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Original Article

Evaluation of Phenotypic Methods to Identify Extended Spectrum Beta-lactamase (ESBL) Producing Gram negative Bacteria

Shikha Paul[1], Sanya Tahmina Jhora[1], Prashanta Prasun Dey[2]

[1]Department of Microbiology, Sir Salimullah Medical College, Dhaka
[2]Department of Endocrinology, Kumudini Hospital, Tangail

Abstract

Extended spectrum beta-lactamase (ESBL) producing Gram negative bacteria are usually multiple drug resistant and their cephalosporin and aztreonam resistance is not reliably detected by susceptibility tests.This study was carried out to find out a cost effective easy standard method to identify ESBL producing bacteria in a laboratory associated with tertiary care hospital and to determine the incidence of ESBL positive bacteria isolated from different clinical specimens. Thereby isolated 124 Gram negative bacteria from various samples were subjected to screening test, double disc synergy test (DDST)and E test ESBL method.Screening test detected 69.35%, DDST identified 37.1% and E test ESBL method confirmed 55.65% ESBL producing strains. Screening test and DDST was compared to E test ESBL method. Considering E test as standard the sensitivity and specificity of screening test were 97.10% and 65.45% respectively and that of DDST were 62.31% and 94.55% respectively. Low specificity of screening test reflects detection of many false positive strains and low sensitivity of DDST signals many missed identification. This study suggested the use of E test ESBL method to confirm screening positive ESBL isolates at microbiology laboratory.

Key words: ESBL, DDST, Etest.

Introduction:

Beta-lactamases are the primary cause of bacterial resistance to beta-lactam antibiotics[1]. These are heterogeneous group of resistance enzymes. Till now more than 890 distinct beta-lactamases have been identified[2].

Resistance to beta-lactam antibiotics is prevalent among many Gram negative bacteria mostly because they inherently produce beta-lactamases enzymes which hydrolyze the beta-lactam ring and inactivate antibiotics[1]. Many new beta-lactam antibiotics have been developed that are specifically designed to be resistant to the hydrolytic action of beta-lactamases. With development of each new class to treat patients, new beta-lactamases are emerged which are resistant to that class of new drug [3].

✉ ***Correspondence:***
Dr. Shikha Paul
Associate Professor
Department of Microbiology, SSMC, Dhaka
Email:shikhapaul2013@gmail.com
Phone:01732806772

Increasing incidence of extended spectrum beta lactamase (ESBL) producing strains is being reported all over the world. Higher incidences are reported in intensive care units, renal failure, burn, urinary catheter[3]. Several studies of Bangladesh reported significant number ESBL producing strains among gram negative bacilli[4,5]. Identification of ESBL producing Gram negative bacteria provides valuable information to the clinicians and raises demand for contact precautions to avoid hospital transmission[6]. ESBL producers can be falsely susceptible to identification discs in routine tests making its recognition difficult[7].

ESBLs can be detected by phenotypic and genotypic methods. Routine identification at clinical laboratory depends on phenotypic methods. The recommended phenotypic strategy for ESBL detection includes initial screening followed by confirmation. Screen tests are Disk diffusion and Broth microdilution. Disk diffusion utilizes cefotaxime or cefodoxime or ceftadizime or aztreonam disk. Use of more than one antimicrobial disk for screening improves the sensitivity of ESBL detection. Phenotypic confirmation can also be done by Disk diffusion and Broth microdilution.

Confirmatory testing requires the use of either cefotaxime or ceftadizime alone and in combination with clavulanic acid. These tests are standardized for *Escherichia coli, Klebsiella pneumonia, Klebsiella oxytoca* and *Proteus mirabilis*. Members of *Enterobacteriaceae* with inducible AmpC lactamase producing capability need cefepime alone and in combination with beta lactamase inhibitor to confirm as ESBL producer[8]. Other confirmation methods are combined disk test(CDT), Double disk synergy test(DDST), ESBL gradient test, E test for ESBL, Three dimensional test etc[3,9].

