

**STUDY ON THE INCIDENCE OF URINARY TRACT  
INFECTION IN DIABETIC PATIENTS AND  
THE PREVALENCE OF MULTIDRUG RESISTANT  
STRAINS AMONG THE BACTERIAL PATHOGENIC  
ISOLATES**

**A**

**DISSERTATION**

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

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

**BY**

**NISHA PURI**

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

**2006**

## RECOMMENDATION

This is to certify that **Ms. Nisha Puri** has completed this dissertation work entitled “**STUDY ON THE INCIDENCE OF URINARY TRACT INFECTION IN DIABETIC PATIENTS AND THE PREVALENCE OF MULTIDRUG RESISTANT STRAINS AMONG THE BACTERIAL PATHOGENIC ISOLATES** ” as a partial fulfillment of Masters of Science Degree in Microbiology under our supervision. To our knowledge this thesis work has not been submitted for any other degree.

---

**Prof. Bharat Jha**  
Professor  
Department of Biochemistry  
T.U. Teaching Hospital  
Maharajgunj, Kathmandu

---

**Mr. Binod Lekhak**  
Assistant Professor  
CDM  
Tribhuvan University  
Kirtipur, Kathmandu

---

**Dr. Ram Chandra Adhikari**  
Lecturer  
Department of Pathology  
T.U. Teaching Hospital  
Maharajgunj, Kathmandu

---

**Ms. Reshma Tuladhar**  
Assistant Professor  
CDM  
Tribhuvan University  
Kirtipur, Kathmandu

**Date:** .....

## **CERTIFICATE OF APPROVAL**

On the recommendation of **Prof. Bharat Jha, Mr. Binod Lekhak, Dr. Ram Chandra Adhikari** and **Ms. Reshma Tuladhar** this dissertation work by **Ms. Nisha Puri**, entitled **“STUDY ON THE INCIDENCE OF URINARY TRACT INFECTION IN DIABETIC PATIENTS AND THE PREVALENCE OF MULTIDRUG RESISTANT STRAINS AMONG THE BACTERIAL PATHOGENIC ISOLATES”** has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirement for Masters of Science Degree in Microbiology.

**Anjana Singh, Ph. D**  
Head of the Department  
Central Department of Microbiology  
Tribhuvan University  
Kirtipur, Kathmandu

Date:-

## **BOARD OF EXAMINERS**

**Recommended by:**

**Prof. Bharat Jha**  
Supervisor

**Mr. Binod Lekhak**  
Supervisor

**Dr. Ram Chandra Adhikari**  
Supervisor

**Ms. Reshma Tuladhar**  
Supervisor

**Approved by:**

**Anjana Singh, Ph. D**  
Head of the Department

**Examined by:**

**Internal Examiner**

**External Examiner**

**Date: .....**

## ACKNOWLEDGEMENT

Respectfully, I am indebted to my supervisor, **Mr. Binod Lekhak**, Assistant Professor of Central Department of Microbiology, Tribhuvan University for his guidance and cooperation for the completion of this dissertation. I am equally obliged to my supervisor, **Ms. Reshma Tuladhar**, Assistant Professor of Central Department of Microbiology, Tribhuvan University for her guidance and constant encouragement for the accomplishment of this dissertation

I am grateful to my supervisor **Prof. Bharat Jha**, Assistant Dean of Tribhuvan University Teaching Hospital for providing a rewarding workplace and his inspiration and support. I would like to express my sincere gratefulness to my supervisor **Dr. Ram Chandra Adhikari**, Pathologist, Pathology Department, “OM” Hospital and Research Center for his inspiration, tremendous support, invaluable suggestions and continuous guidance during my research work.

I am much obliged to honorable Head of Department **Dr. Anjana Singh, Prof. Dr. Sheetal Raj Basnyat, Dr. Prakash Ghimire** and **Ms. Shaila 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 indebted to teachers especially **Mr. Nabaraj Pokhrel** and staffs especially **Mr. Dinesh Pandey** of National college (NIST) for their overwhelming support during the work, in absence of which I would have been unable to complete the work.

