STUDY OF BACTERIA CAUSING URINARY TRACT INFECTION AND THEIR ANTIMICROBIAL RESISTANCE TREND AT NATIONAL PUBLIC HEALTH LABORATORY

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 PADMA SHRESTHA

CENTRAL DEPARTMENT OF MICROBIOLOGY TRIBHUVAN UNIVERSITY KIRTIPUR, KATHMANDU, NEPAL 2007

RECOMMENDATION

This is to certify that **Miss PADMA SHRESTHA** has completed this dissertation work entitled "**Study of bacteria causing urinary tract infection and their antimicrobial resistance trend at National Public Health Laboratory**" 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.

Ms. Shaila Basnyat Assistant Professor Central Department of Microbiology Tribhuvan University Kirtipur, Kathmandu Prof. Dr. Sarala Malla, M.D. Director General Department of Health Services Ministry of Health and Population and Coordinator M. D. Pathology Subject Committee NAMS, Bir Hospital

Date:

CERTIFICATE OF APPROVAL

On the recommendation of Ms. Shaila Basnyat and Prof. Dr. Sarala Malla, this dissertation work of Miss Padma Shrestha, entitled "Study of bacteria causing urinary tract infection and their antimicrobial resistance trend at National Public Health Laboratory" 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. Sarala Malla Supervisor

Approved by:

Examined by:

Dr. Anjana Singh Head of the Department

Dr. Chandrika Shrestha Chief Consultant Pathologist Bir Hospital External Examiner

> Mr. Binod Lekhak Assistant Professor Internal Examiner

Date:

ACKNOWLEDGEMENT

It gives me immense pleasure to express my heartfelt appreciation to all the people who helped me in one way or another to complete this research work.

I would like to express my sincere gratitude and heartfelt appreciation to my respected supervisor **Prof. Dr. Sarala Malla, Director General, Department of Health Services** for her inspiration, proper guidance, invaluable suggestions and tremendous support during this research work. I am equally indebted to my internal supervisor, **Ms. Shaila Basynat**, Assistant Professor of Central Department of Microbiology, Tribhuvan University for her valuable suggestions, continuous guidance and tremendous support in completion of this project work.

I am much obliged to honorable **Dr. Anjana Singh, Head of the Department, Central Department of Microbiology, Dr. Prakash Ghimire, Prof. Dr. Sheetal Raj Basnyat** and all the teachers and staffs of Central Department of Microbiology, Tribhuvan University, all of whom have been very generous and motivating.

I am indebt to **Mrs. Palpasa Kansakar, Mr. Bishnu Raj Upadhya**, **Mr. Gokarna Raj Ghimire** and **Mr. Shyam Prakash Dumre** for their overwhelming support during the whole laboratory work at National Public Health Laboratory. I am also thankful to **Mr. Purna Kumar Shrestha** and all staffs of National Public Health Laboratory for their kind help and cooperation during laboratory analysis.

I wish to express my admiration and special thanks to all my friends especially **Sujata** Lamichane, Shova Khanal and Rojita Tuladhar for their helpful supportive contributions and excellent companionship.

I wish to reiterate my acknowledgement to my brother **Ashim Shrestha** for his help during computer settings.

Finally, I admire my parents for their silent inspiration, encouragement and moral support without whom this work would not have been possible and last but not the least, the patients who provided the urine samples for the success of this project.

Date:

Padma Shrestha

ABSTRACT

The present study was conducted at National Public Health Laboratory, Teku from May to September 2006 with the objectives to isolate bacteria causing urinary tract infection and to determine their antimicrobial resistance trend. During this period, three hundred and fifty two mid-stream urine samples collected were investigated by conventional semi-quantitative culture technique, microscopy and antibiotic susceptibility test.

Only 22.7% (80/352) of the samples showed significant bacterial growth. Status of bacteriuria was found higher in females (29.8%) than in males (15.2%). Association of significant bacteriuria and gender of patients was found to be statistically significant (P<0.05). Status of bacteriuria was found higher in age group 21-30 (21.6%) followed by 31-40 (18.8%).

Altogether 11 different bacteria were isolated among which *Escherichia coli* (48.8%) was found the most predominant organisms followed by *Klebsiella pneumoniae* (18.8%), *Proteus mirabilis* (7.5%), *Proteus vulgaris* (6.3%), Coagulase-negative Staphylococci (5.0%), *K. oxytoca* (3.8%), *Enterobacter* spp. (3.8%), *Citrobacter freundii* (2.5%), *Acinetobacter* spp. (1.3%), *Alcaligenes* spp. (1.3%) and *Staphylococcus aureus* (1.3%).

