ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

AmpC β-lactamases among *Pseudomonas* and *Acinetobacter* species isolates, from a tertiary hospital of North India

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Manuscript Info

Manuscript History:

Received: 10 December 2014
Final Accepted: 26 January 2015
Published Online: February 2015

Key words:
AmpC, *Pseudomonas, Acinetobacter,* beta-lactamases

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Abstract

Background: Production of AmpC beta-lactamases in isolates of *Pseudomonas* and *Acinetobacter* species have resulted into therapeutic failure and consequently increased mortality and morbidity. **Objective:** As the reports regarding the prevalence of AmpC beta-lactamases in *Pseudomonas* and *Acinetobacter* are fragmentary from India, the present study was conducted to determine occurrence of AmpC beta-lactamases in these pathogenic non-fermenters. **Materials and methods:** Ninety non-duplicate *Pseudomonas* and *Acinetobacter* isolates, resistant to any of the third-generation cephalosporin were collected during October 2010 to September 2011. Antibiotic-susceptibility test was performed by disc diffusion method. Cefoxitin-resistant isolates were subjected to modified-three dimensional extract test for confirmation of AmpC beta-lactamases. **Results:** *Pseudomonas* spp. showed highest resistance to cefoxitin (91.78%) followed by ceftriaxone (78.05%), cefepime (72.41%) and then cefoperazone/sulbactam (56.31%); while in case of *Acinetobacter* spp. maximum resistance was noted for cefoxitin (94.12%) and cefotaxime (94.12%). Resistance against cefepime was noted in 88.24% isolates followed by cefoperazone/sulbactam (71.43%). 83 (92.22%) isolates showed resistance against cefoxitin. Out of total 83 cefoxitin-resistant isolates, 66 (79.52%) were tested positive by modified three-dimensional extract test. **Conclusion:** Study shows high prevalence of AmpC beta-lactamases in cefoxitin-resistant isolates of *Pseudomonas* and *Acinetobacter* species.

INTRODUCTION:

Over past decade, non-fermenters have emerged as important opportunistic pathogens in increasing population of patients.[1, 2] The most commonly occurring non-fermenting Gram-negative bacilli belong to the genus *Pseudomonas* and *Acinetobacter*.[3] They are widely distributed in nature and colonize on the healthy and damage tissue. Antimicrobial resistance among nosocomial isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, complicates the treatment of infections and adversely affects clinical outcomes and patient treatment costs.[4, 5]

Production of beta-lactamases is the predominant mechanism of resistance to β-lactam antibiotics in Gram-negative bacteria.[6] Among the β-lactamases, the production of ESBLs and AmpC β-lactamases are the most common.[7]

AmpC β-lactamases are class C or group I cephalosporinases that mediate resistance to a wide variety of β-lactam antibiotics including alpha methoxy β-lactams such as cefoxitin, narrow- and broad-spectrum cephalosporins, aztreonam, and are poorly inhibited by β-lactam inhibitor combinations.[8] As AmpC beta-lactamases exhibit a wide range of resistance to many currently available antibiotics, their early detection is crucial, the benefits of which include implementation of proper antibiotic therapy and infection control policy. Lack of standard CLSI guidelines for detection of AmpC beta-lactamases also hinders their proper identification.

Since the information on the documentation of AmpC β-lactamases among *Pseudomonas* and *Acinetobacter* spp. are fragmentary in India; this study was designed to analyze the occurrence of AmpC β-lactamases in isolates of *Pseudomonas* and *Acinetobacter* spp., from a tertiary care hospital in North India.

MATERIALS AND METHODS:

The present prospective study was conducted in the department of Microbiology, J. N. Medical College, Aligarh Muslim University, Aligarh, during a period of one year (October 2010 to September 2011). A total of ninety isolates (73 *Pseudomonas* and 17 *Acinetobacter* spp.), that were found resistant to any of the third-generation cephalosporin, were randomly selected from clinical specimens received for routine culture and susceptibility testing in the clinical microbiology lab. These samples included pus, CSF, endotracheal tip, ear swab, conjuctival swab, and semen. Highest number of samples included in the study were received from surgery (34.33%), followed by orthopaedics (33.33%), paediatric (12.22%), medicine (6.67%), gynaecology (3.33%), ENT (3.33%) and ophthalmology (2.22%). Least number of samples was received from patients suffering from pulmonary infection (1.11%) and those admitted to intensive care unit (1.11%). Orthopaedic patients were the major source of pus samples (40.85%).