This study employed screening test, double disc synergy test (DDST) and E test ESBL methods to identify ESBLs producing isolates. E test was taken as standard and screening test and DDST were compared to E test ESBL method to evaluate them against E test ESBL method.

Methods:

Total 124 Gram negative bacteria were isolated from different biological samples such as urine, pus, wound swab, stool, blood and High vaginal swab (HVS)of patients attending microbiology laboratory of Sir Salimullah Medical College(SSMC), Dhaka during the period of March 2013 to August 2013.Cultures of these samples were advised by physicians ofinpatient and outpatient departments of SSMC & Mitford Hospital.Standard methods were employed for collection of samples, isolation and identification of the organisms. MaConkey's agar and blood agar media were used for primary isolation. Triple sugar iron (TSI), Motility indole urea(MIU), Oxidase, Gram stain from colony wereused for identification of Gram negative bacilli. Finally extended spectrum beta lactamase (ESBL) producing strains were recognized by Screening test,[2,7] Double disc synergy test (DDST)[2,7,10] and E test ESBL method[3,11,12].

Reference strains:

Klebsiella pneumoniae ATCC 700603 was taken as positive control and it was kindly provided by the department of microbiology, BIRDEM, Dhaka. *Esch. coli,* which was sensitive to ceftazidime, ceftriaxone, cefotaxime and aztreonam was used as negative control.

Screening Test[7]:

Standard inoculum of bacterial suspension matched to 0.5 McFarland was made and Muller Hinton agar (MHA) plate was inoculated properly with bacterial suspension. Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) disks (Oxoid, England) were placed onto MHA plate and incubated overnight at 37°C in air. When inhibition zone of any isolate to Ceftazidime ≤22 mm or Aztreonam ≤27 mm, or Cefotaxime ≤27 mm or Ceftriaxone ≤25 mm alone or in combination was found then the isolate was taken as screening test positive[8].

DDST[4,10]:

The MHA plate was inoculated with bacterial suspension matched to 0.5 McFarland. Ceftazidime (30µg), Ceftriaxone(30µg), Cefotaxime (30µg) and Aztreonam (30µg) disks were placed 15 mm distance centre to centre from amoxiclav disk (20mg amoxicillin and 10mg of clavulanic acid) which was placed at middle. Any extension of inhibition zone of antimicrobial disks(one or more) towards amoxiclav disk confirmed the presence of ESBL.

E test ESBL Method[8,10,11]:

Triple ESBL detection (Ezy MICTM from HIMEDIA, India) strip was used. One side of the strip is calibrated with ceftazidime, cefotaxime and cefepimemixture (0.032-4 mcg/ml) plus fixed concentration of beta lactamase inhibitor mixture (clavulanic acid and tazobactum) having highest concentration tapering downwards. Reverse side carries ceftazidime, cefotaxime and cefepime mixture (0.125-16 mcg/ml) having concentration gradient in opposite direction. Standard bacterial suspension was made and MHA plate was inoculated properly with prepared bacterial suspension.Triple ESBL detection strip was placed on Muller Hinton agar plate. Plates were incubated overnight at 37°C in air. The presence of ESBLwas confirmed by the appearance of a phantom zone or when the minimum inhibitory concentration (MIC) of antibiotic mixture side was reduced by ≥ 8 times in the presence of beta lactamases inhibitor.

Results:

A total of 124 Gram negative bacteria were subjected to ESBL detection methods. E test was taken as standard for ESBL detection. Screening test detected 69.35% (n=86) ESBL producing strain, DDST identified 37.1% (n=46)ESBL positive strain and E test ESBL method determined 55.65% (n=55) ESBL producing isolates.

