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Diagnostic Validity of Cica Beta Test 1 for the Detection of Extended Spectrum Beta-Lactamase (ESBL) Producing Gram Negative Bacteria by Comparing with Phenotypic Method

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Abstract

Background: Detection of extended spectrum beta lactamase producing bacteria is an important issue in the clinical settings. **Objective:** The purpose of the present study was to validate the Cica Beta Test 1 for detection of extended spectrum beta-lactamase (ESBL) producing bacteria. **Method:** This analytical type of cross-sectional study was carried out in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from January 2006 to December 2006 for a period of one (01) year. All the patients presented with the clinical features of urinary tract infection and surgical as well as burn wound infection at any age with both sexes were selected as study population. All bacteria were isolated and identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests. Phenotypic confirmation of ESBLs producing isolates were done by inhibitor potentiated disc diffusion test according to CLSI recommendation. The Cica Beta Test 1 was performed according to the manufacturer's instructions. **Result:** A total number of 288 Gram negative bacteria were isolated. Among these isolates Cica Beta test 1 was positive in 97 strains and phenotypic confirmatory test was positive in 89 strains. The test sensitivity of Cica Beta Test 1 was 100% (95% CI 95.9% to 100.0%). Specificity of the test was 96.0% (95% CI 92.2% to 98.2%). The positive predictive value (PPV) and

negative predictive value (NPV) were 92.7% (95% CI 84.5% to 95.7%) and 100.0% (95% CI 98.0% to 100.0%) respectively. The accuracy of the test was 97.2% (95% CI 95.1% to 99.1%). Area under ROC curve = 0.980 (95% CI 0.964 to 0.996); p value 0.0001. **Conclusion:** In conclusion, Cica Beta Test 1 is very high sensitivity and specificity for the detection of ESBL from Gram negative bacteria.

Keywords

Diagnostic Validity, Cica Beta Test 1, Extended Spectrum Beta-Lactamase, ESBL, Gram Negative Bacteria, Phenotypic Method

1. Introduction

This is a challenge for the laboratory to detect ESBL-containing Gram-negative bacilli because they can appear susceptible in vitro to certain beta-lactam antimicrobial agents yet result in clinical treatment failure [1]. Several ESBL detection tests that have been proposed are based on Clinical Microbiology Techniques are—Screening for ESBL, NCCLS phenotypic confirmatory method, double disc synergy/Disk approximation method, Etest ESBL strips, three dimensional tests, and the Cica Beta Test 1 and Vitek system [2]. Several Molecular Methods also have been proposed for detection of ESBLs including isoelectric point, DNA probes, PCR, oligotyping method, PCR-RFLP, PCR-SSCP, LCR, Nucleotide sequencing, Pulsed field gel electrophoresis (PFGE) and so on [3]. Till now, there is no gold standard test for detection of ESBLs. Clinical laboratory standard institute (CLSI) recommends the phenotypic method as confirmatory test. In a study [4] a total 304 strain of *Klebsiella* spp., *E. coli* and *Proteus mirabilis* were tested using chromogenic cephalosporin kit and phenotypic confirmatory method. Out of these strains 199 was ESBL positive confirmed by phenotypic confirmatory method and 190 positive with chromogenic cephalosporin kit. Two (2) strains were positive with chromogenic cephalosporin but negative by phenotypic confirmatory method. Therefore, 95.5% sensitivity and 98.1% specificity was found by comparing with phenotypic confirmatory disc test.

However, the traditional methods need much labor and time for cultivation and require at least overnight incubation after isolated colonies are available from primary culture. In this context, about 48 hours is required for ESBLs reporting by traditional methods. Molecular characterization of the isolated ESBL was also not possible in maximum laboratories due to lack of facilities. However, rapid detection of ESBLs from the patient with severe infection like septicemia, meningitis with gram negative rods is urgently required; otherwise it may be fatal. The Cica Beta Test 1/HMRZ-86/Chromogenic cephalosporin can rapidly detect ESBLs in Gram negative rods within 15 minutes directly with isolated colonies from primary culture [4]. The great advantage of the kit remains its rapid tur-

naround time, which facilitates reporting of clinically relevant information 24 hours earlier then phenotypic confirmatory test and other tests. Handling the kit is very simple and can be used without any complications.

Rapidly detection of ESBL producing bacteria directly from primary culture can save the time in the laboratories. Thus it reduces the duration of hospital stay, which reduces the treatment cost of both patient and hospital authority. Rapid detection of ESBLs also prevents unnecessary use of antibiotics and benefit the patient by administration of appropriate antibiotic [4]. Therefore this present study was undertaken to compare of the phenotypic confirmatory method by NCCLS and Cica Beta Test 1 for rapid detection of extended spectrum beta lactamases in Gram negative rods.

