Prevalence & antimicrobial resistance pattern of extended spectrum β-lactamase producing Klebsiella spp isolated from cases of neonatal septicaemia

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Background & objectives: Extended spectrum β-lactamase (ESBL) producing Klebsiella spp led to serious concern about septicaemic neonates in neonatal intensive care units (NICU) due to high resistance against commonly used antimicrobial agents. Knowledge of disease burden and information on resistance to antimicrobials are required for proper management of such cases in NICUs. Here we report the prevalence and resistance pattern of ESBL producing Klebsiella spp isolated from cases of neonatal septicaemia at a tertiary care hospital from north India.

Methods: A total of 100 clinical isolates of Klebsiella spp isolated from 2995 blood samples of suspected cases of neonatal septicaemia were studied. Antimicrobial susceptibility was determined by Kirby- Bauer’s disc diffusion method. All isolates were screened for ESBL production on the basis of inhibition zone against cephrotaxime (<27 mm) and ceftazidime (<22 mm) and a breakpoint of minimum inhibitory concentration (MIC) (<2 μg/ml for cephotaxime and <8 μg/ml for cefpodoxime) by agar dilution method. Resistance pattern of ESBL producers and non-ESBL producers was compared.

Results: Of the 100 Klebsiella isolates, 58 were positive for ESBL production, which was much lower than 86.6 per cent reported in 2003. Almost all the isolates were sensitive to imipenam and meropenam. Drug resistance was found to be significantly more common in ESBL producing isolates than in non-ESBL producers.

Interpretation & conclusion: We found that 56 per cent of Klebsiella spp isolates were ESBL producers. There is a need to carefully formulate therapeutic strategies to control infections in NICUs. The high percentage of drug resistance in ESBL producing Klebsiella spp suggests that routine detection of ESBL is required by reliable laboratory methods.

Key words Extended spectrum β-lactamase (ESBL) - Gram-negative septicaemia - Klebsiella spp - neonatal septicaemia
Septicaemia is a leading cause of neonatal mortality and a significant proportion of newborns are infected with *Klebsiella* spp in NICUs\(^1,2\). Extended spectrum \(\beta\)-lactamase (ESBL) producing *Klebsiella* spp have been frequently implicated in outbreaks in NICUs and account for a vast range of resistance against antimicrobial agents\(^1,2\).

ESBL producing *Klebsiella* spp were first reported in 1983 from Germany\(^3\), and since then a steady increase of strains resistant to cephalosporins has been seen. The emergence of ESBL producing strains derived from mutation in TEM and SHV enzymes, which are present in 75 per cent of enterobacteriaceae isolates, is documented\(^4\). ESBLs are more prevalent in *Klebsiella* spp than any other enterobacterial species and outbreaks of infection caused by ESBL producing *Klebsiella* spp have been widely reported\(^4,8\). From India, the high prevalence of ESBL producing *Klebsiella* spp. is reported varying from 6 to 87.0 per cent\(^9,10\). In our previous study in a tertiary care centre in north India\(^1\), we found that 11.8 per cent of neonatal septicaemia was caused by *Klebsiella* spp, and of the total *Klebsiella* isolates, 86.6 per cent were ESBL producers. This study was undertaken to see if there was any change in the prevalence of ESBL producing *Klebsiella* spp. isolated from cases of neonatal septicaemia in the same centre. The resistance pattern of these isolates was also determined.

**Material & Methods**

A total of 2995 blood samples from the same number of suspected cases of neonatal septicaemia, sent to the Department of Microbiology, K.G. Medical University, Lucknow, India, for blood culture during a period of two years (January 2004 - December 2005) were included in the study. Briefly, 1-2 ml of blood was collected into 10 ml of brain heart infusion broth with 0.05 per cent sodium polyanethol sulphonate. The broth was incubated at 37°C, overnight. A blind subculture on MacConkey agar plate, chocolate agar and blood agar plate (Hi-media, Mumbai) was done after 18 h. If no growth was obtained, the bottles were examined daily for seven days. Any sign of growth was followed by subculture and identified by Gram staining. Gram-negative rods were identified by relevant biochemical test *i.e.*, motility test, Methyl Red-Voges-Proskauer test, and sugar fermentation test\(^11\).

