MULTIDRUG RESISTANCE AND EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING STRAINS AMONG CLINICAL ISOLATES OF PATIENTS FROM SHREE BIRENDRA HOSPITAL, CHHAUNI

A

Dissertation

Submitted to the Central Department of Microbiology

Tribhuvan University

In partial fulfillment of the Requirement for the Award of the Degree of Master of
Science in Microbiology
(Environment and Public Health)

Submitted by
Sushila Maharjan
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu, Nepal
2010

RECOMMENDATION

This is to certify that **Ms. Sushila Maharjan** has completed this dissertation work entitled "**MULTIDRUG RESISTANCE AND EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING STRAINS AMONG CLINICAL ISOLATES OF PATIENTS FROM SHREE BIRENDRA HOSPITAL, CHHAUNI** " as a partial fulfillment of Master of Science Degree in Microbiology under our supervision. To our knowledge this thesis work has not been submitted for any other degree.

……	……..	…….....
Dr. Anjana Singh, Ph.D	**Ms. Shaila Basnyat**	**Dr. Sunil Kumar Singh**
Professor	Asst. Professor	Colonel, Pathologist
Central Department of Microbiology	Central Department of Microbiology	Shree Birendra Hospital Chhauni, Kathmandu
Tribhuvan University	Tribhuvan University	
Kirtipur, Kathmandu	Kirtipur, Kathmandu	

Date:…….

CERTIFICATE OF APPROVAL

On the recommendation of **Prof. Dr. Anjana Singh, Ms. Shaila Basnyat,** and **Dr. Sunil Kumar Singh,** this dissertation work by **Ms. Sushila Maharjan**, entitled has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirement for Master of Science Degree in Microbiology.

Dr. Dwij Raj Bhatta
M.Sc., Ph.D in Microbiology
Head of Department
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu

Date:- .....

BOARD OF EXAMINERS

Recommended by:

Prof. Dr. Anjana Singh
Supervisor

Ms. Shaila Basnyat
Supervisor

Col. Dr. Sunil Kumar Singh
Supervisor

Approved by:

Dr. Dwij Raj Bhatta
Head of the Department

Examined by:

Prof. Dr. Bharat Mani Pokhrel
Professor and Head of
Department of Microbiology,
Institute of Medicine
Tribhuvan University,
Maharajgung

Mr. Komal Raj Rijal
Internal Examiner

Date: ……

ACKNOWLEDGEMENT

Respectfully, I am indebted to my supervisors, **Prof. Dr. Anjana Singh and Ms. Shaila Basnyat**, Assistant Professor of Central Department of Microbiology, Tribhuvan University for their guidance and co-operation for the completion of this dissertation.

I am grateful to my supervisor **Col. Dr. Sunil Singh**, Pathologist of Shree Birendra Hospital for providing a rewarding workplace and his inspiration and support. I am equally obliged to **Ms. Puja Shrestha**, Microbiologist of Shree Birendra Hospital for her guidance and constant encouragement for the accomplishment of this dissertation.

I am much obliged to Head of Department **Dr. Dwij Raj Bhatta,** Central Department of Microbiology, Tribhuvan University, for his invaluable assistance during the preparation of this thesis.

Furthermore, I am very thankful for all the rest of the faculties and staffs of Central Department of Microbiology, Tribhuvan University, all of whom have been very generous and motivating. I wish to extend my sincere gratitude towards **Major Mr. Khagendra Sijapati,** Medical Technologist of Shree Birendra Hospital and all the staffs of Bacteriology department of the hospital, for their support, cooperation and encouragement in the process of completing this thesis work. I am also thankful to all the patients for their participation and cooperation during the work.

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.

Sushila Maharjan

Date:……..

