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Comparative Evaluation of Phenotypic Method and Hicrome ESBL Agar in Detecting ESBL Producing *Enterobacteriaeceae*

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Abstract

Background and Objective: Beta lactamases are responsible for numerous outbreaks of infection in the world. The occurrence of multiple β-lactamases among bacteria not only limits the therapeutic options but also poses a challenge for microbiology laboratories to identify them. This study has been taken to screen and confirm the production of ESBL by phenotypic method and to compare it with the Crome agar for its efficacy, which is essential for infection control and antimicrobial therapy. *Methodology:* A total of 209 isolates belonging to the family *Enterobacteriaceae*obtained from different clinical samples, received in the Department of Microbiology, Adichunchanagiri Institute of Medical Sciences. B.G. Nagara formed the study group. ESBL screening test was done, followed by phenotypic confirmatory test, for ESBL detection asper CLSI guidelines. This method was compared with thechromogenic media (HiCrome ESBL agar) for detecting ESBL producers. *Results:* On assessing the sensitivity and specificity of HimediaCrome agar with that of the phenotypic method to detect ESBL producers, Crome agar detected 87 isolates as ESBL producers. Sensitivity and specificity of Crome agar considering combination disc method as gold standard was 84.2% and 93.8% respectively. Positive and negative predictive value was 91.95% and 87.7% respectively. *Conclusion:* Cefotaxime/clavulanate disc potentiation test detected maximum number of ESBL compared to Ceftazidime/Clavulunate. HiCrome ESBL agar has high sensitivity and specificity in screening for ESBLproducers and can be used routinely in the laboratory for rapid detection of ESBL producers.

Keywords: ESBL; Combination Disc Method; Hicrome ESBL Agar; *Enterobacteriaeceae*.

Introduction

Enterobacteriaceae may exhibit a reduced susceptibility to the beta lactam antibiotics by a number of mechanisms which includes reduced outer membrane permeability, target site modification and efflux of beta-lactams out of the cell. However by far the most common mechanism of resistance is the enzymatic inactivation of the betalactams by a beta-lactamase [1]. Betalactamases are the enzymes produced by microorganisms which can hydrolyse/open the betalactam ring of beta lactam antibiotics like Penicillins and cephalosporins [2].

Extensive use of third generation cephalosporin has led to the evolution of newer beta-lactamases such as extended spectrum beta-lactamases (ESBL).

Organisms producing ESBLs hydrolysepenicillins, cephalosporins and monobactams [3], and are inhibited by clavulanic acid, tazobactamand sulbactam. They are plasmid coded and are easily transmissible from one organism to the other. They are generally derived from TEM and SHV type [4].

The aimof the present study, was to study thephenotypic method for the detection of ESBL producing *Enterobacteriaceae* and to compare the advantage of chromogenic media with the conventional method for the detection of ESBL producing *Enterobacteriaceae.*

Materials and Methods

The present study was carried out in the department of Microbiology, AIMS, B.G. Nagar, for a period of one year. Ethical committee clearance has been taken from the institution. A total of 209 isolates belonging to family *Enterobacteriaceae* from 200 different clinical samples like urine, pus, blood, sputum, high vaginal swab collected from out-patients and in-patients admitted in the hospital were included in the study. Gram negative bacilli other than *Enterobacteriaceae* were excluded. Cultures yielding no growth or contaminants, and urine samples with insignificant/mixed growth were excluded. Non-repetitive 200 samples of urine, sputum, pus, blood, fluid, stool, high vaginal swab and other samples received in the Microbiology laboratory, AIMS, B.G. Nagar were processed. The specimens were brought to laboratory within 20min of sample collection. Wet films of urine samples were done and examined for pus cells and organisms. Samples were processed as per standard protocols and organisms isolated were identified based on the standard procedures.

Methodology for Detection of ESBL

All 209 *Enterobacteriaceae* isolated from clinical specimens were subjected to screening tests for ESBL. After adjusting the bacterial suspension to 0.5 MacFarland's unit, lawn culture was done on MHA. Amoxicillin- clavulanic acid disc (20µg+10µg) was placed in the centre of the petridish and Cefpodoxime 10µg, Ceftazidime 30µg disc were placed on either side of Amoxicillin- clavulanic acid disc at a distance of 20mm. Extension of zone of inhibition of cefpodoxime or ceftazidime towards Amoxyclav disc was taken as ESBL screening positive.

Chromogenic Medium for ESBL Screening

Bacterial suspension was adjusted to 0.5 Mc Farland's unit and streaked onto HiCrome ESBL agar (Hi media Mumbai) . Plates were incubated at 37^0C for 18-24 hours.

Interpretation [5]

- *Escherichia Coli:* pink to burgundy coloration of β−glucuronidase producing colonies
- *Klebsiella, Enterobacter, Serratia, Citrobacter (KESC):* Green/ blue to browny green coloration of ß-glucosidaseproducing colonies
- *Proteae (Proteus, Providencia, Morganella)*: dark brown /light brown coloration of deaminase expressing strains.

All *Enterobacteriaceae* are subjected to ESBL confirmation.

