Yonsei Medical Journal
Vol. 39, No. 6, pp. 514-519, 1998

☐ Review Article ☐

# The Characteristics of Extended-Spectrum β-Lactamases in Korean Isolates of *Enterobacteriaceae*

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*Extended-spectrum β-lactamases (ESBLs) in gram-negative organisms have been implicated as the enzymes responsible for resistance to oxyimino-cephalosporins. The incidence of ESBL-producers in Korean isolates of* Escherichia coli *and* Klebsiella pneumoniae *were in the range of 4.8-7.5% and 22.5-22.8%, respectively. The ESBL-producing isolates revealed variable levels of resistance to cefotaxime, ceftazidime and aztreonam. They also showed the elevated MIC values of non-β-lactam antibiotics. SHV-12 and SHV-2a were the enzymes most frequently found in* K. pneumoniae *strains, but TEM-52 was the most prevalent in* E. coli *isolates. About 15% of ESBL-producing isolates of* Enterobacteriaceae *produced CMY-1 enzyme, which conferred resistance to cephamycins such as cefoxitin as well as oxyimino-cephalosporins. Thus, the most common types of ESBLs in Korea are TEM-52, SHV-12, SHV-2a, and CMY-1.*

**Key Words:** Extended-spectrum β-lactamase (ESBL), TEM-52, SHV-12, SHV-2a, CMY-1, *Escherichia coli, Klebsiella pneumoniae*

β-lactam antibiotic has been one of the most commonly prescribed antibiotics worldwide. In our hospital, β-lactam antibiotics have accounted for approximately 58.6% of the total antibiotics used (Rheem *et al.* 1998). With the widespread use of β-lactam antibiotics, resistance to β-lactams has been increasing. Although there are various mechanisms of bacterial resistance to β-lactam antibiotics, the most important is the production of β-lactamases, which hydrolyze the β-lactam ring of penicillins, cephalosporins, and related antimicrobial drugs, rendering them inactive. There are dozens of β-lactamases, which vary in substrate specificity. Among them, extended-spectrum β-lactamases (ESBLs) in gram-negative organisms have recently been causing major clinical problems.

ESBLs, such as plasmid-mediated class A TEM or SHV-type enzymes, have developed by stepwise mutations in their structural genes, resulting in either single or multiple amino acid changes in the encoded enzymes. They confer variable levels of resistance to cefotaxime, ceftazidime and other broad-spectrum cephalosporins, as well as to monobactams such as aztreonam, but they have no detectable activity against cephamycins and carbapenems (Jacoby and Medeiros, 1991). Since these enzymes were first identified in Western Europe, more than 50 ESBLs have been found worldwide. More than 36 TEM variants were identified resulting from substitutions of several amino acids in 19 sites of the TEM-1 enzyme, and at least 10 SHV and 5 OXA enzyme variants were discovered (Jacoby and Bush, 1998). Recently, new plasmid-mediated ESBLs, not derived from the TEM or SHV family but related to cephalosporinases of *Enterobacteriaceae* (AmpC enzymes), and that confer resistance to all cephalosporins, including cephamycins such as cefoxitin, have been reported (Bauernfeind *et al.* 1989; Sirot, 1995; Bauernfeind *et al.* 1996).

Received November 10, 1998
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While the incidence of resistance to extended-spectrum β-lactam antibiotics is increasing in Korea, little data on the β-lactamases has been available until now. We will briefly review the prevalence and characterization of extended-spectrum β-lactamases (ESBLs) produced by Korean isolates of *Enterobacteriaceae*.

## Antimicrobial susceptibilities of gram-negative organisms isolated in Korea

The antimicrobial resistance rate of gram-negative organisms was determined from 30,599 clinical isolates collected from 67 hospitals in 1994 and 1995 (Chong *et al.* 1996). The report showed that 5%, 6%, and 3% of *E. coli* expressed resistance to cefotaxime, ceftazidime, and aztreonam, respectively. For *K. pneumoniae,* 15%, 14%, and 17% of the isolates were resistant to cefotaxime, ceftazidime, and aztreonam, respectively. Although the disk diffusion method with the usual 30 μg antibiotic disks sometimes fail to detect ESBL-producing isolates, the approximate prevalence of ESBL-producing isolates can be speculated with these resistance rates. More interestingly, 7% of *E. coli* and 13% of *K. pneumoniae* were revealed to be resistant to cefoxitin. Because *E. coli* and *K. pneumoniae* do not have inducible AmpC β-lactamases, the cefoxitin resistance rate of these species could be considered relatively high.

