

# CONTENTS

Chapters	Page no.
<b>List of Figures</b>	<b>vii</b>
<b>List of Tables</b>	<b>xi</b>
<b>List of Abbreviations</b>	<b>xii</b>
<b>Abstract</b>	<b>xiv</b>
<b>CHAPTER 1: INTRODUCTION &amp; REVIEW OF LITERATURE</b>	
1.1	The genus <i>Salmonella</i> 1
1.2	Epidemiology 1
1.3	Pathogenesis 3
1.4	Clinical features 4
1.5	Drug resistance 5
1.6	Diagnosis 6
1.6.1	Conventional diagnostic methods 7
1.6.2	Modified diagnostic methods 8
1.6.3	Molecular techniques 9
1.6.3.1	Polymerase Chain Reaction (PCR) 10
1.6.3.2	Multiplex PCR 11
1.7	First research objective 14
1.8	Vaccines against typhoidal <i>Salmonella</i> 15
1.8.1	Parenteral inactivated whole-cell vaccine 15
1.8.2	Oral live attenuated Ty21a vaccine 15
1.8.3	Vi polysaccharide vaccine 16
1.9	Bacterial polysaccharide-protein conjugate vaccines 16

1.9.1	Bacterial polysaccharides	17
1.9.2	Immunological properties of polysaccharides	17
1.9.3	Conjugation of polysaccharides with carrier protein	18
1.9.3.1	<i>Haemophilus influenzae</i> type b (Hib) conjugate vaccines	19
1.9.3.2	<i>Neisseria meningitides</i> conjugate vaccines	20
1.9.3.3	<i>Streptococcus pneumoniae</i> conjugate vaccine	21
1.9.3.4	Vi Conjugate vaccine for <i>S. Typhi</i>	22
1.9.3.5	O-Specific polysaccharide conjugate vaccines	22
1.10	Second research objective	26

## **CHAPTER 2: MATERIALS AND METHODS**

<b>PART-A</b>	<b>Materials and methods for nested multiplex PCR work</b>	<b>27</b>
2.1	Bacterial isolates	27
2.2	Purification of the strains	27
2.3	Biochemical identification of the bacterial strains	27
2.3.1	Stab and streak method	28
2.4	Studies on patients	28
2.4.1	Selection of patients	28
2.4.2	Blood samples	28
2.4.3	Blood culture	28
2.4.4	Serology	29
2.5	Molecular analysis	29
2.5.1	DNA extraction from bacterial isolates	29
2.5.2	DNA extraction from blood samples	30
2.6	Polymerase chain reaction (PCR)	31
2.6.1	Primers for multiplex PCR	31
2.6.2	Regular multiplex PCR	31
2.6.3	Nested multiplex PCR	32
2.6.4	Sensitivity of regular and nested PCR	32

2.6.5	PCR for <i>Salmonella</i>	32
2.7	Agarose gel electrophoresis	33
<b>PART B</b>	<b>Materials and methods for <i>Salmonella</i> O-specific polysaccharides (OSP) conjugate work with diphtheria toxoid (DT)</b>	<b>35</b>
2.8	Bacterial strains	35
2.9	Fermentation	35
2.9.1	Inoculum and growth	35
2.9.2	Killing and harvesting of bacteria	35
2.10	Phenol extraction of lipopolysaccharides (LPS) from <i>S. Typhi</i> and <i>S. Paratyphi A</i>	36
2.10.1	Estimation of nucleic acids contamination in polysaccharides	37
2.11	Acid hydrolysis of <i>S. Typhi</i> LPS to purify O-specific polysaccharides (OSP)	38
2.11.1	Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	39
2.11.2	Silver staining for detection of lipopolysaccharides after SDS-PAGE	39
2.11.3	Zinc-imidazole staining for detection of lipopolysaccharides after SDS-PAGE	40
2.11.4	Limulus Amebocyte Lysate (LAL) assay for determination of endotoxin level	41
2.12	Acid hydrolysis of <i>S. Paratyphi A</i> LPS to purify O-specific polysaccharides (OSP)	42
2.12.1	Anthrone assay	43
2.12.2	Immuno-diffusion assay	44
2.13	Derivatization of <i>S. Typhi</i> OSP with adipic acid dihydrazide (ADH)	45
2.13.1	Trinitrobenzene sulfonic acid (TNBS) assay for determination of hydrazide group	46

