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Original Research Article

ESBL Producing *Pseudomonas Aeruginosa*: A Threat to Patient Care

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ABSTRACT

Background- *Pseudomonas aeruginosa*, well known as opportunistic pathogen, has been implicated in life threatening nosocomial infections in recent years. Resistance of *Pseudomonas aeruginosa* strains to the oxyimino cephalosporins may be caused by production of extended-spectrum β-lactamases (ESBLs).
Aim and objective- To detect the incidence of ESBL producing *Pseudomonas aeruginosa* strains isolated from different clinical specimens and to study the antibiotic susceptibility profile of ESBL producing *Pseudomonas aeruginosa* strains.
Material and methods- Various clinical specimens received in Microbiology laboratory were processed and *Pseudomonas aeruginosa* was identified as per standard procedure. A total number of 150 clinical isolates of *Pseudomonas aeruginosa* were included in the study. Antimicrobial susceptibility test was performed for all strains by Kirby – Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI, 2016) Guidelines. All 150 isolates were subjected for ESBL screening test and confirmatory Combined Disc method and E Test.
Observation and results- Out of 150 *Ps. aeruginosa* strains 90 (60%) were ESBL producers. The highest antibiotic susceptibility was observed with Imipenem (87.8 %) and the maximum 41.4% of ESBL producer strains were isolated from pus and wound swab.
Conclusion- Early detection of ESBL producing *Pseudomonas aeruginosa* is the need of the hour for effective treatment of patients and prevention of spread of ESBL producing strains in health care set up.

Key words: Pseudomonas aeruginosa, antibiotic susceptibility test, ESBL production, combined disc method, E test.

INTRODUCTION

Pseudomonas aeruginosa is the 4th most commonly isolated nosocomial pathogen causing 10% of all hospital acquired infections. [1] They are commonly responsible for infections like ventilator associated pneumonia (VAP), burn wound infections, urinary tract infections, surgical site infections etc. Recently, it has been observed that *Pseudomonas aeruginosa,* a well known opportunistic pathogen, has been implicated in life threatening nosocomial infections due to its inherent resistance to many antibiotics. Not only that, *Pseudomonas aeruginosa* can also develop acquired or adaptive resistance to different antibiotics especially by producing different β lactamases, efflux pumps or porins. The infections can be particularly severe and difficult to treat in patients with impaired immune systems, such as neutropenic or cancer patients. [2]

Ps. Aeruginosa can produce all major classes of β lactamases (A, B, C & D). Extended Spectrum β-lactamases (ESBLs) belong to Amber class A according

to molecular classification. [3] ESBLs are plasmid mediated β-lactamases that mediate resistance to extended-spectrum cephalosporins (ESCs) such as Cefotaxime, Ceftriaxone and Ceftazidime and the monobactam aztreonam [4] and have no effect on Cephamycins and Carbapenems. The ESBLs hydrolyze oxyimino cephalosporins by cleaving structural β-lactam ring but ESBLs are inhibited by beta lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam. They have been reported increasingly to be produced by the members of Family Enterobacteriaceae. In fact, ESBLs were first reported in *Klebsiella pneumoniae* from Germany in 1983. [5] Being plasmid mediated, ESBLs are easily transmitted to other bacteria that would favor not only the dissemination of resistance to beta-lactams but also to other commonly prescribed antibiotics e.g. Aminoglycosides, Sulphonamides, Quinolones etc.. This is due to the fact that plasmid carrying ESBLs often carries resistance genes to various antipseudomonal antibiotics along with ESBL gene. [6] Hence, there is limitation of therapeutic options because of increased incidence of ESBL producing strains among clinical isolates. Though 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 *Ps. aeruginosa* and other nosocomial pathogens, it is proved to have spread to organisms other than *Enterobacteriaceae* also. [6-8] ESBL producing *Ps. aeruginosa* strains are probably more prevalent than currently recognized, because of false sensitive zone in routine antibiotic susceptibility test). [9]

The rationale of this study was to detect the ESBL producing strains to treat the patients effectively and to prevent the spread of these strains in our hospital.

Hence, the present study was conducted to detect the incidence of ESBL producing *Ps. aeruginosa* strains isolated from different clinical specimens and to study the antibiotic susceptibility profile of ESBL producing *Ps.* aeruginosa strains.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology. Various clinical specimens e.g. urine, blood, sputum, pus and wound swab, CSF, medical devices and other body fluids received from patients attending Indoor patient Department (IPD) and Outdoor Patient Department (OPD) of our hospital and *Pseudomonas aeruginosa* was identified as per standard microbiological procedure. [10] A total number of 150 characterised strains of *Pseudomonas aeruginosa* isolated from different clinical specimens were included in the study. *Pseudomonas aeruginosa* ATCC 27853 was used as control strain.

Antibiotic susceptibility test for Amikacin (AK-30 µg), Ciprofloxacin (CIP-10 µg), Netilin (NET-30 µg), Imipenem (IPM-10µg), Meropenem (MRP-10 µg) and Ceftazidime(CAZ-30µg) were done by Kirby –Bauer disc diffusion method [11] as per CLSI guidelines, 2016 [12]. Lawn culture of test strains (turbidity adjusted to 0.5 McFarland) was done on Mueller Hinton (MH) agar plate. Then with all aseptic precaution the antibiotic discs were put and the plates were incubated at 37^0C overnight and the results were noted.

