AmpC β-lactamase producing multidrug resistant strains of *Klebsiella* spp. & *Escherichia coli* isolated from children under five in Chennai

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Received July 18, 2002

**Background & objectives:** AmpC β-lactamases are Group I cephalosporinases that confer resistance to a wide variety of β-lactam drugs. Plasmid mediated AmpC β-lactamases has been discovered most frequently in isolates of *Klebsiella pneumoniae*, *K. oxytoca*, *Salmonella*, *Proteus mirabilis* and *Escherichia coli*. The present study was undertaken to study the occurrence of multidrug resistant and AmpC β-lactamase producing *Klebsiella* spp. and *Escherichia coli* in children less than five years of age as this age group is very susceptible to intestinal and extraintestinal infections.

**Methods:** A total of 116 isolates of *Klebsiella* species and 32 isolates of *Esch. coli* were tested for resistance to cefoxitin, third generation cephalosporin (3GC) antibiotics (ceftazidime, cefotaxime, ceftriaxone), ampicillin, amikacin, cephalexin, cefuroxime, co-trimoxazole, gentamicin, imipenem and tetracycline by disc diffusion method. Isolates found resistant to cefoxitin were tested for the production of AmpC β-lactamases by three dimensional extract method. Transconjugation experiments were done to study the transfer of drug resistance and AmpC β-lactamase production from AmpC producing *Klebsiella* and *Esch. coli* isolates to a recipient *Esch. coli* strain (K12 J62-2).

**Results:** Twenty eight isolates (24.1%) of *Klebsiella* species and 12 (37.5%) of *Esch. coli* were found to be AmpC β-lactamase producers; 66.6 per cent and 81 per cent of *Klebsiella* and *Esch. coli* isolates respectively showed resistance to all the 3GCs. All the strains were found to be sensitive to imipenem. Eighty four (72%) of *Klebsiella* isolates and 20 (62.5%) of *Esch. coli* were found to be resistant to cefoxitin. Transfer of cefoxitin resistance to the recipient strain was observed in all the AmpC producing strains of *Klebsiella* spp. Of the 12 AmpC producing strains of *Esch. coli*, only 4 (33.3%) showed the transfer of cefoxitin resistance to the recipient strain.

**Interpretation & conclusion:** This study has shown the occurrence of AmpC β-lactamase producing *Klebsiella* and *Esch. coli* strains in children in Chennai. Since AmpC β-lactamase production is frequently accompanied by multiresistance to antibiotics, therapeutic options become limited resulting a need for new measures for the management of *Klebsiella* and *Esch. coli* infections. Also failure to identify AmpC β-lactamase producers may lead to inappropriate antimicrobial treatment and may result in increased mortality. Detecting plasmid mediated AmpC β-lactamase producing strains is technically difficult and the phenotypic tests for AmpC detection are not well defined. If an investigational AmpC β-lactamase inhibitor was made available for diagnostic testing, it could be useful in combination with a suitable cephamycin to confirm AmpC production.

**Key words** AmpC β-lactamases - cefoxitin - *Escherichia coli* - *Klebsiella* spp. - multidrug resistance - third generation cephalosporins (3GC)
broad spectrum cephalosporins, aztreonam, and are poorly inhibited by β-lactamase inhibitors such as clavulanic acid\(^1\). Genes for AmpC β-lactamases are commonly found on the chromosomes of the several members of the family Enterobacteriaceae, including Enterobacter, Shigella, Providencia, Citrobacter freundii, Morganella morgani, Serratia marcescens and Escherichia coli. Plasmid mediated AmpC β-lactamases has arisen through the transfer of chromosomal genes for the inducible AmpC β-lactamases on to plasmids. This transfer has resulted in plasmid mediated AmpC β-lactamases in isolates of Esch. coli, Klebsiella pneumoniae, Salmonella species, Citrobacter freundii, Enterobacter aerogenes and Proteus mirabilis\(^2\).

Plasmid mediated AmpC β-lactamases represent a new threat since they confer resistance to cephamycins and are not affected by β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems. This resistance mechanism has been found around the world, can cause nosocomial outbreaks, appears to be increasing in prevalence, and merits further study to define the best options for detection and treatment\(^3\).

