Correspondence

Evaluation of CIVA agar for rapid detection of extended spectrum β-lactamases (ESBL) among isolates of Enterobacteriaceae

Sir,

The emergence of plasmid mediated extended spectrum β-lactamases (ESBLs) in members of family Enterobacteriaceae has become a worldwide problem. Many clinical microbiology laboratories make no effort to detect ESBL production by Gram negative bacilli and have problem to detect ESBL mediated resistance. The choice of optimal laboratory method for detection and surveillance of ESBL producing organisms is still not clear. ESBLs are encoded on conjugative plasmids, transposons or integrons and these elements are readily spread under selective antibiotic pressure. Failure to detect ESBL mediated resistance has contributed to uncontrolled spread of ESBL producers and related treatment failures. Identification of ESBLs, based on phenotypic test is the strategy most commonly used in clinical laboratories. Standard methods for detection of ESBL in clinical isolates namely double disk synergy test, National Committee for Clinical Laboratory Standards (NCCLS) phenotypic confirmatory test (using cephalosporin/clavulanate combination disks), broth micro dilution assay and E test ESBL strip are time consuming and not easily performed. Since a simple method for rapid detection of ESBLs from clinical specimens of patients with severe infection is urgently needed, the present study was undertaken to evaluate the use of a screening medium CIVA agar (ceftazidime, inositol, vancomycin, amphotericin B) for rapid detection of ESBLs among isolates of Enterobacteriaceae.

The study was carried out between January and April 2006 in the Department of Microbiology, PSG Institute of Medical Sciences and Research; using clinical specimens obtained from inpatients of PSG Hospitals, Coimbatore. A total of 150 clinical specimens including urine (82), pus (39), tracheal aspirate (18) and sputum (11), all of which yielded pure growth of lactose fermenters within 24 h on MacConkey agar were included in the study. The above specimens were reinoculated next day on CIVA agar and incubated in ambient air at 35-37°C for 16-18 h to screen for ESBL producing isolates of Enterobacteriaceae.

CIVA agar was prepared as described in an earlier study. CIVA agar is a selective as well as differential medium. Presence of vancomycin (10 mg/l), amphotericin B (4.5 mg/l) and ceftazidime (2 mg/ml) suppresses the Gram positive bacteria, yeast and ceftazidime susceptible organism respectively. Moreover, presumptive identification of the Enterobacteriaceae isolates is also possible due to inositol fermentation.

Bacterial colonies from both plates viz., MacConkey and CIVA agar were identified using standard microbiological methods and were also tested for ESBL production by NCCLS phenotypic confirmatory test and results were interpreted according to guidelines of NCCLS. Plates demonstrating no growth on CIVA agar were further incubated for 24 h. In plates demonstrating growth, nature of colony, size and colour were recorded. Escherichia coli ATCC 25922 (β-lactamase negative) and Klebsiella pneumoniae ATCC 700603 (ESBL positive) strains were used as controls throughout the study.

Of the 150 lactose fermenting isolates, the Gram negative bacteria most commonly isolated were E. coli, (110), followed by K. pneumoniae (34) and Enterobacter spp (6). Upon testing for ESBL production by NCCLS phenotypic confirmatory test, 90 were found positive. ESBL was detected in 69 (62.7%) isolates of E. coli, 19 (55.9%) isolates of K. pneumoniae and 2 (33.3%) of Enterobacter spp. (Table).

On CIVA agar, 91 of 150 specimens yield growth. They were presumptively identified as E. coli (69), K.
*pneumoniae (19) and *Enterobacter* spp. (3) based on colony characteristics on CIVA agar (Table), and the presence of ESBL was confirmed in 90 isolates by NCCLS phenotypic confirmatory test. CIVA agar additionally detected a false positive isolate.

In contrast to previous study ³ the CIVA agar used in our study detected a false positive isolate of *Enterobacter* spp. that failed to show enhanced zone of inhibition between ceftazidime and ceftazidime clavulanic acid. This isolate was found to harbour AmpC β-lactamase upon testing by modified AmpC disc method ⁶. Similar false positivity due to natural AmpC hyper-producing *Enterobacter* spp. has been documented in a recent study ⁷. The sensitivity, specificity, positive predictive value, and negative predictive value, of this test to identify ESBLs were found to be 100, 98.3, 98.9, and 100 per cent respectively as compared with conventional NCCLS phenotypic confirmatory test. Clinical and financial benefits of early determination of antibacterial susceptibility have been discussed earlier ⁸. Though several studies ⁹-¹¹ have documented the use of various selective media and rapid protocol for detecting ESBL producing organism from faecal samples and blood, data on rapid detection of ESBL producing organism on CIVA agar are limited. Compared to several automated system such as Vitek ESBL cards, Microscan walkway ESBL system, BD phoenix ESBL detection method and E test¹ use of CIVA agar to detect ESBLs from clinical samples was found to be a simple and cost-effective method.

The advantage of CIVA agar was the direct identification of ESBL producing organisms from a single primary isolation plate without a need for subculture within 24 h compared with conventional method. It can be easily incorporated in the clinical microbiology laboratories along with the routine media for early detection of ESBL producing *Enterobacteriaceae*. Since the average incubation period required in detecting ESBL mediated resistance was approximately 16-18 h, use of this medium may lead to fast detection of ESBL phenotype thereby facilitating the early administration of appropriate antibiotic of choice.

To conclude, CIVA agar was found to be a sensitive and specific medium for rapid and presumptive identification of ESBL producing *Enterobacteriaceae* from clinical samples and could be used in routine microbiology laboratories.

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**References**


**Table. Detection of ESBL producing strains by phenotypic test and screening on CIVA agar**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colony-characteristics on CIVA agar</th>
<th>ESBL producers</th>
<th>Phenotypic test</th>
<th>Screening on CIVA agar</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Flat colourless</td>
<td></td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Mucoid yellow</td>
<td>19</td>
<td>19</td>
<td></td>
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<tr>
<td><em>Enterbacter</em> spp.</td>
<td>Non mucoid yellow</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>90</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

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