The Optimum Agent for Screening and Confirmatory Tests for Extended-Spectrum Beta-Lactamases in *Escherichia coli* and *Klebsiella pneumoniae* in Ramathibodi Hospital, Thailand

Suthan Srisangkaew, M.D.
Malai Vorachit, D.Sc.

**ABSTRACT**

The purpose of this study was to determine the appropriate antibiotic for extended-spectrum beta-lactamases (ESBLs) screening and confirmatory tests in *Escherichia coli* and *Klebsiella pneumoniae*. The sensitivity of each antibiotic recommended by the National Committee for Clinical Laboratory Standards (NCCLS) to screen for ESBL production was compared. A total of 271 *E. coli* and 185 *K. pneumoniae* isolated in clinical microbiology laboratory, Ramathibodi Hospital, Thailand were tested for ESBL by screening and confirmatory disc diffusion methods recommended by the NCCLS. There were 182 isolates that were positive for ESBL confirmatory test. The sensitivity of the screening methods of aztreonam, cefpodoxime, ceftriaxone, cefotaxime, and ceftazidime were 96.70, 97.25, 97.25, 97.25, and 74.18 percent, respectively. Almost all isolates (45/47) that were false negative for ESBL screening by ceftazidime still effectively hydrolyzed the other agents, and were thus presumed to be CTX-M-type-ESBL-producing strains. We conclude that ceftazidime alone is not sensitive enough to be used for screening and confirmation for ESBL production in our hospital. A genotypic method to determine the presence of CTX-M-type ESBL is thus needed. (*J Infect Dis Antimicrob Agents* 2004;21:1-5.)

**INTRODUCTION**

Extended-spectrum beta-lactamases (ESBLs) produced by gram-negative bacilli are major causes of the bacterial resistance to penicillins and cephalosporins. Screening and confirmatory testing for ESBL production are important in assisting clinicians to select the appropriate antibiotics to treat infections.1-3 The disc diffusion method is widely used in most clinical microbiology laboratory because of its low cost and convenience to perform. The National Committee for Clinical Laboratory Standards (NCCLS) recommends the standard methods for screening and confirmation for ESBL.1,4 In screening test, bacterial isolates were tested with at least one of five antibiotic

---

Clinical Microbiology Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

Received for publication: November 12, 2003.

Reprint request: Suthan Srisangkaew, M.D., Clinical Microbiology Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

**Keywords:** ESBL, CTX-M
discs, cefpodoxime (10 g), ceftazidime (30 g), cefotaxime (30 g), ceftriaxone (30 g), and aztreonam (30 g). The isolate is suspected to produce ESBL, if at least one of the zone diameters falls under the cut-off points specified for each antibiotic. ESBL production is further confirmed by the combined-disc method. The antibiotics used for the combined-disc method were ceftazidime and cefotaxime with and without clavulanic acid. The inhibition zone diameters surrounding the same antibiotic with and without clavulanic acid are compared to determine the inhibitory effect of clavulanic acid on the enzyme, which is the characteristic of ESBL.

Although many previous publications reported that ceftazidime was the most sensitive agent for ESBL screening\textsuperscript{1,2,5-10}, some reports did not show the supporting results.\textsuperscript{11-13} This difference may be due to different types of ESBLs epidemic in different environments. Furthermore, the recent outbreak report of CTX-M-type ESBL made us reluctant to continue using ceftazidime alone for ESBL screening as well as many clinical microbiology laboratories in the United Kingdom.\textsuperscript{3,14} This study was conducted to determine the appropriate agents for ESBL screening and confirmatory tests in our hospital.

MATERIALS AND METHODS

Bacterial isolates
A total of 271 \textit{Escherichia coli} and 185 \textit{Klebsiella pneumoniae} isolated from clinical specimens from the Department of Medicine and all intensive care units (ICUs) of Ramathibodi Hospital, Bangkok, Thailand during August 2002 and February 2003 were collected in this study.

Antimicrobial agents
Antibiotic discs including aztreonam (30 g), cefpodoxime (10 g), ceftriaxone (30 g), cefotaxime (30 g), cefotaxime (30 g) plus clavulanic acid (10 g), ceftazidime (30 g), and ceftazidime (30 g) plus clavulanic acid (10 g) (Oxoid, USA) were studied.

Methods
Bacterial isolates were subcultured on blood agar overnight. A few colonies were suspended into 0.9 percent NaCl solution to reach the 0.5 McFarland standard and confirmed by turbidometer. The bacterial suspension was tested with antibiotic discs listed above as recommended by the NCCLS\textsuperscript{4} with \textit{E. coli} ATCC 25922 as the control. The inhibitory zone diameters were interpreted according to the NCCLS for screening and confirmatory testing of ESBL in \textit{K. pneumoniae} and \textit{E. coli}. The cut-off points of screening and confirmatory tests are shown in Table 1.

Data analysis
The sensitivity of aztreonam, cefpodoxime, ceftriaxone, cefotaxime, and ceftazidime was determined using phenotypic confirmatory test as the gold standard.

