

**ANTIBIOGRAM OF *SALMONELLA* SEROVARS FROM THE BLOOD
OF CLINICALLY SUSPECTED ENTERIC FEVER PATIENTS VISITING
ALKA HOSPITAL**

A

**DISSERTATION SUBMITTED TO THE
CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY
KIRTIPUR, KATHMANDU, NEPAL**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE AWARD OF DEGREE OF
MASTER OF SCIENCE IN MICROBIOLOGY
(Environment and Public Health)**

**BY
HARI JUNG CHAND
2013**

© TRIBHUVAN UNIVERSITY

RECOMMENDATION

This is to certify that **Mr. Hari Jung Chand** has completed this dissertation work entitled "**ANTIBIOGRAM OF *SALMONELLA* SEROVARS FROM THE BLOOD OF CLINICALLY SUSPECTED ENTERIC FEVER PATIENTS VISITING ALKA HOSPITAL**" as a partial fulfillment of the requirements of M. Sc. degree in Microbiology (Environment and Public Health) under our supervision. To our knowledge, this work has not been submitted for any other degree.

-----	-----	-----
Prof. Bharat Jha	Dr. Vijay Kumar Sharma	Mr. Komal Raj Rijal
Professor and Head	MD. M. Phil	Lecturer
Department of Biochemistry	(Clinical Biochemistry)	Central Department of Microbiology
Institute of Medicine, TUTH	Assistant Professor	Tribhuvan University
Maharajgunj, Kathmandu	Department of Biochemistry	Kirtipur, Kathmandu
Nepal	Institute of medicine, TUTH	Nepal
	Maharajgunj, Kathmandu	
	Nepal	

Date: -----

CERTIFICATE OF APPROVAL

On the recommendation of **Prof. Bharat Jha, Dr. Vijay Kumar Sharma** and **Mr. Komal Raj Rijal**, this dissertation work of **Mr. Hari Jung Chand** is approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for M. Sc. degree in Microbiology (Environment and Public Health).

Prof. Dr. Anjana Singh, PhD
Head of the Department
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu
Nepal

Date: -----

BOARD OF EXAMINERS

Recommended by:

.....

Prof. Bharat Jha

Supervisor

.....

Dr. Vijay Kumar Sharma

Supervisor

.....

Mr. Komal Raj Rijal

Supervisor

Approved by:

.....

Prof. Dr. Anjana Singh, PhD

Head of the Department

Examined by:

.....

Ms. Basudha Shrestha

Senior Microbiologist

Department of Pathology

Kathmandu Model Hospital

Exhibition road, Kathmandu

External Examiner

.....

Ms. Shaila Basnyat

Lecturer

Central Department Microbiology

Tribhuvan University

Internal Examiner

Date:

ACKNOWLEDGEMENT

It gives great pleasure to express my deepest gratitude to my respected supervisors, **Prof. Bharat Jha**, Professor and Head, **Dr. Vijay Kumar Sharma**, Assistant Professor, Department of Biochemistry, Institute of Medicine, TUTH, Maharajgunj, Kathmandu, Nepal and **Mr. Komal Raj Rijal**, Lecturer, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal for their valuable guidance, continued supervision, constructive suggestion and constant inspiration and encouragement throughout the research period that enabled me to accomplish this dissertation work.

I owe my deepest gratitude to **Prof. Dr. Anjana Singh** Head of the Department, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal for her continuous encouragement and guidance that enabled me to carry out this research work. Similarly I am thankful to my respected teachers and staffs of Central Department of Microbiology for their generosity, constant inspiration, valuable guidance and moral support.

I heartily thank **Mr. Krishna Thapa**, Director of Alka Hospital, for providing me the opportunity to use laboratory facilities of the Alka Hospital and wish to thank all the laboratory staffs of the hospital for their kind support. I am indebted to my all friends and other individuals who help in one or another way with their abilities to accomplish this research work.

Especially I would like to express my profound gratitude to my father, **Mr. Top Jung Chand** and my mother, **Mrs. Uma Chand** for their endless love, continued support, and encouragement throughout my life to achieve the present academic degree. My brother **Mr. Sushil Chand** and sister **Ms. Gita Chand**, other family members and relatives deserve wholehearted thanks as well.

