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# Pseudomonas Aeruginosa and Newer β-Lactamases: An Emerging Resistance Threat

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## 1. Introduction

The discovery of penicillin, the Magic bullet in 1928 and its clinical use in 1941 led the people to think that mankind has won the war against microbes. With a short span of seventy years, antimicrobial discovery from Penicillin to Tigecycline, mankind is facing the problem with some hospital strains resistant to almost all antimicrobials, and is busy in writing the obituary for antimicrobials.

Infact, the rising trend of developing resistance to multiple antibiotics in microbes, leading to therapeutic failure is a serious problem of global magnitude. P.aeruginosa, Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin resistant Enterococci(VRE), Glycopeptide Intermediate Staphylococcus aureus (GISA), Glycopeptide Resistant Staphylococcus aureus (GRSA), Acinetobacter baumani, Stenotrophomonas maltophila etc. need special attention as they are commonly isolated from Health Care Associated Infections(HAI) and belong to Multidrug resistant Organism (MDRO) i.e. they are resistant to one or more classes of antibiotics (Harrison & Lederberg, 1998). P. aeruginosa is responsible for 10-15% of nosocomial infections worldwide. The β-lactam group of antibiotics which include Penicillins, Cephalosporins, Monobactams and Carbapenems are mainly used to treat infections caused by Gram negative bacteria. The widespread use of antibiotics put tremendous selective pressure on bacteria which develop new mechanisms to escape the lethal action of the antibiotics. These infections are difficult to treat because of emergence of newer β-lactamases such as Extended Spectum β-lactamases (ESBL), AmpCβ-lactamases and Carbapenemases. The β-lactamases inactivate β-lactam antibiotics by cleaving the structural β-lactam ring. Failure to detect these enzymes producing strains has contributed to their uncontrolled spread in Health Care setup and therapeutic failure.

Major mechanisms causing resistance to the β-lactam antibiotics in P.aeruginosa are the production of β-lactamases, reduced outer membrane permeability and altered affinity of targetPenicillin binding proteins. (Washington et al, 2006; Pitt, 1990).

### 1.1 Classification of β-lactamases

β-lactamase can be classified according to Functional or Bush Jacoby Mederious classification ( Bush et al, 1995), into group 1, 2a, 2be, 2br, 2c, 2d, 2e, 2f and according to molecular or Ambler

classification (Ambler, 1980) into Ambler class A,B,C and D. Detection of β-lactamase production has been achieved in the past by measuring the production of penicilloic acid, which is produced when benzyl penicillin is hydrolysed. The acid production can be detected by acidometric method, iodometric method and chromogenic cephalosporin (nitrocephin) method (Miles & Amyes, 2008). The first plasmid mediated β-lactamase was described in early 1960. The TEM1 enzyme was named after the patient Temoniera from whom it was originally found in isolated strains of E.coli, (Medeiros, 1984) whereas β-lactamase SHV-1 (Sulphydryl variable) is chromosomally encoded in most isolates of Klebsiella pneumoniae but is usually plsmid mediated in E.coli (Tz-ouvelekis, 1999).

### 1.1.1 Extended spectrum β-lactamases (ESBL)

ESBLs were first reported in 1983 in Klebsiella pneumoniae from Germany. Typically ESBLs are mutant plasmid mediated β-lactamases derived from older broad-spectrum β-lactamases. The mutations alter the amino acid configuration around active site of β-lactamases (Thomson, 2001). The first ESBL to be described in 1983 was actually TEM3 ( Soughakoff et al, 1980) and now over 130 additional TEMs have been isolated. ESBLs have an extended substrate profile that cause hydrolysis of cephalosporins, penicillins and aztreonam and are inhibited by β-lactamase inhibitors, such as clavulanate, tazobactam and sulbactam. ESBLs are commonly produced by Klebsiella species and Escherichia coli; but also occur in other Gram negative bacteria, including Enterobacter, Salmonella, Proteus, Serratia marcescens, Pseudomonas aeruginosa, Burkholderia, Acinetobacter species, etc.

### 1.1.2 AmpC β-lactamases

Molecular class C or AmpC primarily hydrolyses cephems (cephalosporins and cephamycins) but also hydrolyze penicillins and aztreonam. These enzymes are resistant to the currently available β-lactamase inhibitors such as clavulanate, tazobactam and sulbactam ( Philippon et al, 2002). With rare exceptions, the hydrolysis of cephamycins, such as cefotetan and cefoxitin, is a property that can help to distinguish AmpCs from ESBLs. Genes encoding inducible chromosomal AmpC β-lactamases are part of the genomes of many Gram negative bacteria specially P.aeruginosa. High level production of AmpC may cause resistance to the first, second and third-generation cephalosporins and cephamycins, penicillins and β-lactamase inhibitor combination. Higher level AmpC production may occur as a consequence of mutation or when the organism is exposed to an inducing agent. Cephamycins (e.g. cefoxitin and cefotetan ), ampicillin, and carbapenem are good inducer ( Moland et al, 2008). AmpC β-lactamases producing organisms are on rise and leads to therapeutic failure if 3rd Generation cephalosporins are given empirically or not tested in the laboratory for AmpC β-lactamases production ( Basak et al, 2009). The chromosomally mediated AmpC β-lactamases are only inducible.

### 1.1.3 Carbapenemases

These include β-lactamases which cause carbapenem hydrolysis, with elevated carbapenem MICs and they belonged to molecular classes A, B and D. Molecular classes A, C and D include the β-lactamases with serine at their active site, whereas class B β-lactamases are all metalloenzymes with an active site zinc ( Queenan & Bush, 2007).

Metallobetalactamases – They belong to molecular class B β-lactamases, and have 3 characteristics –

1. Hydrolyze carbapenems
2. Resistant to clinically used β-lactamase inhibitors and
3. Inhibited by EDTA, a metal ion chelator.