**Antimicrobial susceptibility test:** Antimicrobial susceptibility was determined by Kirby-Bauer’s disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations\(^12,13\). Antimicrobial discs (\(\mu\)g) used were ampicillin (10), amoxycillin/clavulanic acid (10/20), piperacillin (100), piperacillin/tazobactem (100), ticarcillin (75), ticarcillin/clavulanic acid (75/10), cefixime (5), cefuroxime (30), cefpodoxime (10), cephoxime (30), ceftazidime (30), aztreonam (30), netilmicin (30), amikacin (30), gentamycin (30), chlorphenicol (30), cotrimoxazol (30), tetracycline (30), imipenam (10) and meropenam (10). All these antibiotics were purchased from Hi-media Laboratories, Mumbai. Quality control was achieved by using standard strain of *Klebsiella* ATCC70063 (gifted by Christian Medical College, Vellore). Isolates showing inhibition zones <27 mm for cephoxime and <22 mm ceftazidime was identified as potential ESBL producers and again tested on the basis of minimum inhibitory concentration (MIC) and confirmatory test.

MIC was determined by agar dilution methods for cephoxime (0.25-128 \(\mu\)g/ml) and cefpodoxime (0.25-128 \(\mu\)g/ml) using series of dilution according to NCCLS-2003 guidelines\(^13\). Inoculated plates were incubated in ambient air at 35°C for 16-20 h. The MIC of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism. A breakpoint of MIC, <2 \(\mu\)g/ml for cephoxime and <8 \(\mu\)g/ml for cefpodoxime was identified as marker of ESBL production\(^13\). Quality
control was achieved by using standard strain of *Klebsiella* ATCC70063.

**Confirmatory test for ESBL production:** The combined disk method was used to confirm the presence of ESBL on all the isolates of *Klebsiella* spp by placing a disk (μg) of ceftazidime (30) alone and ceftazidime (30) in combination with clavulanic acid (10) on a Muller-Hinton agar plate. The discs were placed at least 20 mm apart from each other. Two parameters were taken as indicator of ESBL production. *(i)* The zone diameter around ceftazidime + clavulanic acid disc is >5 mm larger than that around ceftazidime disc, confirms the presence of ESBL. *(ii)* If ratio of zone diameter around discs with ceftazidime + clavulanic acid and ceftazidime alone is >1.5, it confirms ESBL production.

**Quality control for ESBL detection:** *K. pneumoniae* ATCC700603 (ESBL positive) was used as quality control for ESBL test. On disk diffusion testing the zone diameter (mm) ranges for *K. pneumoniae* ATCC700603 were as follows; cefpodoxime 9-16 mm, ceftazidime 10-18, aztreonam 9-17 and cefotaxime 17-25. In disc diffusion phenotypic testing, *K. pneumoniae* ATCC700603 shows >5mm increase in ceftazidime/ clavulanic acid zone diameter.

**Statistical analysis:** Chi-square test was used with appropriate correction for the observation. Where the cell frequency was less than five, Fisher exact tests was applied to see the significance between the resistance level of various drugs in ESBL producer and non-ESBL producer *Klebsiella* spp using STATA 8.2 software. *P* ≤ 0.05 was considered significant.

