

**COMPARATIVE EVALUATION OF MICROSCOPIC AND
CULTURAL EXAMINATION IN BACTERIAL
MENINGITIS AMONG THE PATIENTS ATTENDING
KANTI CHILDREN HOSPITAL**

**A
DISSERTATION
SUBMITTED TO THE CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD
OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY
(MEDICAL)**

**BY
ROJITA TULADHAR**

**CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY
KIRTIPUR, KATHMANDU, NEPAL**

2007

RECOMMENDATION

This is to certify that **Miss Rojita Tuladhar** has completed this dissertation work entitled “**COMPARATIVE EVALUATION OF MICROSCOPIC AND CULTURAL EXAMINATION IN BACTERIAL MENINGITIS AMONG THE PATIENTS ATTENDING KANTI CHILDREN HOSPITAL**” as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To our knowledge, this work has not been submitted for any other degree.

Dr. Aparna Singh Shah

Assistant Professor

Dept. of Clinical Microbiology

Chief, Foreign Students Cell

Institute of Medicine, TUTH,

Kathmandu

Prof. Dr. Bharat Mani Pokhrel

Head of Department

Department of Microbiology

Institute of Medicine, TUTH,

Kathmandu and

Academician

Nepal Academy of Science and

Technology (NAST)

Ms. Shaila Basnyat

Assistant Professor

Central Department of Microbiology

Tribhuvan University, Kathmandu

Date:

CERTIFICATE OF APPROVAL

On the recommendation of **Ms. Shaila Basnyat, Prof. Dr. Bharat Mani Pokhrel and Dr. Aparna Singh Shah**, this dissertation work of **Miss Rojita Tuladhar**, entitled **“COMPARATIVE EVALUATION OF MICROSCOPIC AND CULTURAL EXAMINATION IN BACTERIAL MENINGITIS AMONG THE PATIENTS ATTENDING KANTI CHILDREN HOSPITAL ”** has been approved for the examination and is submitted to the Tribhuvan University in the Partial fulfillment of the requirements for **Master of Science Degree in Microbiology (Medical)**.

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

Date:

BOARD OF EXAMINERS

Recommended by:

Ms. Shaila Basnyat
Supervisor

Prof. Dr. Bharat Mani Pohkhrel
Supervisor

Dr. Aparna Singh Shah
Supervisor

Approved by:

Dr. Anjana Singh
Head of Department

Examined by:

Dr. Basista Rijal, MBBS, M.Phil.
Associate Professor, TUTH
External Examiner

Mr. Binod Lekhak
Assistant Professor, CDM, TU
Internal Examiner

Date:

ACKNOWLEDGEMENT

I am extremely grateful to my respected supervisor **Prof. Dr. Bharat Mani Pokhrel, Ph. D. Post Dr. Fellow (Fullbright), Head of Department, Department of Microbiology, Institute of Medicine, TUTH** for his constant inspiration, proper guidance and valuable suggestions during this research work. Without his help and guidance, this research work wouldn't have completed. I am also equally thankful to **Dr. Aparna Singh Shah, Assistant Professor, Department of Clinical Microbiology, Institute of Medicine, TUTH** for her constructive suggestion, proper guidance and incredible support from the beginning of this research work.

I would also like to express my sincere gratitude to **Ms. Shaila Basynat, Assistant Professor, Central Department of Microbiology, Tribhuvan University** for her proper guidance, invaluable suggestions and advices which has helped a lot for the completion of this research.

I am also obliged to honorable **Dr. Anjana Singh, Head of the Department, Central Department of Microbiology, Dr. Prakash Ghimire, Prof. Dr. Sheetal Raj Basnyat, Mr. Binod Lekhak** and all the teachers and staffs of Central Department of Microbiology for their support and generosity.

I heartily thank **Prof. Nhucche Ratna Tuladhar, Institute of Medicine, TUTH** for providing me the opportunity to work in Laboratory of Kanti Children Hospital.