Other MBL inhibitors used are 2-mercaptoethanol, sodium mercapto acetic acid (SMA), 2-mercaptopropionic acid, copper chloride and ferric chloride (Arkawa et al, 2000). MBLs have a broad substrate spectrum and in addition to carbapenems, they can hydrolyze cephalosporin and penicillins but cannot hydrolyse aztreonam. Interestingly, not all of the MBLs readily hydrolyze nitrocefin. The first MBL detected were chromosomally encoded and was detected in Bacillus cereus ( Lim et al, 1988). Since then there has been a dramatic increase in detection and spread of acquired or transferable families of these MBLs. There are 5 major families of acquired MBLs (IMP, VIM, SPM, GIM and SIM) ( Toleman et al, 2007). In 1990, IMP-1, the 1st MBL encoded on plasmid, was discovered in Japan ( Watanabe et al, 1991). The MBLs are located on integrons and are incorporated as gene cassettes.When these integrons become associated with plasmids or transposons, transfer between bacteria is facilitated.

**Classification of MBLS**

MBLS are classified into 3 subclasses—B1,B2 and B3. Subclass B1 and B3 are divided by aminoacid homology, bind 2 zinc atoms for optimal hydrolysis and have broad hydrolysis spectrum. Subclass B2 are inhibited when a second zinc atom is bound and preferentially hydrolyse carbapenem ( Free et al, 2005).

Molecular class A carbapenemase – Class A serine carbapenemases belong to functional group 2f include chromosomally encoded NMC( not metalloenzyme carbapenemase), IMI (Imipenem hydrolyzing β-lactamase) and SME(Serratia marscenscens enzyme) and plasmid mediated KPC (Klebsiella pneumoniae carbapenemase) and GES/IBC(integron borne cephalosporinase) ( Queenan & Bush, 2007). All have the ability to hydrolyse carbapenems, cephalosporins, penicillins and aztreonem and all are inhibited by clavulanate and tazobactam. The chromosomal class A carbapenemase are infrequently found and can be induced by imipenem and cefoxitin. The KPC ( Klebsiella pneumoniae carbapenemase) producing strains are found in Klebsiella pneumoniae, Enterobacter species, Salmonella species and other Enterobacteriaceae ( Hossain et al, 2004; Miriagou et al, 2003).

Class D Serine carbapenemases: The OXA (Oxacillin hydrolysing) β-lactamase with carbapenemase activity was detected by Patow et al in 1993 and the enzyme was purified from Acinetobacter baumani (Queenan & Bush, 2007). They have been also found in Enterobacteriaceae and P.aeruginosa and were described as penicillinase capable of hydrolyzing oxacillin and cloxacillin ( Bush & Sykes, 1987; Naas & Nordmann, 1999). They were poorly inhibited by clavulanic acid and EDTA and were designated as ARI-1 (Acinetobacter Resistant to Imipenem) and reside on large plasmid. The OXA carbopenemases have hydrolytic activity against penicillins, some cephalosporins and imipenem. The widespread use of reserved antibiotics such as β-lactam /β-lactamases inhibitor combinations, monobactams and carbapenem has caused persistent exposure of bacterial strains to a multitude of β-lactam leading to overproduction of β-lactamases (Goossens et al, 2004; Manoharan et al, 2010; Lee et al, 2003). Consequently the emergence of

carbapenem resistance is a world-wide public health concern since carbabapenems are used as last resort to treat serious infections caused by ESBL producing organisms. Approximately 40% strains of P.aeruginosa are resistant to anti-pseudomonal drugs including carbapenems. Therefore, early detection of of ESBL, AmpC β-lactamase & MBL producing P. aeruginosa strains is of crucial importance for prevention of their inter and intra hospital dissemination.

### 1.2 Aims and objectives

The present study was undertaken with the aim to study Pseudomonas aeruginosa with special reference to β-lactamase production isolated in the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha ( M. S.), India.

#### 1.2.1 To fulfill the aim the following objectives were taken

- To study the prevalence of Extended Spectrum β-lactamases (ESBL), Amp C β-lactamases, Metallobetalactamases (MBL) producing Pseudomonas aeruginosa strains, isolated from different clinical samples of patients attending the Hospital
- To study the antibiotic susceptibility profile of Extended Spectrum β-lactamases (ESBL), Amp C β-lactamases and Metallobetalactamases (MBL) producing Pseudomonas aeruginosa strains isolated.

## 2. Material and methods

The study was conducted from 1st September 2008 to August 2010 (2 year period). A total number of 250 P.aeruginosa strains were isolated from different clinical samples e.g. urine, pus and wound swab, blood, catheter tips, endotracheal tube secretions, different body fluids etc. received from indoor as well as outdoor patients departments (IPD &OPD) of our hospital, which is a tertiary care hospital in a rural set-up. P.aeruginosa strains were characterized according to conventional identification tests. P.aeruginosa ATCC 27853 were used as positive control for all conventional tests. All antibiotic disks and culture media used in the study were procured from HiMedia laboratories Pvt. Limited, India. Ethylene Diamine Tetraacetic acid (EDTA) and 3-amino phenylboronic acid (APB) were procured from Sigma-Alderich.

### 2.1 Antibiotic susceptibility testing

All 250 P.aeruginosa strains were subjected to antibiotic susceptibility testing to different antimicrobial agents using Mueller-Hinton agar plates by Kirby-Bauer disk diffusion method according to CLSI guidelines (CLSI Document M2-A9, 2006). Using sterile swab, lawn culture of the test strain (turbidity adjusted to 0.5 McFarland standard) was made on Mueller Hinton Agar plate. With all aseptic precaution, the antibiotic disks were put on that inoculated plate. Six antibiotic disks were put on a 90mm diameter plate. The antibiotic sensitivity tests were put for aminoglycosides such as amikacin (Ak-30µg), netilimicin (Nt-30µg); cephalosporin such as ceftazidime(Ca-30µg), cefepime(Cpm-30µg); fluoroquinolones i.e. ciprofloxacin(Cf-5µg); monobactams i.e. aztreonam (Ao-30 µg); carbapenems such as imipenem(I-10µg), meropenem(Mr-10µg); piperacillin/tazobactam (Pt-100/10µg ), ceftazidime/clavulanic acid (Cac-30µg /10 µg) and polymyxin B (Pb-300µg) etc. (Fig. 1)

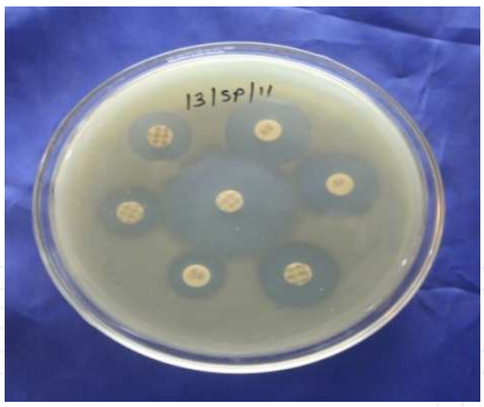


Fig. 1. Antibiotic susceptibility test.

