Original Article

Microbiology Section

ESBL and MBL Mediated Resistance in *Pseudomonas aeruginosa*: An Emerging Threat to Clinical Therapeutics

PRASHANT DURWAS PESHATTIWAR, BASAVARAJ VIRUPAKSAPPA PEERAPUR

ABSTRACT

Purpose: The present study was undertaken to detect the extended spectrum β lactamases (ESBL) and metallo β lactamases (MBL) in isolates of *Pseudomonas aeruginosa* which were isolated from wound infections and to evaluate their susceptibility patterns.

Materials and Methods: One hundred and twenty six isolates of *P.aeruginosa* were analyzed to study their sensitivity patterns. The presence of the ESBL enzyme was detected by the Phenotypic confirmatory test and the MBL enzyme was detected by the Imipenem – EDTA Double Disk Synergy test.

Result: Out of 126 isolates of *P.aeruginosa*, 28 (22.22%) were ESBL producers and 10 (7.8%) were MBL producers. None of the isolates showed the coexistence of ESBL and MBL in the same isolate. All the ESBL producing isolates were sensitive to Imipenem, while the MBL producing isolates showed widespread resistance to aminoglycosides, ciprofloxacin and the piperacillin with tazobactum combination.

Conclusion: The present study underlines the unique problem that the presence of ESBL has led to the widespread use of Imipenem, but that the emergence of MBLs and their broad spectrums and unrivalled drug resistance is creating a therapeutic challenge for clinicians and microbiologists. Hence, we suggest that the detection of ESBL and MBL in *Pseudomonas aeruginosa* should be a routine practice. We recommend a routine surveillance on antibiotic resistance in the hospital.

Key Words: *P.aeruginosa* , Third generation cephalosporins, Imipenem

INTRODUCTION

Pseudomonas aeruginosa is reported to be amongst the leading causes of nosocomial infections. It is known to exhibit intrinsic resistance to several antimicrobial agents [1]. In addition to the intrinsic resistance of *P. aeruginosa* , it also produces the enzymes, namely ß-lactamases, which are responsible for the wide-spread ß-lactam resistance. These ß -lactamases hydrolyse the amide bond of the four-membered characteristic ß - lactam ring, thus rendering the antimicrobial ineffective [2]. The ESBL enzymes encoded by the genes, SHV2a and the genes, SHV2a and TEM have been found in *P. aeruginosa* and the Enterobacteriaceae family, which suggests that these organisms are widespread reservoir of the ESBL enzymes [3].

MBLs are class B enzymes which hydrolyze carbapenems and are encoded by genes like IMP, VIM, etc [4]. They have been described as the enzymes which require divalent cations, usually zinc, as metal co-factors for their enzymatic activity. In recent years, the MBL genes have spread from *P. aeruginosa* to Enterobacteriaceae. Given the fact that we are several years away from the implementation of a therapeutic inhibitor of MBLs, their continued spread is going to be a major therapeutic challenge [5].

Hence, the present study was conducted with an objective to know the antibiogram of *P. aeruginosa*. We also aimed to detect the presence of ESBL and MBL producing *P. aeruginosa*, so as to help in formulating an effective antibiotic strategy and to plan a proper hospital infection control strategy to prevent the spread of these strains.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology of BLDEU's Shri BM Patil Medical College, Bijapur, over a period from Nov 2008 to Sept 2010. 126 isolates of *P. aeruginosa* were obtained from the pus samples of wound infection cases. The cases which yielded a growth of *P. aeruginosa* from cultured wound swabs on blood agar and MacConkey's agar were included in the study and they were further identified as per the standard microbiological procedures [6].The isolates were further tested by employing the Kirby Bauer disc diffusion method to study their antimicrobial susceptibility pattern for cephotaxime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), gentamicin (10µg), amikacin (30µg), tobramycin (10µg), piperacillin (100 µg), piperacillin/tazobactum (100 µg/ 10 µg) , meropenem (10µg) and imipenem (10µg). The results were recorded and interpreted as per the CLSI guidelines [7].