ESBL confirmatory test- Combination disc method/ Disc diffusion test [6]:

Bacterial suspension was adjusted to 0.5 Mc Farland's unit and lawn culture was done on MHA. Ceftazidime 30µg (CAZ), ceftazidime +clavulanic acid 30µg+10µg (CAZ+CAC) and cefotaxime 30µg (CTX) and cefotaxime+clavulanic acid 30µg+10µg (CTX+CEC) were placed >30mm apart as shown in Figure 1. Plates were incubated at 37^0C for 16-18 hours.

Interpretation

≥ 5 mm increase in the zone of inhibition of Ceftazidime+clavulanicacid and/or cefotaxime+ clavulanic acid discs as compared to Ceftazidime and/or cefotaximediscs alone was taken as ESBL positive.

Results

A total of 209 clinical isolates of *Enterobacteriaceae* from different clinical samples like urine, pus, blood, sputum, high vaginal swab collected from out-patients and in-patients admitted in the hospital were included in the study. Out of 200 clinical samples, majority of the isolates were from urine (55.98%), followed by pus (19.13%), sputum (11.48%), high vaginal swab (6.22%), blood (3.82%), stool (2.87%) and fluid (0.47%). Various Gram negative bacilli belonging to family *Enterobacteriaceae*isolated from different clinical specimens (Table 1) and majority were from female patients. Table 2 shows gender distribution from various clinical specimens.

All the 209 isolates were screened for ESBL production. 84 isolates were detected to be ESBL

screening positive and remaining 125 were negative. Irrespective of screening test results, all the isolates were subjected for phenotypic confirmatory test. 102 isolates were detected to be ESBL positive. Out of 209 isolates inoculated onto Hicrome ESBL agar, 87 were detected to be ESBL producers (Table 3). Table 4 shows various methods that detected ESBL producers of *Enterobacteriaeceae*.

Table 1: Various *Enterobacteriaceae* isolated from different clinicalspecimens

Organisms	Urine	Sputum	Pus	Blood	Fluid	HVS	Stool	Total
Esch.coli	71(70.29%)	5(4.95%)	9(8.91%)	2(1.98%)	1(0.99%)	7(6.93%)	6(5.94%)	101(48.31%)
Klebsiellaspp	22(38.59%)	18(31.57%)	10(17.54%)	4(7%)	0	3(5.26%)	0	57(27.27%)
Citrobacterspp	9(60%)	0	4(7%)	0	0	2(13.33%)	0	15(7.17%)
Enterobacterspp	5(38.46%)	1(7.69%)	5(38.46%)	2(15.38%)	0	0	0	13(6.22%)
Proteus spp Vulgaris/mirabilis	2(20%)	0	8(80%)	0	0	0	0	10(4.78%)
Providenciaspp	6(66.66%)	0	2(22.22%)	0	0	1(11.11%)	0	9(4.3%)
Morganellaspp	2(50%)	0	2(50%)	0	0	0	0	4(1.91%)
Total	117(55.98%)	24(11.48%)	40(19.13%)	8(3.82%)	1(0.47%)	13(6.22%)	6(2.87%)	209

Table 2: Gender wise distribution of various clinical samples

Sample	Gender		Total
	Male	Female	
Urine	39(34.5%)	74(65.48%)	113(56.5%)
Sputum	16(69.5%)	7(30.4%)	23(11.5%)
Pus	24(64.9%)	13(35.1%)	37(18.5%)
Blood	2(25%)	6(75%)	8(4%)
Fluid	0	1(100%)	1(0.5%)
HVS	0	11(100%)	11(5.5%)
Stool	4(57.1%)	3(42.9%)	7(3.5%)
Total percentage	85(42.5%)	115(57.5%)	200

Table 3: Number of ESBL isolates detected by phenotypic method and HiCrome agar

Result	Phenotypic method	Hi Crome agar
Positive	102	87
Negative	107	122

Table 4: Comparison of various methods in detecting ESBL among *Enterobacteriaceae*

Methods for ESBL detection	Esch. coli	Klebsiellaspp	Enterobacterspp	Citrobacterspp	Proteus spp	Providenciaspp	Morganellaspp
Screening test	48	12	5	4	7	6	3
Combination disc method	55	19	8	6	6	5	3
Chrom agar	47	14	5	4	7	5	3

Table 5: Sensitivity and specificity of HiCrome ESBL agar considering combination disc method as gold standard

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Hi Crome ESBL agar	84.2%	93.8%	91.95%	87.7%

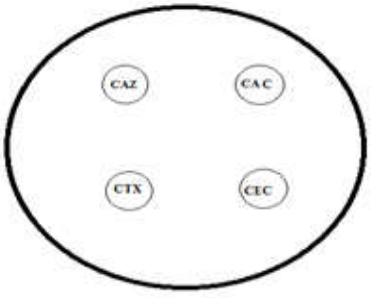

Fig. 1: Template for combination disc test for ESBL detection

Fig. 2: HiCrome ESBL agar
ESBL positive isolate shows growth on Chrom agar. Growth in the tertiary streak line and beyond is considered ESBL positive.

