Occurrence & detection of AmpC β-lactamases at a referral hospital in Karnataka

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Background & objectives: AmpC β-lactamases confer resistance to a wide variety of β-lactam drugs except for cefepime, cefpirome and carbapenems. They are known to be responsible for nosocomial outbreaks, therapeutic failures and multidrug resistance. Although reported with increasing frequency the true rate of occurrence of these β-lactamases in Enterobacteriaceae is not known. Hence the present study was undertaken to determine the occurrence of AmpC enzymes among clinical isolates.

Methods: A total of 520 consecutive, non-repeat clinical isolates were included in the present study. Twenty eight strains resistant to cefoxitin were tested for AmpC β-lactamases by the modified 3-dimensional extract method. Isolates harbouring AmpC β-lactamases were tested for inducible β-lactamases by disc diffusion.

Results: Sixteen (3.3%) isolates were positive for AmpC β-lactamases. Based on the species 9 (3.3%) Escherichia coli, 4 (2.2%) Klebsiella pneumoniae, 2 (5%) Citrobacter freundii and 1 (5.5%) isolate of Enterobacter aerogenes harboured AmpC enzymes. Nine (56.3%) of AmpC harbouring strains, were urinary isolates. All the isolates were sensitive to imipenem and variably sensitive to aminoglycosides and co-trimoxazole.

Interpretation & conclusion: Our findings document the presence of AmpC enzymes in this region. Hence AmpC β-lactamase detection should be undertaken in clinical isolates showing resistance to broad-spectrum cephalosporins.

Key words AmpC β-lactamases - drug resistance

Inactivation of β-lactam antibiotics by enzymes is a major mechanism of resistance in the Gram negative bacteria. Although a variety of β-lactamases have been described, classes A and C have been the most important.

AmpC β-lactamases are clinically significant, since they confer resistance to cephalosporins in the oxyimino group (cefotaxime, ceftazidime, ceftriaxone), 7-α methoxy cephalosporins (cefoxitin or cefotetan) and are not affected by available β-lactamase inhibitors (clavulanate, sulbactam). Plasmid mediated AmpC β-lactamases differ from chromosomal AmpCs in being uninducible and are typically associated with broad multidrug resistance.

Many clinical laboratories are not fully aware of the importance of plasmid mediated AmpC β-lactamases. This lack of understanding is responsible
for a continuing failure to prevent the rapid world-wide dissemination of pathogens possessing these $\beta$-lactamases. Prevalence of this resistance mechanism appears to be increasing and has been responsible for nosocomial outbreaks, avoidable therapeutic failures (sometimes fatal) and outbreaks of multidrug resistant Gram negative pathogens that require expensive control efforts.

Although reported with increasing frequency from different countries, there is not much information available from India. The occurrence of AmpC $\beta$-lactamases in Delhi was reported by Manchanda & Singh, who found 20.7 per cent of the clinical isolates harbouring AmpC $\beta$-lactamases. Hence a prospective study was undertaken to determine the occurrence of AmpC $\beta$-lactamases at Khaja Banda Nawaz private teaching hospital attached to the KBN Medical College, Gulbarga (Karnataka).

**Material & Methods**

A total of 520 consecutive clinical isolates of *Esch. coli* (n=276), *K. pneumoniae* (n=184), *E. aerogenes* (n=40) and *C. freundii* (n=20) obtained over a period of 11 months from February 2002 to December 2002 at KBN hospital, Gulbarga (Karnataka) identified by standard methods were included in the present study. Sources of the isolates were urine (n=322), pus (n=117) and sputum (n=81).

Susceptibility to antibiotics (conc in µg) viz., ampicillin (10), gentamicin (10), co-trimoxazole (25), ciprofloxacin (5), cefotaxime (30), ceftazidime (30), ceftriaxone (30), cefoxitin (30) and imipenem (30) (Hi-Media) were tested by Kirby Bauer’s disc diffusion method and interpreted as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations. *Escherichia coli* ATCC 25922 strain was used for quality control.

