in other hospital units. The retrospective survey showed that the tap water of the aquarium containing leeches in Hand Surgery Unit was contaminated with *Aeromonas* species, thus causing the infection.

Alan and Sriram (2005) reported that *Pseudomonas aeruginosa* was associated with disease primarily in immuno-compromised patients, nosocomial pneumonia amongst the patients on mechanical ventilation, urinary tract infection due to presence of urinary catheter. They observed that *P. aeruginosa* so often infected hospitalized patients, was capable of metabolizing an impressive list of compounds for the generation of energy and thus often contaminated intravenous solutions, hospital equipment, and even disinfectants. Such contamination had led to epidemics in which patients had been infected with a single strain that originated primarily from a single source.

(i) Genus Enterobacter:

Wang *et al.* (1991) reported two outbreaks of nosocomial infections which were due to multi-drug resistant *Enterobacter cloacae* occurring in September 1987, December 1988 to January 1989, in a pediatric intensive care unit. On the bases of plasmid analysis the organism involved in both outbreaks were of different origin. Zhang (1991) reported an incidence of 13.1% nosocomial infections among 1826 hospitalized patients at Hua, Shan Hospital in 1985 to 1987. The incidence of nosocomial infections, due to infection with Enterobacter species was 7.7%.

Ismail *et al.* (1997) studied, retrospectively, nosocomial and community acquired infection in a consecutive series of 191 patients aged over 60 years admitted in 1995 in Singapore. In 57.5% of patients the infection was acquired from the community, in 33% cases it was of nosocomial origin and in 9.5% cases it was result of long-term hospitalization. *Enterobacter* species were isolated from 14.6% cases of nosocomial infections.
Review of Literature

Harbarth et al. (1999) conducted a one-week prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Different species belonging to the family Enterobacteriaceae were isolated from 28% of the cases.

Honderlick et al. (1999) observed various mutant strains of Enterobacter cloacae, which were found resistant to the action of broad-spectrum cephalosporins. They noticed an unusual number of isolates of these strains and to answer the question whether these Enterobacter strains had a common source, they retrospectively studied 56 strains collected from 11 wards of the hospital. Using PFGE with Spe1 restriction analysis it was observed that a clone of Enterobacter cloacae infecting 11 patients was spread over 8 wards. It was proved that cross-contamination from patients to patients with other clones was common. PFGE allowed us to point out that clonal Enterobacter cloacae has taken place in the hospital, even if there is no real outbreak.

Kaminska et al. (2002) studied 42 multi-resistant Enterobacter cloacae isolates which were considered responsible for urinary tract infections (UTIs). Those isolates were obtained from children hospitalized in the dialysis and transplantation unit. E. cloacae isolates obtained between 1994 and 1996 were found susceptible to the aminoglycosides, carbapenems and fluoroquinolones, whereas E. cloacae isolates obtained between 1997 and 1998 were susceptible only to carbapenems and fluoroquinolones. All the isolates were characterized by the polymerase chain reaction-based random amplification of polymorphic DNA and pulsed-field gel electrophoresis. It was found that the majority of multiresistant E. cloacae isolates obtained during 1997-1998, and all isolates obtained during 1994-1996, belonged to the predominant clones A or B. These strains were responsible for gastrointestinal tract colonization and UTI of renal transplant recipients for several years, and persisted as endemic E. cloacae strains in the dialysis and transplantation unit during that period.
(ii) Genus Salmonella

Hammami et al. (1991) isolated *Salmonella wien* from the stools of 27 babies and from blood of 4 babies suffering from acute gastroenterities in an intensive care unit of a Tunisian hospital during the period from January to May 1988. It was also observed that the isolates were of the same biotype and produced TEM-1 and SHV-2 beta-lactamase that was not transferable to *E.coli* by conjugation. TEM-1 may lead to the emergence of resistance to new beta-lactamase. Karim et al (1991) isolated Salmonella strains from 9% cases of one hundred immune compromised patients suffering from bacteremia at Agha Khan University Hospital in Karachi.

(iii) Genus Escherichia

Farmer et al. (1985) reported that majority of the organisms isolated from clinical specimens belonged to the genus *Escherichia coli*. Meyers et al. (1989) isolated *Escherichia coli* from 22% of 100 patients of nosocomial bacteremia in patients over 65 years of age at the Mount Sinai Hospital during a period from October 1984 to October 1986. Schaberg et al. (1991) determined trends in the microbial etiology of nosocomial infections in 1980’s based on documented nosocomial infection cases reported to the National Nosocomial Infections Surveillance System (NNISS) and from the University of Michigan Hospital. It was observed that the *Escherichia coli* infections which were 23% in 1980 had decreased to 16% in 1988-1989. Aoun and Klastersky (1991) had described the isolation of some strains of Escherichia from severe nosocomial pulmonary infections. Watanakunakorn and Jura (1991) reviewed 196 episodes of nosocomial infection including 194 patients between the age group of 70-84 years during 1980-1989 in a community teaching hospital in the USA and isolated *Escherichia coli* from a number of cases.

Zhang (1991) reported that, among 1826 patients hospitalized at Hua, Shan Hospital from 1985 to 1987, 271 developed nosocomial infections. Gram-negative organisms were isolated from 66.4% of the patients with *Escherichia coli* being
isolated form 8.9% of such patients.

Senerwa et al. (1991) isolated 78 enteropathogenic *Escherichia coli* (EPEC) strains and 151- non-EPEC from preterm neonates during an outbreak of gastroenteritis in a hospital in Nairobi. Most of the isolates showed a similar plasmid profile and were multiple-antibiotic resistance. Of the EPEC isolates 90% were resistant to gentamicin while only 37% of the non-EPEC strains were resistant to this antibiotic. Ismail et al. (1997) in Singapore isolated *Escherichia coli* from 11.8% of the cases of nosocomial infections.

Richards et al. (1998) described the epidemiology of nosocomial infections from data collected using the standard protocols of the National Nosocomial Infections Surveillance (NNIS) Intensive Care Unit (ICU) surveillance component. Data on 227, 451 patients with 6,698 nosocomial infections were analyzed. *Escherichia coli* was the most common (27%) isolate from cases of nosocomial urinary tract infections. Valero and Saenz (1998) isolated *Escherichia coli* strains from 20.6% cases of NI in patients of Coronary Care Units (CCUs) in the United States between 1992 and 1997.

**(iv) Genus Klebsiella**

Klebsiella species are the common cause of nosocomial infection and in almost all cases the infection arises from a focus either in the respiratory tract or in the urinary tract (Watanakunakorn and Jura, 1991).

Farmer et al. (1985) reported that common species of bacteria isolated from clinical specimens from general hospital belonged to *Klebsiella pneumoniae*, and they isolated Klebsiella species from 11% cases of nosocomial infections in patients over 65 years of age at the Mount Sinai Hospital during October 1984 to October 1986. Klastersky (1991) reported to have isolated *Klebsiella pneumoniae* from severe cases of nosocomial lung infections. On the bases of data collected at University of Michigan Hospital under the National Nosocomial Infections Surveillance System (NNISS), he reported that that members of genus Klebsiella
were the frequent cause of nosocomial infections in 1980’s but their incidence dropped from 7% to 5% by 1989. Zhang (1991) observed that incidence of nosocomial infections due to *Klebsiella pneumoniae* in hospitalized patients of Hua, Shan Hospital from 1985 to 1987 was 12.2%.

Watanakunakorn and Jura (1991) reviewed 196 episodes of Klebsiella infection during 1980 to 1989 in a community teaching hospital in the USA. They studied 194 patients between the age group of 70-84 years. The prevalence was found 0.76 episodes/1000 admissions. Nosocomial bacteremia occurred in 43% of episodes, *Klebsiella pneumoniae* accounted for 86% and *Klebsiella oxytoca* for 13% of the episodes. Almost 28% of the episodes were found to be poly-microbial among them *Escherichia coli* and Enterococci along with Klebsiella were the most common isolates.

Banerjee et al. (1993) attributed an outbreak of nosocomial neonatal septicemia due to *Klebsiella pneumoniae* in a nursery to environmental dissemination. Multi drug resistant strain of *Klebsiella pneumoniae* was isolated from blood of 33 (70.2%) of 47 neonates suffering from septicemia. The same strain was recovered from the neonates and environment of nursery and labor room as well.

Ismail et al. (1997) conducted a retrospective study on nosocomial and community acquired infections in 191 patients, aged over 60 years, admitted in 1995 in Singapore hospital. In 57.5% of the patients the infection was acquired from the community, in 33% cases it was of nosocomial origin and in 9.5% cases it was result of long-term hospitalization. Klebsiella species were isolated from 19.8% cases of nosocomial infections.

(v) **Genus Serratia:**

Wong et al. (1991) described that *Serratia mercescens* infection observed as hospital and community acquired infections has become ubiquitous and cannot be treated as insignificant. They reviewed 23 episodes of *Serratia mercescens*
bacteremia in 1985 and reported that among them 17(74%) were hospital acquired and 6 (26%) were community acquired. Eleven patients (48%) had no apparent source of infection, 5 (22%) patients had urinary tract infection, 3 (13%) had pneumonia, 2 (9%) had biliary tract infection, 1 (4%) patient had intra-abdominal infection and 1 (4%) had skin and soft tissue infection. They also observed that the isolates were resistant to many antibiotics. Amikacin and the third generation cephalosporins were found to be superior to gentamicin for the treatment of nosocomial *Serratia mercescens* bacteremia. Karim *et al.* (1991) isolated organisms belonging to Genus *Serratia* and *Acinetobacter* from some cases of immune-compromised patients suffering from bacteremia at Agha Khan University Hospital in Karachi. Aoun and Klastersky (1991) isolated *Serratia mercescens* from some severe cases of nosocomial pulmonary infections. Zaidi *et al.* (1989) isolated *Serratia mercescens* from the infants during an epidemic of bacteremia, and meningitis in the neonatal intensive care unit and special care nursery of general hospital in Mexico City, Mexico.

Pegues *et al.* (1994) observed the nosocomial bloodstream infections, caused by Gram-negative bacteria in 26 neonates, in a 1000-bed hospital, in which 23 patients had died. It was noted that during this epidemic the hospital’s water chlorinating system malfunctioned; the chlorine levels were undetectable and tap water contained elevated microbial levels. *Serratia mercescens* was identified in 81% of patient’s blood cultures and from 57% of the staff hand-washings. It was concluded that the incidence of Gram-negative bacteremia increased after the use of invasive procedures on neonates whose skin became colonized through bathing or from hands of hospital personnels.

**(vi) Genus Acinetobacter:**

Villers *et al.* (1998) observed that *Acinetobacter baumannii* is an important opportunistic pathogen that is rapidly evolving toward multidrug resistance and is involved in various nosocomial infections that are often severe. It is difficult to prevent *A. baumannii* infection because it is ubiquitous organism and the
epidemiology of infections it causes is complex. Three case-control studies and a retrospective cohort study were carried out in a 20-bed medical and surgical intensive care units. *Acinetobacter baumannii* was isolated from urine of 45 (31%) patients; the lower respiratory tract (26.7%), wounds (17.8%), blood (11.1%), skin (6.7%), cerebrospinal fluid (4.4%), and sinus specimens (2.2%). One death was due to infection with this organism. Antimicrobial resistance pattern and molecular typing were used to characterize the isolates. Initially, a total of 28 patients developed *A. baumannii* infection. Eleven isolates had the same antimicrobial susceptibility profile, genotypic profile, or both (epidemic cases), and 17 were heterogeneous (endemic cases). Epidemiological work confirmed that fluoroquinolone is an independent risk factor and intravenous use of fluoroquinolone favored the selection of antibiotic-resistant strains of *Acinetobacter baumannii* in endemic area. Karim *et al.* (1991) at Agha Khan University Hospital in Karachi, isolated organisms belonging to Genus Acinetobacter from some cases of immune compromised patients suffering from bacteremia. Zhang (1991) studied nosocomial infections in hospitalized patients of Hua, Shan Hospital from 1985 to 1987. Among 1826 patients, the incidence of nosocomial infection due to Acinetobacter species was 7.7%.

Okpara and Maswoswe (1994) observed nosocomial colonization of *Acinetobacter baumannii* in 87 patients of surgical intensive care unit. Clinical manifestations of pneumonia were observed in 36 patients, bacteremia in 8, wound infections in 6 and urinary tract infections in 2 patients. Sputum was the most common site of bacterial isolation. The medium time between admission and first isolation of drug-resistant strains of *Acinetobacter baumannii* was 11 days. All of the 107 isolates were resistant to cephalosporin, extended spectrum penicillins, quinolones, and Aztreonam and were susceptible to imipenem-cilastatin. Only 9 strains were susceptible to aminoglycosides. Chang *et al.* (1994) reported the clinical features and therapeutic outcomes of four patients who suffered from nosocomial multi-antibiotic-resistant *Acinetobacter meningitis*. All the four patients acquired nosocomial *Acinetobacter meningitis*, and developed multi-antibiotic resistance after
treatment with imipenem/cilastatin. The diagnosis was confirmed by bacterial culture.

Ismail et al. (1997) isolated *Acinetobacter baumanii* from 9.2% cases of nosocomial infections among 191 patients aged over 60 years admitted in 1995 in Singapore hospitals.

Webster et al. (1998) reported that sporadic infections with *Acinetobacter* spp., punctuated with prolonged outbreaks of infection, involving larger numbers of patients, and particular epidemic strains of *Acinetobacter baumannii*, have occurred in the adult intensive care unit (ICU) of Nottingham University Hospital since 1985. Almost 20% of the ICU patients, screened during 1994-1995, became colonized with *Acinetobacter* spp. Five different strains of the *Acinetobacter baumannii* were identified by random amplified polymorphic DNA fingerprinting, including the epidemic strain responsible for outbreaks of infection in 1985-1986 and 1992-1993. Environmental sampling yielded *Acinetobacter* spp. From one or more samples on four occasions; *Acinetobacter radioresistens* was the commonest isolate, and *Acinetobacter baumannii* (not the epidemic strain) was isolated on only one occasion from the environment. The long-term persistence of a potentially epidemic strain in the ICU, even during a non-outbreak period, indicates a need for the continued vigilance.

Jawad et al. (1998) observed increasing frequency in outbreaks of cross-infection with *Acinetobacter* spp, important nosocomial pathogens, during the past 2 decades in Germany. The majority of such outbreaks are caused by *Acinetobacter baumannii*. They compared the desiccation tolerance and survival time of 39 clinical isolates of *A. baumannii* with 17 sporadic strains, not involved in outbreaks, but isolated from inpatients in the same geographic area. Each of the sporadic isolates was genetically different from other by PFGE. There was no difference in the survival times of sporadic strains of *A. baumannii* and outbreak strains (27.2 versus 26.5 days, respectively; P ≤0.44) by the Wilcoxon-Mann-Whitney test. *A. baumannii*
strains have the ability to survive for a long time on dry surfaces. The outbreak strains of *A. baumannii* were significantly more resistant to various broad-spectrum antimicrobial agents than the sporadic strains.

Traub *et al.* (1999) isolated multiple antibiotic-resistant (MAR) strains of *Acinetobacter baumannii* from 3 patients in a surgical intensive care unit. The patients had developed nosocomial cross infections. Corbella *et al.* (1999) evaluated antimicrobial activity of sulbactam, from March 1995 to March 1997, in patients with non-life-threatening multi-resistant *Acinetobacter baumannii* infections. The *in-vitro* activity of the sulbactam/ampicillin combination was by virtue of the antimicrobial activity exhibited by sulbactam. Antibacterial curves showed that sulbactam was a bacteriostatic; no synergy between ampicillin and sulbactam was observed. Their study indicated that sulbactam may prove effective against non-life-threatening *A. baumannii* infections.

Gulati *et al.* (2001) studied an intensive care unit of neuro surgery and found that *Acinetobacter baumannii* was the causative agent for nosocomial infection.

**Genra Aerobacter and Aeromonas:**

Dryden and Munro (1989) reported the isolation of *Aeromonas sorbia* from 10 patients (77%) and *Aeromonas hydrophila* from 3 (23%) case of septicemia from thirteen patients at Westmead hospital from between 1983 to 1987.

**Genus Flavobacterium:**

Bawn *et al.* (1989) isolated strains of *Flavobacterium meningosepticum* from the patients in medical and surgical intensive care units facing outbreak of respiratory colonization and infection caused by this organism. In their opinion *Flavobacterium* is an uncommon cause of adult nosocomial infection. Lin and Huang (1991) observed 11 cases of neonatal meningitis caused by *Flavobacterium meningosepticum* from January 1981 to December 1988. Of the 11 cases 7 were of
nosocomial origin. The isolates were susceptible to the action of piperacillin, and resistant to ampicillin, aminoglycosides and cephalosporins. It was concluded that the meningitis in neonates was more frequent in premature babies and usually appeared following nosocomial infections.

**Genus Clostridium:**

Organisms of the Genus Clostridium rarely cause nosocomial infections. Ladeas *et al.* (1983) examined rectal swabs from 122 patients, and 497 environmental swabs from several hospital wards. Their study indicated that environmental contamination is important in the spread of *Clostridium difficile* in hospital patients, and implementation of isolation procedure may limit that spread. Bennett and Brackman (1986) isolated *Clostridium perfringens* from the skin infections in patients and traced the source back to plastic bandages. Barbut *et al.* (1994) investigated first ever nosocomial outbreak of *Clostridium difficile* associated diarrhea among patients with AIDS and isolated this organism from the stool specimens of patients. They isolated 25 identical strains from those 15 patients. Their work also indicated that the transmission of this highly resistant spore-forming Clostridium occurs by direct patient-patient to contact and was not limited to distance and space.

Barbut and Petit (2001) reported that *Clostridium difficile* was responsible for 15-25% of cases of antibiotic-associated diarrhea (AAD) and for virtually all cases of antibiotic-associated pseudomembranous colitis (PMC). This anaerobic bacterium had been identified as the leading cause of nosocomial infectious diarrhea in adults. Nosocomial *Cl. difficile* infection results in an increased length of stay in hospital ranging from 8 to 21 days. Risk factors for *Cl. difficile*-associated diarrhea included antimicrobial therapy, older age (>65 years), antineoplastic chemotherapy and the length of hospital stay. They observed that patients could be contaminated from environmental surfaces, shared instrumentation, hospital personnel hands and infected roommates. In an outbreak, *Cl. difficile* could spread rapidly throughout the hospital environment where spores could persist for months.
Yassin et al. (2001) observed that *Clostridium difficile* was a spore-forming toxigenic bacterium that caused diarrhea and colitis, typically after the use of broad-spectrum antibiotics. The clinical presentation ranged from self-limited diarrhea to fulminant colitis and toxic megacolon. The incidence of this disease was increasing, resulting in major medical and economic consequences. Although most cases responded quickly to medical treatment, infection with *Cl. difficile* colitis could be serious, especially if its diagnosis and treatment were delayed. Recurrent disease represented a particularly challenging problem. Prevention was best accomplished by limiting the use of broad-spectrum antibiotics and following good hygienic practices and universal precautions to limit the transmission of bacteria.

**Genus Haemophilus:**

Hekker et al. (1991) reported a nosocomial outbreak of acute bronchitis due to amoxycillin-resistant, non-typable *Haemophilus influenzae* in a 23-bed unit, housing patients with respiratory disorders. Within a period of one month, 13 patients and two, previously healthy, members of staff were affected. A detailed strain analysis, based on serotyping, biotyping, major outer membrane protein (MOMP) profiling, SDS-polyacrylamide gel electrophoresis, indicated that 13 of the isolates belonged to the same biotype MOMP type, indicating a nosocomial transmission and cross infection.

**Genus Mycobacterium:**

Nardell (1991) reported an increasing incidence of nosocomial tuberculosis among AIDS patients in the developed countries. Person et al. (1992) investigated the factors associated with the development of multidrug resistant tuberculosis among patients at a New York City Hospital using retrospective case study and tuberculin test survey. Twenty-three patients with tuberculosis whose isolates were resistant to isoniazid and rifampin were compared with patients with tuberculosis whose isolates were susceptible to all agents tested. Health care workers, assigned duties in wards where these patients were admitted, were also subjected to tuberculin
Review of Literature
test. *Mycobacterium tuberculosis* isolates were typed by restriction fragment
polymorphism analysis; the drug-resistant isolates (14 of the 16 isolated) showed
similar banding pattern while the drug sensitive isolates showed distinct patterns in
every case. Health care worker assigned to wards with tuberculosis patients were
more likely to develop a positive tuberculin skin test as compared to workers
performing duties in other wards. It was concluded that nosocomial transmission of
multi-drug resistant tuberculosis occurred from patient to patient and from patients
to health care workers.

Edlin et al. (1992) observed that tuberculosis due to multidrug resistant
*Mycobacterium tuberculosis* is readily transmitted among hospitalized AIDS
patients. *Mycobacterium tuberculosis* isolates from 15 of 16 cases had identical
pattern on restriction endonuclease fragmentation analysis indicating a nosocomial
transmission. Negative pressure ventilation and other measure to prevent nosocomial
transmission were recommended. Beck-Sauge et al. (1992) observed that HIV
patients and health care workers (HCW) assigned duties in HIV patient wards were
at a higher risk of getting infected with multidrug resistant (MDR) strains of
*Mycobacterium tuberculosis*. Nosocomial transmission of MDR *Mycobacterium
tuberculosis*, as determined by tuberculin test and AFB sputum smear positivity
occurred in these wards more frequently. MDR strains of *Mycobacterium
tuberculosis* were more invasive as compared to the drug susceptible strains.

Coronado et al. (1993) studied the transmission of multi-drug-resistant
strains of *Mycobacterium tuberculosis* as nosocomial infection in 16 patients. They
reported that ambulation on the wards of inadequately masked TB patients and lack
of negative pressure in isolation room probably facilitated transmission. Bouvet et
al. (1993) identified risk factors in a nosocomial outbreak of multidrug resistant
*Mycobacterium bovis* [MDRMB] tuberculosis [TB] among HIV infected patients
using a case control study with three control groups, in Paris France. Since MDRMB
is extremely rare they assumed that a single strain was responsible for all six cases.
The index case was an AIDS patient who was hospitalized in September 1989
because of MDRMB TB while other cases were five HIV infected patients who
developed MDRMB TB between January 1990 and October 1991. Nosocomial
transmission was suspected, therefore respiratory isolation precaution, for all
patients suspected of having active TB, were taken; the occurrence of new cases of
TB infection, in HIV infected patients and health care workers ceased, after the
introduction of isolation precaution.

Segal and Kalkut (1994) reported a dramatic increase in the incidence of
nosocomial multidrug-resistant tuberculosis among healthcare workers. The Center
for Disease Control and Prevention (CDC) has formulated certain recommendations
for the safety of healthcare workers from this hazard. The recommendations
included modalities like improvement of ventilation, air filtration, and ultraviolet
irradiation, use of personal protective devices for prevention of disease. Jarvis et al.
(1995) observed that after the implementation of these regulations the incidence of
MDR-TB has considerably decreased among the healthcare workers. Van-Eer et al.
(1994) reported an outbreak of multidrug-resistance Mycobacterium tuberculosis
(MDR-TB ) in hospitals in the United States. Rapid spread of these bacilli and high
mortality among immunocompromised patients, i.e. HIV-infected individuals and
AIDS patients, were observed. Factors that play a role in these outbreaks and the
prevention of MDR-TB are discussed in this article. Awareness of a possible M.
tuberculosis infection and an early introduction of measures to reduce the spread of
these micro- organisms are steps that can prevent a nosocomial outbreak. The use of
more rapid methods for culturing mycobacterium and determining their sensitivity to
antimicrobial drugs can accelerate the diagnostic process and the recognition of
multi-resistance. In view of the poor results of treatment of MDR-TB, prevention
should be the first requirement.

Waxman et al. (1995) observed that tuberculosis, after decades of decline,
has emerged as a global health challenge. In the setting of HIV immuno-
compromise, the tuberculosis occurs frequently, early, and often atypically.
Increased prevalence of treatment failure drug-resistant strains, and nosocomial
transmission are complicating the diagnosis and control of TB. They also described methods for early diagnosis, isolation, appropriate therapy, and environmental control that will protect staff and patients from this disease. Cole and Telenti (1995) observed a marked increase in the number and gravity of tuberculosis cases in the developing countries and the industrialized nations that may in part be attributed to the pandemic of AIDS and in part to the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis*.

Brewer and Colditz (1995) reviewed the available studies on the efficacy of BCG vaccine in health care workers in USA. The 60 years old practice of mandatory BCG vaccination was discontinued in 1988 but due to nosocomial epidemic of tuberculosis and the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis*, the Health Committee on Immunization has reconsidered its decision.

Stroud *et al.* (1995) evaluated the infection control measure implemented after the outbreak of multidrug-resistant (MDR) tuberculosis in a New York hospital. The disease was seen in 38 AID patients before and only 1 AID patient after the implementation of control measures. Tuberculin test conversion rate, among the health care workers, who became positive before the implementation of control measures, remained unchanged. Maloney *et al.*, (1995) and Wenger *et al.*, (1995) evaluated the efficacy of the control measures recommend by Center for Disease Control and Prevention. They looked for evidence of exposure to HIV ward MDR-TB patients positive for acid-fast bacilli in sputum during initial and follow up periods. Among MDR-TB patients and healthcare workers with tuberculin-skin-test conversions on the HIV ward, exposure before implementation of control measures to infectious MDR-TB patients on the HIV ward, was recorded in 12 of 15 (80%) MDR-TB patients during the initial period, and 5 of 11 (45%) MDR-TB patients during follow up. After implementation of control measures, no episodes of MDR-TB could be traced to contact with infectious MDR-TB patients on HIV ward. Skin test conversions among workers on the HIV ward declined from 7 of 25 (28%)
during the initial period to 3 to 17 (18%) in the early and 0 of 23 in the late follow-up periods. Skin-test conversions among health care workers were not associated with increased exposure to MDR-TB patients, and were not significantly higher among workers on HIV ward.

Ikeda et al. (1995) studied infection control practices and skin test results of health care workers (HCW) in a New York hospital. Medical reports and bacteriology of isolated strains was studied. The infection source was a single patient suffering from tuberculosis due to a seven-antibiotic-resistant strain of Mycobacterium tuberculosis. The MDR-TB strain recovered from this patient was also recovered from other inmate and non-inmate patients. Skin test conversion occurred among 46 out of 696 health care workers (HCW). The risk factors included prolonged stay of source of infection in the hospital and inadequate environmental control. Franzetti et al. (1999) observed a high incidence (27.77%) of isoniazid and rifampin resistant Mycobacterium tuberculosis in AIDS patients reducing the medium survival time to 94 days.

Genus Corynebacterium:

Lagrou et al. (1998) characterized, all clinical isolates of catalase-positive Coryneform organisms, isolated during the routine processing of clinical specimens, in the laboratory of the 1800-bed University Hospital of Leuven, during a 6-month period. The species isolated were Corynebacterium amycolatum 70 (53%) samples, Corynebacterium jeikeium 16 (12%), Corynebacterium stratum 11 (8%), Corynebacterium afermentans 10 (7%), Corynebacterium minutissimum 9 (6%), CDC coryneform group G 4 (3%), Corynebacterium urealyticum 4 (3%), Corynebacterium glucuronolyticum 1 (0.7%), and Corynebacterium xerosis 1 (0.7%) sample. Of the 150 isolates, 37 (25%) were considered to be infection related and the remaining 113 (75%) isolates were of questionable clinical significance.

Yeast and Fungi:

Schaberg et al. (1991) using the information of National Nosocomial
Infections Surveillance System (NNISS) from the University of Michigan Hospital reported that *Candida albicans* was isolated from a number of cases of nosocomial infections and that the incidence of Candida infection increased from 2% in 1980 to 5% by 1989. Joshi and Hamory (1991) reviewed literature from 1965 to 1989 during which 6 documented and 1 non-documented cases of Endophthalmitis caused by non-albican species of Candida (NAC) have been reported. These infections are more common in immunocompromised patients.

Krcmery *et al.* (1998) analyzed 41 episodes of breakthrough fungaemia occurring over a 7.5 year period in the National and St Elizabeth’s Cancer Institutes in Bratislava, Slovakia. Five of the episodes occurred during prophylaxis with fluconazole (one *Torulopsis glabrata*, one *Hansenula anomala*, two *Candida Krusei* and one *Candida parapsilosis*), ten with itraconazole (three *Trichosporon pullulans*, one *Trichosporon beigelii*, one *Cryptococcus laurentii*, three *Candida albicans* and two *T. glabrata*), 11 during prophylaxis with ketoconazole (one *Candida norvegensis*, one *C. parapsilosis*, one *C. krusei*, one *Candida tropicalis*, five *C. albicans*, one *Candida stellatoidea* and one *C. laurentii*) and 15 during empirical therapy with amphotericin B (ten *C. albicans*, two *T. beigelii* and three *Candida lusitaniae*). The most frequent risk factors for breakthrough fungaemia were neutropenia, previous therapy with multiple antibiotics and recent catheter insertion. Comparing these episodes with 38 non-breakthrough fungaemias (appearing at the same institute in the same period) differences in certain risk factors were noted: breakthrough fungaemias were more frequently observed in patients with acute leukaemia (39.0% vs 5.2%, *P*<0.001), mucositis (34.2% vs 13.1%, *P*<0.05), prophylaxis with quinolones (58.5% vs 15.8%, *P*<0.0001) and catheter-associated infections (29.3% vs 2.6%, *P*<0.003). In this subgroup overall mortality (36.6% vs 28.8%) or early attributable mortality (22.0% vs 23.6%) were not significantly different (*P*>0.05).

Richards *et al.* (1998) described the epidemiology of nosocomial infections in Coronary Care Units (CCUs) in the United States on the basis of data collected between 1992 and 1997 using the standard protocols of the National Nosocomial
Infections Surveillance (NNIS) Intensive Care Unit (ICU) surveillance component. Data on 227,451 patients with 6,698 nosocomial infections showed that the *Candida albicans* was isolated from the urine of 10% cases of nosocomial infections.

Valero and Saenz (1998) studied the etiologic variations of nosocomial infections (NI) in the surgery department of a university hospital. Active surveillance of NI in the departments of general, vascular and urologic surgery was undertaken in 1988 and 1996. The frequency of the presentation of different microorganisms was globally calculated and based on the localization of the infection. Candida species were isolated from 4.3% of the cases.

Harbarth *et al.* (1999) conducted a one-week prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Standard definitions by the Centers for Disease Control and Prevention were used except that the asymptomatic bacteriuria was not classified as a nosocomial infection. Candida species were isolated from 10 percent of the cases.

**UnCommon Organisms**

Farmer *et al.* (1985) isolated *Proteus mirabilis* from a number of clinical specimens obtained from a general Hospital. Aoun and Klasterisky (1991) reported the isolation of *Pneumocystis carinii* nosocomial pulmonary infections. Mounib *et al.*, (1994) reported the isolation of *Pneumocystis carinii* from Human Immunodeficiency Virus (HIV)-seronegative patients. A telephonic survey of 19 hospitals revealed the presence of 9 patients with *Pneumocystis carinii* nosocomial pulmonary infections; all these cases were observed in AIDS patients wards. This adds to the growing concern for hospital acquired-infections, including drug-resistant tuberculosis, and other opportunistic pathogens.

Bjerke *et al.* (1991) isolated multi-resistant *Xanthomtous maltophilia* from very sick surgical ICU patients who acquired nosocomial infections. This infection not only prolonged the hospital stay but also increased the mortality rate.
Implementation of infection control measures and use of gloves and gowns by the hospital staff reduced the incidence of infection. Amano *et al.* (1999) reported two case of nosocomial pneumonia in polymyositis patients caused by multi drug-resistant strains of *Stenotrophomonas maltophilia*.

**Drug Resistance:**

The era of chemo and antibiotic therapies is marked with continuous developments and introduction of new antimicrobial agents (Wise *et al.*, 1989). These drugs are highly effective against a variety of microbial agents and are therefore known as Broad Spectrum Antibiotics, life saving drugs. On the basis of survival of the fittest, the bacteria has also developed an inverse response against these drugs, and evolved into the multi drug resistant strains. Simultaneously, significant changes have occurred in the character of nosocomial infections. Antimicrobial therapy has greatly improved the prognosis of infectious diseases. Antibiotics are also being used for the preventive purpose, during surgical procedure, to cover short periods of increased risk of getting infection (Alpuche *et al.*, 1989; Uthley *et al.*, 1989). Wide spread use of antibiotics may lead to the suppression of drug sensitive strains and selection of drug resistant bacterial strains. Murtaugh and Mason (1989) observed that pandemic of nosocomial infections due to various species of Staphylococci in 1950’s was triggered by this selective mechanism. The scientists are fighting for better action of drugs and the bacteria for their survival.

Sulphadrugs were discovered in 1930 and Penicillin in 1940. The actual use of these drugs, in human medicine, was started in the middle of twentieth century. It was presumed that these drugs are cure for all the bacterial diseases but the later clinical observations proved the futility of this hypothesis (Wise *et al.*, 1989; Mayer and Opal, 1989; Gentry, 1991). Bacteria become superbugs by "learning" to secrete enzymes that destroy the antibiotics that once used to kill them. Bacteria and viruses are simple and primal organisms that can change themselves very fast for their survival. It was observed that many infectious agents
were failing to respond to antibiotic therapy to which they had good responses in the past; and the strains of bacteria resistant to these agents were repeatedly isolated from many patients (Bennet and Brachman, 1986; Mayer and Opal, 1989; McGowan et al., 1989; Karim et al., 1991). Work on the development of new drugs lead to the discovery of broad-spectrum antibiotics, and examination of drug resistant strains lead to the discovery of plasmids and other factors.

Yates (1999) reported that the increasing antibiotic resistance rates among bacterial pathogens have resulted in increased morbidity and mortality from nosocomial infections. Widespread use of certain antibiotics, particularly third-generation cephalosporins, had shown to foster development of generalized beta-lactam resistance in previously susceptible bacterial populations. Reduction in the use of these agents (as well as imipenem and vancomycin) and concomitant increases in the use of extended-spectrum penicillins and combination therapy with aminoglycosides had shown to restore bacterial susceptibility. Cooperative interaction among infectious-disease physicians, clinical pharmacists, microbiology-laboratory personnel, and infection-control specialists was essential to provide useful suggestions regarding antibiotic choice and its dose to the prescribing physician. Several hospitals had implemented antimicrobial resistance management programs based on these findings. The results of these programs validate the use of a multi-disciplinary, education-based, antibiotic-resistance management approach.

A brief review of literature on the fight amongst the pathogen host and the clinician is presented below.

Most of the drug resistant bacterial strains were isolated from in-patients receiving various kinds of antibiotic therapy (Takesue et al., 1989). These bacterial strains flourished in the hospital environment (McGowan et al., 1989), found their way into the indoor patients and caused severe and sometimes fatal nosocomial infections (Crossley et al., 1979; Ryan, 1989).
Crossley et al (1979) reported that the antibiotic resistant strains of *Staphylococcus aureus* might cause serious infections and significant mortality. These strains first appeared in the Dublin Hospitals in 1976, rapidly increased especially in 1979 and 1980 and became widely disseminated. Cafferky et al. (1983) isolated two new strains of methicillin and gentamicin resistant *Staphylococcus aureus* (MGRSA), from the hospital in which a total of 65 patients were affected and subsequently the same type of infection spread to another Dublin Hospital. Laboratory findings demonstrated that the “new” MGRSA organisms termed as Phenotype III Dublin isolates were completely different and unrelated to those which were isolated prior to 1985. These Phenotype III isolates were found very much similar to MGRSA isolated from a Baghdad Hospital during 1984.

Lyon et al. (1984) investigated the multiple antibiotic resistant *Staphylococcus aureus* isolated from outbreaks of nosocomial infections in Australia. They reported that their isolates possessed, essentially, similar patterns of antibiotic resistance and related plasmids that were observed in all isolates from the hospitals present in the same geographical area. The homogeneity of these organisms suggested that the dissemination of a multi-resistant, plasmid bearing strain of *Staphylococcus aureus* or its derivatives, among geographically related hospitals, in Australia, was from some single source.

Duval (1985) presented a paper on the present state of knowledge regarding the sensitivity and resistance of various bacterial species to macrolides, lincosamides and streptogramins (MLS). This paper was limited to a description of the evolution of different types of resistance in the light of decisive factors described in previous papers, in order to deduce, trends for future strategy in therapeutics.

Bennet and Brachman (1986) reported that 25% of 35% of hospitalized patients received systemic antibiotics at any given time. They further stated that there was a trend towards increasing rather than decreasing antibiotic use within the
hospitals. They presented data of four prevalence surveys, carried out at the Boston City Hospital during January and February 1964, 1967, 1970 and 1973. In 1964, 26% of patients in that hospital were receiving, at least, one systemic antibacterial agent: in 1967 the figure was 27%, in 1970 it was 34%, and in 1973, 36%. In the 1973 survey, 76% of patients receiving antibiotics were considered to have an active infection at the time.

Werk and Schneider (1988) reported that Ciprofloxacin has a reduced activity against various anaerobic pathogens. Therefore, a combination of ciprofloxacin with an antimicrobial agent active against anaerobes, such as metronidazole, seems to be interesting for the treatment of mixed aerobic/anaerobic infections. High metronidazole concentrations (10 mg/l or 40 mg/l) neither affected the bactericidal efficacy of ciprofloxacin on aerobic pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, nor on the anaerobic pathogens *Clostridium perfringens* and *Clostridium difficile*, as demonstrated by kill-kinetic curve of the antibiotics. The same high concentrations, as well as lower therapeutically achievable concentration (2 mg/l or 5 mg/l) of metronidazole in combination with ciprofloxacin were slightly more potent for the tested clostridia than the potency of ciprofloxacin or metronidazole alone.

Gruneberg *et al.* (1988) evaluated the antibacterial activity of ofloxacin, against a wide range of clinical bacterial isolates and compared its activity with that of nalidixic acid, norfloxacin, eneroxacin, pefloxacin and ciprofloxacin by determining minimum inhibitory concentrations (MICs). Ofloxacin was found very active against the nalidixic acid-susceptible isolates of the Enterobacteriaceae (MIC ≤ 0.12 mg/l) and was also active against strains resistant to nalidixic acid (MIC ≤ 2 mg/l). The activity was similar to norfloxacin, enoxacin and pefloxacin but some four-fold less than that of ciprofloxacin. All of the fluoroquinolones were highly active against *Vibrio cholerae* (MIC ≤ 0.015 mg/l), *V. parahaemolyticus* (MIC ≤ 0.12 mg/l) *Aeromonas hydrophila* (MIC ≤ 0.03 mg/l), *Plesiomonas shigelloides*
(MIC ≤ 0.015 mg/l), *Campylobacter jejuni* (MIC ≤ 0.5 mg/l), Neisseria spp., *Haemophilus influenzae*, *H. ducreyi*, *Bordetella pertussis* and *Legionella pneumophila* (MIC ≤ 0.06 mg/l for all species). Ofloxacin, ciprofloxacin and pefloxacin (MIC ≤ 1, 2 and 2 mg/l, respectively) showed similar activity against the *Staphylococcus* spp. and were somewhat more active than the enoxacin (MIC ≤ 4 mg/l) and norfloxacin (MIC ≤ 8 mg/l). Ofloxacin was moderately active against beta-haemolytic *Streptococcus* spp. (MIC ≤ 2 mg/l), *Corynebacterium diphtheriae* (MIC ≤ 1 mg/l) and *Cory. jeikeium* (MIC ≤ 2 mg/l) and somewhat less active against alpha- and non-haemolytic *Streptococcus* spp., *Str. pneumoniae* and *Listeria monocytogenes* (MIC ≤ 4 mg/l for all species) and *Str. faecalis* (MIC ≤ 8 mg/l). The activity of ofloxacin, against these species, was similar to ciprofloxacin and four to eight times greater than norfloxacin, enoxacin and pefloxacin. Ofloxacin, and all of the fluoroquinolones, were less active against anaerobes than the aerobes. *Clostridium perfringens* (MIC ≤ 1 mg/l) was more susceptible to ofloxacin than were other anaerobic species and the *Cl. difficile* (MIC ≤ 16 mg/l) was more resistant. Ofloxacin was the most active compound tested against *Chlamydia trachomatis* SA2f (MIC ≤ 0.5 mg/l) with only ciprofloxacin (MIC ≤ 1 mg/l) approaching similar activity.

Mayer and Opal (1989) discussed the factors responsible for the emergence of constant changes in the nosocomial microflora for the selection of highly resistant and virulent bacteria. These factors included the widespread use of antibiotics, the spread of bacterial resistance plasmids, transposons and the increased use of invasive procedures. They reported that the introduction of new technologies, including enhanced life support techniques ranging from blood products to parental nutrient supplementation also provide opportunities for non-pathogenic organisms to become true pathogen. In addition, the changes in the inanimate environment, such as ventilator system and changes in the personnel and infection control protocols also alter the resident nosocomial flora. They suggested improved awareness of prevailing pathogen and susceptibility pattern in local and
community hospitals. Better information about the new antimicrobial agents that have activity against multi-drug resistant strains was also recommended.

Murtaugh and Mason (1989) reported implementation of various nosocomial infection control programs after the staphylococcal pandemic of 1950’s. In veterinary medicine the prevalence of hospital infection is also expected to increase with the use of invasive monitoring techniques, prolong hospital stay, and widespread use of antimicrobial agents. They proposed the establishment of a nosocomial infection control committee especially at the larger teaching and referral centers. Which should monitor activities like standards of hygiene at the level of hospital, personnel hygiene of health-care workers, handling of infected patients, and antisepsis of surgical and other instruments. They laid down a strict policy for antibiotics use in the hospital pharmacy as the careless use of antibiotics significantly increased the resistant microflora and predisposed hospitalized patients to nosocomial infections. They also suggested continued staff education for successful infection control programs.

Nakagomi et al. (1989) reported considerable increase in the incidence of methicillin resistant Staphylococcus aureus strains in cases reported as hospital-acquired infections. It was observed that the methicillin resistant strains have developed resistance against all forms of beta-lactams, aminoglycosides and many other antibiotics. They suggested continuous efforts to solve the problem of multiple drug resistance strains of MRSA that circulate within the hospitals and their wards, and control over foci of infection and their routes of transmission.

Ryan (1989) observed a change in the pattern of infections from 1980’s and advised appropriate use of antibiotics in the treatment of nosocomial infections because the incidence of infections by gram-positive and gram-negative bacteria, resistant to antibiotics, was increasing and many traditional drugs or their combination had lost their efficacy against those organisms.

Uttley et al. (1989) isolated vancomycin resistant strains of Enterococci
Review of Literature

from 41 patients suffering from renal disease. The emergence of transferable high level vancomycin resistance strains in Enterococci was of great clinical significance, because vancomycin is regarded as drug of choice in the management of infections with multi-drug resistant strains of gram-positive organisms.

Wise et al (1989) reported that penicillin resistant strains of *Staphylococcus aureus* emerged between 1946 – 1966; these strains were the cause of Staphylococcus -pandemic that appeared in the mid twentieth century. Hospital personnel became carriers of antibiotic resistant staphylococcus strains and contaminated the new-born infants and hospitalized patients both adult and young; in turn these patients themselves became carriers of antibiotic resistant epidemic strains and spread them in to communities resulting in an out break. They further reported that local, national and international programs emerged for the development of epidemiological research, hospital surveillance and education in methods of prevention and control. With implementation of measures the carrier rates of *Staphylococcus aureus* among hospital personnel’s dropped to 33% and the incidence of nosocomial Staphylococcal infection also declined.

Takesue et al. (1989) studied 214 strains of *Staphylococcus aureus* that were isolated form a surgical ward, and in addition 62 airborne isolates collected from operation rooms from 1983 to 1988. They observed a significant increase in the incidence and frequency of highly methicillin-resistant strains of *Staphylococcus aureus*, not encountered before 1983. It was observed that the use of disinfectants like chlorohexidine and alcohol significantly decreased the MRSA incidence in 1988. It was further observed that all the airborne isolates were methicillin sensitive. It was concluded that cross infection with MRSA took place in the surgical ward rather than in the operation theatres. Alpuche et al. (1989) reported that of the *Staphylococcus aureus* isolated from nosocomial infections, 24.2% were MRSA while of the community acquired strains 5% were MRSA. Khalifa et al. (1989) reported that all the 93 isolates of *Staphylococcus aureus* were methicillin resistant. Nakagomi et al (1989) reported an increased incidence of
methicillin resistant *Staphylococcus aureus* strains in the cases suspected as hospital-acquired infection.

Voutsinas *et al.* (1989) studied resistance of 173 strains of *Pseudomonas aeruginosa*, isolated from the nosocomial infections, against Cefepime (a cephalosporin) and compared it with ceftazidime and cefotaxime, piperacillin, imipenem, gentamicin, amikacin and ciprofloxacin. It was concluded that cefepime antibacterial activity was comparable with ceftazidime and its antibacterial action was superior to that of cefotaxime, piperacillin, gentamicin and amikacin but was inferior to imipenem and ciprofloxacin.

Zaidi *et al.* (1989) studied an epidemic of bacteremia and meningitis caused by *Serratia mercescens* in the neonatal intensive care unit and special care nursery of general hospital in Mexico City, Mexico. They recorded an incidence of 19.9% of bacteremia and meningitis. The catheters and intravenous fluids, prepared during the wars, were found contaminated with bacteria. In case of asymptomatic neonates 68% carried bacteria while hand cultures of 16.7% of personnel were found contaminated. All the isolates were found to be resistant to all aminoglycosides and Broad Spectrum Penicillins.

Patterson and Zervos (1990) reported the isolation of methicillin-resistant Staphylococci along with other bacteria. Berk and Verghese (1989) reported that the organisms responsible for nosocomial pneumonia are continuously evolving and methicillin-resistant *Staphylococcus aureus* has taken new significance in the respiratory infections.

Hammami *et al.* (1991) observed 27 babies, in an intensive care unit, suffering from the acute gastroenteritis, during a period from the January to May 1988, in a Tunisian hospital. *Salmonella wien* was isolated from stools of all babies and from blood of 4 babies. The same organism was isolated from the stool of one nurse and from a mattress. All the isolates were of the same biotype and showed the same antibiotic resistance pattern and were susceptible to a combination of
Review of Literature

cefotaxime and clavulanic acid. The outbreak was stopped by implementing infection control measures.

Hekker et al. (1991) reported a nosocomial outbreak of acute bronchitis due to amoxycillin-resistant, non-typable Haemophilus influenzae in a 23-bed unit, housing patients suffering from respiratory disorders. The epidemic was controlled by segregation of patients and infected nurses and treatment of patients with cotrimoxazole.

Veyssier (1991) suggested the use of wide-spectrum antibiotics that are not susceptible to bacterial resistance mechanism, for the treatment of very sick ICU patients, it was feared that the use of such drugs may lead to the colonization of patients with drug-resistant nosocomial organisms.

Aoun and Klastersky (1991) observed that mortality and morbidity of nosocomial pneumonia remained high and successful treatment of pulmonary infections depended on several factors including type of infection, offending pathogen, status of host defense and adequate choice of antibiotic therapy. The most frequent causes of nosocomial pneumonia were organisms like Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus and Streptococcus pneumoniae. Pneumocystis carinii infections were common in the immuno-compromised patients. They reported that fluoroquinolones, cephalosporins, imipenem, aztreonam and ticarcillin were comparatively effective in treatment of nosocomial pneumonia. They advised that aminoglycosides should not be used alone, as the combination therapy reduced the risk of selection of Gram-negative resistant mutants. Routine use of such treatment should be limited against Pseudomonas aeruginosa, Enterobacter cloacae and Serratia mercescens infections.