***Antimicrobial susceptibility testing (AST)*:**
Antimicrobial susceptibility testing was performed and results were interpreted as per CLSI guidelines.[9] Antibiotic discs were procured from HiMedia Laboratories Pvt. Ltd, India.

Following antibiotic discs were used:

(i) For *Pseudomonas* spp.: ceftazidime (30µg), cefoperazone (75µg), gatifloxacin (5µg), cefepime (30µg), cefoxitin (30µg), ceftriaxone (30µg), levofloxacin (5µg), aztreonam (30µg), cefoperazone/sulbactam (75/75µg), tobramycin (10µg), amikacin (30µg), gentamicin (10µg), ceftriaxone/sulbactam (30/15µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg), polymixin B (300 units) and imipenem (10µg).

(ii) For *Acinetobacter* spp.: ceftazidime (30µg), cefoperazone (75µg), gatifloxacin (5µg), cefepime (30µg), cefoxitin (30µg), ceftriaxone (30µg), cefotaxime (30µg), levofloxacin (5µg), aztreonam (30µg), cefoperazone/sulbactam (75/75µg), tobramycin (10µg), amikacin (30µg), gentamicin (10µg), ceftriaxone/sulbactam (30/15µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg) and imipenem (10µg).

***Screening for AmpC producers*:**
Cefoxitin discs were used to screen AmpC producers, by disc diffusion method as described previously by Shahid *et al.* [10]

Phenotypic detection of AmpC-producers* by *Modified three-dimensional extract test (MTDET):
MTDET as described by Shahid *et al.* was performed on all cefoxitin-resistant isolates.[10] In this method, 10-15 mg of bacterial wet weight was scraped from culture plate and inoculated into 0.5 ml of peptone water in a sterile micro-centrifuge tube. Tubes containing bacterial suspension were kept in incubator at 37°C for 1 hour. After incubation, repeated freezing thawing the tubes, at least five times, did crude enzyme extract preparation. The surface of Mueller-Hinton plate was inoculated with *E. coli* strains ATCC 25922, as described for the standard disc diffusion.

A 30 µg cefoxitin disc was placed on the inoculated dried agar plate. With a sterile scalpel, a linear trench (3 cm × 1mm) beginning at a distance of 5 mm from the edge of the disc was cut in the agar in an outward radial direction. By using a pipette 50 µl of enzyme preparation was dispensed into the trench, beginning near the disc and moving outward, avoiding overfilling. The inoculated media was incubated overnight at 37°C. Enhanced growth of the surface organism at the point where the trench intersected the zone of inhibition or indentation in the zone of inhibition was considered a positive test result and was interpreted as evidence for the presence of AmpC β-lactamase.

RESULTS:

The number of infections caused by AmpC β-lactamase-producing organism, particularly *P. aeruginosa*, is on the rise and poses a threat to patients due to treatment failure.[11] Failure to detect these AmpC beta-lactamase producing strains by routine susceptibility tests, performed by clinical laboratories, may lead to irrational usage of antibiotics and consequently therapeutic failure.[12] This emphasizes the need for detection of isolates that produce this enzyme so as to overcome therapeutic challenge caused by these drug-resistant isolates.

Antimicrobial susceptibility testing was performed for all the 90 isolates of *Pseudomonas* and *Acinetobacter* spp. Apart from imipenam, most of the isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were sensitive to polymixin B and piperacillin/tazobactam respectively. Among cephalosporins, *Pseudomonas* spp. showed highest resistance to cefoxitin (91.78%) followed by ceftriaxone (78.05%), cefepime (72.41%) and cefoperazone/sulbactam (56.31%). 72.22% isolates of *Pseudomonas* spp. were resistant to amikacin while 68.50% isolates showed resistance against gentamicin. Among fluoroquinolones, resistance against gatifloxacin was noted in 68.19% isolates. Furthermore, only 2.74% isolates were resistant to imipenam. Details of antimicrobial resistance pattern of the tested isolates of *Pseudomonas* spp. is shown in **Figure 1**.

In case of *Acinetobacter* spp., maximum resistance was noted for cefoxitin (94.12%) and cefotaxime (94.12%). Resistance against cefepime was noted in 88.24% isolates followed by cefoperazone/sulbactam (71.43%). In *Acinetobacter* spp. too, resistance against aminoglycosides was significant. Resistance against amikacin and gentamicin was 87.5% and 85% respectively. Resistance to gatifloxacin was also observed in 69.23% isolates. Apart from this, during the study period, none of the isolates of *Acinetobacter* spp. showed resistance to imipenam. Graphical representation is shown in **Figure 2**.