Other *Enterobacteriaceae* such as *Enterobacter cloacae, Citrobacter freundii,* and *Serratia marcescens* had a higher resistance rate to oxyimino-cephalosporins and cephamycins; The resistance rates to cefoxitin, ceftazidime, cefotaxime or ceftriaxone, and aztreonam were: *E. cloacae,* 89%, 32%, 40%, and 25%; *C. freundii,* 90%, 39%, 37%, and 30%; and *S. marcescens,* 34%, 10%, 24%, and 7%, respectively.

## Incidence of ESBL-producing isolates in Korea

There have been several reports of the prevalence of ESBL-producing *Enterobacteriaceae* in Korea. In 1994, Lee *et al.* found that 7.5% of *E. coli* and 22.8% of *K. pneumoniae* isolated at Yonsei Medical Center produced ESBLs (Lee *et al.* 1994). Another report (Pai *et al.* 1997) described that 20 of 90 clinical isolates of *K. pneumoniae* which were collected from Seoul National University Hospital and Dankook University Hospital produced ESBLs, thus the prevalence rate of ESBL-producing *K. pneumoniae* was 22.5%. However, the clinical strains from Seoul National University Hospital harbored ESBLs more commonly than those from Dankook University Hospital (30% vs 12.5%). Among *E. coli* strains, the frequency of ESBL producers was much less, at 4.8% (Pai *et al.* 1998). Considering those reports, approximately 20−30% of *K. pneumoniae* and 5−10% of *E. coli* strains isolated in Korea were thought to produce ESBLs. Recently, another report revealed that *E. cloacae* collected from ICU patients harbored ESBLs in much higher frequencies (Lee *et al.* 1998). Thus, further studies on the ESBL-producing *E. cloacae* became necessary.

## In vitro activities of antibiotics against ESBL producing isolates

ESBLs vary considerably in the level of resistance conferred to cefotaxime, ceftazidime, or aztreonam. Some types of ESBLs such as TEM-3, TEM-4, TEM-20, and others conferred more resistance to cefotaxime, but other enzymes showed more resistance to ceftazidime. Thus, the in vitro activities of various β-lactam antibiotics against the ESBL-producing isolates vary from country to country and between institutions within a country. We determined the MIC values of β-lactam and non-β-lactam antibiotics against 53 ESBL-producing isolates and 63 non-ESBL-producing isolates of *K. pneumoniae* (Lee *et al.* 1997). For the ESBL-producing isolates, the $MIC_{50}$ of cefotaxime, ceftazidime, and aztreonam were 16, 128, and 128 μg/mL respectively (Table 1). The organisms with ESBLs also showed the elevated MIC values of non-β-lactam antibiotics: the $MIC_{50}$ of gentamicin, amikacin, and ciprofloxacin were 8, 8, and $<0.5$ μg/mL, and the $MIC_{90}$ values of gentamicin, amikacin, and ciprofloxacin were 128, 32, and 8 μg/mL respectively. For the isolates which did not produce ESBLs, the $MIC_{50}$ and $MIC_{90}$ of gentamicin, amikacin and ciprofloxacin were $<1$ and 64 μg/mL, $<1$ and 2 μg/mL, $<0.5$ and $<0.5$ μg/mL, respectively ($p<0.001$) (Table 2). These data clearly showed that

**Table 1. In vitro activities of β-lactam antibiotics against ESBL-producing and non-ESBL-producing *K. pneumoniae***

| Organsims (No. of strain) | Antimicrobial agents | MIC (μg/mL) | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| With ESBL (53) | Cephalothin | >512 | >512 | 64−>512 |
| | Cefoxitin | 8 | 128 | 2−>512 |
| | Cefotaxime | 16 | 128 | <1−>512 |
| | Ceftazidime | 128 | >512 | 2−>512 |
| | Ampicillin | >512 | >512 | 8−>512 |
| | Piperacillin | 64 | >512 | 4−>512 |
| | Aztreonam | 128 | 256 | <1−>512 |
| | Imipenem | <1 | 2 | <1−32 |
| Without ESBL (63) | Cephalothin | <1 | 16 | <1−128 |
| | Cefoxitin | 4 | 32 | <1−>512 |
| | Cefotaxime | <1 | <1 | <1−16 |
| | Ceftazidime | <1 | <1 | <1−4 |
| | Ampicillin | 128 | >512 | 8−>512 |
| | Piperacillin | <1 | 8 | <1−256 |
| | Aztreonam | <1 | 2 | <1−128 |
| | Imipenem | <1 | <1 | <1−4 |