Predictors concerning pus cell count (5/HPF) and RBC count (3/HPF) were analyzed to determine the positive predictive value (PPV) in relation to the significant bacteriuria. Positive predictive value for pus cell count was found to be higher (77.8%) than that of RBC count (39.6%).

Gram negative bacilli showed best susceptibility towards gentamicin (80.0%) followed by ceftriazone (76.0%) whereas ampicillin was found out to be the least effective drug. Nitrofurantoin (100.0%) was found to be the most effective against Gram positive bacteria.

Multidrug resistance (MDR) was observed in 45.0% (36/80) of total bacterial isolates. Multidrug resistance was found to be 51.3% (20/39) in *E. coli* and that in *Klebsiella pneumoniae* was 33.3 % (5/15). Higher rate of MDR was found in males (57.7%, 15/26) than in females (38.8%, 21/54). Among the MDR *E. coli* isolates, 100.0%, 90.0% and 65.0% were resistant to ampicillin, norfloxacin and cotrimoxazole respectively. Among the MDR *K. pneumoniae* isolates, 100.0% were resistant to ampicillin, cotrimoxazole and norfloxacin.

Key words: bacteriuria, urinary tract infection, mid-stream urine, pyuria, multidrug-resistance

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 Abbreviations	X
List of Tables	xii
List of Figures	xiii
List of Photographs	xiv
List of Appendices	XV
CHAPTER I INTRODUCTION	1-3
CHAPTER II OBJECTIVES	4
2.1 General Objective	4
2.2 Specific Objectives	4
CHAPTER III LITERATURE REVIEW	5-32
3.1 Urinary tract infection	5
3.2 Pathogenesis related to UTI	6
3.3 Predisposing factors to UTI	6
3.4 Etiological agents of UTI	10
3.5 Categorization of UTI	11-14
3.5.1 Primary or Recurrent UTIs	11
3.5.2 Uncomplicated and complicated UTIs	11
3.5.3 Classification based on source of infection	13
3.5.4 Classification based on symptoms and levels of infection	14

3.6 Laboratory diagnosis of UTI	14-19
3.6.1 Methods of specimen collection and transport	15
3.6.2 Screening procedures	16
3.6.3 Urinalysis	16
3.6.4 Chemical examination of urine	17
3.6.5 Bacteriological examination of urine	18
3.6.6 Antibiotic susceptibility testing	18
3.7 Bacterial resistance to antibiotics	19-23
3.8 Global Scenerio	23-29
3.9 Nepalese Scenerio	29-32
CHAPTER IV MATERIALS AND METHODS	33-37
4.1 Materials	33
4.2 Methods	33
4.2.1 Data collection	33
4.2.2 Specimen collection	33
4.2.3 Specimen evaluation	33
4.2.4 Macroscopic examination	34
4.2.5 Microscopic examination	34
4.2.6 Chemical examination	34
4.2.7 Culture of specimen	34
4.2.8 Identification of isolates	35
4.2.9 Antibiotic susceptibility test	36
4.2.10 Quality control	37
4.2.11 Data analysis	37
CHAPTER V RESULTS	38-51
5.1 Clinical Pattern of Results	38-39

5.3 Antibiotic susceptibility pattern of the bacterial isolates	44-49

39-44

5.2 Microbial Pattern of Results

5.4 Analysis of MDR isolates	50-51
CHAPTER VI DISCUSSION AND CONCLUSION	52-62
6.1 Discussion	52
6.2 Conclusion	62
CHAPTER VII SUMMARY AND RECOMMENDATIONS	63-64
7.1 Summary	63
7.2 Recommendations	64
REFERENCES	65-74
APPENDICES	i-xxvi

LIST OF ABBREVIATIONS

μg	:	Microgram
A/A	:	Acid/ Acid
Alk/A	:	Alkali/ Acid
AUC	:	Acute Uncomplicated Cystitis
BA	:	Blood Agar
CA-UTI	:	Community Acquired Urinary Tract Infection
CFU	:	Colony Forming Units
CoNS	:	Coagulase Negative Staphylococci
DNA	:	Deoxyribonucleic Acid
DoHS	:	Department of Health Services
EC	:	European Commission
ESBL	:	Extended spectrum beta-lactamases
GISA	:	Glycopeptide-intermediate Staphylococcus aureus
Gm	:	Gram
H_2S	:	Hydrogen Sulphide
HPF	:	High power field
Hrs	:	Hours
LF	:	Lactose fermenting
MA	:	MacConkey agar
MDR	:	Multidrug Resistance
MHA	:	Mueller Hinton Agar
MIC	:	Minimum Inhibitory Concentration
Min	:	Minutes
ml	:	Milliliter
MoPH	:	Ministry of Public Health
MR	:	Methyl Red
MRSA	:	Methicillin-resistant Staphylococcus aureus
MSU	:	Mid-stream urine
NA	:	Nutrient agar

