

Original Research Article

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Prevalence of ESBL and MBL Producing *Pseudomonas aeruginosa* in a Tertiary Care Hospital in Tirunelveli, India

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ABSTRACT

Keywords

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The present study was done to detect Extended spectrum β lactamase and metallo- β -lactamase producing isolates of *Pseudomonas aeruginosa* from various clinical samples from patients admitted in Tirunelveli Government medical college hospital. In this study we studied the prevalence of ESBL and MBL among 112 *Pseudomonas aeruginosa* isolates, following standard methods of isolation and identification of these bacteria from clinical materials. Out of 112 isolates of *Pseudomonas aeruginosa*, 32 were producing extended spectrum β lactamase enzyme producing isolates, 11 isolates were resistance to Imipenem. Of the 11 imipenem resistant isolates, 7 were metallo- β -Lactamase enzyme producing isolates. The present study underlines the unique problem that the presence of ESBL has led to the widespread use of Imipenem which in turn led to the emergence of MBLs. Infection caused by MBL (metallo- β -lactamase) positive isolates of *Pseudomonas aeruginosa* is important to identify because it poses not only therapeutic problem due to broad spectrum drug resistance, but also a serious concern for infection control.

Introduction

Pseudomonas aeruginosa is a clinically troublesome gram negative pathogen that causes a wide range of opportunistic infections and nosocomial outbreaks (Senda *et al.*, 1996). *Pseudomonas aeruginosa* is responsible for 10–15% of the nosocomial infections worldwide.

Pseudomonas aeruginosa is a uniquely problematic pathogen because of its natural resistance to many drug classes and its ability to acquire resistance (via mutations) against all relevant treatments. Multi-drug resistance

in *Pseudomonas aeruginosa* makes treatment of infections caused by this organism both difficult and expensive (Strateva and Yordanov, 2009).

Extended-spectrum β -lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime, and the monobactam aztreonam (Jiang *et al.*, 2006). The drug of choice for such ESBL producing *Pseudomonas aeruginosa* are carbapenems. However, reports of carbapenemases have been increasing in recent years (Nordmann and Poirel, 2002).

Carbapenemases are enzymes that have the common property of hydrolyzing, at least partially, imipinem or meropenem. Class B or the metallo-enzymes (MBL) are the most clinically threatening carbapenemases because they are capable of hydrolyzing all β lactams except aztreonem (Yong *et al.*, 2002). Metallo beta lactamase is a binuclear zinc-dependent enzyme whose activity is susceptible to EDTA, where it is not inhibited by clavulanic acid, tazobactam, and sulbactam (Nordmann and Poirel, 2002).

The IMP and VIM genes responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria (Nordmann and Poirel, 2002; Varaiya *et al.*, 2008). So the occurrence of MBL positive isolates in a hospital setting not only pose a therapeutic problem, but also it is a serious concern for infection control management (Pitout *et al.*, 2005). In some countries *Pseudomonas aeruginosa* possessing MBL constitute nearly 20% of all nosocomial isolates, In India, published reports indicate the prevalence of MBLs to range from 7-65% (Manoharan *et al.*, 2010).

Hence, the present study was conducted to detect the presence of ESBL and MBL producing *Pseudomonas aeruginosa* among clinical isolates from Tirunelveli medical college hospital, so as to help in formulating an effective antibiotic policy and to plan a proper hospital infection control strategy to prevent the spread of these strains.

Materials and Methods

A total of 112 isolates of *Pseudomonas aeruginosa* were obtained from various clinical samples like pus, sputum, urine, blood, CSF, pleural fluid and aural swabs in Tirunelveli medical college hospital from January 2012 to June 2012. *Pseudomonas aeruginosa* isolates were identified as per the

standard microbiological procedures (Collee *et al.*, 2008).

Antimicrobial susceptibility testing was performed on Mueller hinton agar plates by Kirby-Bauer disc diffusion method according to CLSI guidelines. Following antibiotic discs were used gentamicin (GM) 10 μ g, amikacin (AK) 30 μ g, ciprofloxacin 5 μ g, imipinem (IMP) 10 μ g and ceftazidime (CAZ) 30 μ g. The zones of inhibition were interpreted as per CLSI guidelines (Clinical and Laboratory Standards Institute, 2006). The quality control strains used for the study were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Test for detection of extended spectrum beta lactamase

All the isolates of *Pseudomonas aeruginosa* which showed resistance to ceftazidime were evaluated for ESBL production. Briefly, a 0.5 MacFarland's suspension of each isolate was spread on a Muller – Hinton agar (MHA) plate and ceftazidime (30 μ g) and ceftazidime / clavulanic acid (30 μ g/ 10 μ g) discs were placed aseptically on the agar plate at a distance of about 15mm from edge to edge and the cultures were incubated at 37°C overnight (Figure 1). The observation of a \geq 5mm increase in the zone diameter for ceftazidime with clavulanic acid compared to ceftazidime alone was considered positive (Clinical and Laboratory Standards Institute, 2006). The increase in the zone diameter was due to the inhibition of the β lactamase by clavulanic acid.