Begue (1991) reported that the severe neonatal infections with Streptococcus group B and Escherichia coli are derived from mother. In the tropical areas the infections with members of Enterobacteriaceae, Pseudomonads and
*Staphylococci* are derived from the hospital environment, and the infected staff helping in delivery of newborns. He further stated that antibiotics of choice for treatment were third generation cephalosporin as the causative pathogens are resistant to commonly used antibiotics.

Chou *et al.* (1991) observed that the *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most important nosocomial pathogens causing 56.2% of the nosocomial infections in new-born nursery, particularly, the skin infection. They adopted three disinfection procedures to determine and to decrease the neonatal staphylococcal colonization rate in neonatal umbilical cords. They collected 1578 swabs from neonatal nares and umbilical cords. During 1st sampling no disinfectant was applied, while during the 2nd sampling beta-iodine in alcohol and during the 3rd sampling bacitracin ointment was applied. The result showed a significant difference in the staphylococcal colonization rate using different disinfectants even after the first day. The same results were obtained for neonatal nares after the application of disinfectants especially after their third application. No cure of skin infection was found. The isolated nosocomial pathogens were found resistant to ampicillin, erythromycin, tetracycline and penicillin, among neonatal nares isolates the resistance was observed as 44% and among umbilical cord isolates the resistance against said antibiotics was found to be as 56%. Choice of an effective disinfectant (especially like bacitracin ointment) to reduce the staphylococcal colonization in newborn nurseries was suggested.

Gentry (1991) discussed the high adaptability of pathogenic bacteria to increasingly hostile environment and a wider variety of more potent antibiotics. Organisms acquired resistance to newer antimicrobial agents. Plasmids and transposons are particularly important in the evolution of antibiotic resistant bacteria. In hospitals, where antibiotic are commonly used to combat infection, the nosocomial flora has evolved many resistant strains, as such the nosocomial infections are more severe and stubborn than the community acquired infections. Archer (1991) observed increased drug-resistance in the coagulase-negative
Review of Literature

Staphylococci, isolated from the skin of same patient, before and after the administration of anti-microbial therapy.

Wong et al. (1991) reported an increased incidence of nosocomial infections due to Serratia mercescens. They observed that these isolates were resistant to many antibiotics. Amikacin and third generation cephalosporins were found to be superior to gentamicin for the treatment of nosocomial Serratia mercescens infection.

Deng and Wang (1991) studied the cause of bacterial sepsis in 70 patients from July 1988 June 1989. They reported that the incidence of sepsis was 0.7% of the whole admitted patients as compared to another similar previous study which was conducted during April 1982 to March, 1983. They observed that the incidence of sepsis has decreased and incidence of nosocomial sepsis remained unchanged whereas its mortality rate has decreased. Antibiotic susceptibility testing revealed that some bacterial strains were resistant to new beta-lactam infection.

Karchmer (1991) discussed the risk of nosocomial prosthetic valve endocarditis (PVE) and reported that PVE varied from 1.4%-3.0% with cases occurring throughout the year after surgery. He reported that 60% of the nosocomial PVE cases were due to infection with methicillin resistant coagulase-negative Staphylococci. Intra-operative contamination of wounds with Staphylococcus epidermidis led to the epidemic of PVE. Studies have failed to identify the elements of intra-operative or postoperative care that might be modified to reduce the incidence of sporadic cases of nosocomial PVE. He observed that prophylactic use of cefamendole or cefuroxime has become essential to prevent the nosocomial PVE.

Nettleman et al. (1991) developed a simple, inexpensive program based on feedback to physicians that resulted in the significant reduction (50%) of nosocomial methicillin-resistant Staphylococcus aureus infections. Nishi et al (1991) while studying the incidence of methicillin resistant S. aureus (MRSA) and
methicillin sensitive *S. aureus* (MSSA) reported that 43.6% of their isolates were MRSA type and were isolated mostly from the respiratory tract specimens obtained from sick individuals.

Lin and Huang (1991) observed 11 cases of neonatal meningitis caused by *Flavobacterium meningosepticum* from January 1981 to December 1988. The isolates were susceptible to piperacillin and resistant to ampicillin, aminoglycosides, and cephalosporins.

Ortega *et al.* (1991) reported nosocomial bacteremia in two patients which was caused by vancomycin resistant *Enterococcus faecalis* in a Spanish hospital. The review of medical records of patients indicated that none of them had previously received vancomycin treatment clearly indicating that the infections were acquired from the hospital.

Karim *et al.* (1991) isolated species of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella*, *Serratia* and *Acinetobacter* from the immune-compromised patients at Agha Khan University Hospital in Karachi. Of *Pseudomonas aeruginosa* species, isolated from nosocomial cases, 54.8% were resistant to carbenicillin, and 9.6% were resistant to gentamicin, 30.2% were resistant to ceftoxamine and none showed resistance to ofloxacin and ceftazidime. Of the *Staphylococcus aureus* isolates, 23% were susceptible to penicillin, 26.6% showed resistance to erythromycin and none of the strains was resistant to methicillin.

Kodoya *et al.* (1991) studied enterococcal infection from April 1983 to March 1990 in Nagoya University Hospital. They reported that like MRSA and *Pseudomonas aeruginosa*, enterococci were also important causes of nosocomial infections. Of the 34 cases, 24 were due to *Enterococcus faecalis* and 10 due to *Enterococcus faecium*. Twenty-seven cases of infection were mono-microbial septicemia and 7 were mixed infections. Among these cases 21 were of intra-
abdominal infection, 4 of urinary tract infection, 2 of respiratory tract, 1 case of diabetes, 1 of intra-muscular catheter and remaining were of unknown sources. They concluded that *Enterobacter faecium* and *Enterococcus avium* were more resistant to antimicrobial agents than the *Enterococcus faecalis*. Among the *Enterococcus* isolates 35% of the 26 strains showed high level of resistance to gentamicin.

Schaberg *et al.* (1991) determined trends in the microbial etiology of nosocomial infections in 1980’s based on documented nosocomial infection reported to the National Nosocomial Infections Surveillance System (NNISS) and from the University of Michigan Hospital. Antimicrobial susceptibility testing data from both the sources were also analyzed. They observed an increase in the infections due to antibiotic resistant strains of *Escherichia coli*, *Klebsiella*, coagulase-negative *Staphylococci*, *Candida albican*, coagulase positive *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter* and *Enterococcus* species. The isolates showed resistance to a variety of antibiotics in medical practice.

Tenover (1991) observed an increase in the incidence of nosocomial infections due to multi-antibiotic resistant strains and the methicillin resistant strains of *Staphylococcus aureus* continued to be a major problem in many hospitals. He observed several new types of resistance determinant including multiple resistance genes among organisms causing hospital acquired infections. New strains of beta-lactamase producing Gram-negative bacilli have emerged. *Enterococci* with high resistance to beta-lactams, glycopeptides and high levels of aminoglycosides have appeared. Methicillin resistant *S. aureus* have developed resistance to quinolone drugs. The evolution of drug resistance in nosocomial pathogens is complicating treatment of these infection.

Zhang (1991) studied nosocomial infections in hospitalized patients of Hua, Shan Hospital during 1985 to 1987. Among 1826 patients, the incidence of
nosocomial infection was 13.1%. Of the 271 nosocomial pathogens, the Gram-negative organisms were 66.4%, *Pseudomonas aeruginosa* 13.3%, *Klebsiella pneumoniae* 12.2%, *E. coli* 8.9%, *Acinetobacter* spp. 7.7% and *Enterobacter* spp. 7.7%. On the other hand Gram-positive pathogens accounted for 22.9% and fungi 10.7% of the isolates. He also reported that nosocomial strains were more resistant to commonly used antibiotics than the community isolates. *E. coli* isolates were resistant to ampicillin and *P. aeruginosa* isolates to polymyxin B and *S. aureus* isolates to Lincomycin and Gentamicin.

Archer (1991) isolated antibiotic resistant strains of *Staphylococcus epidermidis* from the patients of cardiac surgery. Menzis *et al.* (1991) reported that the nosocomial strains of *Staphylococcus epidermidis* isolated from the patients of prosthetic valve endocarditis, skin of eight general surgical patients and hospital environment were resistant to oxacillin, gentamicin, kanamycin and tobramycin. Etienne *et al.* (1989) reported an increased incidence of *Staphylococcus epidermidis* from 1986 to 1988 at the Cardiology and Neurology Hospitals in Lyon due to intensive use of pefloxacin (a new fluoroquinolone). Lee *et al.* (1991) reported 148 nosocomial patients infected with methicillin resistant *Staphylococcus aureus* during 1988 in the department of survey, University Hospital in Koalalumpur, Malaysia.

Watanakunakorn and Jura (1991) reviewed 196 episodes of *Klebsiella* infection during 1980-1989 in a community teaching hospital in the USA. They studied 194 patients between the age group of 70-84 years. The prevalence was found to be 0.76 episodes/1000 admissions. Nosocomial bacterial infections occurred in 43% of episodes, *Klebsiella pneumoniae* accounted for 86% and *Kleb. oxytoca* for 13% of the episodes. 28% of the episodes were due to mixed infection of *Escherichia coil* and *Enterococci* alongwith *Klebsiella*. Major route of infection were found to be the respiratory and urinary tracts. Most of the isolates were found resistant to ampicillin and carbenicillin.
Senerwa et al. (1991) isolated 78 strains of enteropathogenic *Escherichia coli* (EPEC), and 151 non-EPEC isolates from preterm neonates during an outbreak of gastroenteritis in a hospital in Nairobi. Majority of the strains showed resistance to trimethoprim-sulfamethoxazole, chloramphenicol, oxytetracycline, and ampicillin while only few of them were resistant to cephazolin, cefamandole, cefotaxime, amikacin and nalidixic acid. The resistance was almost uniform and plasmid oriented.

Ben-Hassen et al. (1992) isolated 221 strains of oxacillin-resistant *Staphylococcus aureus* from nosocomial patients. Of the isolated strains 53% showed resistance phenotype MLSBc (constitutive resistance to macrolides, lincosamine, streptogramine) and 61% showed resistance phenotype of OS + KGT (resistance to streptomycin, kanamycin, gentamicin and tobramycin). All the isolates were susceptible to pristinamycin and vancomycin.

Tanaka et al. (1992) isolated 282 strains of drug-resistant *Staphylococcus aureus* from the ward environment of a University hospital. The analysis revealed that 84 of the isolated strains were *Staphylococcus epidermidis*, 65 were *Staphylococcus aureus* and 58 isolates were *Staphylococcus haemolyticus*. The results further showed that 136 (48%) isolated staphylococcal strains and 13 of 65 strains were methicillin resistant. The DMPPC resistant strains of *Staphylococcus aureus* were also resistant to many antibiotics.

Huang et al. (1993) devised a special medium for testing the antibiotic susceptibility of Staphylococci to oxacillin using agar dilution, broth dilution for E. (Epsilometer) test and disk diffusion test. The recommended medium contained 0, 2, 4 or 5% sodium chloride. A total of 223 Staphylococcus strains of which 128 were mec gene positive were tested. Seven of the 128 mec gene positive strains were coagulase-negative Staphylococci with 24-hour oxacillin MIC 2 microgram/ml. Ninety-five isolates were mec gene negative including 7 strains of *Staphylococcus aureus* with oxacillin MIC of 4 microgram/ml. The oxacillin MIC
of mec gene positive, oxacillin-resistant strains of Staphylococci increased two-to-four folds with the addition of sodium chloride in the test medium while the MICs for mec gene negative strains did no change with the addition of salt. The addition of salt reduced the major error rates considerably for the mec gene positive strains.

Del-Favero and Menichette (1993) reported the efficacy of ciprofloxacin in febrile neutropenic cancer patients suffering from nosocomial gram-negative infections. The prophylactic use of ciprofloxacin was more effective than that of norfloxacin in the prevention of infections with gram-negative bacteria. These drugs were not effective in the treatment of febrile episodes with streptococcal or coagulase-negative staphylococcal infections.

Banerjee (1993) reported a nosocomial outbreak of neonatal septicemia due to multiple drug resistant *Klebsiella pneumoniae* in a nursery. Pegues et al. (1994) studied nosocomial bloodstream infections caused by Gram-negative bacteria in 26 neonates in a 1000-bed hospital. Of those 23 patients died of infection. During this epidemic *Serratia marcescens* was identified in 81% of patient’s blood cultures and from 57% of the staff hand-washings. Most of the *Serratia marcescens* isolates were ampicillin (100%) and gentamicin (77%) resistant.

Swartz (1994) reported that about 5% of the patients, admitted to the hospitals, acquired nosocomial infections. The infection contributing factors include increasing age, presence of untreatable diseases, extensive medical and intensive use of surgical therapies, frequent use of antimicrobial drugs and capability of selecting a resistant microbial flora. By 1960, aerobic Gram-negative bacilli had assumed increasing importance as nosocomial pathogens, and many strains were found resistant to the available antimicrobial drugs. During the 1980s the principal organism causing nosocomial blood stream infections were coagulase-negative *Staphylococcus* species, aerobic Gram-negative bacilli, *Staphylococcus aureus*, Candida species and the Enterobacter species. Coagulase-negative Staphylococci
and *Staphylococcus aureus* strains are often methicillin resistant but vancomycin sensitive. Increasing number of Enterococcal isolates from intensive care patients were however, vancomycin resistant.

Okpara and Maswoswe (1994) studied the antimicrobial resistance patterns during an outbreak of nosocomial infections caused by *Acinetobacter baumannii*. All the patients were surgical intensive care unit residents and had some predisposing factors for *Acinetobacter* infection. A total of 107 isolates were cultured from various sites; sputum was the most common source. Of these 107 isolates, all were resistant to formulary cephalosporins, extended-spectrum penicillins, quinolones, and aztrenam. Only nine isolates were sensitive to one or more aminoglycosides. All the isolates were susceptible to imipenem-cilastatin.

De-Champs et al. (1994) studied the effects of change of first line antibiotic treatment in a neonatal unit. A group of 238 neonates was treated with gentamicin and compared with a group of 389 neonates (G2) treated with amikacin in combination with ampicillin plus aminoglycosides. The change had no effect on the incidence of nosocomial infections that was 19.7% in G1 and 16.3% in G2. Strains of *E. cloacae* were associated with nosocomial infections. Proportion of *E. aerogenes* and *Enterococci* increased in G2 and that of gentamicin-resistant strains of *E. cloacae* and *Staphylococci* decreased. The study also illustrated the effects of antibiotic therapy on the species and resistance of strains of bacteria isolated in nosocomial infections.

Rotimi et al. (1994) studied the antibiotic susceptibility of some bacterial isolates in Lagos. With the exception of *Streptococcus pyogenes* all other isolates were resistant to the action of penicillin. Penicillin-resistant strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* species showed variable resistance to synergistic effects of various combinations of gentamicin, ampicillin, clindamycin, colistin, cefoxitin, and ceftriazone.
Go-Es et al. (1994) reported that an outbreak of imipenem-resistant *Acinetobacter baumannii* occurred after an increased use of imipenem for treatment of cephalosporin-resistant Klebsiella infection in a New York hospital. Bacteriological studies revealed that 59 patients harbored imipenem-resistant *Acinetobacter baumannii* while 18 acquired this infection. Increased use of imipenem against cephalosporin-resistant Klebsiella may lead to emergence of imipenem resistance in Acinetobacter and other bacterial species.

Sepra and Farber (1994) reported increased prevalence of enterococci as nosocomial pathogens and that the isolates had become increasingly resistant to agents traditionally useful in the treatment of invasive diseases due to enterococci. Vancomycin resistance, first described in clinical isolates in 1988, has disseminated worldwide, and mortality caused by vancomycin-resistant strains of enterococci bacteremia was over 50%. They recommended that since there was no uniformly effective antimicrobial therapy for patients infected with vancomycin-resistant enterococci, preventing the spread with vigorous application of barrier precautions and other infection control techniques is of paramount importance.

Witte et al. (1994) investigated two outbreaks of nosocomial infections with MRSA, one in a urology unit in connection with Tranurethral prostatectomy and the other in an orthopedic clinic with infections after implantation of prosthetic hips. They reported inadequate hospital hygiene and inappropriate antibiotic prophylaxis as the reasons of these outbreaks. Venditti (1994) reviewed routine clinical laboratory records from January 1990 to March 1993 to evaluate the rate of oxacillin-resistance among nosocomial isolates of *Staphylococcus aureus*. Of the 265 clinically significant isolates, 174 (65%) were oxacillin-resistant. Most ORSA isolates proved resistant to ciprofloxacin, gentamicin and rifampicin and susceptible to vancomycin, netilmicin and cotrimoxazole.

Venditti et al. (1994) identified 17 (20%) of 83 consecutive enterococci isolates from hospitalized patients with documented infection with high-level
ampicillin-resistant enterococci. These strains of Enterococci were found frequently resistant to imipenem, ciprofloxacin and streptomycin.

Takesue et al. (1994) investigated nosocomial infections by the comparison of MRSA and Pseudomonas aeruginosa. Strains of both these group were found resistant to several antibiotics.

Grayson et al. (1994) evaluated the efficacy of treatment with ampicillin-sulbactum versus imipenem-cilastatin, using double blind randomized trial, in limb threatening foot infection in diabetic patients. Both the treatments were equally effective. The treatment failures were associated with the presence of antibiotic-resistant pathogens, and possible nosocomial acquisition of infection.

Saunders et al. (1994) studied the efficacy of selective decontamination of digestive tract (SDD) for prevention of respiratory nosocomial infections in respiratory intensive care unit patients and placebo group. The practice reduced the digestive tract colonization with gram-negative bacilli (ANGB) and totally eliminated the Candida species in both the groups. The incidence of colonization of digestive tract with enterococci, coagulase-negative staphylococci and methicillin-resistant staphylococci increased in SDD group. There was no change in the incidence of Acinetobacter infection of the respiratory tract in both the groups. The incidence of Pseudomonas aeruginosa and cefotaxime- and tobramycin-resistant Enterobacteriaceae increased in placebo group.

Cheong et al. (1994) reported that 905 out of 2583 isolates of Staphylococcus aureus, 905 isolates from different hospitals in Malaysia, during a period from August 1990 to November 1991, were methicillin resistant. Majority of the resistant isolates were obtained from patients in surgical / orthopedic or pediatric wards, and the special care units. Vancomycin was the drug of choice followed by ciprofloxacin, rifampicin and fusidic acid. Nonoyama et al. (1994) surveyed 387 clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) obtained from 26 hospitals of Japan to determine whether they became
resistant to arbekacin (ABK). Of the isolates 25 strains of Staphylococci were ABK-MR resistant (6.5%) from 9 hospitals. They concluded that ABK-resistance spreads through nosocomial MRSA infections.

Voustsinas et al. (1994) in their work on in vitro activity of FCE 22101 against 97 Gram negative aerobic nosocomial isolates compared it with that of ceftriaxone using the agar dilution method. Thirty four (79%) of the 43 ceftriaxone resistant (MIC > 16 MG /l) isolates were found to be FCE 22101 susceptible (MIC ≤ 8 mg /l), while of the ceftriaxone susceptible isolates, only seven Enterobacter cloacae and one Serratia spp strain were FCE 22101 resistant. The new penem may offer an alternative for treating nosocomial infections due to aerobic Gram negative strains resistant to third generation cephalosporins, provided that clinical cases confirm its clinical usefulness.

Low et al. (1995) observed that during the last 5 years, the appearance and rapid dissemination of Enterococcus strains with high levels of resistance to vancomycin, ampicillin, gentamicin and streptomycin have been reported. In some cases no effective antimicrobial therapy was available to patients infected with these drug resistant strains. Enterococci in addition to their intrinsic and acquired tolerance to beta-lactamase have acquired the ability to inactivate penicillin and ampicillin via beta-lactamase production. Prompt recognition of such multi-resistance enterococci, the implementation of effective infection control precautions, and rational use of antimicrobials may limit or even prevent the spread of such strains in the hospitals.

Sakumoto et al. (1996) observed that methicillin-resistance in Staphylococcus aureus (SA) was due to mec A gene. This gene was not present in methicillin-susceptible Staphylococcus aureus (MSSA). All the 72% methicillin-resistant strains of Staphylococcus epidermidis (MRSE) possessed this gene. The methicillin-resistant Staphylococcus aureus (MRSA), methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin–resistant Staphylococcus aureus (MSSA) and methicillin–resistant Staphylococcus aureus (MSSA)
*epidermidis* (MRSE), isolated from patients with complicated urinary tract infection showed low sensitivity to imipenem, ceftazidine, flomoxef, amikacin, ciprofloxacin and ofloxacin. It was observed that while dealing with control of nosocomial infection it must be kept in mind that the MRSE may act as mec A gene reservoir for the MRSA.

Brucker (1996) described the situation of hygiene in hospitals in France and nosocomial infection rate of approximately 6 to 16%. These infections in France, as in South Europe, are caused by multiple antibiotic-resistant strains of bacteria and that 40% of the cases are due to methicillin resistant *Staphylococcus aureus* strains.

Ismail et al. (1997) studied, retrospectively, nosocomial and community acquired infection in a consecutive series of 191 patients that aged over 60 years and were admitted in 1995 in Singapore. In 57.5% of the patients the infection was acquired from the community, in 33% cases it was of nosocomial origin and in 9.5% cases it was result of long-term hospitalization. Methicillin-sensitive *Staphylococcus aureus* bacteremia was associated with a mortality rate of 35.3%, followed by *Klebsiella* species (mortality) 28.6%, *Pseudomonas aeruginosa* (mortality) 28.6%, methicillin-resistant *Staphylococcus aureus* (mortality) 25%, *Proteus mirabilis* (mortality) 25% and *E. coli* (mortality) 19.1 percent.

Montorsi and Germiniani (1997) compared the efficacy of intra-venous administration of single dose of Pefloxacin 800 mg with ceftiraxone 2 g administered 1-2 hours before biliary surgery and gastrectomy in 297 patients to reduce the incidence of post-surgical nosocomial infections. In the Pefloxacin group (128 patients), no cases of wound infections were observed, except one case of wound sterile secretion, without dehiscence, (0.81%), one case of urinary infection (0.81%) and three cases of respiratory infections (2.34%). In the ceftriaxone group (131 patients), three cases of wound sterile secretion without dehiscence (2.36%), one case of urinary infections (0.76%) and four cases of respiratory infections
(3.05%) were observed. It was concluded that single-shot of Pefloxacin or Ceftriaxone, is able to prevent post-surgical nosocomial infections.

Rotimi et al. (1998) reported that about 99% of all the isolates belonging to Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacteraeae group, and Acinetobacter spp. were susceptible to ciprofloxacin at both the centers (Jeddah and Kuwait), whereas 87 and 96% were susceptible to imipenem, 69 and 64% to ceftazidime, 59 and 52% to cefotaxime, and 25 and 67% to piperacillin, respectively, in Jeddah and Kuwait. Prior antibiotic usage was more common among patients in Jeddah than those in Kuwait. The higher number of resistant bacteria reported from Jeddah than Kuwaiti may be a reflection of the higher antibiotic consumption, in particular higher usage of broad-spectrum cephalosporins in Jeddah ICU.

Tabe et al. (1998) examined a total of 55 Staphylococcus haemolyticus isolates from patients and neonatal intensive care unit staff for susceptibility to 12 antimicrobial agents. There were 34 isolates that were resistant to oxacillin, ampicillin, cefazolin, cefmetazole, imipenem, and gentamicin. These isolates had a higher frequency of resistance to tobramycin and Ofloxacin, and relatively high MICs (2 to 4 micrograms/mL) for vancomycin, although none of the isolates was vancomycin resistant. To investigate hospital-acquired colonization and infection by multi-resistant S. haemolyticus, they examined all isolates by pulsed-field gel electrophoresis (PFGE) after digestion of restriction endonuclease and detected an endemic PFGE pattern in those multi-resistant isolates. Their results suggested that local spread of multi-resistant S. haemolyticus was hospital acquired, and that the hospital staff acted as a reservoir for that organism.

Lagrou et al. (1998) evaluated drug susceptibility of 150 strains of Corynebacteria to 12 antibiotics active against Gram-positive organisms. They observed that Corynebacterium amycolatum, Corynebacterium jeikeium, and Corynebacterium urealyticum were multiresistant, but all the isolates were susceptible to teicoplanin and vancomycin. Most of the C. amycolatum strains, and
all strains of *C. jeikeium* and *C. striatum*, were susceptible to the vibrocidal compound 0/129.

Pillay *et al.* (1998) reported that nosocomial *Klebsiella pneumoniae* infection is associated with a high mortality in neonates and antimicrobial therapy of these infections has been complicated by the emergence of multi-resistant strains. These organisms remain susceptible to only a few antimicrobial agents, and some of these are not recommended for use in children. During an outbreak, they used piperacillin / tazobactam, imipenem / cilastatin, cefotaxime and ciprofloxacin in the treatment of 33 neonates with *Klebsiella pneumoniae* infection. Extended-spectrum beta-lactamase production was detected in *K. pneumoniae* isolates from 18 of 33 (54.5%) neonates. All-cause mortality was 13 of 33 (39.4%) and there was no significant difference in mortality between neonates treated with imipenem / cilastatin (6 of 17 or 35.3%) and the neonates treated with piperacillin / tazobactam (6 of 13 or 46.2%). The duration of antimicrobial therapy and the total hospital stay was similar between neonates who received imipenem / cilastatin and those that received piperacillin/tazobactam. This report suggests that piperacillin / tazobactam may be a useful antimicrobial agent in neonatal infections caused by beta-lactamase-producing organisms.

Hsueh *et al.* (1998) observed long-term colonization of various body sites, in six patients at National Taiwan University Hospital from April 1996 to May 1997, with a multidrug-resistant *Pseudomonas aeruginosa* clone that was resistant to piperacillin, cefoperazone, ceftazidime, azteonam, imipenem, cefepime, cefpirome, ofloxacin, ciprofloxacin, minocycline, and aminoglycosides with subsequent severe infections in burn patients.

Raad *et al.* (1998) observed that *Staphylococcus epidermidis* and other coagulase negative Staphylococi had emerged among the leading causes of nosocomial blood infections. These pathogens are multi drug resistant. The isolated strains are resistant to action of ciprofloxacin and tolerant and occasionally resistant to vancomycin and are susceptible to minocycline, rifampin, quinupritin/
Review of Literature

dalfopristin and the oxazolidinones.

Brady et al. (1998) reported that the percentage of nosocomial vancomycin-resistant enterococci (VRE) is increasing, rapidly, in the United States. This has recently resulted in recommendations to reserve vancomycin used for cases with proven resistance to other antimicrobials. The incidence of VRE on their campus is almost 10%, which is similar to US data. They studied 50 chronic hemodialysis (HD) patients and 50 peritoneal dialysis (PD) patients. Each patient had a rectal swab test performed and cultured for the presence of enterococci. Antimicrobial exposures over the 6 months, before the initial swab test, were reviewed in each patient. At least one repeated swab test was performed in 30 CRI, 45 HD, and 37 PD patients. From the initial swab culture, vancomycin-sensitive enterococci (VSE) were isolated in 65% of CRI, 54% of HD, and 70% of PD patients. No CRI or HD patients had VRE isolated and 2% of PD patients had VRE isolated. The remaining patients had no enterococci infection. Review of antimicrobial exposures in the 6 months before the initial swab test showed 0% of CRI, 32% of HD, and 36% of PD patients that had received vancomycin. Other antimicrobials were administered to 40% of CRI, 46% of HD, and 78% of PD patients during the same period. In the month immediately preceding the initial swab test, 0% of CRI, 12% of HD, and 22% of PD patients had received vancomycin and 18% of CRI, 20% of HD, and 36% of PD patients had received other antimicrobials. Results from repeated cultures showed that 57% of CRI, 40% of HD, and 38% of PD patients changed their culture status related to VSE, VRE, or no enterococci were present. Cultures of 342 swabs from 140 patients yielded three VRE isolates in two patients. It was concluded that despite the frequent use of vancomycin and other antimicrobials, the incidence of VRE in renal population is less than that reported. Given this lack of VRE isolates, it was recommended that the judicious use of vancomycin in treating renal patients be continued and enterococcal sensitivity surveillance be also continued.

French (1998) reported that the frequency of infections with multiple
antibiotic-resistant gram-positive bacteria is increasing, and in some cases these organisms remain susceptible only to the glycopeptides vancomycin and teicoplanin. The appearance of transferable high-level glycopeptide resistance in Enterococci, producing some strains that are now resistant to all available antibiotics, is thus a cause for concern. The enterococci readily colonize the bowel, spread rapidly among hospital patients, and transfer their antibiotic resistances widely among themselves and other gram-positive species. Glycopeptide resistance has not yet been transferred in vivo to other significant pathogens, but experimental transfer to Staphylococcus aureus has been achieved in vitro. The emergence of glycopeptide-resistant enterococci has been encouraged by the increasing use of aminoglycosides, cephalosporins, and quinolones for the treatment of infections due to gram-negative bacteria and glycopeptides for infections due to staphylococcus spp. and Clostridium difficile. In Europe this antibiotic pressure has been aggravated by the use of the glycopeptide avoparcin in animal feeds. The enterococci may now be poised to disseminate glycopeptide resistance among other more pathogenic gram-positive bacteria.

Heggers et al. (1998) treated 56 pediatric burn patients with ciprofloxacin when bacterial cultures proved resistant to other antibiotics. The burn area was 65% of the total body surface area. The average patient age was 8.4 years. All the patients showed unequivocal reduction in quantitative bacterial counts, and susceptibility to ciprofloxacin remained stable without the development of resistance. Of the 56 patients treated, 42 had a major reduction in their quantitative wound biopsies from 10^6 to less than 100 colonies per gram of tissue, while the remaining 14 were observed to have a 2- to 3-log decrease. On the basis of the data, ciprofloxacin therapy for the treatment of immunosuppressed pediatric burn patients is efficacious and does not cause arthropathy in those patients.

Araque and Velazco (1998) evaluated the in vitro activity of fleroxacin against nosocomial gram-negative organisms, 263 multi- drug resistant gram-negative bacilli (203 Enterobacteriaceae and 60 non-fermenting gram-negative
bacilli) isolated from adult patients with nosocomial infections. The different patterns of resistance to eight different antimicrobial agents (ampicillin, carbenicillin, piperacillin, cephalothin, cefamandole, ceftazidime, gentamicin and amikacin) were determined by minimum inhibitory concentration (MIC), using the agar dilution method. The most prevalent multi-resistant species isolated were *Klebsiella pneumoniae* (28.9%), *Escherichia coli* (24%) and *Pseudomonas aeruginosa* (12.2%). All these bacterial strains showed three to five resistance patterns to at least three different antibiotics. Resistance to ceftazidime was observed in at least one of the resistance patterns of isolated bacteria. The activity of fleroxacin against multi-resistant enteric bacteria was excellent; these strains showed a susceptibility of 79-100%. The susceptibility of *P. aeruginosa* to antipseudomonal agents was low; however, the activity of fleroxacin against these strains was higher than 60% (MIC ≤ 2 microg/ml), broadly comparable with ciprofloxacin. The resistance to fluoroquinolones detected in this study was no cause for alarm (3%). Consequently, fleroxacin maintains a remarkable activity against Enterobacteriaceae and remains highly active against other gram-negative bacilli. Nevertheless, actions directed at preventing or limiting resistance will be crucial to maintain the viability of fluoroquinolones as important therapeutic agents.

Patel *et al.* (1999) reported that linezolid, *in vitro*, completely inhibited organisms like vancomycin-resistant Enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*.

Brett *et al.* (1999) determined the current antibiotic susceptibility patterns of 386 clinical strains of *Streptococcus pneumoniae* collected by four laboratories (Auckland, Wellington, Hamilton and Christchurch) from general practice or inpatients. Eighty-three-percent of those isolates were penicillin susceptible, 12% showed intermediate resistance to penicillin and 5% were penicillin resistant. Overall, 93 and 91% of the isolates were susceptible to amoxicillin/clavulanic acid and ceftriaxone, respectively. Erythromycin and tetracycline had similar rates of susceptibility (88 and 87%, respectively). Resistance to cotrimoxazole was
common, with only 57% of the isolates susceptible to this combination. Wellington Laboratory reported lower resistance rates than the Auckland, Christchurch and Hamilton. Isolates from children had consistently higher resistance rates (two-to five fold greater for beta-lactams and 1.2 to 1.3-fold for other agents) compared with isolates from the adult patients.

Austin and Anderson (1999) reported that epidemic strains of methicillin-resistant *Staphylococcus aureus* (EMRSA) and vancomycin-resistant Enterococci (VRE) 16 are becoming endemic in hospitals of England and Wales in the United Kingdom. An epidemic of VRE is still at an early stage, and the number of hospitals newly affected by VRE is growing exponentially.

Krediet *et al.* (1999) reported that the coagulase-negative staphylococci (CONS) are most common causative agents in neonatal nosocomial septicemia. They evaluated the efficacy of antibiotic regimen for CONS septicemia, in relation to methicillin-resistance and the carriage of mec A gene, encoding methicillin-resistance, among CONS blood isolates. The diagnosis of septicemia was made in 60 patients on the basis of clinical symptoms of septicemia in the presence of a positive a blood culture test. Cephalotin was found to be clinically efficacious in the treatment of neonatal CONS septicemia with high methicillin- and oxacillin-resistant strains.

Oshima *et al.* (1999) reported that methicillin-resistant *S. aureus* is an important causative pathogen of scleral buckling infections, particularly in patients with retinal detachment associated with atopic dermatitis. Preoperative evaluation and intra operative attention to contamination are recommended to prevent methicillin-resistant *S. aureus* infections in these patients.

Parr *et al.* (1999) studied on nosocomial bacteria exposed to various concentration of lidocaine with and without epinephrine. The gram-negative bacteria showed a high sensitivity to lidocaine while the *Staphylococcus aureus*
showed the least sensitivity. The study showed the additional benefits of this local anaesthetic in treatment of wounds which were contaminated with the methicillin- and vancomycin-resistant bacteria.

Smith et al. (1999) isolated strains of methicillin-resistant *Staphylococcus aureus* that were also resistant to vancomycin. The infection responded to a therapy with rifampin and trimethoprim-sulphamethoxazole. Klekamp et al. (1999) suggested that care should be used while prescribing vancomycin otherwise resistant strains are likely to emerge.

File Jr. (1999) reviewed the situation arising out of the emergence of increasingly resistant strains of microbes that is threatening the tremendous therapeutic advantage afforded by the antibiotics. Selective pressure favoring resistant strains arises from misuse and overuse of antimicrobials (notably extended-spectrum cephalosporins), increased number of immune compromised hosts, lapses in infection control, increased use of invasive procedures and devices, and the widespread use of antibiotics in agriculture and animal husbandry. Outside the hospitals, penicillin-resistant *Streptococcus pneumoniae* is of greatest concern; recent reports also indicate the appearance of outpatient methicillin-resistant *Staphylococcus aureus* (MRSA) infections. MRSA is a significant problem in the hospital, as are vacomycin-resistant *Enterococcus*, oxacillin-resistant *S. aureus*, and multidrug-resistant Gram-negative bacilli. Owing to the high rate of antibiotic use and other risk factors, a person is more likely to acquire an antibiotic-resistant infection in the ICU than from anywhere else, either inside or outside the hospital. Reasonable antibiotic use and stringent infection-control policies are needed to discourage the development of resistant strains of micro-organisms.

Santos et al. (1999) tested 103 MRSA isolates, cultured between Sept. 1994 and Sept. 1995, from 62 patients in two teaching hospitals (hospital 1, in Rio de Janeiro; hospital 2, in Minas Gerais) for antimicrobial resistance, and the genomic DNA was analysed by pulsed-field gel electrophoresis (PFGE). MRSA
isolates were resistant to the majority of antimicrobial agents tested. However, susceptibility to vancomycin alone was recorded in 32% of the isolates from hospital 1, whereas 48% of the isolates from hospital 2 were susceptible to both vancomycin and mupirocin, and 34% of the isolates demonstrated susceptibility to vancomycin, mupirocin and chloramphenicol. Thirty-nine percent of all the isolates were mupirocin-resistant. Main risk factors were: hospitalization for more than 7 days (95%), very dependent patients (84%), invasive procedures (79%) and recent antimicrobial therapy (79).

Ramos et al. (1999) reported that mupirocin can be used as topical antimicrobial for the successful eradication of methicillin-resistant *Staphylococcus aureus* from the anterior nares and other sites of patients and health care personnel. This report describes the acquisition of a novel mupirocin resistance gene (ileS) by an epidemic MRSA clone that is geographically widespread in Brazil.

Mokaddas and Sanyal (1999) reported that *Pseudomonas aeruginosa* is a major problem, as a multi-resistant nosocomial pathogen, especially in burns and other immune-compromised patients in hospital. Of the 357 *P. aeruginosa* isolates tested from 188 patients 37 (10.4%) were found resistant to imipenem, 21 (5.9%) to meropenem and 50 (14%) to piperacillin/tazobactam. Cross resistance between the two carbapenems was observed in 5.9% of the isolates. Sixteen (43%) of the imipenem-resistant isolates were susceptible to meropenem but the reverse was observed in none. Amongst the 50 piperacillin/tazobactam-resistant isolates cross-resistance with the two carbapenems was observed in 18 (36%) and in 9 (18%) only with imipenem; 23 (46%) were susceptible to both. Their results indicate that *P. aeruginosa* is the least resistant organism to meropenem followed by imipenem and piperacillin/tazobactam. Cross resistance between the carbapenems and between carbapenems and piperacillin/tazobactam was also observed.

Levin et al. (1999) studied sixty cases of nosocomial infections caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* resistant to the action of
aminoglycosides, cephalosporins, quinolones, penicillins, monobactams, and imipenem treated with colistin (one patient had two infections that are included as two different cases). The infections recorded were pneumonia (33% of patients), urinary tract infection (20%), primary bloodstream infection (15%), central nervous system infection (8%), peritonitis (7%), catheter-related infection (7%), and otitis media (2%). A good outcome occurred for 35 patients (58%), and three patients died within the first 48 hours of the treatment. The poorest results were observed in cases of pneumonia: only five (25%) of 20 patients had a good outcome. A good outcome occurred for four of five patients with central nervous system infections, although no intrathecal treatment was given. Colistin may be a good therapeutic option for the treatment of severe infections caused by multidrug-resistant *P. aeruginosa* and *A. baumannii*.

Kays (1999) compared the time above the minimum inhibitory concentration (T>MIC) for five parenteral beta-lactam antibiotics against common nosocomial bacterial pathogens at different creatinine clearances (Clcr). Serum concentration-time profiles were simulated for cefepime, ceftazidime, piperacillin, piperacillin-tazobactam, and imipenem at Clcr ranging from 120-30 ml/minute. The MIC data for 90% of organisms (MIC90) like *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and oxacillin-susceptible *Staphylococcus aureus* were collected, and a weighed and geometric mean MIC90 was calculated. The T>MIC was calculated as percentage of the dosing interval in which free concentrations exceeded the weighed geometric mean MIC90. A T>MIC of 70% or greater was considered desirable for all the organisms except *S. aureus* (≥ or 50%). Cefepime 2 g every 12 hours (Clcr ≥ 70 ml/min) and every 24 hours (Clcr ≤ 60 ml/min) achieved desirable T>MIC for all *Enterobacteriaceae* and *S. aureus* at every Clcr. Imipenem 0.5 g achieved desirable T>MIC for *E. coli*, *K. pneumoniae*, *C. freundii*, and *S. aureus* at every Clcr. However, imipenem T>MIC was less than 70% for the following regimens and organisms: *S. marcescens* 0.5 g every 6 hours (Clcr ≥ 90 ml/min), *E. aerogenes* 0.5 g every 6 hours (Clcr ≥ 80
ml/min), *E. cloacae* 0.5 g every 6 hours (Clcr ≥ 100 ml/min), *S. marcescens* 0.5 g every 8 hours (Clcr 60-70 ml/min), *E. cloacae* 0.5 g every 8 hours (Clcr 60-70 ml/min), and *E. aerogenes* 0.5 g every 8 hours (Clcr 50-70 ml/min). Ceftazidime 2 g every 8 hours (Clcr 60-100 ml/min) and every 12 hours (Clcr 40-50 ml/min) achieved desirable T>MIC for *E. coli, K. pneumoniae, S. marcescens,* and *S. aureus* only. At every dose and Clcr, piperacillin tazobactam achieved desirable T>MIC for *S. aureus* but not for any *Enterobacteriaceae* at Clcr > 50 ml/minute. Piperacillin did not achieve desirable T>MIC for any organism, and none of the beta-lactams attained a T>MIC of 70% or above for *P. aeruginosa* at any Clcr.

Lelievre et al. (1999) observed that some gentamicin-sensitive methicillin-resistant *Staphylococcus aureus* (GS-MRSA) and gentamicin resistant methicillin-resistant *Staphylococcus aureus* (GR-MRSA) strains had closely related PGFE (Pulsed Field Gel Electrophoresis) profile. These two are the predominant strains of staphylococcus encountered in a mainland hospital in France. GM-MRSA strains possess aac6’-aph2” gene that confers resistance against aminoglycosides and GS-MRSA possess ant4’gene that confers resistance against kanamycin, tobramycin and amikacin.

Bonomo and Rice (1999) reported that managing patients, infected with antibiotic resistant bacteria, is becoming one of the major clinical obstacles for physicians who treat patients in long-term care facilities (LTCFs). Penicillin-resistant pneumococci (PRP), vancomycin-resistant enterococci (VRE), gram-negative bacteria that produce extended-spectrum and ampC-type beta-lactamase enzymes, and quinolones-resistant gram-positive and gram-negative bacteria are the major resistant pathogens that are emerging in these settings. The mechanisms responsible for the evolution of these antibiotic resistant organisms (molecular rearrangement of penicillin binding protein genes, acquisition of a mobile genetic element, and point mutation that alter the active site) are reviewed. Vacomycin intermediate *Staphylococcus aureus* (VISA) and multidrug efflux pumps in gram-negative bacteria are also threatening the most potent available antimicrobials.
Kantzanou et al. (1999) reported that all 105 non-replicate consecutive *Staphylococcus aureus* strains isolated in 1997 from seven Greek hospitals, were found to be susceptible to vancomycin, teicoplanin and chloramphenicol, but only five (8%) were susceptible to all the 16 antibiotics tested. Forty-three (41%) isolates were methicillin-resistant, 58% homogeneously (homMRSA) and 42% heterogeneously (hetMRSA). Resistance of homMRSA strains to other antibiotics was generally high (88-100%), although only one strain was resistant to netilmicin. Resistance in hetMRSA (6-39%) or in MSSA (5-11%) was significantly lower. Consequently, the majority (76%) of homMRSA were multi-drug resistant, while the dominant phenotype of hetMRSA and MSSA was resistance to penicillin (50% and 76%, respectively). Comparison of these strains with isolates from 1994 showed higher resistance rates to erythromycin among MSSA, to erythromycin and amikacin among hetMRSA and to rifampicin among homMRSA strains.

Guyot et al. (1999) isolated multi drug-resistant *Escherichia coli* from chronic urinary tract infection (UTI) and terminal patients in a French hospital. It was observed that 20 days treatment with ciprofloxacin 250mg bid or 3 days treatment with Broad spectrum antibiotics favors the selection of multi drug-resistant strains of *E. coli* in terminally ill patients. The prolonged treatment resulted in the changes in the plasmid contents of resident flora.

Traub et al. (1999) isolated multiple antibiotic-resistant (MAR) strains of *Acinetobacter baumannii* from nosocomial patients in a surgical intensive care unit. The isolates were resistant to netilmicin, tobramycin, imipenem, meropenem, polymyxin B and trovafloxacin. All these strain showed high level of resistance against combination of rifampin and polymyxin B. Wright et al. (1999) studied the composite transposon Tn4001 and a related chromosomal Tn4001-like elements, encoded resistance to the aminoglycosides Gentamicin, tobramycin, and Kanamycin (GmTm Kmr) in Australian strains of *Staphylococcus aureus*. Southern hybridization analysis of GmTmKmr S. aureus strains isolate from various hospitals
in the UK between 1975 and 1985 indicate that they predominantly encoded chromosomal copies of Tn4001 or a Tn4001 like elements. However, a strain isolated in 1985 was to carry Tn4001 on a plasmid related to pSK1, the prototypical multi-resistance plasmid commonly detected in S. aureus strains from Australian hospitals. Cross and Campbell (1999) isolated drug resistant strains of *Strep. pneumoniae* and *H. influenza* from community acquired and hospital acquired cases of pneumonia. They observed that these pathogens have often acquired resistance to traditional antimicrobial therapy in a very short period.

Franzetti *et al.* (1999) observed that two drug (isoniazid and rifampin) resistant cases of *Mycobacterium tuberculosis*, in AIDS patients, responded to therapy with isoniazid, rifampin, ethambutol, and pyrazinamide but produced no significant effects; it also could not improve the average survival time of 94 days.

Perry and Jarvis (2001) reported that Linezolid is the first of a new class of antibacterial drugs, the oxazolidinones. It has inhibitory activity against a broad range of gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-intermediate *S. aureus* (GISA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*. The drug also shows activity against certain anaerobes, including *Clostridium perfringens*, *C. difficile*, various Peptostreptococcus spp. and *Bacteroides fragilis*. In controlled phase III studies, linezolid was as effective as vancomycin in the treatment of patients with infections caused by methicillin-resistant staphylococci and also demonstrated its efficacy against infections caused by VRE. Phase III studies have further demonstrated that linezolid is an effective treatment for patients with nosocomial pneumonia, for hospitalized patients with community-acquired pneumonia, and for patients with complicated skin or soft tissue infections (SSTIs). In these studies, linezolid was as effective as established treatments, including third-generation cephalosporins in patients with pneumonia, and oxacillin in patients with complicated SSTIs. Oral linezolid 400 or 600mg twice daily was as effective as clarithromycin 250mg twice daily or cefpodoxime proxetil 200mg twice daily for the treatment of patients with uncomplicated SSTIs or community-
acquired pneumonia. Linezolid is generally a well tolerated drug. The most frequently reported adverse events in linezolid recipients were diarrhea, headache, nausea and vomiting. Thrombocytopenia was also documented in a small proportion (about 2%) of patients treated with the drug. It was concluded that Linezolid has a good activity against gram-positive bacteria, particularly multidrug resistant strains of *S. aureus* (including GISA), *Enterococcus faecium* and *E. faecalis* (including VRE). In controlled clinical trials, linezolid was as effective as vancomycin in eradicating infections caused by methicillin-resistant Staphylococcus spp. and has demonstrated its efficacy against infections caused by VRE. As the level of resistance to vancomycin increases among *S. aureus* and enterococci, linezolid is poised to play an important role in the management of serious gram-positive infections.

