

Prevalence of Extended Spectrum Beta-Lactamases and AmpC Beta-Lactamases Producing *Pseudomonas Aeruginosa* in an Intensive Care Unit of Tertiary Care Hospital of North India

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ABSTRACT

Introduction: The present study was designed to detect the extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases producing *Pseudomonas aeruginosa* and to evaluate the antimicrobial susceptibility pattern.

Materials and Methods: One hundred fifty nine isolates of *P. aeruginosa* were analyzed for the presence of ESBL and AmpC enzymes by double disc diffusion and AmpC disc methods. Antibiotic resistance pattern of ESBL and AmpC positive *P. aeruginosa* were determined.

Results: Of the 159 isolates tested, 17 (11%) and 4 (3%) were found to be positive for ESBL and AmpC production respectively. All the ESBL and AmpC producing *P. aeruginosa* isolates were multi-drug-resistant. Isolates were 100% sensitive to polymixin B and showed good susceptibility to imipenem.

Conclusion: From this study, we conclude the presence of ESBL and AmpC positive *P. aeruginosa* in our hospital. This has important implications as carbapenems remain the only

choice of treatment for infections caused by these organisms. The control measures include judicious use of antibiotics and implementation of appropriate infection control measures to control the spread of these strains in the hospital.

Keywords: Extended Spectrum Beta Lactamases, *Pseudomonas Aeruginosa*, Amp C Beta Lactamases.

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INTRODUCTION

Pseudomonas aeruginosa is most common opportunistic pathogen and has ability to propagate on medical devices, disinfectants and hospital environments leading to hospital acquired infections.¹ *P. aeruginosa*, is intrinsically resistant to many antimicrobial agents, thus the infections caused by it causes serious therapeutic challenges both in the community and healthcare centers. In addition, it acquires resistance to multiple classes of antimicrobial agents, even during the course of treatment.^{2,3} Resistance may arise to extended spectrum cephalosporins from the over expression of the naturally occurring cephalosporinase or acquired beta-lactamases such as extended-spectrum β -lactamases (ESBL) and AmpC β -lactamases (AmpC).⁴ Among the various β -lactamases, Ambler class A extended-spectrum β -lactamases (ESBLs) are reported as rapidly growing enzymes in clinical isolates of *P. aeruginosa*. ESBLs are capable of hydrolyzing penicillins, cephalosporins, and aztreonam (except for cephamycins or carbapenems). These enzymes are inhibited by β -lactamase inhibitors such as clavulanic acid.^{5,6} AmpC β -lactamases hydrolyze cephamycins (e.g. cefoxitin and cefotetan), oxyimino-cephalosporins (e.g. ceftazidime, cefotaxime,

and ceftriaxone), and monobactams (e.g. aztreonam).⁷ In *P. aeruginosa*, decreased susceptibility to the extended-spectrum cephalosporins such as ceftazidime mostly results from over expression of the naturally occurring AmpC β -lactamases.⁸ With the increase in occurrence and types of these multiple beta-lactamase enzymes, early detection is crucial for the initiation of proper antibiotic therapy and infection control policy. Therefore, this prospective study was conducted over a period of one year from February 2012 to January 2013 to determine the prevalence of ESBL and AmpC β -lactamases production in *Pseudomonas aeruginosa* isolated from the patients admitted in intensive care unit of a tertiary care hospital of north India.

MATERIALS AND METHODS

A total of 159 clinical isolates *P. aeruginosa* were obtained from various clinical samples such as respiratory, blood, pus, drain fluid, body fluids, central line tip and tissues were received in the department of microbiology. They were processed as per standard microbiology protocols. All the *P. aeruginosa* isolates that were obtained as a pure and predominant growth from the clinical

specimens were only considered for the present study. The organisms were identified based on the colony morphology and biochemical reactions.⁹

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antibiogram of the isolates were determined by the standard Kirby Bauer's disc diffusion method. The following antibiotics discs (Hi-Media, India) were used such as, gentamicin (10µg), amikacin (30µg), ceftazidime (30µg), ciprofloxacin (5µg), piperacillin (100µg), piperacillin-tazobactam (100µg/10µg), cefoperazone-sulbactam (75µg/30µg), imipenem (10µg), cefepime (30µg), polymixin B (300 µg), aztreonam (30µg), tobramycin (10µg) disc were used. For the quality control *E.coli* ATCC 25922 and *Pseudomonas* ATCC 27853 were used as control organisms as per CLSI recommendation.^[9-10]

DETECTION OF ESBL

Double Disc Diffusion Test^{9,10}

The lawn culture of test organism was done on MHA. The discs applied of ceftazidime alone (30 µg) and in combination with clavulanic acid (10 µg) were applied on the plate. The discs were placed in such a way that the centre to centre distance between the discs is 30 mm. The MHA plate was incubated at 35°C for 24 hours as per the method given for Enterobacteriaceae for detection of ESBL production in CLSI guidelines.

Interpretation - An expansion of zone of inhibition ≥ 5 mm around the combination disc was considered a positive result.