Date: -----

Hari Jung Chand

ABSTRACT

Enteric fever is endemic in Nepal having a significant public health burden and includes typhoid fever caused by *Salmonella* serotype Typhi and paratyphoid fever caused by *Salmonella* serotypes Paratyphi A, B, and C. A cross sectional study was carried out in clinically suspected enteric fever patients visiting Alka Hospital during July 2011 to February 2012 to determine the prevalence of enteric fever and antibiogram pattern of *Salmonella* isolates.

Out of 1202 blood samples collected, only 86 (7.2%) showed positive blood culture result among which 56 (65.1%) isolates were *Salmonella* Typhi and 30 (34.9%) isolates were *Salmonella* Paratyphi A. Prevalence of enteric fever was higher in female patients (40/542; 7.4%) than in male patients (46/660; 7.0%), in age group 11-21 years (28/220; 12.7%), and in the months of July and August. *Salmonella* Typhi isolates were 100% sensitive to chloramphenicol, cotrimoxazole, cefixime, ceftriaxone, ciprofloxacin and gentamicin followed by 98.2% sensitive to amoxicillin, ofloxacin and 89.3% sensitive to azithromycin. Similarly *Salmonella* Paratyphi A isolates were 100% sensitive to amoxicillin, cotrimoxazole, ceftriaxone and gentamicin followed by 96.7% sensitive to chloramphenicol, ciprofloxacin, ofloxacin, cefixime and 86.7% sensitive to azithromycin. None of the *Salmonella* isolates were multi drug resistant (MDR). About 91.1% *S.* Typhi and 90.0% *S.* Paratyphi A isolates were nalidixic acid resistant (NAR). None of the nalidixic acid resistant *S.* Typhi were ciprofloxacin resistant whereas 1 (3.7%) nalidixic acid resistant *S.* Paratyphi A isolate was resistant to ciprofloxacin. This study revealed the increasing frequency of nalidixic acid resistant *Salmonella* isolates and re-emergence of susceptibility to conventional first line drugs.

Keywords: Enteric fever, Multi drug resistant (MDR), Nalidixic acid resistant, *Salmonella*

TABLE OF CONTENTS

Title Page	i
Recommendation	ii
Certificate of Approval	iii
Board of Examiners	iv
Acknowledgement	v
Abstract	vi
Table of Contents	vii
List of Tables	x
List of Figures	xi
List of Photographs	xii
List of Appendices	xi
List of Abbreviations	xiv
CHAPTER-I INTRODUCTION AND OBJECTIVES	1-5
1.1 Introduction	1
1.2 Objectives	5
1.2.1 General Objective	5
1.2.2 Specific Objectives	5
CHAPTER-II LITERATURE REVIEW	6-24
2.1 Enteric Fever	6
2.2 History of enteric fever	6
2.3 Epidemiology of Enteric Fever	7
2.4 Enteric Fever in Nepal	8
2.5 Etiological Agent of Enteric Fever	9
2.5.1 Genus <i>Salmonella</i>	9
2.5.2 Taxonomy and Nomenclature	9
2.5.3 Habitat	10
2.5.4 Antigenic structure of <i>Salmonella</i> sps.	11

2.5.5	Kaufmann-White Scheme Classification	13
2.5.6	Cultural and Biochemical Characteristics	13
2.6	Transmission of Enteric Fever	14
2.7	Risk factors of Enteric Fever	14
2.8	Pathogenesis of enteric fever	15
2.9	Clinical manifestation of enteric fever	16
2.10	Laboratory diagnosis of enteric fever	17
2.10.1	Culture	18
2.10.2	Serological diagnosis	19
2.11	Antibiotic susceptibility test	21
2.12	Treatment, prevention and control	22
2.13	Antimicrobial susceptibility pattern	22
 CHAPTER-III MATERIALS AND METHODS		25-29
3.1	Materials	25
3.2	Inclusion and exclusion criteria	25
3.3	Methods	25
3.3.1	Blood specimen collection	25
3.3.2	Processing of blood specimen	25
3.3.3	Identification of <i>Salmonella</i> serotypes	26
3.3.4	Purity plate	27
3.3.5	Serotyping	27
3.3.6	Antibiotic susceptibility test	27
3.3.7	Quality control	27
3.3.8	Data analysis	28
3.4	Flowchart for methodology	29
 CHAPTER-IV RESULTS		30-38
4.1	Culture positivity	30
4.2	Age wise distribution of blood culture positive cases	31
4.3	Distribution pattern of bacterial isolates	32
4.4	Distribution of <i>Salmonella</i> isolates	32
4.5	Seasonal distribution of <i>Salmonella</i> isolates	34
4.6	Antibiogram of <i>Salmonella</i> Typhi	35
4.7	Antibiogram of <i>Salmonella</i> Paratyphi A	36