Tsai and Kondo (2001) investigated improved agar diffusion method for determination of residual antimicrobial agents, and the sensitivities of various combinations of test organisms and assay media were determined using 7 types of organisms, 5 media, and 31 antimicrobial agents. *Bacillus stearothermophilus* and synthetic assay medium (SAM) showed the greatest sensitivity for screening penicillins (penicillin G and ampicillin). The combination of *Bacillus subtilis* and minimum medium (MM) was the most sensitive for tetracyclines (oxytetracycline and chlortetracycline), *B. stearothermophilus* and SAM or *Micrococcus luteus* and Mueller-Hinton agar (MHA) for detecting tylosin and erythromycin, *B. subtilis* and MHA for aminoglycosides (streptomycin, kanamycin, gentamicin, and dihydrostreptomycin), *B. stearothermophilus* and SAM for polyethers (salinomycin and lasalocid), and *B. subtilis* and MM or *Clostridium perfringens* and GAM for polypeptides (thiopeptin, enramycin, virginiamycin, and bacitracin). However, gram-negative bacterium *Escherichia coli* ATCC 27166 and MM were better for screening for colistin and polymixin-B. For detecting the synthetic drugs tested, the best combination was *B. subtilis* and MM for sulfonamides, *E. coli* 27166 and MM for quinolones (oxolinic acid and nalidixic acid), *B. subtilis* and MM for furans (furazolidone), and the bioluminescent bacterium *Photobacterium phosphoreum*.
and luminescence assay medium for chloramphenicol and oxolinic acid. The results showed that the use of four assay plates, B. stearothermophilus and SAM, B. subtilis and MM, M. luteus and MHA, and E. coli 27166 and MM, was superior to the currently available techniques for screening for residual antimicrobial agents in the edible animal tissues.

**EPIDEMIOLOGY OF NOSOCOMIAL INFECTIONS:**

Nosocomial infections strike man, when his immuno-competency is compromised due to some disease process or surgical procedure, his body resistance is lowered and his normal defensive mechanisms are overburdened with variety of antigens and possibly he is also receiving some sort of antibacterial therapy. Administration of antibiotics, in majority of circumstances, is empirical and not based on scientific principles, a process that in many cases may do more harm than the good. Studies have revealed that the pathogens acquired from community are less likely to show the antibiotic resistance as compared to the hospital-acquired infections. The organisms picked up from hospitals may acquire resistance against a number of antibiotics. Hospital acquired infection may be severe in nature, unpredictable in course and may occasionally terminate fatally. The epidemiology of nosocomial infections becomes all the more important in understanding the disease and in handling its victims. Many standard protocols have been developed to study the epidemiology of nosocomial infections at national levels and interpretation of results (Richards et al., 1998). Nettleman et al., (1991) proposed a simple, economical program for reducing the incidence of nosocomial infections in hospitals. The hygienic measures included hand-washing, cultures of staff and the other hospital worker; the pathogen is identified and information regarding the characteristic of pathogen is passed on to the physicians. There are monthly presentations of information regarding the pathogens and improvements achieved after the implementations of recommended measures. This practice resulted in significant reduction in nosocomial methicillin resistant *Staphylococcus aureus* (MRSA) infections. After implementation of these suggestions, in the first 15 months, nosocomial MRSA decreased from 1.025 to 0.508 cases per 1000
patient days. It was concluded that feedback of information and assignment of responsibility resulted in the 50% decrease in the nosocomial MRSA infection.

Fujita (1994) described nosocomial infection as an infection acquired by patients while they are in hospital, or by members of hospital staff. It will occur in various modes (1) Cross–infection (infection acquired from either patients or staff). (2) Self-infection (infection caused by resident flora or acquired from infected areas of his own body). (3) Infection acquired from other people or the hospital environment. The most important preventive measure for cross-infection would be isolation of the infected or colonized patients and his practice of hand washing. To prevent self-infection it is essential to maintain the defensive ability of patient including normal flora, and active immunization or prophylactic antibiotic treatment in some patients. Administration of antibiotics often permits selection and overgrowth of multiple resistant microorganisms and result in serious infections.

Rose et al. (1981) suggested that the direct contact with contaminated hands of hospital personnels leads to the occurrence and spread of Meningococcal pneumonia.

Bergler (1991) reported that the nosocomial infections are very common in German hospitals. These infections occur due to hygiene deficiencies such as lack of space and storage room, no separation of septic and non-septic patients, poor hygiene in toilet and bathrooms, inadequate personal hygienic behavior of hospital staff, lack of protective clothing or irregular change of clothing, shortcomings in disinfection, incorrect use of syringes and stethoscopes, non sterile dressing for wounds, no systematic hygiene control and no official consequence for wrong behavior of the hospital staff.

Bengen et al. (1991) carried out epidemiological investigation on 16 nosocomial vancomycin resistant Enterococcus faecium strains isolated from 15 patients in four different wards of a children hospital over a period of 17 months. They reported an increasing frequency of vancomycin resistant enterococci. Pathogens were typed by analysis of restriction fragment length polymorphisms.
(RFLP) of total DNA and of ribosomal DNA regions (ribotyping). This testing showed genetic un-relatedness of the nosocomial strains under study and exclusive transmission between the patients either in the same ward or between wards of the same hospital.

Pfaller et al. (1991) discovered clusters of MRSA at medical center at Iowa USA. They carried out microbiologic surveillance to study the point prevalence, source, and nosocomial acquisition of methicillin-resistant *Staphylococcus aureus*. Epidemiological study revealed 10 distinct subtypes of MRSA and out of 24 colonized patients 9 carried the same subtype, eight of which were receiving treatment in the surgical intensive care unit.

Nardell (1991) observed nosocomial transmission of tuberculosis (TB), from person to person among HIV-infected patients. He reported that it is difficult to prevent spread by conventional control measures and that its incidence is increasing in developed countries. In HIV-infected patients the TB may rapidly progress from infection to disease. Pozniak and Watson (1992) reported the outbreaks of tuberculosis in AIDS patients with drug-resistant strains of Mycobacterium, from many countries. It was observed that the procedures, used in handling of AIDS patients, usually facilitate transmission of Mycobacteria.

Jacobs (1991) reported that the nosocomial pneumonia remains to be one of the leading fatal diseases in adult as well as in pediatric patients. Success of the clinician depends upon rapid diagnosis. He reported that the pediatric nosocomial pneumonia were usually of viral or fungal etiology.

Rossello et al. (1992) reported 74 *Pseudomonas aeruginosa* infection cases, of which 35 (47.3%) were of nosocomial origin in the urology service in a period of one year (March 1987-March 1988) in a large city hospital at Barcelona, Spain. The peak incidence (10.5%) occurred during December declining to 2.2 in the following months. The epidemic cases stayed longer in the hospital and isolated strains showed resistance to ticarcillin and gentamicin. Andersen (1992) observed
Review of Literature

that in Norway the incidence of nosocomial infections was 5-20%. The consequences are serious for the individual patients and his family. It was also a serious problem for the concerned hospital and the Norwegian Government.

Occhipinti et al. (1992) observed that the nosocomial infections are responsible for large percentage of morbidity and mortality in intensive care units in hospitals. Conventional control measures are successful in reducing the exogenous infections but their success in controlling nosocomial infections is variable. Selective decontamination of digestive tract (SDD) with non-absorbable antibiotics attempts to reduce intestinal mucosal colonization with pathogenic bacteria. These antibiotics are directed against the gram-negative bacteria and yeasts.

Boyce et al. (1994) isolated multidrug-resistant nosocomial strains of Enterococcus faecium from 37 patients during an outbreak involving a 250-bed university-affiliated hospital. Risk factors for acquiring the epidemic strain of Enterococcus faecium included proximity to another patient and exposure to a nurse who cared for another case patient. Contamination of the environment by the epidemic strain occurred significantly more often when case patients had diarrhea. Because extensive environmental contamination may occur when affected patients develop diarrhea, barrier precautions, including the use of both gowns and gloves, should be implemented as soon as these pathogens are encountered.

Pegues et al. (1994) reported 23 deaths in 26 neonates caused by bloodstream infections with nosocomial epidemic strain of Serratia marcescens. The disease spread from water when the chlorinating system of a water supply malfunctioned. The chlorine levels were undetectable and tap water contained elevated microbial levels, including total and fecal bacteria. The disease occurred in babies that had undergone some invasive procedure, and later received bath from the infected water or from the hands of hospital workers.

Moroni et al. (1994) observed that Mycobacterium tuberculosis infection is one of the most frequent complication of AIDS patients. The incidence of
tuberculosis has dramatically increased in all countries as a result of HIV epidemic. Apparently HIV has accelerated the incidence of *Mycobacterium tuberculosis* infection. HIV infected patients are contracting nosocomial tuberculosis infection with drug-sensitive or drug-resistant strains. Inadequate therapy has lead to the emergence of multidrug-resistant tuberculosis that is causing increased mortality in AIDS patients. Occurrence of *Mycobacterium avium* infections in AID-tuberculosis patients, have kindled new interests in this disease. Disseminated *Mycobacterium avium* infections occur in a high population of HIV patients with low CD4+ cell count.

Witte *et al.* (1994) observed that intensive care units (ICUs) are more often affected by MRSA than other clinical settings and that ICUs play a special role in intra hospital spread of MRSA. Transfer of patients between hospitals leads to inter regional clonal and inter hospital dissemination of MRSD in Germany. Pre warning of the hospital of destination and a number of hygiene measures can prevent further spread of MRSA.

Ichiyama (1994) recommended the use of DNA fingerprinting for the study of epidemiology of nosocomial MRSA strains. He studied genomic fingerprinting of 563 strains of methicillin-resistant strains of *Staphylococcus aureus* (MRSA), collected from 43 National University Hospitals in Japan, by using pulsed–field gel electrophoresis (PFGE) and identified 136 types.

Webster *et al.* (1994) evaluated hand washing products in terms of user acceptability and effectiveness against MRSA as part of a long-term strategy to eliminate endemic MRSA from the neonatal intensive care unit at the Royal Women Hospital, Brisbane. Following the introduction of a new hand wash disinfectant (triclosan 1% wt / vol), new cases of MRSA colonization were monitored for 12 months. In addition, the use of antibiotics, the incidence of multi-resistant Gram-negative cultures and neonatal infections were noted. No changes were made to any procedures or protocols during the trial. All the babies colonized with MRSA had been discharged from the nursery within 7 months of the
introduction of triclosan and in the subsequent 9 months no new MRSA isolates were reported.

Payne et al. (1994) reported that nosocomial infection in neonates increases morbidity, hospital costs, and mortality. These infections occur most commonly in very low birth weight infants, who frequently required plastic intravascular catheters and parenteral nutrition. Diagnosis often relies on a combination of laboratory tests and nonspecific clinical signs. For treatment the antibiotic coverage of both Gram positive and negative bacteria is necessary and usually multiple antibiotic therapy is recommended to avoid complications and development of resistant strains of micro-organisms.

Emmerson (1994) observed that *Staphylococcus aureus* was responsible for over 20% epidemics during the years 1954-59. MRSA strains emerged rapidly after the introduction of methicillin. Risk factors in epidemic included admission or transfer of a patient or health care worker, suffering from or colonized with MRSA, from community or another hospital. Infection control program is dependent upon staff education and surveillance. Iwahara et al. (1994) evaluated the clinical features of 32 patients with pulmonary infection caused by MRSA. Most of the patients were elderly, postoperative, and had severe underlying diseases. Using chromosomal DNA pattern 32 strains were classified into 20 different types and 5 epidemic strains were observed. The possible route of transmission was microbial colonization of hospital personnel.

Swartz (1994) reported that about 5% of the patients, admitted to the hospitals, acquire nosocomial infections. The infection contributing factors include increasing age, availability for treatment of formally untreatable diseases extensive medical and intensive use of surgical therapies, frequent use of antimicrobial drugs, capability of microbial flora for developing drug-resistance. By 1960 aerobic Gram-negative bacilli had assumed increasing importance as nosocomial pathogens, and many strains were found resistant to available antimicrobial drugs. During the 1980s the principal organism causing nosocomial blood stream infections were coagulase-negative Staphylococci, aerobic Gram-negative bacilli, *Staphylococcus*
Review of Literature

*aureus, Candida species* and *Enterobacter species*. Coagulase-negative *Staphylococci* and *Staphylococcus aureus* strains are often methicillin resistant but vancomycin sensitive. Increasing number of Enterococcal isolates from intensive care patients are vancomycin resistant.

Lee *et al.* (1996) collected from the medical charts of 585 patients admitted in a community based 148 bed nursing facility and reported that overall 41% of the patients developed at least one presumptive nosocomial infection, and 54% of the patients received one or more antibiotic treatment. The overall nosocomial infection rate was 7.2 /1000 patients. The most common sites of infection were urinary tract 38% and respiratory tract 28%. The most common pathogen were *Escherichia coli*. The most frequently used antibiotic group was quinolones. Thirty nine percent of the Staphylococcus isolates, associated with suspected infection, were found resistant to methicillin and of these 94% were resistant to ciprofloxin. Most of the resistant *Staphylococcus aureus* strains were isolated from indwelling catheters associated with urinary tract infections. Infections associated with quinolone resistant strains of gram-negative bacilli were more frequent.

Regnier (1996) reported high prevalence of multi-drug resistant bacterial strains in the intensive care units in hospitals in France. These strains spread to other units of these hospitals. The risk factors involved in transmission were misuse of antibiotics and cross-colonization via the agency of health care workers. High prevalence of resistant strains lead to increased antibiotic use and emergence of new resistant factors. The overall clinical picture was increased morbidity, and increased nosocomial infections. Control measures recommended were the proper use of antibiotics, identification of reservoirs and effective implementation of isolation precautions.

Ismail *et al.* (1997) studied, retrospectively nosocomial and community acquired infection, in a consecutive series of 191 patients, which aged over 60 years and were admitted in 1995 in Singapore. Bacteremia was acquired from the
community in 57.5% of patients and in 33% patients it was of nosocomial in origin. In 9.5% cases it was result of long-term care facilities. The common sources of bacteremia were chest (27.5%), urinary tract (24.5%), skin (12.5), hepatic (8.8%), gut (4.3%), cardiovascular system (1%) and others (3.6%). In 12.5% of cases, the sources were multiple and in 5.3% of cases, the source could not be identified. Twenty-one per cent of the patients with bacteremia died. The factors associated with increased mortality rate were: older age (median age of those that died was 78.5 years compared to survivors with a median age of 73 years, $P = 0.011$), patient's place of origin (patients in nursing home at higher risk of death, $P = 0.04$), patient's mobility status (immobile patients at higher risk, $P = 0.00297$), source of bacteremia--respiratory infection at increased risk of death ($P = 0.00009$) but urinary tract infection had a better survival rate ($P = 0.03935$) and multiple sites of infection (patients with multiple sites of infection had higher risk, $P = 0.00897$).

Methicillin-sensitive *Staphylococcus aureus* infection was associated with a mortality rate of 35.3%, followed by *Klebsiella* species (mortality) 28.6%, *Pseudomonas aeruginosa* (mortality) 28.6%, methicillin-resistant *Staphylococcus aureus* (mortality) 25%, *Proteus mirabilis* (mortality) 25% and *E. coli* (mortality) 19.1%.

Rotimi *et al.* (1998) analyzed the prevalence and antibiotic susceptibility pattern of consecutive gram-negative bacterial isolates in two intensive care units (ICUs) in Saudi Arabia (Jeddah) and Kuwait. From Jeddah 106 strains and from Kuwait 101 strains were isolated, respectively. The most common bacterial isolates in Jeddah versus Kuwait ICUs were *Pseudomonas aeruginosa* (26%, 26%), *Escherichia coli* (23%, 3%), *Klebsiella pneumoniae* (20%, 17%), inducible *Enterobacteraeae* group (17%, 14%), and *Acinetobacter* spp. (9%, 33%).

Nivin *et al.* (1998) reported an increase in the cases of multidrug-resistant tuberculosis (MDRTB) at a large urban facility where a prior nosocomial outbreak of MDRTB had occurred. Nosocomial transmission appeared to account for this outbreak as well, including a cluster of cases in a newborn nursery. Seven of 24 patients (29%) described in this investigation might have been exposed in the
hospital nursery during an approximately 2-week period. It was believed to be the first documented outbreak of MDRTB in a hospital nursery. The transmission in the nursery demonstrates that the possibility of exposure to unrecognized active tuberculosis in nursery and hospital personnel is always present. Infection and active disease in the infants developed after a relatively short period of exposure. These findings underscore the need for adherence to published infection control guidelines in health care settings.

Hsueh et al. (1998) recovered 39 isolates of multidrug-resistant *Pseudomonas aeruginosa* from various clinical samples obtained from three patients in an intensive care burn unit from April 1997 to May 1997 and 7 preserved isolates recovered from six patients in other medical wards at National Taiwan University Hospital from April 1996 to May 1997. They observed long-term colonization of various body sites with these strains. The epidemic strain persisted in the three patients for weeks to months; in the meantime, these patients had received multiple antimicrobial agents for the management of intervening episodes of invasive infections (bacteremia, ventilator-associated pneumonia, and/or catheter-related sepsis) caused by this strain as well as concomitant infections due to other organisms. This strain of bacteria was isolated only once previously, it was from a burn patient who was on the unit in December 1996.

Dennesen et al. (1998) reported that since the introduction of antibiotics for clinical use, bacteria have protected themselves by developing various antibiotic resistance mechanisms. Currently, there are increasing problems worldwide with multi-resistant bacteria. These problems are especially evident within hospitals, where they frequently present as nosocomial epidemics. Methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococcus spp. and members of Enterobacteriaceae, with plasmid-encoded extended-spectrum beta-lactamases, cause the most important nosocomial resistance problems, on global scale. They described the characteristics of nosocomial epidemics of these three groups of multi-resistant nosocomial pathogens. Despite the differences in bacterial species, the differences in mechanisms of resistance, the different ecological niches and the
different infections caused by these pathogens, there are striking similarities in the variables determining nosocomial spread. The existence of each of these multi-resistant microorganisms and their concurrent spread seem to result from extensive antibiotic use and lapses in compliance with infection control measures. Problems with these bacteria became evident as monoclonal outbreaks, soon followed by establishment of endemicity especially in intensive care units. Finally, endemicity seems to be established on general hospital wards and in chronic care facilities and nursing homes, creating a continuous influx of colonized patients into special care wards. High compliance with infection control measures and a prudent and more restrictive use of antibiotics are the key measures to prevent these epidemics.

Paradisi and Corti (1998) observed that *Streptococcus pneumoniae* is the most prominent cause of community-acquired infections of the respiratory tract, central nervous system, and bloodstream, but there is an increasing interest in its role in the epidemiology of hospital-acquired infections. Penicillin-resistant pneumococcus strains appeared 3 decades ago and now are present worldwide, often displaying multiple resistance due to antibiotic selective pressure. Horizontal spread can cause either sporadic cases or hospital outbreaks, primarily in younger children and elderly patients. Pneumococcus transmission from one patient to another can be documented by polymerase chain reaction or pulsed-field gel electrophoresis typing. Nosocomial acquisition of infection, along with pediatric age, previous hospitalization, and previous beta-lactam therapy, are the main risk factors significantly associated with penicillin-resistant pneumococcus infections. Nosocomial acquisition also is associated with higher mortality from pneumococcus disease. The importance of penicillin resistance as a risk factor significantly associated with higher mortality from pneumococcus infection is found in some studies, but not in others. Mortality from pneumococcus pneumonia is approximately the same for human immunodeficiency virus (HIV)-infected patients without acquired immunodeficiency syndrome (AIDS) as for HIV-negative subjects, but it is significantly higher in AIDS patients. Penicillin-resistant bacterial strains are involved in the vast majority of hospital disease outbreaks.
Goossens (1998) observed major differences in the epidemiology of vancomycin-resistant enterococci (VRE) prevalent between the United States and Europe. In contrast with Europe, VRE in the United States are resistant to many antibiotics, and there appears to be less genetic variability among these isolates. European VRE of human origin, are usually susceptible to many other antibiotics and are highly polyclonal. These clinical isolates have the same susceptibility profiles as VRE isolated from the animals. The differences in the spread of VRE between the United States and Europe might be explained by the over consumption of glycopeptides and other antibiotics in hospitals in the United States and the use of avoparcin as a growth promotor in animals in Europe.

Brett et al. (1998) assessed the prevalence of nosocomial infections at a rural government hospital from 1992 to 1995. The hospital was a 653-bed rural facility providing primary and tertiary health care. Data of patients, admitted to the hospital between 1992 and 1995, who developed hospital-acquired infections during stay was studied. Over the 4-year period, 7,158 cases of nosocomial infections were identified from 72,532 patients (10.0/100 admissions). High nosocomial infection rate was found in the intensive-care unit (67/100 admissions), urology (30/100 admissions), neurosurgery (29.5/100 admissions), and newborn nursery (28.4/100 admissions). Urinary tract infections (4.1/100 admissions) accounted for most of the nosocomial infections (42%), followed by postoperative wound infections (26.8%) with a rate of 2.6/100 admissions. Nosocomial pneumonitis and bloodstream infections also were common with 13.2% and 8.0% rates, respectively. The highest incidence occurred in the intensive-care unit for both pneumonia (26.4/100 admissions) and bloodstream infection (7.0/100 admissions). Poor infection control practices, inadequate hand-washing facilities, lack of supplies, and nonexistent garbage cans in most wards were quite evident.

Richards et al. (1998) described the epidemiology of nosocomial infections in Coronary Care Units (CCUs) in the United States from data collected between 1992 and 1997 using the standard protocols of the National Nosocomial
Review of Literature

Infections Surveillance (NNIS) Intensive Care Unit (ICU) surveillance component. Data on 227,451 patients with 6,698 nosocomial infections was analyzed. Urinary tract infections (35%), pneumonia (24%), and primary bloodstream infections (17%) were almost always associated with use of an invasive device (93% with a urinary catheter, 82% with a ventilator, 82% with a central line, respectively). The mean overall patient infection rate was 2.7 infections per 100 patients. Device-associated infection rates for bloodstream infections, pneumonia, and urinary tract infections did not correlate with length of stay, number of hospital beds, number of CCU beds, or the hospital teaching affiliation, and were the best rates for comparisons between units. Overall patient infection rates were lower than in other types of ICUs, which was largely explained by lower rates of invasive device usage.

Blahova-Kralikova et al. (1998) obtained 2 high frequency transduction (HFT) phage isolates, from seriously ill patients, transducing individual determinants of antibiotic resistance to antibiotic-susceptible strain of *Pseudomonas aeruginosa*. The appearance of such phages in clinical conditions with an unusually high frequency of transduction might contribute to the dissemination of antibiotic resistance genes among nosocomial strains of *P. aeruginosa*, thus an increased risk of spread of antibiotic resistance to recently introduced anti-pseudomonal antibiotics and unwanted epidemiological consequences in hospital conditions.

Valero and Saenz (1998) studied the etiologic variations of nosocomial infections (NI) in the surgery departments of a university hospital. Active surveillance of NI in the departments of general, vascular and urologic surgery was undertaken in 1988 and 1996. The frequency of the presentation of different microorganisms was globally calculated and based on the localization of the infection. Microorganisms isolated were *E. coli* (20.6%), *Enterococcus* spp. (15.6%), *S. epidermidis* (8.8%), Streptococcus spp. (8.5%), other negative coagulase staphylococci (NCS) (5.7%), *Pseudomonas* spp. (5.5%), *S. aureus* (5.2%), and *Candida* spp. (4.3%). On analysis of the temporal evolution an increase was observed in gram positives (27.4% in 1988 and 46.4% in 1996). *Enterococcus* spp. increased in surgical infections (5.8% in 1988 and 15.8% in 1996) and in the
Review of Literature

urinary tract (8.5% in 1988 and 25.6% in 1996). Contrary to the *S. epidermidis*, the NCS increased in importance mainly in infections at the site of surgery (0% in 1988 to 5.1% in 1996). The appearance of *Klebsiella* spp., *Enterobacter* spp. and *Proteus* spp. decreased during the same period.

Husni et al. (1999) investigated an outbreak of *Acinetobacter baumannii* in medical intensive care unit (MICU) from March to September 1995. There were 15 cases of nosocomial pneumonia due to *A. baumannii*, with 50% showing evidence of bacteremia, with chart missing in one case. Twenty-nine patients were identified as control patients. The mean age for case patients was 50 (range, 21 to 84). The mean duration of time from admission to the ICU to infection was 12.8 days (range, 4 to 40). Sepsis developed in 35% of the patients. Forty-three percent of the case patients died during their hospitalization, with two of those deaths attributed to Acinetobacter infection. Pulsed-field gel electrophoresis revealed two strains to be responsible for the outbreak. Hand washing was performed before patient contact by only 10% of health-care workers, and only 32% washed their hands after patient contact. Ceftazidime was associated with an increased risk of nosocomial pneumonia with resistant strains of Acinetobacter and contaminated hands of health-care workers were incriminated with transmission of disease.

Gutierrez et al. (1999) genetically characterized multidrug-resistant *Mycobacterium tuberculosis* complex strains that had caused a nosocomial outbreak of tuberculosis affecting 6 HIV-positive patients and one HIV-negative staff member. Study revealed that the outbreak was due to a single dysgonic, slow-growing *M. tuberculosis* strains mimicking *Mycobacterium bovis* phenotype, probably as a consequence of cellular alterations associated with the multi drug resistance. The strain, isolated from the last patient diagnosed three years after the index case, differed slightly from the patterns of the other six strains; the divergence is attributed to genetic event. This study stresses the value of using several independent molecular markers to identify multidrug-resistant tubercle bacilli.

Buttery et al. (1999) reported that the nosocomial outbreaks of
*Pseudomonas aeruginosa* in pediatric hospitals frequently involve neonates and immunosuppressed patients and can cause significant morbidity and mortality. Specimens were collected from infected patients and the ward environment. Bacterial isolates were characterized, by antibiotic susceptibility patterns, and bacterial DNA fingerprinting performed by pulsed-field gel electrophoresis (PFGE). A case-control study was carried out to assess possible risk factors for infection. Eight patients had clinical illnesses including bacteremia (n=5) and infections of skin (n=2), central venous catheter site (n=1) and urinary tract (n=1). The environmental ward survey yielded isolates of multi-resistant *P. aeruginosa* from a toy box containing water-retaining bath toys, as well as from three of these toys. PFGE demonstrated identical band patterns of the isolates from patients, toys and toy box water.

Rello *et al.* (1999) carried out a retrospective multi-center study comparing microorganisms documented by quantitative cultures from bronchoscopic samples in episodes of ventilator-associated pneumonia (VAP) from three different institutions in Barcelona (B), Montevideo (M), and Seville (S). The observations were compared with the findings reported by Trouillet and coworkers (AJRCCM 1998; 157:531-539) in Pairs (P). Significant variations in etiologies (P≤0.05) were recorded in all of the microorganisms isolated from VAP episodes across three treatment sites when compared with the reference site (P). In Group 1 (<7 d and absence of antibiotics), *Pseudomonas aeruginosa* remained extremely infrequent (3 of 89, 3.3%) in the joint category, whereas the incidence of *Acinetobacter baumannii* was significantly higher. On the other hand, one site (B) had a significantly lower incidence of multi-resistant pathogens (Meticillin-resistant *Staphylococcus aureus* and non-fermenters other than *P. aeruginosa*). It was concluded that causes of VAP varied markedly across four treatment sites. Instead of following general recommendations, anti-microbial prescribing practices for VAP should be based on up-to-date information of the pattern of multi-resistant isolates from each institution.

Fridkin and Gaynes (1999) reviewed the unique nature of the intensive
care unit (ICU) environment that makes this part of the hospital a focus for the emergence and spread of many antimicrobial-resistant pathogens. There are ample opportunities for the cross-transmission of resistant bacteria from patient to patient, and patients are commonly exposed to broad-spectrum antimicrobial agents. Rates of drug-resistance have increased for most pathogens associated with nosocomial infections among ICU patients, and rates are almost universally higher among ICU patients compared with non-ICU patients. There are many opportunities, however, to prevent the emergence and spread of these resistant pathogens through improved use of established infection control measures (i.e., patient isolation, hand washing, glove use, and appropriate gown use), and implementation of a systematic review of antimicrobial use.

Alito et al. (1999) studied possible nosocomial transmission of multidrug-resistant (MDR) *Mycobacterium tuberculosis*, in 24 human immunodeficiency virus-positive patients. Isolates from 11 patients had identical IS6110 restriction fragment length polymorphism (RFLP) patterns as well as spoligotype patterns and resistance profiles. Noticeably, nine other isolates from related cases also exhibited identical spoligotypes but slightly different RFLP patterns. These results indicate that some IS6110 MDR-MT strains mutate at a much faster rate.

Harbarth et al. (1999) conducted a one-week period-prevalence survey, aimed at assessing the scale of nosocomial infections, in May 1996 in medical, surgical, and intensive care wards of 4 Swiss university hospitals. Standard definitions by the centers for disease control and prevention were used except that asymptomatic bacteriuria was not classified as a nosocomial infection. A total of 176 nosocomial infections were found among 156 of the 1349 surveyed patients (prevalence 11.6%; inter-hospital range 9.8-13.5%). Surgical site infections were most prevalent (30% of all nosocomial infections), followed by urinary tract (22%), lower respiratory tract (15%), and bloodstream infections (13%). The overall prevalence of nosocomial infections in surgical patients (n = 562) was 16.2% compared to 8.6% for non-surgical patients (prevalence ratio, 1.9; 95% confidence interval. In one center, the in-hospital mortality of patients with nosocomial
Review of Literature

infections was 9.2% (10/109) compared to 3.9% (25/637) for patients without nosocomial infections.

Harbarth et al. (1999) studied the impact and pattern of Gram-negative bacteremia (GNB) at a Swiss University hospital. They conducted a 6 years retrospective cohort study using linear regression and multivariate Cox-proportional hazard analysis. A total of 1766 patients had 1835 episodes of GNB of which 61% were community acquired. The in-hospital mortality of GNB patients decreased from 20% in 1989 to 16% in 1994. The risk ratio for death was higher in GNB patients as compared to patients without GNB. Factors associated with increased hazard ratio (HR) for death were severity of illness [HR 1.5], age 66 to 79 [HR 1.8], GNB due to Klebsiella species [HR 1.7], Pseudomonas species [HR 1.6]; and poly microbial infection [HR 1.6]. Multidrug resistant strains of GNB did not influence the death hazard.

Sacks et al. (1999) reported that the nosocomial multidrug-resistant tuberculosis (MDR-TB) in human HIV-infected people is documented in Europe and America. They reported the first such outbreak in South Africa, in which six hospitalized women were infected with strain of MDR-TB while receiving treatment for drug-susceptible tuberculosis. The putative source of case was identified as an HIV-positive woman who underwent prolonged hospitalization for chronic tuberculosis. Compared with other HIV-positive patients in the hospital, outbreak patients were more immune compromised, had fewer cavitary lung changes, and were less likely to have been treated before. They had high fever episodes, infiltrative patterns on chest radiographs, and a mean survival of 43 days. When individual isolation is not possible, separating highly immunocompromised patients with first-time tuberculosis from previously treated patients with cavitary lesions and from those with established drug resistance may help to reduce nosocomial transmission.

De Galan et al. (1999) reported nosocomial epidemic chronic obstructive pulmonary disease (COPD) involving 36 patients. The source of outbreak was a
76-year old patient, who had been colonized with same strain of *Streptococcus pneumoniae*, from 1993. COPD ceased following the commencement of barrier nursing, a treatment course of ceftriaxone, and a five-day rifampicin eradication therapy for the positive patients. The outbreak resulted from failure to recognize quickly the rapid transmission of this multidrug-resistant pneumococcal clone. They concluded that the patients with COPD are at high risk of acquiring multidrug resistant pneumococci, and suggested that COPD patients who are colonized or infected with multidrug-resistant pneumococci should be isolated to prevent further transmission of pneumococci.

Cors *et al.* (1999) examined 148 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from 13 hospitals in Argentina for antibiotic susceptibility and clonal type, using hybridization with DNA probes specific for mecA and Tn554 polymorphs characteristic of an MRSA clone widely spread in Brazilian hospitals. Similarly to the Brazilian isolates, the MRSA clones recovered in the Argentinean hospitals showed susceptibility to spectinomycin and resistance to the action of numerous antibacterial agents, including beta-lactams, tetracycline, aminoglycosides, macrolides, trimethoprim/sulfamethoxazole, ciprofloxacin, and fosfomycin, and more than 60% of the isolates were also resistant to chloramphenicol and rifampin. The MRSA clone represented the majority of isolates recovered in most of the hospitals, nine of which were located in the city of Buenos Aires, three in the province of Buenos Aires, and one in the province of Tucuman. The observations document further geographic expansion of this South American MRSA clone across national boundaries.

Weber *et al.* (1999) reported that the patients hospitalized in ICUs are 5 to 10 times more likely to acquire nosocomial infections than the other hospital patients. The frequency of infections at different anatomic sites and the risk of infection vary by the type of ICU, and the frequency of specific pathogens varies by infection site. Contributing to the seriousness of nosocomial infections, especially in ICUs, is the increasing incidence of infections caused by antibiotic-resistant pathogens. Prevention and control strategies have focused on methicillin-resistant
Review of Literature

*Staphylococcus aureus*, vancomycin-resistant Enterococci, and extended-spectrum beta-lactamase-producing Gram-negative bacilli, among others. An effective infection control program includes a surveillance system, proper hand-washing, appropriate patient isolation, prompt evaluation and intervention when an outbreak occurs, adherence to standard guidelines on disinfections and sterilization, and an occupational health program for health-care providers. Studies have shown that patients infected with resistant strains of bacteria are more likely than the control patients to have received prior antimicrobials, and hospital areas that have the highest prevalence of resistance also have the highest rates of antibiotic use. For these reasons, programs to prevent or control the development of resistant organisms often focus on the overuse or inappropriate use of antibiotics, for example, by restriction of widely used broad-spectrum antibiotics (e.g., third-generation cephalosporins) and vancomycin. Other approaches are to rotate antibiotics used for empiric therapy and use combinations of drugs from different classes.

Johnson *et al.* (2002) studied the associations of virulence genotype and phylogenetic background with epidemiological factors (primary source of bacteremia, host compromise status, and hospital versus community origin). They assessed 182 *Escherichia coli* blood isolates from adults with diverse-source bacteremia. A continuum of virulence was found, from urinary and pulmonary isolates which were highly virulent as compared to fecal isolates which fed low virulence.
Glassware

Pyrex and Jena glassware, resistant to heat and acids, obtained from local market was used throughout this research investigation. The petri dishes, test tubes, McCartney bottles, screw capped bottles, flasks, beakers, measuring cylinders etc were cleaned and dipped overnight in a mixture of sulfuric acid and potassium dichromate (equal parts of H₂SO₄ and K₂CrO₄, volumes v/w), washed and rinsed several times in sterile distilled water and dried. The petri dishes were wrapped in clean bamboo paper. The test tubes were plugged with sterile cotton wool. The mouth of flasks, beakers and cylinders were wrapped with aluminum foil. All the glassware was sterilized in hot air oven at 180°C for an hour and stored in clean dust free racks till used.

Swabs and Culture tubes

The swabs were prepared from cotton wool and wrapped at one end of a wooden clean applicator and placed in the cleaned sterile test tubes with cotton plugging and sterilized in hot air oven at 180°C for 2 hours, cooled and stored till used.

Chemicals

Analytical grade chemicals manufactured by M/s Merck’s Germany and Sigma Laboratories, USA were used in the study.

Deionized Distilled Water

Deionized distilled water for these investigations was obtained from the Tissue Culture Laboratory of the Microbiology Department, University of Veterinary and Animal Sciences, Lahore.

SPECIMEN

Patients: A total of 32,620 indoor patients during the year 1997-2001 from various wards of the Mayo Hospital Lahore were observed as all of them were
considered to be at risk of contracting the nosocomial infections. The patients who developed various kinds of infections other than their original disease were considered to have contracted the nosocomial infections. Clinical samples were collected from all the superficial and deep seated wounds, closed abscesses, surgical wounds, ulcers, blood, pleural fluids, ascitic fluids, cerebrospinal fluids (CSF), urine, sputum, endotracheal secretions, burn swabs, medical devices present in the body of such patients, fecal and drainage tube material were all included in this study (Table 3.1). A total of 4502 positive cases were selected and processed for bacteriological analysis and the antibiogram of the isolates. The details of various types of samples collected from patients in various wards are shown in Table-3.2.
Table-3.1: Types of samples collected from patients

<table>
<thead>
<tr>
<th>Types of samples</th>
<th>Number collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus and wound swabs</td>
<td>1040</td>
</tr>
<tr>
<td>Blood.</td>
<td>109</td>
</tr>
<tr>
<td>Pleural fluids</td>
<td>115</td>
</tr>
<tr>
<td>Ascitic fluids</td>
<td>286</td>
</tr>
<tr>
<td>Cerebrospinal aspirates</td>
<td>37</td>
</tr>
<tr>
<td>Urine</td>
<td>1398</td>
</tr>
<tr>
<td>Sputum</td>
<td>988</td>
</tr>
<tr>
<td>Burns swabs</td>
<td>329</td>
</tr>
<tr>
<td>Body devices</td>
<td>99</td>
</tr>
<tr>
<td>Fecal and drainage samples</td>
<td>101</td>
</tr>
<tr>
<td>Total sampling from patients</td>
<td>4502</td>
</tr>
</tbody>
</table>
Materials and Methods

Table-3.2: Details of samples collected from various wards of the Mayo Hospital, Lahore

<table>
<thead>
<tr>
<th>Hospital Units</th>
<th>Types of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pus &amp; wound swabs</td>
</tr>
<tr>
<td>Medical Units</td>
<td>15</td>
</tr>
<tr>
<td>Surgical Units</td>
<td>775</td>
</tr>
<tr>
<td>Intensive Care Units (ICU)</td>
<td>25</td>
</tr>
<tr>
<td>Cardiology Deptt.</td>
<td>14</td>
</tr>
<tr>
<td>Cardiac Surgery</td>
<td>31</td>
</tr>
<tr>
<td>Chest Surgery</td>
<td>24</td>
</tr>
<tr>
<td>Orthopedic Surgery</td>
<td>90</td>
</tr>
<tr>
<td>Pediatric Deptt</td>
<td>26</td>
</tr>
<tr>
<td>Burn Unit</td>
<td>-</td>
</tr>
<tr>
<td>Ophthalmology Deptt</td>
<td>4</td>
</tr>
<tr>
<td>Neuro surgery</td>
<td>10</td>
</tr>
<tr>
<td>Urology Deptt</td>
<td>22</td>
</tr>
<tr>
<td>Dermatology Deptt</td>
<td>2</td>
</tr>
<tr>
<td>Oncology Deptt</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1040</td>
</tr>
</tbody>
</table>
Hospital Staff (Health Care Workers)

Swab samples were collected from the nasal and throat areas of hospital staff, their hand surfaces, gloves and aprons. A total of 635 samples were collected and analyzed in this study (Table-3.3). In addition, a total of 25 throat and nasal swabs from apparently healthy male and female persons residing in nearby localities of hospital were collected and processed as control samples.

<table>
<thead>
<tr>
<th>Specimen*</th>
<th>Number Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat and nasal swabs.</td>
<td>127</td>
</tr>
<tr>
<td>Hand swabs.</td>
<td>254</td>
</tr>
<tr>
<td>Gloves and aprons</td>
<td>254</td>
</tr>
<tr>
<td>Total</td>
<td>635</td>
</tr>
</tbody>
</table>

*In addition 25 nasal and throat swab samples were collected from localities nearby hospitals and processed as control samples.

Hospital Environment:

Since environment of a hospital is constantly exposed to extraneous contamination; the healthcare workers, patients, attendants, hospital animates and inmates are always exposed to this atmosphere. Using the air-sampling device the air samples of the operation theatres and hospital wards were collected and processed for bacterial analysis. The sterilized water samples used during surgical procedures were also collected and processed for bacteriology. The surgical instruments used during surgery were examined for bacterial contamination. A total of 2677 samples from the hospital environment were collected and investigated (Table-3.4).
Table-3.4: Details of samples from hospital environment

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Number of collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation theater air</td>
<td>126</td>
</tr>
<tr>
<td>Ward environment</td>
<td>65</td>
</tr>
<tr>
<td>Sterilized water</td>
<td>610</td>
</tr>
<tr>
<td>Surgical instruments.</td>
<td>1876</td>
</tr>
<tr>
<td>Total</td>
<td>2677</td>
</tr>
</tbody>
</table>

a = A total of 25 air samples outside operation theater were also collected and processed as control samples.

Non-human inmates

The cats living in the hospital premises were trapped, carefully secured, and their nasal and rectal swabs were collected. The cockroaches were caught, dissected and bacteriology of their legs and viscera (intestine) was studied. A total of 226 samples were collected from these sources and analysed for bacterial isolation (Table-3.5).

Table-3.5: Number of specimens collected from non-human inmate sources

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Number Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats (nasal swabs)</td>
<td>25</td>
</tr>
<tr>
<td>Cats (fecal swabs)</td>
<td>25</td>
</tr>
<tr>
<td>Cockroaches (legs)</td>
<td>88</td>
</tr>
<tr>
<td>Cockroaches (intestine)</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
</tr>
</tbody>
</table>
COLLECTION OF SAMPLES

Pus Samples:

Samples of pus were collected from abscesses, pustules and other deep seated wounds. Attempts were made to collect the samples of pus before dressing the lesions early in the morning. The pus specimen from the open wound was collected with the help of a sterilized swab which was transferred into sterilized culture tube. In deep seated wounds, the affected area was wiped and cleaned with sterilized cotton swabs. Pus was aspirated in 5 ml. disposable sterilized syringe and then transferred to appropriately labeled sterilized culture tubes. The pustules were washed with soap and luke warm water, the seal of the pustule was broken using a sterile needle and the pus was collected and transferred to a sterilized culture tube.

Exudates from burn wound:

Burn wound samples were collected with the help of a dresser; a sterile swab was gently applied and twisted to the effected area and transferred into sterilized culture tube.

Specimen from deep-seated ulcers and wounds:

The morbid materials from deep ulcers and wounds were collected in a culture tube by the help of a Medical Officer or an experienced nurse. Pus from the abscess was best collected at the time when the abscess was incised and drained or after it had ruptured naturally. When collecting pus from abscess, wounds or other body sites special care was taken to avoid contamination of the morbid tissues with the commensal organisms from the skin.

A total of 1040 pus samples were collected from the indoor patients for bacteriological examination (Table 3.2).

Pleural Fluids:

The collection of pleural fluid was carried out with the help of a medical officer by needle aspiration technique. After aspiration the fluid was aseptically dispensed into sterile screw capped tubes, The labeled and processed soon after its collection. A total of
115 patient samples were collected and used for detailed bacteriological analysis.

**Ascitic Fluids:**

Ascites or ascitic fluid from peritoneal (abdominal) cavity was obtained by the help of a medical officer using needle aspiration technique. The fluid was collected and dispensed aseptically into a sterile screw capped tubes. The specimen were labeled and processed immediately after collection. A total of 286 ascitic fluid samples were collected and analyzed for bacterial isolation and characterization.

**Cerebrospinal Fluid (CSF)**

Cerebrospinal fluid was collected by the help of an experienced medical officer. It was collected aseptically to prevent organisms being introduced into the central nervous system. The fluid was collected mostly from the arachnoid space. A sterile wide-bore needle was inserted between the fourth and fifth lumber vertebrae and the CSF was allowed to drip into a dry sterile container. A ventricular puncture was some times performed to collect the CSF from infants. The collected specimen was labeled and processed immediately. A total of 37 specimens of CSF were collected and analyzed for various bacterial species.

**Urine:**

Mid stream urine samples (10-20 ml) were collected from the patients in sterile, dry, wide necked, leak proof containers by explaining the patient to avoid as little contamination as possible i.e. clean-catch specimen. The containers were labeled with the date, name and time of collection and processed soon after sample collection.

From catheterized patients, urine samples were collected by disinfecting the wall of catheter at its juncture with the drainage tube, and urine was aspirated by a sterile disposable syringe and was processed as explained above.

A total of 1398 urine samples were collected from the patients suspected to be suffering from bacterial urinary tract infections.
Blood:

Blood culture was carried when patients were suspected of suffering from bacteremia or septicemia. For blood collection the venipuncture site was washed with soap, rinsed with sterile distilled water, dressed with pyodine (commercial preparation containing 1% iodine), cleaned with a swab dipped in 70% alcohol, and dried. Blood was drawn with 5 ml sterilize disposable syringe and transferred to blood culture bottle containing thioglycolate broth and tryptic soya broth and the bottles were gently rotated to ensure mixing of broth with the blood. The procedure was adopted with great care to avoid contamination of the specimen and culture medium.

A total of 109 blood samples were collected from the patients, showing evidence of bacteremia or septicemia and were processed for bacterial isolation.

Sputum and Other Samples:

Sputum samples were collected from the patients of indoor and the intensive care units. The variety of 988 samples included pulmonary discharge, sputum, throat swabs and tracheal aspirates. The patient was advised to clean his/her teeth and gargle with sterilized distilled water to remove excessive saliva and food debris. The patients were advised to cough deeply, and the expectorated sputum was collected in sterilized screw capped open mouth bottles/containers. Pulmonary discharges were obtained from the drainage tubes using all necessary aseptic precautions. The specimen was collected with the help of sterilized disposable syringes and transferred to a sterilized screw capped bottle. Tracheal aspirates were collected with the assistance of a trained professional during the procedure. These specimens were collected with a sterilized disposable syringe attached to the aspirator. The material was transferred to sterilized screw capped bottle and subjected to bacteriological examination.

Throat and Nasal Swabs:

A total of 127 samples were collected for detailed bacteriological analysis. Care was taken to avoid the possible contamination with lips, tongue or gums. Using a tongue depressor, the sterilized cotton swab was rubbed firmly over the back of throat, and exposed area of inflammation or ulceration. The swabbing of nostrils was done gently by
introducing and rotating the swab in the nostrils of infected individuals. The swab was immediately transferred in sterile culture tubes.

**Patient Body Devices (Medical Devices):**

A total of 99 samples were collected from catheters, CVP lines, shunts, intravascular cannulae, and joint prosthesis, and were added to McCartney bottle containing enriched broth for bacteriological examination.

**Fecal and Drainage Specimens:**

A total of 99 fresh stool specimens and rectal swabs were collected from the patients and drainage tube specimens were also collected and transferred to a sterilized McCartney bottles containing liquid nutrient media.

**Sampling of Health Care Workers**

*(Throat, Nasal & Hand swabs, Gloves & Aprons)*

The nasal and throat swabs were collected with the help of a medical officer. Using a sterile swab the specimen from the infected area of the throat was collected avoiding any contamination. In the same way the nasal swabs were also collected using aseptic technique. The nails of finger and palms of hands of health care workers were firmly rubbed with swabs soaked in nutrient broth and were transferred to sterilize culture tubes. The swabs of gloves and aprons were also processed for bacteriological analysis (Table-3.3).

**Sampling of Hospital Environment**

**Surgical Instruments:** A total of 1876 samples from surgical instruments were collected and processed for the bacteriological analysis. Swabs soaked in nutrient broth were rubbed on the cutting edge, surface of the instrument and transferred to the sterilized McCartney bottle containing liquid nutrient media.

**Operation Theaters and Wards:** Using air sampling device, a total of 126 air samples were collected from operation theatres, and 65 air samples were collected from the hospital wards and subjected to detailed bacteriological analysis.
**Samples of Sterilized Water:** A total of 610 samples were collected and processed for bacteriological examination. The contamination in sterilized water was evaluated using standard bacteriological technique as the water was sucked through filtering device fitted with a sterilized disc, and the bacteriology of the disc was studied.

