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

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ABSTRACT

The aim of the present study was to detect the extended-spectrum β-lactamase (ESBL) production among *Pseudomonas aeruginosa* isolates from various samples. A total of 125 *Pseudomonas aeruginosa* isolates from various samples in a tertiary care hospital from June 2014 to November 2014 were included in the study. The diagnosis was confirmed using standard bacteriological techniques and antibiotic susceptibility testing was done by Kirby Bauer disc-diffusion method. The isolates resistant to the third generation cephalosporins were tested for ESBL production using double-disc synergy test (DDST) using ceftazidime alone and combined ceftazidime-clavulanic acid discs. It was found that out of the 125 isolates tested, 17 (13.6%) were found to produce ESBL. Also, all the ESBL-producing *P. aeruginosa* were resistant to third generation cephalosporins and they were 100% susceptible to meropenem followed by 60% susceptibility to amikacin and 50% susceptibility to ofloxacin. 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.

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is becoming one of the most widespread gram-negative opportunistic pathogens particularly causing nosocomial infections. It causes ventilator associated pneumonia, complicated urinary tract infections in intensive care units and also causes infection in burns patients. (Collee, Fraser *et al*., 1996) It is ubiquitous in nature and it is a saprophyte found in water, soil or decomposing organic material. It is intrinsically resistant to antiseptics, disinfectants and to a large number of commonly used antimicrobial agents. (Ana Lucia and Afonso, 2002) In the past, infections due to *P. aeruginosa* were only confined to immuno-compromised patients but recently there has been an increasing prevalence in immuno-competent subjects. (Bonomo and Szabo, 2006). With the emergence of acquired resistance due to plasmids in addition to the intrinsic resistance to almost all antimicrobial agents, the therapeutic options has posed a major challenge in combating the infections caused by multidrug resistant *P. aeruginosa* (MDRPA). (Neu, 1983) (Ami Varaiya *et al*., 2008) Various mechanisms of resistance have been identified in *P. aeruginosa*, ESBL (Extended Spectrum Beta Lactamase) production being one among them. (Poirel *et al*., 2000) (Winn, W. Allen *et al*., 2006) 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*. (Naas *et al*., 1999) (Mugnier *et al*., 1996) Thus, this study was undertaken, to understand its prevalence and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates from in-patients and out-patients of the hospital which will help to initiate appropriate infection control measure thereby reducing the morbidity and mortality.

MATERIALS AND METHODS

A total of 125 *Pseudomonas aeruginosa* isolates obtained from various samples in a tertiary care hospital from June 2014 to November 2014 were identified by standard bacteriological methods.

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Kirby-Bauer's disc diffusion method was employed to detect the antibiotic susceptibility pattern of the isolates. The panel of antibiotics used were ampicillin (10 µg), gentamicin (10 µg), amikacin (10 µg), cotrimoxazole (25 µg), cefotaxime (30 µg), ceftriaxone (30µg), ceftazidime (30µg) and meropenem (10µg). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates which showed decreased susceptibility or resistance to third generation cephalosporins (3GC) such as ceftriaxone, cefotaxime, and ceftazidime by disc diffusion test were further tested by double-disc synergy test.

Double-disc synergy test

The Muller Hinton agar was inoculated as a lawn culture with the test strain (0.5 McFarland Standard). Two discs containing ceftazidime (30µg) alone and combined ceftazidime (30µg) + clavulanic acid (10µg) were placed on the surface of dried MHA plate at a distance of 15-20mm (center to center), and incubated at 37^0C overnight. The isolate was considered to be an ESBL producer if the difference in zone size between ceftazidime alone and ceftazidime + clavulanic acid was ≥5mm.

RESULTS

Of the 125 isolates tested, 17 (13.6%) were found to produce ESBL. (Figure 1) Of the 99 (79.2%) 3GC-resistant isolates, 11 (11.11%) produced ESBL; whereas of the 26 (20.8%) 3GC-sensitive isolates, 6 (23.07%) were found to be producers of ESBL enzyme. (Figure 2) Hence, it was found that ESBL production was found in both resistant and susceptible groups; along with resistance to other antimicrobials.

Table 1: Number and Percentage of ESBL producers in 3GC resistant and sensitive isolates.

3GC / ESBL	Resistant 99(79.2%)	Sensitive 26(20.8%)	Total 125(100%)
Positive	11 (11.11%)	6(23.07%)	17(13.6%)
Negative	88(88.89%)	20(76.93%)	108(86.4%)

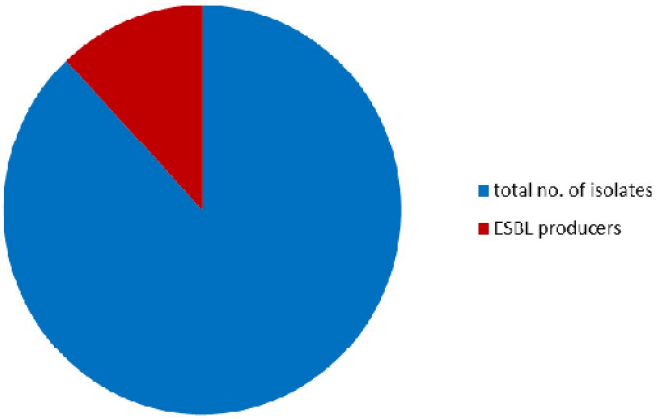

Fig. 1: Percentage of ESBL producers.

All the ESBL-positive *P. aeruginosa* were resistant to more than three drugs (multi-drug resistant). They were 100% susceptible to meropenem; followed by amikacin, which showed good sensitivity (60%) and 50% susceptibility to ofloxacin.

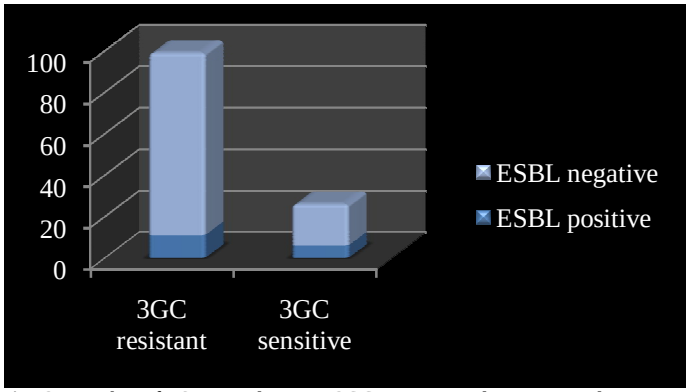

Fig. 2: Number of ESBL producers in 3GC resistant and sensitive isolates.

DISCUSSION

This study detected 13.6% ESBL production among *P. aeruginosa* isolates. ESBL-mediated resistance to 3GC in *P. aeruginosa* in this study is lower than the study by Mathur *et al.* (Mathur *et al.*, 2002) 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. (Nathisuwan *et al.*, 2001) In this study, meropenem was the only drug effective against all ESBL producers. This is similar to various other studies, which also reported a rate of 100% sensitivity to meropenem. (Thomson and Sanders 1992) (Abigail *et al.*, 1995) 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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