**Detection of newer β-lactamases**

Though several methods both phenotypic and genotypic have been described for detection of newer β-lactamases, we restricted our study only to phenotypic methods. There is no CLSI guideline given for detection of ESBL, AmpC β-lactamases ans MBL producing P.aeruginosa.

### 2.1.1 Detection of extended spectrum β-lactamases (ESBL)

Screening test: ESBL production was detected by reduced susceptibility to Ceftazidime, Cefotaxime.

Confirmatory tests: As per Clinical and Laboratory Standard Institute (CLSI) guidelines for Enterobacteriaceae (Waynepa CLSI, 2008; Storenburg, 2003), we used the same combined disk method as confirmatory test for Pseudomonas aeruginosa also, as the principle remains the same.

1. **Combined Disk Method** (Carter et al, 2000)
   Broth cultures of test strains were adjusted to McFarland 0.5 standard and used to inoculate Mueller Hinton agar plates with a sterile swab. Commercialized disks containing ceftazidime (Ca) 30 µg and ceftazidime plus clavulanate (Cac) 30µg plus 10µg respectively were used in this method. An increase in diameter of ≥5mm with ceftazidime plus clavulanate (Cac) disk as compared to ceftazidime(Ca) disk alone was considered positive for ESBL detection. All 250 P. aeruginosa strains were also tested using piperacillin (Pc)100 µg & piperacillin-tazobactam (Pt) 100 µg plus 10 µg respectively in combination.
2. **E test ESBL strip** (Washington et al, 2006)
   The E-test ESBL confirmatory test strips are based on the CLSI dilution method. The strip has concentration gradients of ceftazidime (TZ) 0.5 to 32 µg/ml on one half and ceftazidime 0.064 to 4 µg/ml plus 4 µg/ml clavulanic acid (TZL) on another half . The ESBL E-test was performed and interpreted using test strains and Quality Control strains according to the manufacturerer's instructions. In this method lawn culture of test strain was done on a Mueller Hinton agar plate. With a sterile forceps the ESBL E-test strip was placed onto the inoculated plate. After overnight incubation at 37°C, the zone of inhibition was read from two halves of the strip. MIC ratio of ceftazidime/ceftazidime clavulanic acid (TZ/TZL) ≥ 8 or deformation of ellipse or phantom zone present was considered as positive for ESBL production.

### 2.1.2 Detection of Amp C β-lactamases

For detection of AmpC class of β-lactamases, no satisfactory technique has been established till date as per CLSI guidelines. Induction of C β-lactamase synthesis was Amp based on the disc approximation assay using several inducer substrate combinations.

Screening test: Several inducer/substrate combinations disks like Cefoxitin/Piperacillin, Imipenem/Ceftazidime,Imipenem/Cefotaxime, Imipenem/Cefoxitin, Imipenem/Piperacillin -Tazobactum were used as described by Dunne and Hardin et al. Imipenem and cefoxitin were used as inducers of AmpC β-lactamases (Dunne & Hardin, 2005).

**Interpretation:** Strains were considered inducible if a positive test was obtained with any of the inducer/substrate combinations. A test was considered positive if the zone of inhibition was reduced by ≥2 mm on the induced side of the substrate disc or even blunting of substrate zone of inhibition adjacent to inducer disc. Also, if the zone of inhibition produced by ceftazidime/ceftazidime-clavulanic acid (Cac) disk was ≥2mm less than the zone produced by a ceftazidime (Ca) disk, the strain was considered to be inducible Amp C positive. Similarly, same criteria was used for piperacillin & piperacillin/tazobactam (Pc/Pt) disks.

Confirmatory test: Disk potetiation(DP) test and Double disk synergy test (DDST) using 3-aminophenylboronic acid (APB) (100mg/ml dissolved in DMSO) (Yagi et al, 2005). An increase in zone size of ≥5mm around the Ceftazidime- APB disk compared to ceftazidime only disk was recorded as a positive result for disk potentiation test. In DDST, the presence of change in the shape of growth inhibitory zone around ceftazidime or cefotaxime disk through the interaction with the 3- Aminophenyl boronic acid containing disk was interpreted as positive for AmpC production.

### 2.1.3 Detection of metallobetalactamases (MBL)

All imipenem resistant strains were screened for Carbapenemase activity by Classical Hodge Test and Modified Hodge Test (MHT) (Lee et al, 2001a; 2003b). Pseudomonas aeruginosa strains which were positive by Classsical Hodge Test(IHT) and Modified Hodge Test (MHT) were tested for metallobetalactamase (MBL) production by Imipenem/EDTA double disk synergy test (Lee et al, 2001)and disk potentiation test or imipenem-EDTA combined disk test (Yong et al, 2002) using Di-potassium EDTA (10µl of 0.5 M).

**Imipenem-EDTA double disk synergy test (DDST) ( Lee et al, 2001)**

The IMP-EDTA double disk synergy test was performed for detection of metallobetalactamases. Test strains i.e. Pseudomonas aeruginosa (turbidity adjusted to 0.5 McFarland standard ) were inoculated on to Mueller Hinton agar plate. After drying, a 10µg Imipenem disk and a blank sterile filter paper disk (6mm in diameter, Whartman filter paper no.2) were placed 10mm apart from edge to edge. 10 µl of 50mM zinc sulfate solution was added to the 10 µg imipenem disk. Then, 10µl of 0.5 M EDTA(Sigma, USA) solution was applied to the blank filter paper disk. As disodium-EDTA is difficult to be solubilised in sterile water, we had used dipotassium-EDTA which is easily soluble in sterile water. Enhancement of the zone of inhibition towards the EDTA disk was interpreted as a positive result.