**Sampling of Hospital Inmates:**

**Cockroaches:** Cockroaches were trapped from different wards of the hospital, kitchen facilities, sinks, drains etc. To study the bacteriology of external parts of the body, the insect was caught with a sterilized pincer and the legs were carefully dissected and dipped in broth for further processing. To study the bacterial flora of internal parts of the body, the insects were slightly anaesthetized; and their abdominal contents (intestines) were emulsified and used for bacteriological studies. A total of 88 cockroach legs samples and 88 visceral samples (intestines) were subjected to bacteriological analysis.

**Cats:**

Cats roaming about in various wards were trapped with the help of an expert and trained veterinary assistant and were secured for the collection of nasal and rectal swabs. A total of 25 nasal and 25 rectal swabs were transferred to sterilized culture tubes and immediately processed for bacteriological isolation and characterization.

**Processing of samples:**

All the samples were processed within one hour of collection under strict aseptic conditions during their handling and processing.

Primary Bacterial isolation, from all specimens was attempted on various types of agar media. In cases where liquid media were used for enrichment/primary isolation, the inoculated tubes were examined for the evidence of growth/turbidity after prescribed time, and loopful of material from growth positive tubes, was streaked on solid media for further evaluation of the bacterial growth.
Examination of Body Fluids (Pleural and Peritoneal):

The pleural and peritoneal fluids were examined for the evidence of any bacterial contamination. The suspected materials were processed as under:

- Under the strict aseptic conditions the fluid was transferred to sterilized centrifuge tubes and spun @ 3000 rpm for 15 minutes. The supernatant was discarded and sediments were used for inoculation on media and from the sediment were prepared stained and examined under the oil immersion lens for the presence of any bacterial species.

- Smears were prepared from each specimen by placing a loopful of sediment material on clean glass slide, and spreading on the surface of slides with circular movements of loop; the smears were air-dried, and stained with Gram, Acid fast and Cotton blue stain and examined under oil-immersion lens for the presence of microbes; bacterial morphology, and microbial reaction to differential stains.

- A loopful of the sediment was inoculated on various media such as:
  - Chocolate (heated blood) agar, blood agar and MacConkey agar. The Chocolate agar plates were incubated in a CO₂ atmosphere at 35-37°C for 48 hours. Blood agar and MacConkey agar plates were incubated aerobically at 35-37°C upto 72 hours.
  - Additionally the specimen was cultured on Lowenstein Jensen (L-J) medium for detecting mycobacteria.

- Duplicate sets of plates and tubes were inoculated with each specimen suspected of containing bacterial contamination and the inoculated plates/ tubes from each specimen were incubated aerobically and anaerobically, at 37°C for 24-48 hours and examined for the evidence of growth. The inoculated plates showing no growth after 48 hours were incubated further for 24 hours.

- The plates and tubes inoculated on solid media for fungi and yeasts isolation were incubated at room temperature.
Blood Agar plates were also inoculated, simultaneously, to study the hemolytic properties of the infecting bacteria.

Selective, enriched and differential media, where necessary, were used for primary isolation for enhancing isolation and identification of bacteria.

The bacterial growth was further purified on Brain Heart Infusion Agar for obtaining typical growth characteristics and colonial morphology.

**Examination of Cerebrospinal Fluid (CSF)**

Cerebrospinal fluid was collected with special care because a lumber puncture was always required to collect the specimen:

- The specimen was centrifuged @ 1000 rpm for 5-10 minutes.

- The supernatant fluid was transferred to another tube and the sediment was mixed and used for smear preparation and inoculation.

- Few drops of the sediments were transferred to the slides for the preparation of smears.

- The smears were prepared and stained by Gram stain, Zeil Neelson (ZN) stain, India ink stain and Fungal stain.

- The smears were examined for pus cells bacteria, AFB and fungal cells using Oil immersion lens.

- The supernatant fluid of the CSF was cultured immediately and it was inoculated on chocolate agar, blood agar (in a carbon dioxide atmosphere) and MacConkey’s agar. The inoculated media was incubated for 24-72 hours at 35-37°C.

- A loop of the morbid specimen was also inoculated on LJ media tubes for mycobacteria and the tubes were incubated for six weeks at 35-37°C.
Examination of Urine Samples

Urine specimens collected were processed as under:

- The specimen was centrifuged @ 1000 rpm for 5-10 minutes.
- The supernatant fluid was transferred to another tube and the sediment was mixed and used for smear preparation and inoculation.
- Few drops of the sediments were transferred to the slides for the direct examination of smears.
- The smears were prepared and stained by Gram stain, Zeil Neelson (ZN) stain, India ink stain and Fungal stain.
- The smears were examined for pus cells, bacteria, AFB and fungal cells using Oil immersion lens.
- The freshly collected urine samples were cultured immediately and it was inoculated on blood agar, MacConkey’s agar and CLED agar. The inoculated media was incubated for 24-72 hours at 35-37°C.
- A loop of the morbid specimen was also inoculated on LJ media tubes for mycobacteria and the tubes were incubated for six weeks at 35-37°C.

Blood:

The blood specimens were examined for the evidence of bacteremia, septicemia or pyemia according to standard techniques described by Cheesbrough (2000).

Aseptically drawn, 5-10 ml of blood was added to sterile blood culture bottles containing BHI/Thioglycolate media and the inoculated media bottle were incubated at 35°C to 37°C for 7 days. The bottles were examined daily, using magnifying lens, for the evidence of any growth.

Inocula from media bottles indicating growth was sub-cultured on Blood Agar, Chocolate Agar and MacConkey agar plates and the plates containing blood agar and
Materials and Methods

MacConkey agar were incubated aerobically. The Chocolate Agar plates were incubated in CO₂ incubator.

Examination of Fecal Specimens

- The fecal material for microbiological examination was collected during the acute stage of diarrhea in a clean, dry, disinfectant free wide necked container advising the patient to avoid mixing of individuals feces with urine.

- A portion of the fecal material which contained mucus, pus or blood was transferred into a clean, dry, leak proof container, labeled and processed within one hour of collection.

- Rectal swabs were also obtained and unnecessary contamination of the sample with bacteria from the anal skin was avoided.

- The smears from morbid samples were prepared and stained by Gram’s method, ZN stain, basic fuchsin and fungal stain.

- The smears were examined for pus cells, bacteria, AFB and fungal cells under Oil immersion lens.

- The specimen of fecal material for bacterial isolation was processed as follows:
  
  i) a loopful of fresh emulsified stool material was inoculated on XLD agar plates which were incubated aerobically at 35-37°C over night.

  ii) Several loopfuls of specimen were inoculated in alkaline peptone water and incubated at 35-37°C for 5-8 hours. Several loopfuls of incubated peptone water were sub-cultured on thiosulphate citrate bile-salt sucrose (TCBS) agar and incubated aerobically at 35-37°C over night.

  iii) A loopful of stool sample was inoculated on sorbitol MacConkey agar and incubated aerobically at 35-37°C for 24-48 hours.

- Blood agar plates were also inoculated, simultaneously, to study the hemolytic
Materials and Methods

properties of the infecting bacteria.

- Selective enriched and differential media, where necessary, were used for primary isolation and identification of bacteria.

- The bacterial growth was purified on Brain Heart Infusion Agar for studying the typical growth characteristics and colonial morphology of the isolates.

Examination of Pus, Ulcers and Skin Specimens

Pus, wound swabs and other purulent materials collected from various sources were processed and examined using the following procedure:

- A loopful of pus or purulent material was placed in a drop of sterile distilled water on a clean glass slide and spread on the surface of the slide to achieve a thin smear.

- At least 3 smears were prepared from each specimen, one smear was stained with Gram’s Stain, other with the Acid Fast Stain, and the third with the Fungal stain. All the stained smears were examined under oil immersion lens.

- The specimens were inoculated on blood agar plate, MacConkey agar plate in cooked meat medium, thioglycollate broth and neomycin blood agar plates.

- Inoculated media from each specimen were incubated aerobically and anaerobically at 35°-37°C for 24-72 hours and periodically examined for the evidence of any bacterial growth.

- The hemolytic properties of the bacteria were observed on blood agar.

- A loopful of specimen was inoculated on Lowenstein Jensen (L-J) medium and incubated for six weeks.

- Selective, enrichment and differential media, were employed for primary isolation and identification of bacteria.

- Swabs from dressing materials, catheters, drain tubes, branulas, surgical
instruments, hands of healthcare workers, sterilizers, floors, sinks, stitching material and gloves etc. were also processed for the bacterial isolation using the procedures described by Lenett et al (1980).

**Materials and Methods**

**Examination of Sputum and Pulmonary Expectorate and Exudates**

Samples of sputum, pulmonary expectorate and exudates were examined using the following protocol.

- The material was pretreated with sterile antiformin or sterile 5% sodium hydroxide solution (5g of NaOH dissolved in 95ml sterile distilled water) to dissolve the phlegm and cellular debris without harming the bacteria thus making isolation easier. A measured quantity of diluting fluids was added to the expectorate and well mixed by gentle shaking or triturating; the mixture was allowed to stand for 30 minutes to allow the chemicals to dissolve the contaminating material. The suspension was added to sterilized centrifugal tubes and spun @ 3000 RPM for 15 minutes, the supernatant was discarded and the sediments were suspended in sterile buffer solution to neutralize excessive acidity or alkalinity and the suspension was re-centrifuged at the speed mentioned above; the supernatant was discarded and the sediments were processed for further testing.

- Antiformin was used only when the material was being processed for the isolation of various Mycobacterium spp.

- Three smears were prepared by placing loopful of sediments on separate glass slides and the material was spread on the surface of slides with circular moments of loop, and the smears were air-dried and fixed. One smear of each specimen was stained with Gram’s method other with acid fast stain and the third with cotton blue stain and examined under oil-immersion lens for the morphology, and staining characteristics of the isolates.

- Loopful of the sediments were inoculated on sterile plates and tubes of culture media. By the gentle movements of loop the material was streaked over the entire
Materials and Methods

surface of the petri dish to achieve a thin growth or individual colonies of bacteria.

- The culturing for Mycobacteria was carried out on sets of Lowenstein-Jensen medium tubes and Dorset Egg Medium with glycerol (for *Mycobacterium tuberculosis*) and without glycerol (for *Mycobacterium bovis*). For isolation of Mycobacteria thick inocula of sediments were streaked on the above media slants and the inoculated tubes were incubated for 2 to 3 weeks at 37°C and examined every 24 hours for the presence of any growth.

- Duplicate sets of plates and tubes were used for culturing each specimen, one set of inoculated plates/tubes from each specimen was incubated aerobically and the other set was incubated anaerobically.

- Where the presence of carbon dioxide (CO₂) was necessary for promoting growth the inoculated media plates and tubes were incubated in CO₂ Incubator.

- The plates and tubes, inoculated for fungi and yeasts, were placed at room temperature (22°C) and examined every 24 hours up to a week for the evidence of any bacterial growth.

Air samples from Operation theatres and Hospital environment.

The filter discs from air sampling device were placed in Brain Heart Infusion Broth or Loeffler’s Serum Medium and the inoculated tubes were incubated at 35-37°C for 24 to 48 hours. The further material processing was the same as explained above.

Cats:

Rectal and nasal swabs of cats were obtained and added to enrichment media like Selenite broth/ MacConkey Broth, and Brain Heart Infusion (BHI) broth, and incubated at 35-37°C for 18 hours and examined for the evidence of turbidity. A loopful of material, from the tubes showing turbidity/evidence of growth, were inoculated on Xylose Lysine Dextrose (XLD) agar, MacConkey agar, TCBS agar, Sorbitol MacConkey agar, SS agar and
Brilliant Green Agar, and the plates that were incubated at 35-37°C for 24 hours. This process was used for isolation of Salmonella, Shigella, Vibrios and other Enteropathogens.

**Cockroaches:**

Legs of cockroaches were dipped in bottles containing selenite broth, BHI or TSB. The media plates were incubated at 35-37°C for 24 hours and examined for evidence of any growth. The viscera of the insect was aseptically dissected out, triturated with a small quantity of sterile Brain Heart Infusion Broth, centrifuged in sterile screw capped tubes @3000 RPM for 15 minutes, the supernatant was discarded, and the sediments were inoculated on media such as Blood agar, MacConkey agar, Chocolate agar and CLED agar for the isolation of bacteria. The inoculated media plates were examined for evidence of growth after incubating at 35-37°C (*Lenett et al., 1980*)

**Examination of Smears:**

Smears were prepared, on clean glass slides, from single colony appearing along the track of streaks, near the margin where the colonial growth was typical. A colony was fished out with sterile platinum loop and spread on to the surface of the slide. The stained smears were examined under oil-immersion lens.

**Isolation of bacteria:**

Single colonies showing evidence of pure growth were fished out and plated on appropriate nutrient/ enriched/differential/selective medium and incubated at 37°C for 24 hours. The growth was examined for purity and then transferred to the maintenance medium.

**Identification of bacteria:**

The identification of isolates was based upon the following characteristics:

- **Bacterial morphology and staining reaction:**
  Differential and special stains were used for studying the staining characteristics of various isolates. Liquid culture and hanging drop preparations were used for studying the motility of isolates. Capsule and spore stains were employed where necessary for identification of special morphological features. The descriptions of
Baron and Fingold (1994) and Cruickshank (1984) were followed for studying the morphology and staining characteristics of various isolated.

Stains and Methods of Staining

Gram differential stain was used for dying the bacteria from the cultures growths or specimens. The organisms retaining the color of principal dye and remaining blue or purple in color, after decolorization were considered as gram positive and those decolorized by alcohol and taking up pink or red colors (counter stain) were considered as Gram negative. The stains were prepared fresh and kept in amber-colored bottle to protect it from sunlight. The diluted Gram Iodine solution was protected from air and heat.

Gram’s Method of Staining

- A thin smear prepared from the bacterial growth, dried and fixed on a flame was flooded with Gram Crystals Violet and allowed to stain for 1 minute and then the excessive crystal violet was washed off with the cold tap water.

- The smear was flooded with Gram’s Iodine and allowed to react for one minute. The excessive Iodine solution was removed by gentle washing with the tap water.

- Excess of iodine was flood off and was treated with Gram Decolorizing agent for 30-60 seconds.
- The slide was gently and thoroughly washed in the cold tap water.
- The smear was then counter-stained with the Gram Safaranin for 30-60 seconds and the excessive stain was washed away with the tap water.
- The smear was blotted and allowed to air dry.
- The smear was examined under oil immersion (100x) lens.

Ziehl-Neelsen’s Method of Staining (Acid fast stain)

Carbol fuschin (stock solution) was used as principal stain, acid alcohol or dilute acids as decolorizing agent and methylene blue as counter stain. The acid-fast bacteria (AFB) were able to resist decolorization with the acid alcohol solution and retained the
color of the principal dye (bright red color) while the non-acid fast bacteria underwent
decolorization and took up the color of the counter stain (blue color).

- The slide was placed on a staining rack and flooded with carbol fuschin (stock
  solution) and heated gently from below to steaming for 5 minutes, the excessive
  stain was poured off.
- The smear was gently washed with the tap water and decolorized with acid alcohol
  for 1-2 minutes or until no more red color appeared in the washing.
- Smear was gently washed with the tap water and counterstained for 30 seconds
  with methylene blue, gently washed with tap water, blotted dry and examined under
  oil immersion (100x) lens.

Staining of yeast and fungi:

The specimens suspected to contain fungus growth were either stained with cotton
blue stain or were negatively stained using India ink. Cotton blue imparted blue color to the
fungal hyphae and spores. India ink gave a black color to the background leaving hyphae
and spores unstained.

- Colony Characteristics, Hemolytic Properties and Culture Medias
  
  Pure cultures of the isolates were obtained on selective, differential and special
  media for studying their colony characteristics and hemolytic properties (Appendix-1).
The isolates were streaked on media plates and incubated at 35°C±2°C for 24 hours. The
gross colonial morphology was assessed by observing the form, elevation and margins of
the colonies (Koneman et al., 1994). The growth was examined in direct and transmitted
sunlight or UV light. Assessment of gross colony characteristics was performed by
visual growth pattern on the surface of agar plates by holding the plate in one hand and
observing the surface of the agar for the presence of bacterial growth. During
examination, plates were tilted in various directions under bright light from various
angles. The use of a hand lens or a dissecting microscope was preferred to detect tiny or
immature colonies. Blood agar plates were examined by colony illuminator to detect the
hemolytic reactions.

**Bacteriological Culture Media:**

Various bacteriological culture media manufactured by the Difco, Oxide, USA were used for the processing and identification of isolates. The detail of culture media used in the study are briefly discussed as under:

**Nutrient Agar:**

Nutrient agar a general purpose medium was used for the cultivation of the majority of the microorganisms. It composed of beef extract, peptone and agar.

**Nutrient Broth:**

Nutrient broth a general purpose medium was used for the cultivation of microorganisms from various clinical samples. An infusion of meat and a peptone constituted the nutrients of this medium.

**Blood Agar:**

This was a general purpose medium used for the isolation of fastidious pathogenic organisms and for the study of haemolysin production by bacteria. Sterile defibrinated sheep blood or horse blood @ 5% was added to the medium after autoclaving and cooling to 45°C. The growth characteristics of various types of microorganisms were compared on blood agar plates according to the following criteria:

**Hemolytic reactions on blood agar plates**

<table>
<thead>
<tr>
<th>Hemolysin</th>
<th>Type of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha. (α)</td>
<td>Narrow greenish zone surrounding the bacterial colony</td>
</tr>
<tr>
<td>Beta. (β) (narrow-zone)</td>
<td>Narrow clear colorless zone surrounding the bacterial colony.</td>
</tr>
<tr>
<td>Beta (β) (wide-zone)</td>
<td>Wide clear colorless zone around the bacterial colony.</td>
</tr>
<tr>
<td>Gamma. (γ)</td>
<td>Area around the colony slightly depressed</td>
</tr>
<tr>
<td>Alpha-Beta (α-β) hemolysis</td>
<td>A clear narrow greenish zone surrounded by a broader colorless zone, 24 hours post refrigeration.</td>
</tr>
<tr>
<td>Delta. (δ)</td>
<td>Wide clear colorless zone around the colony.</td>
</tr>
</tbody>
</table>
The δ-haemolysin produced wide clear colorless zone around the bacterial colony. Its hemolytic action resembled that of β-haemolysin and was usually masked by the later.

**Chocolate agar with GC medium base:**

This medium was used for the isolation of Haemophilus, Neisseria and other fastidious organisms. Addition of crystal violet inhibited the growth of many contaminating bacteria. The basal medium was prepared, autoclaved and cooled to 45°C; 500 ml of sterile 2% Hemoglobin solution (Difco), 10 ml of Basic Supplement A (Difco) and 10 ml of antimicrobial solution CNVT (Difco) were added and thoroughly mixed and the medium was dispensed into sterile petri plates. Pathogenic bacteria like *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* were isolated on this medium.

**MacConkey Agar:**

A highly selective medium, MaConkey agar was used for the identification of Gram negative enteric bacilli. Lactose fermenting bacteria produced a localized acidic change in the medium; the colonies absorbed neutral red imparting a red color to the colony. Bile present in the medium was also precipitated around the colony. The colonies of non-lactose fermenting bacteria remained colorless or translucent.

**Characteristics of Isolates on MacConkey Medium**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Color of colony.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterob. Aerogenes.</em></td>
<td>Pink to red.</td>
</tr>
<tr>
<td><em>Escherichia coli.</em></td>
<td>Pink to red with bile (ppt)</td>
</tr>
<tr>
<td><em>Proteus vulgaris.</em></td>
<td>Colorless.</td>
</tr>
<tr>
<td><em>Sal. enteritidis.</em></td>
<td>Colorless.</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Colorless.</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>Nil</td>
</tr>
</tbody>
</table>

**Sorbitol MacConkey agar culture**

Verotoxigenic strains of *E. coli* were identified by growing them on sorbitol MacConkey’s agar. The colourless colonies along with the non sorbitol fermentation
Materials and Methods

confirmed the presence of VTEC. Most other *E. coli* strains fermented sorbitol, forming pink colonies.

**Bromo Thymol Blue agar (BTB Agar):**

This was a general purpose medium used for the isolation of microaerophillic and anaerobic bacteria. The following criteria was used to differentiate various organisms:

**Characteristics of isolate on BTB Agar**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Color of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Yellow.</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Blue to colorless</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Golden Yellow.</td>
</tr>
<tr>
<td><em>Staph. Epidermidis</em></td>
<td>Blue/ colorless.</td>
</tr>
</tbody>
</table>

**Bismuth Sulfite Glucose Glycine Yeast Agar (BIGGY Agar):**

This medium was used to differentiate various organisms as it was used as a selective and differential medium. Candida species grew on this medium and produced brown to black color colonies. The following criteria was used to differentiate various isolates:

**Response of isolates on BIGGY agar**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Colonial Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cadida albicans</em></td>
<td>Brown to black, no diffusion</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>Dark reddish brown with yellow halo</td>
</tr>
<tr>
<td><em>Candida pseudotropicalis</em></td>
<td>Reddish brown, flat.</td>
</tr>
<tr>
<td><em>Escherichia coli.</em></td>
<td>Nil</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Nil</td>
</tr>
</tbody>
</table>

**Bismuth Sulfite Agar:**

Bismuth Sulfite and Brilliant Green were complementary in inhibiting Gram positive bacteria and members of coliform group while allowing the Salmonellae to
Materials and Methods

grow luxuriantly. The colonies of H₂S producing bacteria on BSA were differentiated on this agar using the following criteria:

Characteristics Response of Isolates on BSA Agar

<table>
<thead>
<tr>
<th>Organism</th>
<th>Color of the colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>Brown to green</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Brown to green</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>Black with metallic sheen</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>Black with metallic sheen</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>Brown</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>None</td>
</tr>
</tbody>
</table>

Sodium Azide Blood Agar:

Since the sodium azide has bacteriostatic effect on Gram negative bacteria, the chemical was used for permitting the growth of Streptococi and Staphylococci. The hemolysin producing characteristic of the isolates was also highlighted on this medium. Sterile defibrinated sheep blood or horse blood @ 5% was added at temperature of 50-60°C to the medium after autoclaving (15 lbs pressure for 15-30 minutes at 121°C) and cooling to 45°C it. Hemolytic activity was observed as under:

Hemolytic activity on Sodium Azide Blood Agar

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Nil</td>
</tr>
<tr>
<td>Neisseria meningitides</td>
<td>Nil</td>
</tr>
<tr>
<td>Streptococcus epidermidis</td>
<td>None</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>Alpha/gamma</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Alpha</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Beta</td>
</tr>
</tbody>
</table>

Brain Heart Infusion Agar:

The para-aminobenzoic acid neutralizes sulphonamides that could be present in the blood inoculums and inhibit the growth of pathogenic bacteria. It also provides optimum conditions for aerobes, microaerophilics and obligate anaerobes. This
medium was used with or without the addition of sheep blood. Usually 5% sterile defibrinated sheep blood was added to the medium when desired. Brain heart infusion agar is ideal for the culturing of blood bacteria.

**Thioglycollate Borth:**

The most frequently used enrichment broth in clinical specimen was thioglycolate broth, which used 0.075% agar to prevent convection currents from carrying atmospheric oxygen throughout the broth. Thioglycolic acid also acted as a reducing agent. With the addition of many nutrient factors, such as casein, yeast and beef extracts, vitamins, and others, this medium enhanced the growth of most pathogenic bacteria. Other nutrient supplements, an oxidation-reduction indicator (resazurin), dextrose, vitamin K₁, and hemin, had been added in various modified formulas.

**Brilliant Green Bile (BGB) Agar:**

BGB agar was used as a selective and differential medium for the isolation and characterization of coilform organisms from water. The colonies of lactose fermenting bacteria were deep red at the center with a pink halo gist a blue background.

**Eosin Methylene blue Agar (EMB):**

EMB agar was used as a differential medium. The lactose fermenting bacteria produced colonies that were black or had a darker center and a colorless periphery. The bacteria that were lactose or sucrose negative produced colorless colonies.

**Enterococcus Agar Medium:**

This highly selective medium was used for the isolation of enterococci, as the growth of other Gram positive and Gram negative bacteria was inhibited on this medium. This medium was not autoclaved.
Kligler Iron Agar

A differential medium for bacteriological work. The medium was added to tubes that were slanted in such a way as to get a thick butt and a comparatively smaller slant. A red slant and yellow butt with or without gas indicated dextrose fermentation; a yellow slant and yellow butt with or without gas indicates lactose fermentation; no change in color of tube indicated that sugars were not fermented; blackening of the medium indicated Hydrogen sulfide production. The following criteria was used to differentiate various organisms:

Cultural reaction of Various Isolates on KIA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Slant</th>
<th>Butt</th>
<th>Gas</th>
<th>H2S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>A</td>
<td>A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>A</td>
<td>A</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Sal. Enteritidis</em></td>
<td>K</td>
<td>A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>K</td>
<td>A</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Loeffler Blood Serum

Loeffler blood serum a highly selective medium was used for isolation of Corynebacterium species. It was also used for the study of pigment production and proteolytic properties of various bacteria isolates.

MR-VP Medium:

The medium was used to differentiate between the strains of Coliform organisms on basis of Methyl red and Voges Proskaur reaction. This test was carried out to differentiate the following organisms:

MR-VP Identity Reaction

<table>
<thead>
<tr>
<th>Organism</th>
<th>MR</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>Yellow (-)</td>
<td>Red (+)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Red. (-)</td>
<td>No change (-)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Red. (-)</td>
<td>No change (-)</td>
</tr>
</tbody>
</table>
Mannitol Salt Agar:
A highly selective medium, Mannitol Salt Agar was used for the primary isolation of Staphylococci from the contaminated specimens. High salt concentrations were found inhibitory for other pathogenic bacteria. Rabbit plasma coagulased (+) *Staphylococcus aureus* produced yellow colonies while non-pathogenic strains produced colorless colonies. The strains were differentiated according to the following reactions:

**Characteristics of Various Types of Bacteria on Mannitol Salt Agar**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>Nil</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Nil</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Red.</td>
</tr>
</tbody>
</table>

Staphylococcus Medium No. 110:
This selective medium was used for the primary isolation of pathogenic Staphylococci from the contaminated specimens. The following criteria was adopted to differentiate the pathogens:

**Characteristics of Isolates on Staph. Medium No.110**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Pigment production</th>
<th>Gelatinase production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis.</em></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td><em>Escherichia coli.</em></td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus.</em></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><em>Staph. Epidermidis</em></td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(-) Negative reaction  (+) Positive reaction

Pseudomonas Isolation Agar:
This selective medium was used for the isolation of Pseudomonas from the contaminated material. Irgasan, a potent broad-spectrum antibiotic, inhibited growth of other contaminating bacteria and allowed Pseudomonas spp. to grow. This medium also enhanced the production of blue or blue-green pigment by the *Pseudomonas aeruginosa* strains as under:
Pigment production on Pseudomonas isolation agar

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Pigment production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>(-)²</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>(-)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Blue or Blue-green</td>
</tr>
</tbody>
</table>

² = Negative reaction

Lowenstein Jensen Medium:

This selective medium was used for the isolation of Mycobacterium species. The base medium was prepared in accordance with the instructions of the manufacturer, autoclaved and cooled to 45°C and a uniform suspension of fresh eggs was added to the medium according to the manufacturer’s advice. The medium was added to sterilized, screw capped test tubes, which were arranged in a slant position in a rack. The medium was coagulated in an incipissator at 85°C for 45 minutes. Glycerol was not added to the deionized water used for dehydration of basal medium used for the isolation of glycerophobic Mycobacteria e.g. *Mycobacterium bovis*.

- Biochemical Characteristics

The descriptions of Cruickshank et al. (1984) were followed for studying the Biochemical characteristics of the various isolated strains of bacteria.

Catalase test: The catalase test was used to differentiate staphylococci (positive) from streptococci (negative). Observation for the rapid effervescence produced on addition of a drop of 3% hydrogen peroxide to a portion of the test colony transferred to the surface of a glass slide.

Bile solubility test: *Streptococcus pneumoniae* cells lyse in a 10% solution of sodium deoxycholate. In the plate test, one or two drops of 10% sodium deoxycholate were added to the colonies growing on the surface of an agar plate. Disappearance of the colonies was looked for after 15 minutes of incubation.

Slide coagulase test: A colony suspected of being a Staphylococcus species was emulsified in a drop of rabbit plasma on a glass slide. Bacterial clumping within 2 minutes indicates the
presence of bound coagulase and constitutes a positive test result.

**Direct spot indol test:** A small portion of the bacterial colony was transferred from a nonselective medium such as blood or chocolate agar to a strip of filter paper that was soaked with Kovac’s reagent (p-dimethylaminocinnamaldehyde solution). The immediate development of a red colour using Kovac’s reagent indicated the presence of indole positive reaction.

**PYR test:** This test was used for the rapid identification of enterococci by using PYR (L-pyrrolidonyl-naphthylamide) substrate. After 4 hours of incubation following heavy inoculation of the substrate of PYR with a liquid suspension of the unknown organism prepared from a primary isolation plate, the production of a red colour after adding N,N-methyl-aminocinnamaldehyde reagent was indicative of group D enterococci.

**Enterotube-II:** The Enterotube II was a pencil shaped, self-contained, compartmented plastic tube containing chambers of differential media from which differential characteristics can be determined. An inoculating wire was positioned through the center of all the media chambers and extended out from each end of the tube. One end served as the inoculating tip; the other as the handle. Both ends were covered with a screwcap when packaged. Inoculation was carried out by touching the inoculating tip to the surface of a well isolated colony on an agar plate. The tube was hold firmly while the inoculating wire was pulled through all of the chambers and then reinserted into the first four compartments. The caps were replaced and the tube was incubated overnight at 35°C. The system was taken up little space and the risk of contamination was minimal. The color reactions were generally easy to interpret according the biochemical reactions as mentioned in next page.
## Differential Diagnosis of *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ind*</th>
<th>Urease</th>
<th>Motility</th>
<th>Glu Fer†</th>
<th>Lact Fer†</th>
<th>Sucr Fer§</th>
<th>Malt Ferm</th>
<th></th>
<th>Esc Hyd¶</th>
<th>Hyd Sulf TSI#</th>
<th>Oxidase</th>
<th>Orn Dec**</th>
<th>Lys Dec††</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
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<td></td>
<td></td>
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<tr>
<td><em>Serratia marcescens</em></td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indole  
† Glucose fermentation  
‡ Lactose fermentation  
§ Sucrose fermentation  
|| Maltose fermentation  
¶ Esculin hydrolysis  
# Hydrogen sulfite on TSI  
** Ornithine decarboxylase  
†† Lysine decarboxylase  
+++ Oxidative
**Materials and Methods**

- **Serology**
  Difco sera were used for the serological typing of isolated strains, where possible and standard techniques recommended by Difco Laboratories (1985) were adhered to while performing serological tests. Slide agglutination, tube agglutination or both methods were used in this study for bacterial identification and typing.

- **Pure cultures**
  Pure cultures of non-fastidious bacteria were maintained on nutrient agar slants. Pure cultures of fastidious organisms were maintained on Brain Heart Infusion Agar slants. Cultures of anaerobic bacteria were maintained on Cooked Meat Medium.

**Preparation of Media:**

Various bacteriological culture media were prepared and utilized according to the requirements for the growth of a pathogen in the light of manufacturer’s instructions.

**Sterilization:** The bacteriological media were sterilized either by fractional sterilization (Tyndallization) or by autoclaving.

**Preparation:** To avoid extraneous contamination platting and tubing of media all cultural operations were undertaken in biological safety cabinet of class-II type. The prepared media plates and tubes were incubated at 37°C for 24 hours, to check their sterility, and the sterile media dishes and tubes were stored in the refrigerator at 4°C. The shelf life of media, unless otherwise prescribed, was considered for 2 weeks and all plates and tubes, not used during this period, were autoclaved at 15 lbs. pressure at 121°C for 30 minutes and discarded.

Supplementary nutrients were added to the medium, in accordance with the instructions of the manufacturer and needs of the experiment. Sterilization and autoclaving of media was also carried out in accordance with the instructions of the manufacturer.
Materials and Methods

Enrichment media were prepared and used fresh and were not sterilized in autoclave. Addition of antibiotics and other specific chemicals in culture media was done after media had been autoclaved.

The used culture media plates and tubes and all other contaminated/ infectious material, containing bacterial cultures, were autoclaved at 15 lbs pressure psi at 121°C for 30 minutes before being disposed off.

Unless otherwise described the nutrient agar and brain heart infusion agar were used as general purpose media for propagation and storage of isolates during the course of study.

Fractional sterilization (with steam at 100°C):

The process was carried out in a steam sterilizer at 100°C. The media flasks, to be sterilized, were placed in the automatic electrically operated Steam Sterilizer (time and temperature control) and exposed to steam at 100°C for 30 minutes daily, for 3 consecutive days. The sterilized media were added to plates or tubes, as needed, and checked for sterility by incubating it at 37°C up to 24 hours.

Fractional sterilization (water heated to 85°C):

The added media, in screw capped bottles or tubes, were placed on the racks of automatic electric Serum Incipissator (time and temperature control), and were exposed to hot water-vapors at 85°C for 30 minutes daily, for 3 consecutive days, checked for sterility and stored in refrigerator at 4°C.

Autoclaving:

The system utilized super-heated steam up to 30 minutes, with a temperature of 121°C, and under 15 lbs pressure per square inch (psi); at this temperature and pressure steam was found to be the most perfect instrument for sterilization of culture media. The medium was weighed and re-hydrated, in accordance with the instructions of the manufacturers and added to the sterilized flasks; which were autoclaved for 15-30
Materials and Methods

Pouring of media:

The medium was poured in petri plates, screw capped tubes, test tubes, McCartney bottles etc in accordance with the requirements. The glassware was washed, cleaned, dried, plugged, where necessary or placed in baskets and sterilized in hot air oven at 180°C for 1 hour. The pouring of media was carried out in biological safety cabinet class-II type to avoid extraneous contamination. Before pouring into plates or tubes the sterilized hot medium was allowed to cool to 45°C ± 5°C; to avoid condensation of water vapors on the lid of petri dish while pouring the media or during subsequent solidification. The medium plates were placed on an even flat surface for a uniform solidification. The tubes were placed in racks for solidification and slanted to get required slant length and butt thickness, in accordance with needs of the experiment.

Additional nutrients like blood, serum and other supplements were added to the medium before solidification. Additions were made to the medium that had been cooled to 45°C. The plates, in which additional material had been added, were gently rotated on a smooth flat surface to ensure a uniform mixing; tubes were rotated between the palms of hands or slowly swirled to ensure thorough mixing of additional material.

Sterility Testing:

Medium plates and tubes were placed in incubator for 24 hours at 37°C to check their sterility. The tubes and plates showing any evidence of growth were discarded. The sterile medium plates and tubes were stored in refrigerator at 4°C till used.

Antibiotic Sensitivity Testing:

The test was conducted using a 24 hours old culture of the isolates on Nutrient Agar or Brain Heart Infusion Blood Agar. The following protocol was used.

Mueller Hinton Medium was used for this test. The medium was prepared according to the directions of manufacturer and sterilized by autoclaving at 15 lbs.
pressure PSI at 121°C. The melted medium was cooled down to 45°C - 50°C and poured into sterile petri dishes (approximately 25 ml for 100 mm plates and 60 ml for 150 mm plates). The plates were placed on a surface to obtain a uniform depth of 4 mm. The medium was allowed to cool and to solidify. Collection of moisture on the surface of the medium was avoided by pouring medium cooled to or below 50°C. During cooling the top plate was left slightly opened until medium solidified. This procedure minimized moisture collection on the surface of the medium. In case moisture did collect, plates were incubated at 35°C - 37°C for 30 minutes and then refrigerated at 4°C till used. The plates were kept to room temperature for 1 hour prior to use.

**Antibiotic Sensitivity Discs**

Disc diffusion technique of Bauer *et al.* (1996) as approved by the National Committee for Clinical Laboratory Standard (NCCLS) and World Health Organization (WHO, 1977) was used for determination of antibiotic sensitivity of isolate. Discs of commonly available antibiotics manufactured by Difco Laboratories, USA were employed during the studies. Sensitivity discs were used for determining the *in vitro* susceptibility of microorganism to a variety of antimicrobial agents. The discs were removed from the refrigerator and placed at room temperature for 1-2 hours before use, to minimize possibility of water condensation.

**Preparation of McFarland Standards (Turbidity Standards)**

A standard (McFarland) for visual comparison of the turbidity of bacterial suspension was prepared to measure the density of inocula. A 0.5 ml amount of 0.048 M BaCl₂ (or 0.5 ml of 1.175 % BaCl₂·2H₂O solution) was added to 99.5 ml of 0.35 N H₂SO₄ and distributed in 4-6 ml amount into screw cap tubes of the same size as those being used to grow the broth culture inocula. The tubes were sealed tightly and stored at room temperature in the dark. The turbidity meter was vigorously agitated on a mechanical vortex mixer just before use. New standards were prepared at least every 6 months (NCCLS, 1991).
Test culture suspension:

This suspension was prepared by transferring at least 3-5 well isolated colonies which were emulsified in 3-4 ml of nutrient broth. Using a good light the turbidity of the suspension was matched to the turbidity standard.

**Antibiotic susceptibility of bacteria** (Modified Kirby-Bauer Technique, 1966)

- Within 15 minutes of standardization, a sterile nontoxic cotton swab on a wooden applicator was dipped into the standardized culture suspension and excessive fluid was removed by exposing the swab and rotating it firmly against the inside wall of the test tube.

- The entire surface of the medium was inoculated by streaking it with above swab, in satisfactorily inoculated / streaked plate a confluent lawn of growth after 16-18 hours incubation was visible

- The inocula were allowed to dry for 5-15 minutes.

- A variety of susceptibility discs were applied with a multi disc dispenser (Oxide) on the isolate inoculated surface, and each disc was gently pressed on the surface with sterile forceps, flaming and cooling it between each disc application, to ensure complete contact on disc with the agar.

- Within 15 minutes of applying the antibiotic discs, the plates were aerobically incubated at 35°C for 16 to 18 hours or till the clear appearance of zones of growth inhibition around certain discs.

- The zones of complete inhibition were measured in mm with a ruler for this purpose.

- When blood agar medium had been used, the cover was removed and the surface medium was examined under the reflected light.
Materials and Methods

Antibiotic susceptibility of fastidious bacteria isolates was also carried out according to the procedure described above. In this test Mueller Hinton Medium containing 1% Hemoglobin and 1 % Supplement XV (Difco) was used.

Methicillin Resistance among Staphylococcus spp:

In this study Methicillin Resistance was assessed by using antibiotic discs of Oxacillin, Cloxacillin, Nafcillin and Methicillin against the isolates of Staphylococcus spp. The plates were incubated at 35°C in a CO₂ incubator and the clearance zone smaller than 20 mm indicated methicillin resistance.

Interpretation of antibiotic susceptibility results

Using the interpretative chart the zones of each antimicrobial agent was reported as “Resistant”, “Intermediate/moderately sensitive”, and “Sensitive”. The interpretation was made according the criteria reported by Kirby and Bauer (1966).

Control strains

To maintain the quality of the test, the known characterized following reference bacterial strains were also used for comparing the characteristics of isolates of this study.

- Staphylococcus aureus ATCC 25923
- Escherichia coli ATCC 25922
- Pseudomonas aeruginosa ATCC 27853

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and Least Significant Different (LSD) tests were applied for collecting and analysis of information on nosocomial infections (Steel and Torrie, 1982).
CHAPTER-4
RESULTS

During the present study attempts were made to isolate and characterize species of different pathogens from the ailing persons, hospital staff, hospital environment, and non-human inmates at the Mayo hospital, Lahore, Pakistan.

For this a total of 8040 samples of morbid material obtained from 4502 patients suffering from various infectious disorders, 635 hospital staff samples, 2677 hospital environment samples, and 226 Non-human inmate samples were collected and processed for bacteriological analysis.

The routine techniques for isolation and identification and biochemical and biological characterization were used for the analysis of those samples. The agents from the morbid material were isolated, identified, typed (Baron and Finegold, 1990; Monica Cheesbrough, 2000) and analyzed. Antibiograms of the isolates were determined using disc diffusion method as described by Bauer and Kirby (1966) and NCCL (1991).

SOURCES OF MATERIALS

(i) Patients: A total of 32,620 patients hospitalized in various wards of Mayo Hospital Lahore, Pakistan during the years 1997-2001 were included in this study. Of the total 4502 patients were found to have acquired different types of nosocomial infections. Samples obtained from these 4502 patients consisted of 1040 pus and wound swabs, 109 blood samples, 115 pleural fluid samples, 286 peritoneal fluid samples, 37 cerebrospinal fluid samples, 1398 urine samples, 988 sputum and endotracheal secretion samples, 329 burn swabs, 99 patient body devices (catheters, CVP lines, shunts, IV canulae, joint prosthesis etc.), and 101 fecal and drainage tube material samples (Table 3.1).

On bacteriological examination of 4502 samples, 1287 isolates of Staphylococcus, 429 isolates of Streptococcus, 328 of Enterococcus, 781 of Pseudomonas, 349 of Enterobacter, 41 of Acinetobacter, 266 of Klebsiella, 140 of Proteus, 1031 of Escherichia, 67 of Serratia, 93 of Haemophilus, 119 of other uncommon Gram positive bacteria, 13 of uncommon Gram negative bacteria, and 189 isolates of yeasts and fungi were recovered and characterized (Table 4.1). Detail of isolations of the above
mentioned organisms in various wards is provided in Appendix-1. Isolations of various bacterial species from morbid material samples of patients were significantly different from each other (P<0.05) using LSD test (Table 4.1).

(ii) Hospital staff:

A total of 635 samples consisting of 127 throat and nasal swabs, 254 hand swabs, 254 gloves and apron swabs were collected from the hospital staff (Table 3.3) and tested using routine laboratory techniques such as cultural, staining, purification, identification and biochemical testing. Each sample was subjected to bacteriological analysis (Table 4.2). Of the total morbid samples collected from the hospital staff a total of 119 isolates of Staphylococcus, 60 isolates of Streptococcus, 73 isolates of Enterococcus, 33 of Pseudomonas, 15 of Enterobacter, 24 isolates of Klebsiella, 25 of Escherichia, 13 of Haemophilis, 11 of uncommon Gram positive bacteria, and one of uncommon Gram negative bacteria were recovered (Table 4.2). The difference in recovery of various kinds of bacteria was not significant using LSD test (P>0.05).

Of the 25 throat and 25 nasal swab samples from the clinically healthy individuals, a total of 4 throat samples and 7 nasal swab samples were positive for Staphylococcus; 3 throat and 4 nasal swab samples were positive for Streptococcus and one throat and two nasal swab samples were positive for E. coli isolation. No throat or nasal swab sample was indicative of contamination with Enterococcus, Pseudomonas, Enterobactero, Klebsiella, Haemophilus, uncommon Gram positive or Gram negative agent. The relative isolation of bacteria from throat and nasal swabs from healthy individuals was significantly lower than that of hospital staff (P<0.05).
### Results

Table-4.1: Detailed bacteriology of samples collected from the patients

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Specimen</th>
<th>Pus and wounds swabs</th>
<th>Blood</th>
<th>Pleural fluid</th>
<th>Ascitic fluid</th>
<th>Cerebrospinal fluid</th>
<th>Urine</th>
<th>Spumum</th>
<th>Burns</th>
<th>Patient Body devices</th>
<th>Facenal &amp; drainage Specimen</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td></td>
<td>606</td>
<td>32</td>
<td>24</td>
<td>85</td>
<td>11</td>
<td>67</td>
<td>237</td>
<td>135</td>
<td>63</td>
<td>27</td>
<td>1287</td>
<td>128.3a</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>104</td>
<td>8</td>
<td>21</td>
<td>20</td>
<td>5</td>
<td>14</td>
<td>205</td>
<td>30</td>
<td>13</td>
<td>9</td>
<td>429</td>
<td>42.92abc</td>
</tr>
<tr>
<td>Enterococcus</td>
<td></td>
<td>37</td>
<td>14</td>
<td>5</td>
<td>44</td>
<td>5</td>
<td>55</td>
<td>82</td>
<td>39</td>
<td>35</td>
<td>12</td>
<td>328</td>
<td>32.8cd</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td>205</td>
<td>9</td>
<td>20</td>
<td>26</td>
<td>4</td>
<td>203</td>
<td>97</td>
<td>187</td>
<td>15</td>
<td>15</td>
<td>781</td>
<td>78.1abc</td>
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<tr>
<td>Enterobacter</td>
<td></td>
<td>33</td>
<td>12</td>
<td>7</td>
<td>32</td>
<td>6</td>
<td>115</td>
<td>68</td>
<td>16</td>
<td>33</td>
<td>27</td>
<td>349</td>
<td>34.9bcd</td>
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<tr>
<td>Acinetobacter</td>
<td></td>
<td>30</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>4.0d</td>
</tr>
<tr>
<td>Klebsiella</td>
<td></td>
<td>27</td>
<td>7</td>
<td>10</td>
<td>21</td>
<td>2</td>
<td>62</td>
<td>120</td>
<td>13</td>
<td>0</td>
<td>4</td>
<td>266</td>
<td>26.6cd</td>
</tr>
<tr>
<td>Proteus</td>
<td></td>
<td>22</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>48</td>
<td>21</td>
<td>32</td>
<td>5</td>
<td>0</td>
<td>140</td>
<td>14.8abd</td>
</tr>
<tr>
<td>Escherichia</td>
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<td>99</td>
<td>12</td>
<td>6</td>
<td>30</td>
<td>4</td>
<td>742</td>
<td>65</td>
<td>23</td>
<td>35</td>
<td>15</td>
<td>1031</td>
<td>103.1ab</td>
</tr>
<tr>
<td>Serratia</td>
<td></td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>20</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>6.7d</td>
</tr>
<tr>
<td>Haemophilus</td>
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<td>0</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>79</td>
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<td>0</td>
<td>93</td>
<td>9.3cd</td>
</tr>
<tr>
<td>Other G (+)</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>112</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>119</td>
<td>11.9cd</td>
</tr>
<tr>
<td>Other G (-)</td>
<td></td>
<td>3</td>
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<td>0</td>
<td>4</td>
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<td>6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>1.3d</td>
</tr>
<tr>
<td>Yeast &amp; Fungi</td>
<td></td>
<td>10</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>95</td>
<td>33</td>
<td>10</td>
<td>20</td>
<td>6</td>
<td>189</td>
<td>18.9cd</td>
</tr>
<tr>
<td>Total isolates</td>
<td></td>
<td>1186*</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1435*</td>
<td>1134*</td>
<td>493*</td>
<td>219*</td>
<td>119*</td>
<td>5133</td>
<td>-</td>
</tr>
</tbody>
</table>

Different superscripts = significant (P < 0.05)
* = indicates multiple infection
Table 4.2 Detailed Bacteriology of Hospital Staff Samples

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Specimen</th>
<th>Throat and nasal swabs*</th>
<th>Hands</th>
<th>Gloves &amp; Aprons</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td></td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td>39</td>
<td>55</td>
<td>25</td>
<td>119</td>
<td>39.6a</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>45</td>
<td>10</td>
<td>5</td>
<td>60</td>
<td>20.0a</td>
</tr>
<tr>
<td>Enterococcus</td>
<td></td>
<td>51</td>
<td>18</td>
<td>4</td>
<td>73</td>
<td>24.33a</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td>2</td>
<td>10</td>
<td>21</td>
<td>33</td>
<td>11.0b</td>
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<tr>
<td>Enterobacter</td>
<td></td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>15</td>
<td>5.0c</td>
</tr>
<tr>
<td>Klebsiella</td>
<td></td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>24</td>
<td>8.0c</td>
</tr>
<tr>
<td>Escherichia</td>
<td></td>
<td>5</td>
<td>16</td>
<td>4</td>
<td>25</td>
<td>8.33c</td>
</tr>
<tr>
<td>Haemophilis</td>
<td></td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>4.33c</td>
</tr>
<tr>
<td>Other G (+)</td>
<td></td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>3.66c</td>
</tr>
<tr>
<td>Other G (-)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.33c</td>
</tr>
<tr>
<td>Total isolates</td>
<td></td>
<td>184</td>
<td>122</td>
<td>68</td>
<td>374</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts = significant (P<0.05) difference.
Results

(iii) Hospital’s Environment:

A total of 2677 samples from various locations of hospital such as 126 operation-theatre air samples, 65 ward environment samples, 610 sterilized water samples used for various procedures and 1876 swabs from surgical instruments were collected (Table-3.4) and examined. Of the samples collected from the hospital environment a total of 110 isolates of Staphylococcus, 34 isolates of Streptococcus, 59 isolates of Enterococcus, 53 isolates of Pseudomonas, 25 isolates of Enterobacter, 35 isolates of Klebsiella, 22 isolates of Proteus, 55 isolates of Escherichia, 41 isolates of other gram positive bacteria, 19 isolates of other gram negative bacteria, and 18 species of yeast and fungi were isolated (Table 4.3). The differences in isolation of various pathogen from various specimens of morbid material were significant \( (P<0.05) \) using LSD test.

