

**EXTENDED SPECTRUM -LACTAMASE AND METALLO -  
LACTAMASE PRODUCING MULTIDRUG RESISTANT GRAM  
NEGATIVE BACTERIA AMONG PATIENTS WITH RENAL  
FAILURE**

**A DISSERTATION SUBMITTED TO  
THE CENTRAL DEPARTMENT OF MICROBIOLOGY  
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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
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DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY  
(Medical)**

**BY  
ANIL PANT  
CENTRAL DEPARTMENT OF MICROBIOLOGY  
TRIBHUVAN UNIVERSITY  
KATHMANDU  
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## RECOMMENDATION

This is to certify that **Mr. Anil Pant** has completed this dissertation work entitled "**Extended spectrum  $\beta$ -lactamase and metallo  $\beta$ -lactamase producing multidrug resistant gram negative bacteria among patients with renal failure**" as a partial fulfillment of M.Sc. degree in Microbiology. To the best of our knowledge, this is his original research work and has not been submitted for award of any other degree.

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**Prof. Dr. Anjana Singh**

Head

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

---

**Dr. Anil Dev Pant**

MBBS, MD

National Kidney Centre

Kathmandu, Nepal

Date:.....

## CERTIFICATE OF APPROVAL

On the recommendation of **Prof. Dr. Anjana Singh** and **Dr. Anil Dev Pant**, this dissertation work by **Mr. Anil Pant** entitled “**Extended spectrum - lactamase and metallo -lactamase producing multidrug resistant gram negative bacteria among patients with renal failure**” has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for M.Sc. Degree in Microbiology.

---

**Prof. Dr. Anjana Singh**

Head

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

Date: .....

**BOARD OF EXAMINERS**

**Recommended by:**

-----  
**Prof. Dr. Anjana Singh**

Supervisor

-----  
**Dr. Anil Dev Pant (MBBS, MD)**

Supervisor

**Approved by:**

-----  
**Prof. Dr. Anjana Singh**

Head of Department

**Examined by:**

-----  
**Assoc. Prof. Dr. Keshab Parajuli**

External Examiner

-----  
**Ms. Supriya Sharma**

Internal Examiner

Date: .....

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Date:

Anil Pant

## ABSTRACT

Patients with renal failure are at greater risk of infection because of the abnormality in their immune system and the direct exposure of circulatory system to the microorganisms. The infection in such vulnerable population by drug resistant bacteria may lead to life-threatening consequences. The purpose of this study was to isolate and identify the multiple drug resistant Gram negative bacteria (MDRGNB) from kidney patients and hemodialysis patients and to screen those producing Extended-Spectrum  $\beta$ -Lactamases (ESBLs) and Metallo  $\beta$ -Lactamases (MBLs). During the study period (March-August, 2013), 496 samples of urine and 21 samples of blood collected from patients visiting and undergoing hemodialysis at National Kidney Centre (NKC) were processed. The isolates were identified by standard microbiological procedures and subjected to antimicrobial susceptibility testing by modified Kirby Bauer disc diffusion methods. Production of ESBL and MBL was determined using MASTDISCS<sup>TM</sup> ID ESBL detection discs and imipenem EDTA combined disc assay respectively. The Minimum Inhibitory Concentration (MIC) of ciprofloxacin against the MDR isolates was also determined using agar dilution method.

Total 103 (19.92%) samples showed significant growth and 85.43% of them were multidrug resistant. The higher rate of growth among female patients was found statistically significant ( $p < 0.05$ ). Imipenem was found to be most effective drug against the isolates whereas ceftazidime, with sensitivity and positive predictive value of 94.2% and 76.7% respectively was found to be more effective in screening ESBL producers among MDR strains than cefotaxime. Of the 59 MDR isolates suspected 35 (66.66%) were confirmed to produce ESBL with at least one Combined Disc (CD) assay used. Ceftazidime-clavulanate combined disc correctly identified all of the ESBL isolates whereas cefotaxime failed to confirm two isolates. Only one of the 11 isolates tested for MBL production was confirmed to produce MBL. The majority of ESBL isolates were *Escherichia coli* 29 (82.85%), whereas 2/2 (100%) of suspected *Pseudomonas aeruginosa* isolates were ESBL. None of the *Proteus vulgaris* were found to produce ESBL. Of note was the co-existence of ESBL and MBL in *Pseudomonas aeruginosa*. However, the production of MBL and resistance to carbapenems was statistically insignificant ( $p > 0.05$ ). The statistical pattern of resistance of ciprofloxacin, norfloxacin, ceftazidime and cefotaxime and ESBL production was found significant ( $p < 0.05$ ). Most of the isolates showed MIC value of 8 $\mu$ g/ml towards ciprofloxacin.

Use of single screening agent for ESBL screening may result in false positive results, hence use of more than one of the screening agents and combined disk assay improves the sensitivity of detection of ESBL.

Key Words: Hemodialysis, Chronic Kidney Patients, Multidrug Resistance, ESBL, MBL, MIC

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## LIST OF ABBREVIATIONS

3GCR	Third Generation Cephalosporin Resistance
AMR	Anti Microbial Resistance
ASM	American Society for Microbiology
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
BA	Blood Agar
BHI	Brain Heart Infusion Agar
CD	Combined Disc Assay
CDC	Centre for Disease Control and Prevention
CFU	Colony Forming Unit
CKD	Chronic Kidney Disease
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
EC	Enzyme Commission
EDST	EDTA Disc Synergy Test
EDTA	Ethylene Diamine Tetra Acetic acid
ESBL	Extended Spectrum $\beta$ -Lactamase
HAI	Healthcare Associated Infection
HD	Hemodialysis
KMC	Kathmandu Medical College
MBL	Metallo $\beta$ -Lactamase
MDR	Multidrug Resistant
MDRGNB	Multidrug Resistant Gram Negative Bacteria
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
NIH	National Institutes of Health
NKC	National Kidney Centre
NPHL	National Public Health Laboratory
OMP	Outer Membrane Proteins
OPD	Out Patient Department
PBP	Penicillin Binding Protein

PCR	Polymerase Chain Reaction
PDR	Pan Drug Resistant
SIM	Sulfide Indole Motility Medium
SPSS	Statistical Package for Social Sciences
TSI	Triple Sugar Iron Agar
TUTH	Tribhuvan University Teaching Hospital
VP	Voges-Proskauer Test
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
XDR	Extreme Drug Resistant

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