**Disk Potentiation test or Imipenem-EDTA combined disk test (Young et al, 2002)**

The test was performed for detection of metallobetalactamases. Test strains (turbidity adjusted to 0.5 McFarland standard ) were inoculated on to Mueller Hinton agar plate. Two

imipenem disk (10 µg) were placed on the plate wide apart and 10 µl of 50mM zinc sulphate solution was added to each of the imipenem disks. Then 10µl of 0.5 M EDTA solution was added to one of the disk to obtain the desired concentration. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16-18 hours of incubation at 35°C. If the increase in inhibition zone with the Imipenem and EDTA disk was ≥7 mm than the imipenem disk alone, it was considered as MBL positive.

The MBL producing strains were further confirmed by using MBL – E test strip (AB bioMerieux) (Walsh et al, 2002).

MIC ratio of Imipenem /Imipenem-EDTA (IP/IPI) of ≥8 or deformations of ellipse or phantom zone indicate MBL production by MBL E-test.

### 2.2 Observations and results

Maximum 204(81.6%) P.aeruginosa strains were isolated from Indoor Patient Department (IPD). No newer β-lactamase producing strains were isolated from patients attending Out Patient Department

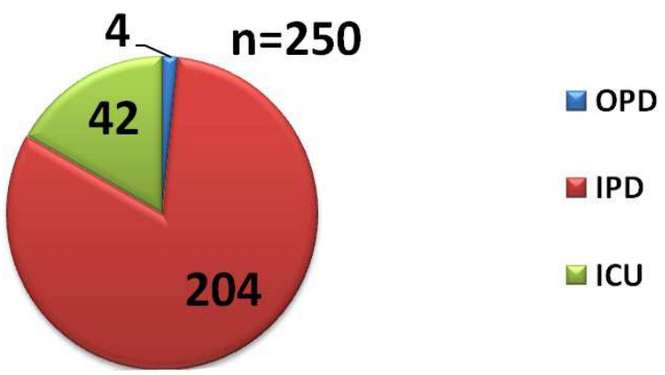



Fig. 2. Isolation of Pseudomonas aeruginosa strains from OPD, IPD and ICU patients.

Fig. 3 shows 165 (66%) P.aeruginosa strains were ESBL, AmpC β-lactamases and MBL producers.

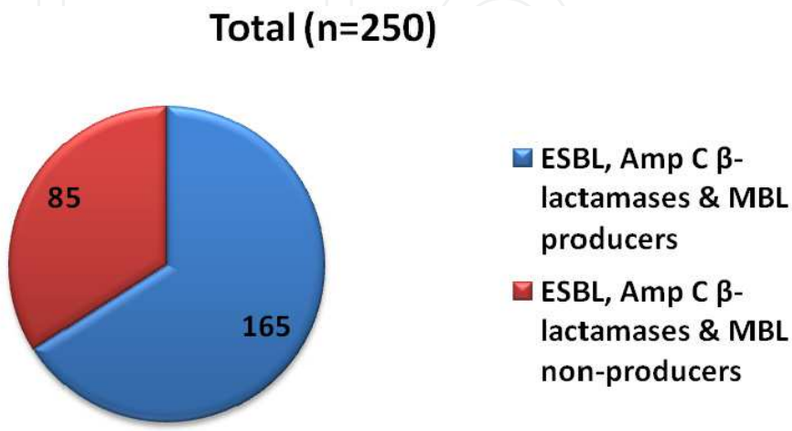



Fig. 3. Prevalence of newer β-lactamases producing P. aeruginosa strains (n=250).

| P.aeruginosa | ESBL | | AmpC | | MBL | |
|---|---|---|---|---|---|---|
| | No. | Percentage | No. | Percentage | No. | Percentage |
| n=250 | 100 | 40 | 105 | 42 | 28 | 11.2 |

Table 1. Prevalence of ESBL, AmpC β-lactamase and MBL producing P. aeruginosa (n=250)

Amongst the 100 ESBL (Fig. 4) and 105 Amp C β-lactamase producers (Fig. 5 & 6), 68 (27.2%) P. aeruginosa strains had produced both ESBL as well as AmpC β-lactamases. 28 (11.2%) P. aeruginosa strains were Metallobetalactamase (MBL) producers.

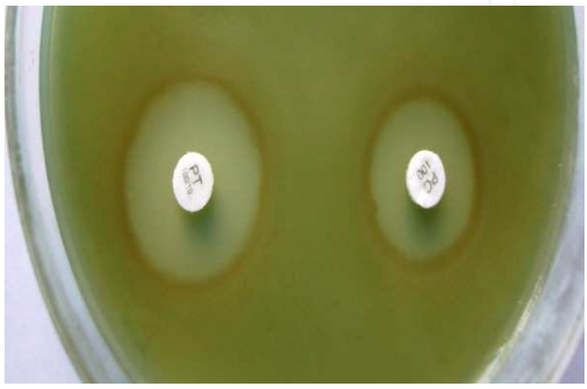


Fig. 4. Detection of ESBL( Combined disk method )

**Detection of AmpC β-lactamases (Figure 5&6)**

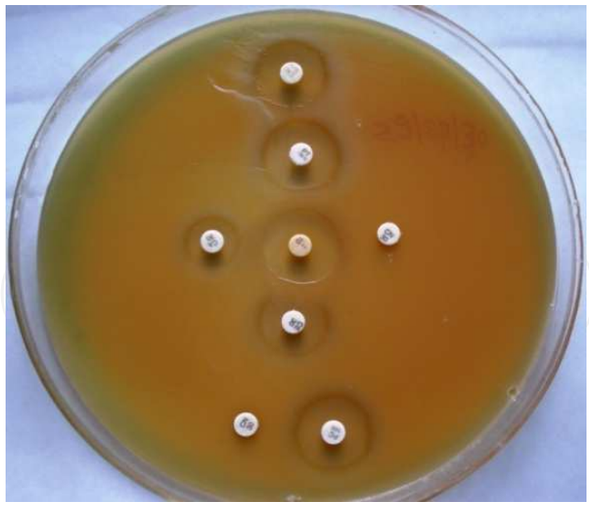


Fig. 5. Inducer-substrate combination disk test.

Fig. 7 shows amongst 100 ESBL and 105 AmpC β-lactamase producers 68 (41.2%) strains had produced both ESBL as well as AmpC β-lactamases. There was no strain which produced all the 3 types of β-lactamases. Similarly, no strain produced ESBL or AmpC β-lactamase along with MBL.