I would also like to thank **Mrs. Maiya Thapa, Laboratory Incharge, Kanti Children Hospital**, for providing me the facilities in the laboratory during the whole research work. I am particularly indebted to **Mr. Samsheer Nepali** who has given me invaluable help, support and generosity during my research work. I am also thankful to **Mrs. Gyani Singh, Mrs. Ishowri Nepali, Mr. Purna Kumar Shrestha** and all the staffs of Kanti Children Hospital for creating such a friendly environment and for their help and cooperation during the laboratory work.

I must extend my sincere gratitude to **Ms. Punita Gauchan, Health Research Officer** whose love, help, guidance and suggestions added a lot in the completion of this thesis. I would also like to acknowledge **Dr. Shailesh Manadhar** and **Dr. Prabesh Shrestha** for their silent help.

It is a pleasure to acknowledge my debt to my friend **Deena Shrestha** whose support and cooperation from the beginning of this thesis is remarkable. I am also thankful to all my friends especially **Sujata Lamichhane, Padma Shrestha, Kamil Prajapati, Nisha Puri, Bikash Shakya and Anup Muni Bajracharya** for their constant support and suggestions.

My acknowledgement goes to my brother **Manish Kansakar** and my sister **Rojina Tuladhar** who have been supportive throughout thesis work and assisted me during the computer settings.

My acknowledgement would be incomplete without **my parents** whose moral support, inspiration and encouragement have made me the person I am today. Last but not the least, I would like to thank all the patients who provided the CSF samples for the success of this project.

Rojita Tuladhar

Date:

ABSTRACT

Present study was conducted in Kanti Children's Hospital, Kathmandu, Nepal among the children suspected of meningitis from June 2006 to September 2006. The main aim of this study was to compare and correlate the microscopic findings of CSF samples with the cultural findings and to find out the incidence of bacterial meningitis among the children visiting Kanti Children's Hospital.

In this study, a total of 431 CSF samples were studied. These samples were subjected for microscopic investigations, biochemical investigation and also cultured on appropriate media.

In this study, out of 431 CSF samples, only 21 (4.87%) showed positive culture result. Gram negative isolates were found to be predominant among the study group (52.58%). Highest percentage of the isolates (33.33%) was obtained from the age group of 0-45days (neonates) and 45d-1 year.

Among the isolates, *Escherichia coli* and *Staphylococcus aureus* were found to be most predominant (28.57% each) followed by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (9.53% each). Only one (4.76%) *Neisseria meningitidis* was isolated during this study.

Higher percentage of isolates (85.71%) was obtained from CSF having cell count more than 100 cells/mm³. The association of the cell count with the culture result was found to be statistically significant (P<0.05). Similarly, Gram stain of CSF samples detected more organisms (7.89%) than culture (4.87%). The sensitivity and specificity of the Gram stain was found to be 100% and 96.82% respectively. Highest percentage of isolates was obtained from the CSF with low glucose level (95.23%) and high protein level (95.23%).

Antibiotic susceptibility pattern of the isolates showed that most of the isolates were susceptible to Chloramphenicol (85.71%). Ceftriazone was found to be resistant among most of the Gram negative isolates.

Key words: Meningitis, CSF (Cerebrospinal fluid), cell count, Gram Stain.

TABLE OF CONTENTS

Title Page	i
Recommendation	ii
Certificate of Approval	iii
Board of Examiners	iv
Acknowledgement	v
Abstract	vii
Table of Contents	viii
List of Abbreviations	xi
List of Tables	xiii
List of Figures	xiv
List of Photographs	xv
List of Appendices	xvi
CHAPTER I INTRODUCTION	1-4
CHAPTER II OBJECTIVES	5
2.1 General Objective	5
2.2 Specific Objectives	5
CHAPTER III LITERATURE REVIEW	6-46
3.1 Meningitis as a Disease	6
3.2 Anatomy and Physiology	6
3.2.1 Anatomy of Meninges	6
3.2.2 Blood Brain Barrier	7
3.2.3 Ventricles of Brain	8
3.2.4 Cerebrospinal Fluid	8
3.3 Possible Pathogens	13
3.4 Routes of Infection	14
3.5 Pathogenesis	15
3.6 Clinical Manifestations	18