DETECTION OF AmpC¹¹

AmpC Disc Test

A lawn culture of *E.coli* ATCC 25922 was prepared on a MHA plate and a cefoxitin disc (30 µg) was placed on the plate. AmpC disc was moistened with 20 µl of sterile saline & inoculated with colonies of test organism. This disc was then placed beside the cefoxitin disc (almost touching) with the inoculated side facing downwards. The MHA plate was incubated at 37°C for 24 hours.

Interpretation – If there was flattening or indentation of cefoxitin inhibition zone, it was considered as an AmpC producer.

RESULTS

The results have been depicted in Table 1 and figure 1-3.

Out of 159 isolates tested, 65 (41%) were resistant to ceftazidime and 8 (5%) resistant to cefoxitin, were tested for ESBL and AmpC production by Double Disc Diffusion Test and AmpC disc test. ESBL and AmpC production was seen in 17 (11%) and 4 (3%) respectively. Co-production was seen in 0.6% of isolates, 10% and 1.2% were pure ESBL and AmpC producers. ESBL producing *P. aeruginosa* showed 100% resistance toward third generation cephalosporins, piperacillin and aztreonam, whereas showed high degree of resistance toward ciprofloxacin (53%) as compared to non ESBL producers. AmpC producing *P. aeruginosa* showed high degree of resistance toward third generation cephalosporins, aminoglycosides, piperacillin and beta-lactamases combination, whereas least resistance was shown toward imipenem (25%).

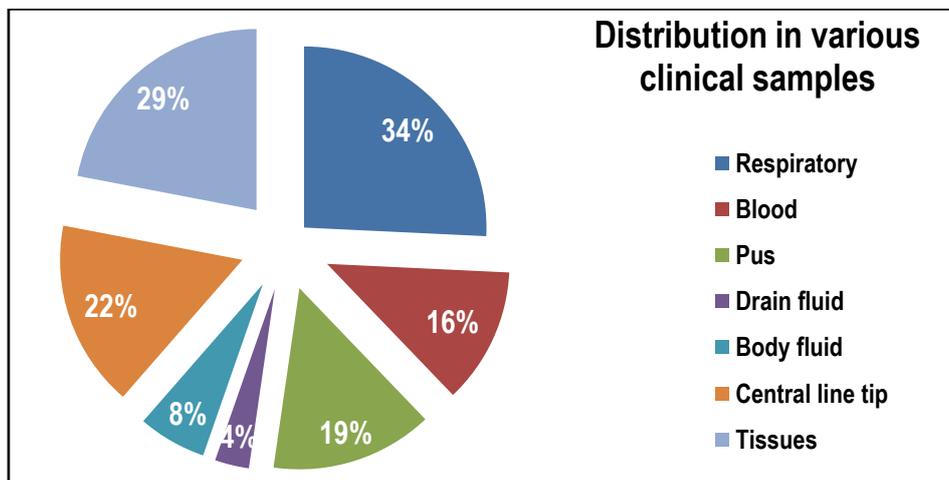


Fig 1: Distribution in various clinical samples

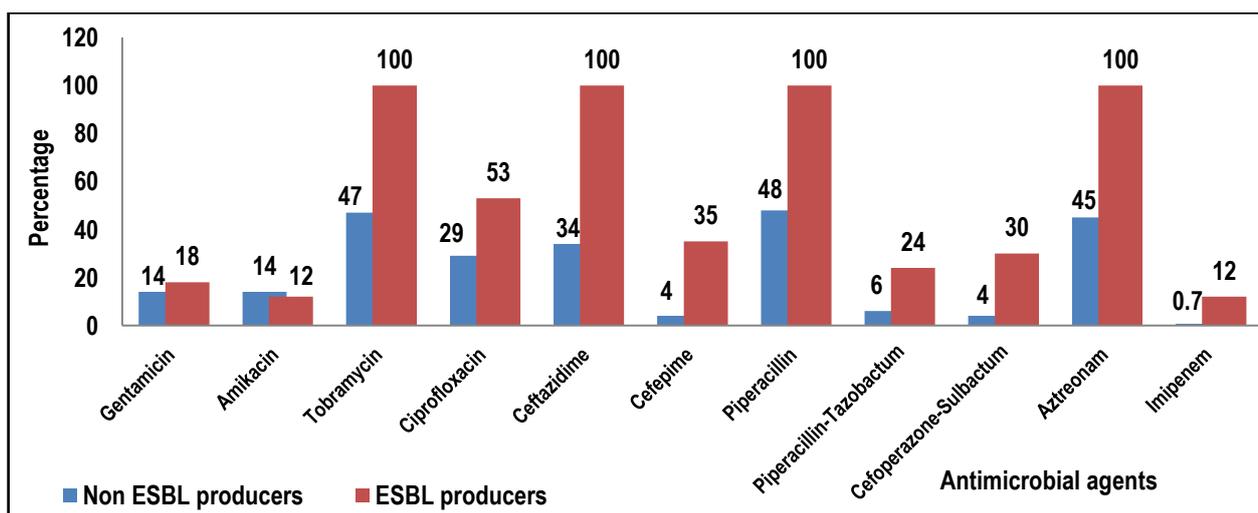


Fig 2: Showing comparison of antimicrobial resistance patterns between ESBL and Non ESBL producing isolates

