Detection of Inducible AmpC \(\beta\)-Lactamase-Producing Gram-Negative Bacteria in a Teaching Tertiary Care Hospital in North India

Uma Chaudhary, M.D., FIAMS*,
Ritu Aggarwal, M.D.*
Sanjeev Ahuja, M.D.**

ABSTRACT

AmpC \(\beta\)-lactamase enzymes confer resistance to a wide variety of \(\beta\)-lactam antibiotics, except carbapenems. AmpC \(\beta\)-lactamase-producing bacteria are known to be causative pathogens of nosocomial infections, which are difficult to be treated. Hence, this study was aimed out to determine the prevalence of AmpC \(\beta\)-lactamase enzyme production among Gram-negative bacteria. A total of 500 multidrug-resistant isolates were tested for cefoxitin susceptibility during a one-year period of study. All the isolates were tested for AmpC \(\beta\)-lactamase production by both the disk approximation and AmpC disk methods. The isolates positive for AmpC \(\beta\)-lactamase production were also tested for antimicrobial susceptibility by the disk diffusion method. AmpC \(\beta\)-lactamase production was noted in 101 (20.20%) isolates. All the isolates were 100-percent susceptible to imipenem and meropenem with variable susceptibilities to other antimicrobial agents. (*J Infect Dis Antimicrob Agents 2008;25:129-33.*)

INTRODUCTION

\(\beta\)-lactamase enzyme production is one of the most common mechanism of drug resistance to \(\beta\)-lactam antibiotics in Gram-negative bacteria, and class C (AmpC) enzymes are very important and clinically significant.¹

AmpC \(\beta\)-lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. They can be differentiated from the extended-spectrum \(\beta\)-lactamases (ESBLs) by their ability to hydrolyze cephemycins and other extended-spectrum cephalosporins. AmpC \(\beta\)-lactamases, either chromosomally or plasmid mediated, have been described in various pathogens including *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp., *Proteus mirabilis*, *Citrobacter freundii*, *Acinetobacter* spp., *Enterobacter* spp., and *Pseudomonas aeruginosa.*² The enzyme production can be constitutive or inducible.³

*Department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak, Pt. B.D. Sharma, PGIMS, Rohtak, India.

**Senior Resident, Super Specialty Hospital Janakpuri, New Delhi, India.

Received for publication: July 4, 2008.

Reprint request: Dr. Ritu Aggarwal, M.D., H. No. 717, Sector-1, HUDA, Rohtak-124001, Haryana, India.

E-mail: drritu252@yahoo.com

Keywords: AmpC \(\beta\)-lactamase, multidrug resistance, carbapenems, Gram-negative bacteria
The prevalence of AmpC β-lactamase-producing Gram-negative bacteria appears to be increasing, and these bacteria has been responsible for some nosocomial infections. It has been stated that the detection of AmpC β-lactamase production is challenging since the hyperproduction of chromosomal AmpC in associated with OMP F porin loss in *E. coli* or porin deficiency in *K. pneumoniae* can produce similar resistance phenotypes.1

There is no clear consensus regarding the guidelines for performing tests for the phenotypic screening or confirmatory tests for the bacterial isolates that harbour AmpC β-lactamases.4 In addition, the Clinical Laboratory Standards Institute (CLSI) do not recommend any method for the detection of AmpC β-lactamase production.5 This study was aimed to determine the occurrence of inducible AmpC β-lactamase-producing Gram-negative bacteria in Pt. B.D. Shama PGIMS, Rohtak, North India, a tertiary hospital, using the standard phenotypic methods presently available for their detection.

**MATERIALS AND METHODS**

A total of 500 multidrug-resistant Gram-negative bacteria, non-repetitive clinical isolates obtained from the pus (100 isolates), the urine (100 isolates), the blood (100 isolates), the cerebrospinal fluid, the other body fluids (100 isolates), and the sputum (100 isolates) were studied over a one-year period from April 2005 to March 2006. All isolates were identified, using the standard conventional microbiological techniques.6 All isolates were resistant to one or more than 3 classes of antibiotics on antimicrobial susceptibility, testing determined by the disk diffusion method. The isolates were also tested for cefoxitin susceptibility, using the 30-μg cefoxitin disk diffusion method. The results were interpreted as suggested by the CLSI 2006 guidelines, and the inhibition zone diameter of less than 18 mm was considered as resistant.5

All isolates of both groups were tested for AmpC β-lactamase production by both the disk approximation7 and AmpC disk methods.2

Regarding the disk approximation method, the isolate was inoculated onto a Mueller-Hinton Agar (MHA) plate as recommended by the CLSI. The cefoxitin (inducer) disk was placed at 2.5 cm (center-to-center) distance from the cefotaxime or ceftazidime disc for Enterobacteriaceae and *P. aeruginosa*, respectively. After an overnight incubation, a flattening of the inhibition zone around the antibiotic disk towards cefoxitin disk equal or more than 1 mm was recorded as the positive test for AmpC β-lactamase production.