3.7 Types of Meningitis	20
3.7.1 Acute Pyogenic (purulent) Meningitis	20
3.7.2 Aseptic Meningitis	21
3.7.3 Tuberculous Meningitis (TBM)	23
3.7.4 Fungal Meningitis	25
3.7.5 Neurosyphilis	27
3.8 Bacterial Meningitis	29
3.8.1 Meningococcal Meningitis	30
3.8.2 <i>Haemophilus influenzae</i> Meningitis	31
3.8.3 Pneumococcal Meningitis	32
3.8.4 Meningitis caused by other Gram Positive Organism	33
3.8.5 Meningitis caused by other Gram Negative Organism	34
3.9 Laboratory Investigations of Meningitis	35
3.10 Global Scenario of Meningeal Infection	40
3.11 National Scenario of Meningeal Infection	44
CHAPTER IV MATERIALS AND METHODS	47-53
4.1 Materials	47
4.2 Methods	47
4.2.1 Collection of Samples	47
4.2.2 Sample Processing	47
4.2.3 Macroscopic Examination	47
4.2.4 Cytological Examination	48
4.2.5 Estimation of Sugar and Protein	49
4.2.6 Microscopic Examination	49
4.2.7 Culture	50
4.2.8 Identification of Isolate	50
4.2.9 Antibiotic Susceptibility Pattern	52
4.2.10 Quality Control	52
4.2.11 Data Analysis	53

CHAPTER V RESULTS	54-67
CHAPTER VI DISCUSSION AND CONCLUSION	68-75
6.1 Discussion	68
6.2 Conclusion	75
CHAPTER VII SUMMARY AND RECOMMENDATIONS	76-78
7.1 Summary	76
7.2 Recommendations	77
REFERENCES	79-89
APPENDICES	I-XXIV

LIST OF ABBREVIATIONS

ABM	:	Acute Bacterial Meningitis
AFB	:	Acid Fast Bacilli
AIDS	:	Acquired Immune Deficiency Syndrome
BBB	:	Blood Brain Barrier
BCG	:	Bacillus Calmette- Guerin
CDC	:	Center for Disease Control and Prevention
CFR	:	Case Fatality Rates
CIE	:	Counter Current Immunoelectrophoresis
cm	:	Centimeter
CMV	:	Cytomegalovirus
CNS	:	Central Nervous System
CO ₂	:	Carbondioxide
CRP	:	C-reactive Protein
CSF	:	Cerebrospinal Fluid
DIC	:	Disseminated Intravascular Coagulation
dl	:	Deciliter
ELISA	:	Enzyme Linked Immunosorbent Assay
GBS	:	Group B Streptococci
GI	:	Gastrointestinal Tract
Hib	:	<i>Haemophilus influenzae</i> type b
HIV	:	Human Immuno Deficiency Virus
HSV	:	Herpes Simplex Virus
IgA	:	Immunoglobulin A
IL-1	:	Interleukin-1
IV	:	Intravenous
JAMA	:	Journal of the American Medical Association
L3	:	Third Lumbar Spine
L4	:	Fourth Lumbar Spine
L5	:	Fifth Lumbar Spine
LAT	:	Latex Agglutination Test
LDH	:	Lactate Dehydrogenase

MA	:	MacConkey Agar
µl	:	Microliter
mg	:	Milligram
ml	:	Milliliter
mm	:	Millimeter
mm ³	:	Cubic millimeter
MHA	:	Mueller Hinton Agar
MHBA	:	Mueller Hinton Blood Agar
MR	:	Methyl Red
NA	:	Nutrient Agar
No.	:	Number
NPV	:	Negative Predictive Value
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
PPV	:	Positive Predictive Value
RBC	:	Red Blood Cells
rpm	:	Revolution Per Minute
SDA	:	Sabouraud Dextrose Agar
SIADH	:	Syndrome of Inappropriate Antidiuretic Hormone
SIM	:	Sulphide Indole Motility
TB	:	Tuberculosis
TBM	:	Tuberculous Meningitis
TSI	:	Triple Sugar Iron
TUTH	:	Tribhuvan University Teaching Hospital
UK	:	United Kingdom
US	:	United States
VDRL	:	Veneral Disease Research Laboratory
VP	:	Voges Proskauer
VZV	:	Varicella Zooster Virus
WBC	:	White Blood Cells
WHO	:	World Health Organization
ZN	:	Ziehl-Neelsen