2.14	Derivatization of <i>S. Paratyphi</i> A OSP with adipic acid dihydrazide (ADH)	47
2.14.1	Hestrin assay for measurement of O-acetyl content	48
2.15	Diphtheria toxoid (DT) as a carrier protein for conjugation with <i>Salmonella</i> polysaccharides	49
2.15.1	Coomassie assay for protein measurement	49
2.16	Conjugation of <i>S. Typhi</i> OSP-AH with diphtheria toxoid	50
2.17	Conjugation of <i>S. Paratyphi</i> OSP with diphtheria toxoid	51
2.18	Conjugation of derivatized <i>S. Paratyphi</i> A OSP ( <i>S. Paratyphi</i> A OSP-AH) with diphtheria toxoid (DT)	52
2.19	High performance liquid chromatography (HPLC) analyses	53
2.20	Development of standard hyper immune sera against whole cell of <i>S. Typhi</i> and <i>S. Paratyphi</i> A in mice	54
2.21	Immunogenicity evaluation of the prepared conjugates through mice immunization	55
2.22	Immuno assay for determination of mice serum IgG antibody levels by enzyme linked immuno sorbant assay (ELISA)	56
2.22.1	Data analysis for calculation of titers and statistical analysis	59

## **CHAPTER 3: RESULTS**

<b>PART A</b>	<b>Results of multiplex PCR work</b>	61
3.1	<i>Salmonella</i> strains	61
3.1.1	Isolation of <i>Salmonella</i> strains	61
3.1.2	Biochemical identification of isolated <i>Salmonella</i> strains	61
3.2	Studies on patients	61
3.2.1	Blood culture of suspected typhoid patients	61
3.2.2	Serology	62
3.3	Polymerase chain reaction (PCR)	62
3.3.1	Regular multiplex PCR	62
3.3.2	Nested multiplex PCR	62

3.3.3	Sensitivity of regular and nested PCR	62
3.3.4	PCR of suspected typhoid patients	63
3.3.5	PCR for <i>Salmonella</i>	63
<b>PART B</b>	<b>Results of <i>Salmonella</i> O-specific polysaccharides (OSP) conjugate work with diphtheria toxoid (DT)</b>	<b>64</b>
3.4	Preparation of O-specific polysaccharides (OSP) from <i>Salmonella</i>	64
3.4.1	<i>Salmonella enterica</i> serovar Typhi ( <i>S. Typhi</i> ) OSP preparation	64
3.4.2	<i>Salmonella enterica</i> serovar Paratyphi A ( <i>S. Paratyphi A</i> ) OSP preparation	64
3.4.3	Quality control assays on extracted LPS and OSP samples from both of <i>S. Typhi</i> and <i>S. Paratyphi A</i>	65
3.5	Derivatization of <i>Salmonella</i> OSP with ADH	65
3.5.1	Derivatization of <i>S. Typhi</i> OSP with ADH	65
3.5.2	Derivatization of <i>S. Paratyphi A</i> OSP with ADH	66
3.5.3	Immuno diffusion assay of derivatized OSP samples	66
3.6	Conjugation of <i>S. Typhi</i> OSP-AH with DT	66
3.7	Conjugation of <i>S. Paratyphi A</i> OSP with DT	67
3.7.1	Conjugation of <i>S. Paratyphi A</i> OSP directly with DT without linker	67
3.7.2	Conjugation of derivatized <i>S. Paratyphi A</i> OSP-AH with DT	67
3.8	High performance liquid chromatography (HPLC) analyses	68
3.9	Development of high titer mice antisera (hyper immune sera) against <i>S. Typhi</i> and <i>S. Paratyphi A</i> LPS	68
3.10	Immunogenicity evaluation of the conjugates in mice	68
 <b>CHAPTER 4: DISCUSSION</b>		
4.1	Diagnosis	123
4.2	Vaccines	126

## **CHAPTER 5: REFERENCES**

References	131
------------	-----

## **APPENDICES**

1	Trypticase soy broth (TSB)	i
2	MacConkey agar	i
3	Nutrient agar	ii
4	Triple sugar iron (TSI) agar	ii
5	Salt-saturated phenol (SS phenol)	iii
6	Tris-borate-EDTA (TBE) buffer (5X)	iii
7	Phenol 90%	iv
8	Phosphate buffer saline (10X)	iv
9	Coating buffer for ELISA (1X PBS)	iv
10	Blocking buffer for ELISA	iv
11	Dilution buffer for ELISA	v
12	Washing buffer for ELISA	v
13	Substrate buffer for ELISA	v

## **PUBLICATIONS AND PRESENTATIONS**