NCCLS	:	National Committee for Clinical Laboratory Standards
NLF	:	Non-lactose fermenting
No.	:	Number
NPHL	:	National Public Health Laboratory
NPV	:	Negative Predictive Value
PABA	:	Para-amino benzoic acid
PBP	:	Penicillin binding protein
PNSSP	:	Penicillin Non-Susceptible Streptococcus pneumoniae
PPV	:	Positive Predictive Value
RBC	:	Red Blood Cells
RNA	:	Ribonucleic Acid
rpm	:	revolution per minute
RS	:	Renal Stone
SIM	:	Sulphide Indole Motility
TMP/SMX	:	Trimethoprim-Sulphamethoxazole
TSI	:	Triple Sugar Iron
TUTH	:	Tribhuvan University Teaching Hospital
UK	:	United Kingdom
UPEC	:	Uropathogenic Escherichia coli
US	:	United States
UTI	:	Urinary Tract Infection
VP	:	Voges Proskauer
VRE	:	Vancomycin-resistant Enterococcus
VUR	:	Vesicoureteral Reflux
WBC	:	White Blood Cells
WHO	:	World Health Organization

LIST OF TABLES

Table 1 : Age and gender wise distribution of patients requested for urine culture	38
Table 2 : Gender wise distribution of type of patients requested for urine culture	39
Table 3 : Pattern of urine culture results	39
Table 4 : Gender wise distribution of urine culture results	40
Table 5 : Age and gender wise distribution of significant bacteriuria	40
Table 6 : Pattern of urine culture results according to type of patients	41
Table 7 : Gender wise distribution of bacterial isolates	42
Table 8 : Correlation of pyuria with culture result	43
Table 9 : Correlation of haematuria with culture result	43
Table 10 : Correlation of albuminuria with culture result	44
Table 11 : Antibiotic susceptibility pattern of Gram negative bacteria	44
Table 12 : Antibiotic susceptibility pattern of Gram positive bacteria	45
Table 13 : Antibiotic susceptibility pattern of bacterial isolates causing UTI	46
Table 14 : Resistance Pattern and distribution of MDR bacterial isolates	50
Table 15 : Gender wise distribution of MDR strains	51
Table 16 : Antibiotic resistance pattern of MDR E. coli isolates	51

LIST OF FIGURES

- Figure 1: Flow chart showing processing of urine sample
- Figure 2: Age and gender wise distribution of patients requested for urine culture
- Figure 3: Pattern of urine culture results
- Figure 4: Pattern of urine culture results in male and female patients
- Figure 5: Pattern of urine culture results in asymptomatic and symptomatic patients
- Figure 6: Percentage distribution of bacterial isolates causing UTI
- Figure 7: Distribution of bacterial isolates in male and female patients
- Figure 8: Correlation of pyuria with culture result
- Figure 9: Correlation of haematuria with culture result
- Figure 10: Correlation of albuminuria with culture result
- Figure 11: Antibiotic susceptibility pattern of Gram negative bacteria
- Figure 12: Antibiotic susceptibility pattern of Gram positive bacteria
- Figure 13: Distribution of MDR strains among bacterial isolates
- Figure 14: Gender wise distribution of MDR strains
- Figure 15: Antibiotic resistance pattern of MDR E. coli isolates
- Figure 16: Antibiotic resistance pattern of MDR K. pneumoniae isolates

LIST OF PHOTOGRAPHS

- Photograph 1: Pure culture of *Escherichia coli* isolated on MacConkey agar plate
- Photograph 2: Pure culture of *Klebsiella pneumoniae* isolated on MacConkey agar plate
- Photograph 3: Biochemical tests of *Klebsiella pneumoniae*
- Photograph 4: Biochemical tests of *Proteus mirabilis*
- Photograph 5: Antibiotic susceptibility test of *Escherichia coli*: MDR strain
- Photograph 6: Antibiotic susceptibility test of *Klebsiella pneumoniae*: MDR strain
- Photograph 7: Investigator culturing urine specimen

LIST OF APPENDICES

- Appendix-I Questionnaire Appendix-II List of the equipments and materials used during the study **Appendix-III** 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 Gram-staining Procedure **Appendix-IV** Appendix-V Methodology of biochemical tests used for identification of bacteria. Appendix-VI Method of collection of mid-stream urine **Appendix-VII** Morphological and cultural characteristics of bacteria isolated from urine sample **Appendix-VIII** Distinguishing reactions of the commoner and pathogenic enterobacteriaceae. **Appendix-IX** Zone size interpretative chart Appendix-X Calculation of sensitivity, specificity, positive and negative predictive
 - value and efficiency
- Appendix-XI Data analysis (chi- square test)
- Appendix-XII List of asymptomatic patients included in the study