Isolates that yielded a cefoxitin zone diameter less than 18 mm (screen positive) were subjected to AmpC $\beta$-lactamase detection by the modified 3-dimensional extract method. AmpC $\beta$-lactamase producing isolates were tasted by disc diffusion for inducible $\beta$-lactamases.

**Results & Discussion**

Of the 520 isolates tested, 28(5.4%) were resistant to third generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone) and cefoxitin, and 16(3.3%) isolates were positive by 3-dimensional test, negative for inducible $\beta$-lactamases by disc diffusion and sensitive to imipenem.

Organisms isolated were *Esch. coli, K. pneumoniae, E. aerogenes* and *C. freundii* (Table I). Of the 16 isolates positive by 3-dimensional test obtained from different clinical samples, AmpC producers were isolated from urine [9(56.3%)], pus [4(25%)] and sputum [3(18.7%)] samples. Susceptibility pattern of AmpC $\beta$-lactamase producers to non $\beta$-lactam antibiotics were also studied (Table II). Of the AmpC $\beta$-lactamase producing strains four and two were found to be susceptible to co-trimoxazole and gentamicin respectively.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of organisms isolated</th>
<th>AmpC producing isolates</th>
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</thead>
<tbody>
<tr>
<td><em>Esch. coli</em></td>
<td>276</td>
<td>9 (3.3%)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>184</td>
<td>4 (2.2%)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>40</td>
<td>2 (5.0%)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>20</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>520</td>
<td>16 (3.3%)</td>
</tr>
</tbody>
</table>

**Table I.** Distribution of AmpC producing strains among different organisms isolated

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</thead>
<tbody>
<tr>
<td><em>Esch. coli</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
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R, resistant; S, sensitive; G, gentamicin; Cf, ciprofloxacin; Co, co-trimoxazole
Thus the present study demonstrates the presence of AmpC β-lactamases producing bacteria in this region. AmpC producing isolates have been commonly encountered in patients after prolonged hospitalization in ICU, following surgical procedures or those who had an underlying disease such as leukaemia or who were immunocompromised. Therapeutic options for infections caused by Gram negative organisms expressing AmpC β-lactamases are limited because these organisms are usually resistant to all β-lactam antibiotics except for cefepime, ceftiraxone (4th generation cephalosporins) and carbapenems. This emphasizes the need for detecting AmpC β-lactamase harbouring isolates, so as to avoid therapeutic failures and nosocomial outbreaks.

Information on the prevalence of AmpC β-lactamase producing strains in India is very limited. In a recent study, 20.7% per cent of the isolates were found to harbour AmpC enzymes in contrast to reported values of 1.2 per cent and 4.2 per cent. In the present study, 16(3.3%) isolates resistant to cefoxitin were positive by 3-dimensional test, negative for inducible β-lactamases by disc diffusion and sensitive to imipenem. Due to the above phenotypic characters these isolates were considered to be harbouring plasmid-encoded AmpC β-lactamases. It has been stated that detection of AmpC enzymes is quiet challenging since, hyperproduction of chromosomal AmpC with OMPF porin loss in Esch. coli or porin deficiency in K. pneumoniae can produce similar resistant phenotype. Further, not all strains with AmpC enzymes meet NCCLS criteria for resistance to cephemycins and oxyimino-cephalosporins. Hence, a reference laboratory for β-lactamase isoelectric focussing or gene localization is needed. This, will help us to know the actual prevalence of these enzymes and characterize them (CMY-2, FOX, BIL) for epidemiological surveillance purposes.

AmpC β-lactamases have been reported in Esch. coli, K. pneumoniae, Salmonella spp, C. freundii, E. aerogenes and P. mirabilis. These species belonging to the family Enterobacteriaceae are the most commonly encountered in clinical laboratory. Gazouli et al, reported 2.6 per cent isolates of Esch. coli harbouring AmpC enzymes. The only published report from India shows 33.3 per cent of K. pneumoniae harbouring AmpC β-lactamases in contrast to 1.2 per cent reported by Couldron et al.

The findings of the present study show the presence of AmpC β-lactamases in this region. Similar studies need to be carried out in various parts of India to know the actual prevalence of AmpC β-lactamases.

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References


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