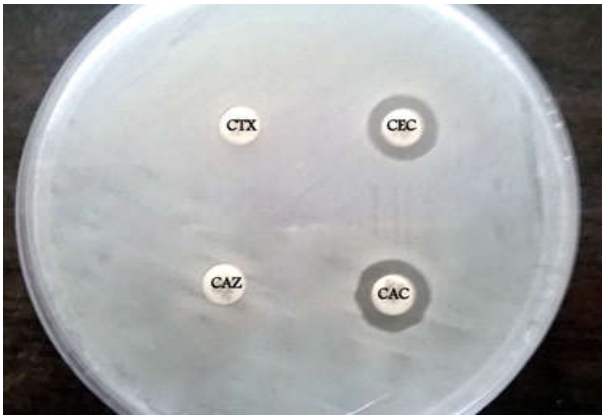

Fig. 3: Combination disc method for ESBL detection.
The isolate is ESBL producer, resistant to CTX and CAZ and the zone of inhibition of CEC and CAC is e" 5mm than CTX and CAZ

Discussion

Members of family *Enterobacteriaceae* accounts for substantial proportion of endemic nosocomial infections. Cephalosporins are the first line drugs used in the treatment of these infections. ESBL detection can be done either by doing confirmatory tests for those isolates which are positive by screening tests or by doing confirmatory tests without prior screening test, as it is notCLSI recommended. Phenotypic ESBL confirmatory test is in routine use [7].

The present study compare the phenotypic method and chromogenic media in detecting the ESBL. In the present study, 209 isolates were screened for the production of ESBL, which showed 84 (40.1%) isolates to be ESBL screening positive and remaining 125 (59.8%) were negative for ESBL production. Irrespective of the screening test all the isolates were put for ESBL confirmatory test. As per CLSI guidelines confirmatory test was put using combination disc method which detected 102 (48.8%) isolates to be ESBL positive, which is comparable with the study of Dalela G in which phenotypic confirmatory disc diffusion test detected 135 (61.6%) out of 219 isolates [8].

In the present study, among the β lactam -inhibitor combination used, CTX and CEC combination detected majority of the ESBL isolates compared to CAZ and CAC. The confirmation of the ESBL production by clavulanic acid inhibition can be difficult in some strains, not only because the activity of the β-lactamase varies with different substrates, but also because the organism may contain additional resistance mechanisms that can mask the presence of the ESBL activity [9]. Shoorashetty RM et al [10] and Sturenberg et al [11] used cefepime/ clavulanate method to detect maximum ESBL.

Apart from phenotypic method, all the isolates were inoculated onto crome agar (HiCrome ESBL agar) for the detection of ESBL. Crome agar detected 87 isolates as ESBL producers.

Sensitivity and specificity of Crome agar considering combination disc method as gold standard was 84.2% and 93.8% respectively. Positive and negative predictive value is 91.95% and 87.7% respectively (Table 5). R.P Helene et al studied the performance of chromID ESBL(bioMerieux) for detection of *Enterobacteriaceae* producing ESBL comparing it with BLSE agar, the sensitivity was 88% for chrom ID ESBL and 85% for BLSE agar [12]. Study by Te-Din Haung et al showed sensitivity of Brilliance ESBL agar (OX; Oxoid, Basingstoke, UK) and Chrom ID ESBL agar (BM; bioMerieux, Marcy l'Etoile, France) 94.9% and specificity was 95.5% and 95.7% respectively [13].

Conclusion

Increased rates of antimicrobial resistance among members of family *Enterobacteriaeceae* which are known to cause clinically significant infections, suggests monitoring mechanisms of antimicrobial resistance as well as simultaneously evaluating the newer antimicrobial agents for their in-vitro activity. Simultaneous production of several β-lactamases in the organisms poses difficulty in detecting each mechanism of resistance as one mechanism can mask the other. Phenotypic methods for detection of these resistant mechanisms are faster, cost effective, easier to perform and less labour intensive.

Combination disc method using cefotaxime/ clavulanic acid detected maximum number of ESBL than ceftazidime/clavulunate but it should be performed with other test using boronic acid for the detection of ESBL in the presence of co-production of AmpC, since the presence of high-level expression of AmpC can mask the recognition of the ESBL's. HiCrome ESBL agar has high sensitivity and specificity. High negative predictive value of this media suggests that this medium though not confirmatory, it can constitute as an excellent screening tool for rapid exclusion of patients for not carrying ESBL producers. Its technically simple, easy to perform, cost effective, can be done routinely in laboratory.

In order to prevent the spread of these multidrug resistant organisms, it is necessary to identify and

detect them routinely in the laboratories using simple phenotypic methods as this helps the clinician to provide appropriate antimicrobial therapy. It is advisable for all the health care settings to have hospital infection control committee with hospital antibiotic policy, with regular updates.

Abbreviations

PCT - Phenotypic confirmatory test, CLSI - Clinical and Laboratory Standard, CAZ- Ceftazidime, CAC- Ceftazidime + Clavulanic acid, CTX- Cefotaxime, CEC- Cefotaxime + Clavulanic acid.

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