CEFOXITIN RESISTANCE MEDIATED BY LOSS OF A PORIN IN CLINICAL STRAINS OF KLEBSIELLA PNEUMONIAE AND ESCHERICHIA COLI

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Abstract

Purpose: Porins are outer membrane protein (OMP) that form water filled channels that permit the diffusion of small hydrophilic solutes like β-lactam antibiotics across the outer membrane. Two major porins that facilitate diffusion of antimicrobials have been described in Klebsiella spp. and Escherichia coli. The present study was carried out to examine the role of porins among Extended Spectrum β-Lactamase (ESBL) and AmpC β-Lactamase positive strains of Klebsiella spp. and E.coli. Methods: Preparation of OMP from phenotypically characterized clinical isolates K.pneumoniae and E.coli and the separation of the proteins by sodium dodecyl sulfate – polyacrylamide gel electrophoresis were performed as per a previously described procedure. Results: OMP analysis revealed that cefoxitin and ceftazidime resistance was mediated by loss of a porin Omp K35 in the isolates of K.pneumoniae and E.coli. Conclusions: Loss of porin mediated resistance mechanism against cefoxitin was observed among the multidrug resistant K.pneumoniae and E.coli.

Key words: Outer membrane proteins, porins, cefoxitin, Klebsiella spp., E.coli

The outer membrane of gram-negative bacteria plays a significant role in a variety of functions and is composed of a bilayer containing phospholipids, lipopolysaccharide and outer membrane proteins (OMPs). One family of OMPs the porins, are present in large amounts in the outer membrane and form water-filled channels that permit the diffusion of small hydrophilic solutes across the membrane.1

Two major non-specific porins, namely Omp K35 and Omp K36 have been described in K.pneumoniae. Omp K352 and Omp K363 porins were found to be the homologues of porins OmpF and OmpC from E.coli respectively. OmpK36 porins allow the diffusion of a variety of a wide variety of molecules, including bacterial nutrients and antimicrobials.

Since β-lactam antibiotics penetrate the outer membrane of gram-negative bacteria, resistance could also be caused by loss or deficiency of specific porins that reduce the outer membrane permeability to β-lactam antibiotic. This might be an important factor in mediating β-lactam resistance in multidrug resistant Klebsiella spp. and E.coli in the presence and absence of broad-spectrum β-lactamase production by these isolates.

The present study was conducted with an objective to examine the presence of porin mediated resistance to β-lactam antibiotics in ESBL and AmpC β-lactamase producing and non-producing strains of K.pneumoniae and E.coli recovered from children under 5 years of age, suffering from extraintestinal infections.

Materials and Methods

Clinical isolates

A total of 76 isolates of K.pneumoniae (14 isolates from cases of septicaemia, 46 from urinary tract infections, 16 from respiratory tract infections) and 22 isolates of E.coli (4 from septicaemia patients, 12 from urinary tract infections and 6 from respiratory tract infections) were obtained from patients between 0 and 5 years of age attending the Institute of Child Health and Hospital for Children, Chennai, during a period of four months from January-April 2002. Isolates that were obtained as a pure and predominant growth from the clinical specimens and considered significant were only considered for the present study. The organisms were identified and speciated based on colony morphology and biochemical reactions.4

Antimicrobial Susceptibility testing

The sensitivity of the isolates to third generation cephalosporins (3GC) viz., ceftazidime, cefotaxime, ceftriaxone each 30 µg/disk and to cefoxitin (30µg) and imipenem (30µg), (Hi-Media, India) was determined by the disc diffusion method.3 The results were interpreted as per National Committee for Clinical Laboratory
Standards (NCCLS) recommendations. E. coli ATCC 25922 was used for quality control.

ESBL detection by Double Disc Diffusion Synergy Test (DDST)

Isolates with resistance or with decreased susceptibility (intermediate by NCCLS criteria) to any of the 3 GC were subjected to the standard DDST. Since all the isolates were found to be resistant to atleast one of the three 3GC antibiotics, they were all tested for ESBL production by DDST.

Detection of AmpC β-lactamase production by 3D extract method

Isolates that were found resistance to cefoxitin (zone diameter <18mm) were considered screen positive for AmpC β-lactamase production. The cefoxitin resistant isolates were then confirmed for AmpC activity by 3D extract method. Twenty-four Klebsiella isolates and 12 E.coli isolates were found resistant to cefoxitin. Clinical isolates of K.pneumoniae and E.coli which contained plasmid derivatives of MCQ-21, CMY-2 AmpC β-lactamases respectively were tested as positive controls (supplied by Dr. Patricia Bradford, Wyeth-Ayerst Pharmaceuticals, New York).

Transfer of resistance and ESBL and AmpC β-lactamase production.

Transconjugation experiments were done with ESBL and AmpC producing K.pneumoniae and E.coli. Mating was performed with E.coli K12 J62-2 (F rif lac) (provided by Dr, Mary V. Jesudason, Christian Medical College and Hospital, Vellore) as the recipient strain. Transconjugants were selected on MacConkey agar supplemented with rifampicin (2.5mg/mL) and ceftazidime (2µg/mL).

Analysis of OMPs

The OMP profile was studied for well characterized strains with the following properties: cefoxitin resistant, ESBL positive K. pneumoniae; cefoxitin sensitive K. pneumoniae; cefoxitin resistant, ESBL and AmpC negative K. pneumoniae; cefoxitin resistant, AmpC positive K. pneumoniae; cefoxitin resistant, AmpC negative K. pneumoniae and cefoxitin sensitive E.coli, AmpC positive E.coli.

