PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF METALLOBETALACTAMASE PRODUCING Pseudomonas aeruginosa ISOLATES AT KENYATTA NATIONAL HOSPITAL.

by Jane Njeri

Submission date: 08-Nov-2018 03:07PM (UTC+0300)
Submission ID: 1035262641
File name: graduate_thesis.docx_pliagirism.doc (3.71M)
Word count: 6857
Character count: 46199

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF METALLOBETALACTAMASE PRODUCING *Pseudomonas aeruginosa* ISOLATES AT KENYATTA NATIONAL HOSPITAL.

Jane Njeri Karuitha BSc,

MSc Medical Microbiology. (Student)

H56/68026/2013

A research dissertation submitted in partial fulfilment of the requirements for the award of a Master of Science degree in Medical Microbiology at the University of Nairobi.

DECLARATION

I Jane Karuitha declare that the work presented in this proposal is my original work and has not been presented in any institution for examination or any other purpose. All sources of information have been acknowledged by means of reference.

Jane Njeri Karuitha

MSc Medical Microbiology (Student)

H56/68026/2013

njerikajane@yahoo.com

Signature………………………………….   Date…………………

+254720 592 640

CERTIFICATION

This is to certify that this project has been submitted with our approval as university supervisors.

M/S Susan Odera. BSc. MSc (Medical Microbiology)

Department of Medical Microbiology University of Nairobi

Signature……………………………………………Date……………………..

Dr. Anne Maina

MBChb: MSc (Medical Microbiology)

Department of Medical Microbiology University of Nairobi

Signature……………………………………………Date…………………….

Dr. Marianne Mureithi BSc. MSc. PhD

Department of Medical Microbiology University of Nairobi

Signature……………………………………………Date……………………

DEDICATION

This work is dedicated to my family and husband for their unwavering support.

ACKNOWLEDGEMENTS

First, I would like to thank God for granting me the ability to successively complete this project.

I would also like to thank my supervisors Ms. Susan Odera, Dr. Anne Maina and Dr. Marianne Mureithi for their help and for their unwavering dedication and encouragement and support.

I would like to thank also Magdaline Burugu and Mr Gitau for their assistance in the laboratory work.

Special thanks to the Microbiology laboratory-in-charge (Beatrice Museve) Kenyatta National Hospital for her invaluable assistance in the isolate collection.

I am also grateful to my classmates Brian, Grace and Naomi for their continued encouragement throughout the Master's program.

Lastly, I would like to thank all who contributed to the success of this study one way or another

God bless you all.

TABLE OF CONTENTS

DECLARATION 1

CERTIFICATION 2

DEDICATION 3

ACKNOWLEDGEMENTS 4

LIST OF TABLES AND FIGURES 8

ABBREVIATIONS 9

ABSTRACT 10

CHAPTER ONE 11

1.0: INTRODUCTION 11

CHAPTER TWO 13

2.0: LITERATURE REVIEW 13

2.1 BACKGROUND 13

2.2 IMPORTANT CLINICAL SIGNIFICANCE OF PSEUDOMONAS AERUGINOSA 13

2.3 PATHOGENESIS 14

2.4 ANTIMICROBIAL SUSCEPTIBILITY 15

2.4.1 TREATMENT 15

2.4.2 RESISTANCE MECHANISM OF Pseudomonas aeruginosa 15

2.4.3 BIOFILMS 15

2.4.4 RESISTANCE MECHANISMS TO VARIOUS DRUGS *15*

2.5 MBL DETECTION 19

2.6 PROBLEM STATEMENT 20

2.7 STUDY JUSTIFICATION 20

2.8 RESEARCH QUESTION 21

2.9 OBJECTIVES 21

2.9.1 BROAD OBJECTIVE *21*

2.9.2 SPECIFIC OBJECTIVES *21*

CHAPTER THREE 22

3.0 METHODOLOGY 22

3.1 STUDY DESIGN 22

3.2 STUDY SITE 22

3.3 SAMPLES USED 22

3.4 SAMPLE SIZE 23

3.5 MATERIALS AND METHODS 24

3.5.1 STORAGE OF ORGANISMS *24*

3.5.2 SUBCULTURING *24*

3.5.3 ANTIMICROBIAL SUSCEPTIBILITY *24*

3.5.4 MBL DETECTION *25*

3.5.5 QUALITY CONTROL *26*

3.6 ETHICAL APPROVAL 26

CHAPTER FOUR 27

4.0 RESULTS **27**

4.1 ISOLATES 27

4.2 SOURCE OF ISOLATES AND THE TYPE OF SPECIMEN 27

4.3 ANTIBIOTIC SUSCEPTIBILITY OF *PS. AERUGINOSA* 28

CHAPTER FIVE **34**

5.1 DISCUSSION 34

5.2 CONCLUSION 35

5.3 RECOMMENDATIONS 35

5.4 STUDY LIMITATIONS 36

REFERENCES **37**

APPENDIX **44**

APPENDIX 1 44

APPENDIX 2 45

LIST OF TABLES AND FIGURES

Table 1: Demographic characteristics of the isolates 27

Table 2: Source of isolates and the type of specimen 28

Table 3: Antibiotic susceptibility of Ps. aeruginosa 28

Table 4: MBL production 30

Table 5: Antibiotic susceptibility by type of specimen 31

Table 6 : Antibiotic susceptibility by source of isolates 33

Figure 1 : Antibiotic susceptibility of P. aeruginosa..... 29

ABBREVIATIONS

AIM..Australian imipenemase

CSF ....Cerebral spinal fluid

EDTA...Ethylene diaminetetraacetic acid

GIM..German imipenemase

IMP ....Imipenemase

MBLs ....Metallobetalactamases

MDR ....Multi drug resistance

MDRPA ....Multi drug resistance Pseudomonas aeruginosa

NDM ....New Delhi metallobetalactamase

PCR..Polymerase chain reaction

SIM ....Seoul imipenemase

SPM..Sao Paolo metallobetalactamase

VIM..Verona integron encoded metallobetalactamase

ABSTRACT

Pseudomonas aeruginosa is a major cause of nosocomial infections with high mortality rates. The organism is highly resistant to most classes of drugs used and can acquire resistance during treatment. One of the resistance mechanisms of *Pseudomonas aeruginosa* is metallobetalactamase (MBL) production. Metallobetalactamase producing *Pseudomonas aeruginosa* is a major health concern given its high resistance to almost all available drugs. The prevalence of this *Pseudomonas aeruginosa* is unknown since there is no standardized method for metallobetalactamase detection.

Study design: This was a laboratory based cross-sectional prospective study that was carried out from September 2015 to March 2016 in Kenyatta National Hospital.

Methodology: 99 isolates of *Pseudomonas aeruginosa* were collected during the period and were tested for antimicrobial susceptibility.Isolates found to be resistant to imipenem were tested for metallobetalactamase production.

Results: The results indicate high resistance of *Pseudomonas aeruginosa* to commonly used drugs. Of the isolates tested 69.7% were resistant to piperacillin 63.6% were resistant to aztreonam, 58.6%were resistant to levofloxacin, 55.6% were resistant to cefipime , 65.7%were resistant to ceftazidime ,68.7% were resistant to ticarcillin – clavulanate ,86.4% of urine isolates were resistant to ofloxacin ,72.2% were resistant to meropenem while 64.9% were resistance to imipenem. 87.3% of the isolates resistant to imipenem were MBL producers.