Test for detection of metallo beta lactamase

Imipenem- EDTA combined disc test (CDT)

Next, 18.61 g of disodium EDTA.2H₂O was dissolved in 100 mL of distilled water, and the

pH was adjusted to 8.0 by sodium hydroxide (NaOH) and autoclave to prepare a sterile 0.5 M EDTA solution to imipenem disks (10 µg imipenem). For each isolate, two imipenem discs (10 µg) were placed at a distance of 25mm centre to centre on the surface of Mueller-Hinton agar plates inoculated with a bacterial suspension equivalent to 0.5 McFarland Standard (Figure 2). To one of the discs 5 µL of EDTA solution was added. After 24 hours of incubation at 37°C, an increase of ≥ 7 mm in the zone diameter of Imipenem with EDTA compared to imipenem alone was considered to be a positive test for the presence of an MBL in imipenem resistant strains (Yong *et al.*, 2002).

Results and Discussion

In this study, out of the 112 *Pseudomonas aeruginosa* isolates 36(32%) were resistant to ceftazidime 32(28%) ESBL producers and, imipenem resistance was found in 11(10%) isolates and out of which 7(6%) were MBL producers. None of the isolates showed coexistence of ESBL and MBL

The occurrence of MBL is significantly higher in Urine samples compared to other samples ($P < 0.05$). Out of the 32 ESBL producers, majority of which were from pus 19(60%), followed by 6(19%) from sputum, 3(9%) from aural swab, 2(6%) from urine, one (3%) from blood and one (3%) from jugular catheter tip. Out of the 32 ESBL producers, 14(44%) were from surgery ward, 4(12%) from medicine ward, 4(12%) from ortho ward, 4(12%) from ENT ward, 2(6%) from ICU, one (3%) from paediatrics, one (3%) from Burns ward, one (3%) from urology ward, one (3%) from CM scheme (Table 1).

Out of the 112 clinical isolates of *Pseudomonas aeruginosa*, 11(10%) were found to be resistant to imipenem. Of the 11 imipenem resistant *Pseudomonas aeruginosa* isolates 7(66%) were found to be metallo-β-

lactamase producers. Of the seven metallo-β-lactamase producers two were from pus sample in surgery ward, two were from aural swab in ENT ward, two were from urine sample in Urology ward and one is from pus from ICU ward. Out of the 112 clinical isolates of *Pseudomonas aeruginosa*, 58(52%) were found to be resistant to Amikacin, 60 (54%) were resistant to Gentamicin, 54(48%) were resistant to ciprofloxacin, 36(32%) were resistant to ceftazidime and 11(10%) were found to be resistant to imipenem (Table 2 and 3).

Pseudomonas aeruginosa infections are hard to treat due to natural resistance of the species as well as its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. In *Pseudomonas aeruginosa*, different enzymic and mutational mechanisms can exist simultaneously conferring combined resistance to many strains. Multidrug resistance in *P. aeruginosa* makes treatment of infections caused by this organism both difficult and expensive. Improved methods for antimicrobial susceptibility testing are needed, including detection of emerging strains producing ESBLs and MBLs (Strateva and Yordanov, 2009). With increasing isolation of ESBL producing isolates in the hospital setting necessitating the use of carbapenems, the problem of MBL production is also increasing (Manoharan *et al.*, 2010).

Our study showed that out of 112 *Pseudomonas aeruginosa* isolates, 32(28%) were ESBL producers, which was higher than 22.22% ESBL producing isolates reported by (Pesattiwat and Peerapur, 2011) and 20.27% by (Aggarwal *et al.*, 2008). Majority of the ESBL producers were from surgery ward (44%), this may be because majority of the isolates (39%) were collected from surgery ward and major ESBL producers are from pus and wound swab (60%).