**Results & Discussion**

A total of 100 isolates of *Klebsiella* spp. were isolated from 2995 blood samples (3.3%). Of these 100 isolates, 58 (58.0%) were ESBL positive. The antimicrobial resistance was significantly (*P* < 0.05) higher in ESBL producers than in non-ESBL producers. All the isolates were sensitive to imipenam and meropenam (except one). ESBL producing *Klebsiella* spp. were almost always resistant to ampicillin, ticarcillin and piperacillin. Monobactem and cephalosporin resistance was also higher in ESBL producing *Klebsiella* spp. Aminoglycosides *i.e.*, amikacin and gentamycin accounted for 58.6 and 70.6 per cent resistance among ESBL producers. Piperacillin/tazobactem showed less resistance as compared to ticarcillin/clavulanic acid and amoxyccillin/clavulanic acid (Table).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ESBL producer (n=58)</th>
<th>Non ESBL producer (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>57</td>
<td>21**</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>57</td>
<td>13**</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>57</td>
<td>14**</td>
</tr>
<tr>
<td>Amox/Clavulanic acid</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Ticarcillin/Clavulanic acid</td>
<td>43</td>
<td>4**</td>
</tr>
<tr>
<td>Piperacillin/Tazobactem</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>49</td>
<td>11**</td>
</tr>
<tr>
<td>Imipenam</td>
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<td>0</td>
</tr>
<tr>
<td>Meropenam</td>
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<td>0**</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>47</td>
<td>8**</td>
</tr>
<tr>
<td>Cephotaxime</td>
<td>52</td>
<td>8**</td>
</tr>
<tr>
<td>Ceufuroxime</td>
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<tr>
<td>Cefixime</td>
<td>53</td>
<td>5**</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>54</td>
<td>6**</td>
</tr>
<tr>
<td>Amikacin</td>
<td>34</td>
<td>8**</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>41</td>
<td>22</td>
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<tr>
<td>Netilmicin</td>
<td>51</td>
<td>32</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>55</td>
<td>34*</td>
</tr>
<tr>
<td>Ciprafloxacin</td>
<td>39</td>
<td>8**</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>48</td>
<td>16*</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>40</td>
<td>26</td>
</tr>
</tbody>
</table>

*P* ≤ 0.05, **P** < 0.001 compared to ESBL producers
The incidence of neonatal septicaemia caused by *Klebsiella* spp. as seen in the current study (3.3%) was much less in comparison of what we reported in 2003 (11.8%) from same NICU. There was also a remarkable decrease in prevalence of ESBL producing *Klebsiella* species as seen in isolates from the cases of neonatal septicaemia (86.6 vs. 58%).

In recent years a significant increase in ESBL producing *Klebsiella* spp. was reported from USA 4.2-44.0 per cent, Canada, 4.9 per cent and China 51 per cent. Focussing on the epidemiology in Europe, there are considerable geographical differences in the occurrence of ESBLs. A recent large survey of 1610 *Escherichia coli* and 785 *K. pneumoniae* isolates from 31 centers in 10 European countries found that the prevalence of ESBL in these organisms ranged from as low as 1.5 per cent in Germany to as high as 39-47 per cent in Russia, Poland and Turkey.

In India, high prevalence of ESBL producing *Klebsiella* strains has been reported by various groups. Reported frequency of ESBL producing *Klebsiella* spp. from India ranged between 6 and 87 per cent. Prevalence of ESBL producing *Klebsiella* spp. as reported by other investigators was 25.6, 25.8, 30.18, 80.0 and 86.6 per cent.

The high percentage of ESBL producing *Klebsiella* spp may be due to the selective pressure imposed by extensive use of antimicrobials. Intensive care unit, in which antibiotic use is heaviest and the potential for patient-to-patient transmission of organisms is greatest, is an important factor. The infection control implications of ESBL producing *Klebsiella* spp. are under-recognized. In most of the cases, molecular genetic evidences indicated patient-to-patient transmission of ESBL producing strains of *Klebsiella* spp. More than 50 hospital outbreaks of infection with ESBL producing *Klebsiella* have now been reported.

In the present study resistance to three or more drugs (multi drug resistance, MDR) was common in ESBL producers than non-ESBL producers. About 95.0 per cent ESBL producers were resistant to penicillins and more than 85.0 per cent to cephalosporins. However, NCCLs documents recommend that ESBL producers should not be reported as susceptible to cephalosporins, since the ESBLs destroy these drugs and ESBL producing bacteria will remain resistant to treatment with these drugs. Carbepenems are considered the last resort in NICU for ESBL producing isolates and resistance to carbepenems is a serious concern and has been reported in certain hospitals. In our study, only one isolate showed the resistance against meropenam. The resistance may be due to reduced levels of drug accumulation or increased expression of pump efflux or may be due to the production of metallo-β-lactamase as seen in *Pseudomonas* spp.

In conclusion, our results showed a decrease in ESBL producing *Klebsiella* spp. in our NICU. The immense use of blood spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of ESBL producing microorganisms. Routine detection of ESBL producing microorganisms is required by reliable laboratory methods and since most of these are multidrug resistant, the therapeutic strategies to control infections in NICUs has to be carefully formulated.

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**References**


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