High Prevalence of Extended-Spectrum β-Lactamase-Producing Strains among Blood Isolates of Enterobacter spp. Collected in a Tertiary Hospital during an 8-Year Period and Their Antimicrobial Susceptibility Patterns

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Of 72 blood isolates of Enterobacter spp. collected over an 8-year period, 50% (36 of 72) were derepressed or partially derepressed AmpC mutants. The extended-spectrum β-lactamase (ESBL) production rate was 43% (31 of 72 isolates), and 67.3% (31 of 46) of extended-spectrum cephalosporin-resistant strains produced ESBLs. Thus, a confirmatory test for ESBL production is necessary for extended-spectrum cephalosporin-resistant Enterobacter spp.

Derepressed production of AmpC β-lactamase has been documented to be a prevalent mechanism of extended-spectrum cephalosporin resistance in clinical isolates of Enterobacter spp. (2, 7, 8), and derepressed AmpC mutants occurred at frequencies of up to 20% when Enterobacter bacteremias were treated with extended-spectrum cephalosporins (7). However, extended-spectrum β-lactamase (ESBL) production is also known to be an another important mechanism for extended-spectrum cephalosporin resistance in Enterobacter, and ESBL-producing Enterobacter has been increasingly reported recently (1, 14, 15, 19). In order to ascertain the prevalence of derepressed AmpC mutants or ESBL-producing organisms in clinical isolates of Enterobacter spp. and to characterize antimicrobial susceptibility profiles, we studied the production of β-lactamases in blood isolates of Enterobacter spp. collected over an 8-year period at a tertiary university hospital.

Of the 434 gram-negative bacteremias identified from October 1994 to October 2001 at the Seoul National University Children's Hospital, Escherichia coli was the most common causative organism, with 146 episodes, followed by Klebsiella pneumoniae with 114 episodes, and Enterobacter spp. were the third most common causative organisms, with 76 episodes (74 E. cloacae isolates, 1 E. aerogenes isolate, and 1 Enterobacter sp. isolate). Of the 76 Enterobacter blood isolates, 72 (71 E. cloacae and 1 Enterobacter sp.) were included in this study.

Species identification was carried out with VITEK-GNI CARDS by standard methods (4), and MICs of antibiotics were determined by the E-test (AB Biodisk, Piscataway, N.J.). To study the inducibility of the AmpC enzyme, the cefotaxime–cefotaxime disk antagonist test was performed as described previously (16), and β-lactamase inducibility was confirmed by the presence of a blunted cefotaxime zone adjacent to the cefoxitin disk. ESBL production was tested by comparing the inhibitory zone diameters of a 30-μg cefepime disk and a 30-μg cefepime–10-μg clavulanic acid disk; an increase of 5 mm or more in the zone diameter was considered positive (3). The strains were also subjected to the double-disk diffusion test with cefotaxime, cefazidime, cefepime, and amoxicillin-clavulanic acid disks as described previously (17, 19).

To characterize ESBLs, isoelectric focusing and inhibition assay with 0.3 mM clavulanic acid or cloxacillin were performed as described previously (10). For isolates which produced β-lactamase with a pI of 7.6 or 8.2 and whose activity was inhibited by 0.3 mM clavulanic acid, SHV-specific PCR and ligase chain reaction were performed as described previously (5), and for those producing β-lactamase with a pI of 5.9, TEM-specific PCR was performed (11), and one of the purified amplicons was sequenced. To characterize the β-lactamase with a pI of 8.0 whose activity was inhibited by 0.3 mM clavulanic acid, CTX-M-14-specific PCR was performed (12).

According to the characteristics of β-lactamase production, we defined the Enterobacter isolates as follows: derepressed AmpC mutants are those with a cefoxitin MIC of ≥32 μg/ml, a cefotaxime MIC of ≥16 μg/ml, a negative cefotaxime–cefotaxime disk antagonist test, and a negative ESBL confirmatory test; partially derepressed AmpC mutants are those with the same characteristics as derepressed AmpC mutants but with a positive cefotaxime–cefotaxime disk antagonist test; derepressed AmpC mutants with ESBL production are isolates showing a cefotaxime MIC of ≥32 μg/ml and a negative cefotaxime–cefotaxime disk antagonist test and producing ESBLs; ESBL producers are isolates which produced ESBLs and for which the cefotaxime MIC was ≤8 μg/ml; and inducible AmpC-producing strains are those with a cefotaxime MIC of ≤8 μg/ml and a positive cefotaxime–cefotaxime disk antagonist test and without ESBL production.

Of 72 blood isolates of Enterobacter spp., 32 isolates were derepressed AmpC mutants and 4 were partially derepressed.

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AmpC mutants; thus, 50% (36 of 72) of the blood isolates were either derepressed or partially derepressed AmpC mutants (Table 1). The ESBL production rate in the blood isolates of Enterobacter was 43% (31 of 72). Among the extended-spectrum cephalosporin-resistant Enterobacter isolates, the ESBL production rate was high; 46 of the 72 isolates (64%) were resistant to extended-spectrum cephalosporins, and 31 of the 46 extended-spectrum cephalosporin-resistant strains (67.3%) produced ESBLs. During the same period at the same hospital, the ESBL production rates of E. coli and K. pneumoniae were 17.9 and 52.9%, respectively (6).