Of the 25 air samples obtained from outside of operation theater and ward environment, a total of 3 samples were positive for Staphylococcus, two for Enterococcus and 3 samples were positive for \( \textit{Escherichia coli} \). None of the samples yielded bacteria like Streptococcus, Enterobacter, Klebsiella, Proteus, Fungi or any other type of Gram negative or positive organism. The isolation rate of various bacterial species from the air outside operation theater and outside ward environment was significantly lower \( (P<0.05) \) than the operation theater and ward environment.

(iv) Non-Human Inmates:

A total of 226 samples of cats and cockroaches collected from various premises of the hospital consisting of 25 nasal and 25 faecal material samples from the cats, 88 samples from the external part of the body of cockroaches, and 88 samples obtained from intestine of cockroaches were collected for the detailed bacteriological analysis (Table-3.5). From the non-human cat inmate samples a total of 15 isolates of Staphylococcus, 27 isolates of Streptococcus, 27 isolates of Enterococcus, 12 isolates of Pseudomonas, 33 isolates of Enterobacter, 8 of Proteus, 32 of Escherichia, none of other gram positive bacteria, 4 of other gram negative bacteria, and 13 isolates of Yeast and Fungi were recovered (Table 4.4). Those isolations of bacteria from fecal and nasal samples of cats differed significantly \( (P<0.05) \).
**Bacteriological studies**

The analysis of various clinical samples from the patients suffering from various infections revealed the presence of various types of bacteria (Table. 4.1).

Similarly, from the legs and intestine samples of cockroaches, a total of 86 isolates of Staphylococcus, 119 isolates of Streptococcus, 139 isolates of Enterococcus, 62 isolates of Pseudomonas, 91 isolates of Enterobacter, 39 of Proteus, 131 of Escherichia, 29 of other isolates of Gram positive bacteria, 82 of Gram negative bacteria and 17 of yeasts and fungi were recovered. The isolation of different bacterial species from samples of legs and intestines differed significantly (P<0.05) as indicated in Table 4.4(a&b).
### Results

Table 4.3  **Bacteriology of samples collected from hospital environment**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Operation theatre air</th>
<th>Ward environment</th>
<th>Sterilizers</th>
<th>Surgical instruments</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>25</td>
<td>48</td>
<td>7</td>
<td>30</td>
<td>110</td>
<td>27.5a</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>12</td>
<td>15</td>
<td>2</td>
<td>5</td>
<td>34</td>
<td>8.5b</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>15</td>
<td>33</td>
<td>6</td>
<td>5</td>
<td>59</td>
<td>14.75b</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0</td>
<td>18</td>
<td>20</td>
<td>15</td>
<td>53</td>
<td>6.25b</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>2</td>
<td>25</td>
<td>10.25b</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>8.75b</td>
</tr>
<tr>
<td>Proteus</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>22</td>
<td>5.5c</td>
</tr>
<tr>
<td>Escherichia</td>
<td>10</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>55</td>
<td>13.75b</td>
</tr>
<tr>
<td>Other G (+)</td>
<td>2</td>
<td>33</td>
<td>5</td>
<td>1</td>
<td>41</td>
<td>10.25b</td>
</tr>
<tr>
<td>Other G (-)</td>
<td>1</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>19</td>
<td>4.75c</td>
</tr>
<tr>
<td>Yeast &amp; Fungi</td>
<td>1</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>4.5c</td>
</tr>
<tr>
<td>Total isolates</td>
<td>69</td>
<td>293</td>
<td>47</td>
<td>62</td>
<td>471</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts = significant (P < 0.05) difference  
Same superscripts = Non significant difference (P>0.05)
## Results

Table 4.4(a) Bacteriology of samples collected from non-human inmates

<table>
<thead>
<tr>
<th>Specimen</th>
<th>CAT</th>
<th>Nasal</th>
<th>Fecal</th>
<th>MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td></td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td>10</td>
<td>5</td>
<td>7.5&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>16</td>
<td>11</td>
<td>13.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Enterococcus</td>
<td></td>
<td>9</td>
<td>18</td>
<td>13.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td>7</td>
<td>5</td>
<td>6.0&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Enterobacter</td>
<td></td>
<td>11</td>
<td>22</td>
<td>16.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proteus</td>
<td></td>
<td>0</td>
<td>8</td>
<td>4.0&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Escherichia</td>
<td></td>
<td>9</td>
<td>23</td>
<td>16.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Other G (+)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Other G (-)</td>
<td></td>
<td>0</td>
<td>4</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeast &amp; Fungi</td>
<td></td>
<td>8</td>
<td>5</td>
<td>6.5&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total isolates</td>
<td></td>
<td>70</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts = significant (P < 0.05)
Table 4.4(b) Bacteriology of samples collected from non-human inmates

<table>
<thead>
<tr>
<th>Specimen</th>
<th>COCKROACHES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Legs</td>
</tr>
<tr>
<td>Observations</td>
<td>88</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>32</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>43</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>56</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>25</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>32</td>
</tr>
<tr>
<td>Proteus</td>
<td>12</td>
</tr>
<tr>
<td>Escherichia</td>
<td>67</td>
</tr>
<tr>
<td>Other G (+)</td>
<td>13</td>
</tr>
<tr>
<td>Other G (-)</td>
<td>33</td>
</tr>
<tr>
<td>Yeast &amp; Fungi</td>
<td>6</td>
</tr>
<tr>
<td>Total isolates</td>
<td>319</td>
</tr>
</tbody>
</table>

Different superscripts = significant (P < 0.05)
(i) Staphylococcus (Staph.)

Staphylococci were isolated from samples of pus and wound swabs, sputum, CSF, blood, body fluids, feces and urine. They were also isolated from the nasal swabs of health care workers, hospital environment and inmates.

Morphology: Staphylococci were found to be Gram positive cocci of uniform size occurring characteristically in groups but also singly and in pairs, they were non-motile and non capsulated.

Characteristics: Staphylococci grew well aerobically and in carbon dioxide enriched atmosphere. Temperature range for their growth was 10-42°C with an optimum of 35-37°C. On blood agar and chocolate agar plates *Staph. aureus* produced yellow to cream coloured colonies after over night incubation. Some isolates were beta hemolytic which grew aerobically, colonies were slightly raised and easily emulsified. On MacConkey’s agar smaller colonies were produced after over night incubation, most isolates were lactos fermenting. Manitol salt agar was used for the isolation of *Staph. aureus* from faecal material which fermented manitol and was able to grow on this agar. They were coagulase, DNase and catalase positive.

*Staph. epidermidis* was isolated from the patients of endocarditis and bacteraemia following infection of cannulae, indwelling catheters, shunts or other appliances positioned in the body. On cultural examination the colonies of *Staph. epidermidis* were found wide and usually non hemolytic.

Isolations: In the present work various species of staphylococci were isolated from a total of 1287 samples, collected from patients, including 606 samples of pus and wound swabs, 32 samples of blood, 24 samples of pleural fluids, 85 samples of ascetic fluids, 11 samples of cerebrospinal fluids, 67 samples of urine, 237 samples of sputum, 135 burn swabs samples, 63 patient body devices and 27 fecal and drainage patient samples (Table 4.5).

In case of samples collected from hospital staff, various Staphylococcus isolates were isolated from 119 of 635 samples including 39 of 127 samples of throat swabs, 55
Results

of 254 samples of hand swabs, and 25 of 254 samples of gloves and aprons (Table 4.6).

Of the samples collected from hospital environment various isolates of Staphylococcus were isolated from 110 of 2677 samples including 25 from operation theatre air, 48 from ward environment, 7 from sterilized water, and 30 from surgical instrument samples (Table 4.7).

Of the 226 non-human inmates samples different isolates of Staphylococcus were isolated from 101 samples including 10 from nasal swabs and 5 from fecal swabs taken from cats, and 32 from legs and 54 from intestines of cockroaches (Table 4.8).

Of the 8040 total samples, 1617 were positive for various staphylococcus isolates including 1287 from the samples of patients, 119 from hands, gloves and aprons of hospital staff, 110 from ward environment, and 101 isolations were from non human inmates (Table-4.9).

The isolates of Staphylococcus were identified and characterized on the basis of their biochemical, hemolytic, and biological characteristics. Of the total 1287 isolates, 1004 (78%) were characterized as \textit{Staphylococcus aureus} and 283 (22%) as \textit{S. epidermidis}. Since the methicillin sensitivity has been adopted as one of the criteria for typing Staphylococcus species therefore all the isolates were subjected to Methicillin sensitivity test using standard methods referred to earlier. Of the total 1287 isolates 555 were found methicillin resistant \textit{Staphylococcus aureus} (MRSA), and the remaining 449 isolates were methicillin sensitive \textit{Staphylococcus aureus} (MSSA). In case of \textit{St. epidermidis} 41 isolates were methicillin resistant (MRSE), and 242 isolates were methicillin sensitive (MSSE) (Fig -1).
### Table 4.5  Isolation of various Staphylococcal species from patient samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Pleural fluid</th>
<th>Ascitic fluid</th>
<th>Cerebrospinal fluid</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burns</th>
<th>Patient Body devices</th>
<th>Faecal and drainage Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Staph. (isolates)</td>
<td>606</td>
<td>32</td>
<td>24</td>
<td>85</td>
<td>11</td>
<td>237</td>
<td>135</td>
<td>63</td>
<td>27</td>
<td>1287</td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>58.26</td>
<td>29.35</td>
<td>20.86</td>
<td>29.72</td>
<td>29.72</td>
<td>4.79</td>
<td>23.98</td>
<td>63.6</td>
<td>26.73</td>
<td>28.58</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4.6  Isolations of Staphylococcal Species from Hospital Staff Samples

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>Throat / Nasal Swabs</th>
<th>Hand Swabs</th>
<th>Gloves and Aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>39</td>
<td>55</td>
<td>25</td>
<td>119</td>
</tr>
<tr>
<td>Percentage</td>
<td>30.7</td>
<td>21.65</td>
<td>9.84</td>
<td>18.74</td>
</tr>
</tbody>
</table>
### Table 4.7  Isolations of Staphylococcal species from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air</th>
<th>Ward Environment</th>
<th>Water from Sterilizers</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>25</td>
<td>48</td>
<td>7</td>
<td>30</td>
<td>110</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>19.84</td>
<td>73.84</td>
<td>1.14</td>
<td>1.59</td>
<td>4.11</td>
</tr>
</tbody>
</table>

### Table 4.8  Isolations of Staphylococcal species from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats Nasal</th>
<th>Cats Faecal</th>
<th>Cockroaches Legs</th>
<th>Cockroaches Intestine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>25</td>
<td>25</td>
<td>88</td>
<td>88</td>
<td>226</td>
</tr>
<tr>
<td>Isolates</td>
<td>10</td>
<td>5</td>
<td>32</td>
<td>54</td>
<td>101</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>40</td>
<td>20</td>
<td>36.36</td>
<td>65.90</td>
<td>44.7</td>
</tr>
</tbody>
</table>

### Table 4.9  Isolations of Staphylococcus species from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Staff hands, gloves &amp; Aprons</th>
<th>Ward Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4500</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>1287</td>
<td>119</td>
<td>110</td>
<td>101</td>
<td>1617</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>28.6</td>
<td>18.76</td>
<td>4.1</td>
<td>44.69</td>
<td>20.11</td>
</tr>
</tbody>
</table>
Colonization of Staphylococcus isolates in various wards

A total of 555 MRSA isolates were isolated from 19 patients under treatment in medical unit, 295 patients in surgical unit, 36 patients in ICU, 3 in cardiology ward, 34 patients in cardiac surgery, 36 patients in chest surgery, 29 patients in orthopedic surgery, 9 in pediatric ward, 90 in Burn Unit, and 4 patients in Neurosurgery of Mayo Hospital, Lahore (Table 4.10).

A total of 449 isolates of methicillin-sensitive Staphylococcus aureus (MSSA) were isolated from 87 patients under treatment in medical unit, 214 in surgical unit, 31 in ICU, 7 in cardiology ward, 20 in cardiac surgery ward, 25 in chest surgery ward, 21 patients in orthopedic surgery ward, 10 in pediatric ward, 11 in burn unit, 2 in ophthalmology ward, 8 in neurosurgery ward, 7 in urology ward, 1 in dermatology ward and 5 patients in oncology ward of Mayo Hospital, Lahore.

A total of 242 isolates of Methicillin Sensitive Staphylococcus epidermidis (MSSE) were isolated from 101 patients under treatment in medical ward, 24 patients in surgical ward, 19 patients in ICU, 33 in cardiology patients, 11 patients in cardiac surgery, 18 patients in the chest surgery, 7 patients in orthopedic, 6 patients in pediatric ward, 10 patients in burn unit, 1 patients in ophthalmology, 2 patients in neuro surgery, 2 patients in urology, and 8 patient in the oncology ward of Mayo Hospital, Lahore.

A total of 41 isolates of coagulase negative methicillin resistant Staphylococcus epidermidis (MRSE) were also isolated from 20 patients under treatment in medical ward, 6 in surgical ward, 3 in ICU, and 12 patients in the urology ward of Mayo Hospital, Lahore.
### Table 4.10 Colonization of Staphylococcus isolates from various wards in Mayo Hospital, Lahore

<table>
<thead>
<tr>
<th>Ward</th>
<th>Isolations</th>
<th>Total Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA(^a)</td>
<td>MSSA(^b)</td>
</tr>
<tr>
<td>Number of Isolates</td>
<td>555</td>
<td>449</td>
</tr>
<tr>
<td>Medical units</td>
<td>19</td>
<td>87</td>
</tr>
<tr>
<td>Surgical Units</td>
<td>295</td>
<td>214</td>
</tr>
<tr>
<td>ICUs</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>Cardiology</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Cardiac Surgery</td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>Chest Surg+Med</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>Orthopedics</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Burn Units</td>
<td>90</td>
<td>11</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Neuro Surgery</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Urology</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Dermatology</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Oncology</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\)MRSA = Methicillin Resistant Staphylococcus aureus.
\(^b\)MSSA = Methicillin Sensitive Staphylococcus aureus
\(^c\)MSSE = Methicillin Sensitive Staphylococcus epidermidis
\(^d\)MRSE = Methicillin Resistant Staphylococcus epidermidis
Antibiogram of Staph. isolates

The nosocomial isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were resistant to Penicillins and Cephalosporins. These isolates showed resistance to other drugs such as 464 (83.6%) to Tetracycline, 382 (68.8%) to Doxycyclin, 394 (70.9%) to Erythromycin, 187 (33.7%) to Klarithromycin, 283 (50.9%) to Augmentin, 166 (30%) to Unasyn, 49 (8.8%) to Tazocin, 93 (16.8%) to Gentamicin, 49 (8.8%) to Amikacin, 127 (22.8%) to Ciprofloxacin, 40 (7.2%) to Sparfloxacin, 38 (6.8%) to Fucidin, 27 (4.9%) to Tienam. However, all the isolates were found sensitive to Vancocin. (Appendix 2-3).

A total of 449 Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates were isolated. Of those 319 (71%) were resistant to Tetracycline, 227 (50.6%) to Doxycycline, 129 (29%) to Cephradine, 82 (18%) to Cefuroxime, 294 (65.5%) to Erythromycine, 83 (18.4%) to Klarithromycin, 91 (20.3%) to Augmentin, 62 (13.8%) to Unasyn, 13 (2.9%) to Tazocin, 48 (10.7%) to Gentamicin, 16 (3.6%) to Amikacin, 71 (15.8%) to Ciprofloxacin, 31 (6.9%) to Sparfloxacin, and 10 (2.2%) to Fucidin. None of the strain was found resistant to Tienam and Vancocin (Appendix 2-3).

Of the 242 Methicillin sensitive *Staph. epidermidis* (MSSE) isolates 105 (43.3%) were found resistant to Tetracycline, 83 (34.3%) to Doxycycline, 103 (43%) to Cephradine, 64 (26%) to Cefuroxime, 130 (53.7%) to Erythromycine, 56 (23%) to Klarithromycin, 62 (26%) to Augmentin, 50 (21%) to Unasyn, 9 (3.7%) to Tazocin, 57 (23.6%) to Gentamicin, 11 (4.5%) to Amikacin, 39 (16%) to Ciprofloxacin, 18 (7.4%) to Sparfloxacin and 6 (2.5%) to Fucidin. None of the isolated strain was resistant to Tienam and Vancocin (Appendix 2-3).

Of the 41 isolates of Methicillin resistant *Staph. epidermidis* (MRSE), 35 (85.4%) were resistant to Tetracycline, 29 (71%) to Doxycycline, 32 (78%) to Erythromycine, 13 (31.7%) to Klarithromycin, 25 (61%) to Augmentin, 19 (46%) to Unasyn, 2 (4.8%) to Tazocin, 9 (22%) to Gentamicin, 3 (7.3%) to Amikacin, 11 (27%) to Ciprofloxacin, 3 (7.3%) to Sparfloxacin, 2 (5%) to Fucidin, and 1 (2.4%) to Tienam. All the isolates were found sensitive to Vancocin (Appendix 2-3).
Fig. 1
(ii) **Streptococcus (Strep.) Species**

Various species of genus Streptococcus were broadly classified using their hemolytic activity whereas in case of beta hemolysis by Lancefield group antigens in their cell wall. *Strep. pyogenese* was isolated from cases of sore throat, tonsillitis, pharyngitis, peritonsillar abscess, scarlet fever, otitis media, cellulitis, impetigo, abscess and septicemia. This organism was using samples of throat swab, pus, fluids and blood.

**Morphology:** Streptococci were observed as gram positive cocci occurring in chains of variable length but also in pairs and singly. This organism was non motile and some of the isolates were found capsulated.

**Characteristics:** On blood agar *Strep.pyogenese* produced beta haemolytic colonies (the colonies were surrounded by a zone of complete haemolysis with decolorization of the haemoglobin). The bacterial colonies were usually small (0.5-1 mm), colourless, dry, shiny or mucoid. Haemolysis was more marked under anaerobic conditions. This organism grew well on crystal violet blood agar. It was catalase negative and PYR (pyrrolidonyl) test positive.

*Strep. pneumoniae* was isolated from cases of lobar pneumonia, bronchitis, meningitis bacteraemia, otitis media, sinusitis and conjunctivitis. *Strep. pyogenese* was isolated from throat swab, pus, fluids and blood specimens. *Strep. pneumoniae* was also isolated from the specimens of sputum, exudates, blood, CSF and pleural fluid. Morphologically *Strep. pneumoniae* was gram positive elongated diplococci which formed short chains. These organisms were non motile and capsulated. On blood agar after overnight incubation *Strep. pneumoniae* formed translucent or mucoid colonies, 1-2 mm in diameter. In young cultures the colonies were raised but later became flattened with raised edges, giving them a ringed appearance. Pneumococci showed alpha haemolysis (i.e. colonies were surrounded by an area of partial haemolysis with a green brown discoloration in the medium. *Strep. pneumoniae* grew on chocolate agar and lyzed blood agar. Its growth was enhanced when incubated in a carbon dioxide enriched atmosphere. *Strep. pneumoniae* was catalase negative and sensitive to optochin.
Isolations: Various species of genus Streptococcus were isolated from a total of 429 samples of material collected from patients (104 samples of pus and wound swabs, 8 samples of blood, 21 samples of pleural fluids, 20 of ascitic fluids, 5 samples of cerebrospinal fluids, 14 samples of urine, 205 samples of sputum; 30 burn swab samples and 13 patient body devices and 9 samples of the fecal and drainage materials; Table-4.11). The identification of 429 isolates from those patient samples were made on the bases of their biochemical and biological characteristics. Of these isolates, 265 were identified as *Strep. pyogenes*, 128 as *Strep. pneumonae* and 36 as belonging to viridan groups of streptococci.

In the present investigation species of Streptococcus were isolated from 60 samples from the hospital staff (45 samples of nasal and throat swabs; 10 samples of hand swabs, and 5 samples of gloves and aprons; Table-4.12).

Various species of Streptococcus were also isolated from 12 operation theatre air samples, 15 ward environment samples, 2 sterilized water samples, and 5 surgical instrument samples (Table 4.13).

*Streptococcus* spp. were isolated from a total of 146 samples obtained from non-human inmates (16 nasal and 11 faecal swab samples from cats; 43 samples from various body parts, and 76 samples from the visceral material of the cockroaches; Table 4.14).

Overall a total of 669 isolates of *Streptococcus* were obtained from 8040 samples collected from various sources (429 samples from patients, 60 samples from hospital staff, 34 samples from hospital environment and 146 non-human inmate samples; Table 4.15).

A total of 429 isolates of *Streptococci* were recovered from 136 patients under treatment in medical unit, 106 in surgical unit, 44 in ICU, 8 in cardiology ward, 28 in cardiac surgery ward, 60 in chest surgery ward, 10 in orthopedic surgery ward, 3 in pediatric ward, 24 in burn unit, 1 patient in ophthalmology ward, 4 patients in neurosurgery ward, 1 patients in urology ward, 1 patient in dermatology ward, and 3 patients in oncology ward (Fig.2).
**Results**

**Antibiogram:** Of the total 429 isolates of Streptococcus, 137 (31.9%) were found resistant to ampicillin, 110 (25.6%) to ampiclox, 119 (27.7%) to cephradine, 69 (16.1%) to cefuroxime, 30 (7%) to cefoparazone, 121 (28.2%) to erythromycin, 47 (11%) to klarithromycin, 59 (13.8%) to augmentin, 41 (9.6%) to unasyn, 9 (2.1%) to tazocin, 55 (12.8%) to gentamicin, 12 (2.8%) to amikacin, 139 (32.4%) to ciprofloxacin, 42 (9.8%) to sparflloxacin and 107 (24.9%) to fucidin. All the isolates were found sensitive to Tienum and Vancocin (Appendix 2-3).
### Table 4.11 Isolation of Streptococcus isolates from patient samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Aseptic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Faecal &amp; drainages Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Strep. Isolates</td>
<td>104</td>
<td>8</td>
<td>21</td>
<td>20</td>
<td>5</td>
<td>14</td>
<td>205</td>
<td>30</td>
<td>13</td>
<td>9</td>
<td>429</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>10</td>
<td>7.33</td>
<td>18.26</td>
<td>6.99</td>
<td>13.5</td>
<td>1</td>
<td>20.74</td>
<td>9.11</td>
<td>13.13</td>
<td>8.6</td>
<td>9.52</td>
</tr>
</tbody>
</table>

### Table 4.12 Isolation of streptococcal isolates from hospital staff

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>Throat / Nasal Swabs</th>
<th>Hand Swabs</th>
<th>Gloves and Aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>45</td>
<td>10</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>35.43</td>
<td>3.93</td>
<td>1.96</td>
<td>9.45</td>
</tr>
</tbody>
</table>
### Table 4.13  Isolation of streptococcus isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air</th>
<th>Ward Environment</th>
<th>Water from Sterilizers</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>12</td>
<td>15</td>
<td>2</td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>9.52</td>
<td>23</td>
<td>0.32</td>
<td>0.26</td>
<td>1.27</td>
</tr>
</tbody>
</table>

### Table 4.14  Isolation of streptococcal isolates from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Legs</td>
<td>Intestine</td>
<td></td>
</tr>
<tr>
<td>Samples Collected</td>
<td>25</td>
<td>88</td>
<td>88</td>
<td>226</td>
</tr>
<tr>
<td>Isolates</td>
<td>16</td>
<td>43</td>
<td>76</td>
<td>146</td>
</tr>
<tr>
<td>Percentage</td>
<td>64</td>
<td>48.86</td>
<td>86.36</td>
<td>64.6</td>
</tr>
</tbody>
</table>

### Table 4.15  Isolation of streptococcal isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Staff hands, gloves &amp; Aprons</th>
<th>Ward Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4500</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolations</td>
<td>429</td>
<td>60</td>
<td>34</td>
<td>146</td>
<td>669</td>
</tr>
<tr>
<td>Percentage</td>
<td>9.53</td>
<td>0.94</td>
<td>1.27</td>
<td>64.6</td>
<td>8.32</td>
</tr>
</tbody>
</table>
Results

Fig. 2
(iii) Enterococcus

Enterococci were isolated from the persons suffering from infection of urinary tract, billiary tract, ulcers, wounds, endocarditis and meningitis.

**Morphology**: Enterococcus species stained gram positive, occurred in pairs or short chains, these isolates were non capsulated and their majority was non motile.

**Characteristics**: Enterococci were aerobic organisms capable of growing over a wide temperature range 10-45°C. Although the isolates were mainly non haemolytic, some isolates showed alpha or beta haemolysis. *Enterococcus faecalis* fermented, lactose, producing small dark red magenta colonies on MacConkey agar and small yellow colonies on cysteine lactose electrolyte deficient (CLED) agar. Enterococcus species grew in the presence of 6.5% sodium chloride and 40% bile. They hydrolyzed the esculin, producing black colonies, reduced litmus milk and were catalase negative.

**Isolations**: Various species of *Enterococcus* were isolated from a total of 328 samples of morbid material collected from patients including 37 samples of pus and wound material, 14 samples of blood, 5 samples of pleural fluid, 44 samples of ascitic fluid, 5 samples of cerebrospinal fluid, 55 samples of urine, 82 samples of sputum, 39 burn swab samples, 35 patient body devices and 12 faecal and drainage material samples (Table 4.16). On the basis of biochemical and biological reactions 272 isolates were found to be *Enterococcus faecalis* and 56 were characterized as *Enterococcus faecium*.

Various species of *Enterococcus* (*Enterococcus faecalis, Enterococcus faecium*) were isolated from 73 samples obtained from the hospital staff consisting of 51 samples of throat swabs, 18 of hand swabs, and 4 samples of gloves and aprons (Table 4.17). In the present work species of *Enterococcus* were also isolated from 59 samples of material obtained from the hospital environment including 15 operation theatre air samples, 33 ward environment samples, 6 sterilized water, and 5 surgical instrument samples (Table 4.18).

Various species of Enterococcus were isolated from 166 samples of material...
Results

from non-human inmates including 9 from throat and nasal swabs and 18 from the fecal swabs from cats, and 56 samples from legs and 83 from visceral material of cockroaches (Table 4.19).

Overall a total of 627 isolates of Enterococci were isolated from 8040 samples including 328 patient samples, 73 hospital staff samples, 59 hospital environment samples and 166 non-human inmate samples (Table 4.20).

In the present work a total of 328 species of Enterococci were isolated from 101 patients under treatment in medical unit, 55 patients in surgical unit, 53 patients in ICU, 10 in cardiology ward, 26 in cardiac surgery ward, 25 in the chest surgery ward, 9 in orthopedic surgery ward, 9 in pediatric ward, 31 in burn unit, 7 in neurosurgery ward, 1 in the urology ward, and 1 in the oncology ward of Mayo Hospital, Lahore-Pakistan (Fig.3).

Antibiogram of Enterococci

Of the 328 isolated isolates of Enterococci 147 (45%) were found resistant to action of ampicillin, 126 (38.4%) to ampiclox, 159 (48.5%) to tetracyclin, 110 (33.5%) to doxycycline, 179 (54.6%) to erythromycine, 130 (39.6%) to klarithromycin, 43 (13%) to augmentin, 32 (9.8%) to unasyn, 18 (5.5%) to tazocin, 52 (16%) to gentamicin, 18 (5.5%) to amikacin, 69 (21%) to ciprofloxacin, 51 (15.5%) to sparfloxacin, 19 (5.8%) to tienam and 8 (2.4%) to vancocin (Appendix. 2-3).
Table 4.16  Isolation of Enterococcal isolates from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Pleural fluids</th>
<th>Ascetic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>37</td>
<td>14</td>
<td>5</td>
<td>44</td>
<td>5</td>
<td>55</td>
<td>82</td>
<td>39</td>
<td>35</td>
<td>12</td>
<td>328</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>3.55</td>
<td>12.84</td>
<td>4.34</td>
<td>15.38</td>
<td>13.51</td>
<td>3.93</td>
<td>8.29</td>
<td>11.85</td>
<td>35.35</td>
<td>12.12</td>
<td>7.29</td>
</tr>
</tbody>
</table>
### Table 4.17  Isolation of Enterococcus isolates from Hospital Staff Samples

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>Throat / Nasal Swabs</th>
<th>Hand Swabs</th>
<th>Gloves and Aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>51</td>
<td>18</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>40.15</td>
<td>7.08</td>
<td>1.57</td>
<td>11.5</td>
</tr>
</tbody>
</table>

### Table 4.18  Isolation of Enterococcus isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air</th>
<th>Ward Environment</th>
<th>Sterilized water</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>15</td>
<td>33</td>
<td>6</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>11.9</td>
<td>50.76</td>
<td>0.98</td>
<td>0.26</td>
<td>2.2</td>
</tr>
</tbody>
</table>
### Table 4.19  Isolation of Enterococcus isolates from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Faecal</td>
<td>Legs</td>
</tr>
<tr>
<td>Samples Collected</td>
<td>25</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>Isolates</td>
<td>9</td>
<td>18</td>
<td>56</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>36</td>
<td>72</td>
<td>63.63</td>
</tr>
</tbody>
</table>

### Table 4.20  Isolation of Enterococcus isolates from various hospital sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Staff Samples</th>
<th>Ward Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4500</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8038</td>
</tr>
<tr>
<td>Isolates</td>
<td>328</td>
<td>73</td>
<td>59</td>
<td>166</td>
<td>627</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>7.28</td>
<td>11.49</td>
<td>2.2</td>
<td>73.45</td>
<td>7.79</td>
</tr>
</tbody>
</table>
Fig. 3
(iv) Pseudomonas

Species of Pseudomonas (*Pseudo. aeruginosa*, and *Pseudo. Cepacia*) were isolated from skin infections, especially the burn sites, wounds, pressure sores, ulcers, urinary tract infections, respiratory infections and post-operative wound infections. Depending on the site of infection, the samples of pus, urine, sputum, effusions and blood were cultured for the isolation of Pseudomonas.

**Morphology:** *Pseudo. aeruginosa* was found to be obligatory aerobic Gram negative, non sporulating motile rod. Some isolates were observed as capsulated.

**Characteristics:** This organism was usually recognized by the pigments, it produced including pyocyanin a blue green pigment and pyoverdin a yellow green fluorescent pigment. A few isolates were found to be non pigmented producing. Cultures of the organism had a distinctive smell due to the production of 2-aminoacetophenon. *Pseudo. aeruginosa* produced large, flat, spreading colonies which were often haemolytic and usually pigment producing. The pigments diffused into the medium giving it a dark greenish blue colour. Some isolates produced small or mucoid colonies. *Pseudo. aeruginosa* produced pale colour colonies on MacConkey agar and green colonies on CLED medium. On blood agar, pigment production was less marked. A characteristic pink red slope (often with a metallic appearance) and pink red butt were produced. No H₂S was produced. The organism was oxidase positive and produced acid only from glucose (no gas). Growth at 42°C differentiated *Pseudo. aeruginosa* from the less commonly isolated pseudomonas (*Pseudo. putido* and *Pseudo. fluorescens*).

**Isolations:** In the present investigation various species of Pseudomonas were isolated from a total of 781 samples collected from patients suffering from various diseases. A total of 205 samples of pus and wound swabs, 9 of blood, 20 of pleural fluid, 26 of ascitic fluid, 4 of cerebrospinal fluid, 203 of urine, 97 of sputum, 187 burn swabs, 15 patient body devices and 15 of fecal and drainage samples were examined (Table 4.21). On the basis of biochemical and biological reactions 693 isolates were identified as *Pseudo. aeruginosa*, and 88 isolates as *Pseudo. cepacia*.
Results

Pseudomonas spp. were isolated from 33 of 635 samples obtained from the hospital staff. Isolation of Pseudomonas was possible from 2 of 127 samples of throat and nasal swabs; 10 of 254 samples of hand swabs, and 21 of 254 samples of gloves and aprons (Table 4.22).

Various Pseudomonas isolates were isolated from 53 of 2677 samples of material obtained from the hospital environment, including 18 of the 65 ward environment samples, 20 of the 610 instrument sterilizer water samples, and 15 of the 1876 surgical instrument samples (Table 4.23).

Various Pseudomonas species were also isolated from 74 samples of morbid material from non-human inmates consisting of 7 nasal and 5 fecal swabs collected from cats; and 25 legs and 37 visceral material samples of cockroaches (Table-4.24).

A total of 941 isolates of Pseudomonas were isolated from 8040 samples from various sources such as 781 patient samples, 33 samples from hospital staff, 53 hospital environment samples and 74 non-human inmate samples (Table 4.25).

Overall 781 isolates of Pseudomonas were isolated from 163 patients under treatment in medical unit, 229 in surgical unit; 62 in ICU, 1 in cardiology ward, 17 in cardiac surgery ward; 41 in chest surgery ward, 35 in orthopedic surgery ward, 12 in pediatric ward, 174 in burn unit, 1 patient in neurosurgery ward, 43 in urology ward, and 3 in the oncology ward (Fig. 4).

Antibiogram: Of the 781 isolates of *Pseudo. aeruginosa*, 320 (41%) were found resistant to action of Cefoparazone, 312 (40%) to Cefrome; 72 (9.2%) to Tazocin; 212 (27%) to Gentamicin; 93 (12%) to Amikacin; 342 (43.8%) to Ciprofloxacin; 359 (46%) to Sparfloxacin; and 63 (8%) to Tienam (Appendix 2-3).
### Table 4.21  Isolation of Psudomonas isolates from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Thoracic fluids</th>
<th>Ascetic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>205</td>
<td>9</td>
<td>20</td>
<td>26</td>
<td>4</td>
<td>203</td>
<td>97</td>
<td>187</td>
<td>15</td>
<td>15</td>
<td>781</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>19.71</td>
<td>8.2</td>
<td>17.39</td>
<td>9.09</td>
<td>10.81</td>
<td>14.52</td>
<td>9.81</td>
<td>56.8</td>
<td>15.15</td>
<td>14.8</td>
<td>17.35</td>
</tr>
</tbody>
</table>

### Table 4.22  Isolation of Psudomonas isolates from hospital staff samples

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>Throat / Nasal Swabs</th>
<th>Hand Swabs</th>
<th>Gloves and Aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>2</td>
<td>10</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>1.57</td>
<td>3.93</td>
<td>8.26</td>
<td>5.19</td>
</tr>
</tbody>
</table>
Table 4.23  Isolation of *Psudomonas* isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air</th>
<th>Ward Environment</th>
<th>Sterilized water</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>18</td>
<td>20</td>
<td>15</td>
<td>53</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>0</td>
<td>27.69</td>
<td>3.27</td>
<td>0.79</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Table 4.24  Isolation of *Psudomonas* isolates from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Fecal</td>
<td>Legs</td>
</tr>
<tr>
<td>Sample Collected</td>
<td>25</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>Isolates</td>
<td>7</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>28</td>
<td>20</td>
<td>28.4</td>
</tr>
</tbody>
</table>

Table 4.25  Isolations of *Psudomonas* isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Hospital staff</th>
<th>Hospital Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>781</td>
<td>33</td>
<td>53</td>
<td>74</td>
<td>941</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>17.34</td>
<td>5.19</td>
<td>1.97</td>
<td>32.74</td>
<td>11.70</td>
</tr>
</tbody>
</table>
Results

Fig. 4
(v) Enterobacter

Enterobacter spp. (*Enterobacter cloacae* and *Enterobacter aerogenes*) were isolated from the samples of urine, pus, ulcers, faeces, blood and other fluids.

**Morphology:** Enterobacter strained as Gram negative motile and non capsulated rods.

**Characteristics:** On blood agar, this organism produced large colonies which resembled to those produced by klebsiellae but were not so mucoid. The organism was MR negative, VP positive, citrate positive and lysine decarboxylase positive.

**Isolations:** In the present investigation Enterobacter were isolated from 349 samples of patients consisting of 33 samples of pus and wound swabs; 12 samples of blood; 7 samples of pleural fluid; 32 samples of ascitic fluid; 6 samples of cerebrospinal fluid, 115 samples of urine; 68 of sputum samples; 16 of burn swabs; 33 patient body devices and 27 fecal and drainage samples (Table 4.26). On the basis of biochemical and biological reactions 212 isolates were characterized as *Enterobacter cloacae* and 137 to *Enterobacter aerogenes*.

Species of Enterobacter were isolated from 15 samples of the hospital staff including 3 samples from throat swabs, 7 from hand swabs, and 5 from gloves and aprons (Table 4.27).

Enterobacter organisms were isolated from 25 of 2677 samples from the hospital environment including 3 from operation theatre air, 15 from ward environment, 5 from sterilized water, and 2 from surgical instruments (Table: 4.28).

A total of 124 Enterobacter isolates were obtained from a total of 226 samples of non-human inmates including 11 nasal swabs and 22 fecal swabs from the cats; and 32 from legs and 59 from intestinal material of cockroaches (Table-4.29).
In the present work 349 isolates of Enterobacter were isolated from 128 patients under treatment in medical unit; 69 in surgical unit; 45 in ICU; 3 in cardiology ward; 20 in cardiac surgery ward; 20 in the chest surgery ward; 6 in orthopedic surgery ward; 10 in pediatric ward; 12 in burn unit; 4 in the neurosurgery ward; 25 in urology ward; and 7 patients in oncology ward (Fig-5).

A total of 513 isolates of Enterobacter were isolated from 8040 samples from various sources including 349 patient samples, 15 samples from hospital staff, 25 hospital environment samples and 124 samples from non-human inmates (Table-4.30).

**Antibiogram of Enterobacter:** Of the total 349 Enterobacter isolates, 301 (86.2%) were resistant to the action of ampicillin; 293 (84%) to ampiclox; 129 (37%) to cefoparazone; 115 (33%) to cefrome; 171 (49%) to augmentin; 131 (37.5%) to unasyn; 23 (6.6%) to tazocin; 55 (15.8%) to gentamicin; 39 (11.2%) to amikacin; 77 (22%) to ciprofloxacin; 52 (14.9%) to sparfloxacain and 16 (4.6%) to tienam (Appendix 2-3).
## Results

### Table 4.26: Isolation of Enterobacter isolates from patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Pleural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>33</td>
<td>12</td>
<td>7</td>
<td>32</td>
<td>6</td>
<td>115</td>
<td>68</td>
<td>16</td>
<td>33</td>
<td>27</td>
<td>349</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>3.17</td>
<td>11</td>
<td>6.08</td>
<td>11.18</td>
<td>16.21</td>
<td>8.22</td>
<td>6.88</td>
<td>4.86</td>
<td>33.33</td>
<td>26.7</td>
<td>7.75</td>
</tr>
</tbody>
</table>

### Table 4.27: Isolation of Enterobacter isolates from hospital staff samples

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>Throat / Nasal Swabs</th>
<th>Hand Swabs</th>
<th>Gloves and Aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>2.36</td>
<td>2.75</td>
<td>1.96</td>
<td>2.36</td>
</tr>
</tbody>
</table>
Table 4.28  Isolation of Enterobacter isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air Ward Environment</th>
<th>Sterilized water</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
</tr>
<tr>
<td>Isolates</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>2.38</td>
<td>23.07</td>
<td>0.81</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 4.29  Isolation of Enterobacter isolates from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats Nasal</th>
<th>Cats Fecal</th>
<th>Cockroaches Legs</th>
<th>Cockroaches Intestine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Collected</td>
<td>25</td>
<td>25</td>
<td>88</td>
<td>88</td>
<td>226</td>
</tr>
<tr>
<td>Isolates</td>
<td>11</td>
<td>22</td>
<td>32</td>
<td>59</td>
<td>124</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>44</td>
<td>88</td>
<td>36.36</td>
<td>67.04</td>
<td>54.86</td>
</tr>
</tbody>
</table>

Table 4.30 Isolation of Enterobacter isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Hospital staff</th>
<th>Hospital Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>349</td>
<td>15</td>
<td>25</td>
<td>124</td>
<td>513</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>7.75</td>
<td>2.36</td>
<td>0.93</td>
<td>54.86</td>
<td>6.38</td>
</tr>
</tbody>
</table>
Results

Fig. 5
(vi) Acinetobacter

Badar In this investigation Acinetobacter were found as a major constituent of the flora of soil, water, sewage and within the hospital environment. These bacteria generally colonized the skin of patients, and were isolated from the cases of wound infections. These organisms were implicated as etiological agent of hospital acquired pneumonia and urinary tract problems. They were isolated from the samples of sputum, pus and wound swabs, urine, blood and other biological fluids.

**Morphology:** Acinetobacter were observed as Gram positive and very plump almost coccoid rods.

**Characteristics:** On blood agar the colonies of Acinetobacter were convex, grey to white, 2-3 mm in diameter and were variably hemolytic were oxidase negative, catalase positive and non lactose fermenting organisms as indicated by their growth on MacConkeys agar.

**Isolations:** A total of 41 isolates of Acinetobacter were obtained from 6 patients under treatment in medical unit, 21 patients in surgical unit, 4 patients in ICU, 3 in chest surgery ward, 2 in orthopedic surgery ward, 2 in urology ward, and 1 in oncology ward (Fig-6). Acinetobacter isolates were obtained from 41 samples of morbid material, consisting of 30 samples of pus and wound swabs, 3 samples of blood, and 8 samples of urine (Table 4.31). On the bases of biochemical and biological reactions all the isolates were characterized as *A. baumannii*.

**Antibiogram:** Of the 41 isolates of Acinetobacter 13 (31.7%) were found resistant to doxycycline; 8 (19.5%) to cefoparazone; 7 (17%) to cefrome; 12 (29.3%) to augmentin; 9 (22%) to unasyn; 2 (5%) to tazocin; 6 (14.6%) to gentamicin; 2 (5%) to amikacin; 9 (21.9%) to ciprofloxacin, and 4 (9.8%) to sparfloxacin (Annexure 2-3).
### Results

#### Table 4.31  Isolation of Acinetobacter species from patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>30</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>2.88</td>
<td>2.75</td>
<td>0</td>
<td>0</td>
<td>0.57</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.91</td>
</tr>
</tbody>
</table>

#### Table 4.32  Isolation of Acinetobacter isolates from various hospital sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients Samples</th>
<th>Hospital staff</th>
<th>Hospital Environment</th>
<th>Non-human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>0.91</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Results

Fig. 6
(vii) Klebsiella Species

Isolates of klebsiella were recovered from the samples of urine, pus, sputum and infected tissues. Klebsiellae were found as Gram negative, non motile, usually capsulated rods.

Characteristics: They were aerobes and facultatively anaerobes on blood agar plates. They produced large grey white usually mucoid colonies. Most of the klebsiellae were lactose fermenting and produced mucoid pink colonies on MacConkey agar and yellow mucoid colonies on CLED medium. Kleb. pneumoniae subspecies were indole negative (Kleb. oxytoca was indole positive), ornithine decarboxylase negative and did not produce H₂S.

Isolations: In the present work Klebsiella species were isolated from a total of 266 samples collected from patients in various wards. Overall the samples consisted of 27 of pus and wound swabs, 7 of blood; 10 of pleural fluid; 21 of ascitic fluid; 2 of cerebrospinal fluid; 62 of urine; 120 of sputum; 13 of burn swabs; and 4 samples of fecal & drainage material (Table 4.33). On the bases of biochemical and biological reactions 96 isolates were characterized as Kleb. Pneumoniae, 131 as Kleb. aerugenese and 39 as Kleb. oxytoca. Klebsiella spp. were also recovered from a total of 24 samples including 18 samples of throat and nasal swabs, 3 of hand swabs, and 3 of gloves and aprons (Table 4.33). Klebsiella were also isolated from a total of 35 samples from hospital environment (Table 4.34).

A total of 325 species of Klebsiella were isolated from 8040 samples from various routes including 266 patient samples, 24 samples from hospital staff and 35 hospital environment samples collected during this (Table-4.35).

A total of 266 isolations of Klebsiella were possible from 113 patients who were under treatment in medical ward, 45 in surgical ward, 29 in ICU, 14 in cardiac surgery ward, 35 in chest surgery ward, 2 in orthopedic surgery ward, 1 in pediatric ward, 9 in burn unit, 16 in the urology ward, and 2 patients in the oncology ward (Fig-7).
Antibiogram: Of the 266 isolates of Klebsiella, 62 (23.3%) were found resistant to the action of cefoparazone; 53 (19.9%) to cefrome; 150 (56%) to augmentin; 102 (38.3%) to unasyn; 16 (6%) to tazocin; 64 (24%) to gentamicin; 25 (9.4%) to amikacin; 83 (31.2%) to ciprofloxacin; 89 (33.5%) to sparfloxacin and 7 (2.6%) to tienam (Appendix: 2-3).
## Results

### Table 4.33  Isolation of Klebsiella isolates from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Pleural fluid</th>
<th>Pericardial fluid</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient body devices</th>
<th>Faecal and drainage specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>27</td>
<td>7</td>
<td>10</td>
<td>21</td>
<td>2</td>
<td>62</td>
<td>120</td>
<td>13</td>
<td>0</td>
<td>266</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>2.59</td>
<td>6.42</td>
<td>8.69</td>
<td>7.34</td>
<td>5.4</td>
<td>4.43</td>
<td>12.14</td>
<td>3.95</td>
<td>0</td>
<td>3.96</td>
</tr>
</tbody>
</table>

### Table 4.34  Isolation of Klebsiella isolates from hospital staff

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>Throat and nasal swabs</th>
<th>Hand swabs</th>
<th>Gloves and aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>14.17</td>
<td>1.18</td>
<td>1.18</td>
<td>3.77</td>
</tr>
</tbody>
</table>
### Results

**Table 4.35  Isolation of Klebsiella isolates from hospital environment**

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>OT air environment</th>
<th>Ward environment</th>
<th>Sterilized water</th>
<th>Surgical instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>0</td>
<td>53.84</td>
<td>0</td>
<td>0</td>
<td>1.31</td>
</tr>
</tbody>
</table>

**Table 4.36  Isolation of Klebsiella isolates from various hospital sources**

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient samples</th>
<th>Hospital Staff</th>
<th>Hospital Environment</th>
<th>Non Human Inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4500</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolations</td>
<td>266</td>
<td>24</td>
<td>35</td>
<td>0</td>
<td>325</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>5.91</td>
<td>3.77</td>
<td>1.31</td>
<td>0</td>
<td>4.04</td>
</tr>
</tbody>
</table>
Fig. 7
Results

viii) Proteus Species

Morphology: Proteus isolates were found as Gram negative motile rods exhibited pleomorphism in their size and shape and varied from coccobacillus to filamentous forms.

