Original Article

Comparison of phenotypic methods for the detection of extended spectrum beta lactamase production among enterobacteriaceae

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Abstract

Introduction: Extended-spectrum β-lactamases (ESBLs) are enzymes which hydrolyse extended-spectrum Cephalosporins and are more common in recent times. **Aim**: We aimed to compare Chrome agar, double disk synergy test (DDST) and potentiated combined disk diffusion test (PCDDT) with ESBL E test for their sensitivity in ESBL detection. **Materials and Methods**: The study was a cross-sectional prospective analytical study involving 100 isolates of Enterobacteriaceae family between May 2014 and December 2014. All the isolates were subjected to ESBL detection by Chrome agar, double disk synergy test (DDST), Phenotypic confirmatory disk diffusion test (PCDDT) and ESBL Estrip method. **Results**: Among the 100 isolates tested, 61 isolates were resistant to one of the third generation Cephalosporins. Among them potentiated disk diffusion test (PCDDT) detected 30/61 isolates (49.18%), DDST detected26 (42.62%) and E strip detected detected35 (57.37%) isolates. Chrome agar showed growth on 42 samples. **Conclusions**: In resource poor settings, Chrome agar along with Phenotypic confirmatory disk diffusion test (PCDDT) can be taken as an effective screening method for ESBL detection.

Keywords: Gram negative bacilli, ESBL detection, double disk synergy test (DDST), Phenotypic confirmatory disk diffusion test (PCDDT).

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INTRODUCTION

Extended-spectrum β-lactamases (ESBLs), are enzymes which hydrolyse extended-spectrum cephalosporins and are inhibited by β-lactamase inhibitors such as clavulanic acid[1]. They are resistant to the third generation cephalosporins like cefotaxime, ceftriaxone, ceftazidime, and monobactams (e.g., aztreonam), but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem)[2]. Increased incidence of resistance among *Enterobacteriaceae*is seen more commonly nowadays. There are various phenotypic methods for the detection of extended spectrum beta lactamase production by*Enterobacteriaceae*. Inappropriate use of antibiotics has led to the present global concern of antibiotic resistance, and the spread of ESBL producing bacteria is rapid worldwide. The detection of extended-spectrum β-lactamase-producing (ESBL) bacteria is of prime importance for infection control and epidemiological surveillance[3]. Hence early detection and efficient infection control practices may prevent its spread and reduce morbidity and mortality in patients. There are many phenotypic methods for ESBL detection like double disk synergy test (DDST), phenotypic confirmatory disk diffusion test (PCDDT), E test, Chrome agar and automated methods like Vitek 2 ESBL, Phoenix ESBL[4]. Chromogenic media is a rapid culture method as they combine presumptive ESBL detection with organism identification[5]. So we aimed to compare Chrome agar, double disk synergy test (DDST) and potentiated

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combined disk diffusion test (PCDDT) with ESBL E test in ESBL detection.

MATERIALS AND METHODS

The study was a cross-sectional prospective analytical study between May 2014 and December 2014. Institutional ethical clearance was obtained for the study. A total of 100 isolates belonging to Enterobacteriaceae family obtained from variousclinical samples like pus, urine, wound swab, sputum were processed. The isolates were identified according to the standard methods and antibiotic sensitivity was performed using Kirby Bauer disk diffusion method. The isolates were further tested for ESBL detection according to CLSI guidelines.

Crome ESBL Agar

The isolates obtained were matched to 0.5 McFarland turbidity and inoculated onto Chrome ESBL agar as per manufacturer' s instructions. The growth along with the colour production determines the isolate and its ESBL producing capacity.

Criteria for the selection of the ESBL producing strains[6]

The isolates were tested for their susceptibility to the third generation cephalosporinslike ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg) by the disc diffusion method according to the CLSI guidelines. The Gram negative bacterial isolates which were resistant to one of the third generation cephalosporins were selected for the study and processed for detection of ESBL production.

Double disk synergy test (DDST)[6]

Discs of Ceftazidime (30 µg), Cefotaxime (30µg) and Amoxyclav (20µg amoxycillin and 10µg clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the amoxyclav disc in the middle on a plate of Mueller Hinton Agar (MHA) being inoculated with the test strain and incubated at 37 ^{0}C aerobically overnight. Isolates that showed an enhancement of the zone of inhibition as greater than 5 mm on the amoxyclav side of the disc as compared to that which was seen on the side without amoxyclav, were confirmed as ESBL producers.