4.8	Ciprofloxacin susceptibility pattern of nalidixic acid resistant <i>Salmonella</i> isolates	38
CHAPTER-V DISCUSSION		39-48
CHAPTER-VI CONCLUSION AND RECOMMENDATIONS		49-50
5.1	Conclusion	49
5.2	Recommendations	50
CHAPTER-VII REFERENCES		51-61
APPENDICES		I-XVIII

LIST OF TABLES

- Table 1: Gender wise distribution of blood culture positive cases
- Table 2: Age wise distribution of blood culture positive cases
- Table 3: Age and gender wise distribution of *Salmonella* Typhi
- Table 4: Age and gender wise distribution of *Salmonella* Paratyphi A
- Table 5: Antibiotic susceptibility pattern of *Salmonella* Typhi
- Table 6: Antibiotic susceptibility pattern of *Salmonella* Paratyphi A
- Table 7: Ciprofloxacin susceptibility pattern of nalidixic acid resistant *Salmonella* isolates.

LIST OF FIGURES

- Figure 1: Proportion of *Salmonella* serovars in positive samples
- Figure 2: Seasonal distribution of *Salmonella* isolates during July 2011 to February 2012

LIST OF PHOTOGRAPHS

- Photograph 1: Non lactose fermenting colonies of *Salmonella* Typhi on MacConkey agar
- Photograph 2: Biochemical tests for *Salmonella* Typhi
- Photograph 3: Antimicrobial susceptibility test for *Salmonella* Typhi
- Photograph 4: Antimicrobial susceptibility test for *Salmonella* Typhi

LIST OF APPENDICES

- Appendix A Clinical and microbiological profile of the patients
 - A. Personal information
 - B. Clinical manifestation
 - C. Microbiological profile
- Appendix B List of equipment, materials and supplies
 - A. Equipment
 - B. Chemicals and reagents
 - C. Antibiotics discs
 - D. Miscellaneous
- Appendix C
 - A. Composition and preparation of different types of culture media
 - B. Composition and preparation of different types of biochemical media
 - C. Composition and preparation of different reagents
- Appendix D Procedure for blood specimen collection
- Appendix E Slide agglutination test for identification of *Salmonella* spp.
- Appendix F
 - A. Procedure of antibiotic sensitivity test (Kirby-Bauer's disc diffusion method).
 - B. Zone size interpretive chart for antibiotics as per CLSI guideline
 - C. Antibiotic susceptibility pattern of *Salmonella* Typhi
 - D. Antibiotic susceptibility pattern of *Salmonella* Paratyphi A
- Appendix G Data analysis (Chi square test)

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ASM	American Society of Microbiology
ATCC	American Type Culture Collection
BA	Blood Agar
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standards Institute
DOHS	Department of Health Services
ESBL	Extended Spectrum Beta Lactamase
HIV	Human Immunodeficiency Virus
MA	MacConkey Agar
MIC	Minimum Inhibitory Concentration
MDR	Multi Drug Resistant
MDRST	Multi Drug Resistant <i>Salmonella</i> Typhi
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MR	Methyl Red
NA	Nutrient Agar
NB	Nutrient Broth
NAR	Nalidixic Acid Resistant
NARST	Nalidixic acid resistant <i>Salmonella</i> Typhi
NPHL	National Public Health Laboratory
QRDR	Quinolones Resistant-Determining Chromosomal Region
SIM	Sulphide Indole Motility
SPIs	Salmonella pathogenicity islands
SPS	Sodium Polyanethol Sulphonate
SPSS	Statistical Package for Social Science
TMP-SMX	Trimethoprim-sulfamethoxazole
TPD	Tetramethyl <i>p</i> -phenylene diamine dihydrochloride
TSI	Triple Sugar Iron Agar
TUTH	Tribhuvan University Teaching Hospital
VP	Voges-Proskauer
WHO	World Health Organization