I wish to extend my sincere gratitude towards **Dr. Yogendra Prasad Deo**, Pathologist and to all the staffs of Pathology laboratory of “OM” hospital and research centre, especially **Pankaj Deo, Anjana Panchakoti** and **Kamala Niraula** for their support, cooperation and encouragement in the process of completing the work. I am also thankful to all the patients for their participation and cooperation during the work.

I wish to express my admiration and special thanks to all the friends for their support and encouragement especially to **Rajdeep Bomjan, Bikash Shakya, Jyoti Pant, Dhan K. Pant** and **Shova Khanal** who assisted me with the completion of this work.

My acknowledgement goes to my brother **Nimesh Puri** and his friends for their help during computer settings.

Finally, I admire my parents for their moral support and attention in achieving the present academic position.

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

**Nisha Puri**

**Date:** .....

## ABSTRACT

Diabetes is a chronic disorder of carbohydrates, fat and protein metabolism due to insulin deficiency and /or insulin resistance. It has been noted that diabetic patients can have severe medical complications. Urinary tract infections (UTI) are very often encountered in patients with diabetes mellitus. They may present themselves as asymptomatic bacteriuria, but may also lead to more serious infection.

Urinary tract infection is a common medical problem; sometimes, leading to the number of deaths either from acute infection or from chronic renal failure. Bacteriological examination of the urine is an important tool in the diagnosis of infection.

This study was conducted in “OM” Hospital and Research Center, Kathmandu among diabetic patients suspected of UTI from March 24, 2006 to June 13, 2006. The aim of this study was to investigate the incidence of UTI in diabetic patients, etiology of UTI, antibiotic susceptibility profile, to isolate the multi-drug resistant strains (MDR-strains) and to compare the results with control patients.

Altogether, 100 samples from diabetic patients as test and 100 samples from nondiabetic patients as control were included in this work, which were investigated by glucose oxidase test for blood sugar level, conventional semi-quantitative culture technique, pus cell count, red blood cell count, epithelial cell count and albumin test for detection of UTI.

Out of 100 test urine samples, 22.0% showed significant growth while 4.0% samples showed mixed growth and 8.0% showed non significant growth. Out of 100 control urine samples, 12.0% showed significant growth while 1 sample showed mixed growth and 2.0% showed non significant growth. The highest number of growth positive samples from test 7 (31.8%) and from control patients 3 (25.0%) belonged to the same age group 51-60 years. Also, among the 22 isolates from test, 13 (59.5%) and among 12 isolates from control, 10 (83.3%) were from female patients. *Escherichia coli* was found to be the most predominant isolate (40.9%) followed by *Citrobacter freundii* (27.2%) and *Staphylococcus aureus* (18.1%) in test and in control it was 83.3%.

Out the total isolates, 20 (90.9%) in test and 9 (75.0%) in control patients were MDR. In case of test out of 9 *E. coli* isolates, 8 were MDR-strains while all the isolates of *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* and out of four *S. aureus* isolates, three were MDR strains. Nitrofurantoin was found to be the most effective drug for all Gram negative isolates followed by Ofloxacin. Erythromycin was the drug of choice for Gram positive isolates.

Microscopy for pyuria showed the sensitivity of 59.0% vs 66.6% and specificity of 93.5% vs 96.5% in diabetic and control patients respectively. Similarly albuminuria showed sensitivity of 59.0% vs 66.6% and specificity of 89.7% vs 92.0%. Higher proportion of positive cases seen among test patients was found to be statistically non significant ( $P>0.05$ ) with control patients, whereas no significant association was found between MDR and non MDR strains and significant and non significant pyuria between test and control patients.