Conclusion: *Ps aeruginosa* is highly resistant to the currently used drugs for treatment and resistance to carbapenems is largely due to MBL production

CHAPTER ONE

1.0: INTRODUCTION

Pseudomonas aeruginosa (*Ps. aeruginosa*) is a motile, non-sporing gram-negative bacilli. It is an opportunistic pathogen causing nosocomial infections and outbreaks with high mortality rates (Vahdani *et al.,*2012, Wirth *et al* .2009, Lambert *et al.,*2011).*Ps. aeruginosa* infections cause a wide array of clinical conditions both local and systemic. It possesses several virulence factors in its structural components and also produces toxins and enzymes which propagate toxigenic and systemic manifestations of infection (Ben *et al.*, 2011).

Most pathogens that cause nosocomial infections exhibit resistance to antimicrobial agents. *Ps. aeruginosa* is a multidrug resistant (MDR) organism and has several resistance mechanisms which include; chromosomal AmpC cephalosporinase depression, loss of permeability of the outer membrane (that is loss of OprD proteins), over expression of active efflux pumps , amino glycoside modifying enzymes synthesis, structural alterations of topoisomerase II and IV and plasmid or integron mediated beta-lactamases. (McGowan.,2006, Hancock and Speert,.2000, Falagasand Bliozoit,.2007, Gooderham and Hancock.,2009).

Carbapenems (meropenem, doripinem, imipenem) are the last line drugs for treatment of MDR *Ps. aeruginosa* (MDRPA) but resistance to these drugs have been detected in some strains (Zhao *et al.,*2010). These strains produce carbapenem hydrolyzing enzymes (carbapenemases) which mediate resistance to carbepenems. Carbapenemases are mostly metallo – beta lactamases (MBLs) and include: Imipenemase (IMP), Australian imipenemase (AIM), Sao Paolo MBL (SPM), Verona integron encoded MBL (VIM), Seoul imipenemase (SIM) German imipenemase (GIM) and most recently New delhi MBL (NDM) (Wang *et al.*, 2010). MBLs have a worldwide

distribution and have been identified virtually in all continents and their spread is continuing. (Poirel *et al.*, 2007).

CHAPTER TWO

2.0: LITERATURE REVIEW

2.1 BACKGROUND

Ps. aeruginosa is a gram negative, bacteria which is widely distributed in nature. It can be found in soil, plants, animals and water. *Ps. aeruginosa* is a non-fastidious organism that has been isolated from sewage, distilled water, swimming pools, disinfectants, water baths, hot tubs, intravenous tubings and medical devices (Richard, 2013). The bacteria is resistant to high temperatures, high salt concentrations and dyes and also to majority of antibiotics .(www.textbookofbacteriology.com). *Ps.aeruginosa* colonises moist areas in hospitals such as food, cut flowers, sinks, toilets, floor mops, respiratory therapy and dialysis equipment and even in disinfectants solutions.(Murray, 2013).

2.2 IMPORTANT CLINICAL SIGNIFICANCE OF *Ps. aeruginosa*

Ps. aeruginosa rarely causes infection in healthy subjects but causes infection where there is disruption of physical barriers such as in the use of invasive devices. The organism causes a wide array of clinical conditions which include: lower respiratory tract infections, primary skin and soft tissue infections mostly in severe burns patients due to moist surface of the burn and neutrophil inability to access the burn wound. It also causes folliculitis, finger nail infection, and osteochondritis. The organism also causes infection of the ear that is external otitis media mostly in children, malignant external otitis media common in the elderly and diabetic and chronic otitis media. It also causes urinary tract infections which are common in patients with long term

indwelling catheters and eye infections which occurs after initial trauma of the eye (Murray , 2013).

2.3 PATHOGENESIS

The bacteria's virulence depends on cell associated and extracellular factors which play a role in colonization, invasion and survival of bacteria in the host. Infections by *Ps. aeruginosa* can be acute or chronic (Ben *et al*., 2011).

In acute infections the virulence factors involved are pili for attachment, exoenzymes S and T and adhesins which cause adherence to epithelial cells, exotoxin A which causes necrosis in tissues, phospholipase C which disrupt the normal organization of the cytoskeleton and destroy immunoglobulin (Ig) IgG and IgA, leading to depolymerization of actin filaments and resistance to macrophages, proteases which cause bleeding and tissue necrosis (Ben *et al*, .2011), elastases; Las A(Serine protease) and Las B(Zinc metalloprotease) which degrade elastin allowing dissemination and also inhibit neutrophil chemo taxis and function facilitating spread and alkaline proteases contribute to tissue destruction and spread of bacteria and interfere with host immune reactions.(Murray, 2013).

Virulence factors that play a role in chronic infections include siderophores (pyoverdin and pyochelin) which allow survival in absence of iron and a pseudo capsule of alginate which protects against phagocytosis, dehydration and antibiotics and also aids in adherence and biofilm formation (Ben *et al* .2011) Chronic infections are characterized by formation of antibodies to Las A and Las B which leads to deposition of immune complexes in infected tissues (Murray, 2013).

2.4 ANTIMICROBIAL SUSCEPTIBILITY

2.4.1 TREATMENT

Ps aeruginosa poses serious challenges in treatment of both nosocomial and community acquired infections and identifying the right drugs for treatment is key. (Bisbe.,1988, Micek *et al*., 2005). Yet selection of the right drug is complicated by the organism's ability to acquire resistance to available drugs even during treatment. Choice of treatment for *Ps aeruginosa* infection is limited. Drugs used for treatment include: betalactams, aminoglycosides, fluoroquinolones with ciprofloxacin being the most active, polymyxins (polymyxin B and colistin whose use is restricted to MDRPA due to toxicity) (Neda *et al*., 2009).

2.4.2 RESISTANCE MECHANISM OF *Pseudomonas aeruginosa*

2.4.2.1 BIOFILMS

These are structures in which bacteria are attached on the surface and are imbedded in a matrix and have a polysaccharide protein and DNA (Lopez et al.,2010). *Ps. aeruginosa* biofilm growth causes it to have marked resistance to antimicrobial agents (Hoiby et al., 2010). Biofilm resistance mechanism are complex and not well understood (Drenkard,2003;Hoiby et al.,2010)

2.4.3 RESISTANCE MECHANISMS TO VARIOUS DRUGS

2.4.3.1 FLUOROQUINOLONES

This class of drugs include: ciprofloxacin, norfloxacin, oflaxacin and levofloxacin. Resistance to these agents is due to mutations in topoisomerase IV enzymes and DNA gyrase which are the targets of the Fluoroquinolones, efflux also contributes to resistance (Jacoby,2005; Drlica et al.,2009) which act together with mutations at the target site(Higgins et al .,2003).

2.4.3.2 AMINOGLYCOSIDES

This class of drugs include: amikacin,tobramycin gentamicin;(Gilbert et al.,2003;Bartlett,2004) However the use of this drugs is associated with resistance development. Resistance to this class is mainly due to endogenous efflux mechanism, rRNA methylases and acquired aminoglycoside-modifying enzymes (AMEs) (Poole ,2005)

Aminoglycoside modifying enzymes

This enzymes lead to inactivation of aminoglycosides. This inactivation is due to actetylation by aminoglycoside acetyltransferase (AACs) or adenylation by aminoglycoside nucleotidyltransferases(ANTs)phosphorylation by aminoglycoside phosphoryltransferases(APHs) and aminoglycoside adenylyltransferase(AAD) (Ramirez and Tolmasky,2010)

Efflux

Aminoglycoside resistance that is not caused by inactivating enzymes has been known for sometime in *Ps. aeruginosa*.It is marked by resistance to all aminoglycoside and brings about reduced aminoglycoside accumulation (Bryan et al.,1976). Which is due to efflux by the MexXY-OprM multidrug system which has been implicated in resistance to aminoglycosides (Hocquet et al.,2006).

16S rRNA methylases

This resistance mechanism is due to methylation of the 16SrRNA of the A site of the 30S ribosomal subunit, , leading to interference of aminoglycoside binding which leads to high-level resistance to aminoglycosides. (Doi and Arakawa,2007).

2.4.3.3 POLYCATIONIC ANTIMICROBIALS

These agents include colistin and polymyxin B. Colistin in particular, is quite effective in treating MDRPA infections (Falagas et al.,2010) Resistance to these drugs have been reported (Landman et al., 2005; Samonis et al.,2010). The resistance mechanism of polymyxin B is not understood but lipopolysaccharide lipid A substitution by aminoarabinose has been shown to contribute to resistance (Moskowitz et al., 2004).

2.4.3.4 BETALACTAMS

This is a broad-spectrum antibiotic class and the antibiotics include;

- Penicillins (Piperacillin,Ticarcillin)
- Cephalosporins (Cefepime,Ceftazidime)
- Monobactams (Aztreonam)
- Carbapenems (Doripinem,Imipinem and Meropenem)

(Paul et al.,2010)

Mechanism of resistance to these drugs include:

Efflux mechanism

Efflux mechanism that mediate resistance to betalactams include 5 families of the efflux system F (Li and Nikaido,2010) It has been shown that the Resistance Nodulation Division (RND)family is the greatest contributor of resistance in *Ps.aeruginosa* and are 12 of which MexAB-OprM,MexCD-OprJ and MexXY-OprM have been shown to mediate resistance β -Lactams (Poole,2004a, 2004b,2007).

Loss of outer membrane porin proteins

This is the most common resistance mechanism to carbapenems (Wang et al .,2010).The outer membrane proteins are the portal of entry of carbapenems (Trias and Nikaido, 1990). This mechanism of resistance does not contribute to resistance in MBL producers but is a major contributor to resistance in non MBL mediated resistance often in combination with other resistance mechanisms such as derepressed *ampC* or MexAB-OprM (Gutierrez et al.,2007; Wang et al.,2010)]. This type of resistance requires AmpC (Livermore,1992).

Betalactamases

Betalactamase enzymes hydrolyze the amide bond of the betalactam ring making the antimicrobial ineffective. These enzymes are grouped into molecular classes of which four of them have been described(A-D) (Helfand and Bonomo, 2003), these enzymes can be divided into endogeneous betalactamases and acquired betalactamases (extended spectrum betalactamases and carbepenamases). (Zhao and Hu, 2010)

Carbapenemases are the most versatile family of betalactamases. These enzymes hydrolyze the beta-lactam ring in carbepenems rendering them ineffective. Betalactamases are divided into three ambler classes: A, D and B (Chen *et al* .2011) MBLs are in class B and are binuclear zinc-dependent enzymes susceptible to inhibition by metal chelators such as EDTA. They hydrolyze a variety of β-lactams agents including cephalosporins,penicillin and carbapenems but not monobactams. The genes responsible for production of MBLs are part of an integron structure, encoded on large transferrable plasmids (Walsh *et al* .2005). These plasmids can be transferred to other bacteria (Poirel *et al* .2007). MBLs are divided into families, according to their

molecular structure. The most common families include the IMP, VIM, SIM, and GIM (Wang *et al.*2010). The first MBL producing *Ps. aeruginosa* was identified in Japan in 1991 and since then virtually in every continent Asia, North America, South America, Europe, Australia and Africa (Poirel *et al* .,2007,Moet et al., 2007).

In Kenya, a study carried out in a private hospital during an outbreak of *Ps. aeruginosa* infection to characterize the betalactamases content of carbapenem resistant *Ps. aeruginosa* found that all carbapenem resistant isolates were MBL producers and the gene isolated was VIM-2(Pitout *et al*., 2008).

Ps. aeruginosa is of great medical importance since it has the ability to acquire resistance during the course of treatment and the fact that it's one of the most prevalent nosocomial pathogen worldwide. There is therefore a need for continued surveillance of the resistance mechanism of the bacteria. MBLs detection is of great importance in control and treatment of MDRPA.

2.4 MBL DETECTION

There is no standardized test for MBL production. MBL detection methods can be phenotypic or genotypic. Polymerase chain reaction (PCR) is a genotypic method which is highly accurate and reliable but it's expensive hence not used in routine laboratories and is only used in reference laboratories (Franklin.,2006). Phenotypic methods include IMP – EDTA combined disc test, IMP – EDTA, double disc synergy test(DDST) EDTA, disc potentiation using Ceftazidime, ceftizoxime, cefipime and cefotaxime and MBL-E test. Of this phenotypic test only, IMP-EDTA combined disc test and MBL – E test are sensitive. (Behera *et al*., 2008)

2.5 PROBLEM STATEMENT

Ps. aeruginosa is a major health concern since it is a common cause of nosocomial infections world-wide and has an ability to develop resistance during treatment (Dharmalingam *et al.*, 2012).Carbepenems are the last drug of choice for treatment of MDR *Ps .aeruginosa.* It is impossible to predict the impact that MBLs will have in future, since there is no drug in the pipeline to replace those that have become ineffective (World Health Organization., 2014). There is need to detect MBL producers in order protect the efficacy of the existing drugs and prevent further spread. Determination of the antibiotic resistance patterns is crucial to the patient and to the society. In patients it is crucial because this information will guide patient management thereby reduce mortality rates, cut cost by reducing the length of hospitalization and also spread of resistant MBL strains. In the society this knowledge would guide policy that would contribute in reducing the health care burden, and for prevention of loss of labor due to death of infected individuals.

Routine surveillance in most countries is only done in patients with severe hospital acquired infections (HAI) and community acquired infections are under-represented leading to a knowledge gap of the big picture. (World Health Organization.,2014) There is no recent data on MBL production in Kenya. A previous study in Kenya in a private hospital showed that all carbapenem resistant isolates were MBL producers (Pitout *et al*., 2008). There is however no data on prevalence of MBL producing isolates of *Ps. aeruginosa* in public hospitals.

2.6 STUDY JUSTIFICATION

There is need for continued surveillance for MBL producing isolates which is necessary for identification of appropriate guide to clinicians in choosing the right treatment drugs and to minimize the rate of resistance and to control spread of resistance. There is need for continued

surveillance of MBL producing *Ps. aeruginosa* given that critical care units are being established in every county in Kenya hence early detection of MBLs will prevent further spread of these genes to other bacteria.

This study was carried out in Kenyatta National Hospital (KNH), the largest public referral hospital in Kenya to identify the resistance patterns of all isolated specimens of *Ps. aeruginosa*

2.7 RESEARCH QUESTION

What is the prevalence, antimicrobial susceptibility pattern and MBL production in *Ps. aeruginosa* isolates in KNH?

2.8 OBJECTIVES

2.8.1 BROAD OBJECTIVE

To determine the antimicrobial susceptibility pattern and production of metallobetalactamase in *Ps. aeruginosa* isolates in KNH.

2.8.2 SPECIFIC OBJECTIVES

i. To determine the antimicrobial susceptibility profile of *Ps. aeruginosa* isolated in clinical specimens in KNH at the microbiology laboratory.
ii. To determine MBL production in *Ps. aeruginosa* isolated in clinical specimens in KNH at the microbiology laboratory.

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

This was a laboratory based prospective cross- sectional study.

3.2 STUDY SITE

The study was carried out in Kenyatta National Hospital Microbiology Laboratory from September 2015 to March 2016. KNH is an 1800 bed capacity facility with 6000 staff. It is the largest referral center in Kenya and a teaching hospital which provides specialized health care to patients referred from other hospitals countrywide. Specimens were processed in the Medical Microbiology laboratory of the hospital. The laboratory process throat swabs, urethral swabs, pus swabs, stool, tracheal aspirates, cerebrospinal fluid, pleural fluid, sputum and urine specimens. The microbiology laboratory undergoes quality assurance by World Health Organization via the National External Quality Assessments Services schemes of the Republic of South Africa. It is a safety level two facility.

3.3 SAMPLES USED

Samples used were *Ps. aeruginosa* isolates from throat swabs, pus swabs, urine and sputum.

3.4 SAMPLE SIZE

The sample size was calculated using Fishers formula (Fisher. 1954)

$$n = z^2pq/d^2$$

Where,

n= sample size

z= standard normal variant corresponding to the 95% confidence interval, and is 1.96

p= Prevalence of *Ps. aeruginosa* 57.9%, expressed as a decimal (Study carried out in Iran. Fallah *et al*., 2013)

q= 1-p

d= the required precision of estimate (0.05)

Thus,

$$n = \frac{1.96^2 * 0.58(0.42)}{(0.05^2)}$$

$$n_0 = 375$$

Since the estimated numbers of Ps. *aeruginosa* isolates in KNH laboratory is few, the formula below is applied to adjust the sample for a finite population;

$$n = \left[\frac{n_0}{1 + \frac{n_0 - 1}{N}}\right]$$

n_0 is the sample size (375)

n = is the final sample size

N = Estimated number of *Ps. aeruginosa* isolates in the two months in the KHN laboratory (140)

Using the above formula our final sample size is **103**

3.5 MATERIALS AND METHODS

Isolates used were those identified as part of the routine laboratory work and had been positively identified as *Ps. aeruginosa.* The isolates were isolated using routine process from September 2015 to April 2016.

3.5.1 STORAGE OF ORGANISMS

All *Ps. aeruginosa* isolates were stocked in vials containing broth with 15% glycerol and incubated overnight to allow them to grow and then frozen at -70°c and pooled to acquire the sample size.

3.5.2 SUBCULTURING

The stocked isolates were removed from the freezer and allowed to thaw and then with a sterile wire loop were subcultured on macconkey agar to revive them and check for viability and were incubated overnight at 37^{0}c.Susceptibility testing was done on mueller hinton agar using the disc diffusion method various drugs were used.

3.5.3 ANTIMICROBIAL SUSCEPTIBILITY

A broth culture of test strain (0.5 Mcfarland opacity) was inoculated on a plate of Mueller Hinton agar. The plate was streaked using a swab in one direction and then the plate was rotated 90° and streaking was repeated in that direction in-order to obtain uniform growth. Rotation was

repeated 3 times and the plate allowed to dry for approximately 5 minutes. Antibiotic discs were then dispensed using an antibiotic dispenser, then using some sterile forceps the discs were pressed gently on the agar to ensure the discs were attached to the agar. The plates were inoculated for 16 – 18 hrs at 37°c. Zone of clearance was measured using a ruler and recorded in millimeters (mm) and was interpreted sensitive, intermediate or resistant according CLSI guidelines. (CLSI 2015)

Antimicrobial agent	Disk content	Zone diameter interpretive criteria to the nearest mm)		
		S	**I**	**R**
Piperacillin	100µg	≥21	15 - 20	≤14
Aztreonan	30µg	≥22	16 - 21	≤15
Levoflaxacin	5µg	≥17	14 - 16	≤13
Cefipime	30µg	≥18	15 - 17	≤14
Ceftazidime	30µg	≥18	15 - 17	≤14
Ticarcillin – Clavulanate	75/10µg	≥24	16 - 23	≤15
Oflaxacin	5µg	≥16	13 - 15	≤12
Imipinem	10µg	≥19	16 - 18	≤15
Meropenem	10µg	≥19	16 - 18	≤15

(CLSI 2015)

3.5.4 MBL DETECTION

A 0.5 M Ethylene diaminetetraacetic acid (EDTA) solution was prepared by dissolving 186.1g of disodium EDTA.2H_2O in 1000ml of distilled water and the pH was adjusted to 8 using NaOH. The mixture was then autoclaved to sterilize it, then using a 4 (micro liters) pipette the EDTA solution was poured on imipenem disks. The disks were then dried in an incubator immediately. A broth culture of test strain (opacity adjusted to0.5McFarland opacity) was inoculated on a plate of Mueller Hinton Agar. A 10 microgram imipenem disk was placed on the agar plate and the

EDTA impregnated disk was placed on the same plate. The plate was incubated at 37°c for 16 – 18 hrs the zone of clearance was measured using a ruler and comparison was made between the imipinem discs and the EDTA impregnated discs. A zone of clearance of at least 7mm around the imipenem-EDTA disk as compared to imipenem disk without EDTA was recorded as an MBL producing strain (Yong *et al*., 2002)

3.5.5 QUALITY CONTROL

Ps. aeruginosa ATCC 27853 was used as a standard positive control and the results were compared with CLSI guidelines for validation. The work was counterchecked by two qualified technologists.

3.6 ETHICAL APPROVAL

Study approval to carry out the research was obtained from KNH/UON (P238/04/2015) ethics review board. Permission to obtain isolates and obtain data on the patient's details was obtained from the Head of Laboratory Medicine KNH. In this study there was no direct interaction with the patients and all the data obtained concerning patients was obtained from the laboratory records. Patient's details were handled with utmost confidentiality.

CHAPTER FOUR

4.0 RESULTS

4.1 ISOLATES

Out of the 104 samples stocked,5 failed to grow so a total of 99 samples were tested for sensitivity. For imipenem only 97 isolates were tested this is because 2 were left out accidentally. The median age of the isolates source was 28 years (IQR 8.8-50.3 years); the isolates source included infants as young as 4 months old and adults up to 86 years of age. More than a half of the isolates (52.5%) were from males and 44.4% were from females.

Table 1: Demographic characteristics of the patients

Variable	**Frequency (%)**
Median age in years (IQR)	28.0 (8.8-50.3)
Gender	
Female	44 (44.4)
Male	52 (52.5)
Not specified	3 (3.0)

4.2 SOURCE OF ISOLATES AND THE TYPE OF SPECIMEN

More than three quarters (77.8%) of the isolates were from the ICU, 15.2% were from other wards while 2% were obtained from outpatient department. The specimens taken were mainly tracheal aspirates (67.7%) while 23.2% were urine samples and 8.1% pus.

Table 2: Source of isolates and the type of specimen

Variable	Frequency (%)
Ward	
ICU	77 (77.8)
Other wards	15 (15.2)
Outpatient	2 (2.0)
Not specified	5 (5.1)
Specimen type	
Pus	8 (8.1)
Sputum	1 (1.0)
TA	67 (67.7)
Urine	23 (23.2)

4.3 ANTIBIOTIC SUSCEPTIBILITY OF *Ps. aeruginosa*

11

Ps. aeruginosa had a high level of resistance to the antibiotics that were tested.

Table 3: Antibiotic susceptibility of *Ps. aeruginosa*

Variable	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Cefepime	37 (37.4)	7 (7.1)	55 (55.6)
Ceftazidime	29 (29.3)	5 (5.1)	65 (65.7)
Piperacillin	27 (27.3)	3 (3.0)	69 (69.7)
Ticarcillinclavulanate	23 (23.2)	8 (8.1)	68 (68.7)
Azetreonam	26 (26.3)	10 (10.1)	63 (63.6)
Levofloxacin	38 (38.4)	3 (3.0)	58 (58.6)
Oflaxicin (n=22)	3 (13.6)	0	19 (86.4)
Meropenem (n=97)	24 (24.7)	3 (3.1)	70 (72.2)
Imipinem (n=97)	34 (35.1)	0	63 (64.9)

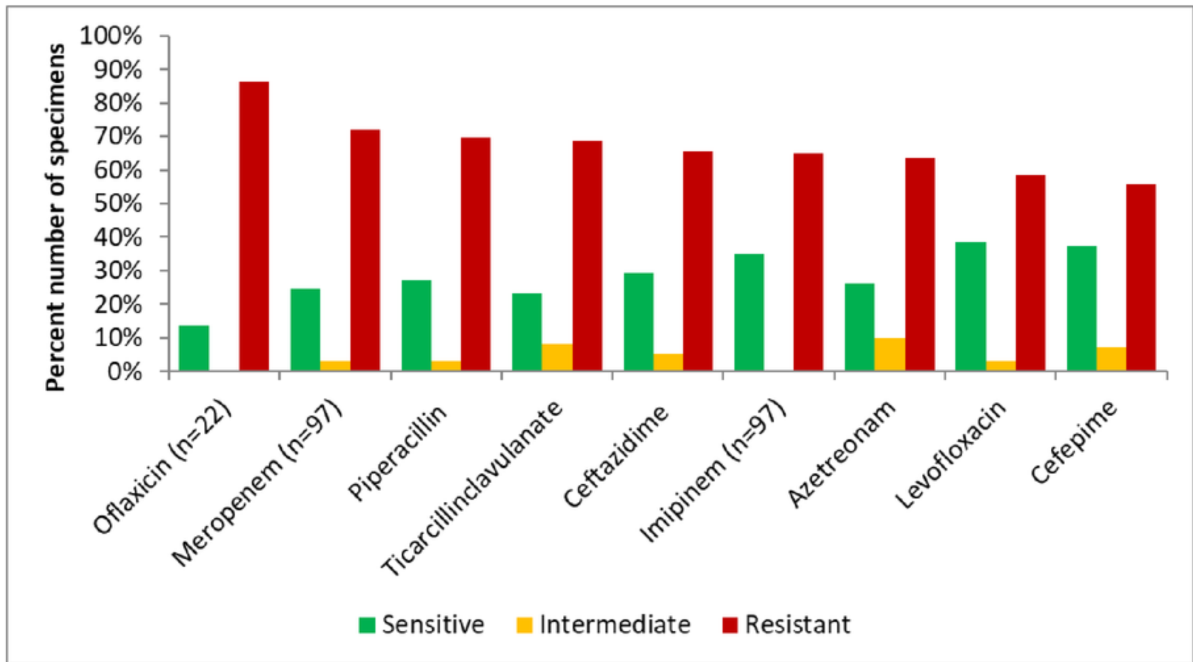


Figure 1 : Antibiotic susceptibility of *Ps. aeruginosa*

MBL production

MBL Production was evaluated in the imipenem resistance isolates and 87.3% of the isolates were MBL producers while 12.7% were non MBL producers

Table 4: MBL production

Variable	Frequency (%)
MBL production (n=63) MBL producers	 55 (87.3)
MBL non producers	8(12.7)

Antibiotic susceptibility by type of specimen

Susceptibility to cefepime and levofloxacin was significantly associated with the specimen type from which the isolates of *Ps. aeruginosa* were obtained. There was a high level of resistant organisms against cefepime isolated from urine samples78.3% (18/30) while about a half (52.2%) were isolated from tracheal aspirates samples; 75%(6/30) of the *Ps. aeruginosa* isolated from pus were sensitive towards cefepime. Similarly, 82.6% (19/30) of isolates obtained from urine samples were resistant to levofloxacin and 53.7% of the tracheal aspirates isolates were resistant while 50%(4/30) of the pus isolates were sensitive.

Table 5: Antibiotic susceptibility by type of specimen

Drug	Specimen type*		
	Tracheal aspirates	**Urine**	**Pus**
Cefepime			
Sensitive	26 (38.8)	4 (17.4)	6 (75.0)
Intermediate	6 (9.0)	1 (4.3)	0
Resistant	35 (52.2)	18 (78.3)	2 (25.0)
Ceftazidime			
Sensitive	20 (29.9)	4 (17.4)	5 (62.5)
Intermediate	5 (7.5)	0	0
Resistant	42 (62.7)	19 (82.6)	3 (37.5)
Piperacillin			
Sensitive	18 (26.9)	4 (17.4)	5 (62.5)
Intermediate	3 (4.5)	0	0
Resistant	46 (68.7)	19 (82.6)	3 (37.5)
Ticarcillin clavulanate			
Sensitive	17 (25.4)	3 (13.0)	3 (37.5)
Intermediate	5 (7.5)	1 (4.3)	1 (12.5)
Resistant	45 (67.2)	19 (82.6)	4 (50.0)
Azetreonam			
Sensitive	19 (28.4)	4 (17.4)	3 (37.5)
Intermediate	9 (13.4)	0	1 (12.5)
Resistant	39 (58.2)	19 (82.6)	4 (50.0)
Levofloxacin			
Sensitive	29 (43.3)	4 (17.4)	4 (50.0)
Intermediate	2 (3.0)	0	1 (12.5)
Resistant	36 (53.7)	19 (82.6)	3 (37.5)
Meropenem			
Sensitive	16 (24.6)	4 (17.4)	4 (50.0)
Intermediate	3 (4.6)	0	0
Resistant	46 (70.8)	19 (82.6)	4 (50.0)
Imipinem			
Sensitive	27 (40.9)	4 (18.2)	3 (37.5)
Resistant	39 (59.1)	18 (81.8)	5 (62.5)

**Sputum specimen excluded due to small numbers (n=1)*

Antibiotic susceptibility by source of isolates

As shown in table 6, the ward in which the isolate was obtained was significantly associated with susceptibility of *Ps. aeruginosa* to ceftazidime, piperacillin, ticarcillin-clavulanate, azetreonam and oflaxicin. In all the instances, organisms isolated from the ICU had a higher level of resistance to the listed drugs than isolates from other wards. ICU isolates were 68.8% resistant to ceftazidime, 74% to piperacillin, 74% to ticarcillin-clavulanate , 66.2% to azetreonam and 100% to oflaxicin . The susceptibility of the other drugs was not significantly associated with the source of the patients.

Table 6 : Antibiotic susceptibility by source of isolates

Variable	Patients' source*	
	ICU	**Other wards**
Cefepime		
Sensitive	26 (33.8)	9 (60.0)
Intermediate	7 (9.1)	0
Resistant	44 (57.1)	6 (40.0)
Ceftazidime		
Sensitive	19 (24.7)	9 (60.0)
Intermediate	5 (6.5)	0
Resistant	53 (68.8)	6 (40.0)
Piperacillin		
Sensitive	17 (22.1)	9 (60.0)
Intermediate	3 (3.9)	0
Resistant	57 (74.0)	6 (40.0)
Ticarcillin-clavulanate		
Sensitive	15 (19.5)	7 (46.7)
Intermediate	5 (6.5)	2 (13.3)
Resistant	57 (74.0)	6 (40.0)
Azetreonam		
Sensitive	17 (22.1)	8 (53.3)
Intermediate	9 (11.7)	1 (6.7)
Resistant	51 (66.2)	6 (40.0)
Levofloxacin		
Sensitive	27 (35.1)	9 (60.0)
Intermediate	3 (3.9)	0
Resistant	47 (61.0)	6 (40.0)
Oflaxicin		
Sensitive	0	3 (42.9)
Resistant	11 (100.0)	4 (57.1)
Meropenem		
Sensitive	15 (20.0)	8 (53.3)
Intermediate	2 (2.7)	0
Resistant	58 (77.3)	7 (46.7)
Imipinem		
Sensitive	25 (33.3)	8 (53.3)
Resistant	50 (66.7)	7 (46.7)

**Outpatients were excluded due to small numbers (n=2)*

CHAPTER FIVE

5.1 DISCUSSION

From this study it's quite evident that *Ps. aeruginosa* is a serious threat in the health care settings. The highest isolates were from the ICU (77.8%), this is because the ICU has one of the highest occurrence rates of nosocomial infections 20 -30% (Hanberger et al, 1999) and the highest resistance was witnessed in isolates from the ICU as compared to other wards.

Of the isolates tested 69.7% were resistant to piperacillin 63.6% were resistant to aztreonam, 58.6%were resistant to levofloxacin ,55.6% were resistant to cefipime ,65.7%were resistant to ceftazidime ,68.7%were resistant to ticarcillin – clavulanate ,86.4% of urine isolates were resistant to ofloxacin while 72.2% were resistant to meropenem while 64.9% were resistance to imipenem.87.3% of the imipenem resistant isolates were found to be MBL producers. A previous study carried out in Kenya in a private hospital showed that 53% of the isolates of *Ps. aeruginosa* were resistant to piperacillin and aztreonam, whereas 100% were resistant to ceftazidime, cefepime, tobramycin, gentamicin, amikacin and ciprofloxacin. (Pitout et al,.2008) of which our results differ slightly. Also majority of our isolates were from the ICU (77.8%) ,15.2% were from other wards while 2% were from outpatients, also from our study the isolates were majorly tracheal aspirates (67.7%) 23.2% were urine samples while 8.15 % were from pus in a similar study carried out Kenya in the Aga Khan University Hospital, of the isolates tested three (5%) were isolated from urine, four (7%) from blood, 17 (30%) from wounds (purulent), 30 (53%) from respiratory tract specimens, and the remaining three (5%) from various other specimens. (Pitout et al,.2008) Variation in results obtained differ according to geographical location and according to the drugs that are prescribed to treat *Ps. aeruginosa* infections.

The results obtained are comparable to a study carried out in Iran (Galvani., 2015) whereby the the rate of resistance to imipenem was 72% while MBL production identified on isolates resistant to imipinem was 88.9% this was slightly lower compared to a study carried out in the In Kenya, in a private hospital during an outbreak of *Ps. aeruginosa* infection to characterize the betalactamases content of carbapenem resistant *Ps.aeruginosa* which found that all carbapenem resistant isolates were MBL producers and the gene isolated was VIM-2(Pitout et al., 2008) . From our study it's quite evident that MBL poses a serious risk in health setups considering that MBL resistant isolates can be resistant to all betalactams posing a serious problem in the treatment of *Ps. aeruginosa* infections.

It's also evident that resistance to carbepenems is not only due to MBLs since all the isolates resistant to imipinem 87.3% were MBL producers, while 12.7% were non MBL producers which could be due to impermeability due to loss of the OprD porin or upregulation of the active efflux pump system in the cytoplasmic membrane of the *Ps. aeruginosa* (Bahar et al .,2010)

5.2 CONCLUSION

Ps aeruginosa is highly resistant to the currently used drugs for treatment and resistance to carbapenems is largely due to MBL production.

5.3 RECOMMENDATIONS

- It's necessary to have a routine surveillance of MBL production to guide the physicians on the most effective treatment regimen and also to prevent further spread of the enzymes to the enterobacteriaceae family

- It's important to test various combinations of drugs for treatment of infections that are resistant to carbapenems
- Use of polycationic antimicrobials (colistin and polymyxin B) should be considered for the carbapenem resistance isolates and also such drugs sensitivity should be tested

5.4 STUDY LIMITATIONS

- Specific MBL genes were not identified due to financial constrain.
- It was impossible to tell the specific mechanism of resistance to the various antibiotics since it was not tested due to cost
- It was also impossible to follow up the patients to obtain the clinical outcome due to the *Pseudomonas aeruginosa* infections since there was no direct interaction with the patients

REFERENCES

1. Bartlett J.G (2004) *Pocket book of infectious Disease Therapy* .Baltimore:Lippincott Williams and Wilkins
2. Behera B, Mathur P, Das A, et al (2008). An evaluation of four different phenotypic techniques for detection of metallobetalactamase producing *Pseudomonas aeruginosa. Indian J Med Micobiol*; *26:233-7*
3. Ben Haj Khalifa A, Moissenet D, Vu Thien H, Khedher M. (2011). Virulence factors in Pseudomonas aeruginosa: mechanisms and modes regulation. *Ann Biol Clin (Paris)*: 69(4); 393 – 403
4. Bryan L. E, Haraphongse R, Van den Elzen H. M. Gentamicin resistance in clinical isolates of *Pseudomonas aeruginosa* associated with diminished gentamycin accumulation and no detectable enzymatic modification *J. Antibiot,29,743-753*
5. Bush K, G.A Jacoby, A.A Medeiros(1995).A functional classification scheme for betalactamases and its correlation with molecular structure.*Antimicrob Agents Chemother*.39:1211-1233
6. Chen Y, Zhou Z, Jiang Y et al (2011): Emergence of NDM- 1 producing *Acinetobacter baumanni* in China. *J Antimicrob Chemother.*66:1255 – 9.
7. Dharmalingam K, Tan B K, Mahmud MZ, et al (2012).Swietenia – macropylla extract promotes the ability of Caenorhabditiselegans to survive Pseudomonas aeruginosa infection *Ethno pharmacology.* 139:657 – 663.
8. Doi Y, ArakawaY(2007) 16S ribosomal RNA methylation:emerging resistance mechanism against aminoglycosides.*Clin infect dis.45,88 – 94*

9. Drenakard E. (2003) Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms. *Microbes Infect,5,1213-1219*

10. Drlica K.., Hiasa H., Kerns R., Malik M., Mustaev A., Zhao X. (2009). Quinolones: action and resistance updated. *Curr. Top. Med. Chem.* 9, 981–998

11. Falagas M E, Bliziotis I A. (2007).Pan drug resistant gram negative bacteria: the dawn of the post antibiotic era? *Int J Antimicrob Agents*.29 :630 – 636.