Characteristics: All the Proteus isolates grew well on all kinds of nutrient medias. On MacConkey’s agar its colonies were pale, small and circular with a fishy smell. It did not ferment lactose, were urease positive, lysine negative and produced hydrogen sulfide gas.

Isolations: Proteus organisms were isolated from 140 samples collected from hospital patients (22 samples of pus and wound swabs; 3 of blood; 9 samples of ascitic fluid; 48 of urine, 21 of sputum, 32 of burn swabs and 5 samples of patient body devices; Table 4.37). On the bases of biochemical and biological observations, 103 isolates were characterized as *Proteus mirabilis*, and 37 as *Proteus vulgaris*. Proteus spp. were also isolated 20 ward environment and 2 surgical instrument samples (Table 4.38). In present work Proteus isolates were obtained from a total of 47 morbid material samples obtained from non-human inmates (8 samples of fecal material from cats, and 12 samples of legs and 27 of visceral material of cockroaches; Table 4.39). A total of 209 species of Proteus were isolated from 8040 samples from various sources (140 patient samples, 22 hospital environment samples, and 47 of non-human inmates samples; Table-4.40). A total of 140 Proteus spp. were recovered from 37 patients under treatment in medical unit, 38 in surgical unit, 16 in ICU, 3 in the chest surgery ward, 7 in orthopedic surgery ward, 2 in pediatric ward, 25 in burn unit, and 12 patients in the urology ward (Fig.8).

Antibiogram of Proteus: Of the 140 Proteus isolates 82 (58.6%) were found resistant to cefuroxime; 54 (38.6%) to cefoparazone; 47 (33.6%) to cefrome; 69 (49.3%) to augmentin; 51 (36.4%) to unasyn; 10 (7%) to tazocin; 28 (20%) to gentamicin; 16 (11.4%) to amikacin; 41 (29.3%) to ciprofloxacin; 36 (25.7%) to sparfloxacin, and 4 (2.9%) to tienam (Appendix 2-3).
Results

Table 4.37  Isolation of Proteus isolates from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage Specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040 109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
<td></td>
</tr>
<tr>
<td>Isolates</td>
<td>22 3 0 9 0 48 21 32 5 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>2.11 2.75 0 3.14 0 3.43 2.12 9.72 5.05 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.11</td>
</tr>
</tbody>
</table>

Table 4.38  Isolation of Proteus isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>OT Air</th>
<th>Ward Environment</th>
<th>Water from Sterilizers</th>
<th>Surgical Instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>0</td>
<td>30.76</td>
<td>0</td>
<td>0.1</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table 4.39  Isolation of Proteus isolates from non-human inmates

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Collected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal</td>
<td>25</td>
<td>88</td>
<td>226</td>
</tr>
<tr>
<td>Fecal</td>
<td>25</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>12</td>
<td>47</td>
</tr>
<tr>
<td>Legs</td>
<td>8</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>12</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>0</td>
<td>25.6</td>
<td>20.79</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>57.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.40 Overall Status of Isolation of Proteus isolates from various hospital sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient</th>
<th>Hospital staff</th>
<th>Hospital environment</th>
<th>Non-human inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4500</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8038</td>
</tr>
<tr>
<td>Isolations</td>
<td>140</td>
<td>0</td>
<td>22</td>
<td>47</td>
<td>209</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>3.11</td>
<td>0</td>
<td>0.82</td>
<td>20.79</td>
<td>2.59</td>
</tr>
</tbody>
</table>
Fig. 8
ix) Escherichia

*E. coli* was the commonest pathogen isolated from patients suffering from ailments of urinary tract, wounds, peritonitis, sepsis and endotoxin induced shock, meningitis, bacteraemia in neonates, infantile gastroenteritis, dysentery and haemorrhagic diarrhoea. Depending on site of infection, sampling included: urine, pus, faeces, cerebrospinal fluid, ascitic fluid and blood.

**Morphology:** *E. coli* were found to be Gram negative usually motile rods, minority of the isolates being capsulated. They grew aerobically and under facultative anaerobic conditions.

**Characteristics:** Optimum temperature for the growth of Escherichia organisms was 36-37°C with most isolates growing over a range of 18-44°C. On blood agar *E. coli* produced 1-4 mm diameter colonies after overnight incubation. The colonies appeared mucoid. Some isolates were haemolytic. *E. coli* fermented lactose, producing smooth pink colonies on MacConkey’s agar and yellow colonies on CLED agar. Some isolates (e.g. inactive isolates) were late or non lactose fermenting. *E. coli* VTEC were non sorbitol fermenting, producing colourless colonies on sorbitol MacConkey’s agar. Yellow colonies were produced on XLD agar. Growth of *E. coli* was usually inhibited on DCA agar. On KIA reaction most isolates of *E. coli* produced an acid deep and an acid slope with gas production and no H₂S blackening (similar to other lactose fermenting coliforms). Most isolates of *E. coli* were indol positive, lysine decarboxylase positive, beta-glucuronidase (PGUA) positive. They reduced nitrates to nitrites, and were citrate and H₂S negative.

**Isolations:** Isolates of Escherichia were isolated from 1031 samples from patients which consisted of 99 samples of pus / wound swabs, 12 samples of blood, 6 of pleural fluid, 30 of ascitic fluid, 4 of cerebrospinal fluid, 742 of urine, 65 of sputum, 23 burn swabs, 35 patient body devices and 15 of fecal and drainage material (Table 4.41).

Escherichia isolates were isolated from a total of 25 samples from hospital staff
including 5 samples of throat and nasal swabs, 16 of hand swabs, and 4 of gloves and aprons (Table 4.42).

Various isolates of *Escherichia coli* were also isolated from 55 samples of the hospital environment including 10 operation theatre air samples and 45 ward environment samples (Table 4.43).

During our investigation various *Escherichia coli* were isolated from 163 samples of material obtained from non-human inmates including 9 samples from nasal swabs, 23 fecal swabs taken from cats; and 67 from legs and 64 samples of visceral material of cockroaches (Table 4.44).

A total of 1274 isolates of *Escherichia coli* were isolated from 8040 samples from various sources including 1031 patient samples, 25 hospital staff samples, 55 hospital environment samples, and 163 of non-human inmate samples (Table-4.45).

A total of 1031 isolates of *Escherichia coli* were isolated from 560 (54.3%) patients which were under treatment in medical unit, 183 (17.7%) in surgical unit, 69 (6.7%) in ICU, 14 (1.3%) in cardiology ward, 31(3%) in cardiac surgery ward, 28 (2.7%) in chest surgery ward, 23 (2.2%) in the orthopedic surgery ward, 19 (2%) in the pediatric ward, 22 (2%) in the burn unit, 4 (0.4%) in the neurosurgery ward, 66 (6.4%) in the urology ward, and 12 (1%) patients in the oncology ward (Fig-9).

**Antibiogram of *E. coli***: Of the total 1031 isolates of *Escherichia coli*, 732 (71%) were found resistant to ampicillin; 710 (69%) to ampiclox; 618 (60%) to tetracyclin; 531 (51%), to doxycycline; 301 (29%) to cefuroxime; 110 (11%) to cefoparazone; 119 (12%) to cefrome; 291 (28%) to augmentin; 208 (20%) to unasyn; 30 (3%) to tazocin; 169 (16%) to gentamicin; 78 (8%) to amikacin; 235 (23%) to ciprofloxacin; 229 (22%) to sparflaxacin, and 32 (3%) to tienam (Appendix 2-3).
### Table 4.41  Isolation of Escherichia isolates from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Samples collected</th>
<th>Isolates</th>
<th>Isolation percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus and wound swabs</td>
<td>1040</td>
<td>99</td>
<td>9.51</td>
</tr>
<tr>
<td>Blood</td>
<td>109</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Pleural fluids</td>
<td>115</td>
<td>6</td>
<td>5.21</td>
</tr>
<tr>
<td>Ascitic fluids</td>
<td>286</td>
<td>30</td>
<td>10.48</td>
</tr>
<tr>
<td>Cerebrospinal fluids</td>
<td>37</td>
<td>4</td>
<td>10.81</td>
</tr>
<tr>
<td>Urine</td>
<td>1398</td>
<td>742</td>
<td>53.07</td>
</tr>
<tr>
<td>Sputum</td>
<td>988</td>
<td>65</td>
<td>6.57</td>
</tr>
<tr>
<td>Burn</td>
<td>329</td>
<td>23</td>
<td>6.99</td>
</tr>
<tr>
<td>Patient Body devices</td>
<td>99</td>
<td>35</td>
<td>35.35</td>
</tr>
<tr>
<td>Fecal and drainage material</td>
<td>101</td>
<td>15</td>
<td>14.85</td>
</tr>
<tr>
<td>Total</td>
<td>4502</td>
<td>1031</td>
<td>22.9</td>
</tr>
</tbody>
</table>
### Results

Table 4.42  Isolation of Escherichia isolates from hospital staff

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>Throat / Nasal swabs</th>
<th>Hand swabs</th>
<th>Gloves and aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>5</td>
<td>16</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>3.93</td>
<td>6.29</td>
<td>1.57</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Table 4.43  Isolation of Escherichia isolates from hospital environment

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>OT air environment</th>
<th>Ward environment</th>
<th>Sterilized water</th>
<th>Surgical instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>10</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>7.93</td>
<td>69.23</td>
<td>0</td>
<td>0</td>
<td>2.05</td>
</tr>
</tbody>
</table>
Table 4.44  Isolation of Escherichia isolates from non-human inmates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Fecal</td>
<td>Legs</td>
</tr>
<tr>
<td>Sample Collected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolates</td>
<td>9</td>
<td>23</td>
<td>67</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>36</td>
<td>92</td>
<td>76.13</td>
</tr>
</tbody>
</table>

Table 4.45  Isolation of Escherichia isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients</th>
<th>Hospital staff</th>
<th>Hospital environment</th>
<th>Non-human inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>1031</td>
<td>25</td>
<td>55</td>
<td>163</td>
<td>1274</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>22.9</td>
<td>3.93</td>
<td>2.05</td>
<td>72.12</td>
<td>15.84</td>
</tr>
</tbody>
</table>
Results

Fig. 9
x) Serratia Species

Serratia spp. were mostly isolated from the soil and water samples. Clinically they were associated with the pulmonary, urinary and cross infections as those isolates were obtained from the samples of sputum, pus, urine and blood. The Serratia spp. were Gram negative and motile rods.

**Characteristics:** Serratia species grew well on blood and MacConkey agars, and did not ferment lactose. Some isolates produced a red pigment in the nutrient agar at room temperature’s incubation.

**Isolations of Serratia spp.:** Serratia spp. were isolated from 67 samples (10 samples of pus and wound swabs, 8 of blood, 6 of ascitic fluid, 20 of urine, 15 of sputum, and 8 of burn swabs; Table 4.46). On the bases of their biochemical and biological reactions all the isolates were recognized as the *Serratia marcescens*.

**Antibiogram:** Of the 67 isolates of Serratia, 23 (34.3%) were found to the action of cefoparazone; 17 (25%) to cefrome; 3 (4.5%) to tazocin; 16 (24%) to gentamicin; 6 (9%) to amikacin; 41 (61%) to ciprofloxacin; 39 (58.2%) to sparfloxacin and 2 (3%) to tienam (Appendix 2-3).
### Table 4.46  Isolation of Serratia isolates from the patients

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Body devices</th>
<th>Fecal and drainage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples collected</strong></td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td><strong>Isolates</strong></td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>20</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td><strong>Isolation percentage</strong></td>
<td>0.96</td>
<td>7.33</td>
<td>0</td>
<td>2.09</td>
<td>0</td>
<td>1.43</td>
<td>1.51</td>
<td>2.43</td>
<td>0</td>
<td>0</td>
<td>1.49</td>
</tr>
</tbody>
</table>

### Table 4.47  Isolation of Serratia isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient samples</th>
<th>Hospital staff</th>
<th>Hospital environment</th>
<th>Non human inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolations</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>1.49</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Results

Fig.10
(xi) Haemophilus species

Morbid samples collected for isolation of Haemophilus species included cerebrospinal fluid, nasopharyngeal exudates, pus and blood. These organisms were stained as small, non-motile, aerobic, Gram negative coccobacilli or short rods.

Characteristics: The capsule was demonstrated by using specific anti serum. Growth of the bacterium was best obtained in a moist carbon dioxide enriched atmosphere at 20-40°C. Media used to isolate *H. influenzae* contained haemin or iron containing porphyrin and nicotinamide adenine dinucleotide (NAD) or its phosphate (NADP). The porphyrin requirement was referred to as growth factor X and the NAD or NADP requirement as growth factor V. On blood agar, after an overnight incubation at 35-37°C in a moist carbon dioxide, atmosphere, capsulated *H. influenzae* isolates produced mucoid colonies, 1.5 mm or more in diameter. The organism grew well on chocolate agar because it contained factors X and V. Heated blood agar to 75°C inactivated serum NADase and released extra factor V from the red cells. Addition of bacitracin (300 mg/liter) provided a selective medium to recover *H. influenzae* from the sputum samples.

Isolation: In the present work 93 isolates of Haemophilus were obtained from a total of 4502 patients (one sample of blood, 13 of plural fluid and 79 of sputum (Table-4.48). On the bases of biochemical and biological reactions all the isolates were demonstrated as *Haemophilus influenzae*. A total of 13 isolates of Haemophilus were obtained from 635 samples such as 13 throat and nasal swabs of the hospital staff (Table-4.49).

A total of 93 *Haemophilus isolates* were isolated from 43 patients under treatment in medical unit, 11 in surgical unit, 15 in ICU, 9 in cardiac surgery ward, 13 in chest surgery ward, 1 in pediatric ward, and 1 patient in the oncology ward (Fig-11).

Antibiogram: Of the 93 (2.27%) isolated isolates of Hemophilus 47 (50.5%) were
Results

found resistant to ampicillin; 32 (34.4%) to ampiclox; 18 (19.4%) to cefuroxime; 18 (19.4%) to cefoparazone; 63 (67.7%) to erythromycin; 21 (22.6%) to klarithromycin; 12 (13%) to augmentin; 8 (8.6%) to unasyn; 14 (15%) to gentamicin; 11 (11.8%) to amikacin; 21 (22.6%) to ciprofloxacin and 14 (15%) to sparfloxacin. All the isolates were susceptible to Tazocin and Tienam (Appendix 2-3).
Table 4.48  Isolation of Haemophilus isolates from patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient body devices</th>
<th>Fecal and drainage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
<td>4502</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>0</td>
<td>0.91</td>
<td>11.3</td>
<td>0</td>
<td>0</td>
<td>7.99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.06</td>
</tr>
</tbody>
</table>

Table 4.49  Isolation of Haemophilus isolates from hospital staff

<table>
<thead>
<tr>
<th>Site of Sampling</th>
<th>Throat / nasal swabs</th>
<th>Hand swabs</th>
<th>Gloves and aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>10.2</td>
<td>0</td>
<td>0</td>
<td>2.04</td>
</tr>
</tbody>
</table>
Table 4.50  Isolation of Haemophilus isolates from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient samples</th>
<th>Hospital staff</th>
<th>Hospital environment</th>
<th>Non human inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolations</td>
<td>93</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>2.06</td>
<td>2.04</td>
<td>0</td>
<td>0</td>
<td>1.32</td>
</tr>
</tbody>
</table>
xii) Uncommon Gram positive bacteria

A total of 119 (2.64%) isolates of various types of Gram positive bacteria were also isolated from the samples of patients including 3 (2.6%) plural fluid, 112 (11.33%) sputum and 4 (3.96%) fecal and drainage specimens (Table 4.51). These Gram positive organisms were also recovered from 11 (1.73%) samples from hospital staff including 8 (6.29%) throat and nasal swabs, 2 (0.78%) hand swabs and 1 (0.39%) gloves sample (Table 4.52).

A total of 41 (1.53%) various isolates of Gram positive organisms were isolated from hospital environment including 2 (1.58%) operation theatre air, 33 (50.76%) ward environment, 5 (0.81%) sterilized water and 1 (0.05%) surgical instruments (Table 4.53). These organisms were also recovered from 29 (12.83%) non human inmates including 13 (14.77%) legs and 16 (18.18%) viscera of cockroaches (Table 4.54).

A total of 200 (2.48%) various Gram positive organisms were isolated from the 8040 samples obtained from various sources such as 119 (2.64%) patient samples, 11 (1.73%) hospital staff samples, 41 (1.53%) hospital environment samples and 29 (12.83%) non human inmates (Table 4.55).

Of the 119 gram positive cocci, 72 (60.5%) were isolated from medical units, 15 (12.6%) from surgical units, 15 (12.6%) from ICU, 17 (14.3%) from chest surgery and medicine units (Fig-12).

A total of 119 isolates of Gram positive bacteria such as 20 isolates of Diphtheroids, 25 isolates of Diplococcoids, 4 isolates of Clostridia and 70 isolates of Anthracoid organisms were isolated from the hospital patients. Most of these isolates appeared as contaminants or as a secondary invader in the nosocomial disease processes.
a) Diplococcus spp.

**Morphology & Characteristics.** Diplococci were found as Gram positive and non-motile cocci generally visualized, all oval and spherical in shape, and were found in pairs or in short chains. Diplococci were non spore-forming and capsulated organisms and their colonies were low, convex, smooth, transparent with depressed centers. All the isolates were facultative anaerobes and grew well in 5-10% carbon dioxide, when media enriched with blood, serum or heated blood, were used for their growth.

**Isolation:** *Diplococcus pneumoniae* were isolated from 25 samples of morbid material of which 20 (80%) samples were obtained from Medical units, and 5 (20%) from ICU of Mayo Hospital, Lahore.

b) Clostridia

**Morphology & Characteristics.** Clostridia were seen as large Gram positive bacilli having terminal elongated and slightly wide spores than the body of the bacillus. They grew well on blood agar without producing hemolysis under anaerobic conditions and their colonies were irregularly circular, and flat to slightly raised.

**Isolation:** *Cl. difficile* were isolated from stool specimens of 4 patients in the Medical ICU who was on prolonged broad spectrum antibiotic therapy.

c) Anthracoids:

**Morphology & Characteristics.** These organisms were found as motile, non-capsulated, bacilli in short chains and these isolates produced beta hemolysis on blood agar.

**Isolation:** Isolates of Bacillus cereus and Bacillus megatherism were obtained from 40 samples of morbid material obtained from patients of medical units, 12 from surgical units, 8 from ICU and 14 from the chest surgery unit. From the hospital environment 27 isolates of Anthracoids organisms (2 from the operation theatre air samples, 19 from the ward environment samples, 5 from the sterilizer water samples, and 1 from
the surgical instrument samples) were obtained. Of the 13 isolates of Anthracoids isolated from non-human inmates 9 were isolated from the legs and 6 from the visceral material of the cockroaches.

**Antibiogram:** Of the 119 isolates of above organisms 45 (38%) were resistant to the action of ampicillin, 31 (26%) to ampiclox, 39 (33%) to tetracyclin, 26 (22%) to doxycycline, 59 (50%) to cephradin, 35 (29%) to cefuroxime, 49 (41%) to erythromycin, 18 (15%) to klarithromycin, 69 (58%) to augmentin, 51 (43%) to unasyn, 6 (5%) to tazocin, 29 (24%) to gentamicin, 4 (3%) to amikacin, 29 (24%) to ciprofloxacin, 12 (10%) to sparfloxacin and 3 (2.5%) to tienam (Appendix 2-3).
### Table 4.51  Isolation of Gram positive isolates from patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Pleural fluids</th>
<th>Peritoneal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>1040</td>
<td>109</td>
<td>115</td>
<td>286</td>
<td>37</td>
<td>1398</td>
<td>988</td>
<td>329</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>112</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Isolation Percentage</td>
<td>0</td>
<td>0</td>
<td>2.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.33</td>
<td>0</td>
<td>0</td>
<td>3.96</td>
</tr>
</tbody>
</table>

### Table 4.52  Isolation of Gram positive organisms from hospital staff samples

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>Throat / nasal swabs</th>
<th>Hand swabs</th>
<th>Gloves and aprons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>127</td>
<td>254</td>
<td>254</td>
<td>635</td>
</tr>
<tr>
<td>Isolates</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>6.29</td>
<td>0.78</td>
<td>0.39</td>
<td>1.73</td>
</tr>
</tbody>
</table>

232
### Table 4.53  Isolation of Gram positive organisms from hospital environment

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>OT air</th>
<th>Ward environment</th>
<th>Sterilized water</th>
<th>Surgical instruments</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected</td>
<td>126</td>
<td>65</td>
<td>610</td>
<td>1876</td>
<td>2677</td>
</tr>
<tr>
<td>Isolates</td>
<td>2</td>
<td>33</td>
<td>5</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>1.58</td>
<td>50.76</td>
<td>0.81</td>
<td>0.05</td>
<td>1.53</td>
</tr>
</tbody>
</table>

### Table 4.54  Isolation of Gram positive organisms from non-human inmates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cats</th>
<th>Cockroaches</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Fecal</td>
<td>Legs</td>
</tr>
<tr>
<td>Samples Collected</td>
<td>25</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>Isolates</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>0</td>
<td>0</td>
<td>14.77</td>
</tr>
</tbody>
</table>

### Table 4.55  Isolation of Gram positive organisms from various sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient samples</th>
<th>Hospital staff</th>
<th>Hospital environment</th>
<th>Non-human inmates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>4502</td>
<td>635</td>
<td>2677</td>
<td>226</td>
<td>8040</td>
</tr>
<tr>
<td>Isolates</td>
<td>119</td>
<td>11</td>
<td>41</td>
<td>29</td>
<td>200</td>
</tr>
<tr>
<td>Isolation percentage</td>
<td>2.64</td>
<td>1.73</td>
<td>1.53</td>
<td>12.83</td>
<td>2.48</td>
</tr>
</tbody>
</table>
Fig. 12
(xiii) Uncommon Gram Negative organisms (Citrobacter and Provedentia spp.)

In the present study 13 (0.29%) isolates of citrobacter and Provedentia spp. Which occurred as Gram negative bacteria were also isolated from a total of 4502 samples. The isolation were possible from 3 (0.28%) samples of pus and wound swabs, 4 (1.39%) of ascitic fluid and 6 (0.42%) samples of urine samples (Table-4.56).

Antibiogram: Of the 7 isolates of Citrobacter 6 (46%) were resistant to Cefparzone, 3 (23%) to Cefrome, 5 (38%) to Augmentin, 3 (23%) to Unasyn, 4 (31%) to Gentamicin, 4 (31%) to Ciprofloxacin, 4 (31%) to Sparfloxacin. However, all the isolates were sensitive to Tazocin, Amikacin and Tienam. (Appendix 2-3). Similarly, of the 6 Provedentia isolates, 5 were resistant to the action of Cefparzone, 3 to cefrome, 6 to augumentin, 5 to unasyn, 6 to gentamycin, 4 to ciprofloxacin and 5 to sparfloxacin. All the isolates were sensitive to amikacin and tienam.
Table 4.56  Isolation of Gram negative organisms from the patient samples

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Pus and wound swabs</th>
<th>Blood</th>
<th>Plural fluids</th>
<th>Ascitic fluids</th>
<th>Cerebrospinal fluids</th>
<th>Urine</th>
<th>Sputum</th>
<th>Burn</th>
<th>Patient Body devices</th>
<th>Fecal and drainage</th>
<th>Total</th>
</tr>
</thead>
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<td>286</td>
<td>37</td>
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<td>0</td>
<td>4</td>
<td>0</td>
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<td>0</td>
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<td>13</td>
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<tr>
<td>Isolation percentage</td>
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<td>0</td>
<td>0</td>
<td>1.39</td>
<td>0</td>
<td>0.42</td>
<td>0</td>
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Yeast & Fungi

In the present work 189 isolates of yeast and fungi were isolated from 89 (47.08%) patients under treatment in medical unit, 33 (17.46%) in surgical unit, 25 (13.22%) in ICU, 2 (1.05%) in cardiac surgery ward, 15 (7.93%) in chest surgery ward, 3 (1.58%) in orthopedic ward, 8 (4.23%) in burn unit, and 14 (7.40%) in urology ward (Fig.13). However, those fungus isolates were not further characterized as work on those isolates did not fall within the ambit of objectives of this study.
Fig. 13
Nosocomial infections have been recognized for over a century as a critical problem affecting the quality of health and a principal source of adverse healthcare outcomes (Nightingale, 1863). These type of infections are usually acquired from the hospital environments. If an individual who is admitted in a hospital, develops an infection, other than the one for which the patient was admitted 48-72 hours following his / her admission in hospital, then the patient is supposed to be in incubation stage at the time of admission, and in such situations their infection is not regarded as nosocomial in origin but is considered as community acquired infections (non-nosocomial). Generally the infections that are manifested within an average of 7 days post hospitalization are considered to be nosocomial in origin. A patient may even develop a nosocomial infection after having been discharged from the hospital provided the organisms causing disease were apparently acquired from the hospital. The immuno-suppressed and immuno-compromised hosts are especially more vulnerable to nosocomial infections. Under certain circumstances even the patients with normal immune system are unsafe from pathogens covering nosocomial infection. Among all major complications of hospitalization, nosocomial infections have accounted for approximately 50% of such cases, and the remaining difficulties are attributed to medication errors, patient falls, and other non-infectious adverse events (Morse, 1993). The potential impact of nosocomial infections is, therefore, considerable when assessed in terms of incidence, morbidity, mortality, and financial burden.

Nosocomial infections have been regarded as a serious problem ever since sick patients were first congregated in the hospitals (Semmelweiss, 1861; Nightingale, 1863; Simpson, 1869). Interest in this kind of infections grew at a very rapid rate from earlier twentieth century, when new bases of hospital infections were found, and there was an alarming increase in the number of serious Streptococcus pyogenes infections in hospitals (Cruickshank, 1935). Introduction of penicillin in the later years of World War II provided considerable benefit to patients (Fraser, 1984) and banished chronic cases of sepsis from hospitals (Fletcher, 1984). The antibiotics replaced the penicillin sensitive
Discussion

Streptococcus species with penicillin resistant Staphylococcus strains, complicating the problem many folds (Williams, 1956). In the mid twentieth century a large number of wide-spectrum antibiotics became available for treatment and prescription of combination-antibiotics therapy was initiated to combat more stubborn infections and to save life. New treatment strategies provided new selection pressures for the pathogen. The bacteria and viruses are simple, primal organisms that can change themselves very fast in order to survive. Under the changing circumstances, the bacteria became super-bugs by “learning" to secrete enzymes that could inactivate the antibiotics that once killed them and in the process multidrugresistant strains of bacteria emerged. Many species of Staphylococcus, Streptococcus, Enterococcus, Enterobacter, Pseudomonas, Acinetobacter, Klebsiella, Clostridium and Mycobacterium evolved strains that were resistant to widely used antibiotics (Clarke et al., 1952) and such resistant strains changed the pattern of nosocomial infections (Ayliffe et al., 1979).

The environment of hospitals in Pakistan continues to be threatening for the visitors as a large and variable number of virulent pathogens are being continuously introduced into it through newly admitted patients and their attendants. The precipitation of these pathogens in hospital environment and personnels working in various wards endanger the health of visiting patients. Increased and indiscriminate use of various broad spectrum antibiotics have provided chances to the bacteria for the emergence of drug resistant strains. The admitted patients are not only exposed to their own resident flora, which may become invasive due to weakened host defense mechanism, but also to the indigenous hospital flora (Wenzel, 1987; McGowan, 1988). Human and non-human animates, living in the hospital environment, and failure of appropriate hygienic measures, help the drug resistant strains of organisms to colonize and thrive in such situations. In large hospitals, therefore, outbreaks of diseases caused by multi drug resistant organisms are quite commonly encountered, placing the visiting patients at greater risk. The intensive care units of hospitals, where more serious patients are received and treated are seats of most severe and difficult nosocomial infections, and the use of parenteral feeding tubes, intravenous infusion devices, various types of catheters and drainage tubes increase the chances of hospital-acquired infectious agents. Patients
in various hospital units especially those in surgical intensive care and pediatric wards are always at a much higher risk. The stubborn infections make the choice of drug difficult, slow down process of recovery, prolong the convalescence and increase the difficulty of the patient, both in terms of health and economics (Hart, 1982; Holmberg et al., 1987). In USA alone, the statistics of nosocomial infections was ascertained by the National Disease Control Center and it was observed that over 2 million patients were annually affected by nosocomial infections, and the cost of treatment of such infections was in excess of $4.5 billion per year (Morse, 1991).

Nosocomial infections are not an uncommon feature in the hospitals throughout the world. Bannett and Brachman (1979) estimated that 5% of all the hospitalized patients developed nosocomial infections and the problem varied in severity from hospital to hospital. Haley and Schaberg (1981) reported that in randomly selected patients of general medical and surgical services during 1975-1976, the nosocomial infection rate was 5.2%. They identified age, sex, underlying disease and immunosuppressive therapy as intrinsic factors increasing the risk of infections while duration of hospitalization, indwelling catheters, duration of catherization and instrumentation were the extrinsic factors that increased the risk of getting infected from the such factors. The nosocomial infections not only contribute, significantly, to morbidity and mortality rates of indoor patients but also cost for hospitalization and treatments. In USA alone nearly 5% of the patients admitted to acute care centers acquired nosocomial infections; their treatment was costing the Federal Government an additional $ 2 billion each year (Dixon, 1978; Holmberg et al., 1987).

During the present investigation an attempt was made to study the prevalence of nosocomial infections, identify and characterize the species of different pathogens which were isolated from the patients, hospital staff, hospital environment and non-human inmates of the Mayo hospital Lahore-Pakistan. In addition, resistant strains isolated from nosocomial origin were also characterized for the attention of research workers and medical practitioners.
SOURCES OF MATERIALS

(i) Patients

Of the 32,620 patients hospitalized in various wards of Mayo Hospital Lahore-Pakistan during the years 1997-2001, a total of 4502 (13.8%) patients were considered to have acquired nosocomial infections from the hospital environment. Samples of the infected patients were examined using routine laboratory techniques and findings were recorded. Samples from 4502 patients consisted of 1040 pus and wound swabs, 109 blood samples, 115 pleural fluid samples, 286 peritoneal fluid samples, 37 cerebrospinal fluid samples, 1398 urine samples, 988 sputum and endotracheal secretion samples, 329 burn swabs, 99 patient body devices (medical devices), and 101 fecal and drainage tube material. All those morbid material samples were processed for detailed bacteriological examinations (Table 3:1). In developed countries 5-10% of admitted patients acquire nosocomial infections from the health care centers. The attack rate for developing countries may, however exceed to 25% (Brewer et al., 2002).

On bacteriological examination of above mentioned 4502 samples, 1287 (28.58%) isolates of Staphylococcus (1004 Staph. aureus; 283 Staph. epidermidis), 429 (9.52%) of Streptococcus (265 Strep. pyogenes; 128 Strep. pneumoniae and 36 Strep. viridians), 328 (7.29%) of Enterococcus (272 Enter. faecalis; 56 Enter. faecium), 781 (17.35%) of Pseudomonas (1693 Pseudo. aeroginosa; 88 Pseudo. cepacia), 349 (7.75%) of Enterobacter (212 Enterobact. cloacae; 137 Enterobact aerogenes), 41 (0.91%) of Acinetobacter baumannii, 266 (5.91%) of Klebsiella (Kleb. pneumoniae; 131 Kleb. aerogenes and 39 Kleb oxytoca), 140 (3.11%) of Proteus (103 Prot. mirabilis; 37 Prot. vulgaris), 1031 (22.9%) of Escherichia coli, 67 (1.49%) of Serratia marcescens, 93 (2.06%) of Haemophilus influenza, 119 (2.64%) of uncommon Gram positive bacteria, 13 (0.29%) of uncommon Gram negative bacteria, and 189 (4.19%) isolates of yeasts and fungi were obtained and characterized (Table-4.1). All these findings are supported by the investigations of Deitch (1988), Janoff and Smith (1988), Klastersky (1989), Brett et al. (1991), Gilligan (1991), Orenstein (1991), Barbut and Petit (2001), Johnson et al. (2002), Yassin et al. (2001), Lodise et al. (2003) and Alan and Sriram (2005), who also reported the isolation of nosocomial pathogens like Staphylococcus, Streptococcus,
Enterococcus, Enterobacter, Pseudomonas, Acinetobacter and Klebsiella from the patients visiting hospitals, applying the techniques and materials used in our investigation.

(ii) Hospital staff:

A total of 635 samples consisting of 127 throat and nasal swabs, 254 hand swabs, and 254 gloves and apron swabs were collected from the hospital staff and tested using the routine laboratory techniques such as cultural, staining, purification and characterization, and biochemical using properties of the isolates. Each sample was subjected to bacteriological analysis (Table 4:2). From the total samples collected from the hospital staff a total of 119 (18.74%) isolates of Staphylococcus (79 Staph. aureus; 40 Staph. epidermidis), 60 (9.45%) of Streptococcus (41 Strep. pyogenes; 15 Strep. pneumoniae), 73 (11.5%) of Enterococcus (51 Enterococc. faecalis; 22 Enterococc. faecum), 33 (5.19%) of Pseudomonas (22 Pseudo. aeruginosa; 11 Pseudo. cepacia), 15 (2.36%) of Enterobacter (13 Enterobact. cloacae; 2 Enterobact aerogenes), 24 (3.77%) of Klebsiella (17 Kleb. pneumoniae; 3 Kleb. aerogenese), 25 (3.93%) of Escherichia coli, 13 (2.04%) of Haemophilus, 11 (1.73%) isolates of other uncommon Gram positive bacteria, and one (0.16%) isolate of uncommon Gram negative bacteria were obtained (Table 4:2). Schaberg et al. (1991), Ben-Hassen et al. (1992), Tanaka et al. (1992) and Coello et al. (1994), and Valero and Saenz (1998) have also reported isolation of Staphylococci, Streptococci, Pseudomonas, Enterococci, Enterobacter, Klebsiella, Escherichia and Haemophilus organisms from the samples of throat and nasal swabs, hand swabs, gloves and aprons of hospital staff. This finding indicates poor hygienic and sanitation practices by the hospital staff. Infact hospital staff must make sure that they do properly wash and disinfect themselves after coming across any infected patient.

(iii) Isolation of bacteria from hospital’s environment:

A total of 2677 samples from various locations of hospital such as 126 operation-theatre air samples, 65 ward environment samples, 610 sterilized water samples and 1876 swabs from surgical instruments were also examined (Table 3:4). Of the 2677 samples collected from hospital environment 110 (4.11%) isolates of Staphylococcus (91 Staph. aureus; 19 Staph. epidermidis), 34 (1.27%) of Streptococcus (14 Strep. pyogenes;
9 Strep. pneumoniae and 11 Strep. viridans), 59 (2.2%) of Enterococcus (37 Enterococ. faecalis; 22 Enterococ. faecium), 53 (1.98%) of Pseudomonas (42 Pseudo. aeruginosa; 11 Pseudo. cepacia), 25 (0.93%) of Enterobacter (16 Enterobact. cloacae; 9 Enterobact aerogenes), 35 (1.31%) of Klebsiella (11 Kleb. pneumoniae; 14 Kleb. aerogenese and 10 Kleb oxytoca), 22 (0.82%) of Proteus (9 Prot. mirabilis; 13 Prot. vulgaris), 55 (2.05%) of Escherichia coli, 41 (1.53%) of uncommon Gram positive bacteria, 19 (0.7%) of uncommon Gram negative bacteria, and 18 (0.67%) isolates of yeast and fungi were recovered (Table 4:3). Takesue et al. (1989), Khalifa et al (1989), Nakagomi et al. (1989), Begue (1991), and Chou et al. (1991), Tanaka et al. (1992), Uehara (1994) and Richards et al. (1998) have also reported the isolation of organisms like Staphylococcus, Streptococcus, Enterococcus, Enterobacter, Pseudomonas, Acinetobacter, Klebsiella and Clostridium from the hospital environment.

(iv) Non-Human Inmates:

A total of 226 samples of cats and cockroaches present in various premises of the hospital were collected. A total of 25 nasal and 25 faecal material samples from the cats, 88 samples from the external body parts of cockroaches and 88 samples obtained from the intestines of cockroaches were collected for the bacteriological analysis (Table 3:5). From the non-human inmate samples a total of 101 (44.7%) isolates of Staphylococcus (25 Staph. aureus; 16 Staph. epidermidis), 146 (64.6%) of Streptococcus (61 Strep. pyogenes; 37 Strep. pneumoniae, 48 Strep. viridons), 166 (73.45%) of Enterococcus (101 Enterococ. faecalis; 65 Enterococ. faecium), 74 (32.74%) of Pseudo. aeruginosa, 124 (54.86%) of Enterobacter (96 Enterobact. cloacae; 28 Enterobact aerogenes), 47 (20.79%) of Proteus (22 Prot. mirabilis; 25 Prot. vulgaris), 163 (72.12%) of Escherichia coli, 29 (12.83%) of uncommon Gram positive bacteria, 86 (38.05%) of uncommon Gram negative bacteria, and 30 (13.27%) isolates of Yeast and Fungi were obtained (Table 4:4). Scott et al. (1988) attributed outbreak of nosocomial infection to a cat which was heavily colonized with various types of infectious agents from the environment and removal of the animal from the hospital premises led to rapid resolution of the outbreak. Cockroach apparently a harmless insect is capable of acting as a biological carrier and mechanical transmitter of a number of bacterial, viral, fungal and parasitic pathogens.
Discussion

(Steinhau, 1941; Okafor, 1981; Steinuous, 1994). The germs present in the environment may stick to the bristles present on its legs and be deposited at the places it visits in search of food (Okafor, 1981).

Bacteriology:

Various kinds of bacterial species are present on the human body; on the normal looking skin, exposed mucous membranes, inside the digestive tract, on the mucus membrane of urogenital tract and respiratory system, as commensals and normal flora (Aoun and Klastersky, 1991; Begue, 1991; Kddoya et al., 1991; Schaberg et al., 1991). Occasionally under certain conditions body may be accidentally exposed to bacterial flora derived from other living beings and its immediate environment (Berk and Verghese, 1989; Meyers et al., 1989; McGowan et al., 1989; Doyden and Munro, 1989). Some of the resident and accidental bacterial species are capable of causing disease and wait for the opportunity of invading the body, although under normal conditions these bacteria do not invade the body and the body also does not mount any resistance against these bacteria thus maintaining an equilibrium (Zhang, 1991). However, under the conditions of stress this equilibrium is disturbed and some of the bacteria find ways to enter the body setting up focal infection. Depending upon the severity of physiological disturbance, and the invasive power and virulence of infecting bacteria, the condition may remain localized or may spread to other parts of the body through blood stream or lymph channels initiating a generalized infection and bacteremia (Richard et al., 1998; Valero and Saenz, 1998). The local reaction ensues as focal infection and is usually characterized by inflammation followed in some cases by pus formation. Invariably all Gram positive and Gram negative types of organism are capable of producing inflammatory changes in the living tissue. Most of the Gram positive organisms like Staphylococcus, Streptococcus and Corynebacterium and Gram negative organisms like Escherichia and Pseudomonas are well known for their pus producing characteristics. These are ubiquitous organisms present on the human body and its immediate surroundings and enter the body through abraded skin, cuts, surgical procedures, piercing wounds, burns, and food and water setting up inflammatory foci, abscess formation, suppuration, purulation and septicemia (Murtaugh and Mason, 1989; Wise et al., 1989;
Discussion

The analysis of various clinical samples from the patients suffering from various lesions, revealed the presence of various types of bacteria (Table: 4:1).

(i) Staphylococcus

Patients: In the present work various species of staphylococci were isolated from 1287 samples of patients, including 606 (58.26%) samples of pus and wound swabs, 32 (29.35%) samples of blood, 24 (20.86%) samples of pleural fluids, 85 (29.72%) samples of ascetic fluids, 11 (29.72%) samples of cerebrospinal fluids, 67 (4.79%) samples of urine, 237 (23.98%) samples of sputum, 135 (41.03%) burn swabs samples, 63 (63.63%) patient body devices and 27 (26.73%) fecal and drainage material samples (Table 4:5). Crossley et al. (1979), Zai Salar et al. (1980), Weinstein et al. (1982), frequently isolated Staphylococcus aureus from various human lesions. Lyon et al. (1984), Cross et al. (1983), Khan et al. (1983), Etienne et al. (1989), Alpuche et al. (1989), Berk and Verghese (1989), Karchmer (1991), Archer (1991), Karim et al. (1991), Kadoya et al. (1991), Chou et al. (1991), Nishi et al. (1991), and Begue (1991), Parras et al. (1991), Nonoyama et al. (1994), Pujol et al. (1994), Huebner et al. (1994), Coello et al. (1994) Witte et al. (1994), Sloos et al. (1998), Richards et al. (1998), and Tabi et al. (1998) also isolated strains of Staphylococci from blood stream of patients with septicemia, and from various types of surgical and burn wounds, urinary tract infections, indwelling catheters, immunocompromized patients, coronary care unit patients, and neonatal patients. The findings of present study are congruent with those reported by above mentioned workers.

Hospital Staff: In case of samples collected from hospital staff, various Staphylococcus species were isolated from 119 (18.74%) of 635 samples of morbid material including 39 (30.7%) of 127 samples of throat swabs, 55 (21.65%) of 254 samples of hand swabs, and 25 (9.84%) of 254 samples of gloves and aprons (Table 4:6). Staphylococci usually spread by contact (Etienne et al., 1989; Meyer et al., 1989). Wise et al. (1989) observed that 33% of the hospital personnels were carriers of Staph. aureus. Nosocomial infections of exogenous origin spread from person to person by direct physical contact,
from hands of nurses, physicians’ aides, and other personnels of hospitals (Crossley et al., 1979; Cross et al., 1982; Khan et al., 1983; Larson, 1988). Etienne et al. (1989) traced back 22% of nosocomial Staph. aureus infections to infected fingers of staff members and 2% infections to the environment. Patterson and Zervos (1990) observed that the most probable way these resistant bacteria are spread among hospital patients is via transient carriage on the hands of personnel, patient-to-patient and inter-hospital transmission. Tanaka et al. (1992) while studying nosocomial transmission of infections in a University Hospital isolated drug-resistant strains of Staph. epidermidis, Staph. aureus and Staph. haemolyticus from the medical staff. Ben-Hassen et al. (1992) isolated Staph. aureus from intensive care unit patients developing nosocomial infections and organisms exhibiting similar bio-characteristics were also isolated from the medical staff of same ward. Coello et al. (1994) reported that nose, throat and perineum of majority of the patients and staff were colonized with various species of Staphylococcus. The findings of the present study are supported by the results reported by Tanaka et al. (1992), Ben-Hassen et al. (1992) and Coello et al. (1994).

**Hospital Environment:** Of the samples collected from hospital environment various species of Staphylococcus (Staph. aureus; Staph. epidermidis) were isolated from 110 (4.11%) of total 2677 samples including 25 (19.84%) of 126 operation theatre air samples, 48 (73.84%) of 65 ward environment samples, 7 (1.14%) of 610 instrument sterilizer water samples, and 30 (1.59%) of 1876 surgical instrument samples (Table 4:7). Menzieas et al. (1991) also isolated strains of Staph. epidermidis from the environment of the operation theaters. Tanaka et al. (1992) isolated drug-resistant strains of Staph. aureus from the ward environment of University hospital. Ben-Hassen et al. (1992) isolated Staphylococcus aureus from intensive care unit patients developing nosocomial infections and organisms exhibiting similar bio-characteristics were also isolated from the hospital environment. The findings of this work were in agreement with those reported by Zai Salar et al. (1980), Khan et al. (1983), Takesue et al. (1989), Khalifa et al (1989), Nakagomi et al. (1989), Begue (1991), and Chou et al. (1991), Tanaka et al., (1992 ), Uehara (1994) and Richards et al. (1998). Who also isolated Staphylococcus epidermidis from the operation theatres which had developed drug
resistance to commonly used antibiotics.

**Non-human Inmates:** Of the 226 non-human inmates samples species of Staphylococcus (*Staph. aureus; Staph. epidermidis*) were isolated from 101 (44.7%) samples including 10 (40%) from 25 nasal swabs and 5 (20%) from 25 fecal swabs taken from cats, and 32 (36.36%) from 88 body and 54 (65.9%) from 88 visceral material of cockroaches (Table 4:8). The findings of this work are in agreement with the reports of Scott *et al.* (1988) who also isolated Staphylococci from cats; Roth and Willis (1957), Hiro and Fuji (1966), Zuberi *et al.* (1972), Bhaumik and Raychandhri (1975), Svec *et al.* (1975), Fisherman and Alcamo (1977), Okafor (1981), Razia and Jafri (1986), and Sarmova *et al.* (1992) who reported isolation of various species of Staphylococci from cockroaches. From these findings it can be concluded that Staphylococci are the major infectious agents colonizing non human inmates such as cats and cockroaches.

**Overall Prevalence:** Of the total 8040 samples, 1617 (20.11%) were positive for *Staph. aureus; Staph. epidermidis*. Of 1617 Staphylococcus isolations, 1287 (28.58%) were from various samples of patients, 119 (18.74%) were from hands, gloves and aprons of hospital staff, 110 (4.1%) from the ward environment, and 101 (44.69%) isolations were possible from non human inmates (Table 4:9). These results indicate a strong relationship amongst various sources of nosocomial infections.

The staphylococci present on or within the body of inanimates are continuously transferred to its immediate environment, thus every living object that happens to pass through the hospital may harbour and become source of pathogens (Crossley *et al.*, 1979; Weinstein *et al.*, 1982). Irrational and inadequate antibiotic therapy provides selection pressure for the evolution and survival of drug resistant staphylococci that thrive and perpetuate in the hospital environment (Cafferky *et al.*, 1983), and through human and non-human agencies may spread to the adjoining areas (Lyon *et al.*, 1984). Staphylococcus infections are usually in hospital acquired (Brucker 1996) or community acquired (Brucker 1996 and long term hospitalization has been considered as one of the major predisposing causes (Klastersky 1989), Alpuche *et al.* (1989) reported that nearly 25% of the nosocomial Staphylococcus infections were hospital acquired and about 5% were community acquired. Murtaugh and Mason (1989)
and Wise et al. (1989) reported that the penicillin resistant strains of Staphylococcus emerged in mid twentieth century and that the methicillin resistant *Staphylococcus aureus* strains appeared on later and caused serious nosocomial infections with significant mortality (Crossley et al., 1979). Cafferky et al. (1983) isolated antibiotic resistant strains of *Staphylococcus aureus* from cases of burns, surgical wounds and traumatic skin lesions. *Staphylococcus aureus* has been reported as the major cause of nosocomial bacteremia in aged patients (Meyers et al., 1989). Cotton et al. (1989) observed that many patients developed nosocomial infections and that the major risk factors for such infections are malnutrition, younger age and prolonged hospitalization and the most common sites of infection were lower respiratory and gastrointestinal tracts. Wu et al. (2000) observed a case of very high mortality due to nosocomial pneumonia caused by MRSA in 3 Chinese hospitals and reported that the underlying diseases, leukocytopenia, malnutrition and secondary infections like fungi, *Klebsiella pneumoniae*, *Pseudomonas maltophilia* and Enterococcus worsened the condition and eventually resulted in high mortality. The results of present study are in concurrence with those reported by Crossley et al. (1979), Weinstein et al. (1982), Cafferky et al. (1983), Murtaugh and Mason (1989), Wise et al. (1989) and Wu et al. (2000), who reported similar observations.

