Chattagram Maa-O-Shishu Hospital Medical College Journal
Volume 15, Issue 2, July 2016

Original Article

Comparison Between Phenotypic Confirmatory Test & Double Disc Synergy Test in Detection of Extended Spectrum -Lactamases Producers Among Gram-Negative Bacilli

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Abstract

Background: Extended-Spectrum -Lactamases (ESBLs) producing bacteria are increasing in number and causing more severe infections because of their continuous mutation and multidrug resistance property which make its treatment difficult. Thus reliable, sensitive and low cost method to detect ESBLs producers, therefore, is of major interest. The present study was undertaken to compare the sensitivity between double disc synergy test & phenotypic confirmatory test to detect ESBLs producing bacteria. **Methods:** All the isolates were identified by standard procedure of identification & isolated gram-negative bacteria initially screened by Minimum Inhibitory Concentration (MIC) ESBLs breakpoints. Then suspected ESBLs producers are confirmed by double disc synergy test & phenotypic confirmatory test. **Results:** In the present study, total 176(74.89%) bacterial strains were isolated from 235 samples of wound swab & pus, and urine. Among the isolates, 150(85.23%) were gram-negative and 26(14.77%) were gram-positive bacteria. The gram-negative bacteria were screened for suspected ESBLs & then subjected to confirmatory test where Phenotypic Confirmatory Test (PCT) detected 89(62.68%) and Double Disc Synergy Test (DDST) detected 74(52.11%) ESBL producers. So 15(10.57%) isolates were missed by double disc synergy test. In this study, we determined sensitivity, specificity, positive predictive value & negative predictive value of Phenotypic Confirmatory Test (PCT) were 100%, 77.9%, 83.1% & 100% respectively and those of Double Disc Synergy Test (DDST) were 83.1%, 100%, 100%, and 77.9% respectively. **Conclusion:** Between these two tests, phenotypic confirmatory test found to be more sensitive procedure than double disc synergy test for the detection of ESBLs producing organisms.

Key words : Extended-spectrum -lactamases; Minimum inhibitory concentration; Phenotypic confirmatory test; Double disc synergy test.

INTRODUCTION

Extended-Spectrum -Lactamases (ESBLs) producing bacteria are becoming a major threat for patients in the hospital, long-term care facilities and community. Inappropriate antibiotic selection in infections caused by these organisms is associated with treatment failures, poor clinical outcomes, increased mortality and longer hospital stays[1].

Extended-Spectrum -Lactamases producing bacteria produce Extended-Spectrum -Lactamase (ESBL) enzymes that mediate resistance to extended spectrum (Third generation) cephalosporins (e.g. Ceftazidime, cefotaxime, ceftrixone etc.) and monobactams (e.g. Aztreonam) but do not affect cephamycins (e.g. Cefoxitin and cefotatan) or carbapenems (e.g. meropenem or imipenem) and are inhibited by -Lactamase inhibitors such as clavulanate, sulbactam and tazobactam[2,3,4]. ESBLs have been found in a wide range of gram-negative rods. Klebsiella pneumoniae seems to remain the major ESBLs producer. Another very important organism is

RESULTS

A total 235 samples were studied, of which 115 were wound swab & pus, and 120 were urine samples. 176 (74.89%) bacterial strains were isolated, of which 105(91.30%) isolated from wound swab & pus, and 71(59.17%) from urine samples.

Table 1 : Distribution of isolated bacteria from different samples (n = 235).

Samples	Number of samples studied	Number of isolated bacteria	Percentage (%)
Wound Swab & Pus	115	105	91.30
Urine	120	71	59.17
Total	235	176	74.89

Table-II shows among the 176 isolates 150(85.23%) were gram-negative bacteria, of which majority were E. coli 70(39.77%), followed by Klebsiella spp. 40(22.73%), Pseudomonas spp. 25(14.21%), Proteus spp. 12(06.82%) & Acinetobacter spp. 03(1.70%) and 26(14.77%) were gram-positive bacteria, of which Staphylococcus aureus 18(10.23%), Enterococci spp. 05(2.84%) & Coagulase negative staphylocci were 03(1.70%).