Bacterial cells were lysed by sonication (Vibra cell, Sonics and Materials, Inc.). The OMPs were obtained after treatment of cell membranes recovered by ultracentrifugation with sodium laurylsarcosinate (1%; Sigma) and subsequent ultracentrifugation following the procedure by Filip et al. The OMP profile was analysed by polyacrylamide gel electrophoresis (30% acrylamide/0.8% bis-acrylamide, 20% SDS). Gels were visualized by staining with Coomassie blue (Sigma).

Results

The ESBL phenotypic screening by double disc diffusion synergy test showed that 18 (23.6%) isolates of K. pneumoniae (3-blood, 9-urine, 6-sputum) and 6 (27.2%) isolates of E.coli (1-blood, 3-urine, 2-sputum) were ESBL producers (Table).

Of the 24 Klebsiella isolates and 12 E.coli isolates, which were found to be resistant to cefoxitin, only 5 (20.8%) isolates of K. pneumoniae (1-blood, 3-sputum, 1-urine) and 2 (16.6%) isolates of E.coli (1-blood, 1-sputum) were found to be AmpC producers by 3D enzyme extract method (Table).

All the ESBL and AmpC positive clinical isolates were subjected to the conjugal resistance transfer experiment, and after repeated attempts they produced transconjugants. All the ESBL positive isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source of clinical specimens</th>
<th>Septicaemia</th>
<th>Urinary tract infection</th>
<th>Respiratory tract infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella Spp.</td>
<td></td>
<td>14</td>
<td>46</td>
<td>16</td>
<td>76</td>
</tr>
<tr>
<td>ESBL +ve</td>
<td></td>
<td>13</td>
<td>9</td>
<td>6</td>
<td>18 (23.6%)</td>
</tr>
<tr>
<td>AmpC +ve</td>
<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5 (20.8%)</td>
</tr>
<tr>
<td>E.coli</td>
<td></td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>ESBL +ve</td>
<td></td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>6 (27.2%)</td>
</tr>
<tr>
<td>AmpC +ve</td>
<td></td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>2 (16.6%)</td>
</tr>
</tbody>
</table>
K. pneumoniae and E. coli showed transfer of ESBL production to the recipient strain. Two cefoxitin resistant, ESBL positive isolates of K. pneumoniae did not conjugally transfer ceftazidime resistance to the recipient strain, and one strain of AmpC positive E. coli, did not transfer cefoxitin resistance.

OMP profile showed the presence of 35 kDa porin protein in cefoxitin sensitive K. pneumoniae and cefoxitin sensitive E. coli (Figure). All the other isolates lacked the protein in the 35kDa region, the loss of which mediated cefoxitin resistance in these isolates. Cefoxitin resistant, AmpC positive K. pneumoniae showed the loss of the similar protein indicating that the resistance to cefoxitin was mediated by both AmpC β-lactamase production and loss of OMP.

Figure: Lanes: M, Marker protein; Lane 1: Cn resistant, ESBL positive K. pneumoniae, did not conjugally transfer Ca resistance; Lane 2: Cn sensitive K. pneumoniae; Lane 3: Cn resistant, ESBL and AmpC negative K. pneumoniae; Lane 4: Cn resistant, AmpC positive K. pneumoniae; Lane 5: Cn resistant, AmpC negative K. pneumoniae; Lane 6: Cn sensitive E. coli; Lane 7: AmpC positive E. coli, did not transfer Cn resistance. (Cn – cefoxitin, Ca – ceftazidime).

Discussion

Plasmid mediated resistance to β-lactam antibiotics was observed in the present study in addition to the ESBL and AmpC β-lactamase mediated resistance. ESBLs are not active against cephapemcid, and most strains expressing ESBLs are susceptible to cefoxitin and cefotetan. However, it has been reported that ESBL producing strains can become resistant to cephapemcid due to the loss of an outer membrane porin protein.12-14 The protein profile analysis carried out in the present study showed that the cefoxitin resistance in ESBL positive and ESBL negative isolates of K. pneumoniae was due to the loss of an outer membrane protein. Loss of porins in K. pneumoniae was found to augment resistance provided by ESBLs and plasmid mediated AmpC β-lactamases, to include resistance to oxyimino-β-lactams and carbapenems.17

Ceftazidime resistance due to porin loss in an ESBL positive isolate of K. pneumoniae, was also observed in the present study (Figure, lane 1). It has been indicated in a previous study that ceftazidime enters the cell through OmpK35 porin14 and OmpK36.15 Reduced permeability of K. pneumoniae due to porin loss was found to increase the MIC of ceftazidime by Rasheed et al.12 In this study, the above mentioned isolate from blood specimen, showed an minimum bactericidal concentration of ≥203/µg/mL. Elevated levels of ceftazidime resistance resulted from a combination of increased enzyme production and minor OMP changes as observed by a previous study16 in a clinical isolate of K. pneumoniae.

OmpK35 and OmpK36 are involved in mediating β-lactam resistance in K. pneumoniae.1 Cefoxitin resistance in K. pneumoniae due to reduced permeability of porins to β-lactam antibiotics has been reported.11 In other study loss porins in K. pneumoniae was found to augment resistance provided by ESBLs and plasmid mediated AmpC β-lactamases, to include resistance to oxyimino-β-lactams and carbapenems.16

Loss of porin mediated resistance mechanism against cefoxitin has been observed among the multidrug resistant Klebsiella spp. and E. coli. Knowledge of the resistance mechanisms in these clinical isolates will provide the people in charge of public health with data on multidrug resistant pathogens which would be helpful in making recommendations on the best use of antibiotics and to formulate therapeutic strategies to control infections. More prudent use of antibiotics and control of the spread of these resistant organisms are necessary. More extensive study related to OMP profiles to resistance patterns should be carried out to emphasize the clinical impact of porin mediated β-lactam resistance among the clinical isolates of Klebsiella spp. and E. coli, in this part of the world.

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References