2. Methods

This analytical type of cross-sectional study was carried out in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from January 2006 to December 2006 for a period of one (01) year. All the patients presented with the clinical features of urinary tract infection and surgical as well as burn wound infection at any age with both sexes were selected as study population. Pus cell less than 5/HPF in a centrifuged urine sample were excluded from this study. Samples were collected from in-patient and out-patient department of Dhaka Medical College Hospital, Dhaka and BSMMU, Dhaka after getting informed verbal consent from the patients or from the attendants. Laboratory work was performed in Department of Microbiology & Immunology, BSMMU, Dhaka. *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) were used for quality control of ESBL tests.

Samples were inoculated on appropriate culture media and plates were incubated at 37˚C aerobically for 24 to 48 hours. Plates were checked for presence of suspected pathogens.

All the organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard methods [5]. Phenotypic confirmation of ESBLs producing isolates were done by inhibitor potentiated disc diffusion test according to CLSI recommendation. The Cica Beta Test 1 was performed according to the manufacturer's instructions. In brief, all Gram negative isolates were tested by using test kit-Cica Beta Test-1/Chromogenic Cephalosporin. This kit originally designed for rapid detection of ESBLs and metallo beta lactamases (MBLs) in Gram negative rods directly from isolated colonies. The kit consists of plastic strip with a paper pad and solution substrate—HMRZ-86 new chromogenic cephalosporin; (7R)-7-[2-(aminothiazol-4-yl)-(Z)-2-(1-carboxy-1-methyl-ethoxyimino) acetamido]-3-(2,4-dinitrostyryl)-3-cephem-4-carboxylic acid trifluoroacetate, E-isomer (**Figure 1**). HMRZ-86 is a new Chromogenic cephalosporin. A carboxypropyl-oxyimino group bonded to the side chain at position 7 in the compound protects the beta

lactam ring (lactamases differentiation ring) from a range of traditional beta lactamases. But this carboxypropyl-oxyimino group can not protect the cephalosporin from hydrolysis by ESBLs or MBLs. A Chromogenic substance-conjugate located at position 3 bonded by double bond. Hydrolysis of the beta lactam ring by these enzymes changes the wavelength absorbed by the conjugated double bond located at position 3, shifting the color of the compound from yellow to red. One drop of kit substrate solution was dropt on the filter strip. Single isolated colony of the organism was then rubbed on the pad surface directly from primary culture and was left to stand at room temperature for 15 minutes and the color of the paper pad was observed with the naked eye. Within 2 - 15 minutes, a change in color from yellow to red was taken as positive result. If the color remains yellow, the strain was considered as ESBL negative. Data was collected as per predesigned Data collection form. The test validity was performed by calculating the sensitivity, specificity, positive predictive value & negative predictive value. The ROC curve was calculated.

3. Results

A total number of 461 specimens were collected from patients with wound infections and urine from suspected cases of urinary tract infection from BSMMU and DMCH of which 280 were urine samples, 87 were wound samples and 94 were burn samples and culture was positive in 320 (69.41%) samples.

Out of 320 isolates 288 (90.0%) were Gram negative bacteria and 32 (10.0%) (Figure 2) were Gram positive bacteria. Among individual samples ESBLs positive strains were highest in urine sample 43 (32.33%) out of 133 urine samples, followed by surgical & other wound 22 (31.42%) out of 70 wound samples, burn wound 24 (28.24%) out of 85 burn wound samples.

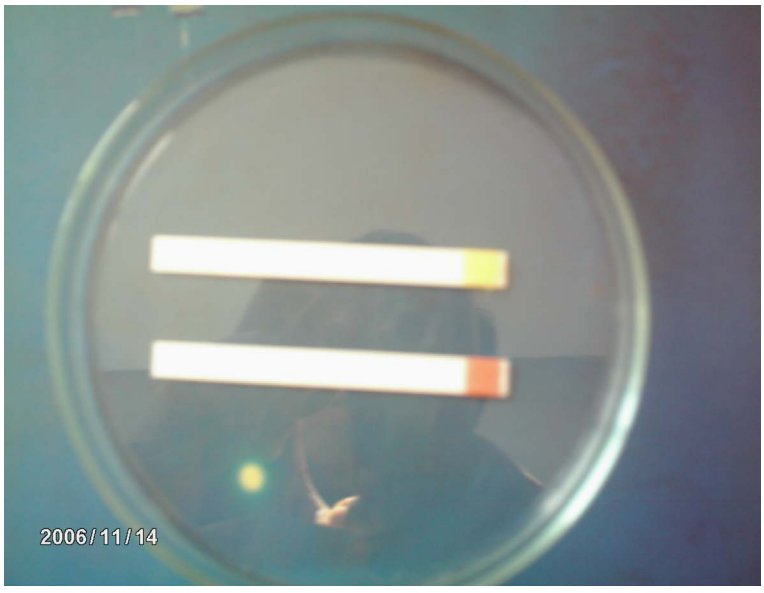

Figure 1. The Cica Beta Test 1; a red color indicate ESBLs positive strain and a yellow color indicate ESBLs negative strain.