**Identification:** The isolated strains of Staphylococcus were identified and characterized on the bases of their biochemical, hemolytic, and biological characteristics. Of the total 1287 isolates, 1004 (78%) were characterized as *Staph. aureus* and 283 (22%) as *Staph. epidermidis*. Since the methicillin sensitivity has been adopted as one of the criteria for typing Staphylococcus species therefore all the isolates were subjected to Methicillin sensitivity test using standard methods referred to earlier. Of the total 1287 isolates 555 (43.1%) were Methicillin resistant *Staphylococcus aureus* (MRSA), and the remaining 449 (34.9%) strains were Methicillin sensitive *Staphylococcus aureus* (MSSA). In case of *St. epidermidis* 41 (3.18%) strains were Methicillin resistant (MRSE), and 242 (18.8%) strains were Methicillin sensitive (MSSE) (Fig.1).

**MRSA:** Parras et al. (1991) and Vindel et al. (1994) observed a serious nosocomial outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in surgical
units of Spanish hospitals. Savitz et al. (1994) reported that in many surgical cases the skin-flora of patients became source of wound infection. Nishi et al. (1991) and Venditti (1994) reported that the most common microorganism isolated from surgical wounds, bloodstream and arterial-venous infections was methicillin-resistant *Staphylococcus aureus*. Parras et al. (1991), Pujol et al. (1994), Nishi et al. (1991) reported that majority of cases of nosocomial bacteremia patients were colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Khan et al. (1983), Nonoyama et al. (1994) and Uehara (1994) isolated methicillin-resistant *Staphylococcus aureus* strains from nosocomial patients, fingertips of medical staff and health care workers, and ward environment of hospitals. Ben-Hassen et al. (1992) reported that the methicillin-resistant *Staphylococcus aureus* strains were a serious problem in hospitals and caused severe nosocomial infection in ICU patients. The results of present study are in agreement with the findings reported by Khan et al. (1983), Nonoyama et al. (1994) and Ben-Hassen et al. (1992) as MRSA strains were isolated from cases of nosocomial infection recorded at the Mayo Hospital, Lahore.

In the present work 555 (43.1%) MRSA were isolated from 19 (3.42%) patients under treatment in medical unit, 295 (53.15%) patients in surgical unit, 36 (6.49%) patients in ICU, 3 (0.34%) of Cardiology patients, 34 (6.13%) patients in Cardiac Surgery, 36 (6.49%) patients in Chest Surgery, 29 (5.22%) patients in Orthopedic Surgery, 9 (1.62%) patients in Pediatric Ward, 90 (16.2%) patients in Burn Unit, and 4 (0.72%) patients in Neurosurgery (Table 4:10). The results of the present study are in agreement with the findings reported by Cafferky et al. (1983), Takesue et al. (1989), Nishi et al. (1991), Parras et al. (1991), Vindel et al. (1994), Emmerson (1994), Venditti (1994), and Valero and Saenz (1998) who also isolated *Staphylococcus aureus* strains from the patients of general surgery; Lee et al. (199_), Archer (1991), Karchmer (1991), Menzis et al. (1991), Cheong et al. (1991) from cases of cardiac surgery; Witte et al. (1994), and Sakumoto et al. (1996) from the cases of orthopedic surgery, Crossley et al. (1979) and Cafferky et al. (1983), Cheong et al. (1994) from the cases of burn cases; Coello et al. (1994) and Sakumoto et al. (1996) from urinary tract infections and urinary tract surgery; Nishi et al. (1991), Ben-Hassen et al. (1992), Coello et al. (1994), and
Harbarth et al. (1999) from the cases of intensive care unit; Kadoya et al. (1991), Karim et al. (1991), Parras et al. (1991), Nishi et al. (1991), Pujol et al. (1994), Cheong et al. (1994) and Richards et al. (1998), Raad et al. (1998), Sloos et al. (1998), and Huebner and Goldmann (1999) from the cases of primary bacteremia, Cross et al. (1983), Nishi et al. (1991) and Venditti (1994) from cases of septicemia; Berk and Verghese, (1989), Nishi et al. (1991), Witte et al. (1994), Sakumoto et al. (1996) and Krediet et al. (1999) from the respiratory tract infection cases; Cotton et al. (1989), Chou et al. (1991), Nishi et al. (1991) and Huebner et al. (1994) from pediatric and neonates ICU; Cheong et al. (1994) from patients of oncology ward; and Karim et al. (1991) from cases in cardiology patients, sclera buckling infections (Oshima et al., 1999), and cases of atopic dermatitis (Oshima et al., 1999). Austin and Anderson (1999) reported that strains of methicillin-resistant Staphylococcus aureus (EMRSA) were becoming endemic in hospitals in England and Wales in the United Kingdom.

MSSA: In the present work a total of 449 (34.9%) strains of methicillin-sensitive Staphylococcus aureus (MSSA) were isolated from 87 (19.37%) patients under treatment in medical unit, 214 (47.66%) patients in surgical unit, 31 (6.9%) patients in ICU, 7 (1.55%) in cardiology ward, 20 (4.45%) patients in cardiac surgery ward, 25 (5.57%) patients in chest surgery ward, 21 (4.68%) patients in orthopedic surgery ward, 10 (2.22%) patients in pediatric ward, 11 (2.44%) patients in burn unit, 2 (0.44%) patients in ophthalmology ward, 8 (1.78%) patients in neurosurgery ward, 7 (1.55%) patients in urology ward, 1 (0.22%) patient in dermatology ward, and 5 (1.11%) patients in oncology ward of Mayo Hospital, Lahore (Table 4:10). MSSA infections are usually community acquired in origin (McGowan et al 1989; Nakagomi et al., 1989). Alpuche et al. (1989), Nishi et al. (1991), Cheong et al. (1994), Sakumoto et al. (1996) Sakumoto et al. (1996) and Brucker (1996) have also reported the isolation of MSSA from patients suffering from various ailments.

Staph. epidermidis: Staph. epidermidis and other coagulase negative Staphylococci colonize skin, nose, ear, axila, umbilicus, and groin of healthy human beings and patients (Sloos et al., 1998) and are the leading cause of nosocomial blood stream infections.
Discussion

(Sloos et al., 1998; Raad et al., 1998; Richards et al., 1998; Huebner and Goldmann (1999). In present work many of the isolated strains indicated resistance to many of the drugs/antibiotics (Archer 1991; Sloos et al., 1998; Raad et al., 1998; Huebner and Goldmann 1999). Menses et al. (1991) and Karchmer, (1991) while studying bacteriology of prosthetic valve endocarditis were able to isolate strains possessing similar bio-characteristic from the skin of patients and the environment of the operation theaters.

**MRSE:** In the present work 41 (3.18%) strains of coagulase negative methicillin resistant *Staph. epidermidis* (MRSE) were also isolated from 20 (48.8%) patients under treatment in medical ward, 6 (14.6%) patients in surgical ward, 3 (7.3%) patients in ICU, and 12 (29.3%) patients in the urology ward of Mayo Hospital, Lahore (Table 4:10). Huebner et al. (1994) reported that *Staph. epidermidis* colonized over many years and played an important role in nosocomial neonatal bacteremia. Huebner and Goldmann (1999) described an increasing pathogenic role of multi-antibiotic-resistant coagulase-negative Staphylococci related to indwelling devices.

**MSSE:** In the present work a total of 242 (18.8%) strains of Methicillin Sensitive *Staphylococcus epidermidis* (MSSE) were isolated from 101 (41.73%) patients under treatment in medical ward, 24 (9.91%) patients in surgical ward, 19 (7.85%) patients in ICU, 33 (13.6%) in cardiology patients, 11 (4.54%) in patients cardiac surgery, 18 (7.43) patients in the chest surgery, 7 (2.9%) patients in orthopedic, 6 (2.5%) patients in pediatric ward, 10 (4.13%) patients in burn unit, 1 (0.41%) patients in ophthalmology, 2 (0.83%) patients in neurology, 2 (0.83%) patients in urology, and 8 (3.3%) patient in the oncology ward of Mayo Hospital, Lahore (Table 4:10).

The observations of the present investigation are supported by those reported by Raad et al. (1998) who also isolated Methicillin Sensitive *Staphylococcus epidermidis* (MSSE) from patients of cardiac and vascular surgery. Archer (1991), Richards et al. (1998), Huebner and Goldmann (1999) and Harbarth et al. (1999) who isolated MSSE from cases of bacteremia; Menzieas et al. (1991), Karchmer, (1991), and Huebner and Goldmann (1999) who also isolated MSSE from cases of prosthetic valve endocarditis,
Richards et al. (1998), Huebner and Goldmann (1999), and Valero and Saenz (1998) isolated MSSE from cases in general surgery, Richards et al. (1998), and Harbarth et al. (1999) isolated MSSE from cases of nosocomial pneumonia, Richards et al. (1998), and Huebner and Goldmann (1999) isolated MSSE from the cases of urologic surgery, Huebner and Goldmann (1999) from cases of central nervous system surgery; Huebner and Goldmann (1999) isolated MSSE from cases of endophthalmitis; Huebner et al. (1994) isolated MSSE from cases in neonatal intensive care units (NICU); Huebner and Goldmann (1999) isolated MSSE from indwelling devices and Sakumoto et al. (1996) and Harbarth et al. (1999) isolated MSSE from the cases of urinary tract infections.

**Antibiogram**

The nosocomial isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were resistant to Penicillins and Cephalosporins. These strains showed resistance to other drugs such as 464 (83.6%) to Tetracycline, 382 (68.8%) to Doxycycline, 394 (70.9%) to Erythromycin, 187 (33.7%) to Klarithromycin, 283 (50.9%) to Augmentin, 166 (30%) to Unasyn, 49 (8.8%) to Tazocin, 93 (16.8%) to Gentamicin, 49 (8.8%) to Amikacin, 127 (22.8%) to Ciprofloxacin, 40 (7.2%) to Sparfloxacin, 38 (6.8%) to Fucidin, 27 (4.9%) to Tienam; all the isolates were sensitive to the treatment with Vancocin. (Appendix 2-3). These findings are in agreement with those observed by Crrol et al. (1983), Cafferky et al. (1983); Ryan (1989), Alpuche et al. (1989), Khalifa et al. (1989), Nakagomi et al. (1989), Takesue et al. (1989), Zaidi et al. (1989), Voutsinas et al. (1989), Patterson and Zervos (1990), Lee et al. (1991), (Tenover 1991), (Zhang (1991), Aoun and Klasterky (1991), and Chou et al. (1991). The MRSA strains were found susceptible to the action of cefotaxime, clavulanic acid, fluoroquinolones, imipenem, aztreonam, ticarcillin, vancomycin, ciprofloxacin, rifampicin, fusidic acid, and linezolid. These observations are in agreement with those reported by Aoun and Klasterky (1991), Hammami et al. (1991), Witte et al. (1994), Swartz (1994), Cheong et al. (1994), Parr et al. (1999) and Patel et al. (1999).

Of the total 449 Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates 319 (71%) were found resistant to Tetracycline, 227 (50.6%) to Doxycycline, 129 (29%)
Discussion

to Cephradine, 82 (18%) to Cefuroxime, 294 (65.5%) to Erythromycine, 83 (18.4%) to Klarithromycin, 91 (20.3%) to Augmentin, 62 (13.8%) to Unasyn, 13 (2.9%) to Tazocin, 48 (10.7%) to Gentamicin, 16 (3.6%) to Amikacin, 71 (15.8%) to Ciprofloxacin, 31 (6.9%) to Sparfloxacin, 10 (2.2%) to Fucidin. However, and none of the isolate was found resistant to Tienam or Vancocin (Appendix 2-3). These findings are in agreement with the observations of Ben-Hassen et al. (1992), Rotimi et al. (1994) and Sakumoto et al. (1996).

Of the 242 strains of Methicillin sensitive *Staph. epidermidis* (MSSE), 105 (43.3%) were resistant to Tetracycline, 83 (34.3%) to Doxycycline, 103 (43%) to Cephradine, 64 (26%) to Cefuroxime, 130 (53.7%) to Erythromycine, 56 (23%) to Klarithromycin, 62 (26%) to Augmentin, 50 (21%) to Unasyn, 9 (3.7%) to Tazocin, 57 (23.6%) to Gentamicin, 11 (4.5%) to Amikacin, 39 (16%) to Ciprofloxacin, 18 (7.4%) to Sparfloxacin, 6 (2.5%) to Fucidin, and none of the strain was resistant to Tienam and Vancocin (Appendix 2-3). The finding was in agreement with those reported by Karim et al. (1991), Karchmer (1991), Archer (1991), Ben-Hassen et al. (1992), Del-Favero and Menichette (1993), Rotimi et al. (1994) and Sakumoto et al. (1996), Raad et al. (1998).

Of the 41 strains of Methicillin resistant *Staph. epidermidis* (MRSE), 35 (85.4%) were resistant to Tetracycline, 29 (71%) to Doxycycline, 32 (78%) to Erythromycine, 13 (31.7%) to Klarithromycin, 25 (61%) to Augmentin, 19 (46%) to Unasyn, 2 (4.8%) to Tazocin, 9 (22%) to Gentamicin, 3 (7.3%) to Amikacin, 11 (27%) to Ciprofloxacin, 3 (7.3%) to Sparfloxacin, 2 (5%) to Fucidin, 1 (2.4%) to Tienam. All the isolates were sensitive to the action of Vancocin (Appendix 2-3). These findings are in agreement with the observation of Karim et al. (1991), Karchmer (1991), Archer (1991), Ben-Hassen et al. (1992), Del-Favero and Menichette (1993), Rotimi et al. (1994), Sakumoto et al. (1996) and Raad et al., (1998).

The health care workers in a hospital play an important role in the perpetuation and transmission of nosocomial infection among the hospitalized patients. The most usual mode of transmission from person to person was through contact which has also been reported by Etienne et al. (1989), Meyer et al. (1989) and Brucker (1996).
organisms isolated from the wounds of patients, their body surfaces, hairs and hands and hand gloves and aprons of healthcare workers, and the operation theatre and ward environment and inanimate objects in the hospitals in majority of the cases were found to be methicillin resistant. Crossley et al. (1979) and Khalifa et al. (1989) have also reported similar observations.

(ii) Streptococcus

**Patients:** Various strains of genus Streptococcus were isolated from a total of 429 (9.53%) samples collected from patients which consisted of 104 (10%) samples of pus and wound swabs, 8 (7.33%) samples of blood, 21 (18.26%) samples of pleural fluids, 20 (6.99%) samples of ascetic fluids, 5 (13.5%) samples of cerebrospinal fluids, 14 (1%) samples of urine, 205 (20.74%) samples of sputum; 30 (9.11%) burn swab samples and 13 (13.13%) patient body devices and 9 (8.6%) samples of the fecal and drainage materials (Table 4:11). Streptococcus was also a very common isolate associated with nosocomial infections. The isolation of species of streptococci from various sites of human beings suffering from nosocomial infection has already been reported (Berk and Verghese, 1989; Klastersky, 1991; De Galan et al., 1999; Brett et al., 1999; and Valero and Saenz, 1998).

**Hospital Staff:** In the present investigation various species of Streptococci were isolated from 60 (9.45%) samples obtained from the hospital staff which consisted of 45 (35.43%) samples of nasal and throat swabs; 10 (3.93%) samples of hand swabs, and 5 (1.96%) samples of gloves and aprons (Table 4:12). Meyers et al. (1989) and Begue (1991) also isolated streptococcus strains from the above mentioned sources.

**Hospital Environment:** Various species of Streptococcus were also isolated from 34 (1.27%) samples obtained from the hospital environment including 12 (9.52%) operation theatre air samples, 15 (23%) ward environment samples, 2 (0.32%) sterilized water samples, and 5 (0.26%) surgical instrument samples (Table 4:13).

**Non human Inmates:** Species of Streptococcus were isolated from a total of 146 (64.6%) samples obtained from non-human inmates consisting of 16 (64%) nasal and 11
(44%) faecal swab samples from cats; 43 (48.86%) samples from legs and 76 (86.36%) samples from visceral material of the cockroaches (Table 4:14). Steinhaus (1941), Roth and Willis (1957), Burgess et al. (1969), Fernandez and Zarror (1972), Zuberi et al. (1972), Svec et al. (1975), Fisherman and Alcamo (1977), Okafor (1981), Razia and Jafri (1986), and Sarmova et al. (1992) have also reported similar observations.

**Overall Prevalence:** A total of 669 (8.32%) strains of Streptococci were isolated from 8040 samples. Those strains were recovered from 429 (9.53%) patient samples, 60 (0.94%) samples from hospital staff, 34 (1.27%) hospital environment samples and 146 (64.6%) non-human inmate samples collected during present study (Table 4:15). Streptococci are considered as the major cause of nosocomial infections. With the introduction of antibiotic therapy in mid twentieth century the penicillin susceptible streptococcal species were replaced by penicillin resistant isolates changing the spectrum of nosocomial infections (Murtaugh and Mason, 1989; Wise et al., 1989). Berk and Verghese (1989) reported that the organisms responsible for nosocomial pneumonia are continuously evolving and some of the Gram positive organisms like enterococci, beta hemolytic group B Streptococci and methicillin resistant *Staph. aureus* have assumed significant role in the nosocomial respiratory infections. Aoun and Klastersky (1991) (1998) isolated *Strept. pneumoniae* from the cases of nosocomial bacteremia and nosocomial pneumonia. In tropical areas mother-linked multi-resistant hospital strains of Streptococcus group B have been reported to cause high mortality in neonates (Begue, 1991). De Galan et al. (1999) isolated multi resistant strains of *Strept. pneumoniae* from the sputum of hospitalized patients in a Dutch medical center. Brett et al. (1999) isolated 386 strains of *Strept. pneumoniae* from general practice or inpatients from 4 laboratories in New Zealand. Paradisi and Corti (1998) observed that nosocomial infections caused by various streptococcus species were community-acquired. Valero and Saenz (1998) reported that on global basis 8.5% nosocomial infections (NI) in the departments of general, vascular and urologic surgery were caused by various Streptococcus species. The results of present study support the findings of above mentioned research workers.

**Wards:** In the present work 429 strains of *Streptococci* were isolated from 136 (31.7%)
patients under treatment in medical unit, 106 (24.7%) patients in surgical unit, 44 (10.2%) patients in ICU, 8 (1.86%) in cardiology ward, 28 (6.52%) patients in cardiac surgery ward, 60 (14%) patients in chest surgery ward, 10 (2.34%) patients in orthopedic surgery ward, 3 (0.7%) patients in pediatric ward, 24 (5.6%) patients in burn unit, 1 (0.23%) patients in ophthalmology ward, 4 (0.9%) patients in neurosurgery ward, 1 (0.23%) patients in urology ward, 1 (0.23%) patient in dermatology ward, and 3 (0.9%) patients in oncology ward (Fig.2). Isolation of streptococcus species from cases of nosocomial infection from patients of general, vascular and urologic surgery has been reported by Rotimi et al. (1994), Valero and Saenz (1998), Brett et al. (1999) and De Galan et al. (1999) from cases of nosocomial pneumonia by Aoun and Klastersky (1991), Paradisi and Corti (1998), De Galan et al. (1999) from cases of nosocomial and bacteremia by Aoun and Klastersky (1991), Paradisi and Corti (1998) from cases of neonate septicemia by Begue (1991), central nervous system infections by Paradisi and Corti (1998) and invasive procedures and devices by File (1999) and Brett et al. (1999). The findings of present work are congruent with those of above named workers.

**Antibiogram:** Of the total 429 strains of Streptococcus, 137 (31.9%) were found resistant to Ampicillin, 110 (25.6%) to Ampiclox, 119 (27.7%) to Cephradine, 69 (16.1%) to Cefuroxime, 30 (7%) to Cefoparazone, 121 (28.2%) to Erythromycin, 47 (11%) to Klarithromycin, 59 (13.8%) to Augmentin, 41 (9.6%) to Unasyn, 9 (2.1%) to Tazocin, 55 (12.8%) to Gentamicin, 12 (2.8%) to Amikacin, 139 (32.4%) to Ciprofloxacin, 42 (9.8%) to Sparfloxacin and 107 (24.9%) to Fucidin. All the isolates were sensitive to Tienum and Vancocin (Appendix 2-3). Rotimi et al. (1994) and Brett et al. (1999) have also reported similar observations. Penicillin-resistant strains of Streptococci were sensitive to third generation cephalosporins and linezolid. These finding are also supported by Begue (1991) and Patel et al. (1999).

(iii) **Enterococcus**

**Patients:** Enterococci are members of the residential flora and frequently find their way into the human tissue under conditions of stress and trauma. These organisms have commonly been isolated from patients with nosocomial diseases. Various species of
**Discussion**

*Enterococcus* were isolated from a total of 328 (7.29%) samples collected from patients such as 37 (3.55%) samples of pus and wound material, 14 (12.84%) samples of blood, 5 (4.34%) samples of pleural fluid, 44 (15.38%) samples of ascitic fluid, 5 (13.51%) samples of cerebrospinal fluid, 55 (3.93%) samples of urine, 82 (8.29%) samples of sputum, 39 (11.85%) burn swab samples, 35 (35.35%) patient body devices and 12 (11.88%) faecal and drainage material samples (Table 4:16). Swartz (1994) reported increasing number of vancomycin resistant *Enterococcus* isolates from intensive care unit patients. Austin and Anderson (1999) reported that strains of vancomycin-resistant *Enterococcus* (VRE) were becoming endemic in hospitals in England and Wales in the United Kingdom. The findings of present work are supported by the observations of Berk and Verghese (1989), Bengen *et al.* (1991), Ortega *et al.* (1991), Kadoya *et al.* (1991), Zhang (1991), Begue (1991), Aoun and Klastersky (1991), Tenover (1991), Watanakunakorn and Jura (1991), Venditti *et al.* (1994), Christie *et al.* (1994), Spera and Farber (1994), Christie *et al.* (1994), Boyce *et al.* (1994), Boyce *et al.* (1994), Nicoletti and Stefani (1995), Low *et al.* (1995), Weinstein *et al.* (1996), Singer *et al.* (1997) and Valero and Saenz (1998).

**Hospital Staff:** Various species of *Enterococcus* were isolated from 73 (11.5%) samples obtained from the hospital staff consisting of 51 (40.15%) samples of throat swabs, 18 (7.08%) of hand swabs, and 4 (1.57%) samples of gloves and aprons (Table 4.17). Boyce *et al.* (1994) isolated multidrug-resistant nosocomial strains of *Enterococcus faecium* from patients suffering from diarrhea, nurse attending the patients, and hospital environment during an outbreak. The findings of the present study were supported by the observations of Uthley *et al.* (1989), Patterson and Zervos (1990), Gentry (1991), Ortega *et al.* (1991), Schaberg *et al.* (1991), and Valero and Saenz (1998).

**Hospital Environment:** In the present work species of *Enterococcus* were isolated from 59 (2.2%) samples obtained from the hospital environment which consisted of 15 (11.9%) operation theatre air samples, 33 (50.76%) ward environment samples, 6 (0.98%) sterilized water, and 5 (0.26%) surgical instrument samples (Table 4:18). Boyce *et al.* (1994) have also reported the isolation of Enterococcus species from the hospital environment.
Discussion

**Non human Inmates:** Various species of Enterococcus were isolated from 166 (73.45%) samples of material from non-human inmates consisting of 9 (36%) samples from throat and nasal swabs and 18 (72%) from the fecal swabs from cats, and 56 (63.63%) samples from legs and 83 (94.31%) from intestines of cockroaches (Table 4:19). Roth and Willis (1957), Fernandez and Zarror (1972), Zuberi *et al.* (1972) and Okafor (1981) also isolated Enterococcus species from cockroaches.

**Overall Prevalence:** Enterococci have emerged as the most common cause of nosocomial infections and on global basis as these organisms have been reported responsible for 8 to 22% cases of nosocomial bacteremia in different countries (Berk and Verghese 1989; Schaberg *et al.* (1991); Begue (1991); Aoun and Klastersky (1991) and Tenover (1991) Venditti *et al.* (1994); Low *et al.* (1995); Valero and Saenz (1998); Zhang (1991). Bengen *et al.* (1991) recorded an increasing frequency of nosocomial Vancomycin resistant strains of *Enterococcus faecium* from patients in a children hospital. Christie *et al.* (1994) concluded that Enterococci were the most important cause of high morbidity and mortality among critically ill children. Ortega *et al.* (1991), Aoun and Klastersky (1991) and Tenover (1991), Spera and Farber (1994), and Nicoletti and Stefani (1995) observed a dramatic increase in the nosocomial isolates including multidrug resistant strains of Enterococcus. They further reported that the vancomycin-resistant enterococcal bacteremia is very difficult to treat and is usually accompanied by high mortality. Christie *et al.* (1994) observed that enterococci were the most important cause of serious morbidity and mortality among critically ill children. The results of present study are congruent with the findings of aforementioned workers. In our findings a total of 627 (7.79%) species of Enterococci were isolated from 8040 samples including 328 (7.28%) patient samples, 73 (11.49%) hospital staff samples, 59 (2.2%) hospital environment samples and 166 (73.45%) non-human inmate samples collected during the study (Table 4:20). Enterococcus species have been reported from the cases of nosocomial respiratory infections (Berk and Verghese 1989; Aoun and Klastersky 1991; Harbarth *et al.* (1999), bacteremia (Meyers *et al.*, 1989; Ortega *et al.*, 1991; Kadoya *et al.*, 1991; Watanakunakorn and Jura, 1991; Spera and Farber, 1994; Christie *et al.*, 1994;
Low et al., 1995; Richards et al., 1998; Harbarth et al. (1999). Meyers et al. (1989), Uthley et al. (1989), Schaberg et al. (1991), Ortega et al. (1991), Kadoya et al. (1991) isolated various species of Enterococci including *Streptococcus fecalis* from patients suffering from nosocomial ailments. Uthley et al. (1989) isolated strains of Enterococci resistant to various drugs from patients with renal diseases. Tenover (1991), Venditti et al. (1994), and Low et al. (1995) Brady et al. (1998) reported that Enterococci with high resistance to beta-lactams, glycopeptides and high levels of aminoglycosides were being increasingly isolated from the cases of nosocomial infections. Kadoya et al. (1991) isolated *Enterococcus faecium* from the cases of nosocomial infections. Aoun and Klastersky (1991) isolated *Enterococcus cloacae* from some cases of nosocomial pneumonia. Venditti et al. (1994) isolated *Enterococcus faecium* and *Enterococcus raffinosus* from the hospitalized patients. Kodoya et al. (1991) isolated antibiotic resistant strains of *Enterococcus faecalis* and *Enterococcus faecium* from the cases of nosocomial bacteremia. De-Champs et al. (1994) and Christie et al. (1994) also isolated *Enterococcus faeclis* and *Enterococcus faecium* from the critically ill children. Boyce et al. (1994) isolated ampicillin-resistant strains of *Enterococcus faecium* from the cases of nosocomial diarrhea. Grayson et al. (1994) and Singer et al. (1997) observed an increase in the incidence of nosocomial infections caused by multidrug-resistant strains of Enterococci in USA since 1989. They implicated species like *E. faecalis*, *E. faecium*, *E. raffinosus* and *E. caseliflavus* and *E. durans* with clusters of infection (Richards et al., 1998). French (1998) observed that enterococci readily colonize the bowel, spread rapidly among hospital patients, and transfer their antibiotic resistance characteristics, widely among themselves and other gram-positive cocci. Austin and Anderson (1999) reported that epidemic strains of vancomycin-resistant enterococci (VRE) are becoming endemic in hospitals in the United Kingdom. The findings of present study are supported with the findings of aforementioned workers.

**Wards:** In the present work a total of 328 strains of *Enterococci* were isolated from 101 (30.8%) patients under treatment in medical unit, 55 (16.8%) patients in surgical unit, 53 (16.1%) patients in ICU, 10 (3%) in cardiology ward, 26 (7.9%) in cardiac surgery ward, 25 (7.6%) in the chest surgery ward, 9 (2.7%) in orthopedic surgery ward, 9 (2.8%) in pediatric ward, 31 (9.5%) in burn unit, 7 (2.1%) in neurosurgery ward, 1 (0.3%) in the
Discussion

urology ward, and 1 (0.3%) in the oncology ward of Mayo Hospital, Lahore-Pakistan (Fig.3). Such isolation studies have also been reported by Low et al. (1995) in general surgery; Valero and Saenz (1998) Harbarth et al., (1999) in vascular and cardiac surgery (Valero and Saenz 1998), urologic surgery (Valero and Saenz 1998; Harbarth et al. (1999), renal diseases (Uthley et al., 1989), enteral tube feeding (Low et al., 1995; Weinstein et al., 1996), neonatal bacteremia (Christie et al., 1994; De-Champs et al., 1994), diarrhea (Boyce et al., 1994; Saunders et al., (1994)), intensive care unit patients (Weinstein et al., 1996), and the cancer patients (Del-Favero and Menichette, 1993). The results of present study partially support the findings of above said workers due to difference in hygienic conditions. It is reported that patients with different kinds of infectious and non-infectious diseases are admitted in various wards of Mayo Hospital, Lahore. All such type of patients contaminated the ward environment through their respiration, sneezing, through contacts being on very close by beds (some times sharing the same bed) and infected and non-infected persons sharing the same eating and drinking utensils etc.

**Antibiogram:** Of 328 isolates of *Enterococci* 147 (45%) were resistant to action of Ampicillin, 126 (38.4%) to Ampiclox, 159 (48.5%) to Tetracyclin, 110 (33.5%) to Doxycycline, 179 (54.6%) to Erythromycin, 130 (39.6%) to Klarithromycin, 43 (13%) to Augmentin, 32 (9.8%) to Unasyn, 18 (5.5%) to Tazocin, 52 (16%) to Gentamicin, 18 (5.5%) to Amikacin, 69 (21%) to Ciprofloxacin, 51 (15.5%) to Sparfloxacin, 19 (5.8%) to Tienam and 8 (2.4%) to Vancocin (Appendix: 2-3). Above findings are in agreement with the findings of (Uthley et al. (1989), Ortega et al. (1991), Swartz (1994), Venditti et al. (1994), Low et al. (1995), Parr et al. (1999) and Patel et al. (1999) who have also reported drug resistance in *Enterococci* against many of above referred antibiotics.

(iv) *Pseudomonas*

**Patients:** In the present investigation various strains of *Pseudomonas* were isolated from a total of 781 (17.34%) samples collected from patients including 205 (19.71%) samples of pus and wound swabs, 9 (8.2%) samples of blood, 20 (17.39%) samples of pleural fluid, 26 (9.09%) samples of ascitic fluid, 4 (10.81%) of cerebrospinal fluid, 203
(14.52%) of urine 97 (9.81%) samples of sputum, 187 (56.8%) burn swabs, 15 (15.15%) patient body devices and 15 (14.8%) samples of fecal and drainage samples (Table 4:21). Hsueh et al. (1998) reported that Pseudomonas could persist at different body sites of burn patients and that the colonization could subsequently lead to various severe infections. Valero and Saenz (1998) calculated that on global basis Pseudomonas species were responsible for over 5.5% cases of nosocomial infections. As secondary invaders the pseudomonas species complicated the disease picture and contributed to high mortality (Wu et al., 2000)). Levin et al. (1984), Bawn et al. (1989), Griffith et al. (1989), Voutsinas et al. (1989), Evy and Katz (1989), Begue (1991), Aoun and Klastersky (1991), Karim et al. (1991), Kadoya et al. (1991), Armstrong et al. (1992), Takesue et al. (1994), Blanc (1997), Traub et al. (1998), Bert et al. (1998), Harbarth et al. (1999), Buttery et al. (1999), Arruda et al. (1999) and Alan and Sriram (2005) also isolated various Pseudomonas species from cases of different infectious lesion findings of present investigation are in agreement to observations reported by above referred scientists.

**Hospital Staff:** In the present study 33 (5.19%) Pseudomonas strains were isolated from a total of 635 samples obtained from the hospital staff which consisted of 2 (1.57%) samples from throat and nasal swabs; 10 (3.93%) from hand swabs, and 21 (8.26%) from gloves and aprons (Table 4:22). Weinstein et al. (1982) remarked that contaminated hands are source of dissemination of infections with gram-negative bacteria. The findings of the present study are supported by the observations of Weinstein et al. (1982), Griffith et al. (1989), Karim et al. (1991), Schaberg et al. (1991) and Harbarth et al. (1999).

**Hospital Environment:** A total of 53 (1.98%) Pseudomonas strains were isolated from 2677 samples from the hospital environment including 18 (27.69%) from ward environment, 20 (3.27%) from sterilized water, and 15 (0.79%) from surgical instruments (Table 4:23). The findings of the present investigation are in agreement with those reported by Levin et al. (1984), Begue (1991), Blanc (1997), and Bert et al. (1998), Alan and Sriram (2005).
**Discussion**

**Non human Inmates**: A total of 74 (32.74%) Pseudomonas strains were isolated from 226 samples of non-human inmates including 7 (28%) nasal swabs and 5 (20%) fecal swabs collected from cats; and 25 (28.4%) from legs and 37 (42.04%) from intestines of cockroaches (Table 4:24). Rodriguez et al. (1993), and Fulton and Walker (1992) isolated pseudomonas from cats; and Roth and Willis (1957), Hiro and Fuji (1966), Burgess et al. (1969), Fernandez and Zarror (1972), Zuberi et al. (1972), Svec et al. (1975), Burgess and Chetwyn (1981), Okafor (1981), and Sarmova et al. (1992) also reported the isolation of Pseudomonas strains from cockroaches.

**Overall Prevalence**: *P. aeruginosa* was amongst one of the most commonly encountered bacteria in patients in hospitals. *Pseudomonas aeruginosa* known as an opportunistic pathogen has been implicated with severe cases of infectious nosocomial problems (Giffith et al., 1989; Bawn et al., 1989; Harbarth et al., 1999). This organism survives in various environment of hospitals and inhibits intrinsic resistance to a wide variety of antimicrobial agents. In normal healthy hosts infection was usually associated with burns, puncture wounds, use of contaminated needles by intravenous drug abusers, eye trauma with contaminated contact lenses and resulted in infections of the skin, bones, heart, or eyes. In the present study a total of 941 (11.70%) species of Pseudomonas were isolated from 8040 various sources including 781 (17.34%) patient samples, 33 (5.19%) samples from hospital staff, 53 (1.97%) hospital environment samples and 74 (32.74%) non-human inmate samples (Table 4:25). Schaberg et al. (1991), while determining the trends in the microbial etiology of nosocomial infections in 1980’s based on documented nosocomial infection reported to the National Nosocomial Infections observed that *Pseudomonas aeruginosa* was the 2nd most common isolate in USA. Takesue et al. (1994) reported emergence of nosocomial infections due to multi-antibiotic-resistant strains of *Pseudomonas aeruginosa*. Evy and Katz (1989) and Karim et al. (1991) observed that *Pseudomonas cepacia* caused severe disease problems in immuno compromised patients and reported that the pathogens were either of nosocomial nature or were community acquired.

**Wards**: In the present work 781 strains of *Pseudomonas* were isolated from 163 (20.87%) patients under treatment in medical unit, 229 (29.3%) in surgical unit; 62
Discussion

(7.9%) in ICU, 1 (0.1%) in cardiology ward, 17 (2.17%) in cardiac surgery ward; 41 (5.2%) in chest surgery ward, 35 (4.5%) in orthopedic surgery ward, 12 (1.5%) in pediatric ward, 174 (22.2%) in burn unit, 1 (0.1%) patients in neurosurgery ward, 43 (5.5%) in urology ward, and 3 (0.4%) in the oncology ward (Fig.4). Isolation of various species of *P. aeruginosa* from the cases of nosocomial infections in patients of medical ICU (Bawn *et al*., 1989; Harbarth *et al*., 1999), surgical ICU (Bawn *et al*., 1989; Valero and Saenz 1998; Harbarth *et al*., 1999), peritonitis (Leviin *et al*., 1999), otitis media (Leviin *et al*., 1999), oncology unit (Giffith *et al*., 1989), keratitis (Evy and Katz, 1989), respiratory tract infections and pneumonia (Saunders *et al*., 1994; Harbarth *et al*., 1999; Leviin *et al*., 1999), neurology and neurosurgery (Leviin *et al*., 1999), bacteremia (Karim *et al*., 1991; Leviin *et al*., 1999; Harbarth *et al*., 1999), urinary tract infections (Karim *et al*., 1991; Harbarth *et al*., 1999; Leviin *et al*., 1999), burn units (Hsueh *et al*., 1998; Leviin *et al*., 1999), catheter related infections (Leviin *et al*., 1999) has been reported and the results of present investigation are congruent with the findings reported by above referred research workers.

**Antibiogram:** Of the 781, isolates of *Pseudo. aeruginosa*, 320 (41%) were found resistant to action of cefoparazone, 312 (40%) to cefrome; 72 (9.2%) to tazocin; 212 (27%) to gentamicin; 93 (12%) to amikacin; 342 (43.8%) to ciprofloxacin; 359 (46%) to sparflloxacin; and 63 (8%) to tienam (Appendix 2-3). Karim *et al*., (1991) also reported isolation of various species of *Pseudo. aeruginosa* from nosocomial cases. Of their isolates 54.8% were resistant to carbinecillin, 9.6% to gentamicin, 30.2% to ceftoxamine. However, none of the isolates was resistant to ofloxacin or ceftazidime. Penicillin-resistant strains of *Pseudomonas aeruginosa* have been reported to have variable resistance to synergistic effects of various combinations of gentamicin, ampicillin, clindamycin, colistin, cefoxitin, and ceftriazone (Rotimi *et al*., (1994). Hsueh *et al*., (1998) isolated *Pseudomonas aeruginosa* strains, from the burn patients, which were found resistant to piperacillin, cefoperazone, ceftazidime, aztreonam, imipenem, cefepime, cefpirome, ofloxacin, ciprofloxacin, minocycline, and aminoglycosides. They observed that 12% nosocomial strains of *Pseudomonas aeruginosa* were resistant to ampicillin, carbenicillin, piperacillin, cephalothin, cefamandole, ceftazdime, gentamicin
and amikacin; and 79 to 100% strains were reported susceptible to fleroxacin (Araque and Velazco, 1998). Leviin et al. (1999) also reported antibiogram of nosocomial isolates of Pseudomonas aeruginosa and reported that their isolates were susceptible to colistin, and resistant to antibacterial / antibiotics like aminoglycosides, cephalosporins, quinolones, penicillins, and monobactams. Findings of the present work are partially in agreement with those reported by Hsueh et al. (1998), Karim et al. (1991), Araque and Velazco (1998) and Levin et al. (1999). Emergence of antibiotic resistance is posing significant threat to human health. It has been observed that antibiotics are prescribed/used without logic. The drugs found effective against one infection are used, indiscriminately by the physician and patients and many times under dosing or over dosing is practiced by the recipi ents. Some times left over antibiotics are used in next episode of infection. All above factors have contributed to emergence of drug resistance strains in Pakistan.

(v) Enterobacter

Patients: In the present investigation a total of 349 (7.75%) strains of Enterobacter were isolated from patients. Those strains were recovered from 33 (3.17%) samples of pus and wound swabs; 12 (11%) samples of blood; 7 (6.08%) of pleural fluid; 32 (11.18%) of ascitic fluid; 6 (16.21%) of cerebrospinal fluid, 115 (8.22%) of urine; 68 (6.88%) from sputum; 16 (4.86%) of burn swabs; 33 (33.33%) of patient body devices and 27 (26.7%) samples of fecal and drainage material (Table 4:26). Swartz (1994) and Richards et al. (1998) reported that factors contributing towards the acquisition of nosocomial infections, by patients admitted to the hospitals are; increasing age, availability of treatment for formally untreatable diseases, extensive medication and intensive use of surgical therapies, frequent empirical use of antimicrobial drugs, indwelling catheters associated with urinary tract infections. Due to cross-contamination from patients to patients (Honderlick et al., (1999) and from ward to ward. The nosocomial species of Enterobacter cloacae can spread widely and can result in frequent outbreaks of infection in different wards of hospital (Harbarth et al., 1999).

Hospital Staff: Various strains of Enterobacter were isolated from 15 (2.36%) of 635
samples obtained from the hospital staff. The material consisted of 3 (2.36%) throat swabs, 7 (2.75%) hand swabs, and 5 (1.96%) gloves and aprons (Table 4:27). The findings of the present study support the results of Wang et al. (1991), Schaberg et al. (1991), Zhang (1991), Saunders et al. (1994), Regnier (1996), Valero and Saenz (1998), Harbarth et al. (1999), and Honderlick et al. (1999), who reported similar observations.

**Hospital Environment**: Of the total 2677 samples, Enterobacter strains were isolated from 25 (0.93%) samples obtained from the hospital environment including; 3 (2.38%) samples of operation theatre air, 15 (23.07%) samples of ward environment, 5 (0.81%) samples of sterilized water, and 2 (0.1%) of surgical instruments (Table-4.28). Begue (1991) and Wang et al. (1991) have also reported similar findings.

**Non human Inmates**: Of the 226 samples of non-human inmates, a total of 124 (54.86%) Enterobacter strains were isolated. The isolates were obtained from 11 (44%) nasal swabs and 22 (88%) fecal swabs from the cats; and 32 (36.36%) from legs and 59 (67.04%) from intestinal material of cockroaches (Table 4:29). The findings of this work are in agreement with results of Roth and Willis (1957), Burgess et al. (1969), Fernandez and Zarror (1972), Zuberi et al. (1972), Dolmierski and ldzkowski (1979), and Sarmova et al. (1992) who also isolated Enterobacter from cockroaches.

**Overall Prevalence**: A total of 513 (6.38%) strains of Enterobacter were isolated from a total of 8040 samples including 349 (7.75%) patient samples, 15 (2.36%) samples from hospital staff, 25 (0.93%) hospital environment samples and 124 (54.86%) non-human inmate samples (Table 4:30). The findings of the present investigation show a close relationship among the sources. It has been reported that the species of Enterobacteria are associated with nosocomial infections (Begue 1991; Sarmova et al., 1992; Lee et al., 1996; Richards et al., 1998; Brett et al., 1998), bacteremia (McGowan et al., 1989; Swartz 1994; Brett et al., 1998; Richards et al., 1998).

**Wards**: In the present work 349 strains of Enterobacter were isolated from 178 (36.7%) patients under treatment in medical unit; 69 (19.8%) patients in surgical unit; 45 (12.9%) patients in ICU; 3 (0.9%) in cardiology ward; 20 (5.7%) in cardiac surgery ward; 20
(5.7%) in the chest surgery ward; 6 (1.7%) in orthopedic surgery ward; 10 (2.9%) in pediatric ward; 12 (3.4%) in burn unit; 4 (1.1%) in the neurosurgery ward; 25 (7.2%) in urology ward; and 7 (2%) patients in oncology ward (Fig.5). Urinary tract infections (Lee et al., 1996; Brett et al., 1998; Richards et al., 1998); medical ICU (Brett et al., 1998; Harbarth et al., 1999); surgical ICU (Brett et al., 1998; Valero and Saenz 1998; Harbarth et al., 1999), CCU (Richards et al., 1998; Harbarth et al., 1999), indwelling catheters (Lee et al., 1996; Richards et al., 1998), neurosurgery (Brett et al., 1998), nosocomial pneumonias (Brett et al., 1998; Richards et al., 1998), and cardiology and cardiac surgery (Valero and Saenz 1998) have been reported as sources of Enterobacter species. Intensive care units (Harbarth et al., 1999) and transplantation and dialysis units (Kaminska et al., 2002) have also served as sources of Enterobacter species (Harbarth et al., 1999, Kaminske et al., 202).

**Antibiotic susceptibility:** Various species of Enterobacter, especially the multi-drug resistant Enterobacter cloacae, are being increasingly incriminated in the Nosocomial infections world wide (Wang et al., 1991; Zhang 1991; Dennesen et al., 1998). Excessive use of antibiotics in medical practice has provided selection pressure for enterobacteria to develop antibiotic resistance, which is commonly exhibited by nosocomial ailments (Regnier, 1996: Dennesen et al., 1998; and Bonomo and Rice, 1999). Lee et al., (1996). Regnier (1996), and Bonomo and Rice (1999) reported that antibiotic resistant gram negative bacteria are emerging as one of the major clinical concerns for physicians who treat patients in long-term care facilities. Rotimi et al. (1998) isolated drug resistant strains of Enterobacter from patients in Saudi Arabia and Kuwait. De-Champs et al. (1994) and Araque and Velazco (1998) also isolated enterobacteria from adult patients suffering from nosocomial infections. Kays (1999) isolated antibiotic resistant strains of *Enterobacter aerogenes* and *Enterobacter cloacae* from the cases of nosocomial infections. In infections caused by multi-drug resistant strains of bacteria like MRSA, the enterococci are common secondary invaders which complicate the clinical disease picture and eventually lead to high mortality (Wu et al., 2000; Kaminska et al., 2002)

Of the total 349 isolates of Enterobacter species, 301 (86.2%) were resistant to
Ampicillin; 293 (84%) to ampiclox; 129 (37%) to cefoparazone; 115 (33%) to cefrome; 171 (49%) to augmentin; 131 (37.5%) to unasyn; 23 (6.6%) to tazocin; 55 (15.8%) to gentamicin; 39 (11.2%) to amikacin; 77 (22%) to ciprofloxacin; 52 (14.9%) to sparfloxacin and 16 (4.6%) to tienam (Appendix 2-3). These findings are in agreement with the observation of Schaberg et al (1991) who reported that isolated nosocomial species of Enterobacter were multi-drug resistant. Aoun and Klastersky (1991) and Begue (1991) reported that species of Enterobacter isolated from cases of nosocomial pneumonia were susceptible to fluoroquinolones, cephalosporins, imipenem, aztreonam and ticarcillin. The findings of the present investigation are quite close to the observations of Schaberg et al. (1991); Klastersky (1991); and Begue (1991). Honderlick et al. (1999) also reported outbreaks of nosocomial infections due to multi drug resistant strain of Enterobacter cloacae.

(vi) Acinetobacter

Patients: Villers et al. (1998) and Jawad et al. (1998) observed that Acinetobacter baumannii was ubiquitous opportunistic organism with a complex epidemiology of nosocomial infections. Jawad et al. (1998) observed that strains of A. baumannii have the ability to survive long on dry surfaces (Traub et al., 1999; Corbella et al., 1999). Webster et al. (1998) and Husani et al. (1999) reported that Acinetobacter species were incriminated in outbreaks of nosocomial infections and that it was difficult to prevent infection with this organism. In the present study 41 (0.91%) strains of Acinetobacter were isolated from a total of 4502 samples of patients which consisted of 30 (2.88%) samples of pus and wound swabs, 3 (2.75%) samples of blood, and 8 (0.57%) of urine (Table 4:31). Acinetobacter species are being increasingly incriminated in nosocomial infections and the Acinetobacter baumannii and Acinetobacter radioresistens have been referred as epidemic strains (Okpara and Maswoswe 1994; Webster et al., 1998). The results of the present work are in concurrence with the findings of Karim et al. (1991), Zhang (1991), Okpara and Maswoswe (1994), Chang et al. (1994), Webster et al. (1998), Jawad et al. (1998), Villers et al. (1998), Traub et al. (1999) and Corbella et al. (1999).
Discussion

Overall Prevalence: A total of 41 (0.51%) species of Acinetobacter were isolated from 8040 samples from 41 (0.91%) patients in various wards analysed during the present of study (Table 4:32). Isolation of Acinetobacter strains from the urine (Okpara and Maswoswe 1994; Villers et al., 1998; Leviin et al., 1999), lower respiratory tract (Villers et al., 1998; Trouillet et al., 1998; Leviin et al., 1999), ventilator-associated pneumonia (Rello et al., 1999), wounds (Okpara and Maswoswe 1994) has been reported.