The phenotypic confirmatory disc diffusion test (PCDDT)[6]

The ceftazidime (30µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10µg discs) were applied onto a plate of Mueller HintonAgar (MHA) which was innoculated with the test strain. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be an ESBL producer.

ESBL E test[6]

Isolates were tested using E test strips containing ceftazidime / ceftazidimeclavulinic acid for testing the synergy between the concentration gradients of ceftazidime and clavulinicacid. The concentration ranges from0.064 to 4 µg/mL for TZ/TZL; If the MIC ratio for TZ/TZL was ≥8 or if there is a ellipse or phantom zone formation, the isolate is identified as an ESBL producer.

RESULTS

Among the 100 isolates, 61 isolates were resistant to one of the third generation Cephalosporins. Among them potentiated disk diffusion test (PCDDT) detected 30/61 samples (49.18%), DDST detected26 (42.62%) and E strip detected 35(57.37%) isolates. Among 100 isolates, 42 isolates (42 %) showed growth and colour production on Chrome agar.

In comparing the different phenotypic methods in ESBL detection, DDST and E strip showed concordant results in 28 isolates, potentiated disk diffusion test (PCDDT) and E strip showed concordant results in 33 isolates Among the 42 isolates showing growth in Chrome agar, 29 isolates showed concordant results with E strip method.

Table 1: Organism wise distribution

Organism	Number
E.coli	30
Klebsiellapneumoniae	17
Citrobactersps	6
Proteus vulgaris	5
Proteus mirabilis	2
Morganellasps	1

Table 2: Comparison of various methods for ESBL detection

ESBL detection methods	Positive (percentage)
Double disk synergy test (DDST)	26 (42.62)
The phenotypic confirmatory disc diffusion test (PCDDT)	30(49.18)
ESBL E test	35(57.37)

DISCUSSION

Extended-spectrum β-lactamases (ESBLs) producing Enterobacteriaceae has emerged as a rapidly evolving pathogen that is spread worldwide. In the present era, these organisms were found to be multidrug resistant, leading to difficulties in the treatment. The identification of ESBL producers helps the clinicians to provide appropriate treatment to the patients. Hence in this study, we assessed four different phenotypic methods in their sensitivity for the detection of ESBL producers. The various organisms isolated in our study were 30 (49.18%) *E. coli*, 17 (27.86%) *K. pneumoniae*, 6 (9.8%) *Citrobacter species*,5 (8.19%) *Proteus vulgaris*, 2 (3.27%) *Proteus mirabilis* and 1 (1.6%) *Morganellasps.*

The prevalence of ESBL production in our study was 61%. The prevalence of ESBL varies among the different geographical areas[7]. Singhal*et al*., reported a prevalence rate of 63.60 % in four different tertiary care hospitals from across India and Mathur*et al*. reported a prevalence rate of 68% for ESBL production among Gram negative bacilli by the NCCLS confirmatory tests[8,9]. Chrome ESBL agar helps in identification and detection of ESBL producer within a day thereby reducing the delay in detection. In our study, Hi chrome ESBL agar gave a higher sensitivity of42%. About 7isolates showed a false positive results in Chrome agar. The screen agar can be used as a method of ESBL production but its higher false positives makes it difficult to use as a single method of detection. But the media can be used as a screening method because of its quick results. Potentiated disk diffusion test (PCDDT) detected 30/61 samples (49.18%). In our study the detection rate of PCDDT was 49.18% compared to DDST which is only 42.62%. This is in accordance with the study done by MKR Khan *et al* and Hawalkar *et al* where PCDDT was more sensitive than DDST[10,11]. DDST detected 42.62% of ESBL producers in our study. In a study done by SridharaRao *et al*, by using the double-disk synergy test (DDST) method 26.1% of the isolates were positive for ESBL production[12]. Some of the isolates that were resistant to the third generation Cephalosporins were not detected by DDST and PCDDT. These isolates could have produced AmpC, as clavulinic acid production can induce this enzyme. Amp C detection was not done in the study which is a drawback of the study. ESBL Estrip method has been used as a gold standard method. By this method we could detect 57.37%. The MIC gradient concentrations can be detected by this method. In a study done byNandagopal *et al* in Vellore, with E-test ESBL strips, 59 (92.2%) were positive for ESBL[13]. The cost limits its usein resource poor settings.

CONCLUSION

ESBL E strip method is an effective method in the detection of ESBL producers, but its cost limits its use. In resource poor settings, Chrome agar along with Phenotypic confirmatory disk diffusion test (PCDDT) can be taken as an effective screening methods for ESBL detection.

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