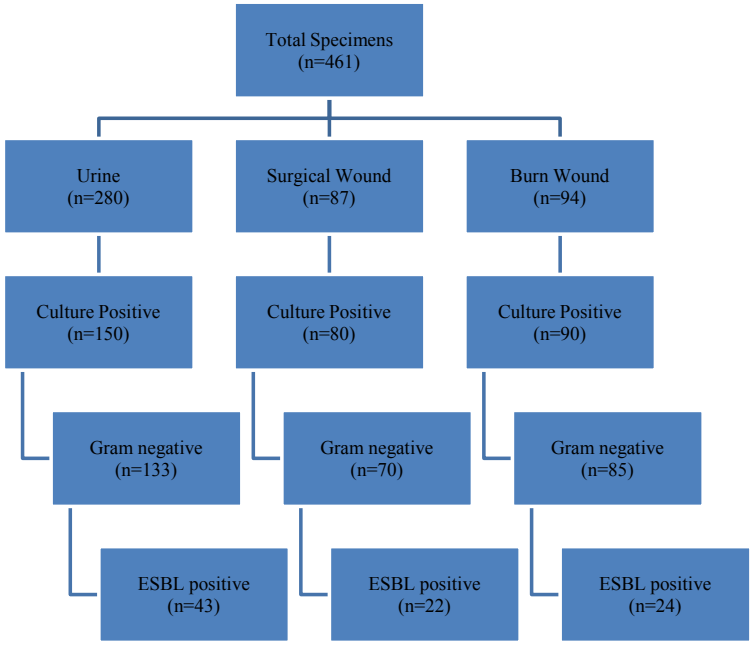

Figure 2. Flowchart showing the isolation and identification of ESBL Gram negative bacteria from different specimens.

Total 288 Gram negative bacteria were tested for ESBLs production by Cica Beta Test 1 and Phenotypic confirmatory test. Cica Beta test 1 was positive in 97 strains and phenotypic confirmatory test was positive in 89 strains. Two strains of *Proteus* species from burn sample and six strains of *Pseudomonas* species from burn sample shows positive reaction by Cica Beta Test 1 which shows negative result by Phenotypic confirmatory test. No strain found phenotypic confirmatory test positive but Cica Beta Test 1 negative (**Table 1**).

During comparison of Cica Beta Test 1 and Phenotypic confirmatory test the true positive and true negative were found in 89 and 191 isolates of Gram negative bacteria. However, false positive Gram negative bacteria were detection in 8 isolates. No false negative gram negative bacteria were found (**Table 2**).

The test sensitivity of Cica Beta Test 1 was 100% (95% CI 95.9% to 100.0%). Specificity of the test was 96.0% (95% CI 92.2% to 98.2%). The positive predictive value (PPV) and negative predictive value (NPV) were 92.7% (95% CI 84.5% to 95.7%) and 100.0% (95% CI 98.0% to 100.0%) respectively. The accuracy of the test was 97.2% (95% CI 95.1% to 99.1%) (**Table 3**, **Figure 3**).

4. Discussion

Bacterial antibiotic resistance has become a major clinical concern worldwide including Bangladesh [6]. Failure to detect these enzymes—ESBLs, AmpC β-lactamases, Metallo-β-lactamases has contributed to their uncontrolled spread and therapeutic failure [7].

In this study out of 461 different samples total 320 (69.41%) bacterial strains were isolated; of which 288 (90%) were Gram-negative and 32 (10%) were

Gram-positive bacteria. Among the 288 Gram-negative bacteria ESBL was detected in 89 (30.90%) strains. Although among the Gram -negative bacteria *E. coli* was isolated in maximum number of patients but the rate of ESBL positivity was highest in *Klebsiella* spp. (43.47%) followed by *E. coli* (35.38%), *Enterobactor* spp. (31.25%), *Proteus* spp. (27.11%), *Acinetobactor* spp. (26.32%) and less in *Pseudomonas* spp. 7 (17.07%). In a study, ESBL was detected in 23.19% Gram negative bacteria, among them *Klebsiella* spp. was highest 40.90%, followed by *Proteus* spp. 40.62%, *E. coli* 26.92% and less in *Pseudomonas* spp. 4.87% [8]. In another study at urban hospital in Dhaka showed (43.21%) *E. coli* and (39.5%) *Klebsiella* species as ESBL producers [9].