Fig. 6. DDST & DP test using 3-Aminophenyl-boronic acid (3-APB).

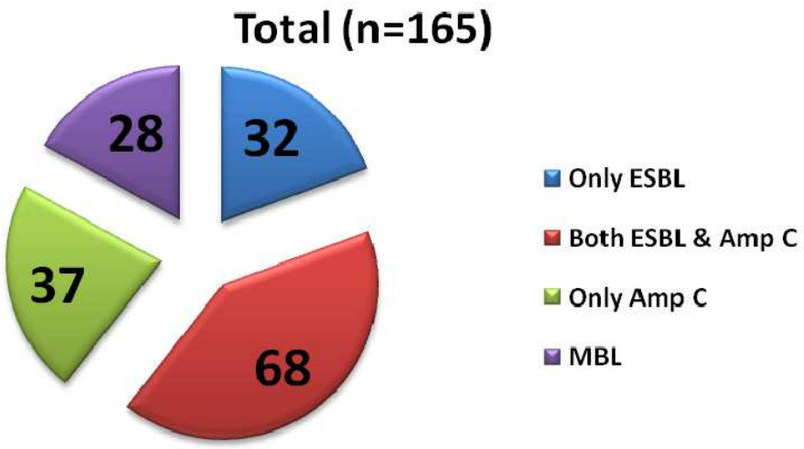



Fig. 7. Incidence of different newer β-lactamases producing P.aeruginosa strains(n=165)

**Table 2** shows that out of 250 P. aeruginosa strains studied, 31 (12.4 %)were imipenem resistant and 28 (11.2 %) were metallobetalactamase (MBL) producers. 31 imipenem resistant strains were screened for carbapenem hydrolysis by Classical Hodge test (HT) & modified Hodge test (MHT). Amongst these 31 imipenem resistant P. aeruginosa strains, 28 (90.3%) were positive for Classical Hodge test (HT) & modified Hodge test (MHT) for carbapenem hydrolysis and these 28 strains were also positive for metallobetalactamase (MBL) production by Double disk synergy test (DDST) and disk potentiation test (DP).

| Imipenem resistant P. aeruginosa | Screening test for carbapenem hydrolysis | | Confirmatory test for MBL | | |
|---|---|---|---|---|---|
| | Classical Hodge test | Modified Hodge test | Double disk synergy test | Disk potentiation test | MBL E-test |
| n = 31 | 28 | 28 | 28 | 28 | 28 |

Table 2. Prevalence of MBL producing P. aeruginosa (n =250)

These 28 strains were also confirmed for metallobetalactamase (MBL) production by using MBL E-test strip (AB bioMerieux). MBL E-test (Fig. 9) shows MIC ratio of imipenem IP/ imipenem-EDTA IPI for test strain P. aeruginosa as 16/1 i.e. 16 and MBL E-test positive. The phantom zone shown in Fig. 9 is another criteria for MBL E-test positivity. MBL E-tests done for those 28 P. aeruginosa strains showed that the MIC ratio of imipenem / imipenem-EDTA i.e. IP/IPI were > 8 such as 16/1 for 9 strains, 24/1.5 for 3 strains, 32/1 for 5 strains, 48/1 for 8 strains, 64/1 for 2 strains and 128/1 for 1 strain.

**Confirmation of MBL by E-test : Figure 8 & 9**

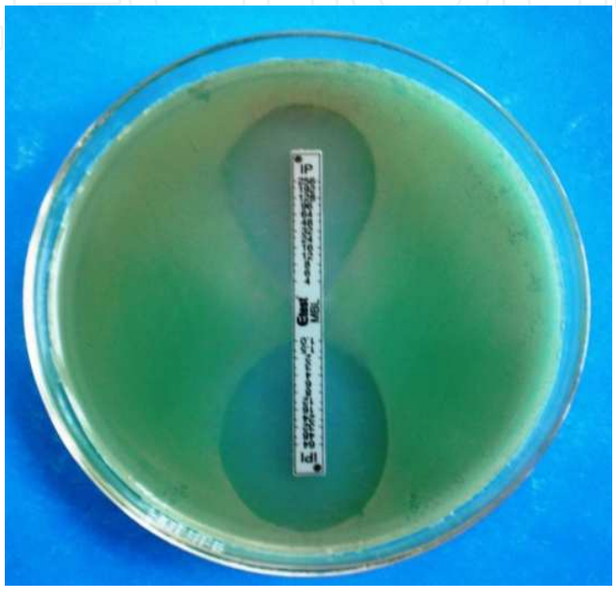



Fig. 8. Quality control: P.aeruginosa ATCC 27853 (MBL E test negative).

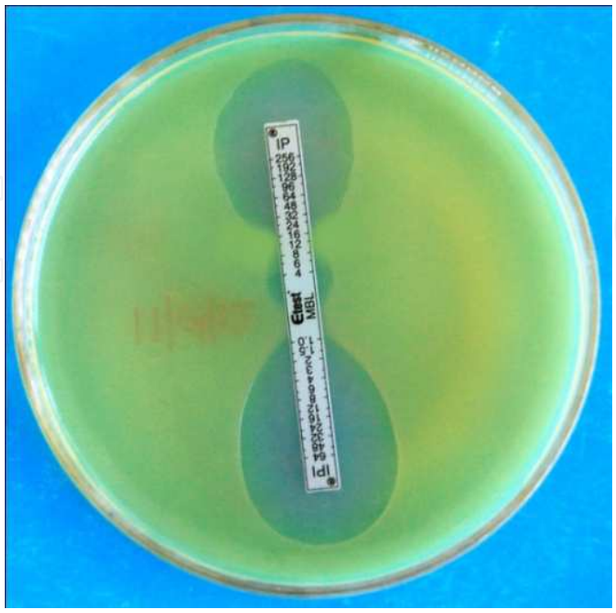



Fig. 9. MBL E-test (positive).

Fig. 10 shows from ICU 6 (14.3%) strains produced both ESBL and AmpC β-lactamases whereas 9(21.4%) strains produced MBL.