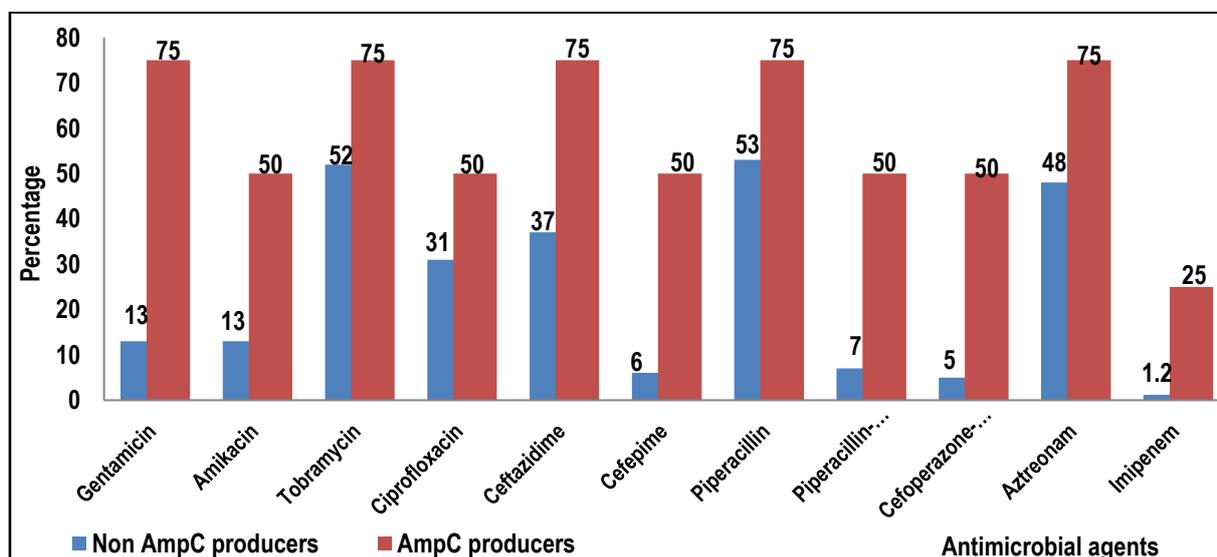


Fig 3: Showing comparison of antimicrobial resistance patterns b/w Amp C and Non Amp C producing isolates

Table 1: Distribution of ESBL And AmpC B-Lactamase And Their Co-Production

Organism	ESBL+ AmpC	Pure ESBL	Pure AmpC	No mechanism
<i>P. aeruginosa</i> (159)	1 (0.6)	16 (10)	2 (1.2)	140 (88)

DISCUSSION

Multiple β -lactamase producing *P. aeruginosa* if remain undetected cause major therapeutic failure and pose significant clinical challenges. The current CLSI guidelines do not describe a method for detection of AmpC β -lactamases. AmpC disc test was originally introduced to detect plasmid-mediated AmpC β -lactamases.¹² However, Black et al. reported the detection of chromosomally mediated inducible AmpC β -lactamases in a number of bacteria including *P. aeruginosa*, by the AmpC disc test.¹¹ In Our study, we reported low incidence of ESBL (11%) production in *Pseudomonas aeruginosa* which was in harmony with the study conducted by Aggarwal et al and Aishwarya J et al, who reported 20.2% and 13.6% of ESBL production in *Pseudomonas aeruginosa*.^{13,14} In *P. aeruginosa* the resistance to ceftazidime is increasing day by day at an alarming rate, complicating the clinical management of patients infected with such resistant strains. ESBL-mediated resistance in *P. aeruginosa* as reported by Goel et al, Mathur et al and Easwaran S et al, was (42.3%), (64%) and 95.7%¹³ was much higher than that reported in the present study.¹⁵⁻¹⁷ AmpC beta-lactamases are largely unknown due to difficulties in the phenotypic detection and often misidentified as ESBLs. In our study the occurrence of AmpC production was only 3% which was quite low similar to Rawat et al who reported 6% of AmpC production in *Pseudomonas*.¹⁸

The coexistence of AmpC and ESBL were observed in 0.6% which corroborates the findings of Upadhyay et al who reported 3% (19) as compared to Easwaran S et al who reported higher incidence of 68%.¹⁵ Our study showed much lower prevalence of ESBL and AmpC co production. The only betalactams active against co-AmpC and ESBL producers are carbapenems.

CONCLUSION

Our study underlines the unique problem of ESBL and AmpC 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. Empirical therapy followed by definitive treatment after the antimicrobial susceptibility testing and local antibiogram would serve as guide for the clinicians for judicious use of antimicrobial agents

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