Table II : Distribution of bacterial isolates (n = 176).

Name of bacterial species	Wound swab & pus (n = 105)	Urine (n = 71)	Total number of bacteria (n = 176)
E. coli	25 (23.81)	45 (63.38)	70 (39.77)
Klebsiella species	26 (24.76)	14 (19.72)	40 (22.73)
Pseudomonas species	23 (21.90)	02 (02.82)	25 (14.21)
Proteus species	10 (09.52)	02 (02.82)	12 (06.82)
Acinetobacter species	00 (00.00)	03 (4.22)	03 (01.70)
Total gram-negative bacteria	**84 (80.00)**	**66 (92.96)**	**150 (85.23)**
Staphylococcus aureus	18 (17.14)	00 (00.00)	18 (10.23)
Enterococci species	00 (00.00)	05 (7.04)	05 (02.84)
Coagulase negative staphylococci	03 (2.86)	00 (00.00)	03 (01.70)
Total gram positive bacteria	**21 (20.00)**	**05 (7.04)**	**26 (14.77)**

It appears total 150 isolated gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC ESBL breakpoints, out of which 142 (94.67%) were found suspected ESBLs producers & 8 (5.33%) gave negative result (Figure-3).

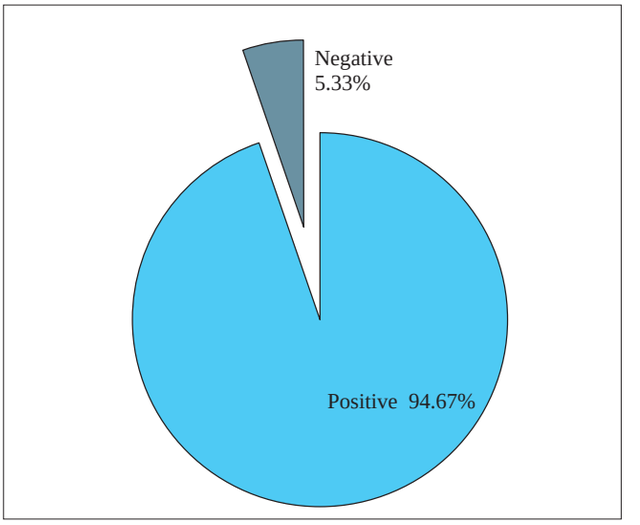

Figure 3 : Detection of ESBL producing bacteria on the basis of MIC (Screening test) by agar-dilution method.

Screening positive 142 suspected ESBLs producing bacteria were subjected to double disc synergy test, it was found 74(52.11%) were positive & 68(47.89%) were double disc synergy test negative (Figure 4).

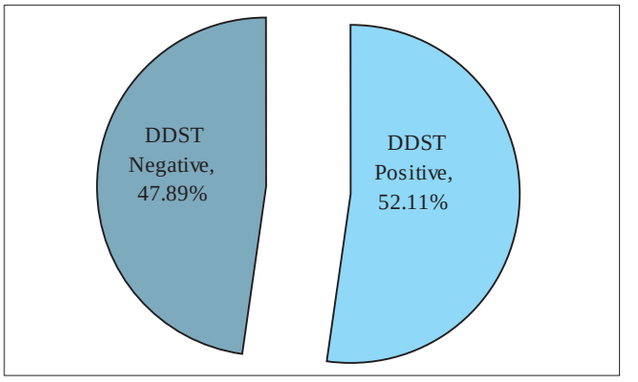

Figure 4 : Detection of ESBL producing organisms by double disc synergy test.

142 suspected ESBLs producing bacteria were further tested by phenotypic confirmatory test where 89(62.68%) found as confirmed ESBL producers & 53(37.32%) showed negative result (Figure 5).