Keywords: diabetes, urinary tract infection, mid-stream urine, multi-drug resistance, pyuria

# TABLE OF CONTENTS

	<b>Page No.</b>
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
<b>CHAPTER I INTRODUCTION</b>	<b>1-3</b>
<b>CHPATER II OBJECTIVES</b>	<b>4</b>
<b>CHPATER III LITERATURE REVIEW</b>	<b>5-39</b>
3.1 Diabetes	5
3.2 Classification of diabetes	6-8
3.2.1 Type I diabetes mellitus	6
3.2.2 Type II diabetes mellitus	6-7
3.2.3 Other specific types	7
3.2.4 Gestational diabetes	7-8
3.3 Diagnostic criteria for diabetes mellitus	8-9
3.4 Symptoms of diabetes	9
3.5 Insulin in relation to diabetes	9-10
3.5.1 Insulin synthesis, secretion and its regulation	10-11
3.5.2 Physiological action of insulin	11-12
3.5.3 Mechanism of insulin action	12
3.5.4 Metabolic effects of insulin deficiency	12-13
3.6 Complications associated with diabetes	13-14
3.6.1 Long term complications	13
3.6.2 Short term complications	13-14
3.6.3 Infectious complications	14
3.6.4 Diabetes and infection	14
3.7 Common Infections	14-16
3.8 Urinary tract infection	16-19
3.9 Microbiology of UTI in diabetic patients	19
3.9.1 Etiological agents of urinary tract infection	19-21
3.9.2 Site of infection	21



3.9.3	Routes of infection	21-22
3.9.4	Factors predisposing to infection	22-23
3.9.5	Categorization of urinary tract infection	23-25
3.9.6	Pathogenesis	25-28
3.9.7	Host defense mechanism	28-29
3.9.8	Establishment and multiplication of bacteria in urine	29
3.9.9	Manifestations of urinary tract infection	30
3.10	Diagnosis of urinary tract infection	30
3.10.1	Methods of specimen collection	30-31
3.10.2	Screening procedures	31
3.10.3	Macroscopic examination of urine	32
3.10.4	Microscopic examination of urine	32-33
3.10.5	Chemical examination of urine	33
3.10.6	Bacteriological examination of urine	33-34
3.11	Urinary Antiseptics	34-35
3.11.1	Bacterial resistance to antimicrobial agents	36-37
3.11.2	Predisposing factors	37
3.11.3	Types of drug resistance	37-38
3.11.4	Mechanisms of antimicrobial resistance	38
3.12	Antimicrobial susceptibility testing	39
<b>CHAPTER IV MATERIALS AND METHODS</b>		<b>40-44</b>
4.1	Materials	40
4.2	Methods	40-44
4.2.1	Blood sample	40
4.2.1.1	Specimen collection	40
4.2.1.2	Detection of blood sugar	41
4.2.2	Urine sample	41
4.2.2.1	Specimen collection	41
4.2.2.2	Macroscopic examination	41
4.2.2.3	Microscopic examination	41
4.2.2.4	Chemical examination of urine	41-42
4.2.2.5	Culture of specimen	42
4.2.2.6	Identification of the isolates	43
4.2.3	Antibiotic susceptibility testing	43-44
4.2.4	Purity Plate	44
4.2.5	Quality Control for test	44
<b>CHAPTER V RESULTS</b>		<b>45-57</b>
5.1	Clinical pattern of results	45-47
5.1.1	Pattern of patient requesting for urine culture	45
5.1.2	Age and gender wise distribution of patients requesting for urine culture	45-46
5.1.3	Growth pattern of bacteria in urine samples	46
5.1.4	Pattern of genderwise significant bacterial growth from	47