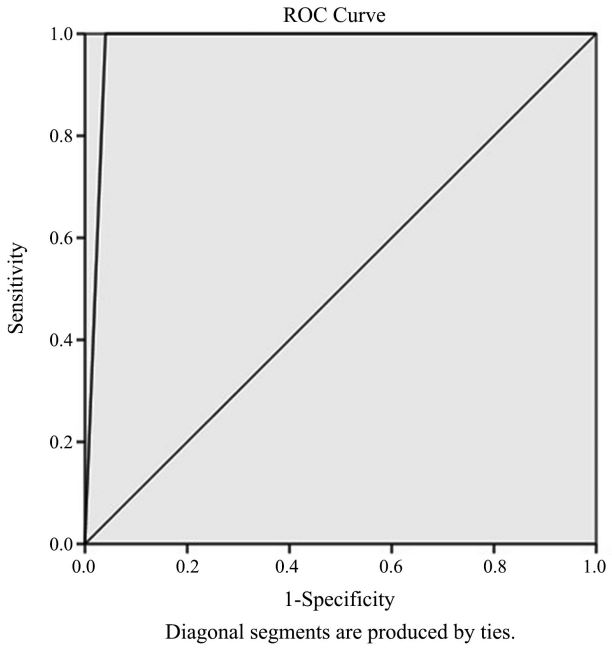

Figure 3. Receiver-Operative Characteristic (ROC) Curve of Cica Beta Test 1. Area under ROC curve = 0.980 (95% CI 0.964 to 0.996); p value 0.0001.

Table 1. Comparison of ESBL positive strain by Cica Beta Test 1 & Phenotypic confirmatory method among total Gram Negative Bacteria (n = 288).

Name of strain	Cica Beta Test 1 +ve	Phenotypic confirmatory test +ve	Phenotypic test –ve but Cica Beta Test 1 +ve
E.coli (n = 130)	46	46	
Klebsiella spp. (n = 23)	10	10	
Proteus spp. (n = 59)	18	16	2 (Burn Wound)
Pseudomonas spp. (n = 41)	13	7	6 (Burn Wound)
Enterobactor spp. (n = 16)	5	5	
Acinetobactor spp. (n = 19)	5	5	
Total (N = 288)	97	89	8

Table 2. Relation between Cica Beta Test 1 and Phenotypic Confirmation of ESBL (n = 288).

Cica Beta Test 1	Phenotypic confirmatory		Total	P value
	Positive	Negative		
Positive	89 (100.0%)	8 (4.0%)	97 (33.7%)	
Negative	0 (0.0%)	191 (96.0%)	191 (66.3%)	0.10
Total	**89 (100.0%)**	**199 (100.0%)**	**288 (100.0%)**	

Table 3. Diagnostic Validity of Cica Beta Test 1 for Detection of ESBL.

Test Validity	Value	95% CI
Sensitivity	100.0%	95.9% to 100.0%
Specificity	96.0%	92.2% to 98.2%
PPV	92.7%	84.5% to 95.7%
NPV	100.0%	98.0% to 100.0%
Accuracy	97.2%	95.1% to 99.1%

Rate of positivity of ESBL of different strains varies from country to country and institution to institution. In India a study done at Jawaharlal Institute, Pondicherry observed (58.06%) *Escherichia coli* and (43.75%) *Klebsiella* spp. In Europe the incidence is 23% - 25% in *Klebsiella* spp. and 5.4% for *E. coli* [10]. In Asia the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies, from 4.85 in Korea to 8.5% in Taiwan and up to 12% in Hong Kong [2]. In a study it has been observed ESBL producing *E. coli* 16.1% and *Klebsiella* spp. 44% [11].

ESBL producing strains were isolated from urine samples, surgical wound and burn wound. Highest rate of ESBLs (32.3%) was found among the bacteria isolated from urinary strains, followed by (31.4%) in surgical & other wound and (28.24%) in burn wound. Among isolated ESBL producing bacteria *Klebsiella* species was highest in all types of sample. In urine sample out of all ESBL positive strains, *Klebsiella* spp. was highest 36.36% followed by *Escherichia coli* 34.12%, *Enterobactor* species 33.33%, *Proteus* species 25%, *Acinetobactor* species 23.08% and *Pseudomonas* species 20%. A study Alim [8] found that in urine sample ESBLs positive *Klebsiella* species 35%, *E. coli* 17.82%, *Proteus* species 28.57%.