**Table 2. In vitro activities of non-β-lactam antibiotics against ESBL-producing and non-producing *K. pneumoniae***

| Organsims (No. of strain) | Antimicrobial agents | MIC (μg/mL) | | |
|---|---|---|---|---|
| | | 50%* | 90% | Range |
| With ESBL (53) | Amikacin | 8 | 32 | <1 −64 |
| | Gentamicin | 8 | 128 | <1 −>512 |
| | Ciprofloxacin | 0.5 | 8 | <0.5−8 |
| Without ESBL (63) | Amikacin | <1 | 2 | <1 −32 |
| | Gentamicin | <1 | 64 | <1 −>512 |
| | Ciprofloxacin | <0.5 | <0.5 | <0.5 |

*: $MIC_{50}$ values of amikacin, gentamicin, and ciprofloxacin were significantly higher in ESBL-producing isolates (Mann-Whitney U test, $p<0.001$).

ESBL-producing isolates were more resistant to non-β-lactam antibiotics, which could limit the therapeutic options for the infections by ESBL-producing isolates.

### Characteristics of ESBLs produced by *Enterobacteriaceae* isolated in Korea

Although a limited number of isolates was studied, the ESBLs from Korean isolates had somewhat different characteristics (Kim *et al.* 1998) (Table 3). In the study with 53 clinical isolates of *K. pneumoniae*, the most prevalent types of ESBLs were SHV-12 and SHV-2a, which have rarely been found in other countries. Furthermore, SHV-12 was only recently found in two Swiss isolates. However, in Korea, 27 of 53 *K. pneumoniae* isolates harbored SHV-12. These enzymes expressed a high level of resistance to ceftazidime and aztreonam. On the other hand, SHV-2a conferring a relatively low level

**Table 3. Distribution of ESBLs produced by Korean isolates of *Enterobacteriaceae***

| Organism | ESBL types | pI value | No. of isolates |
|---|---|---|---|
| *K. pneumoniae* | SHV-12 | 8.2 | 24 |
| | SHV-2 | 7.6 | 10 |
| | SHV-2 + TEM-1 | 5.4, 7.6 | 1 |
| | SHV-12 + CMY-1-like | 8.0, 8.2 | 1 |
| | SHV-2 + CMY-1-like | 7.6, 8.0 | 1 |
| | TEM-52 + TEM-1 | 5.4, 5.9 | 8 |
| | TEM-52 + SHV-12 | 5.9, 8.2 | 2 |
| | TEM-52 + CMY-1-like | 5.9, 8.0 | 2 |
| | CMY-1-like | 8.0 | 4 |
| *E. coli* | TEM-52 | 5.9 | 2 |
| | TEM-52 + TEM-1 | 5.4, 5.9 | 8 |
| | SHV-12 + TEM-1 | 5.4, 8.2 | 1 |
| | SHV-2 | 7.6 | 1 |
| | SHV-2 + CMY-1-like + TEM-1 | 5.4, 7.6, 8.0 | 1 |
| | CMY-1-like + TEM-1 | 5.4, 8.0 | 1 |
| | AmpC Hyperproducer | 8.5, >8.5 | 2 |

**Table 4. Antimicrobial susceptibilities, pIs of β-lactamases, and plasmid sizes of CMY-1 producing *K. pneumoniae* strains**

| Isolate No. | Strain | MIC (μg/mL) | | | | | pI(s) of β-lactamases | Plasmid size (kb) |
|---|---|---|---|---|---|---|---|---|
| | | AMP | CFX | CTX | CAZ | AZT | | |
| 1 | KY02 | >512 | 512 | 32 | 2 | <1 | 8.0 | 130, 77 |
| | pKY2 | 128 | 64 | 16 | <1 | <1 | 8.0 | 77 |
| 2 | KY03 | >512 | 256 | 8 | 4 | <1 | 8.0 | 130, 77 |
| | pKY3 | 128 | 64 | 16 | <1 | <1 | 8.0 | 77 |
| 3 | KY04 | 128 | 64 | 8 | 4 | <1 | 8.0 | 130, 77 |
| 4 | KD08 | >512 | 128 | 16 | 4 | 4 | 8.0 | 130 |
| | pKD8 | 128 | 256 | 32 | 2 | 2 | 8.0 | 130 |
| 5 | KY23 | >512 | 54 | 64 | 512 | >512 | 8.0, 8.2 | 142, 106 |
| 6 | KY29 | >512 | >512 | 256 | 32 | 8 | 8.0, 7.6 | 142, 106 |
| 7 | KY05 | >512 | >512 | >512 | 512 | 512 | 8.0, 5.4, 5.9 | 142, 83 |
| 8 | KS18 | >512 | >512 | 128 | >512 | >512 | 8.0, 5.4, 5.9 | 142, 83 |