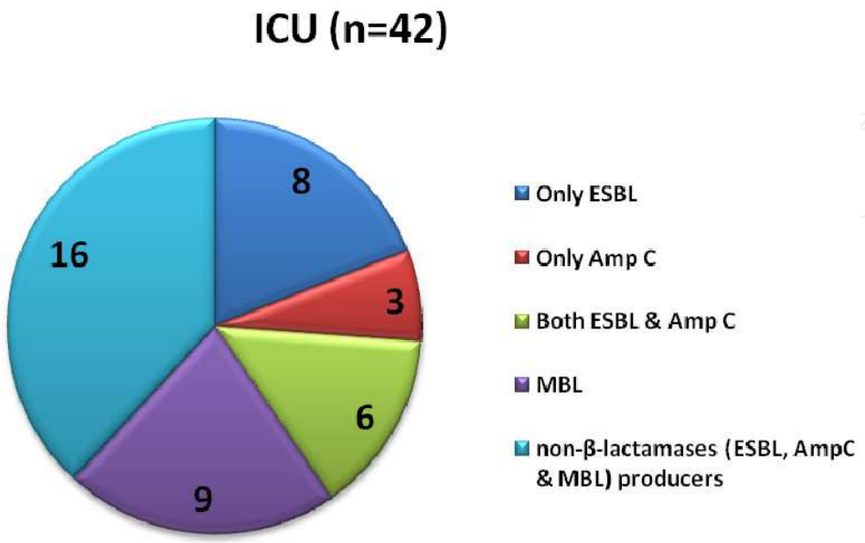
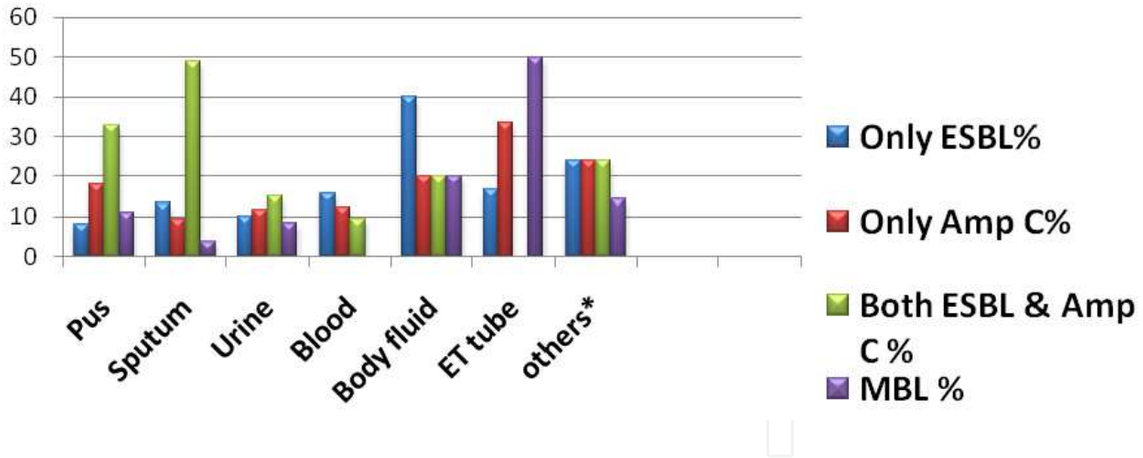



Fig. 10. Isolation of different β-lactamases producing P. aeruginosa strains from ICU.




*others include throat swab, vaginal swab, ear swab, bronchial wash, tip of catheter & drain fluid.

Fig. 11. Isolation of β-lactamases producing P.aeruginosa strains from different clinical specimens.

In the present study, maximum P.aeruginosa strains isolated from pus and wound swab, 73 (29.2%) followed by 60(24%) from urine. The fig. 11 shows 26(49%) P.aeruginosa strains isolated from sputum sample were both ESBL and AmpC β-lactamase producer. 50% P.aeruginosa strains isolated from endotracheal tube secretions were MBL producers. Though no MBL producing strains were isolated from blood culture.

Fig. 12 shows maximum 36.2% strains isolated from Medicine ward produced both ESBL and AmpC β-lactamase. From surgery ward maximum 10(35.7%) strains were MBL producers. No MBL producing P.aeruginosa strains were isolated from Neonatal Intensive Care Unit(NICU) and 3(20%) strains were only ESBL producers.

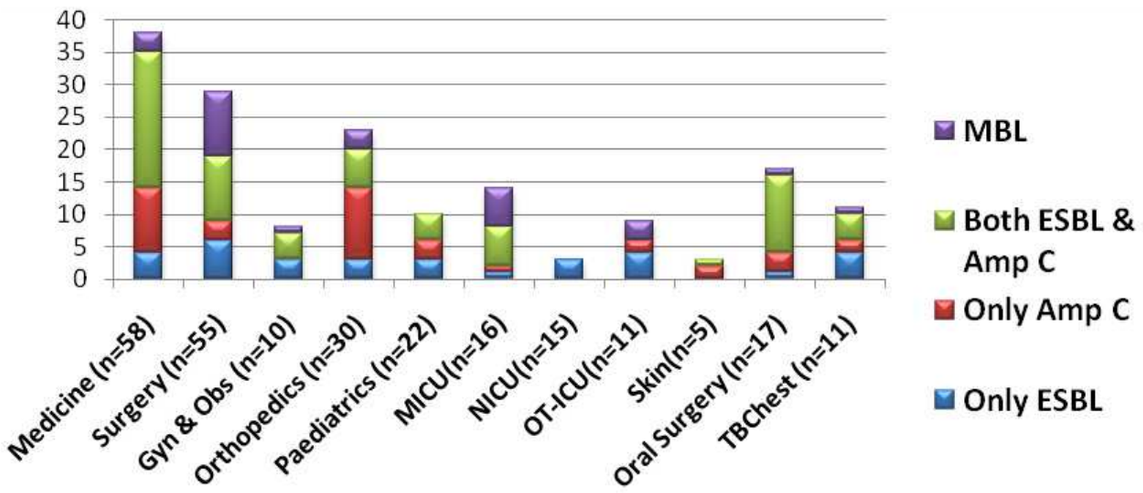



Fig. 12. Isolation of β-lactamases producing P. aeruginosa strains from different clinical specialities

Fig. 13 shows that P.aeruginosa strains showed a high degree of resistance to cefepime (90.4%), cefoxitine(91.6%) and ceftazidime(67.2%). However effective antimicrobial agents were found to be polymyxin B (100%), Imipenem(87.6%) and piperacillin-tazobactum (86%) sensitive.

