METALLO β-LACTAMASE PRODUCTION AND ANTIBIOTIC SUCEPTIBILITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SAMPLES

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

(MEDICAL)

BY

KAMAL BAGALE

CENTRAL DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

KIRTIPUR, KATHMANDU, NEPAL

2013

RECOMMENDATION

This is to certify that **Mr. Kamal Bagale** has completed this dissertation work entitled **"Metallo β-lactamase production and antibiotic suceptibility pattern of *Pseudomonas aeruginosa* isolated from different clinical samples"** 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.

Dr. Megha Raj Banjara

Lecturer

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

Prof. Dr. Narbada Thapa

Principal

College of Nursing

Nepalese Army Institute of Health Sciences

Dr. Sabita Bhatta

Asst. Professor

Nepalese Army Institute of Health Sciences

Date: …….

CERTIFICATE OF APPROVAL

On the recommendation of **Dr. Megha Raj Banjara, Prof. Dr. Narbada Thapa** and **Dr. Sabita Bhatta,** this dissertation work by **Mr. Kamal Bagale** 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 of Department

Central Department of Microbiology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

Date: …….

BOARD OF EXAMINERS

Recommended by: _____

Dr. Megha Raj Banjara

Supervisor

_____

Prof. Dr. Narbada Thapa

Supervisor

_____

Dr. Sabita Bhatta

Supervisor

Approved by: _____

Prof. Dr. Anjana Singh

Head of the Department

Examined by: _____

Mr. Bishnu Prasad Upadhyay

External Examiner

_____

Ms. Shaila Basnyat

Internal Examiner

Date: ……..

ACKNOWLEDGEMENT

First and foremost, I offer my sincere gratitude to my supervisor Dr. Megha Raj Banjara, who has supported me throughout my dissertation work with his expert guidance and constant and untiring inspiration. It is an honor for me to express my gratitude to my other supervisor Brig. Gen. Prof. Dr. Narbada Thapa who gave me the platform to perform my dissertation work. Her inspiration was always a blessing to count. I'm heartily thankful to my supervisor Maj. Dr. Sabita Bhatta for her valuable guidance and immense support during the entire research period. I owe my deepest gratitude to Maj. Dr. Raina Chaudhary for her support and guidance throughout my work.

I would like to express my deepest regards and gratitude to Prof. Dr. Anjana Singh, Head of Central Department of Microbiology, Tribhuvan Universtity for her support.

I wish to express my warm and sincere thanks to all the teachers and staff of Central Department of Microbiology. It gives me great pleasure to acknowledging the support and help of all staff of Microbiology Laboratory of Shree Birendra Hospital, especially to Mr. Ram Kumar Pandit, Mr. Binod Jamarkattel and Mr. Kiran Shrestha.

I would like to acknowledge University Grants Commission for providing fund for this work.

I am equally grateful to my all dear friends and to all the people who directly or indirectly helped me in completing this work.

Finally, I express my gratitude to my parents, who always inspired me for higher studies and are my constant source of inspiration and encouragement in every step of my life.

Kamal Bagale

Date: …….

ABSTRACT

Pseudomonas aeruginosa is one of the leading causes of nosocomial infections. Carbapenems are used as the last resort for treatment of multidrug resistant (MDR) gram-negative bacterial infection. Resistance to this life saving drug has been increasingly reported in *Pseudomonas* which is mainly due to production of metallo β- lactamases. Therefore this present study was conducted with an objective to find the prevalence of *P. aeruginosa* in different samples along with their antimicrobial susceptibility profile, find out the burden of MDR among *P. aeruginosa* and the production of metallo β- lactamases among those isolates.

Total of 5833 samples were analysed, at the microbiology department of Shree Birendra Hospital's, Chauni, Kathmandu from January 2013 to June 2013, for routine culture and antibiotic susceptibility testing. Organisms were identified by conventional microbiological method and antibiotic susceptibility test was performed by Kirby- Bauer disc diffusion method. Metallo β- lactamases (MBLs) were detected by using combined disk test using imipenem with Ethylenediaminetetraacetic acid (EDTA). Out of 5833 samples analysed, 942 showed positive growth among which 114 (12.1%) were *Pseudomonas aeruginosa*. Most of the isolates (95.6%) were sensitive to Imipenem and highest resistance (57.0%) was observed towards Ceftadizime. Only 33.3% of *P. aeruginosa* were multidrug resistant and most were isolated from ICU patients. Prevalence of metallo β- lactamases producing *P. aeruginosa* strains was 4.4% and all isolates were multidrug resistant. The study showed increasing trend of metallo β- lactamase producing isolates indicating the need of routine surveillance and timely control of the spread of these isolates in different units of health institutions.