RESULTS
The phenotypic confirmatory test for ESBL revealed that 39.91 percent (182/456) of all isolates

<table>
<thead>
<tr>
<th>Table 1. Cut-off points of initial screening and confirmatory tests for ESBL production.\textsuperscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial screening test</strong></td>
</tr>
<tr>
<td>Aztreonam (30 g) zone</td>
</tr>
<tr>
<td>Cefpodoxime (10 g) zone</td>
</tr>
<tr>
<td>Ceftriaxone (30 g) zone</td>
</tr>
<tr>
<td>Cefotaxime (30 g) zone</td>
</tr>
<tr>
<td>Ceftazidime (30 g) zone</td>
</tr>
</tbody>
</table>

produced ESBL. The percentage of ESBL production in *E. coli* and *K. pneumoniae* were 37.64 (102/271) and 43.24 (80/185), respectively.

Of 182 ESBL-producing isolates, confirmatory test was positive with cefotaxime plus clavulanic acid in 178 isolates, and positive with ceftazidime plus clavulanic acid in 130 isolates. The sensitivity of cefotaxime and ceftazidime in confirmatory testing were 97.80 and 71.43 percent, respectively. According to the phenotypic confirmatory test as the gold standard, the sensitivity and the false positive rate of each agent to screen ESBL production was shown in Table 2.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity (%)</th>
<th>False-positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam (30 g)</td>
<td>96.70</td>
<td>19.18</td>
</tr>
<tr>
<td>Cefpodoxime (10 g)</td>
<td>97.25</td>
<td>18.43</td>
</tr>
<tr>
<td>Ceftriaxone (30 g)</td>
<td>97.25</td>
<td>19.18</td>
</tr>
<tr>
<td>Cefotaxime (30 g)</td>
<td>97.25</td>
<td>20.27</td>
</tr>
<tr>
<td>Ceftazidime (30 g)</td>
<td>74.18</td>
<td>21.05</td>
</tr>
</tbody>
</table>

Almost all bacterial isolates giving false-negative screening results with ceftazidime (45/47) revealed no additional beta-lactamases inhibitory effect of clavulanic acid when tested with cefotaxime plus clavulanic acid. This implies that ESBL cannot hydrolyze ceftazidime effectively. These isolates were susceptible to ceftazidime in initial screening and no inhibitory effect of clavulanic acid was observed in the confirmatory test. However, they still effectively hydrolyzed astreonam, cefpodoxime, ceftriaxone, and cefotaxime. This characteristic is specific to the newly emergent type of ESBL called CTX-M-type ESBL, which needed further genotypic confirmation. In this study, the suspected CTX-M-type ESBL isolates comprise 24.73 percent of all ESBL isolates and 9.87 percent of all isolates. Mainly of suspected CTX-M-type ESBL isolates were observed in *E. coli* (82.22 percent).

**DISCUSSION**

Detection of ESBL production in *E. coli* and *K. pneumoniae* in clinical practice is not only important in guiding clinicians to select the appropriate antibiotics for patients but also for early implement of appropriate infection control measures. Molecular methods for ESBL detection are sensitive, but they are expensive, time-consuming, and require specialized equipment and expertise. Since disc-diffusion susceptibility testing is widely used, the NCCLS has established the disc-diffusion technique as a standard method for ESBL detection. However, the selection of screening agents for ESBL is left open. Several studies recommend ceftazidime is the most sensitive agent for ESBL screening, while others recommend cefpodoxime or cefotaxime. This difference may be caused by various types of ESBL which may be epidemic in different environments.

In our study, ceftazidime yielded the lowest sensitivity for ESBL screening, while the other agents showed higher sensitivity. The false-positive rates were not significantly different. The low sensitivity of ceftazidime could probably be explained by the presence of CTX-M-type-ESBL-producing *E. coli* and *K. pneumoniae* in our isolation, as reported by other medical centers in the United Kingdom, Japan, Bulgaria, Poland, India, and France.

CTX-M-type ESBL is now considered as an emerging problem because this enzyme can escape from ESBL screening by ceftazidime, the most widely used single agent for ESBL screening. Therefore,
it is probably appropriate not to use ceftazidime as the sole means for screening of ESBL. The sensitivity of ESBL screening test could be improved by adding cefotaxime or cefpodoxime, and the sensitivity of ESBL confirmatory test could be improved by adding cefotaxime plus clavulanic acid or cefpodoxime plus clavulanic acid.

A limitation of the phenotypic confirmatory testing without genotypic confirmation of ESBL gene prevented this study to evaluate efficiency of the phenotypic confirmatory test to identify ESBL production of bacterial isolates in our hospital.

Futhermore, the phenotypic confirmatory test cannot detect ESBL in some strains which also produce other classes of beta-lactamases including AmpCs and inhibitor-resistant TEMs (IRTs). These enzymes can mask the inhibitory effect of clavulanic acid on ESBL in the phenotypic test, resulting in a false-negative result. Hyperproduction of TEM and/or SHV beta-lactamases in accompanied with ESBL can cause a false-negative result by the phenotypic test. A genotypic method to determine the presence of CTX-M-type ESBL in these isolates is thus needed.

**CONCLUSION**

Ceftazidime alone is not appropriate to be used for screening and confirmatory tests of ESBL production in our hospital. The sensitivity of ESBL screening tests could be improved by adding cefotaxime or cefpodoxime, and the sensitivity of ESBL phenotypic confirmatory test could be improved by adding cefotaxime plus clavulanic acid or cefpodoxime plus clavulanic acid.

**ACKNOWLEDGEMENT**

This study was supported by Research Grant from the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand.

**References**

11. Ho PL, Tsang DN, Que TL, Ho M, Yuen KY. Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among