Wards: In the present work 41 (0.91%) strains of Acinetobacter were isolated from 6 (14.6%) patients under treatment in medical unit, 21 (51.2%) patients in surgical unit, 4 (9.7%) patients in ICU, 3 (7.3%) in chest surgery ward, 2 (4.9%) in orthopedic surgery ward, 2 (4.9%) patients in urology ward, and 1 (2.4%) patient in oncology ward (Fig.6). These findings are in agreement with those of Webster et al. (1998) and Husani et al. (1999).

Antibiogram: Of the 41 strains of Acinetobacter 13 (31.7%) were resistant to doxycycline; 8 (19.5%) to cefoparazone; 7 (17%) to cefrome; 12 (29.3%) to augmentin; 9 (22%) to unasyn; 2 (5%) to tazocin; 6 (14.6%) to gentamicin; 2 (5%) to amikacin; 9 (21.9%) to ciprofloxacin, and 4 (9.8%) to sparfloxacin (Annexure 2-3). These findings are in agreement with the observations of research workers like Okpara and Maswoswe (1994), Maswoswe (1994), Go-Es et al. (1994), Levin et al. (1999) and Traub et al. (1999). Villers et al. (1998) and Jawad et al. (1998) observed that Acinetobacter baumannii was rapidly emerging as multidrug resistant organism. Strains of A. baumannii are more resistant to various broad-spectrum antimicrobial agents than the other sporadic isolates (Traub et al., 1999; Corbella et al., 1999).

(vii) Klebsiella

Patients: Schaberg et al. (1991) while determining trends in the microbial etiology of nosocomial infections in 1980’s based on documented incidence of nosocomial infections reported to the Natinal Nosocomial Infections concluded that the 5% cases were due to infection with Klebsiella species and those species were responsible for a significant number of cases of nosocomial infections (Meyers et al., 1989; Rotimi et al., 1998) of respiratory tract and the nosocomial pneumonia (Farmer et al., 1985;
In the present work Klebsiella strains were isolated from 266 (5.91%) samples collected from patients i.e. 27 (2.59%) samples of pus and wound swabs, 7 (6.42%) samples of blood; 10 (8.69%) samples of pleural fluid; 21 (7.34%) samples of ascitic fluid; 2 (5.4%) samples of cerebrospinal fluid; 62 (4.43%) samples of urine; 120 (12.14%) samples of sputum; 13 (3.95%) of burn swabs; and 4 (3.96%) samples of fecal and drainage material (Table 4:33). Isolation of Klebsiella from the patients of nosocomial bacteremia and septicemia has been commonly reported by Meyers et al. (1989); Watanakunakorn and Jura (1991), isolated Klebsiella strains from lower respiratory tract reported by Farmer et al. (1985); Watanakunakorn and Jura (1991); Klastersky (1991); Wu et al. (2000), from the urinary tract it was reported by Watanakunakorn and Jura (1991), from the nosocomial neonatal septicemia reported by Banerjee et al. (1993).

**Hospital Staff:** A total of 325 (4.04%) strains of Klebsiella were isolated from 8040 samples from various sources including 266 (5.91%) patient samples, 24 (3.77%) samples from hospital staff and 35 (1.31%) hospital environment samples collected during the present of study (Table 4:34). Schaberg et al. (1991), Wiener et al. (1999) have also reported similar findings.

**Hospital Environment:** Strains of Klebsiella were isolated from a total of 35 (1.31%) samples obtained from hospital environment (Table 4:35). The findings of this work are in agreement with observations of Callaghan (1988), and Wiener et al. (1999).

**Overall Prevalence:** Klebsiella strains were isolated from a total of 24 (3.78%) samples which included 18 (14.17%) samples of throat and nasal swabs, 3 (1.18%) samples of hand swabs, and 3 (1.18%) samples of gloves and aprons (Table 4:36). Schaberg et al. (1991), Wiener et al. (1999) have also reported similar findings.

**Wards:** A total of 266 isolations of Klebsiella were possible from 113 (42.9%) patients of medical unit, 45 (16.9%) of surgical unit, 29 (10.9%) of ICU, 14 (5.3%) of cardiac
surgery ward, 35 (13%) of chest surgery ward, 2 (0.8%) of orthopedic surgery ward, 1 (0.4%) of pediatric ward, 9 (3.4%) of burn unit, 16 (6%) of the urology ward, and 2 (0.8%) patients of the oncology ward (Fig.7). It was reported that in Malaysia Klebsiella isolates were considered responsible for nearly 20% of the cases of nosocomial infections and affected the patients which were under treatment in the dermatology, medical, surgical, neurology and neurosurgery wards of hospitals. High mortality in neonates was recorded in nosocomial infections caused by multi-resistant strains of *Klebsiella pneumoniae* (Pillay et al., 1998). Banerjee et al. (1993) attributed an outbreak of nosocomial neonatal septicemia due to *Klebsiella pneumoniae* in a nursery. The findings of the above referred scientists are closely related to those of the present investigation.

**Antibiogram:** Of the 266 isolates of Klebsiella, 62 (23.3%) were found resistant to cefoparazone; 53 (19.9%) to cefrome; 150 (56%) to augmentin; 102 (38.3%) to unasyn; 16 (6%) to tazocin; 64 (24%) to gentamicin; 25 (9.4%) to amikacin; 83 (31.2%) to ciprofloxacin; 89 (33.5%) to sparfloxacin and 7 (2.6%) to tienam (Appendix: 2-3). High mortality in neonates in nosocomial infections caused by multi-resistant strains of *Klebsiella pneumoniae* has been reported (Pillay et al., 1998). Banerjee et al. (1993) attributed an outbreak of nosocomial neonatal septicemia due to multi-drug resistant *Klebsiella pneumoniae* in a nursery. Klebsiella isolates from nosocomial problems were resistant to ampicillin and carbenicillin (Watanakunakorn and Jura 1991; Rotimi et al., 1994; Araque and Velazco, 1998). Pillay et al. (1998) successfully used combinations of imipenem/cilastatin (Tienam) and piperacillin/tazobactam (Tazocin) for treatment of nosocomial diseases caused by Klebsiella species.

**viii) Proteus Species**

Proteus organisms are considered as potential secondary invaders in the pulmonary infections and these organisms usually complicate the disease picture caused by other pathogens (Farmer et al., 1985; Wu et al., 2000). Farmer et al. (1985) isolated *Proteus mirabilis* from clinical specimens which were submitted by general Hospitals. Wu et al. (2000) reported high mortality in cases of MRSA nosocomial pulmonary pneumonia.
which was complicated by *Proteus mirabilis*.

**Patients:** In the present work various strains of Proteus were isolated from 140 (3.11%) samples collected from hospital patients. The isolations were possible from 22 (2.11%) samples consisting of pus and wound swabs; 3 (2.75%) of blood; 9 (3.14%) samples of ascitic fluid; 48 (3.43%) of urine, 21 (2.12%) of sputum, 32 (9.72%) of burn swabs and 5 (5.05%) of patient body devices (Table 4:37). The findings of present study support the observations of Farmer *et al.* (1985), McGowan *et al.* (1989), Berk and Verghese (1989) and Klastersky, 1989.

**Hospital Environment:** Proteus strains were isolated from a total of 22 (0.82%) samples [20 (30.76%) ward environment and 2 (0.1%) surgical instrument samples] (Table 4:38). Callaghan (1988) has reported the same observations.

**Non human Inmates:** In present work Proteus strains were isolated from a total of 47 (20.79%) samples of material obtained from non-human inmates including 8 (32%) samples of fecal material taken from cats, and 12 (63.63%) samples of legs and 27 (30.68%) of visceral material of cockroaches (Table 4:39). Fernandez and Zarror (1972), Zuberi *et al.* (1972), and Okafor (1981) have also reported similar observations.

**Overall Prevalence:** A total of 209 (2.59%) species of Proteus were isolated from 8040 samples from various sources including 140 (3.11%) patient samples, 22 (0.82%) hospital environment samples, 47 (20.79%) of non-human inmates samples (Table 4:40). Fernandez and Zarror (1972), Zuberi *et al.* (1972), and Okafor (1981), Callaghan (1988) has also reported isolation of various proteus species. There is increasing evidence of the involvement of Proteus organisms with cases of nosocomial pneumonia and that these bacteria are being commonly isolated from cases of nosocomial blood stream and other hospital derived infections over the past 20 years (Farmer *et al.* 1985; Berk and Verghese, 1989; Klastersky, 1989; and Wu *et al*., 2000).

**Wards:** A total of 140 (2.72%) Proteus strains were recovered from 37 patients under treatment in medical unit, 38 patients in surgical unit, 16 in ICU, 3 in the chest surgery
ward, 7 in orthopedic surgery ward, 2 in pediatric ward, 25 in burn unit, and 12 patients in the urology ward (Fig.8). McGowan et al., 1989; Pegues et al., 1994 have also reported the similar findings.

**Antibiogram:** Of the 140 isolates of Proteus 82 (58.6%) were found resistant to cefuroxime; 54 (38.6%) to cefoparazone; 47 (33.6%) to cefrome; 69 (49.3%) to augmentin; 51 (36.4%) to unasyn; 10 (7%) to tazocin; 28 (20%) to gentamicin; 16 (11.4%) to amikacin; 41 (29.3%) to ciprofloxacin; 36 (25.7%) to sparfloxacin, and 4 (2.9%) to tienam (Appendix 2-3). Araque and Velazco (1998) and File Jr. (1999) studied the drug resistance of nosocomial isolates to eight different antimicrobial agents such as ampicillin, carbenicillin, piperaclillin, cephalothin, cefamandole, ceftazidime, gentamicin and amikacin. They reported that the species of *Proteus mirabilis* were resistant to many antibacterials.

**ix) Escherichia**

**Patients:** Species of Escherichia have been implicated with severe cases of nosocomial bacteremia and septicemia (Farmer et al., 1985; Meyers et al. (1989; Zhang 1991; Bonomo and Rice 1999). Nosocomial *Escherichia coli* are well known to have acquired enhanced virulence, invasiveness and adherence, more often than those by the other community strains (Donnerberg and Kkaper, 1992). In USA nearly 16% cases of nosocomial infections were attributable to infection with Escherichia species (Schaberg et al., 1991), nearly 12% cases were implicated with Escherichia species, which are important members of the intestinal resident flora and are now increasingly emerging as cause of nosocomial infections (Farmer et al., 1985; Meyers et al., 1989; Schaberg et al., 1991). Aoun and Klastersky (1991) and Watanakunakorn and Jura (1991) have reported isolation of strains of Escherichia from cases of severe nosocomial pulmonary infections and bacteremia. Begue (1991) described that in tropical areas high mortality in neonates was due to bacterial infections and severe sepsis was caused by mother-linked hospital strains of *Escherichia coli*. Richards et al. (1998) reported that *Escherichia coli* was the most common species isolated from cases of nosocomial urinary tract infections.
The findings of the present study co-inside with the above mentioned workers. In the present work, strains of Escherichia were isolated from 1031 (22.9%) samples from patients; which consisted as 99 (9.51%) samples of pus / wound swabs, 12 (11%) samples of blood, 6 (5.21%) of pleural fluid, 30 (10.48%) of ascitic fluid, 4 (10.81%) of cerebrospinal fluid, 742 (53.07%) of urine, 65 (6.57%) of sputum, 23 (6.99%) burn swabs, 35 (35.35%) patient body devices and 15 (14.85%) of fecal and drainage material (Table 4:41). The findings of present work are supported by the reports of Farmer et al. (1985), Meyers et al. (1989), Schaberg et al. (1991), Aoun and Klastersky (1991), Watanakunakorn and Jura (1991), Zhang (1991), Senerwa et al. (1991), Richards et al. (1998) and Saenz (1998). The results of present study are also in agreement with those reported by Valero and Saenz (1998).

**Hospital Staff:** In the present study 25 (3.93%) Escherichia strains were isolated from a total of 635 samples from hospital staff [5 (3.93%) samples of throat and nasal swabs, 16 (6.29%) of hand swabs, and 4 (1.57%) of gloves and aprons (Table 4:42). Weightman and Kirby (2000) described the nosocomial transmission of Shiga toxin-producing \textit{Escherichia coli} O157 between staff and patients. Findings of the present work are supported by the observations of Meyers et al. (1989), Begue (1991), Schaberg et al. (1991).

**Hospital Environment:** In the present study 55 (2.05%) strains of Escherichia were isolated from a total of 2677 samples from the hospital environment [10 (7.93%) samples of operation theatre air, and 45 (69.23%) samples of ward environment samples (Table 4:43). Isolation of Escherichic strains from hospital premises has also been reported by Richards et al. (1998), Sedor and Mulholland (1999) and Wiener et al. (1999).

**Non human Inmates:** During our investigation 163 (72.1%) Escherichia strains were isolated from a total of 226 samples from non-human inmates [9 (36%) samples of nasal swabs, 23 (92%) samples of fecal swabs from cats; and 67 (76.13%) samples of legs and 64 (72.72%) samples of intestines of cockroaches (Table 4:44). The findings of this work were in agreement with observations of Steinhaus (1941), Roth and Willis (1957) Burgess et al. (1969), Zuberi et al. (1972), Fisherman and Alcamo (1977), Burgess and Chetwyn (1981), Okafor (1981), and Razia and Jafri (1986) who also isolated
Discussion

Escherichia strains from cockroaches, indicating that cockroaches found in hospital environment could harbor pathogenic Escherichia species.

**Overall Prevalence:** In the present study a total of 1274 (15.84%) strains of Escherichia were isolated from 8040 samples obtained from various sources [1031 (22.9%) samples from various kinds of patients, 25 (3.93%) samples from hospital staff, 55 (2.05%) from hospital environment, 163 (72.12%) from non-human inmates (Table 4:45). Isolation of various species of *Escherichia coli* from nosocomial cases has also been reported by Meyers *et al.* (1989); Aoun and Klastersky (1991); Zhang (1991); Brucker (1996); Araque and Velazco (1998) and Kays (1999). Cases of the nosocomial pneumonia reported by Watanakunakorn and Jura (1991), Aoun and Klastersky (1991); from respiratory tract infections by Saunders *et al.* (1994); and from gastroenteritis in preterm neonates by Senerwa *et al.* (1991). Isolation of Escherichia strains from hospital premises has also been reported by Richards *et al.* (1998), Sedor and Mulholland (1999) and Wiener *et al.* (1999). Burgess and Chetwyn (1981), Okafor (1981), and Razia and Jafri (1986) also isolated Escherichia strains from cockroaches in the hospital environment and other surroundings. The results of present study correlates with the findings of above mentioned research workers.

**Wards:** In the present work 1031 strains of *Escherichia* were isolated from 560 (54.3%) patients which were under treatment in medical unit, 183 (17.7%) patients in surgical unit, 69 (6.7%) in ICU, 14 (1.3%) in cardiology ward, 31(3%) in cardiac surgery ward, 28 (2.7%) in chest surgery ward, 23 (2.2%) in the orthopedic surgery ward, 19 (2%) in the pediatric ward, 22 (2%) in the burn unit, 4 (0.4%) in the neurosurgery ward, 66 (6.4%) in the urology ward, and 12 (1%) patients in the oncology ward (Fig.9). Escherichia strains from urinary tract infections has been reported by Watanakunakorn and Jura (1991), Richards *et al.* (1998), and Guyot *et al.* (1999); and from patients of patients of coronary care units by Valero and Saenz (1998); cases of neonatal septicemia by Begue (1991), and from patients of Coronary Care Units (CCU) by Valero and Saenz (1998). The results of present study are congruent with the findings of above mentioned research workers.
Antibiogram: Of the total 1031 strains of Escherichia, 732 (71%) were found resistant to ampicillin; 710 (69%) to ampiclox; 618 (60%) to tetracyclin; 531 (51%), to doxycycline; 301 (29%) to cefuroxime; 110 (11%) to cefoparazone; 119 (12%) to cefrome; 291 (28%) to augmentin; 208 (20%) to unasyn; 30 (3%) to tazocin; 169 (16%) to gentamicin; 78 (8%) to amikacin; 235 (23%) to ciprofloxacin; 229 (22%) to sparfloxacin, and 32 (3%) to tienam (Appendix 2-3). These observations are in agreement with those reported by Zhang (1991), Senerwa et al. (1991) and Rotimi et al. (1994). Araque and Velazco (1998) reported that 60% of nosocomial strains of Escherichia coli were susceptible to fleroxacin, and 76% to ampicillin, carbenicillin, piperacillin, cephalothin, cefamandole, ceftazdime, gentamicin and amikacin. Guyot et al., (1999) isolated drug-resistant Escherichia coli from a French hospital.

x) Serratia Species

Patients: In the present work 67 (1.48%) Serratia strains were isolated from a total of 4502 tissue samples from patients [10 (0.96%) samples of pus and wound swabs, 8 (7.33%) samples of blood, 6 (2.09%) of ascitic fluid, 20 (1.43%) of urine, 15 (1.51%) of sputum, and 8 (2.43%) of burn swabs (Table 4:46). Zaidi et al. (1989) reported an epidemic of bacteriaemia and meningitis caused by Serratia mercescens in the neonatal intensive care unit and special care nursery of a hospital in Mexico city, Mexico. Karim et al. (1991) isolated species of Serratia from immune-compromised patients at Agha Khan University Hospital, Karachi. Wong et al. (1991) reviewed Serratia mercescens bacteriaemia in 1985, and reported an increase in the incidence due to these organisms. Multi drug resistant Serratia mercescens species were commonly isolated from neonatal and immune-compromised patients suffering from blood stream infections (Wong et al., 1991; Karim et al., 1991). Contaminated hands of hospital personnel and infected bath tubs may also result in the colonization of skin of neonates (Zaidi et al., 1989; Pegues et al., 1994) with Serratia species.

Overall Prevalence: In the present investigation, a total of 67 (0.83%) strains of Serratia were isolated from a total of 8040 samples (Table 4:47). The findings of present work
are supported by the results of Wong et al. (1991), Karim et al. (1991), Aoun and Klastersky (1991), Serratia were also isolated from hand washings of hospital personnels and bath tubs. Pegues et al. (1994) and Zaidi et al. (1989) also isolated Serratia organisms from patients, tubs and skin of neonates. Serratia mercescens species have been reported as associated with the urinary tract infection (Wong et al., 1991), Pneumonia (Wong et al., 1991), biliary tract infection (Wong et al., 1991), intra-abdominal infection (Wong et al., 1991), skin and soft tissue infection (Wong et al., 1991) and bacteremia (Karim et al., 1991; Pegues et al., 1994), neonate bacteremia (Zaidi et al., 1989; Pegues et al., 1994), meningitis (Zaidi et al., 1989). Findings of present work are congruent with the work of Wong et al. (1991), Karim et al. (1991), Zaidi et al. (1989) and Pague et al. (1994).

Wards: In the present work 67 strains of Serratia were isolated from 23 (34.3%) patients under treatment in medical unit, 18 (26.9%) surgical unit, 10 (15%) in ICU, 2 (2.98%) in cardiac surgery ward, 2 (3%) in chest surgery ward, 3 (4.5%) in pediatric ward, 5 (7.5%) in burn unit, 3 (4.5%) in the dermatology ward, and 1 (1.5%) in the oncology ward (Fig.10). Zaidi et al. (1989), Pegues et al. (1994) reported cases of bacteriaemia and meningitis caused by *Serratia mercescens* in the neonatal intensive care unit and special care nursery in Mexico city, Mexico.

Antibiogram: Of the 67 strains of Serratia, 23 (34.3%) were found resistant to cefoparazone; 17 (25%) to cefrome; 3 (4.5%) to tazocin; 16 (24%) to gentamicin; 6 (9%) to amikacin; 41 (61%) to ciprofloxacin; 39 (58.2%) to sparfloxacin and 2 (3%) to tienam (Appendix 2-3). Majority of the *Serratia mercescens* isolates were sensitive to amikacin and third generation cephalosporins. Wong et al. (1991); Aoun and Klastersky (1991); Pegues et al. (1994) and Zaidi et al. (1989) reported that their isolates (*Serratia mercescens*) were resistant to all aminoglycosides and broadspectrum penicillins. Banerjee (1993) found that most of the *Serratia marcescens* isolates were resistant to ampicillin (100%) and gentamicim (77%). The antibiotic sensitivity of isolates of Serratia in the present work to gentamycin was 24% as compared to 77% observed by Banerjee (1993).
(xi) Haemophilus

Patients: In the present work 93 (2.07%) strains of Haemophilus were isolated from a total of 4502 patients samples including 1 (0.91%) from blood, 13 (11.3%) from plural fluid and 79 (7.99%) from specimens of sputum (Table 4:48). Hekker et al. (1991) and Cross and Campbell (1999) have reported that epidemic of nosocomial infections caused by *Haemophilus influenza* were associated with cases of nosocomial pneumonia.

Hospital Staff: A total of 13 (2.04%) strains of Haemophilus were isolated from a total of 635 samples from throat and nasal swabs of the hospital staff (Table 4:49). Hekker *et al.* (1991) and Cross and Campbell (1999) also isolated *Haem. Influenzae* which was associated with the nosocomial pneumonia.

Overall Prevalence: In this study 106 (1.32%) strains of Serratia were isolated from a total of 8040 samples obtained from various sources [93 (2.06%) samples from patients and 13 (2.04%) samples from hospital staff (Table 4:50). These findings are supported by the investigations of Hekker *et al.* (1991) and Cross and Campbell (1999).

Wards: In the present work 43 (46%) Haemophilus strains were isolated from a total of 93 patients under treatment in medical unit; 11 (12%) in surgical unit, 15 (16%) in ICU, 9 (9.7%) in cardiac surgery ward, 13 (14%) in chest surgery ward, 1 (1%) in pediatric ward, and 1 (1%) patient in the oncology ward (Fig-11).

Antibiogram: Of the 93 (2.27%) strains of Hemophilus species 47 (50.5%) were found resistant to ampicillin; 32 (34.4%) to ampiclox; 18 (19.4%) to cefuroxime; 18 (19.4%) to cefoparazone; 63 (67.7%) to erythromycin; 21 (22.6%) to klarithromycin; 12 (13%) to augmentin; 8 (8.6%) to unasyn; 14 (15%) to gentamicin; 11 (11.8%) to amikacin; 21 (22.6%) to ciprofloxacin and 14 (15%) to sparfloxacin. All the isolates were susceptible to Tazocin and Tienam (Appendix 2-3).
xii) **Uncommon Gram Positive Bacteria**

A total of 119 strains of Gram positive bacteria such as 20 strains of Diphtheroids, 25 strains of Diplococcoids, 4 strains of Clostridia and 70 strains of Anthracoids were isolated from the patients.

**a) Diplococcoids**

Strains of *Diplococcus pneumoniae* were isolated from 25 samples of morbid material of which 20 (80%) specimens were obtained from Medical units, and 5 (20%) from ICU. Diplococci are incriminated in pulmonary infections and nosocomial pneumonia. In infants, in the past, the infection due to *Diplococcus pneumoniae* was regarded as community-acquired pulmonary infection but recently there is an increasing evidence of its role in the nosocomial infections (Paradisi and Corti, 1998).

**b) Clostridia:**

*Cl. difficile* strains were isolated from stool specimens of 4 patients in the Medical ICU who were on prolonged antibiotic therapy. These findings are in line with the work of Burdon (1982), Barbut *et al.* (1994) Dupont and Ribner (1998), Barbut and Petit (2001); Yassin *et al.* (2001) who have recorded similar observations.

**c) Anthracoids organisms:**

Seventy strains of Anthracid organisms were isolated from 40 samples of morbid material obtained from medical units, 12 from surgical units, 8 from ICU and 14 from the chest surgery. From the hospital environment 27 strains of Anthracoids including 2 from the operation theatre air samples, 19 from the ward environment samples, 5 from sterilized water samples, and one from the surgical instrument were isolated. The findings of this work were in agreement with results of Callaghan (1988). Of the 13 strains of Anthracoids isolated from non-human inmates 9 were isolated from the legs and 6 from the visceral material of the cockroaches. The findings of present work are in agreement with the observations of Burgess *et al.* (1969), Fernandez and Zorrar (1972), Zuberi *et al.* (1972), Bhaumik and Raychaudhri (1975), Svec *et al.* (1975), and Okafor (1981) who also isolated Bacilli from cockroaches.
**Antibiogram:** Of the 119 strains of above organisms 45 (38%) were resistance to ampicillin, 31 (26%) to ampiclox, 39 (33%) to tetracyclin, 26 (22%) to doxycycline, 59 (50%) to cephradin, 35 (29%) to cefuroxime, 49 (41%) to erythromycine, 18 (15%) to klarithromycin, 69 (58%) to augmentin, 51 (43%) to unasyn, 6 (5%) to tazocin, 29 (24%) to gentamicin, 4 (3%) to amikacin, 29 (24%) to ciprofloxacin, 12 (10%) to sparfloxacin and 3 (2.5%) to tienam (Appendix 2-3). Lagrou *et al.* (1998) observed that multiresistant strains of *Corynebacterium urealyticum* susceptible to teicoplanin and vancomycin. The findings of present work are supported by results of Rose *et al.* (1981) and De Galan *et al.*, (1999).

**xiii) Uncommon Gram Negative Bacteria**

**Patients:** In the present study 13 (0.29%) strains of various types of Gram negative bacteria were isolated from a total of 4502 samples collected from patients including 3 (0.28%) from pus and wound swabs, 4 (1.39%) from ascitic fluid and 6 (0.42%) from urine samples (Table 4:56).

**Hospital Staff:** In this study only one (0.39%) strain of *Salmonella paratyphi* was isolated from hand swab of the hospital staff, from a total of 635 samples. Hammami *et al.* (1991) were also able to isolate Salmonellae from the secretions and excretions of recovered patients for almost one year to fourteen months after recovery from disease. Isolation of Salmonellae from recovered patient apparently healthy individuals is considered indicative of a carrier state.

**Yeast & Fungi**

**Patients:** In the present investigation various types of yeast and fungi were isolated from 189 (4.19%) samples collected from patients including 10 (0.96%) samples of pus and wound swabs, 6 (5.21%) of pleural fluid, 9 (3.14%) of ascitic fluid, 95 (6.79%) of urine, 33 (3.34%) of sputum, 10 (3.03%) of burn patients, 20 (20.20%) of patient’s body devices and 6 (5.94%) fecal and drainage samples. However, the fungus isolates were not further characterized. Findings of present investigation support the observations of Schaberg *et al.* (1991), Hamory (1991), Krcmery *et al.* (1998),
Richards et al. (1998), Valero and Saenz (1998) and Harbarth et al. (1999). Nucci et al. (2002) also reported the isolation of *Nosocomial fungemia*.

**Hospital Environment**: Two types of fungi (*Aspergillus fumigatus*, and *Aspergillus niger*) were isolated from 18 (0.67%) samples of material from the hospital environment; 1 (0.79%) sample of operation theatre air and 17 (26.15%) samples of ward premises. Richards et al. (1998) have also reported similar observations. Warris et al. (2002) isolated *Aspergillus fumigatus* and other molds from hospital water.
CONCLUSIONS

The major sources of nosocomial infectious agents identified in the present study included clinical samples from patients (pus and wound swabs, blood, pleural fluid, ascitic fluid, cerebrospinal fluid, urine, sputum, burn swabs fecal and drainage material, patient body devices), hospital staff including doctors, nurses, technicians, ward attendants, dressers, sweepers etc. (throat and nasal swabs, hand swabs, gowns and aprons), hospital environment (operation theater air, ward environment, surgical instruments, sterilized water), and hospital inmates including cats, cockroaches. The isolation of various types of bacteria taken from different sources included species of Staphylococci, Streptococci, Enterococci, Pseudomonas, Enterobacter, Acinetobacter, Klebsiella, Proteus, Escherichia, Serratia, Haemophilus, and yeast and fungi. The most prevalent organisms isolated from various wards are shown in Fig.14. The emergence of various drug resistant strains of bacteria, observed in the present investigation indicates the irrational use of antibiotics in medical practice, as few antibiotics were found effective against the multi-drug resistant isolates of the nosocomial origin.
Conclusions

Fig. 14
RECOMMENDATIONS / SUGGESTIONS

This study indicates that the rate of occurrence of nosocomial infection is very high at various wards of Mayo Hospital, Lahore. Hence there is an immense need of research and professional activities. In order to reduce the risk of nosocomial infections, being contracted from the clinical and hospital facilities following measures are suggested:

- Consistant surveillance to establish baseline data on the occurrence and sources of nosocomial infections.

- Ten second hand washing and disinfection after all patient encounters or use of medical device on the same patient (e.g. vascular catheter).

- All efforts for minimizing the risk of transmission of any kind of microorganisms from patient to hospital care workers (HCW) or from HCWs to patient through use of proper cleaning, washing and disinfection procedures after dealing with any patient.

- Sterile gloves be worn while touching blood, body fluids, secretions, excretions, contaminated objects, mucous membranes or broken skin and those gloves be changed after treating a patient. Hand washing must be thoroughly performed after removing the used gloves.

- Mask and eye protector should be worn to protect mucous membranes during procedures that are likely to result in splashing of blood, body fluids, secretions or excretions.

- A gown should be worn to protect skin and clothing during procedures that are likely to result in splashing of blood, body fluids, secretions, or excretions. Gowns should also be worn during the care of patients suspected of being infected with pathogens to reduce the probability of
their transmission within the hospital or clinic, or to other patients or
HCWs. Gowns must be removed before leaving the patient’s room and
thorough hand washing must follow after each such patient encounter.

- Soiled linen should be transported in a bag. If the bag is sturdy and the
  articles can be placed in the bag without contaminating outside of the bag.

- Sharp instruments and needles should be handled with care. If possible,
  never recap. If recapping is necessary, use the one handed technique or a
  mechanical device to recap safely. Never remove, bend, break or
  manipulate needles from syringes by hand. Used needles, scalpel blades,
  and other sharp items should be placed in appropriate puncture resistant
  containers, which must be properly closed and subjected to proper
  sterilization.

- Disposable or reusable dishes can be used in patients on isolation
  precautions. Hot water and detergents in hospitals are sufficient to
  decontaminate the dishes, glasses, cups or other eating utensils.

- Rooms, cubicles, and bedside equipment should be appropriately cleaned
  and properly disinfected against certain pathogens such as Clostridium
  difficile and Enterococcus.

- Critical medical devices must be sterilized, and stored in a sterile
  environment.

- Steam sterilization should be used for all items that will not be damaged
  by heat, pressure, or moisture. Biologic monitoring of sterilization
  procedures should be monitored regularly, e.g. once a week.

- Medical devices or patient care equipment that enters the normal sterile
  tissue or the vascular system, or through which blood flows, are the so-
  called critical items requiring sterility. Examples of such items are surgical
instruments, urinary or vascular catheters. All such type of items pose a high risk of infection if they are found contaminated with microorganisms.

- Cleaning, disinfection, and sterilization of patient care supplies should be performed in a central processing department to make quality control easier. The central processing area should be divided into several areas including a cleaning and decontamination area, a packaging area, and areas for sterilization and storage of sterile supplies that are separated by physical barriers. Sterilization of critical medical equipment depends upon reduction of the bio-burden, before beginning the sterilization process all items should be thoroughly cleaned. Manual cleaning of contaminated items can expose personnel to blood borne pathogens and other potentially harmful microorganisms, hence the manual cleaning be undertaken with required precautions and utmost care.

- Both the patient and the HCW need to be educated and protected from contracting or transmitting nosocomial infections using recommended infection control measures.

- All HCWs need to be educated about their personal hygiene, risk areas of infection spread, and the route of transmission of pathogens.

- Immunization of HCWs against various diseases agents is a must to prevent transmission of infections from HCWs to patients. Evaluation and appropriate control measures for patients with signs and symptoms of transmissible infectious diseases can reduce the risk of hospital acquired diseases.

- Managing antibiotic resistance in hospitals requires a multifaceted prevention and control strategy. Key among this are antibiotic restriction and its rational use policies, focused infection control surveillance
activities and isolation policies, education and administrative control programs.

- Establish protocols which should facilitate the rapid identification, isolation and treatment of patients colonized or infected with multiple drug resistant bacteria to prevent transmission.

- The hospital environment should be kept dry, clean, well ventilated and exposed to sunlight. Maintaining surfaces and equipment dry is important, as wet surfaces and equipment promote microbial growth and help in spread of nosocomial pathogens from such sources.

- Cleaning procedures should be defined, applied consistently and compliance to these validated. Cleaning personnel should be properly trained and be made responsible for implementation of cleaning, washing and disinfection practices.

- Products used for cleaning and decontamination of the environment should be selected on the advice of professionals and used as per manufacturer’s instructions at proper dosage levels.

- Good air management is difficult to achieve in many health care facilities. An air maintenance program should be in place and filters should be replaced periodically. Patients with an airborne communicable disease should be isolated in a single room, if possible. Rooms with good airflow and high volume ventilation greater than six air changes per hour including a good fresh air mix, have a much reduced risk of air borne diseases.

- Specific infection prevention measures should be adopted for water system management, superheating, use of bio-cides such as chlorine and air filters are well described and advocated. Hospital authorities should
Recommendations & Suggestions

develop a routine maintenance programme for water filtration equipment to prevent bacterial overgrowth in filters.

- Walls and ceilings should be periodically cleaned and avoid disinfection unless known contamination (e.g. blood splashes) has occurred. A good effective disinfectant be used for cleaning of floors. Levels of bacterial contamination on floors can be brought to their original values within 2 hours of cleaning, regardless of use of disinfectants.

- Disposal of wastes must comply strictly with legislation. Clinical waste must be contained to prevent leakage and sharps must be discarded into puncture resistant containers. Disposal strategies include incineration, autoclaving followed by disposal with regular waste, mechanical/chemical disinfection, microwave decontamination and compacting. Liquid waste such as blood can be poured down a sanitary sewer. Alternatives for disposal of medical waste commonly seen in countries with limited resources include: incineration of small amounts of waste in a metal drum, landfills or burial in refuse pits that are securely fenced off to prevent access to human and animal scavengers.

- The patient environment harbors a number of potential reservoirs for pathogens. Patients need a clean environment for their uncomplicated recovery. They should be cleaned periodically and after each operation which may lead to contamination. Areas should be protected from heavy dust. It has been demonstrated that some parts of the environment have served as reservoirs for outbreaks for nosocomial infections, e.g. air filters, insulation materials, or surfaces. Other objects and surfaces known to harbor bacteria, such as flowers, toilets and medical waste have not been clearly linked to nosocomial infections. Routine cleaning of environmental surfaces with detergents is sufficient in most circumstances. In case of outbreaks, especially outbreaks due to resistant micro-organisms found in
the environment, additional cleaning with a disinfection solution may be required.

- All personnel handling food should understand the sources and transmission routes of food-related pathogenic microorganisms and learn how to handle the food items in a hygienic way, from production or collection until the final preparation and serving of meals.

- Laboratory workers are at occupational risk of exposure to microbiological pathogens that may cause inapparent to life-threatening infections. Laboratory acquired infections are defined as all infections acquired through laboratory activities, regardless of their clinical/subclinical manifestations. Reviews of the incidence, consequences, and control of laboratory acquired infections led to the development of laboratory safety programs. Despite these early guidelines, laboratory acquired infections still occur, probably due to a lack or ignorance and poor compliance with safe laboratory practices.

- Strategies for the prevention and management of laboratory acquired infections should be aimed at containing biohazardous agents and educating laboratory workers about the occupational risks. In general, biosafety programs include recommendations for work practices, laboratory design, personal protective equipment, and safety devices. Adherence to these biosafety guidelines can reduce the risk of exposure and consequent laboratory acquired infections.

- The aim of keeping good infection control practices in the operation threats (OTs) is to decrease surgical site infections (SSIs), which represent an important proportion of nosocomial infections, with associated morbidity and excessive health care costs. Modifying environmental factors associated with SSIs is an important area for infection control interventions.
Optimally, operating rooms should be equipped with positive pressure systems to ensure that air travels from OTs (aseptic zone) to adjacent areas (clean and protective zone).

To remove airborne contaminants generated during surgery by patients or the surgical team in attendance, ventilation should filter air at a minimum of 20 changes/hour, of which at least four should be with fresh air.

Keep the temperature of OTs between 18°C and 24°C, with humidity of 50 to 55%.

If the OT is not so equipped because of limited resources, focus on less expensive strategies to keep air as clean as possible: keep personnel to a minimum in the OT during a procedure, avoid excessive walking, keep doors and windows closed, and keep entries into the OT to a minimum during any procedure.

Intravascular catheters are frequent sources of bloodstream infections. Their prevention is an essential part of any infection control. Health care education, training and monitoring for insertion and maintenance of catheters can reduce the chances of catheters associated infections.

Hospital acquired urinary tract infections (HUTI) are quite common. These infections are associated with non sterile urinary catheters and other urogenital instrumentation. Their incidence can be significantly reduced by applying sterile catheters, and observing disinfection procedures.

Mechanical ventilation is the main risk factor for nosocomial pneumonia in critically ill patients. Ventilator associated pneumonia is the most common nosocomial infection (NI) in intensive care units. Preventive strategies be applied according to the International recommendations for the prevention of nosocomial pneumonia.
On the basis of findings of present research study following recommendations are made for the consideration of Medical practitioners, professionals, scientists and research workers.

- There is a dire need to establish:
  - a) a Directorate of Continuing Education and Extension for Human Resources Development in Biomedical Sciences which should train the staff of hospitals (Doctors, Nurses, Clinical and Laboratory attendants, Technicians etc.) on infection management and control and use of antibiotics and other medicines.
  - b) a Quality Control Wing be made responsible for monitoring the sterility of equipment, environment of operation theatres and disinfection of wards housing various kinds of patients. Sterilized water samples used in clinical and surgical procedures needs periodic testing for various bacterial species.
  - c) The drinking water available in hospitals need to be periodically evaluated for the presence of various kinds of pathogens.
  - d) The fungus / yeast isolates of present study also indicate improper sanitary and unhygienic conditions prevailing at hospitals. Proper inactivation of these infectious agents needs to be periodically carried out using disinfectants effective against them.

**FUTURE QUEST**

Since this study has indicated that many types of bacteria having developed resistance against quite a large number of antibiotics have emerged and are causing serious health problems in the patients (hospital acquired / community acquired and those acquired through other vehicles such as those communicated through unhygienic water semi-cooked food and vectors etc).
There is a need to investigate on the common gene coding for such a resistance in those bacteria and future investigations be on dealing with the transmission of resistance problem from one generation of organisms to another using tools of molecular biology.

Since a large number of pathogens are transmitted through patients suffering from nosocomial and community based infectious diseases to susceptible human population there is need for investigation on the means and methods which could effectively control the spread of pathogens amongst sick and susceptible populations.

The health administration in Pakistan must take every effort to highlight the infectious risk area for the public at large and investigate to develop vaccine against some common pathogens such as *Salmonella, Shigella, Streptococci, Enterococci, Staphylococci, Haemophilus* etc. so as to provide resistance in susceptible population.
The present study was designed to investigate the sources and causes of nosocomial infections in patients admitted in the Mayo Hospital, Lahore, Pakistan. Of the total 32,620 patients examined during 1997-2001, 4502 (13.80%) were reported to have acquired nosocomial infections during their stay at hospital with different types of bacterial pathogen.

Morbid samples collected from various types of patients consisted of 1040 samples of pus and wound swabs; 109 samples of blood; 115 of chest cavity aspirates; 286 of abdominal cavity aspirates; 37 of cerebrospinal fluid; 1398 of urine; 988 of sputum; 329 of burn swabs; 99 of patient body devices and 101 samples of fecal material.

Of the total 4502 samples examined; 1287 isolates of *Staphylococci* (1004 *Staph. aureus* and 283 *Staph. epidermidis*) 429 of *Streptococci* (265 *Strep. pyogenes*, 128 *Strep. pneumoniae* and 36 *Strep. viridans*), 328 of *Enterococci* (272 *Enterococ. faecalis*, 56 *Enterococ. faecium*), 781 of *Pseudomonas* (693 *Pseudo. aeruginosa* and 88 *Pseudo. cepacia*), 349 of *Enterobacter* (212 *Enterobact. cloacae* and 137 *Enterobact. aerogenes*), 41 of *Acinetobacter baumannii*, 266 of *Klebsiella* (96 *Kleb. pneumoniae*, 131 *Kleb. aerugenese* and 39 *Kleb. oxytoca*), 140 of *Proteus* (103 *Prot. mirabilis* and 37 *Prot. vulgaris*), 1031 of *Escherichia coli*, 67 of *Serratia marcescens*, 93 of *Haemophilus influenzae*, 119 isolates of quite uncommon types of Gram positive bacteria, 13 of uncommon Gram negative bacteria, and 189 isolates of yeast and fungi were recovered and characterized.

A total of 635 morbid samples consisting of 127 nasal and throat swabs, 254 hand swabs, and 254 glove and apron samples were collected from the staff working in various hospital wards and tested for bacterial isolation. From the 635 samples, 119 isolates of *Staphylococci* (79 *Staph. aureus* and 40 *Staph. epidermidis*), 60 of
Summary

Streptococci (41 Strep. pyogenes, 15 Strep. pneumoniae and 4 Strep. viridans), 73 of Enterococci (51 Enterococ. faecalis, 22 Enterococ. faecium), 33 of Pseudomonas (22 Pseudo. aeruginosa and 11 Pseudo. cepacia), 15 of Enterobacter (13 Enterobact. cloacae and 2 Enterobact. aerogenes), 24 of Klebsiella (17 Kleb. pneumoniae, 3 Kleb. aerogenes and 4 Kleb. oxytoca), 25 of Escherichia coli and 13 of Haemophilus influenzae were recovered indicating that the staff of Mayo Hospital, Lahore may harbours many types of potential pathogens which are transmitted from them to the patients while they receive treatment and come in contact with the carrier hospital staff.

A total of 2677 samples (126 samples of operation theatre air, 65 of ward environment, 610 of water from sterilizers, and 1876 surgical instrument swabs) were also collected and processed for isolation of bacteria. Of those samples, 110 isolates of Staphylococci (91 Staph. aureus and 19 Staph. epidermidis), 34 of Streptococci (14 Strep. pyogenes, 9 Strep. pneumoniae and 11 Strep. viridans), 59 of Enterococci (37 Enterococ. faecalis, 22 Enterococ. faecium), 53 of Pseudomonas (42 Peudo. aeruginosa and 11 Pseudo. cepacia), 25 of Enterobacter (16 Enterobact. cloacae and 9 Enterobact. aerogenes), 35 of Klebsiella (11 Kleb. pneumoniae, 14 Kleb. aerogenes and 10 Kleb. oxytoca), 22 of Proteus (9 Proteus mirabilis and 13 Proteus vulgaris), 55 of Escherichia coli and 18 isolates of yeast and fungi were obtained and characterized.

During the present study 226 samples from non human hospital inmates were also tested. Of those samples, 88 of each were from the legs and viscera (intestines) of cockroaches, and 25 each of nasal and fecal swabs from the cats in the hospital premises. Of the total 226 samples examined, 101 isolates of Staphylococci (85 Staph. aureus and 16 Staph. epidermidis), 146 of Streptococci (61 Strep. pyogenes, 37 Strep. pneumoniae and 48 Strep. viridans), 166 of Enterococci (Enterococ. fecalis and Enterococ. facium), 79 of Pseudomonas aeruginosa, 124 of Enterobacter (96 Enterobact. cloacae and 28 Enterobact. aerogenes), 47 of Proteus (22 Prot. mirabilis
and 25 Prot. vulgaris), 163 of Escherichia coli, and 30 isolates of yeasts and fungi were obtained and further characterized.

The sensitivity of various bacterial isolates to antibiotics such as penicillin, tetracycline, cephalosporin, betalactams, betalactamase inhibitors, aminoglycosides, quinolones, carbapenam and teicoplanin groups of drugs was determined using disc method. This study revealed that most of the isolates colonizing premises and body surfaces of personnel of Mayo Hospital had developed resistance against the above mentioned commonly used antibiotics. These isolates were found generally resistant to the action of ampicillin, cloxacillin, tetracyclines, cephalosporin, macrolides, betalactamase inhibitors, gentamycin, amikacin and quinolones (Ciprofloxacin and Sparfloxacín).

The isolation of pathogens from above sources indicates loop-holes in implementation of infection control programmes in the hospitals. Short comings in infection management and prevention leads to high incidence of hospital acquired infections. The emergence of various drug resistant bacteria, as observed in the present study is a matter of concern for the biomedical practitioners and necessitates an effective policy on rational use of antibiotics, as very few antibiotics / antibacterials are now left with broad spectrum efficacy against pathogens being commonly isolated from the patients with various ailments. Both the physicians and patients need education on the rational and effective use of antibiotics. However, more investigations in hospitals of Pakistan on the various factors leading to emergence of drug resistance against various bacterial species are suggested.
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Literature Cited


304


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Literature Cited


Literature Cited


## APPENDIX-2
### RESISTANT ORGANISMS
#### Antibiogram of Isolated strains

| Drug Groups | Penicillin’s | Ampicillin | Ampiclox | Ampicillin | Tetracycline’s | Tetracyclin | Doxycylin | Doxycycline | Cephalosporins | Cefuroxime | Cefoparazone | Cefrome | Ceftriaxone | Cefuroxime | Macrolides | Macrolide | Beta-lactams | Beta-lactam | Aminoglycosides | Aminoglycoside | Quinolones | Quinolone | Miscellaneous | Miscellaneous |
|-------------|--------------|------------|----------|------------|--------------|-------------|-----------|------------|---------------|------------|--------------|---------|-------------|------------|------------|-----------|-------------|-------------|----------------|-------------|---------------|---------------|---------------|---------------|---------------|
| S. aureus   |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| MRSA: 555  |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| S. aureus   |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| MSSA: 449   |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| S. epidermidis |        |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| MSSE: 242   |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| MRSE: 41    |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Streptococci: |        |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 429         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Enterococci: |            |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 328         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Pseudomonas: |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 781         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Enterobacter: |        |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 349         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Acinetobacter: |       |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 41          |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Klebsiella: |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 266         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Proteus:    |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 140         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Escherichia: |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 1031        |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Serratia:   |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 67          |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Haemophilus: |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 93          |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Other Gram (+): |      |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 119         |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| Other Gram (-): |     |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
| 13          |              |            |          |            |              |             |           |            |               |            |              |         |             |            |            |            |              |             |                |               |               |               |               |               |               |
## APPENDIX-3
### PERCENTAGE OF RESISTANCE

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329
### APPENDIX-1 COLONIZATION OF NOSOCOMIAL PATHOGENS IN VARIOUS WARDS OF MAYO HOSPITAL

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