



Detection of Extended Spectrum Beta-Lactamases in *Pseudomonas aeruginosa* Isolates in a Tertiary Care Hospital

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Abstract

Background: Extended spectrum β -lactamases (ESBLs) represent a major group of lactamases responsible for resistance, mostly produced by gram-negative bacteria, to newer generations of β -lactam drugs currently being identified in large numbers worldwide. The aim of the present study was to detect the extended-spectrum β -lactamase (ESBL) production among *Pseudomonas aeruginosa* isolates from various samples.

Materials and Methods: A total of 100 *Pseudomonas aeruginosa* isolates from various samples in a tertiary care hospital from January 2011 to December 2011 were included in the study. Kirby-Bauer's disc diffusion method was employed to detect the antibiotic susceptibility pattern of the isolates. The isolates were tested for ESBL production using double-disc synergy test (DDST) using ceftazidime alone and combined ceftazidime-clavulanic acid discs.

Result: Out of the 100 *Pseudomonas aeruginosa* isolates studied, 45 (45%) were ESBL producers. Out of the ESBL producing isolates, 44 (98%) were resistant to third generation cephalosporins, 28 (62%) to Ciprofloxacin, 34 (76%) to Gentamicin, 28 (62%) to Amikacin respectively. 45 (100%) of isolates were susceptible to Imipenem.

Conclusion: This study emphasizes on the need for global control of antimicrobial resistance; and to create awareness among the clinicians and general population thereby reducing the mortality and morbidity associated with multi-drug resistant pathogens.

Keywords: *Pseudomonas aeruginosa*, extended spectrum β -lactamases, third-generation cephalosporins, Kirby-Bauer disc diffusion technique, double-disc synergy test, Multidrug Resistance.

INTRODUCTION

Pseudomonas aeruginosa, an opportunistic and worrisome nosocomial pathogen, is a Gram-negative, aerobic rod belonging to bacterial family Pseudomonadaceae.^{1,2} It causes ventilator associated pneumonia, complicated urinary tract

infections in intensive care units and also causes infection in burns patients.³ It is ubiquitous in nature and it is a saprophyte found in water, soil or decomposing organic material.⁴ Infections caused by *P. aeruginosa* are particularly problematic because the organism is inherently resistant to many drug

classes and is able to acquire resistance to all effective antimicrobial drugs.⁵

Various mechanisms of resistance have been identified in *P. aeruginosa*, ESBL (Extended Spectrum Beta Lactamase) production being one among them.^{1,6} ESBLs were originally considered to be confined to Enterobacteriaceae family but with the detection of genes coding for ESBL production such as TEM-42 and SHV-2a in *P. aeruginosa* and other nosocomial pathogens, it is proved to have spread to organisms other than Enterobacteriaceae.^{7,8} Thus, this study was undertaken, to understand its prevalence and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates which will help to initiate appropriate infection control measure thereby reducing the morbidity and mortality.

MATERIALS & METHODS

A total of 100 *Pseudomonas aeruginosa* isolated from various clinical samples like pus, urine, blood, sputum, endotracheal aspirate, CSF and other body fluids (pleural, ascitic, peritoneal fluid) over a period of one year from January 2011 to December 2011 from hospitals attached to Mysore Medical College and Research Institute, Mysore were included in the study.

The *Pseudomonas aeruginosa* isolated were subjected to antibiotic susceptibility testing by Kirby - Bauer disc diffusion technique according to CLSI guidelines. *P.aeruginosa* ATCC 27853 was used as control.

The susceptibility testing was carried out against the following antibiotics

Antimicrobial agent	Disc conc. µg/disc
Cefotaxime	30
Ceftazidime	30
Cefepime	30
Ciprofloxacin	5
Gentamicin	10
Amikacin	30
Imipenem	10
Piperacillin	100
Piperacillin-Tazobactam	100/10
Aztreonam	30

All the antibiotic disks were procured commercially from Hi -Media laboratories Pvt. Ltd. Mumbai. The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines

Detection of ESBL

Phenotypic confirmatory disk diffusion test:

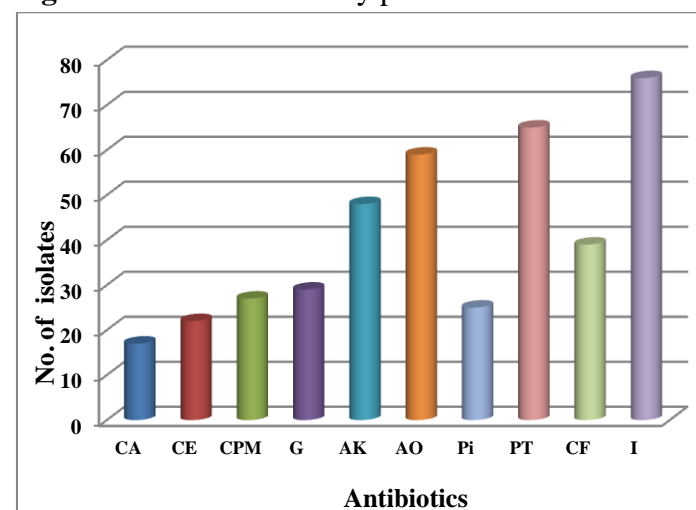
Extended Spectrum β -lactamase detection was done by phenotypic confirmatory disk diffusion test using disks Ceftazidime (30µg) alone and in combination with Clavulanic acid (30/10µg).