ABSTRACT

The microbial resistance to various classes of drugs has increased a multitude of bacterial species which has complicated the therapeutic management of infections. For this reason, a six month study was conducted in order to analyze the prevalence of the Multidrug Resistant strains(MDR) and the Extended Spectrum of β-lactamase(ESBL) producing strains among the organisms, isolated from clinical specimens (urine, sputum and pus samples) received in the laboratory. Microorganisms from 388 clinical specimens were identified by conventional microbiological method and antimicrobial susceptibility of bacterial isolates was determined by CLSI (Clinical and Laboratory Standard Institute) recommended by Kirby-Bauer method. Among 388 clinical samples processed in the study, 207 were urine samples, 79 were sputum samples and 102 were pus samples. Out of 207 urine samples, 95 (45.89%) showed significant growth and among the 95 isolates, 62 (65.26%) were multi-drug resistant. In urine sample, *Escherichia coli* was the most predominant Gram-negative isolate. Out of 68 *E. coli* isolates, 47 (69.12%) were found to be MDR and 11 (16.18%) were ESBL-producers. Out of 79 sputum samples received, 77 (97.47%) met the American Society for Microbiology (ASM) criteria and thus were considered for further processing, whereas 2 (2.53%) of the samples didn't meet the criteria and were not included in this study. Out of 77 processed sputum samples, 20 (25.97%) samples showed significant growth, out of which, 9 (45%) were MDR and 3 isolates of *Klebsiella pneumoniae* among 20 different isolates from the sputum samples were ESBL-producers. Likewise, out of 102 pus samples, 75 (73.53%) showed significant growth with 82 isolates (some samples showed more than one type of significant bacterial growth) and among 82 isolates, 40 (39.22%) were multi-drug resistant, additionally, 5 isolates (3 out of 23 isolates of *Escherichia coli* and 2 out of 8 isolates of *Klebsiella pneumoniae*) were found to be ESBL-producers. In all the urine and pus specimens, the most predominant Gram-negative isolate was *Escherichia coli* while in case of sputum, it was *Pseudomonas aeruginosa*. The most predominant Gram-positive isolates were *Staphlococcus aureus* in all the studied specimens. Erythromycin and cloxacillin were highly effective towards Gram-positive isolates, nitrofurantion towards Gram-negative urinary pathogens while gentamicin followed by ciprofloxacin and amikacin for sputum and pus isolates. Thus total of 386 samples were processed, out of which 190 (49.22%) samples showed positive growth with 197 (51.03%) of total isolates and among those bacterial isolates, 111 (57.21%) were found to be MDR-strains whereas 19 of them were found to be ESBL-producers. Significant association was found between multidrug resistance and hospitalization of patients in different wards ($P<0.05$), whereas no association was seen between multidrug resistance and gender ($P>0.05$).

Key words: Urine, Pus, Sputum, ASM, MDR, ESBL, DDST

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ASM	American Society for Microbiology
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
BA	Blood Agar
BLNAR	Beta-lactamase Negative, Ampicillin Resistant
CA	Chocolate Agar
CAT	Chloramphenicol Acetyltransferase
CFU	Colony Forming Unit
CLED	Cystine Lactose Electrolyte-deficient
CLSI	Clinical and Laboratory Standard Institute
CONS	Coagulase Negative Staphylococci
CRF	Coagulase Reacting Factor
DDST	Double Disk Synergy Test
DHFR	Dihydrofolate Reductase
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
ESBL	Extended Spectrum Beta Lactamase
ICU	Intensive Care Unit
IS	Insertion Sequence
LPS	Lipopolysaccharide
LRTI	Lower Respiratory Tract Infection
MA	MacConkey Agar
MBC	Minimum Bactericidal Concentration
MDR	Multi-drug Resistant
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration

MLS	Macrolide-Lincosamide-Streptogramin
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRVP	Methyl Red Voges Proskauer
NA	Nutrient Agar
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NB	Nutrient Broth
NCTC	National Collection Type Cultures
NFW	New Female Ward
ONPG	*o*-nitrophenyl-β-D-galactopyranoside
PBP	Penicillin Binding Protein
PDA	Phenylalanine Deaminase
QRDR	Quinolone Resistance-determining regions
QREC	Quinolone Resistant *Escherichia coli*
RNA	Ribonucleic Acid
SIM	Sulfide Indole Motility
TPD	Tetramethyl p-phenylene diamine dihydrochloride
TSIA	Triple Sugar Iron Agar
TUTH	Tribhuvan University Teaching Hospital
UTI	Urinary Tract Infection
VRE	Vancomycin Resistant Enterococci
VRSA	Vancomycin Resistant *Staphylococcus aureus*

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES