STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL

By

Dr. PRASHANT D. PESHATTIWAR

Dissertation submitted to the

BLDE UNIVERSITY BIJAPUR, KARNATAKA

In partial fulfillment

of the requirements for the degree of

M. D.

In

MICROBIOOLOGY

Under the guidance of

Dr. B.V.PEERAPUR **M.D.(MICROBIOLGY)**

Professor and Head

DEPARTMENT OF MICROBIOOLOGY

B. L. D. E. U.'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE,

BIJAPUR.

2011

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL &

RESEARCH CENTRE, BIJAPUR.

DECLARATION BY THE CANDIDATE

I, **Dr. PRASHANT D. PESHATTIWAR**. hereby declare that this dissertation Entitled "**STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL."** is a bonafide and Genuine research work carried out by me under the guidance of **Dr. B.V.PEERAPUR** **M.D.** Professor, Department of microbiology at B. L. D. E. U's Shri. B. M. Patil Medical College Hospital and Research Centre, Bijapur.

Date: **Dr. PRASHANT D. PESHATTIWAR**.

Place: Bijapur

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL."** is a bonafide research work done by **Dr. PRASHANT D. PESHATTIWAR,** under my overall supervision and guidance, in partial fulfillment of the requirement for the degree of M.D. in microbiology

Date :

Place : Bijapur

DR. B.V.PEERAPUR
PROFESSOR AND HEAD
DEPARTMENT OF MICROBIOLOGY
B. L. D. E. U's Shri. B. M MEDICAL COLLEGE , HOSPITAL AND RESAERCH CENTRE.
BIJAPUR.

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL."** is a bonafide research work done by **Dr. PRASHANT D. PESHATTIWAR,** under the guidance of **Dr. B.V.PEERAPUR M.D.,** Professor of microbiology at B. L. D. E. U's Shri. B. M. Patil Medical College Hospital and Research Centre, Bijapur.

DR. B.V.PEERAPUR. M.D.
PROFESSOR AND HEAD
DEPARTMENT OF MICROBIOLOGY
B. L. D. E. U's Shri. B. M. PATIL
MEDICAL COLLEGE HOSPITAL
& RESEARCH CENTRE,
BIJAPUR.

Date:
Place: Bijapur

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

ENDORSEMENT BY THE PRINCIPAL

This is to certify that the dissertation entitled **"STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL."** is a bonafide research work done by **Dr. PRASHANT D. PESHATTIWAR,** under the guidance of **Dr. B.V. PEERAPUR M.D,** Professor of microbiology at B. L. D. E.s Shri. B. M. Patil Medical College Hospital and Research Centre, Bijapur.

Date:

Place: Bijapur

DR R.C. BIDRI (M.D. Gen Med)
PRINCIPAL,
B.L.D.E.U'S Shri B.M. PatiL Medical College, Hospital and Research Centre.
Bijapur

COPYRIGHT

Declaration by the candidate

I here by declare that the BLDE UNIVERSITY BIJAPUR , Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic/ research purpose.

Date: **Dr. PRASHANT D. PESHATTIWAR**
Place: Bijapur

ACKNOWLEDGEMENT

With proud privilege and deep sense of respect I express my gratitude and indebtedness to my teacher and guide **DR. B.V.PEERAPUR** , Professor and Head , Department of Microbiology, BLDEU'S SHRI B. M. PATIL MEDICAL COLLEGE, for his constant inspiration, patience, encouragement and support, which he rendered in preparing this dissertation and in pursuit of my post graduate studies.

I am grateful to **Dr. R.C.BIDRI**, Principal of B.L.D.E.U'S Shri. B.M.Patil Medical College Hospital and Research Centre, Bijapur, for permitting me to conduct this study.

I take this opportunity to express my deep sense of gratitude and sincere thanks to **Dr. P.K. PARANDEKAR** Professor , **Dr. APARANA THAKPERE** Associate Professor, **Dr. P.R.SHAHAPUR** Associate Professor.

My sincere thanks to **Dr. A.G. SAJJAN** Associate Professor **, Dr. Basvraj M.C.** Assistant Professor **,Dr. MANJUNATH P S,** Assistant Professor, **Dr.SMITHA BAGALI** Assistant Professor, **Dr. LAXMI KAKHANDKI** Assistant Professor **, Smt. SMITHA MANGALGI** lecturer**, Mr. SANJAY WAVARE** lecturer, **Miss SHIVALI** lecturer , **DR SHRIHARI** tutor for their valuable supervision and good wishes.

I am extremely thankful to Mrs. Vijaya Soraganvi, Statistician for her guidance in statistical analysis.

I am very grateful to Dr JYOTHI P., Dr .NAVEEN G and DR SHYMALA R. for their kind co-operation and encouragement.

I thank all the non teaching staff of my department for their constant encouragement and moral support.

I also thank Mr. NAGNESHAWAR. Chief Librarian and other library staff for their cooperation in bringing out this dissertation.

I wish to express my special , sincere humble pronouns to my Mother SANDHYA and Father DURWAS for their love and affectionate divine blessing , moral and constant support in every walk of my life till I become post graduate.

I can not forget my sister and my brother in law and their daughter, Bhargavi, for being a constant source of energy and inspiration.

Finally I acknowledge with gratitude to all the patients for their co-operation during the study.

Date: **Dr. PRASHANT D.PESHATTIWAR**

Place: Bijapur.

LIST OF ABBREVIATIONS USED

Ak	-	**Amikacin**
Ca	-	**Ceftazidime**
CAC	-	**Ceftazidime-Clavulanic acid**
Ce	-	**Cephotaxime**
Cf	-	**Ciprofloxacin**
CLSI	-	**Clinical and Laboratory standards institute**
DDST	-	**Double disk diffusion synergy test**
ESBL	-	**Extended Spectrum β-lactamase**
G	-	**Gentamicin**
I	-	**Imipenem**
I	-	**Intermediate sensitive**
MBL	-	**Metallo – β –lactamase**
MIC	-	**Minimum inhibitory concentration**
Mr	-	**Meropenem**
NCCLS	-	**National committee for Clinical Laboratory Standards**
PBP	-	**Penicillin binding protein**
PCR	-	**Polymerase chain reaction**
Pi	-	**Piperacillin**
PT	-	**Piperacillin + tazobactum**
R	-	**Resistance**
S	-	**Sensitive**
Tb	-	**Tobramycin**

ABSTRACT

Background and objectives:

Emergence of multidrug resistance in Pseudomonas aeruginosa is a notable threat. Emergence of ESBLs and MBLs has limited the therapeutic options. Objectives: 1) To isolate and identify Pseudomonas aeruginosa in specimens obtained from wound infections. 2)To study antimicrobial sensitivity of P. aeruginosa by Kirby-Bauer disk diffusion method. 3).To determine ESBL and MBL production in multidrug resistant P. aeruginosa isolated from wound infections.

Methods:

126 isolates of pseudomonas aeruginosa from pus samples were tested as per standard microbiological procedures. Antimicrobial susceptibility testing was done by Kirby - Bauer disc diffusion test. ESBL production was detected by Double disk diffusion synergy test and MBL production detected by Imipenem-EDTA double disk synergy test (DDST).

Results:

Of the 126 isolates 28 [22.22%] were ESBL producers while 7.8 % isolates were metallo β lactamases producers.

Interpretation and Conclusion:

Multidrug resistance is common in P. aeruginosa. This study highlights the need for all diagnostic laboratories to perform ESBL and MBL detection as a routine practice among Pseudomonas aeruginosa.

Key words: P. aeruginosa, ESBL, MBL, Multidrug - resistance

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) an opportunistic pathogen of humans, is a Gram negative, aerobic rod belonging to the bacterial family Pseudomonadaceae[1]. It is reported to be a leading cause of nosocomial infections, including pneumonia, urinary tract infections, burns infection, meningitis and bacteremia. Its ability to survive on inert materials, live on minimal nutritional requirement, with its tolerance to a wide variety of physical conditions and antiseptics; has contributed enormously to its ecological success and its role as an effective opportunistic pathogen .It is known to exhibit intrinsic resistance to several antimicrobial agents[2].

In addition to the intrinsic resistance of Gram-negative bacteria, such as P. aeruginosa , these bacteria also produce enzymes namely ß -lactamases, which are responsible for wide-spread ß-lactam resistance. These ß -lactamases hydrolyse the amide bond of the four-membered characteristic ß - lactam ring, rendering the antimicrobial ineffective. Many genera of gram negative bacteria possess a naturally occurring, chromosomally mediated ß lactamases and also some are plasmid mediated ß lactamases. These enzymes are thought to have evolved from penicillin binding proteins [3]. Four molecular classes of ß -lactamases are known, dubbed A-D according to Ambler's classification.[1] Classes A and D include the so-called extended-spectrum ß-lactamases [oxacillinase (OXA)-type; cefotaximase (CTX-M) -type; sulphydryl variable (SHV)-type; Temoneira (TEM)-type and Guiana extended-spectrum(GES) -type] , Class B ß-lactamases include carbapenemases that hydrolyze most ß - lactams, including carbapenems, while AmpC enzymes belong to the class C cephalosporinases [4].

The extended-spectrum ß-lactamases (ESBLs) constitute a major problem in the use of ß -lactams antibiotics to treat infections, given their broad substrate specificity and ability to hydrolyze many of the extended-spectrum third-generation cephalosporins . Extended -spectrum ß-lactamase producing bacteria are typically resistant to penicillins, first- and second-generation cephalosporins as well as the third-generation oxyiminocephalosporins (e.g. Ceftazidime, ceftriaxone) and monobactams (aztreonam) [5].

Based on molecular studies, two types of carbapenems – hydrolyzing enzymes have been described: Serine enzymes possessing a serine at the active site and Metallo β lactamases (MBLs) requiring divalent cations usually zinc as metal co-factors for enzyme activity. Despite avidity of these enzymes for carbapenems they do not always mediate high level resistance and not all are inhibited by clavulanic acid. MBLs, like all β – lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes. In recent years MBL genes have spread from P. aeruginosa to Enterobacteriaceae. Given that MBLs will hydrolyze virtually all classes of β lactamases and we are several years away from the implementation of a therapeutic inhibitor, there continued spread is going to be a major therapeutic challenge[6].

Major risk factors for colonization or infection with ESBL and MBL producing organism are long term antibiotic exposure. Prolonged hospital stay, severe illness, resistance in an institution with high rates of third generation cephalosporin use and instrumentation or catheterization [7].

The present study was conducted with an objective to find out the presence of ESBL and MBL producing P. aeruginosa in multidrug resistant strains and to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

AIMS AND OBJECTIVES OF THE STUDY

1) To isolate and identify Pseudomonas aeruginosa in specimens obtained from wound infections.
2) To study antimicrobial sensitivity of P. aeruginosa by Kirby-Bauer disk diffusion method.
3) To determine ESBL and MBL production in multidrug resistant P. aeruginosa isolated from wound infections.

REVIEW OF LITERATURE

Evaluation and dissemination of ß lactamases:

Fifty years ago the antibiotic era began with the discovery of penicillin.[7] The first ß lactamase was identified in E.coli prior to the release of penicillin for use in medical practice.[3]

The TEM-1, first plasmid mediated ß lactamase was originally found in a single strain of E.coli isolated from a blood culture from a girl named Temoniera in Greece, hence the designation TEM. Being plasmid and transpose mediated has facilitated the spread of TEM-1 to other species of bacteria. Within few years after its first isolation, the TEM-1 ß lactamase spread world wide and is now found in many different species of members of the family Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae, and Neisseria gonorrhoeae.[3]

Over the last 20 years, many new ß lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of ß lactamases. However, with each new class that been used to treat patients, new ß lactamases emerged that caused resistance to that class of drug. Presumably the selective pressure of the use and over use of new antibiotics in the treatment of patients has selected for new variants of ß lactamases. One of these new classes was the oxyiminocephalosporins, which because widely used for the treatment of serious infections due to gram negative bacteria in the 1980s. Resistance to these expanded spectrum ß lactam antibiotics due to ß lactamases also emerged quickly.[3]

The SHV-2 is first of enzymes capable of hydrolyzing the newer ß lactams, it was found in a single strain of Klebsiella ozaenae isolated in Germany. Because of their increased spectrum of activity, especially against the oxyiminocephalosporins these enzymes were called extended spectrum ß lactamases [ESBLs].[7]

Among the types of metallo-β-lactamases (MβLs) reported to date, IMP and VIM variants are the most prevalent and have been described from numerous geographic locations . VIM-2 was first reported from a Pseudomonas aeruginosa strain recovered during 1996 in a hospital located in Marseilles, France . Among VIM enzymes, VIM-2 appears to be the most dominant allelic variant and isolates carrying this enzyme have been described in more than 23 countries, including India.[8] More than 170 ß lactamases have been recognized. Their growth spurt shows no signs of slowing down.[7]

Table 1 : Key dates showing emergence of ß lactamases

Year	Enzyme	Organism	Place
1944	Penicillinase	S.aureus	-
1963	TEM-1	E.coli	Athens
1974	SHV-1	E.coli	Switzerland
1978	OXA-10	P.aeruginosa	-
1982	SME-1	S.marcescens	London
1988	Metalloβ lactamase	P.aeruginosa	Japan
1989	Inhibitor resistant penicillinase	E.coli, K.pneumonia	France, Spain.
1990	NMCA	E.cloacae	Paris
1991	OXA-11 OXA-14	P.aeruginosa	Turkey
1991	PER-1	P.aeruginosa S.typhimurium	Turkey
1992	MEN-1	E.coli, K.pneumonia	France
1994	TOHO-1	E.coli	Japan
1996	PER-2	E.coli, K.pneumonia, S.typhimurium, P.mirabilis	Germany
1997	VEB-1	E.coli	Germany

Classification of ß lactamases :[1]

β lactamases are produced by both gram positive and gram negative bacteria.

Gram positive bacteria:

Among gram positive bacteria, staphylococci are the major pathogens that produce ß lactamases. These ß lactamases preferentially hydrolyze penicillins. Most are inducible and are excreted extracellularly. The genes that determine staphylococcal ß lactamases usually are carried on small plasmids or transposons. Enterococci produced a plasmid determined ß lactamases that seems to be of staphylococcal origin.[9]

Gram negative bacteria:

Gram negative bacteria produce a greater variety of ß lactamases. ß lactamases encoded by plasmid are of special concern because it increases the possibility of spread among different strains and different species of pathogens. Various classification schemes have been proposed by many researchers. Sawai et al in 1968 classified based on response to antisera. Richmond and Sykes scheme in 1973 was on the basis of substrate profile. This scheme was extended by Sykes and Mathew in 1976, which was based on differentiation by isoelectric focusing. Bush in 1989 proposed the grouping based on correlation of substrate and inhibiting properties with molecular structure.[7]

I. Ambler classification: [7]

A more modern scheme based on molecular structure was proposed by Ambler, includes 4 evolutionarily distinct classes of ß lactamases.

a. Class A ß lactamases :

Have molecular weight around 29,000, possess a service residue at their active site and preferentially hydrolyze penicillins. E.g. TEM-1 ß lactamases widely prevalent in gram negative bacilli.

b. Class B ß lactamases :

These enzymes are metallo enzymes that have a zinc binding thiol group required for ß lactamase activity. This class has been reported in Pseudomonas and Serratia. These enzyme producers are resistant to carbapenams, oxyiminocephalosporins ß lactamase inhibitors except aztreonam.

c. Class C ß lactamases:

These enzymes are large proteins (molecular weight 39,000) with mainly Cephalosporinase activity. They also have serine at their active site. The tertiary structure of class C ß lactamases show striking similarities to penicillin binding proteins, from which they might have evolved. E.g. AmpC ß lactamases which are found naturally on chromosomes of E.cloacae, C.freundii or P. aeruginosa. These AmpC ß lactamases spread to E.coli and klebsiella making these isolates resistant not only to the oxyiminocephalosporins but also to cefoxitin and cefotetan and ß lactams inhibitor combinations. But these are sensitive to carbapenems.

d. Class D ß lactamases:

These ß lactamases are oxacillin hydrolyzing enzymes.

II. Bush – Jacoby – Medeiros classification: [7]

Recently a new classification scheme has been developed to integrate functional molecular characteristics .This scheme puts 178 ß lactamases from naturally occurring bacterial isolates into four groups based on substrate and inhibitor profiles.

Table 2 : Bush – Jacoby -- Medeiros classification

Group	Enzyme type	Inhibition by clavulunate	Molecular class	No. of enzymes	Example
1	Cephalosporinase	No	C	53	E.cloacae P 99, MIR-1
2a	Penicillinase	Yes	A	20	S.aureus, S.albus
2b	Broad spectrum	Yes	A	16	TEM-1, SHV-1
2be	Extended spectrum	Yes	A	81	TEM-3, SHV-2, K.oxytoca
2br	Inhibitor resistant	Diminished	A	13	TEM-30, TRC-1.
2c	Carbenicillinase	Yes	A	15	PSE-1, CARB-3, BRO-1.
2d	Cloxacillinase	Yes	D or A	21	OXA-1, PSE-2, Steptomyces cacaoi
2e	Cephalosporinase	Yes	A	19	Proteus vulgaris Bacteriodes fragilis cepA
2f	Carbapenamase	Yes	A	3	E.cloacae, EMI-1, NMC-A
3	Metalloenzyme	No	B	15	Stenotrophomonas maltophilia-L1
4	Penicillinase	No	-	7	Burkholderia cepacia.

Contribution of ß lactamases to drug resistance: [9]

Organisms exhibit resistance to ß lactam antibiotics. It is mainly due to the production of ß lactamases. These enzymes inactivate the antibiotics by splitting the amide bond of the ß lactam ring and the antibiotic targets (PBP).[9]

The level of antibiotic resistance mediated by a particular ß lactamase in a population of bacteria is determined by several variables. The efficiency of the ß lactamase in hydrolyzing an antibiotic depends on –

a) Rate of hydrolysis – Vmax.

b) Its affinity for the antibiotic (Km).

c) Amount of ß lactamase produced by the bacterial cell

d) Susceptibility of the target protein (PBP) to the antibiotic and

e) The rate of diffusion of the antibiotic into the periplasm of the cell

In case of staphylococci, they produce ß lactamase upon exposure to penicillin then two events take place concurrently:

1) Penicillin lyses bacteria
2) ß lactamase hydrolyzes penicillin.

If viable bacterial cells remain after the level of penicillin has fallen below the MIC, re growth of bacteria occurs.

In case of gram negative bacilli, ß lactamase produced remains trapped in the periplasmic space. In case of H. influenzae strains that produce TEM-1 ß lactamase that remain trapped in the periplasmic space and also have no barrier to antibiotic penetration. In both this model and the first one discussed i.e. staphylococcal ß lactamase, marked inoculum effect occurs in that the MIC for a large inoculum (10^6org/ml) may be thousand fold greater than that for a small inoculum (10^2 org/ml).

Another model is exemplified by ampicillin resistance of E. coli strains that produce the TEM-1 ß lactamase. These bacteria have barrier to entry of ß lactams molecules (the outer membrane) and produce a ß lactamase that remains localized to periplasmic space. The enzyme is situated between the barrier to antibiotic penetration and the antibiotic targets (PBP).

In this position the enzyme can sequentially destroy antibiotic molecules as they make their way through the barrier. As a consequence high levels of resistance occur with single bacterial cell, unlike previous example. Sometimes amount of ß lactamase produced increases with exposure to a ß lactam (induction) as occurs in Enterobacter and Pseudomonas species. High levels of ß lactamase are produced only after a period of exposure to the inducing antibiotic and hence resistance may be expressed late. Resistant bacteria are emerging world wide as a threat to this favourable outcome of common infections in community and hospital setting.

ß-lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to Penicillins and Cephalosporins the antibiotics which are commonly used for the treatment in the hospital settings.

Most of the ß- lactamases are plasmid mediated originally confined to one group of bacteria sooner or later may appear in other groups. The widespread use of antibiotics increases the prevalence of resistant organism locally and then spread world wide. An example of this process occurred with the TEM-1 ß lactamase, which has spread from the Enterobacteriaceae to H. influenzae and N. gonorrhoeae and P. aeruginosa. Any clinical isolates may produce two and even three, plasmid determined ß - lactamases.

Sporadic nosocomial outbreaks due to strains producing ß – lactams antibiotics apparently enhance colonization of the digestive or respiratory tracts of other patients and infection follows. Failure to control outbreaks has resulted in this appearance of new types of ß - lactamases, and to determine suitable prevention measures and treatment policies.

Detection of different types of ß - lactamases among clinical isolates still remains a challenge for clinical microbiology laboratory.

Extended spectrum ß-lactamases (ESBL)

The term ESBL refers to ß lactamase produced by gram negative bacteria that encode for resistance to broad spectrum ß lactam antibiotics that normally have activity against gram negative bacilli. These enzymes not active against the cephamycins, and are inhibited by β lactamase inhibitor like clavulanic acid, sulbactum or tazobactum. They belongs to Ambler's molecular class A and functional group 2be.[10]

As these belong to class A enzymes, they are characterized by an active site serine a molecular mass of approximately 290000 Da. This class of ß-lactamase includes enzymes such as TEM-1, SHV-1. Point mutation of these classical plasmid mediated ß-lactamases resulted in emergence of new ß-lactamases like ESBLS. ESBLS represent a major group of ß-lactamases currently being identified world wide in large numbers and are now found in a significant percentage of E. coli and K. pneumoniae strains. They have also been found in other gram negative bacterial strains like Enterobacter, Citrobacter, Proteus, Morganella morganii, Serratia marsescens, Shigella dysenteriae, Pseudomonas aeruginosa, Burkholderia cepacia and Capnocytophaga ochracea.[7]

The currently identified ESBLs in P. aeruginosa include the SHV-, TEM-, PER-, VEB-, and IBC/GES-types .[11] The GES-type ß lactamases are of particular importance since these enzymes possess more than one of the different hydrolysis profiles (expanded-spectrum cephalosporins, carbapenems, cephamycins and monobactams).[12]

Types of ESBLs : [3]

Most ESBLs arose from the TEM or SHV enzymes through point mutations at selected loci giving rise to the extended-spectrum phenotype .The nomenclature of ß -lactamases is not standardized . The ß -lactamase SHV denotes a variable response to sulphydryl inhibitors and TEM was named after the patient Temoneira, from whom the first sample was obtained.

TEM - type ESBLs (class A)

The enzyme, TEM-1, is the most commonly encountered ß -lactamase in Gram-negative bacteria which is responsible for up to 90% of ampicillin resistance in E. coli .There are more than 130 TEM enzymes recognized at present . TEM

enzymes have been reported in genera of the family Enterobacteriaceae such as Enterobacter aerogenes, Proteus spp. and Salmonella spp. The TEM-type ESBLs have been found in non- Enterobacteriaceae Gram-negative bacteria such as TEM-42 in P. aeruginosa and TEM-17 in Capnocytophaga ochracea .

A number of amino acid residues are especially important for producing the ESBL phenotype when substitutions occurs at that position. They include glutamate to lysine at position 104, arginine to either serine or histidine at position 164. Glycine to serine at position 238 and glutamate to lysine at position 240. In addition to ß lactamase TEM-1 through TEM-92, there has been report of naturally occurring TEM like enzyme, TEM – Aq that contained a number of amino acid substitutions and one amino acid deletion that have not been noted in other TEM enzymes. It has been suggested that the naturally occurring TEM type ESBLs are the result of fluctuating selective pressure from several ß lactams within a given institution rather than selection with a single agent.

SHV-Type ESBLs (class A) :

The enzyme SHV-1 is related to TEM-1 with 68% amino acid homology and has a similar structure. There are relatively few derivatives of SHV-1compared to TEM-1 with approximately 50 varieties currently recognized . Amongst the SHV variants many are characterized b y the substitution of a serine (Ser) for a glycine (Gly) at position 238 and a substitution of lysine (Lys) for glutamate (Glu) at position 240 . It has been noted that the Ser at position 238 is critical for efficient hydrolysis of ceftazidime and the Lys residue at position 240 is critical for efficient hydrolysis of cefotaxime. The SHV-type ESBLs are most prevalent in Klebsiella pneumonia but these enzymes have also been identified in Citrobacter diversus , E. coli and P. aeruginosa.

Cefotaximase-Type ESBLs (class A) :

Cefotaximase (CTX-M) exhibits greater activity against cefotaxime than against ceftazidime A unique feature of these enzymes is that they are better inhibited by tazobactum than by sulbactam or clavulanate, probably due to increased active site flexibility. The CTX-M enzymes are the most common group of ESBLs that do not belong to the SHV or TEM families. The CTX-M enzymes share approximately 40% homology with TEM-and SHV-type ESBLs.

Currently mo re than 40 CTX-M enzymes are known with CTX-M-2, CTX-M-3 and CTX-M-14 being most widespread .The CTX-M ESBLs have mainly been identified in strains of Salmonella enteric serovar. Typhimurium , E. coli and other species of Enterobacteriaceae. The CTX-M-type enzymes have not been identified in P. aeruginosa and are so far restricted to the family Enterobacteriaceae.

Oxacillinase -Type ESBLs (class D) :

The Oxacillin -hydrolysing (OXA-type) ESBLs belong to molecular class D and functional group 2d. The OXA type ß lactamase confer resistance to Ampicillin and Cephalothin and are characterized by their high hydrolytic activity against Oxacillin and Cloxacillin and the fact that they are poorly inhibited by Clavulanic acid.

The OXA type ESBLs provide weak resistance to oxyimino cephalosporins when cloned into E. coli, but provide fairly high level resistance in P. aeruginosa transconjugants. In contrast to the majority of the OXA type ESBLs, which confer resistance to Ceftazidime the OXA -17 ß lactamase confers resistance to Cephotaxime and Ceftriaxone but provides only marginal protection against Ceftazidime. While most of the ESBLs have been found in E. coli, K. pneumonia and other Enterobacteriaceae, the OXA type ESBLs have been found mainly in P. aeruginosa.

Metallo-enzyme Type ESBLs (class B) in bacteria

The metallo-enzymes hydrolyse most ß - lactams including carbapenems ceftazidime, cefsulodin , and cefpirome except for aztreonam. Metallo-beta lactamase were first reported as a zinc dependent enzyme in Bacillus cereus in mid 1960s. A few decades later, imipenem-hydrolyzing metallo enzymes were found in Aeromonas hydrophila and Bacteroides fragilis. All these enzymes were produced by chromosomal genes and at first were recovered only from single clinical isolates. In 1988, Japan reported the first plasmid-mediated metallo beta lactamase in P. aeruginosa.[6]

All class B enzymes including metallo- enzymes are not inhibited by clavulanic acid or tazobactam. Metallo enzymes in P. aeruginosa are exemplified by imipenemase (IMP-1). The IMP-1 enzyme is often termed as a carbapenemase since it hydrolyses imipenem and meropenem . The gene coding the IMP-1enzyme is transferred through plasmids and integrons . Currently types of MBls that have been identified are IMP, VIM, SPM, GIM. The most common MBL identified worldwide is VIM -2.[13]

1) Chromosomally encoded MBLs
2) Transferable MBLs : Consists of
    - IMP (imipenem) – Observed in Japan in 1988
    - VIM (Veronese imipenemase) – First described in Verona, Italy
    - GIM (German imipenemase) – First detected in Germany
    - SPM (Sao Paulo MBL) – First detected in Sao Paulo, Brazil[8].

Novel ESBL Group:

In addition to the established enzyme families, unusual enzymes of non-TEM, non-SHV and non-OXA lineages have been described . These novel enzymes have mainly been found in P. aeruginosa a limited number of geographical sites. The enzymes that fall into this group include PER-1 in France, Italy and Turkey; VEB-1 and VEB-2 in Southeast Asia; GES-1, GES-2 and GES-8 (IBC-2) in France, Greece and South Africa . Other uncommon ESBLs such as Brazil extended-spectrum ß-lactamase (BES-1), GES-7 (IBC-1), SFO-1 (named after Serratia fonticola on the basis of the high degree of homology to the ß-lactamase of S. Fonticola).

Problems in ESBL detection and Detection methods :

The increased prevalence of P. aeruginosa producing ESBLs creates a great need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates. Identifying these ESBL producers continues to be a major challenge for the clinical microbiology laboratory. Although most ESBLs confer resistance to one or more of the oxyimino ß- lactam antibiotics, the ß lactamase does not always increase the MICs to high enough levels to be called resistant by CLSI interpretive guidelines .Due to variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin in vitro. However, treatment of infections due to an ESBL producing organism with 3 rd generation cephalosporins may result in clinical failure. For ESBL producing bacteria there is a dramatic rise of MIC for extended spectrum cephalosporins as the inoculum is increased beyond that used in routine susceptibility. Some isolates susceptible at the standard inoculum of 10 CFU/ml but resistant at an inoculum of 10 CFU/ml. Therefore, they may be reported as false sensitive if tested by routine methods.

The sensitivity and specificity of a susceptibility test to detect ESBLs vary with the Cephalosporins tested. A number of investigations have suggested that Cefpodoxime detected more ESBLs than other Cephalosporins such as Ceftazidime, Cefotaxime and Ceftriaxone. However, more recent data suggest that testing with Cefpodoxime can lead to a high number of false positives if the current NCCLs interpretive criteria are used. However, Cefpodoxime and Ceftazidime have been proposed as better indicators of ESBL production as compared to Cefotaxime and Ceftriaxone. Hence an institution where only Cefotaxime and Ceftriaxone are used in the routine sensitivity testing panel may have difficulty in testing ESBLs.[7]

Clinical laboratory detection of ESBLs in P. aeruginosa is difficult.[11] The following factors contribute to this: i) the presence of naturally occurring ß - lactamases, such as the chromosomally encoded AmpC (class C Cephalosporinase) enzymes, which may be over expressed and therefore be responsible for false-negative results; ii) the simultaneous presence of metallo - enzymes with carbapenems-hydrolysing activities or with extended-spectrum oxacillinases; iii) the relative resistance to inhibition by clavulanate; iv) and a combination of resistance mechanisms, such as efflux pump and impermeability of the cell wall. However several tests have been tried to detect the presence of ESBLs in a bacteria in clinical microbiology laboratory.

Methods of detection:

1) Screening Methods: [7]

CLSI has developed broth microdilution and disk diffusion screening tests for ESBL detection.

Table 3 : CLSI screening methods for ESBL detection :

Antibiotic	Zone diameter for possible ESBL producing strain	MIC for possible ESBL producing strain.
Aztreonam	≤ 27 mm	≥ 2 mg / l
Cefotaxime	≤ 27 mm	≥ 2 mg / l
Cefpodoxime	≤ 22 mm	≥ 2 mg / l
Ceftazidime	≤ 22 mm	≥ 2 mg / ml
Ceftriaxone	≤ 25 mm	≥ 2 mg/l

2) NCCLs phenotypic confirmatory methods:[3]

Phenotypic confirmatory test performed by broth microdilution method or by disk diffusion method.

MIC method: A decrease of ≥ 3 doubling dilutions in an MIC for either Cefotaxime or Ceftazidime tested in combination with 4 µg/ml clavulanic acid versus its MIC when tested alone confirms an ESBL producing organism

Disk diffusion method:

≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.

3) Double disk diffusion synergy test:

This is the first detection method described by Jariler et al in 1988.[14] In this method a lawn culture of test organism onto a Mueller-Hinton agar plate is performed. Augmentin disk [Amoxicillin – clavulanate] is placed in the centre of the plate and disk containing one of the oxyimino ß lactam antibiotics is placed 30 mm from centre to centre from Augmentin disk. The test organism is considered to produce ESBL, if the zone size around the test antibiotic disc increases towards the Augmentin disk. The sensitivity of the test can be increased by reducing the distance between the disks to 20 mm, also using more than one oxyimino ß lactams antibiotic.

Modifications of the double disk test:

1) Detection of ESBLs in Ampc producing organisms is problematic. Pitout et al have developed a modification of double disk test for successful detection of ESBLs. The test is performed using a combination of Cefepime and Piperacillin- tazobactam.

MDDT accurately differentiated between ESBL producers and derepressed chromosomal AmpC mutants.[15]

2) The addition of Clavulunate 4 µg/ml to the Mueller-Hinton agar can be used to potentiate the zone of inhibition of one or more disks containing extended spectrum Cephalosporins.
3) Jacoby and Han method – Here 20 µg of Sulbactam was added to susceptibility disks containing one of the oxyimino ß lactam antibiotics. An increase of 5 mm in the zone of inhibition in a disk containing sulbactam compared to the drug alone was considered a positive test. By this method significant numbers of strains were not detected. In addition a number of AmpC producing strains also showed an enhancement of the zone diameter with the addition of sulbactam.[3]
4) Three dimensional Test- Thomson and Sanders suggested this method. In this method, following inoculation of the organism onto the surface of a Muller –Hinton agar plate, a slit is cut into the agar, on to which a broth suspension of the test organism is introduced. Subsequently antibiotic disks are placed on the surface of the plate 3 mm from the slit. Distortion or discontinuity in the expected circular zone of inhibition is considered a positive test.[3]

Modified three dimensional test:

A cylindrical plug of agar (diameter 4 mm) 2 mm from the antibiotic disk was removed. This cup was then filled with 30 µl of the three dimensional test inoculum, consisting of a milky suspension (Mc Farland No 5 turbidity standard) of the test strain in 0.5 ml of brain heart infusion broth, which was pre incubated at 37°C. Test was positive when the inhibition zone around the ß lactam antibiotic was distorted in such a way that growth of the test organism appeared within the zone behind the cup, and fully reaching this cup. This is due to enzymatic inactivation of the test antibiotic by

the ß lactamase diffusing through the agar in the vicinity of the three-dimensional test inoculum, resulting in a gradually decreasing diameter of the inhibition zone towards the cup.

The three dimensional test was determined to be very sensitive in detecting ESBLs, but it is more technically challenging and labor intensive than other methods.

5. ESBL E test Strips: This combines both the principles of dilution and diffusion techniques. E test strip is a thin non porous plastic strip 5 mm wide and 60 mm long. It carries two shorter gradients aligned in opposing directions, on a single strip. One end generates a stable concentration gradient of Ceftazidime, (TZ 0.5 to 32 µg/ml). While the other end generates a gradient of Ceftazidime + Clavulanic acid (TZl 0.125 to 8 µg/ml Ceftazidime + 4 µg/ml Clavulanic acid) when applied to an inoculated agar plate inhibition ellipses may be seen on both ends of the strip, one to Ceftazidime and the other for its combination with Clavulanic acid. A Tz : Tzl ratio of = 8 denotes a positive result.

This test was shown to be more sensitive than double disk approximation test in detecting ESBLs in clinical isolates. This method is convenient and easy to use.

But it is sometimes difficult to read the E -test when the MICs of Ceftazidime are low because the Clavulanate sometimes diffuses over to the side that contains Ceftazidime alone.[3]

6. Vitek method: The automated microbial susceptibility test system Vitek has also produced an ESBL test that utilizes either Ceftazidime or Cefotaxime alone and in combination with Clavulanic acid (4 µg/ml). A predetermined reduction in growth in wells containing Clavulanate compared to those containing drug alone indicates the presence of an ESBL. Factor that mask the ESBL activity includes high level

expression of AmpC ß lactamases that are not inhibited by Clavulanic acid due to mutations in the coding sequences, contributes to difficulties associated with ESBL detection. Vitek system appears to be acceptable for clinical use and is more sensitive, convenient.[3]

While each of these methods has its merits none of these methods can accurately detect all strains producing ESBL to detect and report the presence of ESBLs. A survey in Connecticut found that 21% of laboratories fail to detect ESBL producing isolates. A proficiency testing project for clinical laboratories participating in the National Nosocomial Infections Surveillance system indicated that as many as 58% laboratories failed to detect and report ESBL isolates correctly.[7]. None of the detection tests that are based on phenotype of the ß lactamase produced is 100% sensitive or specific for the accurate detection of ESBLs among clinical isolates of gram negative bacteria.[3]

Molecular detection methods:[3]

The phenotypic methods described above only presumptively identify the presence of an ESBL. Identifying which specific ESBL is present in a clinical isolate is more complicated. In early days determination of the isoelectric point was usually sufficient to identify the ESBL. However, with more than 90 TEM type ß lactamases many of which possess identical isoelectric points, determination of the ESBL by isoelectric point is no longer possible.

DNA probes: Early detection of ß lactamase genes was performed using DNA probes that are specific for TEM and SHV enzymes. However, sometimes is rather labor intensive procedure.

PCR: The easiest and most common molecular method used to detect the presence of a ß lactamase is PCR with oligonucleotide primers that are specific for a ß lactamase gene. Various types of PCR like multiplex PCR, Real time PCR are available, which are more specific.

Oligotyping: The first molecular method for the identification of ß lactamase was the oligotyping method developed by Ouellette et al, which was used to discriminate between TEM-1 and TEM-2. Using this method several new TEM variants were identified within a set of clinical isolates.

LCR: Ligase chain reaction used for the identification of SHV genes. LCR allows the discrimination of DNA sequences that differ by a single base pair by the use of a thermostable ligase with four oligonucleotide primers that are complimentary to the target sequence and hybridize adjacent to each other.

Nucleotide sequencing: It remains the gold standard for determination of the specific ß lactamase gene present in a strain, but labor intensive and can be technically challenging.

But it is sometimes difficult to read the E -test when the MICs of Ceftazidime are low because the Clavulanate sometimes diffuses over to the side that contains Ceftazidime alone.[3]

Treatment of ESBL infections: [7]

The treatment of ESBL producing bacteria remains a problem particularly in low income countries where expensive second line drugs are unavailable or not affordable by the patients.[7]

The drugs with most reliable activity against ESBL producers includes Carbapenems, Cephamycins, Fluoroquinolones and Aminoglycosides. Of all the ß lactams Carbapenems like Imipenem and Meropenem are most effective and reliable drugs. Several new Carbapenems, Ertapenem and Feropenem are being studied in the various phases of clinical trails.[7]

A few ß lactams, 7 methoxy Cephalosporins such as Cefoxitin, Cefmetazole, Cefotetan are often effective in the treatment of infections caused by enzyme producing bacteria. However, Cephamycins like Cefoxitin, Cefotetan should be used with caution because of the relative ease with which clinical strains decrease expression of outer membrane proteins. ß lactamase inhibitors although inhibit ESBL activity the only infection that may be treated safely with ß lactam / ß lactamase inhibitor combination are the urinary tract infection. Clavulanic acid appears more efficient than Sulbactam. Piperacillin / Tazobactam has been successful in the treatment of ESBL producers and remains an option.[16]

Among the non ß lactam antibiotics aminoglycosides like Amikacin, Fluoroquinolones like Ciprofloxacin may be beneficial however co - resistance rates against these agents are frequent.[6] The antimicrobial resistance is a global problem and emphasizes the need for surveillance and promotion of correct and restrictive antibiotic policies to halt the further spread of these multi drug resistant bacteria.

Problems in MBL detection and Detection methods:[6]

Unfortunately, there are no standardized phenotypic methods available and the testing criteria are likely to depend on whether the gene is carried by P. aeruginosa or a member of the Enterobacteriaceae, i.e., the evincible level of resistance. For example most Enterobacteriaceae and some Acinetobacter spp. carrying MBL genes will appear sensitive, with imipenem MICs of between 1 and 2 mg/ml. Therefore, the implementation of a screening plate to detect MBLs, as has been advocated for extended- spectrum β - lactamases, must take account of the genus of the bacterium, i.e., pseudomonads intrinsically have higher carbapenem MICs than Enterobacteriaceae. It is plausible that for screening Enterobacteriaceae for the presence of MBLs, a plate could contain ceftazidime with and without EDTA, but this would only be effective if the bacterium did not also produce an extended-spectrum β-lactamase, which cannot be assumed.

Identification of some β - lactamases has been aided by isoelectric focusing with the aid of counterstaining the gel with the chromogenic substrate nitrocefin to determine the enzymes isoelectric point. This technique is based on the surface charge properties of these enzymes, which are neutralized at a certain pH. For closely related enzymes e.g. TEM and SHV, the isoelectric point represents a valuable tool in the identification process. However, MBLs, even the transferable types, differ considerably from one another, and thus, isoelectric focusing is not recommended as a tool to identify them, although it can provide useful information as to the isoelectric point of unknown MBLs by using EDTA inhibition (preincubated with the enzyme prior to electrophoresis or soaking the gel with EDTA after electrophoresis) as part of the isoelectric focusing process. Given the fact that all MBLs are affected by the removal of zinc from the active site, in principle, their

detection should be straightforward, and studies have seized upon this principle and used a variety of inhibitor-β - lactam combinations to detect strains possessing these clinically important enzymes. However, MBLs vary in their level of inhibition with certain compounds and also vary in their ability to confer resistance to ceftazidime or imipenem, two substrates commonly used in screening MBLs.

Methods of MBL detection :[17]

MBL screening

Screening for MBL production was done in imipenem resistant isolates by the following methods:

Imipenem (IMP)-EDTA combined disc test

The IMP-EDTA combined disk test was performed as described by Yong et al.[18] Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two 10 μg imipenem disks were placed on the plate, and appropriate amounts of 10 μL of EDTA solution were added to one of them to obtain the desired concentration (750 μg). The inhibition zones of the Imipenem and Imipenem-EDTA disks were compared after 16 to 18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive.

Imipenem-EDTA double disc synergy test (DDST)

The IMP-EDTA double disk synergy test was performed as described by Lee et al.[19] Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An Imipenem (10 μg) disc was placed 20 mm centre to centre from a blank disc containing 10 μL of 0.5 M EDTA (750 μg).

Enhancement of the zone of inhibition in the area between Imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result

EDTA disk potentiation using Ceftazidime, Ceftizoxime, Cefepime and Cefotaxime.

Test organisms were inoculated on to plates with Mueller Hinton agar as described for the standard disc diffusion test. A filter paper (Whatmann No. 2) blank disc was placed and the following discs [ceftazidime (30 μg), ceftizoxime (30 μg), cefotaxime (30 μg), cefepime (30 μg)] were placed 25mm center to center from the blank disc. Ten microlitre of 0.5 M EDTA solution was added to the blank disc and the plate was incubated overnight at 35º C. Enhancement of the zone of inhibition in the area between the EDTA disc and any one of the four cephalosporin discs in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.[20]

MBL E-test

The E Test MBL strip containing a double sided seven-dilution range of IPM (4 to 256 μg/ mL) and IPM (1 to 64 μg/mL) in combination with a fixed concentration of EDTA has been reported to be the most sensitive format for MBL detection. The E-test was done according to manufacturer's instructions. MIC ratio of IP(Imipenem) / IPI (Imipenem-EDTA) of >8 or >3 $\log_2$ dilutions indicates MBL production.

Molecular methods of MBL detection :[6]

PCR –

Genes for IMP, VIM can be detected. It requires tailor-made DNA primers, but cannot differentiate between variants, may not detect new variants.

DNA probes –

Probe required for each gene family, and It cannot differentiate between variants.

Cloning and sequencing –

It is a Molecular gold standard procedure but highly Labor intensive and interpretation of data requires experience.

Treatment of MBL positive infections:[6]

The unique problem with MBLs is their unrivalled broad-spectrum resistance profile. In addition, in many cases the MBL genes may be located on plasmids with genes encoding other antibiotic resistance determinants, i.e., amino- glycoside resistance genes. These MBL-positive strains are usually resistant to β-lactams, aminoglycosides, and fluoroquinolones. However, they usually remain susceptible to polymyxins.

No extended survey with a series of human infections with MBL-positive isolates has been performed to determine the optimal treatment. Thus, suitable therapy for treating those infections remains unknown. Using an animal model of pneumonia infection with a VIM-2-positive P. aeruginosa isolate, it was shown that aztreonam at a high dose reduced the bacterial load and may be a useful drug.

The only therapeutic alternative may be the therapeutic administration of polymyxin B or Colistin, which have recently been shown to be efficient for treating multidrug-resistant gram-negative bacilli.[21] It has been claimed recently that polymyxins are not as toxic as previously thought. In any case, these molecules should not be used in monotherapy, and rapid determination of MICs of aminoglycosides by MIC methods (not disk diffusion) may help to choose an aminoglycoside molecule that may have kept some activity. In addition, rifampin may be an interesting agent for treating multidrug resistant P. aeruginosa infections.

Clearly, in the absence of novel agents in the near future, the spread of MBL producers may lead to therapeutic dead ends. Early detection may avoid spread of these multidrug resistant isolates and may help maintain first- and second-line therapies.

Risk factors for selection and spread of ESBLs and MBLs:

ESBL and MBLs producing organisms are often found in areas of the hospital or community where antibiotic use is heavy and the patient's condition is critical. Various risk factors for colonization / infection and spread of ESBLs and MBLs are: [6,7]

- Recent surgery
- Instrumentation
- Admission to an intensive care unit
- Prolonged hospital stay
- Admission to a nursing home

Long term antibiotic exposure, especially to extended spectrum antibiotics. Use of these antibiotics exerts a selective pressure for emergence of ESBL and MBL producing gram negative bacteria. The resistance plasmids can then be transferred to other bacteria, not necessarily of the same species, conferring resistance to them. The lower digestive tract of colonized patients is the main reservoir of these organisms. Gastro intestinal carriage can persist for months. In some cities nursing homes may be an important reservoir of ESBL producing strains.

Use of these antibiotics exerts a selective pressure for emergence of ESBL and MBL producing gram negative bacteria. The resistance plasmids can then be transferred to other bacteria, not necessarily of the same species, conferring resistance to them. The lower digestive tract of colonized patients is the main reservoir of these organisms. Gastro intestinal carriage can persist for months. In some cities nursing homes may be an important reservoir of ESBL producing strains.

Patient to patient transmission of ESBL and MBL producing organisms occurs via the hands of hospital staff.

Control of Spread of ESBL and MBL Isolates: [6, 22]

Following are needed to be adopted to prevent the spread of ESBL and MBL isolates.

1) Improve laboratory detection of ESBLs and MBLs.
2) If an ESBL is detected, the strain should be reported as resistant to all extended spectrum Cephalosporins and Aztreonam regardless of the susceptibility testing result.
3) Judicious use of antimicrobials especially third generation cephalosporins.
4) Re-emphasis of hygienic techniques especially hand washing among personnel involved with patient care.
5) A search for common source of contamination such as respirators, disinfectant solutions and soap dispensers.
6) Isolation of patients with open infection.
7) Educate visiting health care staff on importance of control of ESBLs and MBLs.
8) Prevent outbreaks arising from transfer of patients to other units, hospitals or nursing homes.
9) Ensure that status of patients with regard to ESBL and MBL producing organisms is informed to receiving ward personnel before transfer.

REVIEW OF LITERATURE

Yoshochika Arakawa et al (2000) constructed a simple disk diffusion test for detection of IMP-1-type metallo-b-lactamase producing gram-negative bacteria. Two Kirby-Bauer disks containing ceftazidime (CAZ) and a filter disk containing a metallo- β -lactamase inhibitor were used in this test. Several IMP-1 inhibitors such as thiol compounds including 2-mercaptopropionic acid, heavy metal salts, and EDTA were evaluated for this test. Two CAZ disks were placed on a Mueller-Hinton agar plate on which a bacterial suspension was spread according to the method recommended by the National Committee for Clinical Laboratory Standards. The distance between the disks was kept to about 4 to 5 cm, and a filter disk containing a metallo- β -lactamase inhibitor was placed near one of the CAZ disks within a center-to-center distance of 1.0 to 2.5 cm. For IMP-1-producing strains, the growth-inhibitory zone between the two disks expanded, while no evident change in the shape of the growth inhibitory zone was observed for CAZ-resistant strains producing serine β -lactamases such as AmpC or SHV-12. As a result, 2 to 3 ml of undiluted 2-mercaptopropionic acid or mercaptoacetic acid able to block IMP-1 activity gave the most reproducible and clearest results, and CAZ-resistant strains producing AmpC or extended-spectrum β -lactamases were distinguishable from IMP-1 producers by this test. The specificity and sensitivity of this test were comparable to those of PCR analysis using β la $_{\text{IMP specific}}$ primers. [20]

Shittu A.O. (2002) et al studied wound infections in two health institutions in ILE-IFE, Nigeria, the microbiological analysis of wound infections in 102 patients was undertaken which showed isolation of Staphylococcus aureus (25%) , Escherichia coli (12%) and P. aeruginosa (9%) of cases of wound infection. [24]

Shenoy Set al (2002) obtained 494 strains obtained 494 strains of P. aeruginosa from various clinical specimens. Exudates followed by urine sample accounted for maximum isolates of P. aeruginosa viz. 290 (42.33%) and 74 (34.9%) respectively. Most exudates were obtained from burn patients while all urine samples were obtained from catheterized patients. Amikacin was found to be the most suitable antibiotic for routine use with sensitivity of 68% while high resistance was noted for Netilmicin showing resistance of 70.04%. Gentamicin showed relatively higher sensitivity (58.87%). ceftazidime and cefoperazone showed sensitivity 57.08% and 55.87% respectively. Imipenem and Meropenem showed 100% sensitivity. [26]

B.V. Navaneeth et al (2002) studied the resistance pattern of P. aeruginosa to ß-lactamase inhibitors and carbapenems, and detected the presence of MBL among resistant isolates to both groups of antibiotics. Fifty P. aeruginosa isolates from clinical specimens were tested for susceptibility to ß-lactamase inhibitors and carbapenems by Kirby-Bauer disc diffusion method. Isolates resistant to both groups of antibiotics were screened for the presence of MBLs by disc diffusion method using 2-mercaptoethanol. Of the 50 isolates, 6 (12%) were resistant to both ß-lactamase inhibitors and carbapenems. All 6 isolates were MBL producers and were resistant to all the antibiotics tested. [34]

Arya Mukhanjali et al (2005) obtained 516 bacterial isolates from 502 pus samples collected from post operative wound infection. Pseudomonas aeruginosa (n = 65) was isolated from 12.6% of post operative wound infections. For P. aeruginosa , piperacillin emerges as the most sensitive antimicrobial agent followed by Amikacin , Gentamicin , ceftazidime and Carbenicillin . In her study fluroquinolones were found less effective in treatment of wound infections. [23]

Zarakolu P et al (2005) compared the results of Epsilomer test (E-test), combined disk (CD) and double disk synergy (DDS) methods for the detection of ESBL in a total of 38 P. aeruginosa and 45 A. baumannii strains with ceftazidime or cefotaxime MIC > or = 1 micro g/ml, isolated from different clinical samples. Cefepime was included in the group of indicator antibiotics and the distance between clavulanic acid containing disk and indicator antibiotic disks was reduced to increase the sensitivity of DDS test. Of the P. aeruginosa strains, 78.9% was detected as ESBL producers by ceftazidime/ ceftazidime-clavulanic acid and 21.1% detected by cefotaxime / cefotaxime-clavulanic acid ratio. Among this group of strains, 31.5% was positive by DDS test and 84.2% was positive by CD method. The use of cefotaxime together with ceftazidime as an indicator antibiotic increased the sensitivity of detection of ESBL enzymes. Although, 28.9% of the P. aeruginosa and 13.3% of the A. baumannii strains were susceptible to ceftazidime by in vitro tests, they were ESBL producers. In conclusion, E-test method was found to be the most sensitive phenotypic method for the detection of ESBL in their study. [35]

Shampa Anupurba et al (2006) isolated 301 strains of P. aeruginosa from 940 relevant clinical specimen accounting 32% of various clinical specimens. She showed that the rate of isolation of P. aeruginosa was higher in age group 16-40 years (134 nos.). Highest prevalence rate (29.9%) was from surgery ward with antimicrobial susceptibility for cefoperazone / sulbactum (74%) followed by ciprofloxacin (58%) and ceftazidime (54%) with 18 % of isolates showing resistance to all the eight antibiotics tested in vitro. [25]

Ibukun Aibinu et al (2007) in Nigeria studied occurrence of ESBL and MBL in P. aeruginosa between March and August 2006 isolated from various clinical specimens. ESBL and MBL were detected by using double disk synergy test and

Imipenem EDTA combined disk test respectively. Results of their study showed Carbapenems had the highest activity (95.9%) followed by ceftazidime (79.4%), Among 20 ceftazidime resistant isolates 9 were ESBL producers, while 4 isolates showing resistance to carbapenems were MBL producers. Amikacin was the most potent among Aminoglycosides and they observed high level resistance to fluroquinolones.[2]

Jaykumar S, Appalraju B et al (2007). obtained 245 isolates, out of which 54 strains (22%) and 11 strains (4%) were found to be Multidrug resistant P. aeruginosa and Pandrug resistant P. aeruginosa respectively. Carbapenam resistant isolates showed MIC ranging from 16 to > 64 μg / ml. Thirty eight strains (15.5%) were ESBL producers. [27]

Aggrawal Ritu et al (2008) analyzed one hundred forty eight isolates of P. aeruginosa out of which 30 (20.27%) were found to be positive for Extended spectrum ß - lactamases. Maximum production was found to be in sputum and tracheostomy swabs (28.57%) followed by pus (24.13%). She opined that isolates were 100% sensitive to imipenem. Ofloxacin was second most (70%) effective drug[33]

Behera B et al (2008) detected metallo-β-lactamase (MBL) in nosocomial isolates of Pseudomonas aeruginosa by four different phenotypic methods. Ninety-one consecutive P. aeruginosa isolates were subjected to susceptibility testing by disc-diffusion assay and Vitek 2. Imipenem resistance was determined by three different methods (disc-diffusion, Vitek 2 and E test). Screening for MBL production was done by imipenem- EDTA combined disc test, imipenem-EDTA double-disc synergy test, imipenem-EDTA MBL E test and EDTA disc potentiation using four cephalosporins. Out of 63 imipenem resistant isolates, MBL screening could be done in 56 isolates, of which 48 were MBL positive by combined disc test and 36 by the double disc synergy

test. For confirmation of MBL production, MBL E test was done in 30 isolates. All the 30 isolates were confirmed to be MBL positive by the MBL E test method. EDTA disc potentiation using four cephalosporins was not very useful for MBL detection. [17]

Mariana Castanheira et al (2009) studied 301 Pseudomonas isolates collected from 10 Indian hospitals. The isolates were susceptibility tested against 28 antimicrobial agents according the Clinical and Laboratory Standards Institute (CLSI) broth micro dilution procedure and interpretation criteria. Among those isolates, 107 (35.5%) showed elevated MICs for imipenem or meropenem (MIC, ≥8 μg/ml). Isolates non susceptible to imipenem or meropenem (MIC, ≥8 μg/ml) were tested with a multiplex PCR strategy. Amplicons obtained were sequenced on both strands and analyzed. MβL genes were detected in 57 (53.2%) of the carbapenems - nonsusceptible strains. Five bla_{VIM} variants were detected, and bla_{VIM-2} was detected in 38 (66.7% of the MβL producers) isolates. The second most common VIM-encoding gene was bla_{VIM-6}, found in 12 (21.0%) P. aeruginosa isolates. bla_{VIM-11} was detected in one P. aeruginosa isolate and one P. stutzeri isolate, while bla_{VIM-5} was observed in one P. aeruginosa isolate. Additionally , one P. aeruginosa isolate was found to carry a new VIM variant, named VIM-18. They concluded that VIM producing Pseudomonas spp. were highly prevalent in India with a great diversity of bla_{VIM} types and MBL carrying integrons. [8]

Renata C. Pica˜o et al (2009) conducted a retrospective study in Brazil to characterize β lactamases in a collection of 43 ceftazidime-resistant Pseudomonas aeruginosa isolates recovered from patients with bloodstream infections hospitalized at a Brazilian teaching hospital between January and December 2005. Resistance rates for carbapenems, aminoglycosides , and quinolones were over

80%, with only colistin remaining active against all isolates. Pulsed-field gel electrophoresis analysis identified seven different genotypes. AmpC overproduction was found to be the sole β-lactamase-mediated mechanism responsible for ceftazidime resistance in four isolates (9.3%).[28]

Manoharan A et al (2010) evaluated Combined Disk diffusion test (CDDT) with confirmatory E test and PCR for screening and confirmation of MBLs among carbapenem resistant P. aeruginosa isolates collected as a part of multicenteric study (2005-2007). Combined Disk diffusion test was done by using imipenem, meropenem and ceftazidime with EDTA. MBL positives were further confirmed by IMP + EDTA E tested. They reported 42.6% to be MBL producers among 61 P. aeruginosa isolates. 15 out of 20 MBL producer strains were positive for VIM type MBL. They reported CDDT using IMP + EDTA had the highest sensitivity and specificity of 87.8% and 84. % when compared to E test. CDDT using IMP + EDTA showed specificity 90.9% and sensitivity 93.3% when compared with PCR. They recommended routine use of IMP- EDTA CDDT test for screening of MBL producers among P. aeruginosa.[36]

METHODOLOGY

SOURCE OF DATA:

The patients of both sexes irrespective of age groups with signs and symptoms of wound infection attending B.L.D.E.A'S Shri B.M. Patil medical college hospital and research centre, Bijapur from Nov 2008 to Sept 2010.

INCLUSION CRITERIA

1. All age groups and both sexes having suspected wound infections will be included in present study.
2. Only those cases yielding growth of Pseudomonas aeruginosa from cultured wound swabs will be included in the study and will be further identified and tested for antimicrobial susceptibility pattern.

EXCLUSION CRITERIA

1 Cases of wound infection which do not yield growth of Pseudomonas aeruginosa but yield growth of other bacteria will not be included.

Methods :

Collection of pus sample

Procedure:

Before wound cleaning and dressing conducted by attending Doctor, pus sample will be taken from each wound site by using sterile cotton tiped applicator. And transported immediately to microbiology laboratory.

2) Direct smear study:

Direct smears were made from the first swab and stained with grams stain. The smear was screened for the presence of inflammatory cells and the type of microbial flora.

3) Culture:

The second swab was used for inoculation on to Blood agar, MacConkey agar was incubated aerobically at 37°C for 24 hrs.

4) Identification of Pseudomonas aeruginosa:

Colonies showing greenish pigmentation on MacConkey agar and Blood agar of gram negative bacilli in grams stain smear were further subjected to biochemical tests such as catalase, oxidase, nitrate, indole, urease, citrate, TSI and Arginine dihydrolase test. (Procedures in annexure).

5) Antibiotic susceptibility testing:

The Pseudomonas isolates were subjected for antibiotic susceptibility testing by employing Kirby Bauer disc diffusion technique according to CLSI guidelines. In the present study the susceptibility was tested against Cephotaxime (30µg) ,Ceftazidime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Tobramycin (10µg) , Piperacillin (100 µg) ,Piperacillin/Tazobactum (100 µg/ 10) Meropenem (10µg), Imipenem (10µg). All the discs were procured commercially [Hi-media laboratories limited].The diameter of the zone of inhibition was measured and interpreted according to the guidelines of CLSI.

Table 4 : Zone size interpretative table in accordance to CLSI

Chemotherapeutic Agents	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Cephotaxime(Ce) (30µg)	>23	14-23	< 14
Ceftazidime (Ca) (30µg)	>18	14-18	< 14
Netilmicin (Nt) (30µg)	>15	12-15	< 12
Ciprofloxacin(Cf) (5µg)	>21	15-21	< 15
Gentamicin (G) (10µg)	> 15	12-15	< 12
Tobramycin (Tb) (30µg)	>15	12-15	< 12
Amikacin (Ak) (30µg)	>17	14-17	< 14
Imipenem (I) (10µg)	>16	13- 16	< 13
Piperacillin (Pi) (100 µg)	>18	---	<17
Piperacillin/Tazobactum (PT) (100 µg/ 10 µg)	>18	---	< 17
Meropenem (Mr) (10µg)	>16	13 -16	< 13

ESBL DETECTION METHOD

Double disk diffusion synergy method

PROCEDURE

Two to three colonies of P.aeruginosa strains are subcultured into peptone water and incubated at 37^{o}C for 4 to 6 hours to obtain optical density matching that of 0.5 MacFarland turbidity standard . This suspension is then used to inoculate Mueller – Hinton Agar (MHA) plates by swabbing with a sterile cotton swab. 30 µg disk of ceftazidime, is placed 15 mm (edge to edge) from an ceftazidime/ clavulanic acid (30 µg /10 µg) disk. Inoculated plates are incubated overnight at 37^{o}C. Enhancement of zone of inhibition between the clavulanate disk and any of the ß-lactam disks indicate the presence of an ESBL.

METALLO - ß- LACTAMASE (MBL) DETECTION METHOD

Imipenem-EDTA double disk synergy test (DDST) .

PROCEDURE

Two to three colonies of P. aeruginosa strains are subcultured into peptone water and incubated at 37 ^{o}C for 4 to 6 hours to obtain optical density matching that of 0.5 McFarland turbidity standards. This suspension is then used to inoculate Mueller-Hinton Agar (MHA) plates by swabbing with a sterile cotton swab. By placing imipenem 10 µg disk and a blank filter paper disk at a distance of 10 mm (edge-edge). To these blank filter paper disk 10 µgl EDTA (0.5 M) solutions is added after overnight incubation, the presence of even a small synergistic inhibition zone is interpreted as positive.

Figure 1: Collection of pus sample from wound infection

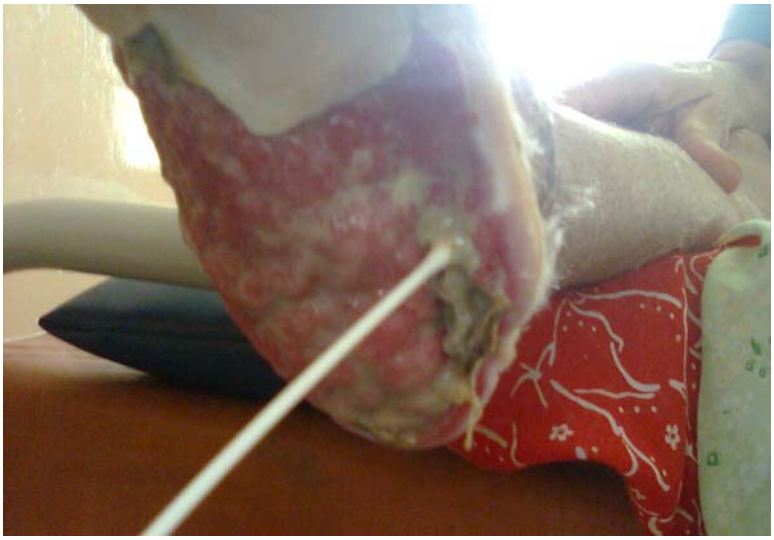


Figure 2 : Gram stain smear

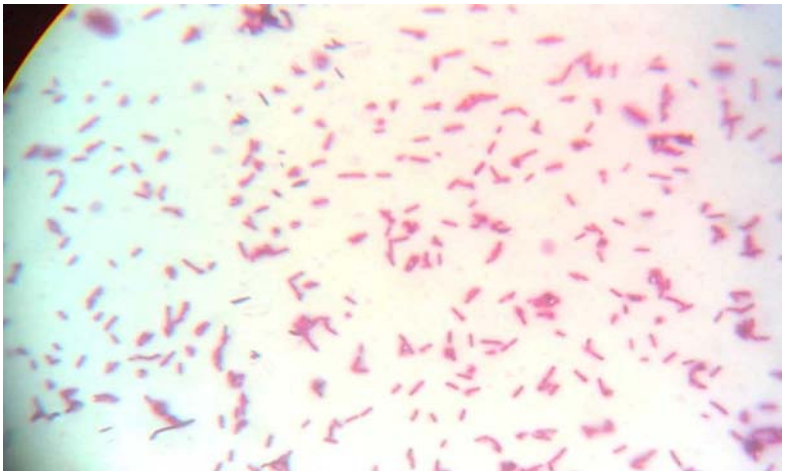


Figure 3: Growth of Pseudomonas aeruginosa on Nutrient Agar

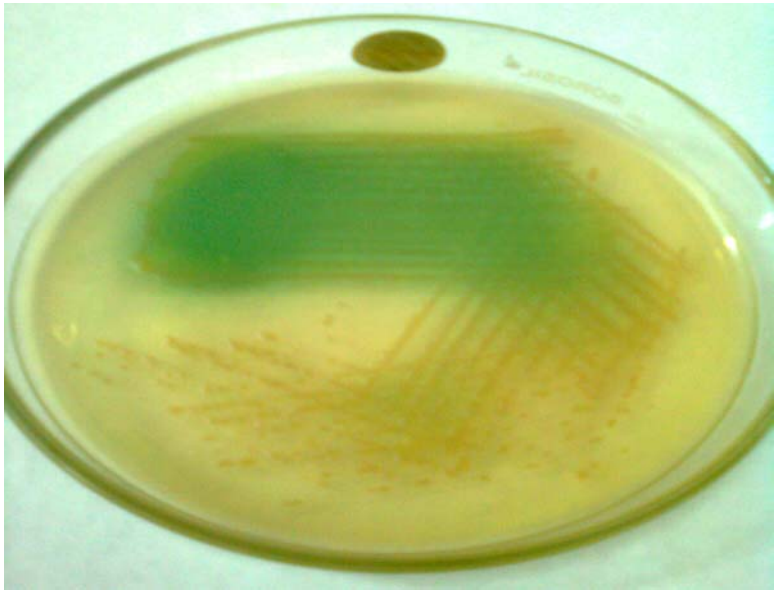
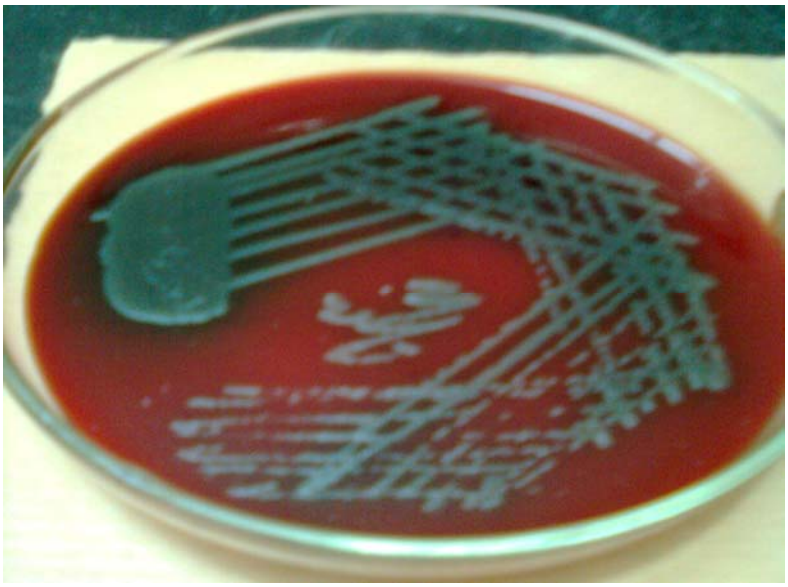


Figure 4: Growth of Pseudomonas aeruginosa on Blood agar



C Figure 5: Growth of P. aeruginosa on MacConkey agar

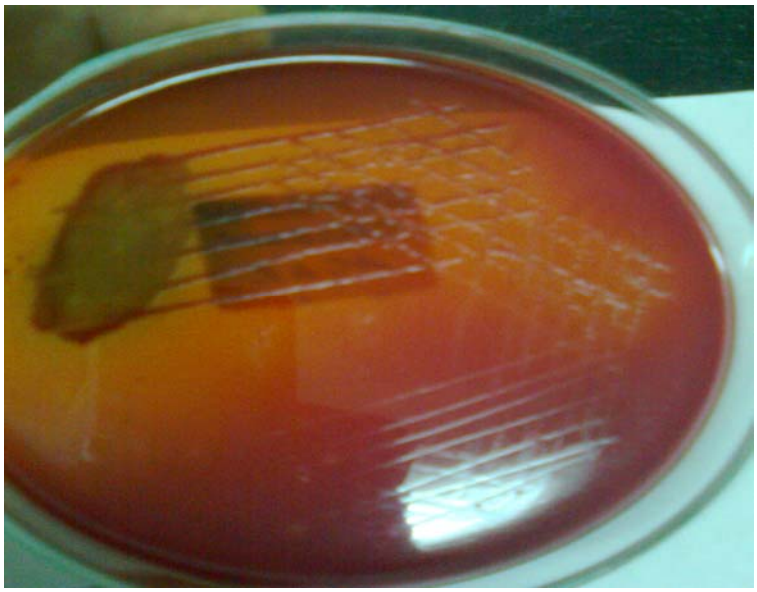
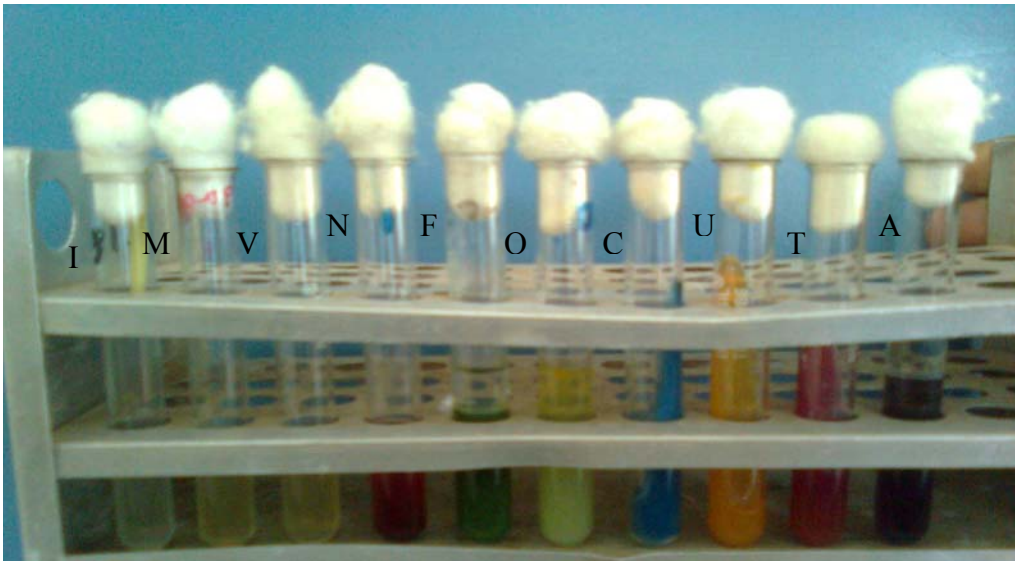


Figure 6 : Tests for the speciation of Pseudomonas aeruginosa



From Left to Right : I- Indole test , M- Methyl red test, V- Voges- Proskauer test, N- Nitrate reduction test, F- OF Glucose test ; fermentive , O – OF Glucose test ;oxidative , C – Citrate test, U – Urease test, T- TSI test , A – Arginine dihydrolase test

Figure 7 : Antibiotic susceptibility test by Kirby- Bauer disc diffusion test

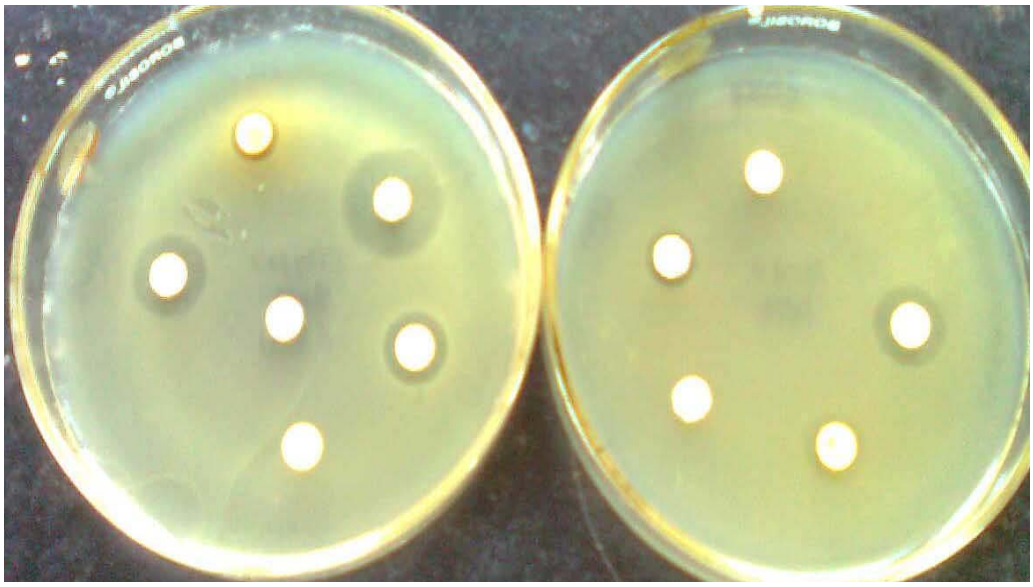


Figure 8: ESBL detection by Double disk synergy test

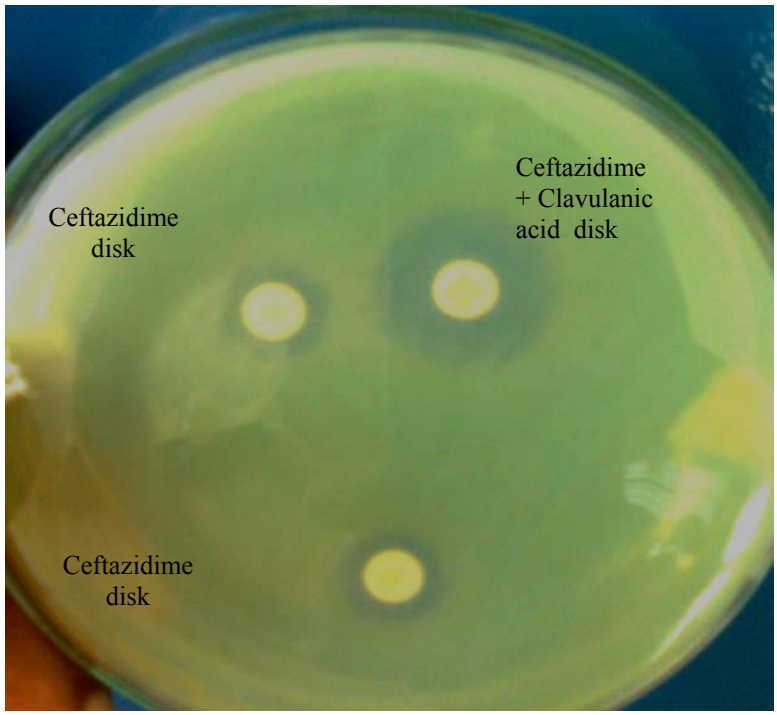
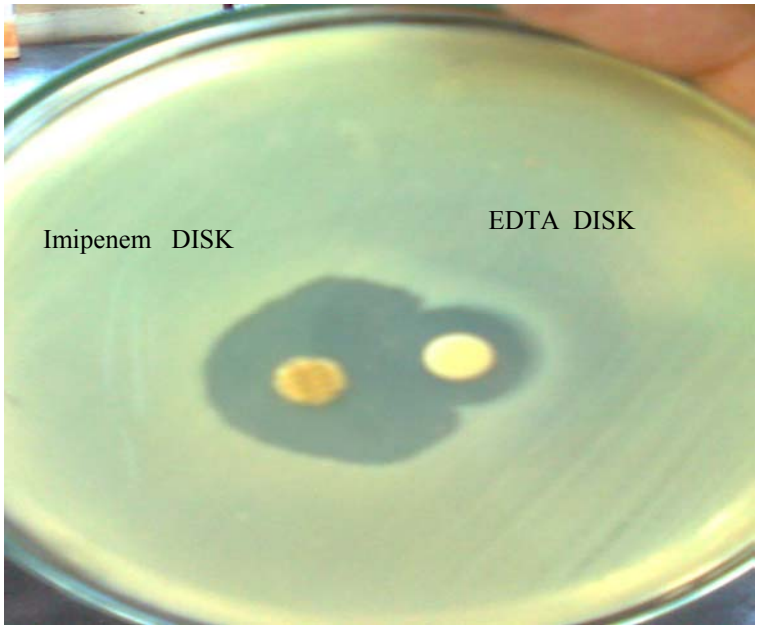


Figure 9 : MBL detection by Imipenem – EDTA synergy test



Statistical Methods applied

Following statistical tests will be used to compare the results in the present study

i) Diagrammatic presentation.

ii) Mean ± S D

iii) Percentage

RESULTS

The present study was carried out at the Dept. of Microbiology, Shri B.M. Patil Medical College, Bijapur between November 2008 and September 2010 to look for the presence of ESBL and MBL producing strains of Pseudomonas aeruginosa isolated from wound infections.

A total of 1628 pus samples were collected from various Pyogenic infections like diabetic ulcer, cellulitis, abscess, traumatic wound, non healing ulcer, CSOM , Burns, Post operative wound infection and Gangrene.

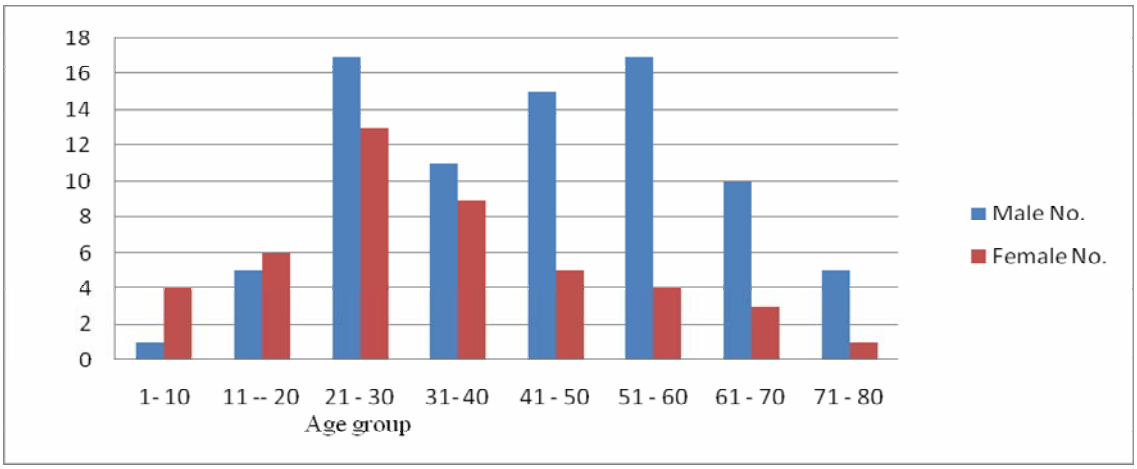
Out of 1628 pus samples cultured, 126 yielded growth of Pseudomonas aeruginosa. 103 samples yielded pure growth of Pseudomonas aeruginosa while 23 had mixed infections due to E. Coli (8 cases), S. aureus (6 cases), Proteus species (3 cases), K. pneumonia (3 cases), Acinetobacter species (2cases), Citrobacter species (1 case).

Out of 126 subjects who showed growth of Pseudomonas aeruginosa, 81 [64.28 %] were male patients and 45 [35.71%] were Female patients. The male to female ratio in the present study was 1.8: 1.

The age and sex distribution of the samples is shown in table.

Table 5 : Age and Sex wise distribution of patients

Sl No.	Age	Male		Female		Total	
		No.	%	No.	%	No.	%
1	1- 10	01	1.23	04	8.88	05	3.96
2	11- 20	05	6.17	06	13.33	11	8.73
3	21 - 30	17	20.98	13	28.88	30	23.80
4	31- 40	11	13.58	09	20	20	15.87
5	41 - 50	15	18.51	05	11.11	20	15.87
6	51 - 60	17	20.98	04	8.88	21	16.66
7	61 - 70	10	12.34	03	6.66	13	10.31
8	71 - 80	05	6.17	01	2.22	06	4.76
9	Total	81	100	45	100	126	100

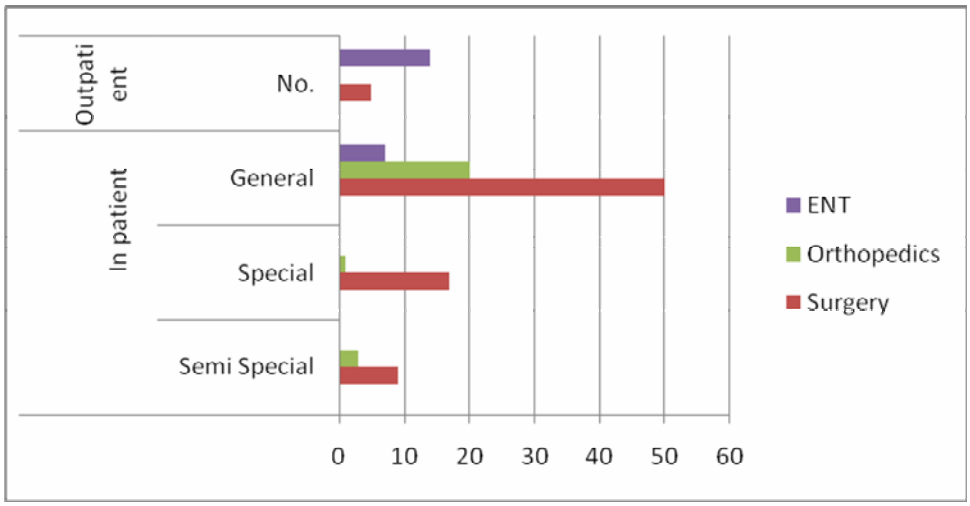


Graph 1: Age and Sex wise distribution of Patients.

Out of 126 isolates 19 [15.07 %] isolates were from outpatients department and 107 [84.92%] were from in patients. Among the isolates from in patients, 76 [71.02] isolates were from surgery ward followed 24 [22.42%] were from orthopedics ward and 7 [6.54 %] patients were from ENT ward. Amongst in patients, majority were from General ward 77 [71.96 %] followed by Special ward 18 [16.82%] and semi special ward 12 [11.21 %] .

Table 6 : Ward wise distribution of Pseudomonas aeruginosa

Sr. No.	WARD	In patient						Outpatient	
		Semi Special		Special		General		No.	%
		No.	%	No.	%	No.	%		
1	Surgery	09	75	17	94.44	50	64.93	05	26.31
2	Orthopedics	03	25	01	5.55	20	25.97	--	
3	ENT	-		-		07	9.09	14	73.68
	Total	12	100	18	100	77	100	19	100



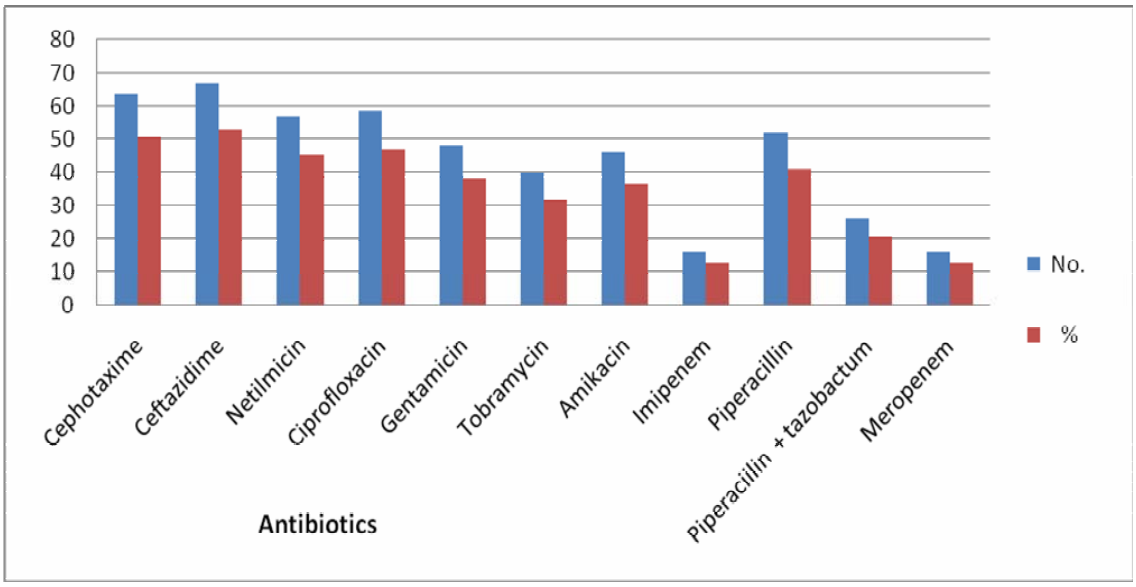
Graph 2 : Ward wise distribution of Clinical cases

More than 53 % of isolates of Pseudomonas aeruginosa were resistant against Ceftazidime, followed by Cephotaxime [50.79%]. Amongst aminogycosides least resistance was noted against Tobramycin [31.74%] while higher resistance was noted against Netilmicin [45.23%], Gentamicin [38.09%] and Amikacin [36.50%] in that order.

While Resistance to Imipenem and Meropenem was noted in 12.69 % cases. Piperacillin + tazobactum combination showed resistance to 20.63 % isolates as compared to Piperacillin alone [41.26 %]. The Resistance pattern of Pseudomonas aeruginosa is shown resistance in Table

Table 7 : Resistance pattern of Pseudomonas aeruginosa

Sr no.	Name of antibiotic	No.	%
1	Cephotaxime	64	50.79
2	Ceftazidime	67	53.17
3	Netilmicin	57	45.23
4	Ciprofloxacin	59	46.82
5	Gentamicin	48	38.09
6	Tobramycin	40	31.74
7	Amikacin	46	36.50
8	Imipenem	16	12.69
9	Piperacillin	52	41.26
10	Piperacillin + tazobactum	26	20.63
11	Meropenem	16	12.69



Graph 3: Resistance pattern of Pseudomonas aeruginosa

Among 126 Pseudomonas aeruginosa isolates 28 [22.22%] were ESBL producers. Percentage of ESBL producer strains in the present study is shown in following table.

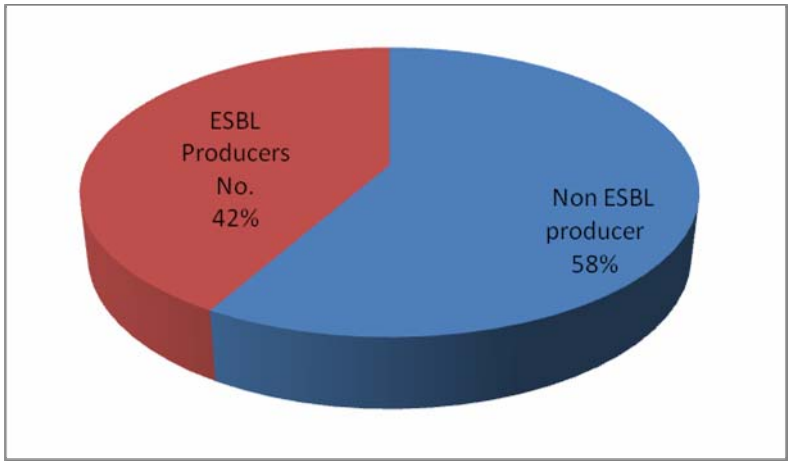
Table 8 : Percentage of ESBL producers among Pseudomonas aeruginosa

Organism	**Total**	**ESBL Producers**	
		No.	**%**
Pseudomonas aeruginosa	126	28	22.22

Sixty seven out of 126 strains of P. aeruginosa showed resistance to ceftazidime of which 28 [41.79%] were found to be ESBL producers . Percentage of ESBL producer among ceftazidime resistant isolates is shown in table.

Table 9: Percentage of ESBL producers among ceftazidime resistant Pseudomonas aeruginosa

Organism	No. of Ceftazidime resistance isolates	ESBL Producers	
		No.	%
Pseudomonas aeruginosa	67	28	41.79

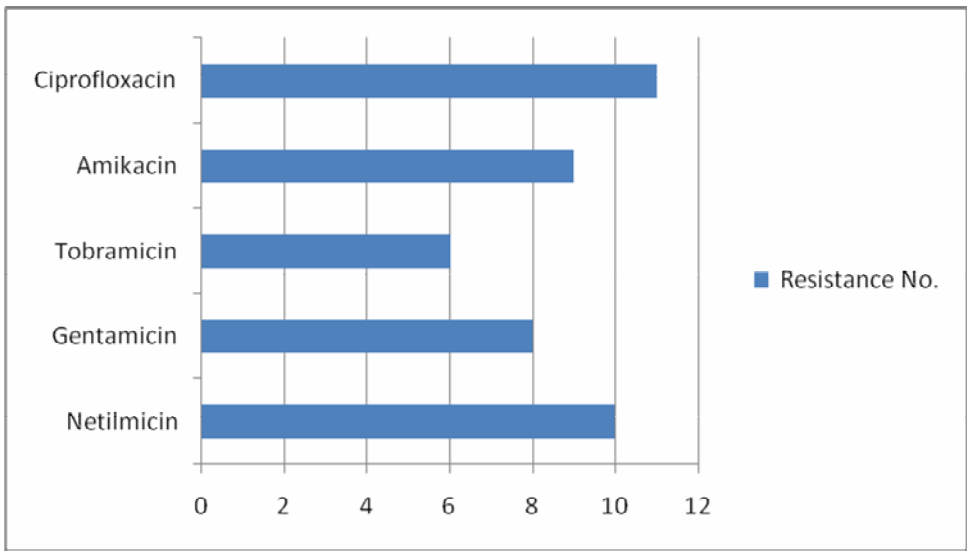


Graph 4: Percentage of ESBL producers among ceftazidime resistant isolates of P . aeruginosa

In the present study all 28 ESBL producers showed variable resistance pattern to aminoglycosides. 35.71 % ESBL producers showed resistance to Netilmicin while 28.17% isolates were resistant to Tobramycin. Resistance to Ciprofloxacin was 39.28 % Resistance pattern of ESBL producers for aminoglycosides and quinolones is shown in table

Table 10: Resistance pattern of ESBLs producers for aminoglycosides and Ciprofloxacin

Antibiotic	Resistance	
	No.	%
Netilmicin	10	35.71
Gentamicin	08	28.57
Tobramycin	06	21.42
Amikacin	09	32.14
Ciprofloxacin	11	39.28



Graph 5 : Resistance pattern of ESBL producers for Amino glycosides and Ciprofloxacin

Among 126 isolates of Pseudomonas aeruginosa, 10 [7.8 %] isolates were metallo β lactamases producers. Percentage of MBL producers among P. aeruginosa is shown in table.

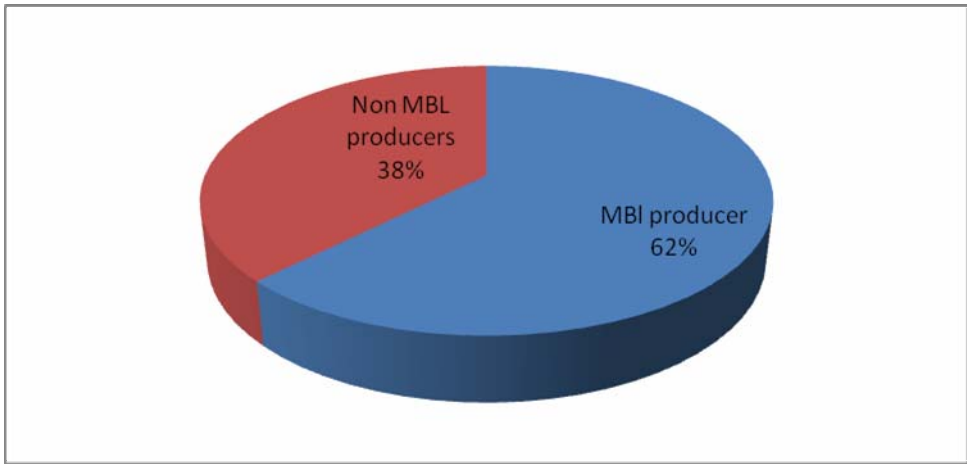
Table 11 : Percentage of MBL producers among P. aeruginosa

Organism	Total	MBL Producers	
		No.	%
Pseudomonas aeruginosa	126	10	7.87

In the present study 10 MBLs were isolated from 16 imipenem resistant isolates. So percentage of MBLs in imipenem resistant isolate is 62.5 %. Percentage of MBL producers in Imipenem resistant isolates is shown in table and graph.

Table 12 : Percentage of MBL producers among Imipenem resistant isolates

Organism	Total of Imipenem resistant isolates	MBL Producers		Non MBL producer	
		No.	%	No.	%
Pseudomonas aeruginosa	16	10	62	06	38

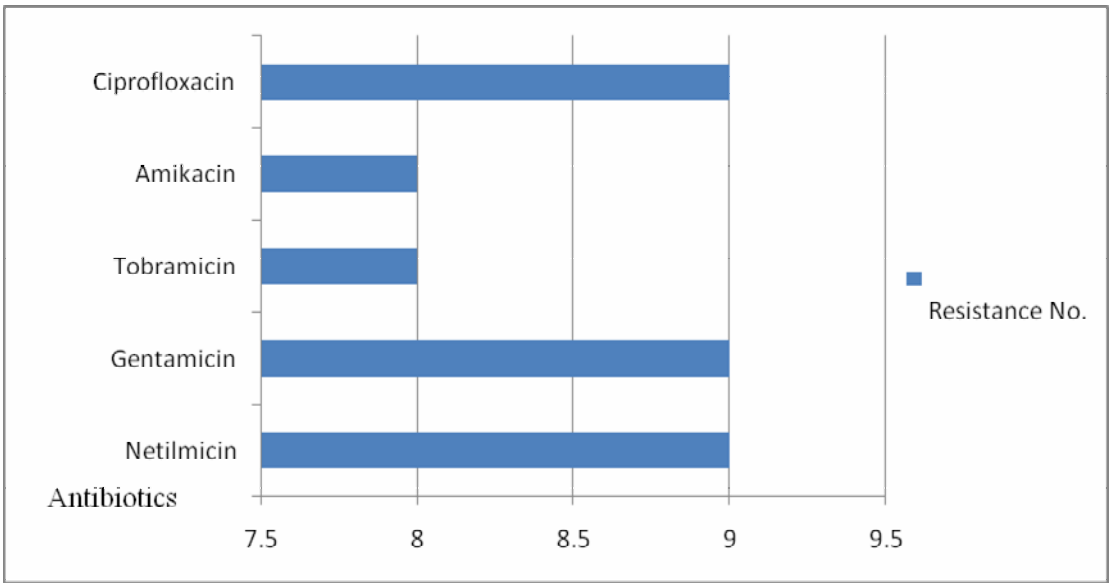


Graph 6: Percentage of MBL Producers among imipenem resistant isolates

In the present study, all 10 MBLs producers showed 90 % resistance to Netilmicin and Gentamicin while 80 % MBL producing isolates showed resistance to Tobramycin and Amikacin each. 90% of resistance was observed for Ciprofloxacin.

Table 13 : Resistance pattern of MBL producers for aminoglycosides and Ciprofloxacin

Antibiotic	**Resistance**	
	No.	**%**
Netilmicin	09	90
Gentamycin	09	90
Tobramicin	08	80
Amikacin	08	80
Ciprofloxacin	09	90



Graph 7: **Resistance pattern of MBL producers for aminoglycosides and Ciprofloxacin**

DISCUSSION

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. Unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. And it has increased the hospital stay of the patients which in turn causes economic burden.

In the present study, an attempt was made to know the rate of ESBL and MBL producing Pseudomonas aeruginosa, isolated from pus samples and to know their antibiogram at BLDEU'S Shri. B.M. Patil medical college, Bijapur.

Patients with Pyogenic infections admitted or attending out patient department between Nov 2008 to Sept 2010 were included in the study. Pus samples were collected from 1628 patients with suspected pyogenic infections. OUT of which 126 (12.92%) yielded growth of P. aeruginosa. The isolates were obtained from 81 male patients and 45 Female patients. A similar observation was made by Shampa Anupurba et al who have reported isolation of Pseudomonas aeruginosa from wound infection in 208 male and 93 female patients.[25]

We observed isolation of P. aeruginosa from wound infection more common in the age group 21-60 years which corresponds to young and middle age group. A similar observation was made by Shampa Anupurba et al who have reported isolation of Pseudomonas aeruginosa more common from age group 16 – 60 years [230 cases] as against 71 cases from infants and elderly age group.[25]

In present study majority of P. aeruginosa isolates were obtained from Cases of Cellulitis [19.04 %] followed traumatic wound infections [16.66%], Diabetic foot [15.07%], CSOM [11.90%] and Burns cases [9.5 2%]. Our findings are on similar lines with Shittu A.O. , et al who reported P. aeruginosa isolation most commonly from traumatic wound infection [37.5%] followed by CSOM [28.7%] and Burns [18.6%].[24]

In the present study, rate of isolation of P. aeruginosa was higher in, indoor patients [84.92%] as compared to out door patients [15.07 %]. Majority of indoor patients were from Surgery ward [71.02%], followed by Orthopedics ward [21.49%]. Majority of outdoor patient cases were from ENT OPD [73.68%]. A similar observation is made by Shampa Anupurba et al who have reported isolation of Pseudomonas aeruginosa more commonly from indoor patients [73.42%] compared to OPD cases [26.57%]. They noted highest prevalence P. aeruginosa in Surgery ward and Burns ward [51.6%] followed by Orthopedics ward [10%]. They had expressed view that duration of hospital stay is directly proportional to the higher prevalence of infection since rate of isolation was higher in indoor patients than in Outdoor patients.[25]

In the present study, antibiogram of 126 P. aeruginosa isolates, had shown more resistance against ceftazidime [53.17%] which is similar to the observation done by Diwivedi et al who had reported ceftazidime resistance around 63%.[45] Arya M et al had reported ceftazidime resistance 55.4% in isolates obtained from post operative wound infections.[23] Our findings differ from Anupurba S et al[25] and Ibukun et al [2] who had reported higher susceptibility for ceftazidime 54% and 79.4% respectively. We reported resistance for Cephotaxime around 51 % which differs from that of Arya M et al who had reported higher resistant i.e. 81.51%.[23]

In the present study Netilmicin [45.23%] had shown high resistance among aminoglycosides tested. This is in agreement with Arya et al who had reported Netilmicin resistance 56.9 %[23] while Aggarwal et al reported higher resistance for Netilmicin [76.66 %]. [33] In the current study Tobramicin [31.7%] had shown least resistance among aminoglycosides which is similar to Ansary et al who had reported Tobramicin resistance around 51% [38]

In present study majority of P. aeruginosa isolates were obtained from Cases of Cellulitis [19.04 %] followed traumatic wound infections [16.66%], Diabetic foot [15.07%], CSOM [11.90%] and Burns cases [9.5 2%]. Our findings are on similar lines with Shittu A.O. , et al who reported P. aeruginosa isolation most commonly from traumatic wound infection [37.5%] followed by CSOM [28.7%] and Burns [18.6%].[24]

In the present study, rate of isolation of P. aeruginosa was higher in, indoor patients [84.92%] as compared to out door patients [15.07 %]. Majority of indoor patients were from Surgery ward [71.02%], followed by Orthopedics ward [21.49%]. Majority of outdoor patient cases were from ENT OPD [73.68%]. A similar observation is made by Shampa Anupurba et al who have reported isolation of Pseudomonas aeruginosa more commonly from indoor patients [73.42%] compared to OPD cases [26.57%]. They noted highest prevalence P. aeruginosa in Surgery ward and Burns ward [51.6%] followed by Orthopedics ward [10%]. They had expressed view that duration of hospital stay is directly proportional to the higher prevalence of infection since rate of isolation was higher in indoor patients than in Outdoor patients.[25]

In the present study, antibiogram of 126 P. aeruginosa isolates, had shown highest resistant against ceftazidime [53.17%] which is similar to the observation done by Diwivedi et al who had reported ceftazime resistance around 63%.[45] Arya M et al had reported ceftazidime resistance 55.4% in isolates obtained from post operative wound infections.[23] Our findings differ from Anupurba S et al[25] and Ibukun et al [2] who had reported higher susceptibility for ceftazidime 54% and 79.4% respectively. We reported resistance for Cephotaxime around 51 % which differs from that of Arya M et al who had reported higher resistant i.e. 81.51%.[23]

In the present study Netilmicin [45.23%] had shown high resistance among aminoglycosides tested. This is in agreement with Arya et al who had reported Netilmicin resistance 56.9 %.[23] while Aggarwal et al reported higher resistance for Netilmicin [76.66 %]. [33] In the current study Tobramycin [31.7%] had shown least resistance among aminoglycosides which is similar to Ansary et al who had reported Tobramycin resistance around 51% .[38]

In our study Amikacin [36.5 %] resistance was low when compared to Gentamicin [38.9 %] . Also Shahid M , Malik A reported less resistance for Amikacin [40.9%] as compared to Gentamicin [45.45%][40] while. Jesudason et al had observed more resistance for Amikacin [67.5%][39]

In present study we reported Ciprofloxacin resistance of 46.82 % which is comparable to Anupurba et al who had reported ciprofloxacin resistance 58%.[25] while Behera et al had reported higher resistance to ciprofloxacin [79%].[17]

In the current study 41.26% strains of P. aeruginosa were resistant to Piperacillin alone while resistance was lower in piperacillin with tazobactam combination [20.63]. Better anti Pseudomonal activity of Piperacillin with tazobatum is reported earlier by MYSTIC study which had reported , piperacillin with tazobactum resistance 6%.[43] and others.[41] Ibukun Aibinu et al had reported piperacillin resistance alone to be 69.1 % while piperacillin with tazobactum combination showing less resistance i.e. 23.7 % .[2]

In the present study Imipemem and Meropenem had shown good antipsuedomonal activity. Similar observation was done by Jaykumar S.[27] We had observed prevalence of 12.69 % carbapenem resistant strains in our study which is comparable with Naveenth et al who had reported 12% carbapenems resistant among P .aeruginosa.[34] While higher carbapenems resistance is noted by Varaiya

et al [25%] [21], Jaykumar S and Appalraju B had noted lower carbapenem resistance [4.5%].[27] .

Out of 126 P. aeruginosa isolates, 28 [22.22 %] isolates were found to be positive for ESBL mediated resistance to 3rd generation cephalosporins. Which is similar to 20.27 % ESBL producer isolates in P. aeruginosa as reported by Aggarwal et al.[33] and 15.5% isolates found to be ESBL producer as described by Jaykumar S and Appalaraju B.[27] ESBL mediated reistance to 3rd generation cephalosporin in P. aeruginosa as reported by Uma et al [77.3%] is much higher than that reported in the present study.[30] Out of 67 isolates showing resistance to ceftazidime we found ESBL production in 28 [41.79%] . 45 % of ESBL mediated resistance to ceftazidime resistant isolates is reported by Ibukun et al is comparable in our study.[2]

As reported earlier [Ibkunu et al[2] and Aggarwal et al[33]] the possible co-existence of drug resistance to quinolones and aminogycosides by ESBL producing strains is observed in the present study. We reported Netilmicin [35.71%], Amikacin [32.71%] , Gentamicin [28.57%] and Tobramycin [21.42%] resistance in ESBL producer isolates , which is much lower to Netilmicin [76.6%], Tobramycin [73.33 %] resistance reported by Aggarwal et al[33] and Amikacin [87.8%],Gentamicin [87.8%] reported by Ibukun et al[2] al among ESBL producers. We had reported ciprofloxacin resistance 39.28 % in the present study which differs from Renato picao et al who had reported higher ciprofloxacin resistance [80%] in ESBL producer isolates.[28]

The findings of the present study highlights the problem of ESBLs producers among the isolates obtained from patients with wound infection as they show decreased susceptibility to the aminogycosides , third generation cephalosporins and Ciprofloxacin.

In the present study out of 126 isolates of Pseudomonas aeruginosa, 10 [7.8 %] isolates were metallo β lactamases producers. Overall percentage of MBLs in present study is consistent with Navaneeth et al[34], Ibukun et al[2], Uma Chudhari et al[30] Percentage of MBLs from various places is shown in table.

Table 14 : Percentage of MBLs from various places

Sl. No.	Author	Year	Place	MBL %
1	Navaneeth et al[34]	2002	Banglore	12
2	Ibukun et al[2]	2007	Lagos, Nigeria	4.12
2	Uma Chudhari et al[30]	2008	Rohtak	6.12
3	Behera et al[17]	2008	New Delhi	39.56
4	Manoharan et al[36]	2010	Vellore	42.6
5	Present Study	2010	Bijapur	7.8

In the present study 10 MBLs were isolated from 16 imipenem resistant isolates. So percentage of MBLs in imipenem resistant isolate is 62.5 %. This suggests that carbapenem resistance in P. aeruginosa is mediated predominantly via MBL production. A similar finding has been observed by SARI study group.[36] Also Behara et al reported MBL production in 64.28% of 56 carbapenem resistant isolates when tested by double disk synergy test.[17]

In the present study 5 isolates [50%] out of 10 MBLs were resistant to all the 11 antibiotics tested. Presence of MBLs in pandrug resistant isolates has been already observed by Ibukun et al.[2] Jaykumar S, Appalaraju B had reported 54.5% MBL producers among pandrug resistant P. aeruginosa isolates.[27]

In the present study resistance pattern of MBL positive isolates among aminoglycosides was as follows, Netilmicin [90%] , Gentamicin [90 %] , Amikacin [80%] and Tobramycin [80%]. Ami et al had shown 100% resistance against Netilmicin , Gentamicin, Amikacin and Tobramycin in MBL producing P. aeruginosa isolates. A similar observation was also made by Jaykumar S, Appalaraju B[27] Ibukun et al[2] and others.[37]

In present study 90 % resistance was seen for Piperacillin / tazobactum , which differs from Ami et al who had reported higher sensitivity with Piperacillin tazobactum [35%] in MBL producing P.aeruginosa. [21] While Uma et al had reported 100% resistance for Piperacillin with tazobactum in MBL producing isolates of P. aeruginosa. [30]

In the present study none of the isolate had coproduced both ESBL and MBL. This is in agreement with findings of Renata Picao et al.[28] and others [27]

Present study underlines the unique problem with MBLs, because of their broad spectrum and unrivalled drug resistance, creating the therapeutic challenge for clinician and Microbiologists. Hence we suggest the detection of ESBL and MBL in Pseudomonas aeruginosa should be a routine practice. To overcome the problem of emergence and the spread of multidrug resistant P. aeruginosa combined interaction and cooperation of Microbiologist and Clinicians and the infection control team is needed. We recommend the routine Surveillance of antibiotic resistance in the hospital.

CONCLUSION

- In the present study a total of 126 isolates of Pseudomonas aeruginosa isolated from wound infections were studied.
- Majority of samples were from male patients, Male to female ratio was 1.8: 1.
- Wound infection was more common in the age group of 21-60 years [72%] which corresponds to young and middle age group.
- Distribution of cases showed Cellulitis [19.04 %] followed traumatic wound infections [16.66%] , Diabetic foot [15.07%] , CSOM [11.90%] and Burns cases [9.5 2%].
- Rate of isolation of P. aeruginosa was higher in, indoor patients [84.92%] as compared to outdoor patients [15.07 %]. This suggests, the rate of isolation of P. aeruginosa is associated with the long duration of hospital stay.
- Majority of indoor patients were from Surgery ward [71.02%] , followed by Orthopedics ward [21.49%].
- Majority of outdoor patients were from ENT OPD [73.68%].
- Cefatazidime [46.83%] was the least sensitive drug while Imipenem and Meropenem each showing 12.69% resistance had shown good antipseudomoanl activity.
- Netilmicin [45.23%] had shown higher resistance among aminoglycosides tested . Tobramycin [31.7%] had shown least resistance among the aminoglycosides tested.
- Less resistance for Amikacin [36.5 %] as compared to Gentamicin [38.9 %] was noted.

- 22.22 % of the isolates were found to be positive for ESBL mediated resistance to 3rd generation cephalosporins.
- Aminoglycoside resistance in ESBL producers was less as compared to previous reports. All the ESBL producers were sensitive to Imipenem in the present study, indicating Imipenem as the drug of choice in infections with ESBL producers. Imipenem can be combined with Tobramycin which was the most potent aminoglycoside.
- Out of 126 isolates 7.8 % of isolates were metallo β lactamases producers.
- Prevalence of MBLs in imipenem resistant isolates is 62.5 %. This is a major mechanism of carbapenem resistance.
- 50% out of 10 MBLs were pandrug resistant P. aeruginosa.
- Metallo beta lactamases producing isolates had shown high degree and widespread resistance for aminoglycosides, Ciprofloxacin, Piperacillin with tazobactum combination.
- This highlights the need for all diagnostic laboratories to perform ESBL and MBL detection as a routine practice among Pseudomonas aeruginosa.
- To overcome the problem of emergence and the spread of multidrug resistant P. aeruginosa combined interaction and cooperation of Microbiologist and Clinicians and the infection control team is needed. We recommend the routine Surveillance of antibiotic resistance in the hospital.

SUMMARY

- The present study was carried out at BLDEU's shri B.M. Patil Medical College, Bijapur between November 2008 and September 2010.
- The totals of 126 pus samples yielding Pseudomonas aeruginosa from various pyogenic infections were studied.
- Majority of samples were from male patients, Male to female ratio was 1.8: 1.It showed male preponderance.
- Rate of isolation of P. aeruginosa was higher in, indoor patients [84.92%] as compared to outdoor patients [15.07 %] suggesting the association between the rate of isolation of P. aeruginosa with the long duration of hospital stay.
- Cefatazidime [46.83%] was the least sensitive drug while Imipenem and Meropenem each showed12.69% resistance. Netilmicin [45.23%] had shown high resistance while Tobramycin [31.7%] had shown least resistance among aminoglycosides tested
- ESBL detection test was carried out by double disk synergy test using ceftazidime and ceftazidime - clavulanic acid disk in isolates showing resistance to ceftazidime. Of the 126 isolates 28 [22.22%] were ESBL producers. All ESBL producers were sensitive to Imipenem.
- Out of 126, isolates showing resistance to imipenem were tested for MBL production 7.8 % isolates were metallo β lactamases producers.
- Prevalence of MBLs in imipenem resistant isolate was 62.5 %.
- 50% out of 10 MBLs were pandrug resistant P. aeruginosa.
- Metallo beta lactamases producing isolates had shown widespread resistance for aminoglycosides, Ciprofloxacin, Piperacillin with tazobactum combination.

- Metallo beta lactamases producing isolates had shown high degree and widespread resistance for aminoglycosides, ciprofloxacin. Piperacillin with tazobactum combination.
- This study highlights the need for all diagnostic laboratories to perform ESBL and MBL detection as a routine practice among Pseudomonas aeruginosa.
- To overcome the problem of emergence and the spread of multidrug resistant P. aeruginosa combined interaction and cooperation of Microbiologist and Clinicians and the infection control team are needed. We recommend the routine Surveillance of antibiotic resistance in the hospital.

BIBLIOGRAPHY

1) Winn W Jr,Allen S, Janda W, Koneman E, Procop G, Scherckenberger P, Woods G (Eds). Koneman's Color Atlas And Textbook of Diagnostic Microbiology, Sixth Edition, Lippincott and Williams, 2006.
2) Ibukun A , Tochukwu N , Tolu O. Occurrence of ESBL and MBL in Clinical Isolates of Pseudomonas aeruginosa From Lagos, Nigeria. Journal of American Science 2007 ; 3(4) : 81-85.
3) Bradford PA. Extended spectrum ß-lactamases in the 21st century : Characterization, epidemiology and detection of this important resistance threat. Clin. Microbiol. Rev 2001; 14: 933-951.
4) Poole K. Resistance to beta-lactam antibiotics. Cell Mol. Life Sci. 2004; 61**:**2200-2223.
5) Rodrigues C, Joshi P, Jani SH et al. Detection of ß-lactamases in nosocomial gram negative clinical isolates. Indian journal of medical microbiology 2004; 22 (4): 247-250.
6) Timothy R. Walsh, Mark A. Toleman, Laurent Poirel and Patrice Nordmann. Metallo-β-lactamases: the quiet before the storm? Clinical Microbilogy Reviews Apr 2005; 306-325.
7) Chaudary U, Aggarwal R. Extended spectrum ß-lactamases (ESBL) – an emerging threat to clinical therapeutics. Indian J Med Microbiol 2004; 22 (2) :75-80.
8) Mariana Castanheira, Jan M. Bell, John D. Turnidge, et al. Carbapenem Resistance among Pseudomonas aeruginosa Strains from India: Evidence for Nationwide Endemicity of Multiple Metallo-²-Lactamase Clones (VIM-2, -5, -6, and -11 and the Newly Characterized VIM-18). Antimicrob. Agents Chemother 2009 ; 53(3):1225-1227.

9) Gernald L. Mandell, John E. Bennett, Raphael Dolin. Principles and practice of infectious diseases. 6 th edition Elsevier Churchill Livingstone 2005; 253-270.

10) Ananthakrishnan Ashwin N, Reba Kanungo, Kumar A, Badarinath S.Detection of extended spectrum ß lactamase producers among surgical wound infections and burns patients in JIPMER. Indian J Med Microbiol 2000; 18 (4): 160-135

11) Weldhagen GF, Poirel L, Nordmann P. Amber class A extended-spectrum beta-lactamases in Pseudomonas aeruginosa : Novel developments and clinical impact. Antimicrob. Agents Chemother (2003) ;.47: 2385-2392.

12) Poirel L, Naas T, Nordmann P. Pyrosequencing as a rapid tool for identification of GES-type extended-spectrum beta-lactamases.J. Clin. Microbiol (2006); 44:3008-3011.

13) Yusuf Yakupogullari , Laurent Poirel , Sandrine Bernabeu , Ahmet Kizirgil and Patrice Nordmann . Multidrug-resistant Pseudomonas aeruginosa isolate co-expressing extended-spectrum b-lactamase PER-1 and metallo-b-lactamase VIM-2 from Turkey. J. Antimicrob Chemother 2007;48:421 -422.

14) Jain Amita, Indranil Roy et al. Prevalence of extended spectrum ß lactamase producing gram negative bacteria in Septicaemic neonates in a tertiary carehospital. Journal of medical microbiology 2003; 52: 421-425.

15) Pitout JDD, Reisbig MD, Venter EC et al. Modification of double disk test fordetection of Enterobacteriaceae producing extended spectrum and Ampc ß-lactamase. Journal of clinical microbiology 2003; (41): 3933-3935.

16) Paterson David L, Nina Singh et al. Fatal infection due to extended spectrum ß-lactamase producing Escherichia coli implications for antibiotic choice for spontaneous bacterial peritonitis. Clin Infect Dis 1999; 28: 683-684.

17) B. Behera, P. Mathur, A. Das, A. Kapil, V. Sharma. An evaluation of four different phenotypic techniques for detecting of metallo-β-lactmases producing Pseudomonas aeruginosa. Indian J Medical Microbiology 2008; 26(3): 233-237

18) Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo- β-lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801.

19) Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiation of metallo- β-lactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol 2003 ; 41: 4623-9.

20) Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamases producing gram-negative bacteria using thiol compounds. J Clin Microbiol 2000;38:40-3.

21) Ami Varaiya, Nikhil Kulkarni, Manasi Kulkarni, et al. Incidence of Metallo beta lactamase producing Pseudomonas aeruginosa in ICU patients. Indian J Med Res 2008; 127: 398-402.

22) Peterson David L, Victor YU. Extended spectrum ß lactamases: a call for improved detection and control. Clin Infect Dis. 1999; 29: 1419-1422.

23) Arya M , Arya P , Biswas D , Prasad R . Antimicrobial susceptibility pattern of bacterial isolates from post operative wound infections . Indian J Pathol Microbiol 2005; 48(2): 266-269.

24) Shitoo O, Kolawole D.O. , Oyedepo E.A.R. . A study of wound infections in two health institutions in ILE – IFE, Nigeria. Afr. J biomed res 2002 ; 5 : 266-269.

25) Anupurba S , Battacharjee A , Garg A, Ranjan sen M. Antimicrobial susceptibility of Psuedomonas Aeruginosa isolated from wound infections. Indian J Dermatol 2006 ; 51(4): 286-288.

26) Shenoy S , Baliga S , Saldhana , Prashanth H . Antibiotic sensitivity pattern of Psuedomonas aeruginosa strains isolated from various clinical specimen. Indian J med sci 2002 ; 56 (9) : 427-430.

27) Jaykumar S, Appalraju B. Prevalence of multi and pan drug resistant Psuedomonas aeruginosa with respect to ESBL and MBL in a tertiary care hospital Indian J Pathol Microbiol 2007; 50 (4) : 922—925.

28) Renata C. Picão, Laurent Poirel, Ana C. Gales, et al. Diversity of ²-Lactamases Produced by Ceftazidime-Resistant Pseudomonas aeruginosa Isolates Causing Bloodstream Infections in Brazil. Antimicrob. Agents Chemother 2009;53(9):3908-3913.

29) Gladstone P., Rajendran P., Brahmadathan K. N. Incidence of Carbapenem resistance non fermenting Gram negative Bacilli from patients with respiratory infections in the Intensive Care Unit. Indian J Med Microbiol 2005; 23: 189-191.

30) Chaudhari Uma , Hemlata Bhaskar , Madhu Sharma. Imipenem –EDTA disk method for rapid identification of metallo β lactamase producing Gram negative bacteria. Indian J Med Res 2008; 127(2):406-407.

31) Wolska K, Jakubczak A, Soszyńska A. Antibiotic susceptibility and occurrence of ESBL, IBL and MBL in Pseudomonas aeruginosa strains. Med Dosw Mikrobiol. 2008; 60(2):111-119.

32) Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo –β- lacatamase producing clinical isolates of Acinetobacter species and Psuedomonas aeruginosa from

intensive care unit patients of a tertiary care hospital. Indian J Med Microbiol 2008; 26(3): 243-245.

33) Ritu Aggarwal , Uma Chaudhari, Kiaran Bala. Detection of extenend – spectrum β lactamas in Psuedomonas aeruginosa. Indian J Pathol Microbiol 2008;51(2): 222-224.

34) Navneeth BV, Sridaran D, Sahay D, Belwadi M. A preliminary study on Metallo betalactamase producing Pseudomonas aeruginosa in hospitalised patients. Indian J Med Res 2002; 112: 264-267.

35) Zarakolu P, Metan G, Hasçelik G, Akova M. Comparison of different phenotypic methods detecting extended spectrum beta-lactamases in Pseudomonas aeruginosa and Acinetobacter baumannii strains. Mikrobiyol Bul 2005 ; 39(3):265-72.

36) Manoharan A, Chatterjee S, Mathai D, SARI study group. Detection and characterization of metallo beta lactamases producing Pseudomonas aeruginosa. Indian J Med Microbial 2010; 28(3): 241-244.

37) Atsushi Nakamura et al. Combined effects of meropenem and aminoglycosides on Pseudomonas aeruginosa in vitro. Journal of Antimicrobial Chemotherapy (2000); 46 : 901-904

38) Ansary SP, Haque R, Faisal AA. Resistance pattern of P. aeruginosa occurring in northen Bangladesh. Tropical Doctor 1994; 24 : 188.

39) Mary V. Jesudason, A.J. Kandathil & V. Balaji Comparison of two methods to detect carbapenemase and metallo-β-lactamase production in clinical isolates. Indian J Med Res 2005; 121: 780-783.

40) Shahid M, Malik A. A plasmid mediated amikacin resistance in clinical isolates of Pseudomonas aeruginosa. Indian J of Med Microbiol 2004; 22: 182-184.

41) Walsh T. R. , Bolstrom A , Qwarnstorm A , Gales A , Evaluation of new E test for detecting metallo β lactamases in routine clinical testing. J Clin Microbiol 2002; 40: 2755 2759.

42) Obritsch MD , Fish DN , Maclaren R, Jung R. National surveillance of Antimicrobial Resistance in Pseudomonas aeruginosa Isolates obtained from Intesive care unit patients from 1993 to 2002. Antimicrob Agents Chemother 2004; 48 : 4606- 10.

43) Diwivedi M , Mishra A., Singh R.K., A. Azim , A.K. Baronia, K.N. Prasad. Nosocomial cross – transmission of Pseudomonas aeruginosa between patients in a tertiary intensive care unit. Indian J Pathol Microbiol 2009;52(4): 509-513

ANNEXURE-I

STUDY OF PSUEDOMONAS AERUGINOSA ISOLATED FROM WOUND INFECTIONS AND THEIR ANTIBIOGRAM WITH RESPECT TO ESBL AND MBL IN A TERTIARY CARE HOSPITAL.

PROFORMA

1) HISTORY

A) PATIENT DETAILS

1) Name	:	CASE NO	:
2) Age/sex	:	IP NO	:
	:	DOA	:
	:	DOD	:
5) Occupation	:	Lab No	:
6) Residential Address	:		

7) Chief Complaints : Wound

Onset

Duration

Progress

: Pain

: Pus

8)Treatment history :

2) **CLINICAL EXAMINATION**

A) GENERAL PHYSICAL EXAMINATION

- Nutritional Status :
- Pallor :
- Icterus :
- Pulse :
- Temperature :
- BP :

B) SYSTEMIC EXAMINATION

- CVS :
- RS :
- PA :
- CNS :

C) Local Examination of wound :

D) ROUTINE

- Microscopy
- Protein
- Sugar

3) **MICROBIOLOGICAL STUDY**

CULTURE STUDY

A) COLONY CHARACTERS

	After 24 hrs	After 48 hrs
Blood agar		
Mac Conkey's Agar		

B) **PRELIMINARY TESTS**

- Gram staining
- Motility
- Catalase test
- Oxidase test
- Nitrate reduction test
- IMViC reactions
- TSI test

C) **BIOCHEMICAL REACTIONS**

- Arginine dihydrolyse test

D) **ANTIBIOGRAM**

The isolated organism will be subjected to susceptibility testing for the following chemotherapeutic agents by Kirby- Bauer disk diffusion method.

Chemotherapeutic agents	Sensitive	Intermediate	Resistant
Cephotaxime Ceftazidime Cefepime Ciprofloxacin Gentamicin Tobramycin Amikacin Imipenem Piperacillin (100 µg) Piperacillin/Tazobactum (100 µg/ 10 µg) Meropenem			

ESBL DETECTION

- Double disk diffusion synergy test

MBL Detection

- Imipenem – EDTA double disk synergy test

Final identification of organism :

ANNEXURE II

GRAMS STAINING

Reagents:

1) Violet dye :	Crystal violet	10g
	Absolute alcohol	100 ml
	Distilled water	1000 ml
2) Iodine Solution:	Iodine	10 g
	Potassium iodide	20 g
	Distilled water	1000 ml
3) Decolorizer :	Absolute alcohol	
4) Counter stain:	Safranine 0.5%	

Procedure

1) The direct smear from the thick and tenacious part of pus and the culture smear from the suspected Pseudomonas aeruginosa colony was prepared, air dried and heat fixed.
2) Smear was flooded with 1% Crystal violet [Primary stain] for 1 minute, and washed with water.
3) Smear was flooded with Gram's Iodine for 1 minute and washed with water.
4) Smear was decolorized with absolute alcohol till no colour was seen to flow out of the preparation.
5) Smear was counterstained with 0.5% safranine for 1 minute.
6) The smear was washed with water, air dried and observed under oil immersion objective for the presence of inflammatory cells and organisms.

Interpretation

The gram positive organism take up violet colour and the gram negative organisms and tissue elements take up pink colour.

ANNEXURE III

CATALASE TEST

Principle:

This test demonstrates the presence of catalase , an enzyme that catalyses the release of oxygen from hydrogen peroxide.

Procedure:

A small amount of the colony to be tested is picked with sterile thin glass rod and it is inserted into 3% hydrogen peroxide solution held in a small, clean tube.

Interpretation

Production of gas bubbles was interpreted as positive catalase test and no bubble formation was interpreted as negative test.

ANNEXURE IV

Oxidase test

Principle: This test demonstrates the presence of cytochrome Oxidase enzyme which catalyses oxidation of reduced cytochrome by oxygen.

Procedure : Filter paper strips soaked in the Oxidase reagent (1% tetramethyl - P- Phenylene diamene hydrochloride) is placed in a petridish and the colony to be tested is smeared on the strip using sterile glass rod.

Interpretation : Development of purple colour within 10 sec is interpreted as positive.

ANNEXURE V

Nitrate test:-

Principle:- This test demonstrates the presence of nitrate reductase enzyme which reduces nitrate to nitrite.

Procedure

Nitrate reagent A	Nitrate reagent B
Sulfanilic acid – 4g	N,N – dimethyl-1-naphthylamine - 3ml
Acetic acid (5M)-500ml	Acetic acid (5M) – 500 ml

Organism is grown in 5 ml of nitrate broth for 24 to 48 hours. Equal volumes of reagent A and reagent B mixed just before use. 0.1ml of the reagent mixture is added to the culture.

Interpretation: Development of red colour with in few minutes is considered positive.

ANNEXURE VI

Indole test

Principle :- This test demonstrates the presence of enzyme trytophanase, that degrade the tryptophan to indole.

Procedure:-

Kovacs reagent ingredients

P- Dimethylaminobenzaldehyde	- 10 g
Isoamyl alcohol	- 150 ml
Concentrated hydrochloric acid	- 50 ml

The test organism is inoculated into peptone water and incubated for 24 hours 0.5 ml of kovacs reagent is added to overnight browth.

Interpretation :- A pink colour ring development indicates a positive test.

ANNEXURE VII

Urease test:-

Principle:- This test demonstrates the presence of urease enzyme which splits urea to ammonia and CO2.

Christensen's urease medium:

Peptone - 1g

Sodium chloride - 5g

Dipotassium hydrogen phosphate: - 2g

Phenol red (in 500 aqueous solutions - 6 ml

Agar - 20 g

10% Glucose solution - 10 ml

Urea 20% solution - 100 ml

Procedure : - Test organism is inoculated on to slope of urease medium and incubated at 37^0C for 24-48 hours.

Interpretation:-

Development of pink colour in the slope is interpreted as positive test.

ANNEXURE VIII

Citrate test

Principle:- This test demonstrates the ability of organism to use citrate as sole source of carbon.

Simmon's citrate medium.

Magnesium sulphate - 0.2g

Ammonium dihydrogen phosphate - 1g

Dipotassium phosphate - 1g

Sodium citrate - 2g

Nacl - 5g

Agar - 15g

Bromothymol blue - 0.08g

Procedure:- Test organism is inoculated onto slope of simmon's citrate medium and incubated at 37^0 C for 24-48 hours.

Interpretation :- Development of blue colour in the medium is interpreted as positive test.

ANNEXURE IX

Methyl red test

Principle:- This test detect the production of acid during the fermentation of glucose and maintenance of pH below 4.5.

Procedure :- Test organism is inoculated into glucose phosphate medium and Incubated at 37^0C for 48 to 72 hours. Few drops of 0.04% solution of methyl red is added to the culture.

Interpretation:- Development of red colour is interpreted as positive test.

ANNEXURE X

Voges -Proskauer test:-

Principle - This test detects the production of acetyl methylcarbinol from pyruvic acid as an intermediate stage in its conversion to 2:3 butylene glycol in presence of alkali and atmospheric oxygen, small amount of acetyl methyl carbinol is oxidized to diacetyl which reacts with peptone in the broth.

Procedure:- Test organism is inoculated into the glucose phosphate medium and incubated at 37^0C for 48 to 72 hours. 0.6 ml of 5% solution of α-naphthol in ethanol and 0.2 ml of 40% koH is added to 1 ml of culture.

Interpretation:- Development of pink colour with in 30 minutes is interpreted as positive test .

ANNEXURE XI

TRIPLE SUGAR IRON AGAR TEST

Triple sugar iron (TSI) agar medium contains 10 parts lactose;10parts sucrose; 1 part glucose and peptone .Phenol red and ferrous sulphate serve as indicators of acidification and H2S production respectively. With a straight inoculation needle, touch the top of a well-isolated colony. Inoculate TSI by first stabbing through the centre of the medium to the bottom of the tube and then streaking the surface of the agar slant. Incubate the tube at 37°C in ambient air for 18 to 24hours.The results are interpreted as follows.

Alkaline slant/No change in butt : Glucose, lactose and sucrose non-utilisers. (K/No change)

Alkaline slant/Acidbut(K/A) : A glucose fermentation only .

Acid slant/acid butt (A/A) : A Glucose , Sucrose and / or Lactose fermenter

A black precipitate in the butt indicates production of ferrous sulphide and H2S gas (H2S+). Bubbles or cracks in the tube indicate the production of CO2 or H2s.

ANNEXURE XII

ARGININE DIHYDROLASE TEST

Preparation of medium

Peptone	5 gm
Meat extract	5 gm
Glucose	0.5 gm
Pyridoxal	5 mg
Bromocresol purple	5 ml (1 in 500 solution)
Cresol red	2.5 ml (1 in 500 solution)
Distilled water	1 litre

Dissolve the solids in water and adjust the pH to 6.0. This is the basal medium and to it is add 1% L – arginine dihydrochloride . No additions done in control tube.

ANNEXURE XIII

Antibiotic sensitivity testing by Kirby Bauer disc diffusion method

- Three to five identical colonies were picked from an overnight grown primary agar plate with a sterile loop and was suspended in 0.5ml of sterile saline. The turbidity was matched with 0.5 Mac Farland turbidity standard.
- A fresh, sterile cotton tipped swab was dipped into this suspension and the excess of inoculums was removed by pressing it against the sides of the tube.
- The surface of Mueller Hinton agar plate was inoculated, by starting at the top and streaking back and forth from edge to edge. The plate was rotated approximately 600 and swabbing repeated three times.

- The antibiotic discs were placed on the plate, so that even contact was ensured using sterile forceps with 15 minutes of inoculation and incubated aerobically at 35^0 C.
- After 18-24 hours of incubation, the diameter of the clear zone around the disc was measured under transmitted light with measuring scale and results interpreted as susceptible, intermediate or resistant as per the CLSI criteria.

Ingredients of: Mueller Hinton agar

Beef infusion	300ml
Casein hydrolysate	17.5 g
Starch	1.5 g
Agar	10 g
Distilled water	1000 ml

ANNEXURE XIV

EDTA solution (0.5 M) for Imipenem – EDTA double disk synergy test

EDTA [extra pure]	186.1 gm
Distilled water	1000 ml

Add the EDTA powder aseptically to the distilled water, adjust the pH to 8. Autoclave the solution at 121^0c for 15 minutes.

KEY TO MASTER CHART

I.P. No.	--	Indoor Patient Number
Yrs	--	Age in years
M	--	Male
F	--	Female
D.O.A.	--	Date of admission
Semi SPL	--	Semi Special ward
RTA	--	Road traffic Accident
CSOM	--	Chronic suppurative ottitis media
Ce	--	Cephotaxime
Ca	--	Cefatazidime
Nt	--	Netilmicin
Cp	--	Ciprofloxacin
G	--	Gentamicin
Tb	--	Tobramycin
Ak	--	Amikacin
I	--	Imipenem
Pi	--	Piperacillin
Pt	--	Piperacillin + tazobactum
M	--	Meropenem
ESBL	--	Extend spectrum beta lactamases
MBL	--	Metallo- beta -lactamases

MASTER CHART

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
1	11302	37 yrs	F	P-3648	2/10/08	Semi spl	Surgery	Burns 40-50%	R	R	R	R	R	R	R	S	R	R	S	N	N
2	201208	27 yrs	M	P-646	3/10/08	OPD	Surgery	Left leg cellulitis	R	S	R	R	R	R	S	S	S	S	S	N	N
3	11638	20 yrs	M	P-695	18/10/08	Semi spl	Surgery	Snake bite	S	S	R	S	S	S	S	S	S	S	S	N	N
4	14552	35 yrs	M	P-532	25/10/08	Special	Surgery	Burns 40-50%	R	R	R	R	S	R	S	S	R	R	S	N	N
5	13331	35 yrs	F	P-733	28/10/08	Special	Surgery	Rt leg cellulitis	S	S	R	S	S	S	S	S	S	S	S	Y	N
6	13336	30 yrs	F	P-731	28/10/08	GENERAL	Surgery	Snake bite	S	S	S	R	S	S	S	S	S	S	S	N	N
7	14425	55 yrs	M	P-740	4/11/08	GENERAL	Surgery	Ulcer	S	S	R	S	R	R	R	S	S	S	S	N	N
8	1088	50 yrs	M	P-736	3/11/08	GENERAL	Surgery	Left leg cellulitis	R	S	R	S	R	R	R	S	S	S	S	N	N
9	17538	70 yrs	M	P-751	8/11/08	GENERAL	Surgery	Rt leg cellulitis	S	S	S	S	S	R	R	S	S	S	S	N	N
10	15392	45 yrs	M	P-760	12/11/08	GENERAL	Surgery	Rt leg cellulitis	S	S	S	R	S	S	S	S	R	S	S	N	N
11	129985	35 yrs	F	P-752	10/11/08	OPD	ENT	Post mastoidectomy	R	R	S	R	S	S	S	S	S	S	S	Y	N
12	15129	28 yrs	M	P-755	7/11/08	Special	Surgery	Head injury	S	R	S	S	S	S	S	S	S	S	S	Y	N
13	15193	45 yrs	M	P-759	12/11/08	GENERAL	Surgery	diabetic foot	S	S	R	R	R	R	R	R	R	R	R	N	Y
14	700	58 yrs	M	P-215	19/01/09	Semi spl	Ortho	Pin tract infection	I	R	S	S	I	I	I	S	S	S	S	N	N
15	17708	61 yrs	F	P-5014	16/01/09	Special	Surgery	diabetic foot	I	I	R	R	R	R	R	S	R	S	S	N	N
16	633	22 yrs	M	P-83	31/01/09	GENERAL	Ortho	RTA	R	R	R	R	R	I	I	S	R	S	S	Y	N
17	17251	75 yrs	M	P-10	5/1/09	GENERAL	Surgery	Cellulitis	S	S	S	S	S	R	R	S	S	S	S	N	N
18	134222	3 yrs	F	p-12	5/1/09	OPD	ENT	Lacrimal sac infection	R	R	R	R	R	R	R	R	R	R	R	N	Y
19	1524	55 yrs	M	P-102	7/2/09	Special	Surgery	Cellulitis	R	R	S	R	S	S	S	S	R	S	S	N	N
20	1034	51 yrs	M	P-440	25/1/09	Special	Surgery	Fournier's gangrene	R	R	S	R	S	S	S	S	R	S	S	N	N
21	1302	30 yrs	M	P-432	6/2/09	Semi spl	Ortho	RTA	R	R	S	I	S	S	R	S	R	S	S	N	N
22	2270	78 yrs	M	P-45	25/02/09	GENERAL	Surgery	Cellulitis	R	R	S	S	S	S	S	S	S	S	S	Y	N

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
23	2266	70 yrs	M	P-648	25/02/09	GENERAL	Surgery	diabetic foot	I	R	S	S	S	S	S	S	S	S	S	N	N
24	1694	55 yrs	M	P-48	11/2/09	GENERAL	Surgery	Ulcer	R	R	R	R	R	R	R	R	R	R	R	N	Y
25	3308	60 yrs	F	P-944	21/03/09	Special	Surgery	Diabetic	I	R	R	R	R	I	R	S	S	S	S	N	N
26	3441	60 yrs	M	P-121	2/4/09	GENERAL	Surgery	Cellulitis	R	R	S	S	S	S	S	S	S	S	S	Y	N
27	2872	30 yrs	M	P-955	21/03.09	Special	Surgery	Burns 80%	I	I	R	R	R	I	R	S	R	S	S	N	N
28	4842	59 yrs	M	P-176	2/5/09	GENERAL	Surgery	diabetic foot	I	R	S	S	S	S	S	S	S	S	S	N	N
29	5130	44 yrs	F	P-195	12/5/09	GENERAL	Surgery	Ulcer	R	R	R	I	S	S	S	S	R	S	S	N	N
30	130519	13 yrs	F	E-133	15/06/09	OPD	ENT	CSOM	R	R	R	S	S	S	S	S	S	S	S	N	N
31	128809	40 yrs	M	ES-156	13/07/09	OPD	ENT	ASOM	I	R	R	R	S	S	S	S	R	R	S	Y	N
32	156320	25 yrs	F	P-410	15/0709	OPD	Surgery	Wound infection	R	R	I	R	S	S	S	S	R	S	S	Y	N
33	9429	65 yrs	M	P-436	27/07/09	GENERAL	Surgery	Cellulitis	R	R	R	R	R	R	R	R	R	R	R	N	Y
34	8938	65 yrs	M	P-2416	26/07/09	Special	Surgery	Abscess	R	R	I	R	I	S	S	S	S	S	S	N	N
35	9252	22 yrs	F	P-122	23/07/09	Special	Surgery	Burns 40%	R	R	R	R	R	R	R	S	R	S	R	N	N
36	5297	28 yrs	F	E-161	10/7/09	GENERAL	Surgery	CSOM	R	R	R	I	S	S	S	S	S	S	S	Y	N
37	11715	1 yrs	M	P-524	29/08/09	GENERAL	Surgery	CSOM	R	R	S	R	S	S	S	S	R	R	S	N	N
38	9428	30 yrs	M	P-513	28/08/09	GENERAL	Surgery	Cellulitis	R	R	R	I	S	S	S	S	R	S	S	Y	N
39	10543	30 yrs	M	P-530	26/08/09	GENERAL	Surgery	Cellulitis	R	R	R	R	R	R	R	S	R	R	I	N	N
40	10643	25 yrs	M	P-505	22/08/09	GENERAL	Surgery	Cellulitis	I	R	I	S	S	S	S	S	S	S	S	Y	N
41	10681	65 yrs	M	P-591	10/8/09	Special	Surgery	Burns	S	R	S	S	S	S	S	S	S	S	S	Y	N
42	9184	30 yrs	F	P-2775	16/07/09	Special	Surgery	Burns	I	R	S	S	S	S	I	S	R	S	S	Y	N
43	197790	16 yrs	F	E-190	31/08/09	GENERAL	ENT	ASOM	I	R	S	S	S	S	S	S	S	S	S	Y	N
44	197769	24 yrs	F	E-3107	2/9/09	OPD	ENT	ASOM	I	R	I	S	S	S	S	S	S	S	S	Y	N
45	12486	45 yrs	F	P-546	O6/09/09	GENERAL	Surgery	Ulcer	I	S	S	S	S	S	S	S	S	S	S	N	N
46	12532	24 yrs	M	P-541	7/9/09	GENERAL	Surgery	Ulcer	I	S	S	S	S	S	S	S	S	S	S	N	N
47	12164	21 yrs	M	P-547	7/9/09	GENERAL	Surgery	Ulcer	S	S	S	I	S	S	S	S	S	S	S	N	N

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
48	205013	20 yrs	F	P-58	8/9/09	OPD	ENT	ASOM	R	R	R	R	R	R	R	S	R	S	R	Y	N
49	184731	24 yrs	M	E-191	10/9/09	GENERAL	ENT	ASOM	I	R	S	S	S	S	S	S	S	S	S	Y	N
50	18237	30 yrs	F	P-3420	16/09/09	Semi spl	Surgery	BURNS	R	S	S	R	R	S	S	S	R	S	S	N	N
51	184823	60 yrs	M	P-3429	16/09/09	GENERAL	Surgery	Cellulitis	R	R	R	S	R	R	S	R	R	R	R	N	Y
52	12913	47 yrs	M	P-3547	27/09/09	GENERAL	Surgery	diabetic foot	S	S	S	I	S	S	S	S	S	S	S	N	N
53	12640	25 yrs	F	P-3581	28/09/09	Special	Surgery	burns	R	R	R	R	R	R	R	S	R	S	R	Y	N
54	171208	45 yrs	F	P-565	29/09/09	GENERAL	Surgery	diabetic foot	S	R	S	S	S	S	S	S	I	S	S	Y	N
55	17351	45 yrs	M	P-4938	19/11/09	Special	Surgery	diabetic foot	I	S	R	S	S	S	S	S	S	S	S	N	N
56	17397	65 yrs	M	P-742	20/11/09	GENERAL	Surgery	diabetic foot	R	R	S	S	S	S	S	S	S	S	S	Y	N
57	17708	50 yrs	F	P-5014	26/11/09	GENERAL	Surgery	Diabetic foot	S	S	S	S	S	S	I	S	S	S	S	N	N
58	275734	6 yrs	F	P-759	28/11/09	OPD	Surgery	Impetigo	R	S	S	S	S	S	S	S	S	S	S	N	N
59	282307	78 yrs	F	p-795	7/12/09	OPD	Surgery	Bed sore	S	S	S	S	S	S	S	S	S	S	S	N	N
60	12532	24 yrs	M	P-779	10/12/09	GENERAL	Surgery	Ulcer	S	S	S	S	S	S	S	S	S	S	S	N	N
61	18015	38 yrs	M	P-774	10/12/09	GENERAL	Surgery	Abscess	S	S	S	S	S	S	S	S	S	S	S	N	N
62	18497	61 yrs	M	MIS-24	12/12/09	GENERAL	Surgery	Cellulitis	I	R	S	S	I	S	S	S	S	S	S	N	N
63	18724	52 yrs	M	P-5857	16/12/09	Semi spl	Ortho	RTA	R	R	R	R	R	R	R	R	R	R	R	Y	N
64	17193	65 yrs	F	P-815	8/11/09	GENERAL	Ortho	RTA	R	R	R	R	R	R	R	R	R	R	R	Y	Y
65	16669	80 yrs	M	P-252	17/12/09	GENERAL	Ortho	RTA	R	R	S	R	R	R	I	S	R	S	R	N	N
66	16455	55 yrs	F	P-817	17/12/09	GENERAL	Surgery	Diabetic foot	R	R	R	R	R	R	R	R	R	R	R	N	N
67	171208	60 yrs	F	P-818	17/12/09	OPD	Surgery	Diabetic foot	R	R	R	R	R	R	R	S	R	R	R	N	N
68	16905	45 yrs	M	P-834	24/12/09	GENERAL	Surgery	Cellulitis	R	R	R	R	R	R	R	R	R	R	R	Y	N
69	2977	35 yrs	M	P-10	4/1/09	GENERAL	Ortho	RTA	R	R	R	R	R	R	R	S	R	R	R	N	N
70	29992	35 yrs	M	P-114	8/2/10	GENERAL	Ortho	RTA	R	R	I	R	S	S	S	S	R	R	S	N	N
71	2253	8o yrs	M	P-434	10/2/10	Semi spl	Surgery	Cellulitis	I	S	S	S	S	S	S	S	S	S	S	N	N
72	36169	32 yrs	F	P-436	10/2/10	Semi spl	Surgery	diabetic foot	R	S	R	S	R	S	R	S	S	S	S	N	N

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
73	2000	22 yrs	M	P-416	9/2/10	GENERAL	Surgery	Cellulitis	S	S	S	I	S	S	R	S	S	S	S	N	N
74	30089	39 yrs	M	ED-15	5/2/10	GENERAL	ENT	ASOM	I	S	S	S	S	S	S	S	S	S	S	N	N
75	2222	55 yrs	M	P-159	1/3/10	GENERAL	Surgery	Cellulitis	I	S	R	R	R	R	R	S	S	S	S	N	N
76	1884	44 yrs	M	P-700	2/3/10	GENERAL	Surgery	Cellulitis	R	S	R	R	R	R	R	S	S	S	S	N	N
77	46206	26 yrs	F	E-3107	2/3/10	OPD	ENT	CSOM	S	S	S	S	S	S	S	S	S	S	S	N	N
78	53916	11 yrs	M	P-164	3/3/10	OPD	ENT	CSOM	I	S	S	S	S	S	S	S	S	S	S	N	N
79	3894	16 yrs	F	P-169	2/3/10	GENERAL	Ortho	osteomyelitis	R	S	S	R	S	S	S	S	S	S	S	N	N
80	46209	40 yrs	F	P-173	2/3/10	OPD	ENT	CSOM	R	R	I	R	S	S	S	S	S	S	S	N	N
81	3942	55 yrs	M	P-252	4/3/10	OPD	ENT	CSOM	S	S	S	S	S	S	S	S	S	S	S	N	N
82	4052	32 yrs	M	P-246	10/3/10	GENERAL	Ortho	RTA	S	S	S	R	S	S	S	S	R	S	S	N	N
83	4092	20 yrs	M	P-263	22/03/10	GENERAL	Ortho	RTA	I	S	I	S	S	S	S	S	S	S	S	N	N
84	2222	55 yrs	M	P-159	1/3/10	GENERAL	Surgery	CELLULITIS	I	S	R	R	R	R	R	S	S	S	S	N	N
85	4098	65 yrs	F	P-269	24/03/10	GENERAL	Surgery	Diabetic foot	S	I	R	S	S	I	S	S	R	S	S	N	N
86	1884	44 yrs	M	P-700	2/3/10	GENERAL	Surgery	CELLUITIS	R	S	R	R	R	R	S	S	S	S	S	N	N
87	46206	26 yrs	F	E-31	2/23/10	OPD	ENT	CSOM	S	S	S	S	S	S	S	S	S	S	S	N	N
88	53916	11 yrs	M	P-164	3/3/10	OPD	ENT	CSOM	I	S	S	S	S	S	S	S	S	S	S	N	N
89	3893	16 yrs	F	P-162	2/3/10	GENERAL	Ortho	osteomyelitis	R	S	S	R	S	S	S	R	S	S	R	N	Y
90	3994	48 yrs	M	P- 240	6/4/2010	GENERAL	Surgery	Diabetic foot	R	R	S	S	S	S	S	S	S	S	S	Y	N
91	11112	46 yrs	M	P-273	22/5/2010	GENERAL	Surgery	Non healing ulcer	I	S	I	S	S	S	I	S	S	I	S	N	N
92	10353	8 yrs	F	P-261	17/5/2010	SPCEAIL	Surgery	BURNS	R	R	R	R	R	S	R	S	R	R	R	N	N
93	10237	30 yrs	M	P-260	17/05/2010	GENERAL	Ortho	RTA	R	R	R	R	R	R	R	S	R	R	R	Y	N
94	10452	40 yrs	F	P-282	25/5/2010	Semi spl	Surgery	BURNS	S	S	S	S	I	R	S	S	S	S	S	N	N
95	10467	23 yrs	M	P-292	27/5/2010	GENERAL	Ortho	RTA	R	R	R	R	R	R	R	S	R	R	R	N	N
96	12267	34 yrs	F	P-324	3/6/2010	GENERAL	ENT	CSOM	R	R	S	R	S	S	S	S	S	S	S	N	N
97	12312	56 yrs	F	P-333	6/6/2010	GENRAL	Surgery	Abscess	R	S	S	S	S	S	S	S	S	S	S	N	N

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
98	11771	50 yrs	F	P-1870	7/6/2010	Semi SPL	Surgery	DIBETIC FOOT	S	R	S	S	S	S	S	S	S	S	S	Y	N
99	11867	30 yrs	F	P-418	12/6/2010	GENERAL	Surgery	Cellulitis	R	R	R	R	R	S	R	S	R	I	R	N	N
100	12327	55 yrs	M	P-1939	14/6/2010	Special	Ortho	RTA	R	R	R	R	I	R	R	R	R	R	R	N	N
101	141621	13 yrs	F	E-70	19/06/2010	GENERAL	ENT	CSOM	R	R	R	R	R	R	R	R	R	R	R	N	Y
102	143989	46 yrs	M	P-430	17/06/10	GENERAL	Ortho	Chr.osteomyelitis	I	R	R	I	S	I	R	R	S	S	R	N	N
103	11563	38 yrs	F	P-434	18/06/10	GENERAL	Ortho	Acetabular #	R	R	R	R	R	R	R	R	R	R	R	N	Y
104	11622	65 yrs	M	P-448	20/6/10	GENERAL	Ortho	FEMUR #	S	S	R	R	R	S	R	S	S	S	S	N	N
105	13523	30 yrs	M	P-469	28/06/10	GENRAL	Ortho	RTA	S	S	R	S	R	R	R	S	S	S	S	N	N
106	155211	42 yrs	M	E-75	29/06/10	OPD	ENT	CSOM	S	S	S	S	S	S	S	S	S	S	S	N	N
107	14136	37 yrs	M	P-460	29/06/10	GENRAL	Ortho	# Tibia	R	S	S	R	R	S	S	S	R	S	R	N	N
108	14489	25 yrs	M	P-473	2/7/2010	GENRAL	Ortho	# Tibia	R	R	R	R	R	S	S	R	R	S	R	N	N
109	14267	34 yrs	M	P-486	16/7/2010	GENERAL	ENT	CSOM	I	S	I	S	S	S	I	S	S	I	S	N	N
110	14288	60 yrs	M	P-492	16/7/2010	GENERAL	Surgery	CELLULITIS	R	S	S	S	R	S	S	S	S	S	S	N	N
111	165221	33 yrs	M	E-86	18/7/2010	OPD	ENT	CSOM	R	S	R	R	R	S	S	S	S	S	S	N	N
112	14328	50 yrs	M	P- 496	19/7/2010	GENERAL	Surgery	CELLULITIS	S	S	S	S	S	S	R	S	R	S	S	N	N
113	14338	11 yrs	M	P- 502	20/7/2010	GENERAL	Surgery	Snake bite	S	S	S	S	S	S	S	S	S	S	S	N	N
114	14372	58 yrs	M	P-504	20/07/2010	GENRAL	Surgery	Cellulitis	R	R	R	R	R	I	R	R	R	R	R	N	Y
115	14804	8 yrs	F	E-79	29/7/2010	GENRAL	Ortho	RTA	S	S	S	S	S	S	S	S	S	S	S	N	N
116	14904	80 yrs	M	P-524	2/8/2010	Semi spl	Surgery	Diabetic foot	R	R	R	I	S	S	S	S	R	S	S	N	N
117	15984	36 yrs	F	P-538	18/8/2010	GENERAL	Ortho	# FEMUR	S	S	S	R	S	R	S	S	S	S	S	N	N
118	14372	58 yrs	M	P-547	24/8/2010	GENERAL	Surgery	Cellulitis	R	R	R	R	R	I	R	S	R	S	R	N	N
119	14371	25 yrs	F	P-647	3/8/2010	Special	Surgery	BURNS	S	S	R	S	R	R	R	S	S	S	S	N	N
120	16734	42 yrs	M	P-680	31/08/2010	GENERAL	Surgery	Cellulitis	S	S	S	S	S	S	S	S	S	S	S	N	N
121	16789	65 yrs	M	P-705	3/9/2010	GENERAL	Surgery	CELLULITIS	R	R	S	S	R	I	R	S	S	S	S	Y	N
122	16814	24 yrs	F	P-707	5/9/2010	Special	Surgery	BURNS	R	S	S	S	S	R	S	S	R	S	S	N	N

Sl No.	I.P. No.	Age	Sex	lab No.	DOA	Category	Ward	Diagnosis	ce	Ca	Nt	Cp	G	Tb	Ak	I	Pi	PT	Mr	Esbl	MBL
123	16875	67 yrs	M	P-715	8/9/2010	Semi spl	Surgery	Diabetic foot	S	S	R	R	S	S	S	S	R	S	S	N	N
124	16902	56 yrs	M	P-718	12/9/2010	GENERAL	Surgery	Diabetic foot	S	R	S	R	S	R	S	S	R	S	S	N	N
125	16949	36 yrs	M	P-723	14/0010	GENERAL	ENT	CSOM	R	R	S	S	S	S	R	S	S	S	S	Y	N
126	17103	42 yrs	M	P- 749	16/09/2010	GENERAL	Ortho	RTA	S	S	R	R	R	S	R	S	S	R	S	N	N