For screening of ESBL producers, we employed the same criteria laid down for Enterobacteriaceae (CLSI, 2016), as the principle remains the same for Pseudomonas also. CLSI 2016 has recommended the use of any of the following antibiotics discs of Ceftazidime, Aztreonam, Cefotaxime and Ceftriaxone for ESBL screening test. All 150 isolates were subjected for ESBL screening test using Ceftazidime (30µg) disc. [12] Isolates were considered a potential ESBL producer if the zone of inhibition for Ceftazidime was <22mm.

As ESBL producing strains can give a false sensitive zone in disc diffusion method, all 150 strains were tested for ESBL

Phenotypic confirmatory test i.e. combined Disc method as recommended by Clinical Laboratory Standards Institute, 2016. [12,13]

Combined disc method

A lawn culture of *Pseudomonas aeruginosa* strain with turbidity 0.5 McFarland was done on Mueller Hinton agar plate. Then Ceftazidime (CAZ-30µg) disc alone and Ceftazidime with clavulanic acid (CAC-30/10µg) were placed at a distance. Then the MH plates were incubated aerobically at 37^{0}C overnight. A ≥ 5mm increase in zone diameter for Ceftazidime with clavulanic acid (CAC) in comparison to the zone diameter of Ceftazidime alone was taken as positive for ESBL production. The increase in the zone diameter was due to the inhibition of the ESBL by clavulanic acid.

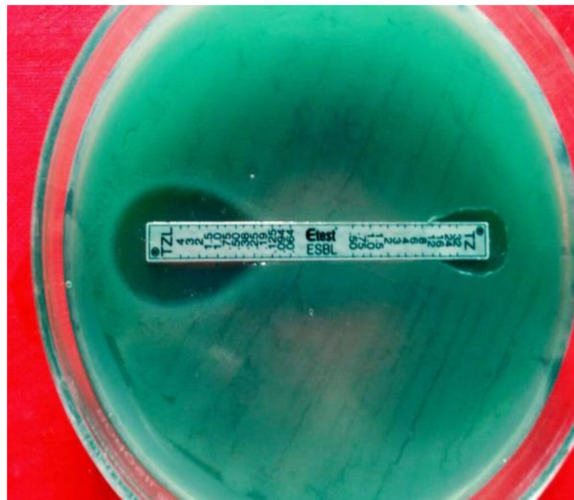
E TEST

All 90 ESBL producing *Pseudomonas aeruginosa* strains detected by combined disc method, were also confirmed by putting E-test ESBL strip (bioMerieux). The Etest 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. In this method lawn culture of test strain (turbidity adjusted to 0.5 McFarland) was done on a Mueller Hinton agar plate. With all aseptic precaution, the ESBL E-test strip was placed onto the inoculated plate. After overnight incubation at 37^{0}C, the zone of inhibition was read from two halves of the strip. As per manufacturer's instruction, MIC ratio of Ceftazidime/ Ceftazidime plus clavulanic acid (TZ/TZL) ≥ 8 or deformation of ellipse or phantom zone present was considered as positive for ESBL production.

OBSERVATION AND RESULTS

Out of 150 *Ps. aeruginosa* isolated, 94 (62.7 %) strains were screen positive by ESBL screening test on the basis of zone of inhibition < 22mm by Ceftazidime (30µg) disc. Out of these 94 screen positive strains, 11 strains were non ESBL producers by confirmatory combined disc method and 7 strains showed false sensitivity zone in routine antibiotic susceptibility test. Hence, 90 (60%) *Pseudomonas aeruginosa* strains were found to be ESBL producers by confirmatory combined disk method (Photograph1). All these 90 *Pseudomonas aeruginosa* strains were also E-Test positive. (Photograph 2).

Photgraph 1- Combined Disc Method



Photgraph 2 - E Test

Photograph 2 shows in Etest, Ceftazidime (Tz) MIC was 12 µg/ml and Ceftazidime/clavulanic acid (TZL) was 0.25 µg/ml.

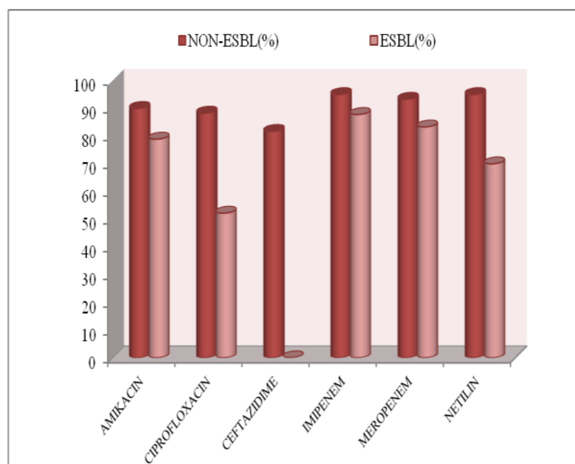


Figure1: Comparison of Antibiotic Susceptibility Profile of ESBL (n=90) & Non-ESBL (n=60) producing *Pseudomonas aeruginosa* strains.

