Correspondence

Detection of ESBLs using third & fourth generation cephalosporins in double disc synergy test

Sir,

For detection of various types of extended spectrum beta lactamases (ESBLs), molecular methods are the best but the facilities are not available in most of the laboratories especially in the developing countries. Therefore, various phenotypic methods are recommended for routine use to detect ESBL production in Gram negative bacilli. Of these, double disk synergy test (DDST) using third generation cephalosporins (3GC) is the simple and reliable method, easily adopted by laboratories for detection of ESBLs among clinical isolates. Recently, co-existence of both Amp C and ESBLs in some Gram negative organisms has been reported. Such strains may give false negative tests for detection of ESBLs. In such cases, fourth generation cephalosporin (4GC)- ceftazidime is found to act as a better indicator drug. We undertook this study with the aim of improving the detection of ESBLs in *Klebsiella pneumoniae* and *Escherichia coli* using both 3GC and 4GC in DDST.

The study was conducted in the Department of Microbiology, Government Medical College Hospital, Chandigarh. A total of 100 consecutive isolates of *K. pneumoniae* (42) and *E. coli* (58) were studied for ESBL production during July 2005 to June 2006. These isolates were obtained from various samples namely urine (58), pus and body fluids (24) and blood (18).

All isolates showing zone diameter of < 27mm for cefotaxime and < 25mm for ceftriaxone were selected for ESBL production. ESBL production was tested by DDST using a disc of ceftazidime-clavulanate along with four cephalosporins; 3GC- cefotaxime, ceftriaxone, cefoperazone and 4GC- ceftazidime. A lawn culture of the organisms was made on Mueller-Hinton agar plate as recommended by CLSI. A disc containing ceftazidime-clavulanate was placed in the centre of the plate. The discs of 3GC and 4GC were placed 15 and 20 mm apart respectively, centre to centre to that of ceftazidime-clavulanate disc. Any distortion or increase in zone towards the disc of ceftazidime-clavulanate was considered positive for ESBL production. *K. pneumoniae* ATCC 700603 was used as a control strain for positive ESBL production and *E. coli* 25922 was used as a negative control for ESBL production.

In our study, 69 isolates (69%) were found to be ESBL producers; 24(34.78%) of these were from ICU patients, 12(17.39%) from surgical ward, 8(11.59%) gynecological ward, 7(10.14%) medicine ward, 3(4.34%) paediatric ward and 15(21.73%) were from out patient department.

ESBL positivity was 63.79 (37), 87.5 (21) and 61.11 per cent (11) in uropathogens, pus and body fluids isolates and blood isolates respectively (Table). Various studies done on Gram negative bacilli isolated from

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>E. coli</em></th>
<th><em>K. pneumoniae</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Total isolates</td>
<td>45</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>ESBL producers</td>
<td>27 (60)</td>
<td>10 (76.92)</td>
</tr>
<tr>
<td>Pus and body fluids</td>
<td>Total isolates</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>ESBL producers</td>
<td>8 (100)</td>
<td>13 (81.25)</td>
</tr>
<tr>
<td>Blood</td>
<td>Total isolates</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>ESBL producers</td>
<td>2 (40)</td>
<td>9 (69.23)</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages.

Table. Percentage isolation of ESBLs in various isolates
clinical specimens have reported varied ESBL production rate. High prevalence of ESBL producing Klebsiella strains has been repeatedly reported\textsuperscript{4,5}. Our study also supported the same except for the isolates from pus and body fluids where ESBL production was seen more in \textit{E. coli} than \textit{K. pneumoniae}. As reported previously\textsuperscript{6} \textit{E. coli} isolates showed higher percentage positivity for ESBLs in wound swabs from admitted patients. Our samples of pus and body fluids showing ESBL producing \textit{E. coli} were also from indoor patients.

The antimicrobial susceptibility testing was done in all the 100 isolates by Stokes disc diffusion method as per the CLSI recommendations by using various antibiotics (in\( \mu \text{g} \)) viz., cefotaxime (30), ceftriaxone (30), cefoperazone (30), cefepime (30), cotrimoxazole (25), amikacin (30), gentamicin (10), ciprofloxacin (5), piperacillin (100), ceftazidime/clavulanate (30/10), ceferazone/sulbactam (75/35) and imipenem (10). ESBL producing organisms (69) showed comparatively high level co-resistance to gentamicin (91.17%), cotrimoxazole (100%) and ciprofloxacin (94.91 %). Resistance to amikacin was comparatively less (64.28%). All the strains were sensitive to imipenem, however, eight (11.59%) of the ESBL producing strains showed resistance to combination drug cefperazone-sulbactam. Similar finding has also been reported earlier\textsuperscript{2}.

In the presence of AmpC along with ESBL in Gram negative organisms, the DDST may not show positivity as Amp C type \( \beta \) lactamase inhibits the action of clavulanate and hence obscures the synergistic effect of clavulanic acid and 3GC. Thus, 4GC -cefepime has been recommended as an alternative cephalosporin for ESBL detection in the presence of AmpC\textsuperscript{7}. Though this drug gets hydrolyzed efficiently by ESBLs, it resists hydrolysis by class C\( \beta \) lactamases\textsuperscript{8}. It has been reported that the use of cefepime increases the sensitivity of DDST with extended spectrum cephalosporins for the detection of ESBLs in \textit{K. pneumoniae} and \textit{Enterobacter} species\textsuperscript{7,8}. We used both 3GC and 4GC in DDST and found that two of our \textit{K. pneumoniae} isolates were positive for DDST with cefepime only, but not with any of the 3GCs used.

In conclusion, the sensitivity of detection for ESBL production can be improved using both 3GC and 4GC for screening purpose. It is important to know the prevalence of ESBL producing organisms in a given area so that judicious use of antibiotics could be done.

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References