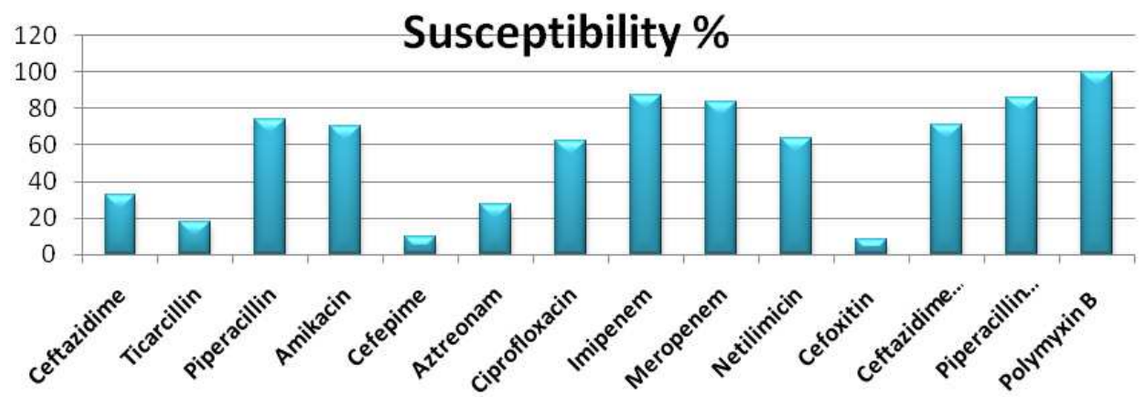



Fig. 13. Antibiotic susceptibility profile of P. aeruginosa strains (n=250)

Fig. 14 shows most effective antimicrobial agent against ESBL andAmpC β-lactamase producing P.aeruginosa strains were Imipenem(100%) and Polymyxin-B(100%). However, sensitivity of ESBL and AmpC β-lactamase producers to piperacillin-tazobactam were 100% and 82.8% respectively.

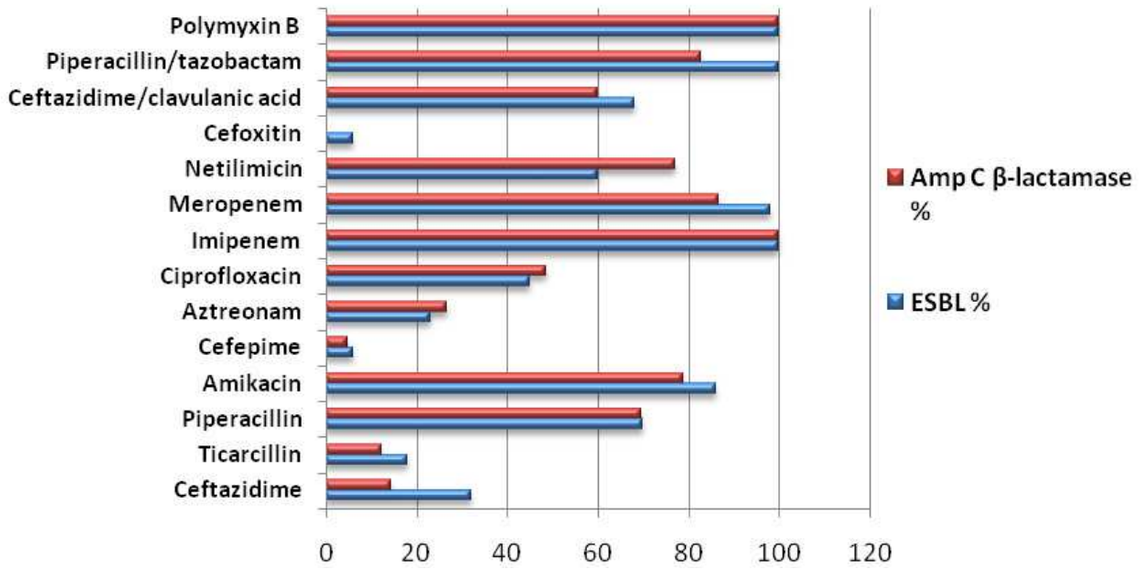



Fig. 14. Antibiotic susceptibility profile of ESBL & Amp C β-lactamases producing P.aeruginosa strains

Fig.15 shows all(100%) MBL positive isolates were sensitive to polymyxin-B. 1(3.7%) each MBL producing strain was sensitive to Amikacin and Netilmicin respectively. But no MBL producing strain was susceptible to Aztreonam.

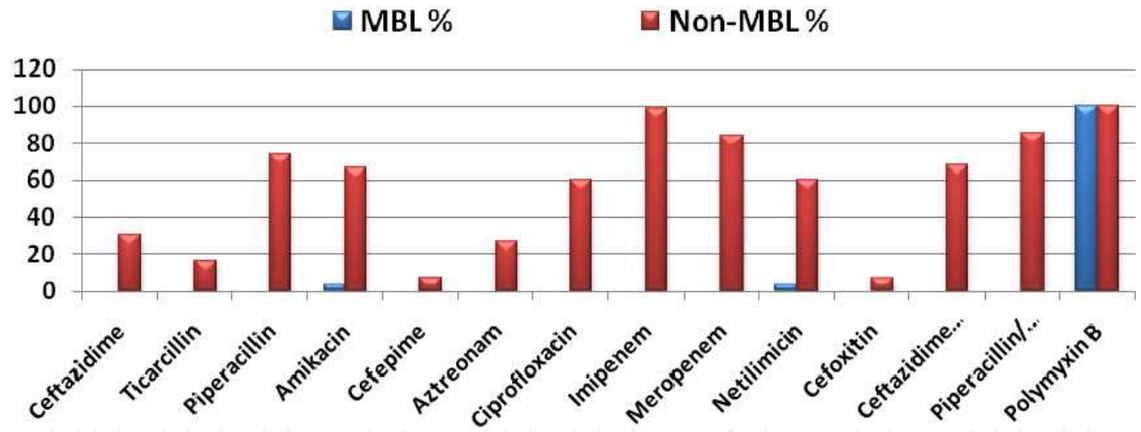



Fig. 15. Antibiotic susceptibility profile of MBL & non-MBL P. aeruginosa strains

### 2.3 Discussion

Pseudomonas aeruginosa is one of the most important microorganisms which causes problems clinically as a result of its high resistance to antimicrobial agents and is therfore a particularly dangerous & dreaded bug. Despite the discovery of ESBL, Amp C β-lactamases and MBL at least a decade ago, there remains a low level of awareness of their importance and many clinical laboratories have problems in detecting ESBL& Amp C β-lactamases. Failure to detect these enzymes has contributed to their uncontrolled spread and commonly to therapeutic failures.

