

Frequency and antibiotic susceptibility pattern of Amp C beta-lactamase producing bacteria isolated from a tertiary care hospital of Rawalpindi, Pakistan

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ABSTRACT

Objective: Amp C beta-lactamases are cephalosporinases which hydrolyze cephamycins and are poorly inhibited by clavulanic acid. Amp C beta-lactamases confer resistance to a wide variety of antibiotics and pose both diagnostic and therapeutic challenges. The objective was to detect the frequency and antibiotic susceptibility pattern of Amp C beta-lactamase producing bacteria isolated from a tertiary care hospital of Pakistan.

Methodology: Organisms were isolated from various clinical specimens. First, the screening of the isolates was done by using cefoxitin disc. Screen-positive organisms were subjected to three dimensional extract test for detection of Amp C beta-lactamases.

Results: From a total of 100 organism tested, 64 organisms were positive on cefoxitin screen test. Out of these 64, 40 (62.5%) showed the presence of Amp C beta-lactamase. (*E. coli* n=18, *K. pneumoniae* n=14, *K. oxytoca* n=1, *Enterobacter species* n=5, *Citrobacter freundii* n=2) by three dimensional extract test. The antibiotics found out to show good activity against these resistant bacteria include meropenem and tigecycline. This is the first study to determine the frequency of Amp C beta lactamases from Pakistan.

Conclusion: This study shows a high frequency of Amp C beta-lactamase producing isolates from a hospital, which may lead to serious therapeutic problems.

KEY WORDS: Amp C beta lactamases, Antimicrobial resistance, Three dimensional extract test.

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INTRODUCTION

AmpC β -lactamases are cephalosporinases which belong to the molecular class C as classified by Ambler in 1980 and Group I under a classification scheme of Bush et al.¹ AmpC β -lactamases are more sensitive to inhibition by sulbactam than by clavulanate or tazobactam.² They are clinically significant as they may confer resistance to cephamycins, narrow-, expanded- and broad-spectrum cephalosporins, aztreonam and beta-lactam/ beta-lactamases inhibitor combinations (ampicillin-clavulanic acid, piperacillin-tazobactam, etc).³ The genes for Amp C beta-lactamases are commonly found on chromosomes of several members of family Enterobacteriaceae including *Enterobacter species*,

Shigella, *Providencia*, *Citrobacter freundii*, *Morganella morganii*, *Serratia* and *Escherichia coli*. Plasmid mediated Amp C beta-lactamases have arisen through the transfer of chromosomal genes for the inducible Amp C beta-lactamases on to plasmids. This transfer has resulted in plasmid-mediated Amp C beta-lactamases in *E.coli*, *K.pneumoniae*, *Salmonella spp*, *Citrobacter*, *Enterobacter* and *Proteus mirabilis*.⁴ There are more than 20 different Amp C beta-lactamases which are mediated by plasmids.⁵ Amp C beta-lactamase production is associated with in vitro resistance to all beta-lactam antibiotics except carbapenems and ceftipime. But resistance to carbapenems may be seen and it is associated with loss of outer membrane porins (OMP).⁶

Currently there are no CLSI recommended tests for detection of Amp C beta-lactamases and there is a need to address this issue as much as the detection of ESBLs. Researchers have used various test methods for Amp C beta-lactamase detection, namely the three dimensional extract test⁷, modified double disk test⁸, inhibitor based method using inhibitors like boronic acids⁹, and cefoxitin agar method.¹⁰ The method used by Coudron et al⁷ is considered standard in identification of AmpC beta-lactamases. He used the standard disk diffusion breakpoint for cefoxitin (zone diameter <18 mm) to screen isolates and used a three-dimensional extract test as confirmatory for identification of the isolates that harbor AmpC beta-lactamases.⁷

These resistant organisms are prevalent in large hospitals leading to therapeutic failure. This prompted us to determine the frequency and antibiotic susceptibility pattern of Amp C beta lactamase-producing bacteria isolated from a tertiary care hospital of Pakistan.