12. Falagas M. E., Rafailidis P. I., Ioannidou E et al (2010). Colistin therapy for microbiologically documented multidrug-resistant Gram-negative bacterial infections: a retrospective cohort study of 258 patients. *Int. J. Antimicrob. Agents* 35, 194–199

13. Fallah F, Taherpour A, Borhan S. R et al (2013) Evaluation of Zataria MultiFlora Boiss and Carum Copticum Antibacterial activity on Imp-Type metallo-beta-lactamase producing *Pseudomonas aeruginosa. Ann Burn Fires*;26(4):193-198

14. Fisher, R.A. (1954). *Statistical Methods for Research Workers*. Oliver and Boyd. ISBN 0-05-002170-2

15. Franklin C, Liolios L, Peleg AY. (2006) Phenotypic detection of carbepenem susceptible metallobetalactamase producing Gram negative bacilli in the clinical laboratory. *J Clin Microbiol;* 44:3139-44.

16. Galvani AA,Tukmechi T (2015) .Determination of the prevalence of metallobetalactamase producing *Pseudomonas aeruginosa* strains from clinical samples by imipenem-Edta combination disk method in Mottahari and Emam Khomaini hospitals of Urmia. *Report of health care ;*1(2);65 -68

17. Gilbert D. N., Moellering R.C., Sande M. A. (2003). The Sanford Guide to Antimicrobial Therapy 2003. *Hyde Park: Antimicrobial Therapy, Inc*

18. Gooderham W J, Hancock REW. (2009)Regulation of virulence and antibiotic resistance by two component regulatory system in Pseudomonas aeruginosa. *FEMS Microbiol Rev* .33:279 - 294.

19. Gutierrez O., Juan C., Cercenado E et al (2007: Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. *Antimicrob. Agents Chemother*. 51, 4329–4335

20. Hancock R E, Speert DP. (2000) Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment. *Drug Resist Updat*.3:247 – 255.

21. Helfand M. S., Bonomo R. A. (2003). β-lactamases: a survey of protein diversity. *Curr. Drug Targets Infect. Disord.* 3, 9–23

22. Higgins P. G., Fluit A. C., Milatovic D et al (2003). Mutations in GyrA, ParC, MexR and NfxB in clinical isolates of *Pseudomonas aeruginosa. Int. J. Antimicrob. Agents* 21, 409–413.

23. Hocquet D., Nordmann P., El Garch F et al (2006): Involvement of the MexXY-OprM efflux system in emergence of cefepime resistance in clinical strains of *Pseudomonas aeruginosa.Antimicrob. Agents Chemother.* 50, 1347–1351

24. Hoiby N., Bjarnsholt T., Givskov M et al (2010). Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents* 35, 322–332

Http://aminj.myweb.uga.edu/KIRBY - BAUER.html.

26. Jacoby G. A. (2005) Mechanisms of resistance to quinolones. *Clin. Infect. Dis. 41(Suppl. 2),* S120–S126

27. Lambert L.M, Suetan .C. Savey et al (2011): Clinical outcome of health care associated infections and antimicrobial resistance in patients admitted European intensive care units: a cohort study. *The lancet infectious diseases* .11(1) 30 – 38.

28. Landman D., Bratu S., Alam M et al (2005). Citywide emergence of *Pseudomonas aeruginosa*strains with reduced susceptibility to polymyxin *B. J. Antimicrob. Chemother.* 55, 954–957

29. Lopez D., Vlamakis H., Kolter R. (2010). Biofilms. Cold Spring Harb. *Perspect. Biol. 2*

30. Li X. Z., Nikaido H. (2009). Efflux-mediated drug resistance in bacteria: *an update. Drugs* 69, 1555–1623

31. Lister P D, Wolter D J, Hanson ND (2009) Antibacterial – resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanism. *Clin Microbiol Rev* 22(4):582 – 610.

32. Livermore D. M. (1992): Interplay of impermeability and chromosomal β-lactamase activity in imipenem-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 36, 2046–2048

33. Moskowitz S. M., Ernst R. K., Miller S. I. (2004). PmrAB, a two-component regulatory system of*Pseudomonas aeruginosa* that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. *J. Bacteriol.* 186, 575–579

34. McGowan, J.E. Jr (2006).Resistance in non-fermenting gram negative bacteria: Multidrug resistance to the maximum. *Am J Infect Control.* 34, S29 – S37

35. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: twenty third informational supplement M100 – S23.Wayne, PA: NCCLS, 2013.

36. Neda G, Scott A, Andrew P, Nader P. (2009) Detection of point mutations associated with antibiotic resistance in *Pseudomonas aeruginosa .Int J Antimicrobial Agents* .34:414-418.

37. Paul M., Yahav D., Bivas A et al (2010). Anti-pseudomonal β-Lactams for the initial, empirical, treatment of febrile neutropenia: comparison of β-Lactams. *Cochrane Database Syst. Rev*.

38. Patrick R Murray, Kens Rosenthal and Michael A Pfaller(2013) 7th edition,*Medical Microbiology*(874pp)Elsevier health sciences.

39. Pitout J D , Revathi G, Chow B L, et al (2008): Metallobetalactamases producing *Pseudomonas aeruginosa* isolated from a large tertiary center in Kenya. *Clin. Microbiol Infect* 14: 755 – 759.

40. Poirel L, Pitout J D, Nordmann P. (2007) Carbapenemases: molecular diversity and clinical consequences.*FutureMicrobiol*.2:501 – 512.

41. Poole K. (2002). Outer membranes and efflux: the path to multidrug resistance in Gram-negative bacteria. Curr. Pharm. Biotechnol. 3, 77–98

42. Poole K. (2004a). "Efflux pumps," in Pseudomonas, Vol. 1. Genomics, life style and molecular architecture, ed. Ramos J. L., editor. (New York, NY: Kluwer Academic, Plenum Publishers;), 635–674

43. Poole K. (2004b). Resistance to β-lactam antibiotics. Cell. Mol. Life Sci. 61, 2200–2223

44. Poole K. (2005). Aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 49, 479–487

45. Poole K. (2007). Efflux pumps as antimicrobial resistance mechanisms. Ann. Med. 39, 162–176

46. Ramirez M. S., Tolmasky M. E. (2010). Aminoglycoside modifying enzymes. *Drug Resist.* 13, 151–171

47. Richard A. Harvey, Cynthia Nau Cornelisen, Bruce D Fisher (2013)3rdedition. *Lippincott's Illustrated Reviews: Microbiology* (450 pp)Lippincott Williams and Wilkins.

48. Samonis G., Matthaiou D. K., Kofteridis D et al (2010). In vitro susceptibility to various antibiotics of colistin-resistant Gram-negative bacterial isolates in a general tertiary hospital in Crete, Greece. *Clin. Infect. Dis*. 50, 1689–1691

49. Trias J., Nikaido H. (1990). Outer membrane protein D2 catalyzes facilitated diffusion of carbapenems and penems through the outer membrane of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother.34, 52–57

50. Vahdani M,Azimi L, Asgzari B et al (2012):Phenotypic screening of extended spectrum betalactamases and metallobetalactamases in multi drug resistant *Pseudomonas aeruginosa* from infection burns. *Ann burns fire disaster,* 25:78 – 82

51. Walsh T R, Toleman M A, Poirel L, Nordmann P. (2005)Metallobetalactamases: the quiet before the storm? *Clin Microbiol Rev*; 18:306 – 325.

52. Wang J, Zhou Y.J, Qu T.T et al (2010) .Molecular epidemiology and mechanism of Carbepenem resistance in *Pseudomonas aeruginosa* isolates from Chinese hospitals. *International journal of antimicrobial agents*. 35(5) 486 – 491.

53. Wirth W F, Picoli U S, Cantarelli VV et al (2009): Metallobetalactamases producing *Pseudomonas aeruginosa* in two hospitals from southern Brazil. *Brazilian journal of infectious disease* .13(3) 170-172.