AMP, ampicillin; CFX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; AZT, aztreonam.

of resistance to cefotaxime, ceftazidime, and aztreonam was discovered in 12 of 53 strains. Thus, 73% of the organisms with ESBLs produced SHV-12 or SHV-2a.

TEM-type ESBLs were also found in 12 of 53 isolates of *K. pneumoniae* (Pai *et al.* 1998). All 12 strains had the same enzymes with a pI of 5.9, and they were shown to be TEM-52 by nucleotide sequencing. TEM-52 is an enzyme only recently found and is not prevalent in other countries. One strain producing this enzyme was found in France (Poyart *et al.* 1998). TEM-52 conferred a relatively low level of resistance to cefotaxime and lesser resistance to ceftazidime and aztreonam. Another report from Korea has also showed that *K. pneumoniae* strains had mostly TEM enzymes with pIs of 5.9 and 5.4. However, $bla_{TEM}$ genes were not sequenced in that report (Chong *et al.* 1997). In *E. coli* strains, TEM-52 was the most prevalent ESBL type in Korea (Pai *et al.* 1998). Ten of 14 ESBL-

producing *E. coli* strains harbored TEM-52. On the other hand, 3 of 14 strains had SHV-12 or SHV-2a.

The most disturbing finding in Korean isolates of *Enterobacteriaceae* was the plasmid-mediated AmpC enzyme. In 1989, CMY-1 was first discovered in *K. pneumoniae* strain CHO in Korea (Bauernfeind *et al.* 1989). The strains harboring this enzyme show resistance to cephamycins such as cefoxitin as well as oxyimino-cephalosporins (Table 4) (Kim *et al.* 1998). These enzymes are not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. In *K. pneumoniae* strains, approximately 15% of ESBL-producing isolates harbored CMY-1 like enzymes, and this enzyme was also found in *E. coli* strains, but less commonly than in *K. pneumoniae*.

From these data we now know that TEM-52, SHV-12, SHV-2a, and CMY-1 are the most common types of ESBLs in Korea. It is interesting that the enzymes rarely found in other countries are the most prevalent in Korea. Another noteworthy finding was that the isolates from different hospitals and during different periods had the same ESBL types. The reason for the common ESBL types in *Enterobacteriaceae* isolated in Korea is not clear. In other countries, such as France and the United States, many kinds of ESBLs were identified in different hospitals, and even in the same institutions. However, a predominant ESBL was present in the clinical isolates from different areas of other countries such as Argentina (Galas *et al.* 1998). The hospitals in which the isolates were collected in our study were located in Seoul, except for Dankook University Hospital, which was located 75 kilometers from Seoul. This could have been the result of frequent shifting of patients among the hospitals. Therefore, the spread of resistant organisms or other movable elements may have played a role in the spread of common ESBLs. Moreover, intrahospital spread of common organisms or similar selective pressure among the institutions could be another possible explanation.

To reduce the prevalence of ESBL-producers in gram-negative organisms, there have been several suggestions (Rice *et al.* 1990; Rice *et al.* 1996; Piroth *et al.* 1998). Decreased use of oxyimino-cephalosporins, especially ceftazidime, has shown a temporary decrease of ceftazidime-resistant *K. pneumoniae*. Some authors have suggested that the use of a β-lactam-β-lactamase inhibitor combination had the effect of preventing acquisition of ESBLs, which has not been fully validated however. In Korea, widespread use of oxyimino-cephalosporins in the hospitals has dramatically increased the prevalence of ESBL-producers in *Enterobacteriaceae*. Therefore, more prudent use of antibiotics is necessary to reduce the spread of these resistant organisms. If necessary, a policy for the strict control of antibiotic usage should be established.

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