EXTENDED SPECTRUM β – LACTAMASE (ESBL) DETECTION

All the isolates of *P.aeruginosa* which showed resistance to ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test [7]. Briefly, a 0.5 MacFarland's suspension of each isolate was spread on a Muller – Hinton agar (MHA) plate and ceftazidime (30 µg) and ceftazidime / clavulanic acid (30 µg/ 10 µg) discs were placed aseptically on the agar plate. A distance of about 15mm was kept between the two discs (edge to edge) and the cultures were incubated at 37^{0}C overnight. The observation of a ≥ 5mm increase in the zone diameter for the antimicrobial agent which was tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed the presence of ESBL production by the organism. The increase in the zone diameter was due to the inhibition of the β lactamse by clavulanic acid.

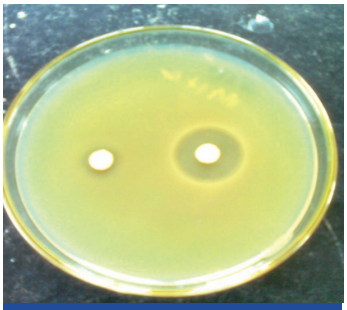
THE METALLO - β- LACTAMASE (MBL) DETECTION METHOD

The Imipenem resistant isolates were tested by the Imipenem-EDTA double disk synergy test (DDST) as described by Lee et al. [8]. The test organism was inoculated onto MHA plates as recommended by CLSI. An Imipenem 10 µg disk was placed 10mm edge to edge from a blank disc which contained 10 µl of EDTA (750 µg), with overnight incubation at 37°C. An enhancement in the zone of inhibition in the area between the Imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.

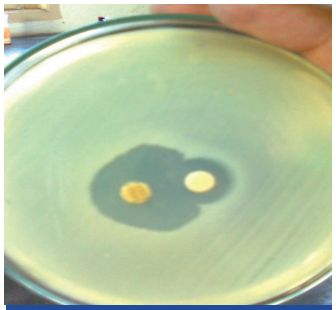
The ESBL Phenotypic Confirmatory test and the Metallo β lactamase production by the Imipenem - EDTA Double Disk Synergy test are shown in [Table/Fig-1 and 2] respectively.

RESULTS

The Department of Microbiology, Shri B.M. Patil Medical College, Bijapur, received a total of 1628 pus samples between November 2008 and September 2010. Out of 1628 pus samples which were cultured, 126[12.92 %] yielded the growth of *Pseudomonas aeruginosa*. 103 samples yielded a pure growth of *Pseudomonas aeruginosa,* while 23 isolates were mixed cultures with *E. Coli, S. aureus, Proteus* species, *K. pneumoniae, Acinetobacter* species and *Citrobacter* species. Out of the 126 subjects who showed the 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. Out of the 126 isolates, 19 [15.07%] isolates were from the Outpatients Department and 107 [84.92%] were from inpatients. Among the isolates from the InPatients Departments, 76 [71.02%] isolates were from the surgery ward, followed by 24 [22.42%] from the orthopaedics ward and 7 [6.54 %] patients from the ENT ward. In the present study, a majority of the *P. aeruginosa* isolates were obtained from cases of cellulitis [19.04 %], followed by traumatic wound infections [16.66%], cases of diabetic foot [15.07%], CSOM [11.90%] and burns cases [9.5 2%]. The resistance pattern of *P.aeruginosa* was noted as follows, ceftazidime -67 [53%], cephotaxime -64 [50.79%], tobramycin -40[31.74%], netilmicin -57[45.23%], gentamicin – 48[38.09%], amikacin- 46 [36.50%] , ciprofloxacin- 59 [46.82%], imipenem and meropenem- 16 each [12.69 %], The piperacillin + tazobactum combination -26[20.63 %] and piperacillin -52 [41.26 %]. Among the 126 *Pseudomonas aeruginosa* isolates, 28 [22.22%] were ESBL producers. A total of sixty seven strains showed resistance to ceftazidime, of which 28 [41.79%] were found to be ESBL producers . Among the 126 isolates of *Pseudomonas aeruginosa*, 10 [7.8 %] isolates were metallo β lactamase producers and these were isolated from 16 imipenem resistant isolates. Hence, the percentage of MBLs in the imipenem resistant isolates was 62.5 %. Also, those 10 MBL producing strains were also resistant to netilmicin, gentamicin and ciprofloxacin (9) each and tobramycin and amikacin (8) each. None of the isolates showed the coexistence of ESBL and MBL.



[Table/Fig-1]: Phenotypic confirmatory test for ESBL detection. Left-Ceftazidime (30µg), Right–ceftazidime clavulanic acid (30µg/10µg), showing increase in zone of inhibition by more than 5 mm.