Table.1 Sample wise distribution

S NO	SPECIMEN	TOTAL NO (%) n=112	ESBL NO (%) n=32	IMP REST NO (%) n=11	MBL NO (%) n=7
1	Pus	67(60)	19(60)	4(36)	3(43)
2	Sputum	17(15)	6(19)	-	-
3	Urine	8(7)	2(6)	3(28)	2(28.5)
4	Aural swab	14(12.5)	3(9)	4(36)	2(28.5)
5	Blood	3(2.5)	1(3)	-	-
6	Pleural fluid	1(1)	-	-	-
7	CSF	1(1)	-	-	-
8	Jugular catheter tip	1(1)	1(3)	-	-

Table.2 Ward wise distribution

S NO	WARD	SAMPLES n=112	ESBL NO (%) n=32	IMP RES NO (%) n=11	MBL NO (%) n=7
1	Medicine	15(13%)	4(12.5)	-	-
2	Surgery	44(39%)	14(44)	3(27)	2(28.5)
3	Orthopaedics	9(8%)	4(12.5)	-	-
4	Obstetrics and Gynaecology	2(2%)	-	-	-
5	Peadiatrics	2(2%)	1(3)	-	-
6	ENT	16(14%)	4(12.5)	4(36)	2(28.5)
7	ICU	12(11%)	2(6.5)	1(12)	1(14.5)
8	Burns	4(3%)	1(3)	-	-
9	Skin	4(3%)	-	-	-
10	CM Scheme	2(2%)	1(3)	-	-
11	Urology	2(2%)	1(3)	3(27)	2(28.5)

Table.3 Antibiotic susceptibility pattern

S NO	ANTIBIOTIC	RESISTANT ISOLATES	
		NO n=112	%
1	Amikacin	58	52
2	Gentamicin	60	54
3	Ciprofloxacin	54	48
4	Ceftazidime	36	32
5	Imipinem	11	10

Fig.1

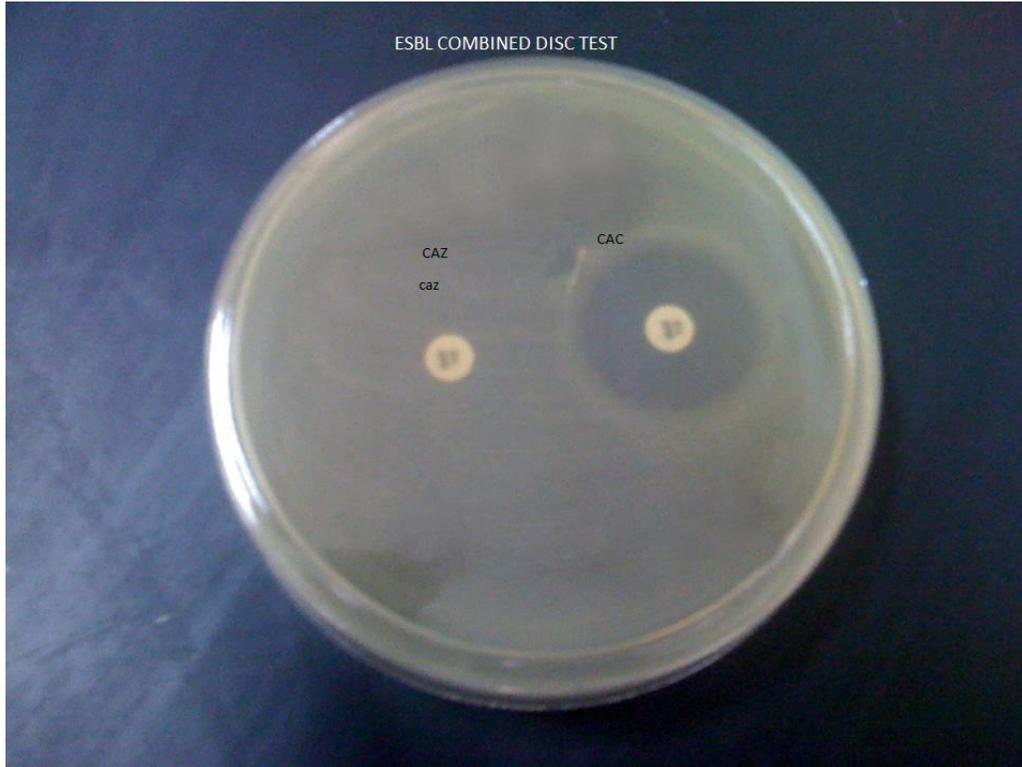
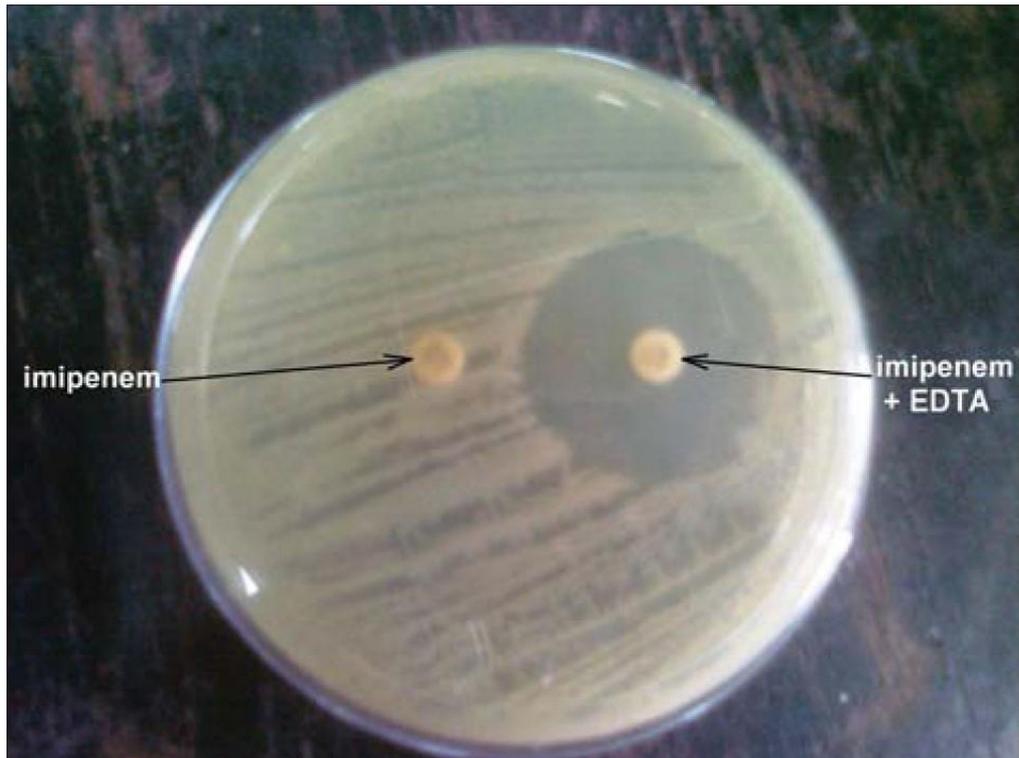