In the present study isolates showing resistance against cefoxitin were assumed to be potential AmpC β-lactamase producer. Out of 90 isolates, 83 (92.22%) showed resistance against cefoxitin. Taking into consideration, *Pseudomonas* and *Acinetobacter* separately, this resistance pattern was 91.78% and 94.12% respectively (**Table 1**). All the cefoxitin-resistant isolates were further tested by modified three-dimensional extract test for confirmatory phenotypic detection of AmpC β-lactamases (**Figure 3**). Out of total 83 cefoxitin resistant isolates, 66 (79.52%) were tested positive by modified three-dimensional extract test (**Table 2)**.

Table 1. Cefoxitin-resistance pattern in *Pseudomonas* and *Acinetobacter* spp.

Organism	cefoxitin disc	
	Sensitive	**Resistant**
***Pseudomonas* spp. (n=73)**	6 (8.22%)	67 (91.78%)
***Acinetobacter* spp. (n=17)**	1 (5.88%)	16 (94.12%)
Total (n=90)	7 (7.78%)	83 (92.22%)

Table 2: Number of AmpC-producers detected by Modified three-dimensional extract test

Organism (cefoxitin resistant isolates)	Test	
	Modified three-dimensional extract test	
	Positive	Negative
***Pseudomonas* spp. (n=67)**	55 (82.09%)	12 (17.91%)
***Acinetobacter* spp. (n=16)**	11 (68.75%)	5 (31.25%)
Total (n=83)	66 (79.52%)	17 (20.48%)

Figure: 1. Antibiotic susceptibility pattern of *Pseudomonas* spp.

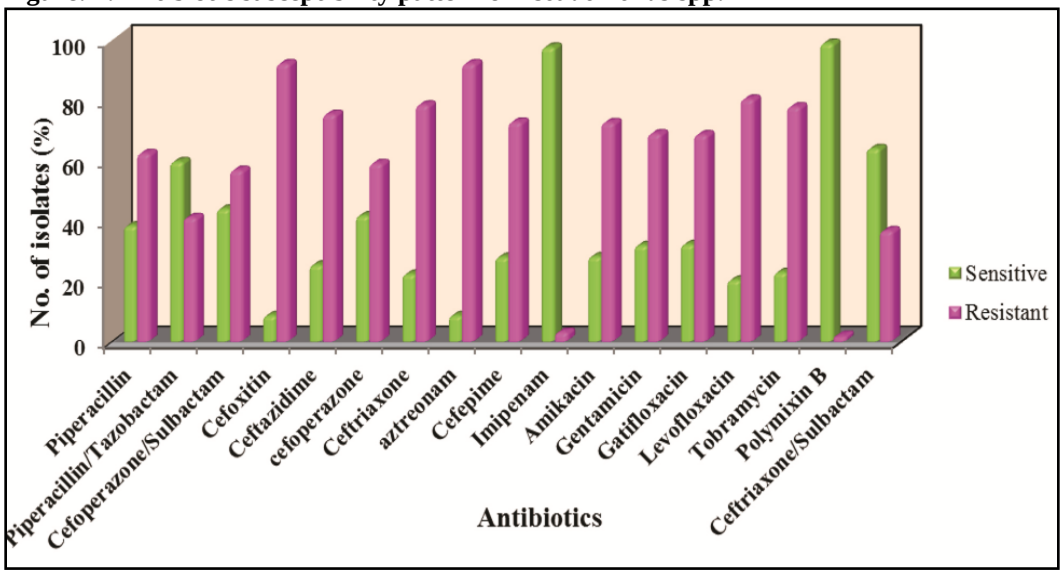

Figure: 2. Antibiotic susceptibility pattern of *Acinetobacter* spp.

