

**ISOLATION, IDENTIFICATION AND PLASMID
PROFILING OF MULTIDRUG RESISTANT BACTERIAL
PATHOGENS ISOLATED FROM UTI PATIENTS**

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

Sanjiv Neupane

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

2008

RECOMMENDATION

This is to certify that **Mr. Sanjiv Neupane** has completed this dissertation work entitled “**ISOLATION, IDENTIFICATION AND PLASMID PROFILING OF MULTIDRUG RESISTANT BACTERIAL PATHOGENS ISOLATED FROM UTI PATIENTS**” as a partial fulfillment of M. Sc. Degree in Microbiology under our supervision. To our knowledge this thesis work has not been submitted for any other degree.

.....
Basudha Shrestha

Microbiologist
Kathmandu Model Hospital
Kathmandu, Nepal

.....
Mr. Binod Lekhak

Assistant Professor
Central Department of Microbiology
Tribhuvan University, Kirtipur
Kathmandu, Nepal

.....
Dr. Shreekant Adhikari, Ph. D.

Associate professor
Central Department of Microbiology
Tribhuvan University, Kirtipur
Kathmandu, Nepal

Date:.....

CERTIFICATE OF APPROVAL

On the recommendation of **Dr. shreekant Adhikari, Mr. Binod Lekhak** and **Mrs. Basudha Shrestha** this dissertation work of **Mr. Sanjiv Neupane**, entitled **“ISOLATION, IDENTIFICATION AND PLASMID PROFILING OF MULTIDRUG RESISTANT BACTERIAL PATHOGENS ISOLATED FROM UTI PATIENTS”** has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of science in Microbiology.

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

Date:-

BOARD OF EXAMINERS

Recommended by:

.....
Dr. Shreekant Adhikari, Ph.D.
Supervisor

.....
Mr. Binod Lekhak
Supervisor

.....
Mrs. Basudha Shrestha
Supervisor

Approved by:

.....
Dr. Anjana Singh, Ph.D.
Head of Department

Examined by:

.....
Prof. Nhuchchhe Ratna Tuladhar
External Examiner

.....
Dr. Dwij Raj Bhatta, Ph.D.
Internal Examiner

Date:-

ACKNOWLEDGEMENT

This research work has been supported by many people whose advice and encouragement were critical throughout the period of this work. It gives me immense pleasure to express my heartfelt appreciation to all the people who helped me to complete this dissertation.

Respectfully, I would like to express my sincere gratefulness to my supervisors **Dr. Shreekant Adhikari**, Associate Professor of Central Department of Microbiology, **Mr. Binod Lekhak**, Assistant Professor Central Department of Microbiology for their erudite guidance, generosity, tremendous support, and valuable suggestions during this research work. I am also indebted to my supervisor, **Mrs. Basudha Shrestha**, Microbiologist, Kathmandu Model Hospital for her guidance and constant encouragement for the completion and accomplishment of this work.

I sincerely like to express my appreciation to **Dr. Anjana Singh**, Associate Professor and Head of Department, Central Department of Microbiology, for her deep understanding, valuable suggestions and kind co-operation during this work.

I am thankful to Dr. Prakash Ghimire, Dr. Dwij Raj Bhatta, Ms. Shaila Basnyat and Mr. Kiran Babu Tiwari for their valuable idea, moral support, and kind co-operation during this study. I am much obliged to honorable Professor Dr. Ronald H. Bauerle, Mr. Megha Raj Banjara and all the teachers and staffs of Central Department of Microbiology, Tribhuvan University.

I acknowledge profound gratitude to Kathmandu Model Hospital for providing me laboratory and other facilities. My deep sense of indebtedness and gratitude goes to Dr. Bharat Pradhan (Director, KMH), Dr. Shambhu Upadhyaya, Dr. Ranga Bahadur Basnet (Pathologist), and all the staff of Pathology Department, KMH, for their support and kind cooperation. I wish to reiterate my acknowledgement to my friends Pankaj, Kashi, and Bishnu, for their insightful help and encouragement.

Finally, I admire my Family for their moral support and attention.

Date:

.....
Sanjiv Neupane

ABSTRACT

A study on the antibiotic susceptibility pattern of the bacteria isolated from Urine samples of suspected UTI patients was carried out. The plasmid profiling of multidrug resistant isolates and antibiotic resistance transfer mechanism was also studied.