Fig.2



In this study, out of the 112 *Pseudomonas aeruginosa* isolates, 11(10%) were imipinem resistant strains, which was less than that observed by (Varaiya *et al.*, 2008) and (Manoharan *et al.*, 2010) This may be due to the minimal use of carbapenems in our hospital. Of the 11 imipinem resistant strains, 7 were found to be MBL producing isolates. The likely reason for the resistance in remaining isolates may be due to other mechanisms like upregulation of an active efflux system¹³ or diminished expression of outer membrane protein (Buscher *et al.*, 1987).

In the present study, the prevalence of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* was found to be 6%, which is similar to the 5% prevalence of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* was observed and reported by (Chaudari *et al.*, 2011) and (Ibukan *et al.*, 2007).

In the present study, there is an increased resistance to antibiotics like Amikacin 52%, gentamicin 54%, ciprofloxacin 48% and ceftazidime 32%. A decreased susceptibility of *Pseudomonas aeruginosa* to commonly used antibiotics have already been noted by previous researchers (Aggarwal *et al.*, 2008; Jaykumar and Appalaraju, 2007; Arya *et al.*, 2005). The emergence of multidrug resistant bacteria is threatening the effectiveness of many antimicrobial agents which increases the hospital stay of the patient, thus leading to an increased economic burden on them (Peshattiwari and Peerapur, 2011).

In recent years MBL genes were found to be spread from *Pseudomonas aeruginosa* to members of *Enterobacteriaceae* family (Behera *et al.*, 2008). The genes encoding IMP, VIM, SIM, GIM-1 and SPM-1 are transferable MBLs (Walsh *et al.*, 2005). In the absence of novel agents for the treatment of

infections caused by multidrug-resistant *Pseudomonas aeruginosa* in the near future, the spread of MBL producers may lead to treatment failures with increased morbidity and mortality. Since these organisms carry multi drug resistance genes, the only viable option that remains is potentially toxic polymyxin B and colistin. The early detection of MBL producers may avoid future spread of these multi drug resistant isolates (Pitout *et al.*, 2005).

Detection of MBL producing isolates by PCR is expensive, requires specialized technicians and instruments, and more importantly, is able to detect only previously described MBL-encoding genes (Picão *et al.*, 2008). The development of simple screening tests to detect acquired MBL production is a crucial step towards large scale monitoring of these emerging resistant determinants (Manoharan *et al.*, 2010). Several phenotypic methods are available for the detection of MBL producing bacteria. All of these methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds, to inhibit the activity of MBLs. There are conflicting reports regarding the performance of MBL E test in the literature. MBL E - Test has been reported to be insensitive to detect carbapenem sensitive MBL carrying organisms.

The combined disk test using EDTA with imipenem is simple to perform and interpret and can be easily introduced into the workflow of a clinical laboratory. This test has been used in several studies where it produced excellent sensitivity and specificity for detecting MBL-producing *P. aeruginosa* strains (Saderi *et al.*, 2010). The detection of ESBLs and MBLs must be followed upon a routine basis in the laboratory, as it will ensure optimal patient care and the timely introduction of appropriate infection control procedures.

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