54. World health organization (2014) Antimicrobial resistance global report on surveillance: 1 – 5.

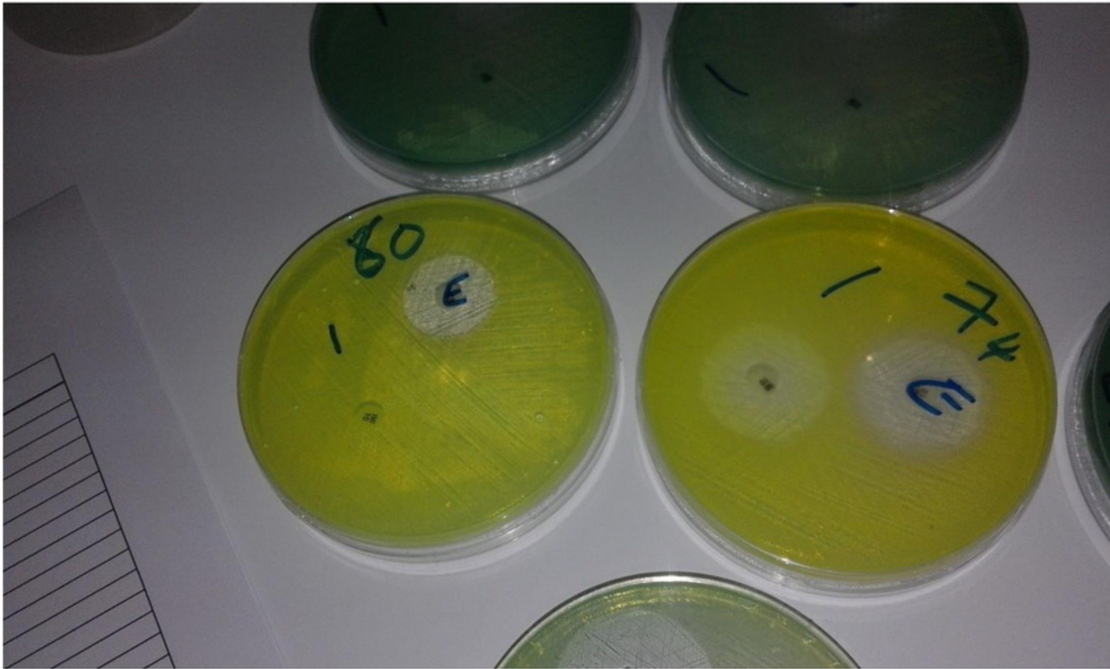
55. www.textbookofbacteriology.net

56. Yong D, Lee K, Yum, JH, Shin H B, Rossolini GM, Chong Y (2002) Imipenem – EDTA disk method for differentiation of metallobetalactamases producing clinical isolates of *Pseudomonas* spp and *Acinetobacter* spp. *J Clin Microbiol.*40:3798- 3801.

57. Zhao W H, ZQ (2010):Betalactamases identified in clinical isolates of *Pseudomonas aeruginosa. Crit Rev microbiology* 36(3) 245 – 258.

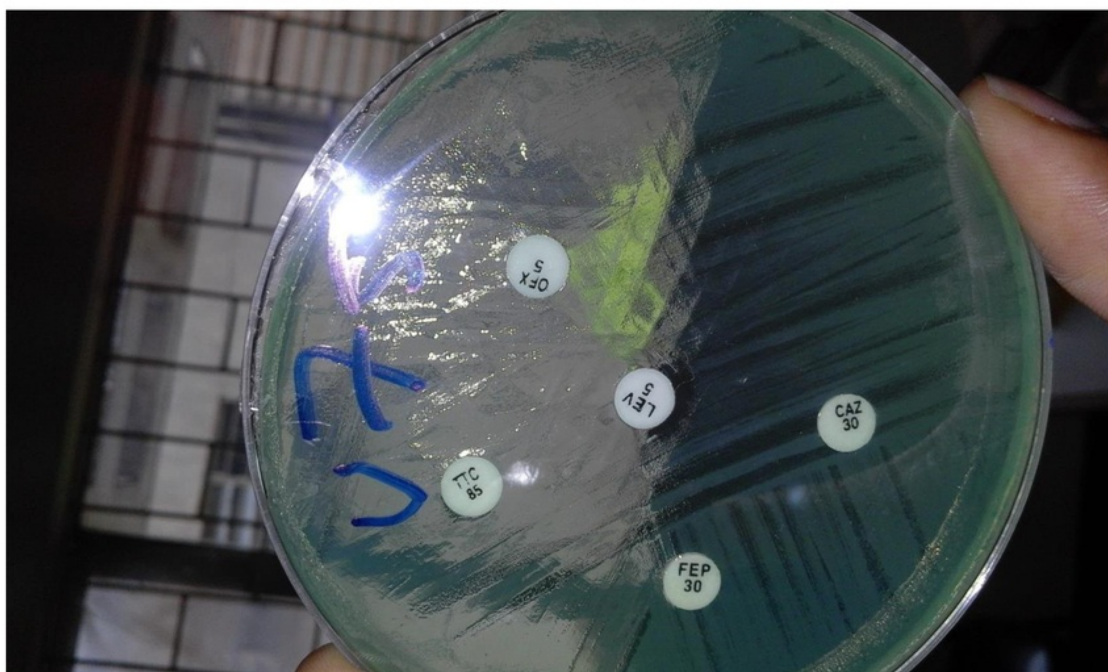
APPENDIX

APPENDIX 1



MBL detection using IMP-EDTA(E) and IMP(I)

APPENDIX 2



Antimicrobial sensitivity test of pseudomonas aeruginosa

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/353

12th August 2015

Jane Njeri Karuitha
H56/68026/2013
Dept.of Med Microbiology
School of Medicine
University of Nairobi

Dear Jane

RESEARCH PROPOSAL – PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND METALLOBETALACTAMASE PRODUCTION OF *PSEUDOMONAS AERUGINOSA* ISOLATED IN KENYATTA NATIONAL HOSPITAL (P238/04/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 12th August 2015 – 11th August 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study.
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF METALLOBETALACTAMASE PRODUCING Pseudomonas aeruginosa ISOLATES AT KENYATTA NATIONAL HOSPITAL.

ORIGINALITY REPORT

12%	12%	13%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	%
1	journal.frontiersin.org Internet Source	3%
2	Poole, Keith. "Pseudomonas Aeruginosa: Resistance to the Max", Frontiers in Microbiology, 2011. Publication	2%
3	J.D.D. Pitout, G. Revathi, B. L Chow, B. Kabera, S. Kariuki, P. Nordmann, L. Poirel. "Metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from a large tertiary centre in Kenya", Clinical Microbiology and Infection, 2008 Publication	2%
4	academicjournals.org Internet Source	1%
5	Antibiotic Discovery and Development, 2012. Publication	1%

6	Submitted to University of Cape Town Student Paper	1%
7	A. A. Ocampo-Sosa. "Alterations of OprD in carbapenem-intermediate and -susceptible strains of Pseudomonas aeruginosa isolated from bacteremia in a Spanish multicenter study.", Antimicrobial Agents and Chemotherapy, 01/30/2012 Publication	1%
8	www.intechopen.com Internet Source	1%
9	130.15.126.37 Internet Source	1%
10	Submitted to University of Sheffield Student Paper	1%
11	digitalcommons.fiu.edu Internet Source	1%

Exclude quotes	Off	Exclude matches	< 1%
Exclude bibliography	Off		