The isolation rate of ESBL producing *Klebsiella* spp. was highest among burn wound (60%), followed by surgical wound (42.86%) and urine (36.36%). Similar higher rate ESBL producing strain s of *Klebsiella* spp. (44%) also observed in Singapore hospital [11]. In the study by Rahman *et al.* [9] ESBL producer *Klebsiella pneumoniae* was highest in pus (54.5%). *Klebsiella* spp. has the ability to spread rapidly in hospital environment and tends to cause nosocomial outbreak [12].

Out of total 130 isolated *E. coli* strains. ESBL producing *E. coli* was 46 (35.38%).

A study by Rahman *et al.* [9] found that ESBLs producing *E. coli* (43.2%) and *K. pneumoniae* (39.5%). ESBLs are most commonly recognized in *Klebsiella* spp. and *E. coli* [13]. ESBL producing *Proteus* spp. was observed in 16 (27.11%) out of total 59 samples of which highest rate was observed in burn wound 28.94%, probably due to high rate of isolation from burn unite. Multi drug resistant Pseudomonas spp. also found in burn unite. Increase number of ESBLs producer is probably due to previously treated with β-lactam drugs, extreme ages, bed retention, immune suppuration, association with other diseases, temporary or permanent urinary catheter [14].

Among *Pseudomonas* species 7 (17.07%) ESBL positive strains were isolated out of total 41 isolates. In a study Alim [14] also found lower rate of *Pseudomonas* spp. 4.87% ESBLs producer. Lower rate of ESBL producing *Pseudomonas* is due to *Pseudomonas* spp. exhibits multiple mechanism of drug resistance simultaneously other than ESBL [10] such as AmpC β-lactamase enzymes, and Metallo β-lactamase. These enzymes are resistant to clavulanic acid that is used to detect ESBL producing bacteria in double disc and phenotypic method [8].

Detection of ESBL producing bacteria was done using chromogenic cephalosporin (Cica Beta Test 1) and Phenotypic confirmatory method by NCCLS. ESBLs was detected by Cica Beta Test 1 within 15 minutes after primary culture. 18 - 24 hours was taken for ESBLs detection by phenotypic confirmatory method after primary culture. Phenotypic confirmatory method needed at least over night incubation. In this study Phenotypic confirmatory method was considered as parameter of ESBLs detection test. Cica Beta Test 1 shows 97 positivity and Phenotypic confirmatory method shows 89 positivity. Cica Beta Test 1 showed 100% sensitivity and 96.13% specificity in comparison with phenotypic confirmatory method. Cica Beta Test 1 also shows positivity in case of AmpC β-lactamase and Metallo-β-lactamase. Cica Beta Test 1 positive but Phenotypic confirmatory method negative 6 *Pseudomonas* spp. from burn; out of these 6 samples 2 were both imipenem and cephamycins-cefotetan resistant but aztreonam sensitive (may be MBL), rest 4 were imipenem sensitive but cephamycin-cefotetan and aztreonam resistant (may be AmpC β-lactamase producer). Cica Beta Test 1 test positive but phenotypic confirmatory method negative 2 *Proteus* spp. from burn, were imipenem sensitive but cephamycins-cefotetan and aztreonam resistant (may be AmpC β-lactamase producer). As ESBLs, AmpC β-lactamases and Metallo-β-lactamases producers all are resistant to extended spectrum cephalosporin, so Cica Beta Test 1 positive strains should be treated by antibiotics other then cephalosporin. Resistance to cefotaxime, ceftazidime with and without clavulanate in NCCLS phenotypic confirmatory method may give an impression about AmpC β-lactamases and Metallo-β-lactamases. Cica Beta Test 1 fails to differentiate ESBLs, AmpC β-lactamases and Metallo-β-lactamases. Now Cica Beta Test C and Cica Beta Test MBL can differentiate AmpC β-lactamases and Metallo-β-lactamases, which was not performed in this study.

It is important for clinical microbiology laboratory to implement one or more

methods to detect ESBLs [12]. Early detection and prompt containment can limit the spread of these multi-resistant pathogens [8].

There are some limitations of the study. The sample size is small. The test is performed in a single center.

5. Conclusion

In the present study, it is concluded that Cica Beta Test 1 is an effective diagnostic kit for the detection of ESBL. The detection of different Gram negative bacteria is possible by this test. This test should be applied in the diagnostic lab for rapid detection of ESBL Gram negative bacteria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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