In view of the increasing reports of AmpC β-lactamase producing strains of Klebsiella spp. and Esch. coli from around the world, and lack of reports about such strains from India, the present study was conducted with an objective to examine the occurrence of AmpC β-lactamase producing strains of Klebsiella spp. and Esch. coli recovered from children under 5 yr of age, suffering from intestinal and extraintestinal infections in Chennai. The present study was conducted in this particular age group, as these children are more susceptible to intestinal and extraintestinal infections.

**Material & Methods**

*Clinical isolates:* A total of 116 isolates of Klebsiella species (24 isolates from patients of septicaemia, 56 from urinary tract infections, 20 from acute diarrhoeal cases and 16 from respiratory tract infections) and 32 isolates of Esch. coli (8 from septicaemia patients, 12 from urinary tract infections and 12 from respiratory tract infections) were obtained from patients 0-5 yr of age attending the Institute of Child Health and Hospital for Children, Chennai during a period of four months from April-July 2001. All 28 patients with respiratory tract infections were more than 3 yr of age and so the sputum samples were collected with induced cough. 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 and speciated based on colony morphology and biochemical reactions\(^4\). Sensitivity to cephamycin such as cefoxitin 30 µg/disc (Hi-Media, India) was tested by disc diffusion method\(^5\) and interpreted as per the National Committee for Clinical Laboratory Standards (NCCLS)\(^6\).

Isolates with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to cefoxitin were selected for further study. Eighty four Klebsiella isolates and 20 Esch. coli isolates were found to be resistant to cefoxitin. Escherichia coli ATCC 25922 strain (culture collection of Department of Microbiology, Dr ALM PGIBMS, Chennai) was used for quality control.

*AmpC β-lactamase detection by three dimensional extract method:* Klebsiella and Esch. coli isolates resistant to cefoxitin were tested for AmpC β-lactamase activity by the standard three dimensional extract method\(^7\).

Clinical isolates of K. pneumoniae and Esch. coli which contained plasmid derivatives of MCQ-21 and CMY-2 AmpC β-lactamases respectively were tested as positive controls (kindly supplied by Dr Patricia Bradford, Wyeth-Ayerst Pharmaceuticals, New York).
Susceptibility to other antibiotics: The sensitivity of the Klebsiella and Esch. coli isolates was determined by the disc diffusion method (concentration/disc in µg) to ampicillin (10), amikacin (30), ceftazidime (30), cefotaxime (30), cephaloridine (30), cefuroxime (30), co-trimoxazole (10), gentamycin (10), imipenem (10), tetracycline (30) (Hi-Media, India). The results were interpreted as per NCCLS recommendations.

Transfer of cefoxitin resistance and AmpC β-lactamase production: Transconjugation experiments were done following the procedure by Abigail et al., with few modifications. Mating was performed with Esch. coli K12 J62-2 (F– rif lac–) (kindly provided by Dr Mary V. Jesudason, Christian Medical College & Hospital, Vellore) as the recipient strain. Overnight brain heart infusion (BHI) broth cultures (0.5 Mac Farland turbidity matched) of the donor and the recipient strains were mixed in the ratio of 1:10. This mixed culture was incubated overnight at 37°C.

Transconjugants were selected on MacConkey agar supplemented with rifampicin (2.5 mg/ml) and ceftazidime (2 µg/ml). 200 µl of the mixed culture after overnight incubation was spread on the selection plates with the help of the L-rod. The plates were incubated overnight at 37°C. The plates were then observed for the presence of the transconjugants, which were then biochemically identified. The transconjugants were then tested for their antibiotic sensitivity pattern by the disk diffusion technique.

Only the AmpC β-lactamase producing isolates of Klebsiella and Esch. coli were subjected to transconjugation.