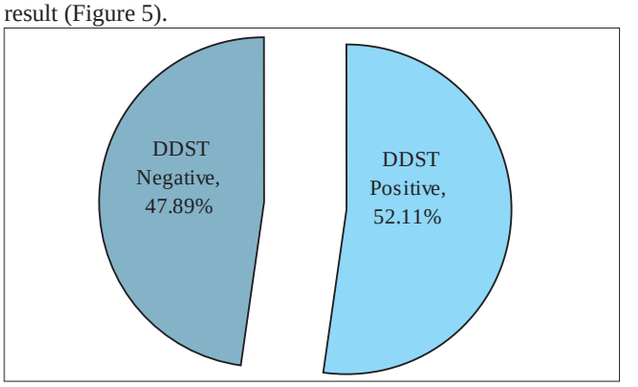

Figure 5 : Detection of ESBL producing organisms by phenotypic confirmatory test.

Table 3 shows comparison of ESBLs positive isolates by double disc synergy test and phenotypic confirmatory test. 5 strains of E. coli, 5 strains of Klebsiella species, 2 strains of Pseudomonas species, 2 strains of Proteus species, 1 strains of Acinetobacter species shows positive reaction by phenotypic confirmatory test but negative result by double disc synergy test.

No strain was found double disc synergy test positive but phenotypic confirmatory test negative. Total 15(10.56%) isolates were missed by double disc synergy test. The difference in ESBLs detection by double disc synergy test and phenotypic confirmatory test were statistically very highly significant ($p<0.001$).

Table 3 : Comparison between Phenotypic Confirmatory Test (PCT) & Double Disc Synergy Test (DDST) on detection of ESBLs producers (n = 142).

Name of strains tested	Phenotypic confirmatory test positive	Double disc synergy test positive	PCT (+) ve DDST (-) ve	PCT (-) ve DDST (+) ve
E. coli (n = 65)	41 (63.08)	36 (55.38)	05 (07.69)	00 (00.00)
Klebsiella spp. (n = 37)	27 (72.97)	22 (59.46)	05 (13.51)	00 (00.00)
Pseudomonas spp. (n = 25)	13 (52.00)	11 (44.00)	02 (08.00)	00 (00.00)
Proteus spp. (n = 12)	07 (58.33)	05 (41.67)	02 (16.67)	00 (00.00)
Acinetobacter spp. (n = 03)	01 (33.33)	00 (00.00)	01 (33.33)	00 (00.00)
Total (n = 142)	* 89 (62.68)	* 74 (52.11)	15 (10.56)	00 (00.00)

● Figures within parentheses indicate percentages

* $^2 = 88.721$; $p < 0.001$. Very highly significant

In this study, sensitivity, specificity, positive predictive value & negative predictive value of phenotypic confirmatory test in comparison to double disc synergy test were 100%, 77.9%, 83.1% & 100% respectively (Table 4).

Table 4 : Sensitivity, specificity, positive & negative predictive value of double disc synergy test and phenotypic confirmatory test.

	Double Disc Synergy Test (DDST)	Phenotypic Confirmatory test (PCT)
Sensitivity	83.1 %	100.0 %
Specificity	100.0 %	77.9 %
Positive predictive value	100.0 %	83.1 %
Negative predictive value	77.9 %	100.0 %

DISCUSSION

In the present study, a total of 235 samples were collected and of which 115 were wound swab & pus, and 120 were urine samples. From these samples, culture positive bacterial isolates were 176(74.89%) and among which 105(91.30%) from wound swab & pus, and 71(59.17%) from urine samples. This result is closely related to that of Rahman in Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka, who found 69.41% culture positive isolates and isolated 93.92% organisms were from wound swab & pus, and 53.57% from urine samples[10].

