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Correspondence

Performance of extended spectrum beta lactamases (ESBL) screening agar in various clinical specimens

Sir,

Extended spectrum beta lactamases (ESBL) producing members of *Enterobacteriaceae* have gained attention in hospital settings because of limited therapeutic options, poor clinical outcome and causation of a significant proportion of hospital acquired infections. Hospitalized patients colonized with these act as reservoir[1]. Early detection of these bacteria is important to control nosocomial outbreaks.

Laboratory methods to detect ESBL producers from clinical specimens by conventional methods are time consuming. Several phenotypic tests have been recommended for initial screening and subsequent confirmation of ESBL producers, but these are usually performed on clinical isolates following culture and antibiotic susceptibility testing. Use of ceftazidime or cefotaxime incorporated into the routine culture medium for detection of ESBL producers from either clinical isolates or directly from the clinical specimens has also been suggested[2]. Though selective culture media such as MacConkey agar with ceftazidime and Drigalski lactose agar are available, these do not specifically detect ESBL producers[3], mainly due to the growth of organism with inducible AmpC beta lactamases. It has been shown that incorporation of cloxacillin to the medium can inhibit AmpC production in some bacteria that interferes with ESBL detection[4]. The present study was done to evaluate the ESBL screening agar incorporated with cloxacillin for detection and presumptive identification of ESBL producing *Enterobacteriaceae* directly from clinical specimens.

The study was performed in the Microbiology department of a tertiary care teaching hospital at Kattankulathur, Tamil Nadu, India, during March to August 2012 after obtaining clearance from the Institute's Ethical Committee. The study was carried out in two parts: in part I, 19 Gram negative bacteria belonging to *Enterobacteriaceae* (*Escherichia coli* -14 and *Klebsiella pneumoniae* -5) and resistant to ceftazidime (third generation cephalosporin) were taken as phenotypically confirmed ESBL producers and tested on ESBL screening agar for growth. Sensitivity for cefpodoxime was tested using disc diffusion technique[5]. In part II, various clinical specimens were screened for ESBL production using in-house preparation of ESBL Screen Agar (ESA) consisting of MacConkey agar I with ceftazidime (1.0mg/l) and MacConkey agar II with cefotaxime (1.0mg/l), cloxacillin (400mg/l) and vancomycin (64mg/l) (Hi-media, Mumbai, India). To assess the commensal flora and to check the selectivity of ESA, all specimens were subjected to bacterial culture and identification by standard procedure using conventional methods[6]. The phenotypic confirmation method by combined disc diffusion [ceftazidime (30 µg) alone and combination of ceftazidime (30µg) + clavulanic acid (10µg)] was carried out on the isolates recovered on ESA according to the CLSI guidelines[6]. Standard ATCC controls such as *E.coli* 25922, *Pseudomonas aeruginosa* 27853 were used as negative controls and *Klebsiella pneumoniae* 700603 as positive control.

Among the 19 phenotypically confirmed ESBL producers, 18 (94.73%) grew on MacConkey agar I and 14 (73.68%) grew on MacConkey agar II. Three isolates were sensitive to cefpodoxime. One isolate of *K.pneumoniae* did not grow on either of the two media but showed resistance for cefpodoxime by disc diffusion technique.

Of the 100 clinical specimens analyzed for ESBL screening using ESA, 23 showed growth of Gram-negative bacilli (GNB), 28 showed growth of other microorganisms [Gram-positive cocci -20, Gram-

positive bacilli - 4 and *Candida* spp -4], and 49 specimens showed no growth. Of the 49 specimens showing no growth, 32 culture positive by conventional method were found culture negative on ESA. Of these 32 culture positive isolates, 14 (43.75%) were normal flora that did not grow on ESA, thus reducing the workload for identification and susceptibility testing of isolates. Also, ESA demonstrated a good inhibitory activity against 56.25 per cent (18 of 32) of the true pathogens susceptible to the third generation cephalosporins.