Procedure

2-3 well isolated colonies were suspended in 0.5 ml of sterile broth and the turbidity matched to 0.5 McFarland standard. Using a sterile cotton swab, the broth culture was uniformly spread on the sterility checked Mueller Hinton Agar plate. Ceftazidime alone and in combination with Clavulanic acid was placed at a distance of 20 mm from centre to centre. Plates were incubated at 37°C overnight. A measurement of ≥ 5 mm increase in zone diameter for Ceftazidime with Clavulanic acid versus Ceftazidime zone when tested alone, confirms an ESBL producing organism.

RESULTS

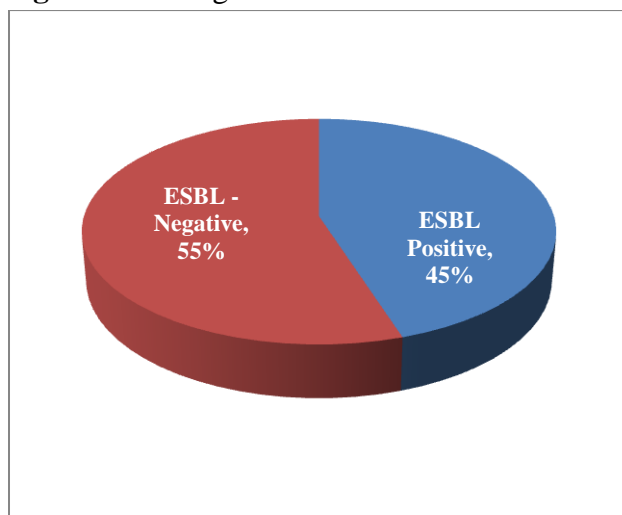
Fig-1: Antibiotic sensitivity pattern of the isolates



CA-Ceftazidime, CE-Cefotaxime, CPM-Cefepime, G-Gentamicin, AK-Amikacin, AO-Aztreonam, Pi-Piperacillin, PT - Piperacillin-Tazobactam, CF-Ciprofloxacin, I- Imipenem.

For Ceftazidime - $\chi^2 = 43.56$; $P = .000$ (HS). Cefotaxime - $\chi^2 = 31.36$; $P = .000$ (HS). Cefepime - $\chi^2 = 21.16$; $P = .000$ (HS). Gentamicin - $\chi^2 = 17.64$; $P = .000$ (HS). Amikacin - $\chi^2 = 0.16$; $P = .69$ (NS). Aztreonam - $\chi^2 = 3.24$; $P = .072$ (NS). Piperacillin - $\chi^2 = 25$; $P = .000$ (HS). Piperacillin-Tazobactam - $\chi^2 = 9.00$; $P = .003$ (Sg), Ciprofloxacin - $\chi^2 = 4.84$; $P = .028$ (Sg). Imipenem - $\chi^2 = 27.040$; $P = .000$ (HS).

Fig -2: Percentage of ESBL Producers



$\chi^2 = 1.00$; $P = .317$ (NS).

Out of the ESBL producing isolates, 44 (98%) were resistant to third generation cephalosporins, 28 (62%) to Ciprofloxacin, 34 (76 %) to Gentamicin, & 28(62%) to Amikacin respectively. 45 (100%) of isolates were susceptible to Imipenem.

DISCUSSION

Antibiotic when first introduced was considered as a magic bullet.⁹ Unfortunately the genes expressing resistance to antimicrobials have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration.²

The percentage of ESBL producers in the present study is 45(45%), which is comparable to the study of Ibukun A et al.² All the ESBL-positive *P. aeruginosa* were resistant to more than three drugs (multi-drug resistant).

The use of β -lactam/ β -lactamase inhibitor combination is effective as ESBLs are inhibited by β -lactamase inhibitors, viz., clavulanic acid and sulbactam but it depends on the subtype of ESBL present. There is emergence of resistance even among the combination agents which emphasizes a cautious use.

As already stated, ESBL-producing pathogens are commonly resistant to other classes of antimicrobials such as aminoglycosides and fluoroquinolones. This is attributed to the co-occurrence of genes on the plasmids encoding resistance to other antimicrobials which code for ESBL.¹⁰ In this study, 45(100%) of isolates were susceptible to Imipenem.

Though the carbapenems may be of use in the treatment of ESBL infection, indiscriminate use may lead to increased carbapenem resistance. Steps should be taken to screen ESBLs in the laboratory as a routine procedure and to isolate the plasmids using molecular techniques; and to imply a nationwide antibiotic policy to minimize the spread of resistance.

CONCLUSION

This study demonstrates that ESBL production continues to be one of the important mechanisms of drug resistance though other resistance mechanisms have emerged in *P. aeruginosa*, particularly in the hospital setup. Infections with MDRPA are associated with prolonged hospital stay, increased expenditure and adverse clinical consequences. Further studies are necessary to detect the risk factors for MDRPA. Hence, vigilant infection control measures and cautious use of antibiotics by clinicians should be encouraged.

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