Key words: *Pseudomonas aeruginosa*, Multidrug Resistance, Metallo β- lactamase

Table of Contents

Page itleT	i
Recommendation	ii
Certificate of Approval	iii
Board of examiners	iv
Acknowledgement	v
Abstract	vi
Table of Contents	-viivii
List of Abbreviations	ix
List of Tables	x
List of Figures	xi
List of Photographs	ixi
List of appendices	iiix

4-1 Introduction and Objectives : CHAPTER I

1.1 Introduction	1
1.2 Objectives	4

Literature Review :CHAPTER II 19-5

2.1 *Pseudomons aeruginosa*	5
2.2 Characteristic of *P.aeruginosa*	6
2.3 Virulence factor	7
2.3.1 Bacterial cell surface virulence factor	8
2.3.2 Secreted virulence factor	9
2.4 Pathogenecity of *P.aeruginosa*	11

2.5 Antibiotic resistance in *P.aeruginosa* 12
2.6 Multidrug resistant *Pseudomonas aeruginosa* 13
2.7 β - lactams and β- lactamases 14
2.7.1 β - lactams 14
2.7.2 β- lactamases 15
2.7.3 Classification β – lactamases 15
2.8 Metallo β – lactamases (MBLs) 16
2.8.1 Types of MBLs 18
2.8.2 Detection of MBLs 18

rials and MethodsMate :III CHAPTER 26-20
MATERIALS 3.1 20
3.2 METHODOLOGY 20
3.2.1 Sample size and sample type 20
specimens of Collection and transportation 3.2.2
20
3.2.3 Culture of specimens 22
3.2.4 Identification of isolates 23
3.2.5 Antibiotics susceptibility testing 24
Detection of metallo 3.2.6 βlactamase producing strain- 25
3.3 Quality control 25
3.4 Data analysis 26

Results :VCHAPTER I 34-27
40-35 nDiscussio :CHAPTER V
on and RecommendationConclusi :CHAPTER VI 41-42
6.1 Conclusion41
Recommendation 6.2 42
References 58-43
Appendices XVI-I

LIST OF TABLES

Table 1:	Distribution pattern of *Pseudomonas aeruginosa* according to gender and age	28
Table 2:	Growth status of the *P. aeruginosa*	30
Table 3:	Antibiotic sensitivity pattern of *P. aeruginosa* isolated from different growth positive specimen	31
Table 4:	Antibiotic resistant pattern of *P. aeruginosa* with reference to ward	32
Table 5:	Distribution and analysis of Multidrug Resistant *P. aeruginosa* in various specimens	33
Table 6:	Distribution of Multidrug Resistant *P. aeruginosa* according different wards	33

LIST OF FIGURES

Fig 1: Flow chart of methodology

Fig 2: Different types of bacterial organisms grown

Fig 3: Distribution of *P. aeruginosa* from various samples

Fig 4: Distribution of *P. aeruginosa* according different wards

Fig 5: Metallo β- lactamases production in *P. aeruginosa*

LIST OF PHOTOGRAPHS

Photograph 1 : Growth of *P. aeruginosa* in MacConkey Agar

Photograph 2 : Detection of MBL by Imipenem- EDTA method.

APPENDICES

Appendix A	List of Equipments and Materials	I
Appendix B	Methodology	III
Appendix C	Microbiological Profile of Patient	V
Appendix D	Composition and Preparation of Different Culture Media	VII
Appendix E	Identification of *Pseudomonas aeruginosa*	XVI

ABBREVIATIONS

ADP	: Adenosine diphosphate
ATCC	: American Type Culture collection
BA	: Blood Agar
CAZ	: Ceftazidime
CDC	: Centre for Disease Control
CLSI	: Clinical and Laboratory Standards Institute
CSF	: Cerebrospinal Fluid
CLED	: Cysteine lactose electrolyte deficient agar
DNA	: Deoxy Ribonucleic Acid
EDTA	: Ethylenediaminetetraacetic Acid
ESBL	: Extended Spectrum Beta Lactamase
ICU	: Intensive Care Unit
IMP	: Imipenemase
IPM	: Imipenem
ITCU	: Intensive Trauma Care Unit
LPS	: Lipopolysaccharide
MA	: Mac Conkey Agar
MBL	: Metallo β- lactamase
MDR	: Multi Drug Resistance
MHA	: Muller Hinton Agar
MHB	: Muller Hinton Broth
NA	: Nutrient Agar
PBP	: Penicillin Binding Proteins
PCR	: Polymerase Chain Reaction
SPSS	: Statistical Package for Social Science
TSI	: Triple Sugar Iron
TUTH	: Tribhuvan University Teaching Hospital
VIM	: Veronese Imipenemase
WHO	: World Health Organization