	urine samples	
5.2	Biochemical pattern of the results	47-49
5.2.1	Blood sugar level verses significant growth pattern of isolates in urine samples of diabetic patients	47
5.2.2	Correlation of albuminuria with culture result	48
5.2.3	Correlation of glycosuria with culture result	49
5.3	Microscopic observation of urine	49-51
5.3.1	Microscopic observation of pus cells against the culture result	49
5.3.2	Microscopic observation of RBC against the culture result	50
5.3.3	Microscopic observation of epithelial cells against the culture result	51
5.4	Microbial Patterns of results	51-53
5.4.1	Pattern of bacteria isolated from culture positive urine samples according to Gram's stain	51
5.4.2	Pattern of microbial isolates from urine sample	52
5.5	Antibiotic susceptibility pattern of the isolates	53-55
5.5.1	Antibiotic susceptibility pattern of Gram positive isolates	53
5.5.2	Antibiotic susceptibility pattern of Gram negative isolates	54
5.6	Antibiotic resistance pattern of the isolates	55-57
5.6.1	Distribution of MDR pathogens in diabetic and nondiabetic patients	55
5.6.2	Antibiotic resistance pattern of the isolates from urine sample	56
	<b>CHAPTER VI DISSCUSSION AND CONCLUSION</b>	<b>58-66</b>
6.1	Discussion	58-66
6.2	Conclusion	66
	<b>CHAPTER VII SUMMARY AND RECOMMENDATIONS</b>	<b>67-68</b>
7.1	Summary	67-68
7.2	Recommendations	68
	<b>CHAPTER VIII REFERENCES</b>	<b>69-82</b>
	<b>CHAPTER IX APPENDICES I–XII</b>	<b>i-xxvii</b>

## LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ASB	Asymptomatic Bacteriuria
ATCC	American Type Culture Collection
BA	Blood Agar
BMI	Basal Metabolic Index
CC-MSU	Clean Catch Mid Stream Urine
CFA	Colonization Factor Antigens
CFU	Colony Forming Units
CONS	Coagulase Negative Staphylococci
CRF	Coagulase Reacting Factor
dl	Decalitre
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
FN	False Negative
FP	False Positive
HPF	High Power Field
IDSA	Infectious Diseases Society of America
IDDA	Insulin Dependent Diabetes Mellitus
L	Litre
LPF	Low Power Field
MA	MacConkey Agar
MDR	Multi-drug Resistant
MHA	Mueller Hinton Agar
mmol	Millimolar
µl	Microlitre
MODY	Maturity Onset Diabetes in Youth
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MRVP	Methyl Red Voges Proskauer

MSU	Mid-Stream Urine
NA	Nutrient Agar
NB	Nutrient Broth
nm	Nano Meter
NCCLS	National Committee for Clinical Laboratory Standards
NIDDM	Non Insulin Dependent Diabetes Mellitus
NNIS	National Nosocomial Infections Surveillance
ONPG	<i>o</i> -nitrophenyl- -D-galactopyranoside
OPD	Out Patient Department
PAF	Prostatic Antibacterial Factor
PAP	Pyelonephritis-Associated Pili
PBP	Penicillin Binding Protein
PDA	Phenylalanine Deaminase
PPG	Post Prandial Glucose
PPN	Predictive Value of Negative Test
PPV	Predictive Value of Positive Test
QREC	Quinolone Resistant <i>Escherichia coli</i>
RBC	Red Blood Cell
RIA	Radio Immuno Assay
SDA	Sabouraud Dextrose Agar
SIM	Sulfide Indole Motility
TMP-SMX	Trimethoprim-Sulfomethaxole
TN	True Negative
TP	True Positive
TPD	Tetramethyl <i>p</i> -phenylene diamine dihydrochloride
TSIA	Triple Sugar Iron Agar
TUTH	Tribhuvan University Teaching Hospital
UTI	Urinary Tract Infection
WBC	White Blood Cell
WHO	World Health Organization



