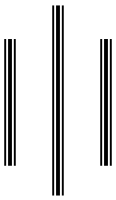
MULTIDRUG RESISTANCE AMONG VARIOUS CLINICAL BACTERIAL ISOLATES AND PRODUCTION OF DIFFERENT TYPES OF β-LACTAMASES WITH SUBSEQUENT TRANSFER MECHANISM BY PLASMID DNA ANALYSIS



A

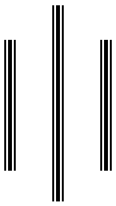
Dissertation

Presented 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)



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2008

RECOMMENDATION

This is to certify that **Mr. Pankaj Baral** has completed this dissertation work entitled "**MULTIDRUG RESISTANCE AMONG VARIOUS CLINICAL BACTERIAL ISOLATES AND PRODUCTION OF DIFFERENT TYPES OF β-LACTAMASES WITH SUBSEQUENT TRANSFER MECHANISM BY PLASMID DNA ANALYSIS**" 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.

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CERTIFICATE OF APPROVAL

On the recommendation of **Mr. Binod Lekhak** and **Mrs.Basudha Shrestha** this dissertation work by **Mr. Pankaj Baral**, entitled "**MULTIDRUG RESISTANCE AMONG VARIOUS CLINICAL BACTERIAL ISOLATES AND PRODUCTION OF DIFFERENT TYPES OF β-LACTAMASES WITH SUBSEQUENT TRANSFER MECHANISM BY PLASMID DNA ANALYSIS**" has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirement for M. Sc. Degree in Microbiology.

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ACKNOWLEDGEMENT

I would like to express my heartfelt appreciation and profound gratitude to all the people who helped me in one way or another to complete this dissertation.

Respectfully, I would like to express my sincere gratefulness to my supervisor **Mr.Binod Lekhak,** Asst.Prof, Central Department of Microbiology, Trhibhuvan University, Kirtipur for his superb guidance, generosity, encouragements, invaluable suggestions and enlightenment during whole of this research work.

I am indebted to my supervisor, **Mrs.Basudha Shrestha**, microbiologist, Kathmandu Model Hospital, Kathmandu for her meticulous guidance and tremendous support for accomplishment of this work. My special gratefulness also goes to Mr.Kiran Babu Tiwari, Asst. Prof. Pokhara University for his expert guidance, moral support and encouragement for doing and accomplishment of this work.

I am much obliged to honorable Head of Department **Dr. Anjana Singh**, Central Department of Microbiology, Mr. Megha Raj Banjara and all the teachers and staffs of Central Department of Microbiology, Tribhuvan University.

I am thankful to Dr.Dwij Raj Bhatta, Dr.Prakash Ghimire, Dr.Shreekant Adhikari, Ms. Shaila Basnyat and all the teachers and staffs of Central Department of Microbiology for their valuable idea, moral support, and kind cooperation during this study. I acknowledge profound gratitude to Prof.Dr.Ronald H.Baeurle, Senior Fulbright professor for his scrupulous guidance, moral support and kind cooperation.

My special thanks goes to colleagues Mr. Sanjiv Neupane, Mr. Kashi Ram Ghimire, Mr.Bishnu prasad Marasini, Mr.Navaraj Dahal and Mr.Girdhari Rijal for their support and cooperation for completion of this work.

I wish to extend my sincere gratitude to all participating patients during the study for their good cooperation and response during the study and my family for their moral support, patience and encouragement.

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Pankaj Baral

ABSTRACT

In the present study 710 urine, 551 blood, 91 pus, 28 fluid, 59 sputum, 13 CSF, 2 tissue, 16 stool, 30 throat swab and 3 perianal swab samples were collected from symptomatic patients visiting Kathmandu Model Hospital from May 2007 to August 2007. The samples were processed for routine culture. The isolated bacteria were subjected for antimicrobial susceptibility testing by modified Kirby-Bauer method. The production of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases (ABLs) and metallo- β-lactamases (MBLs) in the multidrug resistant isolates likely to produce these β –lactamases were determined by Double Disk Synergy test (DDST), AmpC Disk test (ADT) and EDTA-Disk Synergy test (EDST) methods respectively.

Plasmid DNA anlysis of the multidrug resiatant ESBL, ABL and MBL producing isolates of the bacteria were performed. The plasmid encoded β–lactamases and multidrug resistance transfer mechanism/s were also studied by conjugation and transformation method. The work was carried out at the laboratory of Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

All together, 1503 samples were received in the laboratory, of which predominant were urine 710 (47.24%) samples. Out of 1503 samples, 336 (22.35%) showed bacterial growth (urine -30.84 %, blood-9.25 %, pus-61.53 %, fluid-7.14 %, sputum-1.69 %, CSF-7.69 %, tissue-100.0 %, stool-0.0 %, throat swab-3.34 % and perianal swab-100.0 %). The most predominant bacterial pathogen among all these samples was *E. coli* with 193/336 (57.44 %) isolates.

Imipenem was the drug of choice with a susceptibility of 98.43% for all Gram negative isolates, whereas amikacin and vancomycin were the drugs of choice with susceptibility 100.0% each for Gram positive isolates. Out of 336 isolates, 41.07 % (138/336) MDR isolates were found. *E. coli* was found most predominant MDR isolates with 46.12% (89/193) MDR strains among all samples. *E. coli* was found most predominant ESBL producers with 100.0% (27/27) ESBL producing MDR strains, ABL producers with 81.03 % (47/58) ABL producing MDR strains and MBL producers with 75.75% (25/33) MBL producing MDR strains.

All MDR β-lactamase producing isolates of *E. coli* (30) and *C. freundii* (3) selected for plasmid DNA analysis harbored megaplasmid with other small size plasmids. Most of them were found responsible for the transfer of plasmid mediated β-lactamase production with MDR traits frequently via conjugation.The most common plasmid type responsible for different β-lactamase production and mutidrug resistance was 32.5 kb.

Key Words: Double Disk Synergy test, AmpC Disk test, EDTA Disk Synergy test, Plasmid, Conjugation

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Chi –square test
Odds ratio

LIST OF ABBREVIATIONS

ABL	AmpC β-l.aactamase
ATCC	American Type Culture Collection
BA	Blood Agar
CA	Chocolate Agar
CAT	Chloramphenicol Acetyltransferase
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
CONS	Coagulase Negative Staphylococci
CRF	Coagulase Reacting Factor
DDST	Double Disk Synergy Test
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic acid
ESBL	Extended Spectrum of Beta Lactamase
IS	Insertion Sequence
LRTI	Lower Respiratory Tract Infection
MA	MacConkey Agar
MBC	Minimum Bactericidal Concentration
MBL	Metallo β-lactamase
MDR	Multi-drug Resistant
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MRVP	Methyl Red Voges Proskauer
NA	Nutrient Agar
NB	Nutrient Broth
NCCLS	National Committee for Clinical Laboratory Standards
NCTC	National Collection of Type Cultures
PBP	Penicillin Binding Protein

RAPD	Randomely amplitfied polymorphic DNA
RNA	Ribonucleic Acid
SDS	Sodium dodecyl sulfonate
SIM	Sulfide Indole Motility
TPD	Tetramethyl *p*-phenylene diamine dihydrochloride
TSIA	Triple Sugar Iron Agar
TCBS	Thiosulfate Citrate Bile salt Sucrose
TUTH	Tribhuvan University Teaching Hospital
UTI	Urinary Tract Infection
VRE	Vancomycin Resistant enterococci
VRSA	Vancomycin Resistant *Staphylococcus aureus*