METHODOLOGY

Place and duration of the study: The study was carried out from October 2009 to March 2010 at the Department of Microbiology, Army Medical College/ National University of Sciences and Technology (NUST), Rawalpindi, Pakistan, affiliated with a 1100 bedded tertiary care hospital.

Isolation of the organisms: A total of 100 organisms were isolated during the study period. Organisms were identified by standard microbiological procedures (Gram's stain appearance, colonial morphology, catalase test, cytochrome oxidase reaction, motility, routine biochemical tests) and by using API 20 E (Biomérieux).

Screening test for Amp C beta lactamase producers: Isolates were screened for Amp C production by disc

diffusion method using cefoxitin disc (Oxoid, UK). A 30 µg cefoxitin disc was placed on inoculated Mueller-Hinton agar plates (Oxoid, UK). Isolates with zone diameter less than 18mm were selected for Amp C beta lactamase testing.

Three dimensional extract method (3DET): Three dimensional extract method (3DET) as described by Coudron et al (2000) was used to detect for Amp C production.⁷ First, 0.5 McFarland bacterial suspensions were prepared from an overnight culture. 50µl of each of which was inoculated in 10 ml of trypticase soy broth (TSB, Oxoid, UK). TSB along with organisms were incubated at 37°C for 4 hours. The bacterial cells were concentrated by centrifugation and crude enzyme preparations were made by freeze thawing the cell pellets five times. The surface of a Mueller-Hinton agar plate was inoculated with control strain of *Escherichia coli* ATCC 25922. A cefoxitin disc (30µg) was placed in the centre of inoculated agar plates. By using a sterile scalpel blade, a slit beginning 5mm from the edge of the disc was cut in the agar in outward radial direction. 30µl of the enzyme preparation was dispensed into the slit, beginning near the disc and moving in an outward direction, by using a micropipette. Overfill of the slit was avoided. Known Amp C positive strain of *K. pneumoniae* was used as control strain. The inoculated agar plates were incubated at 37°C for 24 hours. The enhanced growth of surface organism at the point where the slit intersected the zone of inhibition was considered a positive three dimensional test and was interpreted as evidence for the presence of Amp C beta lactamases.

All the isolates were simultaneously checked for ESBL production by double disk approximation method of Jarier et al¹¹ and confirmed by Etest (ceftazidime, ceftazidime/clavulanic acid, AB biodisk). Antimicrobial susceptibility of isolates against aminoglycosides, fluoroquinolones, cotrimoxazole, carbapenems, tetracyclines and beta-lactam/beta-lactamase inhibitor combination was tested by using Kirby Bauer disc diffusion technique, according to the CLSI guidelines.¹²

RESULTS

A total of 100 organisms isolated during the study period were tested. The isolates were obtained from the specimens of urine (n=29), pus (n=25), blood (n=15), naso-bronchial lavage (n=12), intravenous catheter tips (n=11) and urinary catheter tips (n=8). Majority of the isolates were recovered from the patients admitted in hospital (78/100), and among them 37 were from patients in intensive care unit.

From a total of 100 organisms tested, 64 isolates (*E.coli* n=32, *K.pneumoniae* n=18, *K.oxytoca* n=6, *Enterobacter species* n=6, *Citrobacter freundii* n=2) were positive on cefoxitin screen test. These 64 isolates were subjected to three dimensional test for Amp C beta lactamase detection. Out of these 64, 40 (62.5%) showed the presence of Amp C beta lactamase (*E.coli* n=18, *K.pneumoniae* n=14, *K.oxytoca* n=1, *Enterobacter species* n=5, *Citrobacter freundii* n=2).

Tigecycline and meropenem were found to be the most active drugs against Amp C beta lactamase producing bacteria. Antibiotics which were least effective included cotrimoxazole, ciprofloxacin, piperacillin-tazobactam and gentamicin. Antimicrobial resistance pattern of Amp C beta lactamase producing isolates is shown in Table-I. Among 40 Amp C beta lactamase producing bacteria, 24 (60%) were co-producing ESBLs.