Table I: Detection of ESBL producing strain by different methods (n=124)

Name of the test	% of ESBL strain (Positive number)	% of non ESBL strain (Negative number)
Screening test	69.35(86)	30.65(38)
DDST	37.1(46)	62.9(78)
E test	55.65 (69)	44.35(55)

p value < 0.001

Table II: The sensitivity and specificity of screening test against E test

Screening test	E test ESBL method			Sensitivity	Specificity
	Positive	Negative	Total		
Positive	67	19	86	97.10%	65.45%
Negative	02	36	38		
Total	69	55	124		

Table III: The sensitivity and specificity of DDST against E test

DDST	E test ESBL method		Total	Sensitivity	Specificity
	Positive	Negative			
Positive	43	03	46	62.31%	94.55%
Negative	26	52	78		
Total	69	55	124		

Discussion:

ESBL producing bacteria are resistant to nearly all beta lactam antimicrobialswhich comprise more than 50% antibiotic uses for systemic infections. Thus ESBL producing Gram negative bacteria make a threat to current beta lactam therapy[13].

In this study, ESBL positive strains had been identified by screening test, DDST and E test ESBL method. E test is reliable and was taken as standard. Other methods were compare with E test[11]. Screening test showed many false positive results and exhibited only 65.45% specificity. Therefore this method could not be suggested to isolate ESBL producing strains[11].

DDST is a recommended phenotypically confirmatory test but it has many limitations[9]. Evaluation of the double-disk diffusion test against genotypic methods revealed its sensitivity ranging from 79% to 97% and specificity ranging from 94%to 100%. Most important advantage of DDST is that it is technically simple and the interpretation of the test is subjective. However, the sensitivity may be reduced when ESBL activityis very low.[2] In this study the sensitivity of DDST was 62.31%, making it clear that it missed a fair number of ESBL positive strains. Hence DDST should not be a recommended method for ESBL positive strain isolation in diagnostic laboratory.

The commercially available ESBL Etest strip is a quantitative technique and is widely regarded as the 'gold standard' forESBL detection in clinical laboratories[14]. E test method of this study identified 55.65% ESBL positive strainamong 124 Gram negative isolates. E test strip carrying combination of cephalosporins has increased its sensitivity. E test technique is an expensive method and this combination makes the technique more expensive. However, it is a recommended method. Clinical laboratorycan use it for confirmation of screening positive isolates rather than as a routine test[15]. In addition, early detection of ESBL positive strains can markedly reduce expenditure related to patient management[3].

Mobile units of Gram-negative bacteria often carry multiple genes for multiple beta-lactamase enzymes such as ESBLs, AmpC beta-lactamases, metallo beta-lactamases and KPC carbapenemases. Simultaneous presence of multiple gene encoded multiple beta-lactamase enzyme in plasmids or transposons warrants that these genes could reach any Gram negative bacterium and can endanger public health[2]. In 2009, a study at Sir Salimullah Medical College detected 36% ESBL positive isolates among all Gram negative pathogens[7]. In 2012, the percentage of ESBL producing isolates reached to 41.3%[8]. Current study detected 55.65% ESBL positive strain from this same institution in 2013. If this upward trend continuesthe acquisition of resistance would go beyond control. To prevent this upward trend of resistant pathogens, routine identification of ESBL producing strains is mandatory by a reliable cost effective phenotypic method from each clinical specimen.

The phenotypic ESBL isolation methods lose their sensitivity if the isolate produces an additional AmpC or metallo beta-lactamases which are not inhibited by clavulanic acid and tazobactam. This can be a significant clinical problem. Therefore, phenotypic detection should be accompanied by genotypic identification of ESBL genes where facilities are available[3].

Conclusion:

The ESBL producing strains are increasing day by day at an alarming rate. These resistant pathogens are one of the important causes of uncontrolled infection. To reduce the dissemination of resistant pathogens specific treatment is urgently needed. As ESBL producing strains are resistant to almost all beta lactam antibiotics except carbapenem early detection is essential. Thereby in clinical laboratory, a reliable cost effective phenotypic method should be applied to confirm screening positive ESBL isolates.

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