Detection problems arise especially with organisms that produce an inducible Amp C β-lactamases, as clavulanate can induce high level production of Amp C , which may obscure recognition of ESBLs ( Moland et al, 2008). According to Clinical & Laboratory Standards (CLSI) interpretive definations, ESBLs do not always increase MICs to levels characterized as resistant ( Livermore, 2002). Not only that ESBL producing organisms may give false sensitive zones in routine disk diffusion test. The number of infections caused by Amp C β-lactamases producing P. aeruginosa is on rise and poses a threat to patients due to therapeutic failure if they remain undetected ( Arora & Bal, 2005). Metallobetalactamase (MBL) producing P.aeruginosa is an emerging threat and a cause of concern for treating physicians as it can hydrolyze carbapenems which are given as a last resort to the patient having infection with ESBL and AmpC β-lactamase producing P.aeruginosa. The MBLs have become more notorious as therapeutically available inhibitors are not available and for their potential for rapid and generalized dissemination to different other Gram negative bacilli. Hence, accurate identification of MBL producing strains are very urgently needed. Though PCR gives specific and accurate results, it's use is limited to few laboratories because of it's high cost and different types of ESBLs, AmpCβ-lactamases and metallobetalactamases (MBLs) present worldwide ( Moland et al, 2008b).

In the present study 81.6% P.aeruginosa strains were isolated from IPD patients Algun et al from Turkey in 2004 reported isolation of P. aeruginosa 61% from IPD patients. Basak et al in 2009 reported 89.3% isolation from IPD and 10.7% from ICU from our hospital.

In the present study, co-existence of MBL producing P. aeruginosa was not observed along with ESBL & Amp C producers. Saha et al in 2010 reported 86% strains producing both MBL and Amp C β-lactamases while only one strain was observed to produce both ESBL and MBL. In the present study 68(27.2%) strains were both ESBL & Amp C β-lactamases producers amongst 250 P. aeruginosa strains studied.

6(22.2%) MBL producing strains were isolated from Medicine ICU. Sarkar et al in 2006 reported 36.4% of imipenem resistance in nosocomially infected patients with P. aeruginosa. In present study, we found 15 (35.7%) P. aeruginosa from NICU and all were from blood cultures of neonates and all were imipenem sensitive. Only 3 (20%) of these were found to be positive for ESBL production. Arkawa et al, 2000 recommended testing ceftazidime-resistant isolates for MBL production because in their study some MBL producing Gram negative bacilli were inhibited by low concentration of imipenem and they were difficult to detect. But Lee et al, 2001 reported that in their study, not a single MBL-producing isolates were detected among imipenem susceptible isolates.

In Japan, Sugino et al, 2001 used only carbapenem non-susceptible isolates for screening of MBL. Hence we also used carbapenem resistant isolates for detection of MBL. Though Arkawa et al, 2000 and other authors have done DDST & Disc potentiation test with ceftazidime and EDTA, in our study we used imipenem and EDTA for DDST & Disc potentiation test. As in our study, even in non MBL producing *P.aeruginosa* strains, the ceftazidime resistance was quite high (69.8%). The MBL producing strains may also have another ceftazidime resistance mechanism (Lee et al, 2003b). With such type of strains, DDSTs using an imipenem disc can show positive results for MBL but a ceftazidime disc can not; just as a cefepime disc but not a ceftazidime disc can detect extended spectrum β-lactamase (ESBL) production in Amp-C β-lactamase producing strains.

Though Franklin et al, 2006 have reported that 87% of their MBL producing Enteobacteriaceae isolates had >30mm of zone with aztreonam, we did not find any MBL producing *P.aeruginosa* strain to be susceptible to aztreonam. This can only be explained by the fact that there are presence of some other mechanisms for aztreonam resistance in P. aeruginosa strains isolated.

Aggarwal et al in 2008 found that polymyxin B was the most effective antibiotic recording 0% resistance, similar was the finding of our study. In our study we found 67.2% resistance against ceftazidime which was quite high and corelated well with the study of Behra et al in 2008 who had reported 70% resistance to ceftazidime.

## 3. Conclusion

Microbial drug resistance is now a global problem due to newer β-lactamases produced by Gram-negative bacteria including Pseudomonas aeruginosa. E-test and Polymerase chain reaction (PCR) can be used for accurate detection of newer β-lactamases , but both are costly and require expertise and cannot be done routinely.

Hence to conclude, for detection of ESBL, combined disk method using piperacillin/piperacillin-tazobactam (Pc/Pt), for detection of Amp C β-lactamases confirmatorty Disk potentiation test using 3-aminophenylboronic acid and for detection of MBL producing P. aeruginosa disk potentiation test using imipenem-EDTA should be done by all clinical Microbiolgy laboratories to prevent its dissemination and also for a good therapeutic outcome as these tests are economical, easy to perform and quite specific.

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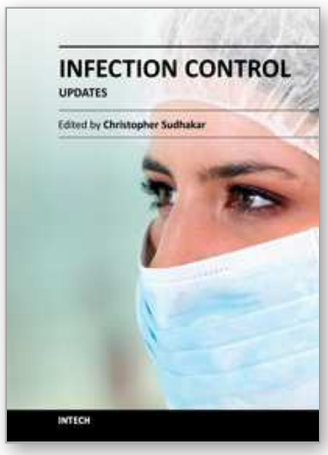


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Health care associated infection is coupled with significant morbidity and mortality. Prevention and control of infection is indispensable part of health care delivery system. Knowledge of Preventing HAI can help health care providers to make informed and therapeutic decisions thereby prevent or reduce these infections. Infection control is continuously evolving science that is constantly being updated and enhanced. The book will be very useful for all health care professionals to combat with health care associated infections.

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