Out of 150 Pseudomonas aeruginosa strains studied, 90 (60%) were ESBL producers and 60 strains were non ESBL producers. The highest sensitivity was observed for Imipenem in ESBL producers (87.8 %) & in non-ESBL producers (95 %) respectively, whereas the lowest sensitivity was observed for Ceftazidime in ESBL producers (0%) and in non-ESBL producers (81.7%) (fig1). Out of 90 ESBL producing strains, 52.2% strains were sensitive to Ciprofloxacin, compared to 88.3% Non-ESBL producing strains sensitive to Ciprofloxacin.

Table1: Isolation of ESBL producing Ps, aeruginosa from different clinical specimens (n=90)

Specimen	ESBL	
	No.	%
Pus & wound swab	37	41.1
Urine	18	20
Blood	17	18.8
Body fluids	9	10
Medical devices	4	4.4
Others*	5	5.5

Others* include sputum (2), tracheal secretion (2), vaginal swabs (1) etc.

In the present study, maximum numbers of ESBL producing *Pseudomonas aeruginosa* strains were isolated from pus and wound swab (41.1%), followed by urine (20%), blood (18.8%), cerebrospinal fluid (6%) and medical devices (4.4%).

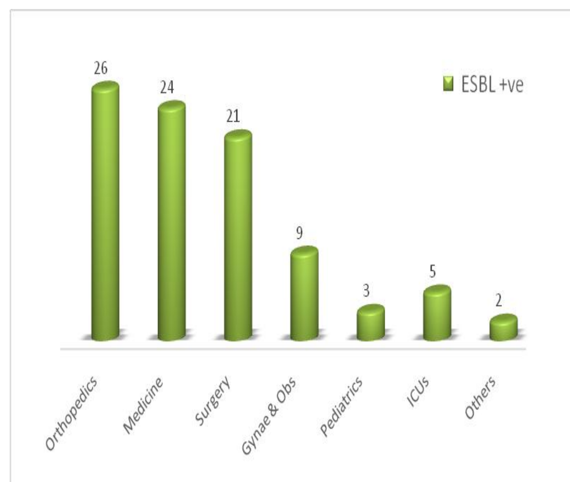


Figure 2: Isolation of ESBL producing *Pseudomonas aeruginosa* from different clinical specialities (n=90)

Though the ESBL producing *Pseudomonas aeruginosa* strains were isolated from most of the wards, the maximum number of strains were isolated from Orthopedics ward (28.8%) followed by Medicine ward (26.6%). Others include 2 OPD patients who were operated in our hospital and had discharge from wound.

DISCUSSION

Pseudomonas aeruginosa has a high resistance to antibiotics and is a common cause of morbidity and mortality in hospitalized and immunocompromised patients. [14] Infections caused by *Pseudomonas aeruginosa* are difficult to treat as the majority of isolates show varying degrees of inherent resistance. Acquired resistance is also reported by the production of newer β-lactamases such as ESBL, AmpC β-lactamase and metallo beta-lactamase enzymes. [15]

In the present study out of 150 *Pseudomonas. aeruginosa* strains studied, 90 (60%) strains were ESBL producers which correlated well with other studies as 57% and 50% respectively [14,16] The incidence of ESBL in other studies conducted by V. Sudha Rani et al. (2016) and Varun Goel et al. (2013) were 37.3 % [17] and 42.3 % [18] respectively. In the present study, imipenem and meropenem showed good antipsuedomonal activity. A similar observation was made by Jaykumar S (19) while a higher degree of carbapenem

resistance was noted by Varaiya et al. [20] The ESBL producing Pseudomonas aeruginosa isolates showed co-resistance against most of the antibiotics tested. This is in harmony with most of the recent findings of Bandekar et al., 2011 [21] and Begum et al., 2013 [22]

Though the carbapenems are kept as last resort for the treatment of patients infected with strains producing ESBLs, their indiscriminate use has caused increased carbapenem resistance. ESBLs should be screened in Clinical Microbiology Laboratory as a routine procedure for effective treatment of the patient. A nationwide antibiotic policy should be implemented to prevent the overuse and misuse of antibiotics. Overuse and misuse of antibiotics have to be stopped to prevent the development of antibiotic resistance. The implication of this study is, if early detection of ESBL producing *Ps. aeruginosa* can be done, the patient can be treated effectively and infection control measures can be taken to prevent their spread in Health care set up. Our study mainly highlights the challenge imposed by *Pseudomonas aeruginosa* as the ESBL production has limited the therapeutic choices. Therefore, the improvement in antibiotic prescription policies and infection control programs are of high necessity to prevent the spread of such resistant infectious strains.

CONCLUSION

Hence, to conclude, all *Pseudomonas aeruginosa* strains, which is one of the most common isolates in Clinical Microbiology Laboratory should be tested phenotypically for ESBL production routinely. Standard precautions must be followed meticulously to prevent the spread of ESBL producing strains in Health care set up.

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