Regarding the AmpC disk method, a lawn culture of *E. coli* ATCC 29522 was prepared onto a MHA plate. The sterile disks (6 mm in diameter) were moistened with the sterile saline (20 mL) and inoculated with several colonies of the test bacteria. The inoculated disk was then placed besides a cefoxitin disk (almost touching) onto the inoculated plate. The plates were incubated overnight at 35 °C. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk.

All the AmpC β-lactamase-producing isolates were tested for antimicrobial susceptibility by the Stoke’s disk diffusion method.8 Briefly, 0.5 McFarland turbidity suspension was prepared from the test isolates and the standard control strains. The control inoculum was spread in two bands on either side of the plate, leaving a central band uninoculated. The test isolate inoculum was streaked onto the central area of the plate. An uninoculated gap of 2-3 mm wide was left between the test and control isolates. The antimicrobial agents tested for susceptibility included levofloxacin (5 μg), sparfloxacin (5 μg), enrofloxacin (5 μg), ofloxacin (5 μg), tobramycin (30 μg), sisomycin (10 μg), netilmicin (30 μg), fosfomycin (50 μg), aztreonam (30 μg),
AmpC β-lactamase production by both phenotypic detection methods. AmpC β-lactamase-producing isolates were obtained from urine (35%), followed by pus (33%), blood (23%), and others (Table 1). AmpC β-lactamase production was most commonly noted in *P. aeruginosa* (25.8%), followed by *Acinetobacter* spp. (21.43%), and *E. coli* (19.05%) (Table 2).

These AmpC β-lactamase-producing isolates were 100-percent susceptible to carbapenems including meropenem and imipenem. The high rate of resistance was observed in all other antimicrobial agents, ranging from 36.63 percent to 99.01 percent (Table 3).

### Table 1. Distribution of inducible AmpC β-lactamase-producing isolates according to different clinical specimens.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Isolates Number</th>
<th>AmpC-producing isolates Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>100</td>
<td>35 (35%)</td>
</tr>
<tr>
<td>Pus</td>
<td>100</td>
<td>33 (33%)</td>
</tr>
<tr>
<td>Blood</td>
<td>100</td>
<td>23 (23%)</td>
</tr>
<tr>
<td>Cerebrospinal fluid and other body fluids</td>
<td>100</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>100</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>101 (20.2%)</td>
</tr>
</tbody>
</table>

### Table 2. Distribution of inducible AmpC β-lactamase-producing isolates in different organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Isolates Number</th>
<th>AmpC-producing isolates Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>183</td>
<td>47 (25.7%)</td>
</tr>
<tr>
<td><em>Escherichai coli</em></td>
<td>126</td>
<td>24 (19.1%)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>79</td>
<td>13 (16.5%)</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>42</td>
<td>9 (21.4%)</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>42</td>
<td>5 (11.9%)</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.</td>
<td>18</td>
<td>3 (16.7%)</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>10</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>101 (20.2%)</td>
</tr>
</tbody>
</table>
DIscussion

Despite the discovery of ESBLs and AmpC β-lactamases at least two decades ago, there remains a low level of awareness regarding their laboratory detection and clinical significance. The confusion exists about the importance of these resistance mechanisms, the appropriate test methods, and the appropriate reporting conventions. The failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.

In this study, 20.20 percent of multidrug-resistant isolates were positive for AmpC β-lactamase production. Our results are in concordance with the previous studies. One study in Delhi, India, reported a 20.7-percent AmpC β-lactamase production rate among Gram-negative bacteria. The higher rate of AmpC β-lactamase production was also reported by several studies. However, some studies described the low rate of AmpC β-lactamase production. This difference may be due to the different selection criteria of isolates, the variation in an ability to produce AmpC β-lactamases among different Gram-negative bacteria, and different clinical specimens.

Of 160 cefoxitin-resistant isolates, 59 (36.97%) were negative for AmpC β-lactamase production. Cefoxitin resistance in these isolates could be due to the lack of permeation porins. This result indicates that even though the screening methods that use the cefoxitin for the detection of AmpC-producing isolates are easily performed, but they are not accurate.

This study had some limitations including the lack of molecular epidemiologic analysis due to our constrains, and no available detection of ESBL production of the same isolates. However, there was no evidence of clonal spread, based on the antimicrobial susceptibility patterns of the isolates. In addition, the antimicrobial susceptibility was carried out by the Stoke’s disk diffusion method which is not suggested by the CLSI. The therapeutic options for infections caused by Gram-negative bacteria expressing AmpC β-lactamases are limited because these organisms are usually resistant to all β-lactam antibiotics except carbapenems. In addition, plasmid-mediated AmpC β-lactamase-producing isolates are typically resistant to multiple classes of antimicrobial agents.

This is a preliminary study designed with an objective to detect the possible occurrence of AmpC β-lactamases in a tertiary care hospital and to institute antibiotic policy to minimize the emergence of antimicrobial resistance. This is perhaps the first report of AmpC β-lactamase production among Gram-negative clinical isolates from this state of India.

References
1. Ratna AK, Menon I, Kapur I, Kulkarni R. Occurrence & detection of AmpC β-lactamases at a referral hospital
AmpC \(\beta\)-Lactamase Production in Gram-Negative Bacilli: Chaudhary U, et al.