Among the bacterial isolates, 150(85.23%) were gram-negative and 26(14.77%) were gram-positive in our study (Table 2). Similar to present study Alim and Rahman of BSMMU, Dhaka found 90.21% gram-negative & 9.79% gram-positive and 90% gram-negative & 10% gram-positive isolates respectively[10,11]. Amongst the isolates in our study, the majority were E. coli 70(39.77%), followed by Klebsiella spp. 40(22.73%), Pseudomonas spp. 25(14.21%), Staphylococcus aureus 18(10.23%), Proteus spp. 12(06.82%), Enterococci spp. 5(2.84%), Acinetobacter spp. 3(1.70%) and Coagulase-negative staphylococci 3(1.70%). Similarly, Haq et al. of Dhaka showed E. coli (37.10%) and Klebsiella spp. (17.60%) as the most prevalent isolates from the clinical samples in a multi-center study, in Bangladesh. In contrast to our findings Rahman revealed E. coil (40.63%) & Proteus spp. (18.44%) and Alim revealed E. coli (42.39%) & Pseudomonas spp. (22.28%) as the prevalent isolates in their study[10,11]. These sorts of variation are not unexpected, because it depends upon some external factors like socioeconomic conditions, hygienic status, environmental factors, level of education, and genetic factors[12].

In the present study, we found 142(94.67%) suspected ESBLs producers from 150 gram-negative isolates, based on Minimum Inhibitory Concentration (MIC) ESBLs screening breakpoints (Figure 3). As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (Ceftriaxone, ceftazidime & cefotaxime) for the screening[8]. Our finding is closely related to that of Metri et al in North Karnataka, India, who found 91.74% suspected ESBLs producers by screening test.[1]

74(52.11%) by Double Disc Synergy Test (DDST).

When these 142 screening positive isolates were subjected to the confirmatory tests, 74(52.11%) were confirmed as ESBL producers by Double Disc Synergy Test (DDST) (Figure 4) and 89(62.68%) by Phenotypic Confirmatory Test (PCT) (Figure 5). Closely similar to the present study, Dalela (2012) of Rajasthan, India detected ESBL producers 61.6% by PCT & 57.5% by DDST and Giriyapur et al (2011) of Karnataka, India detected 63.89% by PCT & 56.23% DDST[13,14].

Comparison of ESBLs positive isolates by double disc synergy test and phenotypic confirmatory test (Table 3) showed that 15(10.56%) isolates were missed by double disc synergy test. 7.69% of E. coli, 13.51% of Klebsiella species, 8% of Pseudomonas species, 16.67% of Proteus species and 33.33% of Acinetobacter species showed positive reaction by phenotypic confirmatory test but negative result by double disc synergy test. No strain was found double disc synergy test positive but phenotypic confirmatory test negative. The difference in ESBLs detection by double disc synergy test and phenotypic confirmatory test were statistically very highly significant ($p<0.001$).

The double disc synergy test lacks sensitivity because of the problem of optimal disc spacing, need of precision, correct storage of the clavulanate containing discs, inability of the clavulanate to inhibit all ESBLs and the inability of the test to detect ESBLs in strains producing chromosomal cephalosporinases[1,15].

In present study, we found sensitivity, specificity, positive predictive value & negative predictive value of PCT are 100%, 77.9%, 83.1% & 100% respectively and those of DDST are 83.1%, 100%, 100% & 77.9% respectively (Table 4). Similarly, Giriyapur et al. (2011) of Karnataka, found sensitivity, specificity, positive predictive value and negative predictive value of DDST were 94.89%, 75.91%, 83.55% & 92.03% respectively[14]. Of the two tests, used in the study, phenotypic confirmatory test found to be more sensitive procedure than double disc synergy test for the detection of ESBLs producing organisms. Similar findings were also reported by some other studies[1,16,17,18]. For this reason, some authors recommended phenotypic confirmatory test for the detection of ESBLs producers[14,18,19].

CONCLUSION

Existing of extended spectrum -lactameses in bacteria and their potential multidrug resistance will create serious problem in the future as their continuous mutation and limited therapeutic option. Indiscriminate use of antibiotics especially 3rd generation cephalosporins and monobactams should be avoided. The regular detection of ESBLs producing organisms by conventional methods should be carried out in every laboratory where molecular methods cannot be performed.

DISCLOSURE

All the authors declared no competing interest.

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