When we categorized the 72 blood isolates of Enterobacter according to the definition described above, 15 isolates were derepressed or partially derepressed AmpC mutants, 21 strains were derepressed AmpC mutants with ESBL production, 3 strains produced ESBLs only, and 26 isolates were inducible AmpC-producing strains. Seven isolates that showed a positive cefoxitin-cefotaxime antagonist test and also produced ESBLs were derepressed or partially derepressed AmpC mutants, 21 strains with ESBL production were twofold higher than those for the derepressed mutants without ESBLs. For cefepime, MICs were higher for the derepressed AmpC mutants with ESBLs than for those without ESBLs. All isolates of derepressed or partially derepressed AmpC mutants except one showed MICs of 8 μg/ml or less; however, 9 of 21 isolates (42.8%) of those with ESBLs showed MICs higher than 8 μg/ml (Fig. 1). Nevertheless, MICs for ESBL-producing strains and non-ESBL-producing strains overlapped each other.

Cefepime is considered one of the treatment choices for infections by Enterobacter spp. because of its consistent activity against Enterobacter spp., even against derepressed mutants (9, 13). However, it still remains uncertain whether to use cefepime for infections caused by ESBL-producing gram-negative bacteria, for which the MIC of cefepime is low (18). Therefore, a confirmatory test for ESBL is necessary for extended-spectrum cephalosporin-resistant Enterobacter spp.

Concerning the MICs of cephemycins, the MIC of cefoxitin for the derepressed or partially derepressed AmpC mutants with or without ESBLs was \( \geq 32 \) μg/ml, and for 34 of 36 isolates (94%) the MIC of cefotetan was \( \geq 32 \) μg/ml. However, 28 of 29 inducible strains (96.5%) showed cefotetan MICs of \( \leq 16 \) μg/ml regardless of ESBL production, although 23 of 29 isolates (79.3%) showed cefotaxime MICs of \( \geq 32 \) μg/ml. The MIC\(_{50}\) of cefotetan for the derepressed or partially derepressed AmpC mutants was 128 μg/ml, whereas that for the inducible strains was 0.5 μg/ml. Therefore, these different resistance profiles of cefoxitin and cefotetan would help differentiate ESBL-producing strains from derepressed AmpC mutants. However, our data showed that most ESBL-producing Enterobacter spp. were already derepressed AmpC mutants (21 of 31 strains [67%]), thus limiting the use of resistance profiles of cefoxitin and cefotetan for discriminating ESBL production in Enterobacter spp.

Thirty-one isolates which produced β-lactamases other than AmpC β-lactamase with or without a TEM-1-like enzyme with a pI of 5.4 were further characterized for β-lactamases. The results showed that 14 strains produced SHV-12, 3 strains produced SHV-2a, 7 isolates produced a TEM-52-like ESBL, and 1 strain produced a CTX-M-14-like enzyme. Two isolates produced a TEM-related ESBL with a pI of 5.4, one isolate produced a SHV-related ESBL with a pI of >8.2, and

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**TABLE 1.** Antimicrobial susceptibilities, cefoxitin-cefotaxime antagonism test, and confirmatory test for ESBL production for blood 71 E. cloacae isolates and 1 Enterobacter sp. isolate

<table>
<thead>
<tr>
<th>Group (no. of strains)</th>
<th>Cefoxitin-cefotaxime antagonism</th>
<th>ESBL testa</th>
<th>MIC(_{50}): MIC range (μg/ml)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derepressed or partially derepressed mutants (15)</td>
<td>+, 1; +, 4</td>
<td>–, 15</td>
<td>&gt;256, 32–256</td>
</tr>
<tr>
<td>Derepressed mutants with ESBL production (21)</td>
<td>+, 21</td>
<td>+, 18, –, 3</td>
<td>&gt;256, 48–256</td>
</tr>
<tr>
<td>Strains with ESBL production (3)</td>
<td>+, 3</td>
<td>+, 3</td>
<td>8, 4–12</td>
</tr>
<tr>
<td>Inducible strains without ESBL production (26)</td>
<td>+, 26</td>
<td>NDc</td>
<td>&gt;256, 0.75–256</td>
</tr>
<tr>
<td>Undetermined strains (7)</td>
<td>+, 6, –, 1</td>
<td>+, 7</td>
<td>&gt;256, 32–256</td>
</tr>
</tbody>
</table>

a The numbers of isolates with negative (–) and positive (+) results are shown.

b The confirmatory test for ESBL production was performed by enhancement of the inhibitory zone of cefepime (30 μg) with addition of 10 μg of clavulanic acid and by the double-disk synergy test with cefotaxime, ceftazidime, cefepime, and amoxicillin-clavulanic acid disks.

c Abbreviations: FOX, cefoxitin; CTT, cefotetan; CPD, cepodoxime; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; FEP, cefepime; PIP, piperacillin; TZP, piperacillin-tazobactam; TIM, ticarcillin-clavulanic acid; IPM, imipenem; GEN, gentamicin; AMK, amikacin; OFX, ofloxacin.
three strains produced an OXA-related β-lactamase with a pI of 7.5.

In summary, production of ESBLs is an important resistance mechanism in Enterobacter spp. In order to use cefepime safely for the treatment of infections by Enterobacter spp., an ESBL confirmatory test is necessary for clinical isolates of Enterobacter spp.

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REFERENCES