Results

Out of the 116 isolates of Klebsiella spp. 96 were speciated as K. pneumoniae (20 isolates from blood, 4 from sputum, 56 from urine and 16 from stool specimens) and 20 as K. oxytoca (4 isolates from blood, 12 from sputum, 4 from stool). Of the 32 isolates of Esch. coli, 8 isolates were from blood, 12 from sputum and 12 from urine.
The drug resistance pattern of the *Klebsiella* and *Esch. coli* isolates is given in the Figure. *Klebsiella* and *Esch. coli* isolates showed high rates of resistance against 3GC antibiotics (ceftazidime, cefotaxime, ceftriaxone), ampicillin and cefoxitin. Among the 116 *Klebsiella* isolates and 32 *Esch. coli* isolates tested against all 12 antimicrobials, 28 (24%) isolates of *Klebsiella* and 8 (25%) of *Esch. coli* were resistant to all the antimicrobial agents studied except for imipenem. A majority of (90; 77.6%) isolates of *Klebsiella* and all the *Esch. coli* (100%) isolates were found resistant to at least one 3GC antibiotic used for the study. All the *Klebsiella* and *Esch. coli* isolates showed multidrug resistance (MDR). The MDR phenotypes identified in the study showed that 77 (66.6%) *Klebsiella* and 26 (81.1%) *Esch. coli* isolates were resistant to all the antimicrobial agents studied. A total of 110 (94.8%) *Klebsiella* isolates and all the 32 *Esch. coli* isolates were resistant to at least one non-β-lactam antibiotic (amikacin, gentamycin, co-trimoxazole, tetracycline).

Eighty four (72%) *Klebsiella* isolates and 20 (62.5%) *Esch. coli* isolates that were found to be resistant to cefoxitin (zone diameter <18 mm) were considered a surrogate marker for AmpC β-lactamase production. In this study, 20 (20.8%) isolates of *Klebsiella pneumoniae* and 8 (40%) isolates of *Klebsiella oxytoca* and 12 (37.5%) isolates of *Esch. coli* resistant to cefoxitin showed the production of AmpC β-lactamases by three-dimensional extract test.

Of the total 28 AmpC producing strains of *Klebsiella* spp. 12 (42.8%) isolates were from sputum specimen, 8 (28.6%) from blood and 8 (28.6%) from urine specimens. Eight of the 12 AmpC producing *Klebsiella* spp. obtained from sputum were found to be *K. oxytoca*. Among the total 12 AmpC producing strains of *Esch. coli*, 8 (66.6%) were from urine specimens and 4 (33.3%) from sputum. All the cefoxitin resistant isolates from stool were found to be non-AmpC producers. As shown in the Table, all the MDR and AmpC β-lactamase producing isolates were 100 per cent sensitive to imipenem.

The transconjugation studies (Table) done for the AmpC positive strains of *Klebsiella* spp. and *Esch. coli* showed that the resistance to β-lactam antibiotics and gentamycin was transferred to the recipient strain thus indicating that the resistance to β-lactam antibiotics and gentamycin coexisted. Transfer of cefoxitin resistance to the recipient strain was observed in all the AmpC producing strains of *Klebsiella* spp. Out of the 12 AmpC producing strains of *Esch. coli* only 4 strains (33.3%) showed the transfer of cefoxitin resistance to the recipient strain.

**Discussion**

The study has revealed the occurrence of AmpC β-lactamase producing strains of *Klebsiella* spp. recovered from children with urinary tract infections, septicaemia and respiratory tract infections and *Esch. coli* strains recovered from cases of urinary tract infections and respiratory tract infections. The comparatively less percentage of AmpC producing *Klebsiella* spp. indicates that this species harbours AmpC enzymes less frequently than *Esch. coli*. The higher incidence of AmpC producing *Esch. coli* may reflect 2 modes of production: hyperproduction of chromosome mediated AmpC and plasmid mediated AmpC β-lactamases. However, *Klebsiella* spp. does not possess a chromosomal AmpC. The transconjugation studies confirm that only plasmid mediated AmpC β-lactamases is seen in *Klebsiella* spp. whereas both chromosome mediated AmpC and plasmid mediated AmpC β-lactamases are seen in *Esch. coli*.

Occurrence of a large percentage of multidrug resistant strains has been observed in our study. Clinical isolates of *Klebsiella* spp. are often known to produce other β-lactamases in addition to an AmpC enzyme. The *bla* genes coding for other β-lactamases may be on different plasmids, but often they coexist on the same plasmid. Conjugative dissemination of
these AmpC β-lactamase encoding plasmids is thought to facilitate the spread of resistance against a wide range of antibiotics among different members of Enterobacteriaceae.