LIST OF TABLES

Table 5.1: Age and gender wise distribution of the patients suspected of meningitis

Table 5.2: Pattern of CSF culture results

Table 5.3: Gender wise distribution of the no. of bacterial isolates from CSF samples

Table 5.4: Age and gender wise distribution of no. of bacterial isolates from CSF samples

Table 5.5: Pattern of bacterial isolates from the CSF positive cultures

Table 5.6: Gender wise distribution of bacterial isolates from CSF samples

Table 5.7: Age wise distribution of types of bacterial isolates from CSF samples

Table 5.8: Correlation of the no. of isolates with the appearance of CSF

Table 5.9: Correlation of the no. of isolates with cell count of CSF

Table 5.10: Correlation of no. of bacterial isolates with the protein level of CSF samples

Table 5.11: Correlation of no. of bacterial isolates with the Glucose level of CSF samples

Table 5.12: Correlation of Gram staining with the no. of isolates of CSF samples

Table 5.13: Antibiotic susceptibility pattern of individual Gram Negative organism isolated from CSF samples

Table 5.14: Antibiotic susceptibility pattern of Gram positive organism isolated from CSF samples

LIST OF FIGURES

- Figure 1:** Age and gender wise distribution of patients suspected of meningitis
- Figure 2:** Pattern of CSF culture results
- Figure 3:** Gender wise distribution of isolates from CSF samples
- Figure 4:** Age and gender wise distribution of no. of bacterial isolates from CSF samples
- Figure 5:** Pattern of bacterial isolates from CFS positive cultures
- Figure 6:** Correlation of appearance of CSF with culture result
- Figure 7:** Correlation of cell count with culture result
- Figure 8:** Correlation of Protein level of CSF with culture result
- Figure 9:** Correlation of Glucose level of CSF with culture result
- Figure10:** Correlation of Gram stain with culture result

LIST OF PHOTOGRAPHS

Photograph 1: Collection of CSF sample

Photograph 2: Gram stain result of CSF sample showing Gram positive diplococci with pus cells

Photograph 3: Gram stain result of CSF sample showing Gram negative rods with pus cells

Photograph 4: *Streptococcus pneumoniae* colonies on chocolate agar with zone of inhibition around Optochin discs (ethyl hydrocupreine hydrochloride)

Photograph 5: Antimicrobial susceptibility test for *Streptococcus pneumoniae* on MHBA

Photograph 6: Satellitism of *Haemophilus influenzae* with *Staphylococcus* colonies on blood agar

Photograph 7: Antimicrobial susceptibility test for *Haemophilus influenzae* on MHBA

Photograph 8: Antimicrobial susceptibility test for *Neisseria meningitidis* on MHBA

Photograph 9: Investigator performing the laboratory work

LIST OF APPENDICES

- Appendix-I** List of the equipments and materials used during the study
- Appendix-II** A. Composition and preparation of different culture media
B. Composition and preparation of different biochemical media
C. Composition and preparation of different staining and test reagents
- Appendix-III** Gram staining procedure
- Appendix-IV** Ziehl-Neelsen staining procedure
- Appendix-V** Methodology of biochemical test used for the identification of bacteria
- Appendix-VI** Morphological and cultural characteristic of bacteria isolated from CSF sample
- Appendix-VII** Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae
- Appendix-VIII** Zone size interpretation chart
- Appendix-IX** Zone size interpretation chart of *Streptococcus pneumoniae* and *Haemophilus influenzae*
- Appendix-X** Calculation of Sensitivity, Specificity, Positive and Negative Predictive values and Efficiency
- Appendix-XI** Data Analysis (Chi square test)
- Appendix- XII** Guidelines for the interpretation of results following Haematological and Chemical analysis of CSF from children and adults