The study was carried out during May 2007 to January 2008. Seven hundred and ten mid-stream urine samples received in the laboratory of Kathmandu Model Hospital, Kathmandu were processed for routine culture. The antimicrobial susceptibility of bacterial isolates was determined by Kirby-Bauer disk diffusion technique. The isolates which showed resistant to more than two different structural classes antibiotics were considered as multidrug resistant. The plasmids of multidrug resistant isolates were extracted and electrophoresed. The antibiotic resistance transfer study was carried out by conjugation and transformation experiment in the laboratory of Central Department of Microbiology, Kirtipur, Kathmandu.

Only 30.85% (219/710) of the samples showed significant bacterial growth. Status of bacteriuria was found higher in females (33.52%) than in males (23.71%). 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 20-29 (34.25%). Altogether 16 different bacterial genera were isolated from all culture positive urine samples. *Escherichia coli* (81.28%) was found to be the most predominant organism.

In all the *E.coli* isolates, 38.20% were Multidrug resistant. Among the MDR *E. coli* 95.58% isolates were resistant to Norfloxacin, 94.11% to Ampicillin, 92.64% to Ciprofloxacin and 86.76% to Co-trimoxazole .

Conjugation experiment showed that 100% (10/10) resistant isolates possessed the conjugative types of plasmids. The plasmid profile showed that the donors having 51, 38, 32.5 Kb plasmids were found to be transferred completely. Most of the isolates and their transconjugants had high degree of resistance against Ampicillin, Ciprofloxacin, Cefixime, Trimethoprim and Norfloxacin (MIC >1024).

Key words: Bacteriuria, Urinary Tract Infection, Mid-stream urine, Multidrug-resistance, Plasmid profiles, MIC

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	1-5
CHAPTER II OBJECTIVES	6
2.1 General Objective	6
2.2 Specific Objectives	6
CHAPTER III LITERATURE REVIEW	7-41
3.1 Urinary tract infection	7
3.2 Pathogenesis related to UTI	9
3.3 Urinary tract and bacterial multiplication in urine	9
3.4 Predisposing factors to UTI	11-16
3.5 Etiological agents of UTI	16-18
3.6 Categorization of UTI	18-20
3.7 Laboratory diagnosis of UTI	20
3.7.1 Methods of specimen collection and transport	21
3.7.2 Screening procedures	22
3.7.3 Urinalysis	22

3.7.4 Chemical examination of urine	23
3.7.5 Bacteriological examination of urine	24
3.7.6 Antibiotic susceptibility testing	24
3.8 Bacterial resistance to antibiotics	25-30
3.9 Global Scenario	30-36
3.10 Nepalese Scenario	36-40
3.11 Electrophoresis	40
3.12 Plasmid profiling	40
CHAPTER IV MATERIALS AND METHODS	40-46
4.1 Materials	42
4.2 Methods	42
4.2.1 Urine sample collection	42
4.2.2 Data collection	42
4.2.3 Urine sample evaluation	43
4.2.4 Macroscopic examination	43
4.2.5 Microscopic examination	43
4.2.6 Culture of specimen	43
4.2.7 Identification of isolates	44
4.2.8 Antibiotic susceptibility test	45
4.2.9 Quality control	46
4.2.10 Purity plate	46
4.2.11 Preservation of MDR <i>E.coli</i>	46
4.2.12 Extraction of plasmid DNA from MDR <i>E.coli</i>	47
4.2.13 Conjugation	47
4.2.14 Transformation	47
4.2.15 Determination of MIC of Donors, Transconjugants and Transformants	47
4.2.16 Extraction of plasmid DNA from transconjugants and transformants	48
4.2.17 Electrophoresis of plasmid DNA	48

4.2.18 Determination of size of plasmid by semi log plot	48
4.2.19 Decontamination of ethidium bromide	48
4.2.20 Data analysis	48
CHAPTER V RESULTS	49-69
5.1 Microbial analysis of urine samples	49
5.2 Antibiotic susceptibility pattern	53-63
5.3 Analysis of MDR isolates	63-66
5.4 Plasmid analysis	66-69
5.5 Statistical pattern of the results	69
CHAPTER VI DISCUSSION AND CONCLUSION	70-84
6.1 Discussion	70
6.2 Conclusion	84
CHAPTER VII SUMMARY AND RECOMMENDATIONS	86-88
7.1 Summary	86-87
7.2 Recommendations	87-88
REFERENCES	89-113
APPENDICES	i-xlvi