DISCUSSION

Detection of Amp C production is critical in order to optimize the antibiotic therapy and clinical outcomes. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.¹³ For clinical microbiologists, detection of AmpC-mediated resistance in Gram-negative organisms poses a problem because the phenotypic tests may be misleading resulting in misreporting and treatment failures. The current CLSI does not indicate the screening and confirmatory tests that should be used for the detection of Amp C beta-lactamases.

According to our study, 62.5% of the isolates were positive for Amp C beta lactamases. This shows a very high occurrence of Amp C beta lactamase producing bacteria in our set up. There is paucity of data on frequency of Amp C beta lactamase producing bacteria from our country. Regional data from India reported that among the 135 clinical isolates of Gram-negative bacilli tested, 20.7% were found to harbour Amp C enzymes using a modified three-dimensional test.¹⁴ Hemalatha et al (2007) reported in their study that out of 76 screen-positive 36 (47.3%) were positive for Amp C production.⁹ In a study conducted in China, plasmid-mediated AmpC β -lactamases were found in 10.1% of *K. pneumoniae* (64/637) and in 2.0% of *E. coli* (10/494) strains. According to them, the proportion of plasmid-mediated AmpC-producing strains significantly increased from 2005 (2.6%) to 2006 (9.3%). 24.3% of AmpC β -lactamase producing isolates were co-producing ESBL.¹⁵ Coudron et al reported that out of 683 *E.coli* and 371 *Klebsiella pneumoniae* isolates from US veterans medical

Table-I: Resistance pattern of positive Amp C beta lactamase isolates n=40

Antibiotic	Resistant n (%)
Amikacin	26 (65)
Gentamicin	30 (75)
Ciprofloxacin	30 (75)
Minocycline	27 (67.5)
Tigecycline	11 (27.5)
Cotrimoxazole	38 (95)
Cefoperazone-sulbactam	13 (32.5)
Piperacillin-tazobactam	32 (80)
Meropenem	0

centers 1.6% and 1.1% respectively produced AmpC beta-lactamases.⁷ In a study conducted at the intensive care unit of the Children's Memorial Hospital in Warsaw, Poland, greater than 40% of the isolates tested produced AmpC beta-lactamases, which was higher than their incidence of ESBL producing isolates.¹⁶

It has been stated that the Amp C beta lactamases when present along with ESBLs can mask the phenotype of the latter.⁵ In our study, we found that 24 isolates were equally expressing Amp C beta lactamase and ESBLs, suggesting a possible low level expression of Amp C enzymes. Very few antibiotics can be reliably used against such resistant bacteria. The antibiogram of AmpC beta-lactamase producing bacteria in our study showed that none of the isolates was resistant to meropenem. Highest resistance was seen against cotrimoxazole, followed by piperacillin-tazobactam, ciprofloxacin and gentamicin. According to our study, meropenem and tigecycline can be effectively used against infections caused by Amp C beta lactamase producing bacteria. 100% efficacy of meropenem against Amp C beta lactamase producing bacteria was also reported in a study by Mohamudha et al.¹⁷

CONCLUSION

The detection of plasmid mediated Amp C beta lactamases is necessary for infection control. The occurrence of AmpC beta-lactamase producing isolates was quite high (62.5%) in our setup. The three-dimensional extract test can be applied as a phenotypic screening method for detection of Amp C harbouring organisms. Pakistan has limited resources with a low health care budget. Such resistant organisms will be

a further drain on the already meager resources and added cost for patients in the public sector. A suggested solution lies in stringent infection control measures.

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Authors Contribution:

Afreenish Hassan: I found the idea of this topic, and performed the experiment and wrote the article.
 Javaid Usman: He helped in providing the necessary equipment and writing the manuscript.
 Fatima Kaleem: She helped in performing the experiment and interpretation of results.
 Maria Omair: She helped in collecting and identification of the isolates, helped a lot in writing the article.
 Ali Khalid: He helped in performing the experiment. Worked a lot in analyzing the data.
 Muhammad Iqbal: He being senior technician in the laboratory, helped a lot in the practical procedure.