[Table/Fig-2]: Imipenem – EDTA double disk synergy test. Left – Imipenem disk (10µg), Right-EDTA (10µl) disk. A synergistic zone between two disks.

DISCUSSION

The emergence of antibiotic resistant bacteria is threatening the effectiveness of many antimicrobial agents. It has increased the hospital stay of the patients, thus leading to an increased economic burden on them.

In the present study, the rate of isolation of *P. aeruginosa* was higher in indoor patients [84.92%] as compared to that in the outdoor patients [15.07 %]. A similar observation was made by Shampa Anupurba et al, who reported the isolation of *Pseudomonas aeruginosa* to be more common in indoor patients [73.42%] as compared to that in the OPD cases [26.57%]. They expressed their view that the duration of the hospital stay was directly proportional to a higher prevalence of the infection, since the rate of isolation of the organisms was higher in indoor patients than in outdoor patients [9].

In the present study, the antibiogram of the 126 isolates of *P. aeruginosa* showed more resistance against ceftazidime [53.17%], which was similar to the observations which were by Diwivedi et al, who reported the ceftazidime resistance to be around 63%, and Arya M et al who reported a ceftazidime resistance of 55.4% in isolates obtained from post operative wound infections [10-11]. Our findings differed from those of Ibukun et al, who reported a higher susceptibility for ceftazidime of 79.4% [1]. In the present study, we observed an increased resistance of this organism to various antibiotics like cephotaxime- 50.79%, netilmicin- 45.23%, gentamicin - 38.09%, amikacin -36.50%, ciprofloxacin- 46.82% and piperacillin- 41.26 %. A decreased susceptibility of *P.aeruginosa* to the commonly used antibiotics has already been noted by previous researchers [3,4,11,12] Our study showed that among the 126 *Pseudomonas aeruginosa* isolates, 28 [22.22%] were ESBL producers, which was similar to 20.27 % ESBL producing isolates of *P. aeruginosa* which was reported by Aggarwal et al. [3]. The ESBL mediated resistance of *P. aeruginosa* to the third generation cephalosporins as reported by Uma et al [77.3%], was much higher than that reported in the present study [14]. We observed that only 12.69 % strains were resistant to carbapenem in our study. In the present study, imipenem and meropenem showed good antipsuedomonal activity. A similar observation was made by Jaykumar S. [4], while a higher degree of carbapenem resistance was noted by Varaiya et al [25%] [15]. This difference could be attributable to the study environment under which the study was performed. A study by Varaiya et al from Mumbai involved patients from the ICU, where the use of broad spectrum antibiotics was common [15]. In our study, out of the 126 isolates of *Pseudomonas aeruginosa*, 10 [7.8 %] isolates were metallo β lactamases producers. The prevalence of MBLs in the present study was consistent with the findings of Ibukun et al, Navaneeth et al and others [1,16,17]. The percentage of MBLs in the imipenem resistant isolates was 62.5%. This suggested that the carbapenem resistance in *P. aeruginosa* was mediated predominantly via MBL production. A similar finding was observed by the SARI study group and by Behara et al. [17,13]. In the present study, 5 isolates [50%]

out of 10 MBLs were resistant to all the 11 antibiotics which were tested. The presence of MBLs in the pandrug resistant isolates was already observed by Jaykumar S [4]. In our study, none of the isolates had coproduced both ESBL and MBL. This was in agreement with the findings of Renata Picao et al [18].

Our study underlines the unique problem of ESBL and MBL mediated resistance, which has created a therapeutic challenge for the clinicians and microbiologists. To overcome the problem of emergence and the spread of multidrug resistant *P. aeruginosa,* a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed. We recommend the routine surveillance of antibiotic resistance in the hospital.

REFERENCES

[1] 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.

[2] Bradford PA. Extended spectrum ß-lactamases in the 21st century: The characterization, epidemiology and the detection of this important resistance threat. *Clin. Microbiol. Rev* 2001; 14: 933-951.

[3] Aggarwal R, Chaudhari U, Bala K. Detection of extended – spectrum β lactamases in Psuedomonas aeruginosa. *Indian J Pathol Microbiol* 2008;51(2): 222-24.

[4] Jaykumar S, Appalraju B. The 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) : 922-25.