LIST OF TABLES

- Table 1: Bacteriuric syndromes and their definition
- Table 2: Classification of urinary tract infection
- Table 3: Urine culture result
- Table 4: Gender and source of UTI positive samples
- Table 5: Distribution of microorganisms
- Table 6: Distribution of bacterial isolates on source and gender of patients
- Table 7: Correlation of pyuria with culture result
- Table 8: Antibiotic susceptibility pattern of Gram Negative bacteria
- Table 9: Antibiotic susceptibility pattern of Gram Positive Bacteria
- Table 10: Antibiotic susceptibility pattern of *E.coli*
- Table 11: Antibiotic susceptibility pattern of *Citrobacter* spp.
- Table 12: Antibiotic susceptibility pattern of *Klebsiella* spp.
- Table 13: Antibiotic susceptibility pattern of *Enterobacter* spp. and *Salmonella* Typhi
- Table 14: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*
- Table 15: Antibiotic susceptibility pattern of *Proteus mirabilis*
- Table 16: Antibiotic susceptibility pattern of *Morganella morganii* and *Acinetobacter* spp.
- Table 17: Antibiotic susceptibility pattern of CONS AND *Staphylococcus aureus*
- Table 18: Antibiotic susceptibility pattern of *Streptococcus faecalis*
- Table 19: Distribution of MDR on bacterial isolates
- Table 20: Distribution of MDR on gender and type of patients
- Table 21: Distribution of MDR on age of patients
- Table 22: Antibiotic resistance pattern of MDR *E. coli* isolates
- Table 23: Plasmid profiles and resistance patterns of donors and transconjugants
- Table 24: Conjugation Frequency of Transconjugants on Different Selective media
- Table 25: Antibiotic profile of transformants
- Table 26: Statistical pattern of the results

LIST OF FIGURES

Figure 1: Percentage distribution of pattern of culture results

LIST OF PHOTOGRAPHS

Photograph 1: Significant bacterial growth on MacConkey agar plate

Photograph 2: Biochemical tests of *E.coli*

Photograph 3: Antibiotic susceptibility test of *Escherichia coli*: MDR strain

Photograph 4: Plasmid profiles of MDR *E.coli*

Photograph 5: Plasmid profiles of MDR *E.coli* and
their transconjugants

LIST OF APPENDICES

Appendix-I	Questionnaire
Appendix-II	List of the equipments and materials used during the study
Appendix-III	I. Composition and preparation of different culture media II. Composition and preparation of different biochemical media III. Composition and preparation of different staining and test reagents
Appendix-IV	Gram-staining Procedure
Appendix-V	Methodology of biochemical tests used for identification of bacteria.
Appendix-VI	Method of collection of mid-stream urine
Appendix-VII	Biochemical characteristics of different bacteria.
Appendix-VIII	Zone size interpretative chart
Appendix-IX	Protocols
Appendix-X	Data analysis
Appendix-XI	Susceptibility of donors and their transconjugants
Appendix-XII	Plasmid profiles of selected MDR <i>E.coli</i>
Appendix-XIII	Causative organisms of UTI
Appendix-XIV	1. Age and gender wise distribution of growth positive culture 2. Age wise distribution of bacterial isolates
Appendix-XV	No. of samples and culture results

LIST OF ABBREVIATIONS

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
CLSI	:	Clinical and Laboratory Standards Institute
DNA	:	Deoxyribonucleic Acid
EC	:	European Commission
ESBL	:	Extended spectrum beta-lactamases
EtBr	:	Ethidium Bromide
GISA	:	Glycopeptide-intermediate <i>Staphylococcus aureus</i>
H ₂ S	:	Hydrogen Sulphide
HPF	:	High power field
Hrs	:	Hours
Kb	:	Kilo base
Kbp	:	Kilo base pair
LF	:	Lactose fermenting
MA	:	MacConkey agar
MDR	:	Multidrug Resistance
MHA	:	Mueller Hinton Agar
MIC	:	Minimum Inhibitory Concentration
Min	:	Minutes
ml	:	Milliliter
µg	:	Microgram
µl	:	Microliter
MR	:	Methyl Red

MRSA	:	Methicillin-resistant <i>Staphylococcus aureus</i>
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
PABA	:	Para-amino benzoic acid
PBP	:	Penicillin binding protein
PNSSP	:	Penicillin Non-Susceptible <i>Streptococcus pneumoniae</i>
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
TUTH	:	Tribhuvan University Teaching Hospital
UK	:	United Kingdom
UPEC	:	Uropathogenic <i>Escherichia coli</i>
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