## LIST OF TABLES

Table 1	Pattern of clinical samples and prevalence of MDR strains
Table 2	Growth pattern in urine sample
Table 3	Pattern of patients requesting for urine culture
Table 4	Age and gender wise distribution of patients requesting for urine culture
Table 5	Pattern of significant growth and MDR strains from urine sample
Table 6	Pattern of bacteria isolated from culture positive urine samples according to Gram's stain
Table 7	Blood sugar level (mmol/L) verses significant growth pattern of isolates in urine samples of diabetic patients
Table 8	Correlation of albuminuria with culture result in diabetic patients.
Table 9	Correlation of glycosuria with culture result in diabetic patients.
Table 10	Microscopic observation of pus cells against the culture result in diabetic patients
Table 11	Microscopic observation of RBC against the culture result in diabetic patients
Table 12	Microscopic observation of epithelial cells against the culture result in nondiabetic patients
Table 13	Pattern of different species of bacteria isolated from infected urine of diabetic patients
Table 14	Pattern of different species of bacteria isolated from infected urine of nondiabetic patients
Table 15	Distribution of MDR pathogens in diabetic and nondiabetic patients
Table 16	Antibiotic sensitivity pattern against gram positive bacteria isolated from urine samples of diabetic patients
Table 17	Antibiotic sensitivity pattern against gram negative bacteria isolated from urine samples of diabetic patients
Table 18	Antibiotic sensitivity pattern against gram negative bacteria isolated from urine samples of nondiabetic patients
Table 19	Antibiotic resistance pattern of isolates from urine samples of diabetic patients
Table 20	Antibiotic resistance pattern of the isolates from urine sample of nondiabetic patients

## LIST OF FIGURES

- Figure 1      Flow diagram for processing urine sample
- Figure 2      Pattern of patients requesting for urine culture
- Figure 3      Age wise distributions of patients requesting for urine culture
- Figure 4      Growth pattern of bacteria in urine sample
- Figure 5      Correlation of albuminuria with culture result in diabetic patients
- Figure 6      Microscopic observation of pus cells against the culture result in nondiabetic patients
- Figure 7      Microscopic observation of RBC against the culture result in nondiabetic patients
- Figure 8      Distribution of bacteria in culture positive samples according to Gram's stain
- Figure 9      Percentage distributions of isolates from urine culture of diabetic patients
- Figure 10     Percentage distributions of isolates from urine culture of nondiabetic patients
- Figure 11     Antibiotic sensitivity pattern of Gram positive bacteria from urine sample of diabetic patients
- Figure 12     Antibiotic sensitivity pattern of Gram negative bacteria from urine sample of diabetic patients
- Figure 13     Number of MDR strains among total isolates from urine sample of diabetic patients

## **LIST OF PHOTOGRAPHS**

- Photograph 1      Significant growth of *Escherichia coli* on MacConkey agar
- Photograph 2      Antibiotic susceptibility test of *Escherichia coli* isolate showing inhibition zones on Mueller-Hinton agar
- Photograph 3      Antibiotic susceptibility test of *Staphylococcus aureus* isolate showing inhibition zones on Mueller-Hinton agar
- Photograph 4      Biochemical tests for *C. freundii*
- Photograph 5      Transferring the isolated bacterial colonies for studies



# LIST OF APPENDICES

**APPENDIX-I** Questionnaire

**APPENDIX-II**

- I. Composition and preparation of different culture media
- II. Biochemical test media
- III. Staining and test reagents

**APPENDIX-III**

- A. Gram staining procedure
- B. Standardization of loop

**APPENDIX-IV** Method of collection of midstream urine

**APPENDIX-V** Method of estimation of blood sugar level

**APPENDIX-VI** Biochemical tests for identification of bacteria

**APPENDIX-VII** List of equipments and materials used during the study

**APPENDIX-VIII** Morphology and cultural characteristics of bacteria isolated from urine sample

**APPENDIX-IX** Distinguishing reactions of members of Enterobacteriaceae

**APPENDIX-X** Statistical analyses of screening tests

**APPENDIX-XI** Chi-square tests

**APPENDIX-XII** Antibiotic sensitivity pattern of individual bacterial isolates