Of the 23 specimens showing growth of GNB on ESA, 18 yielded growth on both the media and five showed growth only on MacConkey agar II. The GNB grown on ESA were recovered from urine (*E.coli* -7), blood (*K. pneumoniae* -1, *E. coli* - 1, *P. aeruginosa* -1, *Acinetobacter* spp. - 2), tracheal aspirate (*Citrobacter* spp. - 1, *E.coli* - 1), pus (*E. coli*-2, *Proteus* spp. -1, *K. pneumoniae* -1, *P. aeruginosa* - 1), vaginal swab (*Proteus* spp. - 1), stool (*E. coli* - 3).

The percentage of ESBL producer on ESA in our study was lower than that reported in other studies[7,8]. This could be due to the use of cefotaxime and ceftazidime. It was suggested that those isolates producing ESBL enzyme CTX-M type are sensitive to ceftazidime giving false negative results and lowers the percentage[9]. Cefpodoxime is considered the best for screening all types of ESBL producers in clinical specimens[2]. In the present study, *Escherichia coli* was the predominant ESBL producer obtained from MacConkey agar -I (n=12) and MacConkey agar -II (n=14).

The phenotypic confirmation method (combined disc diffusion test) demonstrated only 36.84 per cent (7/19) of ESBL screen agar positive GNB as potential ESBL producer. The remaining 63.15 per cent (12/19) ESA positive isolates were found to be negative by phenotypic confirmation method. The low sensitivity was due to the use of only ceftazidime and ceftazidime+clavulanic acid in phenotypic confirmation method whereas ESA contained cefotaxime in addition to ceftazidime. It has been suggested that ceftazidime alone is not a preferred substrate to demonstrate the ESBL activity resulting in low sensitivity[8].

The present results show that the ESA is particularly useful when detection of resistance is urgently required in the patients admitted in high risk units. It reduces the turn around time and may also reduce the cost of establishing the ESBL diagnosis. Moreover, screening is only the initial step for presumptive identification of ESBL production and final diagnosis is made by confirmation testing according to CLSI. Further evaluation of ESA is needed on a large number of isolates originating directly from clinical specimens.

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References

1. Pena C, Ricart A, Ardanuy C, Ayats, Linares J, Pujol M, Dominguez MA, *et al*. Risk factors for feacal carriage of *Klebsiella pneumoniae* producing extended spectrum β- lactamases (ESBL-KP) in the intensive care unit. *J Hosp Infect* 1997; *35* : 9-16.
2. Pfaller MA, Segreti J.Overview of epidemiological profile and laboratory detection of extended spectrum β- lactamases. *Clin Infect Dis* 2006; *42* (Suppl 4): 153-63.
3. Overdevest IT, Willemsen I, Elberts S, Verhulst C, Kluytmans JA. Laboratory detection of extended - spectrum β – lactamase producing Enterobacteriaceae: evaluation of two screening agar plates and two confirmation techniques. *J Clin Microbiol* 2011; *49* : 519-22.
4. Al Naiemi N, Murk JL, Savedkoul PH, Vandenbroucke – Grauls CM, Debets-Ossenkopp YJ. Extended spectrum beta – lactamases screening agar with AmpC inhibition. *Eur J Clin Microbiol Infect Dis* 2009; *28* : 989-90.
5. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*, 22nd informational supplement. Wayne, PA: CLSI; 2012.
6. Fraser AG, Collee JG, Marmion BP, Simmons A. *Mackie and Mccartney practical medical microbiology,* 14th ed. Oxford, UK: Elseiver Health Sciences; 2011.
7. Reglier-Poupet H, Naas T, Carrrer A, Cady A, Adam JM, Fortineau N, *et al*. Performance of Chrom ID ESBL, a chromogenic medium for detection of *Enterobacteriaceae* producing extended - spectrum β- lactamases. *J Med Microbiol* 2008; *57* : 310-5.
8. Glupczynski Y, Berhin C, Bauraing C, Bogaerts P. Evaluation of a new selective chromogenic agar medium for detection of extended - spectrum β- lactamase - producing *Enterobacteriaceae*. *J Clin Microbiol* 2007; *45* : 501-5.
9. Canton R, Coque TM. The CTX-M beta-lactamase pandemic. *Curr Opin Microbiol* 2006; *9* : 466-75.