[5] Walsh T R., Toleman M A., Poirel L Nordmann P. Metallo-β-lactamases: the quiet before the storm? *Clinical Microbiology Reviews* Apr 2005; 306-25.

[6] Colle JG, Miles RS, Wan B. Tests for the identification of bacteria. *In*: Colle JG, Fraser AG, Marimon BP, Simmons A, Editors. Mackei and McCartney Practical Medical Microbiology, 14th ed. Edinburg: Chuchill Livingstone; 1996; 131-50.

[7] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk tests ; Approved Standards , 9th ed. CLSI Document M2-A9 , Vol. 26 No.1 Wayne PA 2006.

[8] Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for the differentiation of the metallo- β-lactamase producing clinical isolates of the Pseudomonas spp and the Acinetobacter spp. *J Clin Microbiol* 2003 ; 41: 4623-29.

[9] Anupurba S, Battacharjee A, Garg A, Ranjansen M. The antimicrobial susceptibility of *Psuedomonas aeruginosa* isolated from wound infections. *Indian J Dermatol* 2006 ; 51(4): 286-88.

[10] Diwivedi M , Mishra A., Singh R.K., A. Azim, A.K. Baronia, K.N. Prasad. The nosocomial cross – transmission of Pseudomonas aeruginosa between patients in a tertiary intensive care unit. *Indian J Pathol Microbiol* 2009;52(4): 509-13.

[11] Arya M , Arya P , Biswas D , Prasad R . The antimicrobial susceptibility pattern of the bacterial isolates from post operative wound infections . *Indian J Pathol Microbiol* 2005; 48(2): 266-69.

[12] Obritsch MD , Fish DN , Maclaren R, Jung R. The national surveillance of antimicrobial resistance in the Pseudomonas aeruginosa isolates obtained from intensive care unit patients from 1993 to 2002. *Antimicrob Agents Chemother* 2004; 48 : 4606-10.

[13] Behera B., Mathur P., Das A., Kapil A., Sharma V. An evaluation of four different phenotypic techniques for detecting the metallo-β-lactamases producing Pseudomonas aeruginosa. *Indian J. Medical Microbiology* 2008; 26(3): 233-37.

[14] Chaudhari U, Bhaskar H, Sharma M. The Imipenem–EDTA disk method for the rapid identification of metallo β lactamase producing gram negative bacteria. *Indian J Med Res* 2008; 127(2):406-07.

[15] Varaiya A, Kulkarni N, Kulkarni M, et al. The incidence of metallo beta lactamase producing Pseudomonas aeruginosa among ICU patients. *Indian J Med Res* 2008; 127: 398-402.

[16] Navneeth BV, Sridaran D, Sahay D, Belwadi M. A preliminary study on the metallo betalactamase producing Pseudomonas aeruginosa in hospitalised patients. *Indian J Med Res* 2002; 112: 264-67.

[17] Manoharan A, Chatterjee S, Mathai D. The SARI study group. Detection and characterization of metallo beta lactamases produced by Pseudomonas aeruginosa. *Indian J Med Microbial* 2010; 28(3): 241-44.

[18] Picão R C., Poirel L, Gales A C., et al. The diversity of β-lactamases produced by ceftazidime-resistant pseudomonas aeruginosa isolates causing bloodstream infections in Brazil. Antimicrob. *Agents Chemother* 2009;53(9):3908-13.

AUTHOR(S):

1. Dr. Prashant Durwas Peshattiwar
2. Dr. Basavaraj Virupaksappa Peerapur

PARTICULARS OF CONTRIBUTORS:

1. MD (Microbiology), Asst. Prof, Konaseema institute of medical sciences and research foundation, Amalpuram, East Godavari, AP.
2. MD (Microbiology) Prof and head Department of Microbiology, BLDEU's Shri B M Patil Medical college Bijapur, Karnataka.

PLACE OF STUDY:

BLDEU's Shri B M Patil Medical college Bijapur, Karnataka.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Basavaraj Virupakshappa Peerapur
MD (Microbiology) Prof and head Department of Microbiology,
BLDEU's Shri B M Patil Medical college Bijapur,
Karnataka, India.
Phone: 09448139438
E-mail: peerapur_2003@yahoo.co.in

DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: **Oct 11, 2011**
Date of peer review: **Nov 21, 2011**
Date of acceptance: **Dec 08, 2011**
Date of Publishing: **Dec 25, 2011**