Since AmpC β-lactamase production is frequently accompanied by multi-resistance to antibiotics, therapeutic options become limited, leading to a demand for new measures for the management of *Klebsiella* and *Esch. coli* infections. Also failure to identify AmpC β-lactamase producers may lead to inappropriate antimicrobial treatment and may result in increased mortality. But it is encouraging to note that imipenem retained its potent activity against these highly resistant organisms encountered in this study.

Cephamycin resistance in non AmpC producing *Klebsiella* spp. is often due to porin deficient mutants. Hernandez-Alles *et al* demonstrated that interruption of a porin gene by insertion sequences is a common type of mutation that causes the loss of porin expression and increased cefoxitin resistance in *K. pneumoniae*.

Widespread use of antimicrobial therapy has often been held responsible for the occurrence of multiple resistant *Klebsiella* spp. and *Esch. coli* strains in hospitals. Because these undesired effects might be reversed by strict control of antibiotic use, demands for strategies to avoid the overuse of antibiotics are increasingly being expressed. Detecting plasmid mediated AmpC β-lactamase producing strains is technically difficult and the phenotypic tests for AmpC detection are not well defined, hence an investigational AmpC β-lactamase inhibitor if made available for diagnostic testing could be useful in combination with a suitable cephamycin to confirm AmpC production.

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Antibiotic resistance pattern of the AmpC positive donor strains</th>
<th>Antibiotic resistance pattern of the transconjugants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Klebsiella</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spp. (n=28)</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak A G T</td>
<td>Ca Ce Ci Cu Cr Cn A G</td>
</tr>
<tr>
<td>8</td>
<td>Ca Ce Ci Co Cu Cr Cn A G T</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ca Ce Ci Co Cu Cr Cn A G</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak A G</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak A G</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak</td>
<td></td>
</tr>
<tr>
<td><strong>Esch. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12) isolates</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak A G</td>
<td>Ca Ce Ci Cu Cr Cn A G</td>
</tr>
<tr>
<td>8</td>
<td>Ca Ce Ci Co Cu Cr Cn Ak A G</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ca Ce Ci Co Cu Cr Cn A G</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ca Ce Ci Co Cu Cr Cn A T</td>
<td></td>
</tr>
</tbody>
</table>

A, ampicillin; Ak, amikacin; Ca, ceftazidime; G, ceftriaxone; Ce, cefotaxime; Cn, cefoxitin; Cr, cephaloridine; Cu, cefuroxime; Co, co-trimoxazole; G, gentamycin; I, imipenem; T, tetracycline

Our study has shown the occurrence of multidrug resistant and plasmid mediated AmpC producing *Klebsiella* and *Esch. coli* isolates in Chennai also. Hence it is suggested that, routine diagnosis of AmpC producing strains should be carried out in hospitals in Chennai in order to avoid the effects of multidrug resistant and AmpC producing *Klebsiella* spp. and
The findings of our study have important implications for the control of AmpC β-lactamase producing *Klebsiella* spp. and *Esch. coli* which are likely to be overlooked in hospitals and suggest that both formulary restriction of broad spectrum antimicrobial agents and rigorous attention to infection control procedures will be necessary. But no NCCLS guidelines and recommendations exist for detecting and reporting plasmid mediated AmpC β-lactamases.

Plasmid mediated AmpC β-lactamases represent a new threat since they confer resistance to cephamycins and are not affected by commercially available β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems. This resistance mechanism has been found around the world, can cause nosocomial outbreaks, appears to be increasing in prevalence, and merits further study to define the best options for detection and treatment.

**Acknowledgment**

The authors gratefully acknowledge Dr Patricia Bradford, Wyeth-Ayerst Pharmaceuticals, New York, for kindly providing the clinical isolates of AmpC β-lactamase producing *K. pneumoniae* and *Esch. coli*. The authors acknowledge Dr S.K. Amsavathani, Professor and Head, Department of Microbiology, Institute of Child Health & Hospital for Children, Chennai, for providing clinical specimens. The authors thank Lady Tata Memorial Trust for financial support.

**References**


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