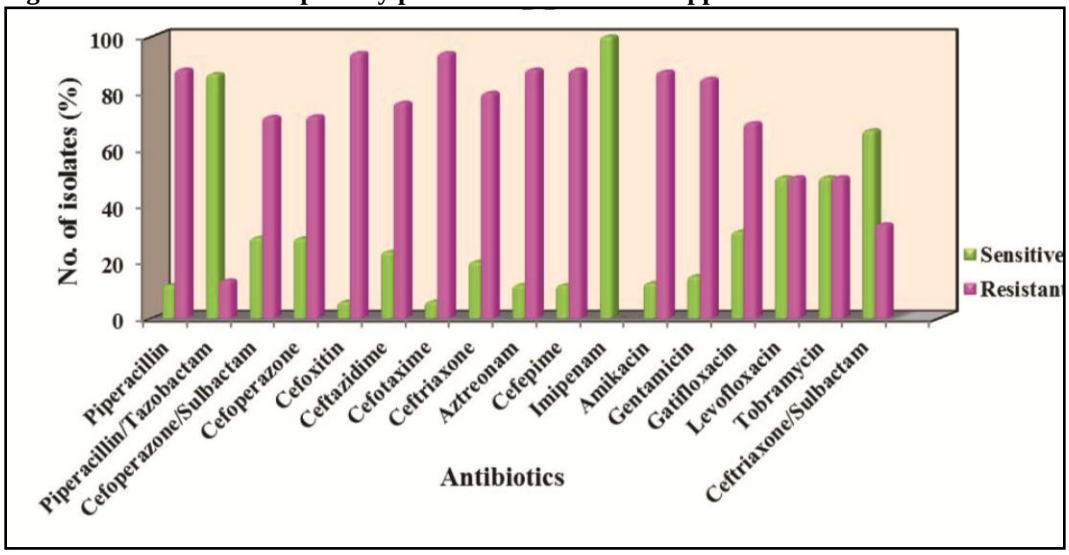

Figure: 3. A representative isolate showing distortion of the zone of inhibition, indicating AmpC-producer by modified three-dimensional extract method

DISCUSSION:

There are few reports regarding the prevalence of AmpC beta-lactamases in *Pseudomonas* and *Acinetobacter* spp. Apart from fragmentary reports, the actual prevalence is still unknown. One important fact is that, there is currently no clear consensus regarding guidelines for phenotypic screening or confirmatory tests for AmpC β-lactamase-producing organisms.[10] However, surprisingly, we found a high prevalence of AmpC β-lactamase-producing isolates (79.52%) at our centre as compared to earlier studies in India. From North India, 20 % of *P. aeruginosa* (Delhi) and 20.7 % of Gram-negative organisms (Aligarh) and 47.8% *E. coli*, 17.3% *P. aeruginosa*, 13% *K. pneumoniae* (Kolkata) were reported as AmpC β-lactamase producers.[13, 14, 15] 37.50% isolates have been reported as AmpC-producers from Chennai.[16] In another study performed by Singhal *et al*., prevalence of AmpC β-lactamases was reported as 8%.[17] They found 36% of cefoxitin-resistant isolates as AmpC-producers which were further confirmed by three-dimensional extract test and also by AmpC disc test. In 2007, Hemlatha *et al*. from Chennai reported 47.3% AmpC-producers in *Escherichia coli* and *Klebsiella* isolates.[18] One year later, Sinha *et al*. came out with the finding of 24% AmpC-producers in *E. coli* isolates from tertiary care hospital of Jaipur.[19] Bhattacharjee *et al*. reported 22% isolates of *P. aeruginosa* as AmpC β -lactamase-producers.[20] In 2010, Upadhyay *et al*. from Varanasi, reported 59.4% isolates of *P. aeruginosa* as AmpC-producers.[21] In the same year, Mohamudha *et al*. reported 93.6% Gram-negative clinical isolates as AmpC-producers, based on three-dimensional extract method.[22] They reported 66.6% and 55.5% plasmid-mediated AmpC-producers in *Acinetobacter* and *Pseudomonas* respectively.

Based on phenotypic detection methods, Tan *et al*. reported AmpC activity in 49.8% isolates.[23] In Algeria and England, the prevalence of AmpC was reported to be 1% and 7.15% respectively.[24, 25]

Therapeutic failure followed by increased mortality and morbidity in patients infected with beta-lactamase producing strains is a matter of great concern. For treatment of multidrug resistant *A. baumannii*, sulbactam usually as ampicillin/sulbactam is still the most effective therapy.[26]

Our study shows an increasing pattern of resistance towards β-lactam antibiotics, by production of AmpC β-lactamases. The present study emphasizes that phenotypic methods should be carried out to know the prevalence of beta-lactamases. So early identification of isolates harbouring these beta-lactamases is need of time, to halt the indiscriminate use of antibiotics and further spread of these resistant strains.

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