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The Comparison of DDST and NCCLS Methods in Detecting ESBL Production of *E. coli* and *K. pneumonia.*

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ABSTRACT

There are few gram-negative bacteria which possess resistance to the antimicrobial drugs like extended spectrum cephalosporins and aztreonam. This has been attributed to the production of the extended spectrum beta-lactamases enzyme by these bacteria. There are few methods available from which the production of ESBL can be detected. We have performed earlier, the studies about the detection of ESBL separately by using the double disk synergy test (DDST) and the national committee for clinical laboratory standards phenotypic confirmatory tests (NCCLS-PCT). The objective of the present investigation was to tabulate and compare the results of the DDST and NCCLS-PCT methods. From the comparison, it has been observed that the NCCLS-PCT test is more efficient in detecting ESBL production than the DDST method. We also opine that the resistant strains of E. coli and K. pneumoniae should be confirmed for ESBL production by using the NCCLS-PCT phenotypic confirmatory test.

Keywords: comparison; DDST; E. coli; ESBL; K. pneumonia; NCCLS-PCT

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INTRODUCTION

There are few gram negative bacteria which exhibit resistance to the higher antibiotics like extended spectrum cephalosporins and aztreonam [1, 2]. Perhaps this is because of the production of extended spectrum beta-lactamases (ESBLs) enzyme by the bacilli. ESBLs are usually produced by the Klebsiella species and Escherichia coli species; however they are also seen in other Pseudomonas aeruginosa and Enterobacteriaceae family. ESBLs are difficult to detect based on the antibiotics which are tested [2]. There are various investigation methods available, which can detect and confirm the production of ESBL. The list includes the double disk synergy test (DDST), the national committee for clinical laboratory standards phenotypic confirmatory test (NCCLS-PCT), inhibitor potentiated test, three dimensional test, E-test, Vitek system. It is believed that, the each and every investigation test has its own advantages and disadvantages. The DDST test, in which the third generation cephalosporin is commonly used, is a reliable and simple test [3]. We have reported in our earlier studies [4, 5] about the detection of ESBL by separately using the NCCLS-PCT and DDST methods. The present study aims at comparing the results of the NCCLS-PCT and DDST methods in detecting ESBL.

MATERIALS AND METHODS

The data from our previous studies [4, 5] about the ESBL detection from the DDST [4] and NCCLS-PCT [5] methods were collected and tabulated. The DDST and NCCLS tests were compared with respect to the detection of production of ESBL in E. coli and K. pneumonia strains. The data comparisons has been done and are represented by the bar charts (Figs. 1 & 2).

RESULTS

In our previous studies [4, 5], it was observed that, about 53 E.coli strains and 26 K. pneumoniae strains were producing the ESBL. By the NCCLS-PCT method [5], 98.1% of E.coli and 100% of K. pneumoniae were detected as the producers of ESBL. However, only 7.6% of E.coli and 15.3% of K. pneumoniae were detected as producer of ESBL by the DDST method [4]. It is also observed that, only 3 E. coli and 4 K. pneumoniae were positive by both the DDST and NCCLS-PCT test.

The comparison of the results of our previous reports [4, 5] is given in Table 1 and Table 2. Table 1 shows the results of ESBL detection and their frequency by using the DDST and NCCLS-PCT methods separately in E. coli and K. pneumoniae strains respectively. The number of E. coli and K. pneumoniae strains which are positive or negative by both the methods and a single method are represented in Table 2.

It is obvious from the Table 2 that, only 3 strains of E.coli which were DDST positive were also positive by the NCCLS-PCT method. Only 1 strain of E. coli which was DDST positive was observed negative by the NCCLS-PCT. The remaining 49 E. coli strains were NCCLS-PCT method positive and the same were DDST negative. There was no K. pneumoniae strain which was positive only by the DDST method. However there were 22 strains which were positive only by the NCCLS-PCT method. The K. pneumoniae strains had positive result in both the DDST and NCCLS-PCT methods in only 4 isolates.

The comparison between DDST and NCCLS methods in detecting ESBL production of E. coli and K. pneumoniae of the present study are also represented by the bar chart in Fig. 1. Fig. 2 shows the frequency of positive and negative results in DDST and NCCLS methods in detecting the ESBL production of E. coli and K. pneumoniae strains.

Table 1: showing the frequency of ESBL detection by using the DDST and NCCLS-PCT methods in E. coli and K. pneumoniae strains

	E. coli (n=53)		K. pneumoniae (n=26)	
method	positive	Negative	Positive	negative
DDST	4 (7.6%)	49 (92.4%)	4 (15.3%)	22 (84.7%)
NCCLS-PCT	52 (98.1%)	1 (1.9%)	26 (100%)	0 (0%)

DDST - double disk synergy test; NCCLS-PCT – the national committee for clinical laboratory standards- phenotypic confirmatory test

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Table 2: showing the number of E. coli and K. pneumoniae strains, which are positive or negative by DDST and NCCLS-PCT methods and same result in both methods

		E. coli (n=53)			K. pneumoniae (n=26)		
		DDST			DDST		
		positive	negative	total	positive	negative	Total
NCCLS-PCT	positive	3	49	52	4	22	26
	negative	1	0	1	0	0	0
total		4	49	53	4	22	26

DDST - double disk synergy test; NCCLS-PCT – the national committee for clinical laboratory standards- phenotypic confirmatory test

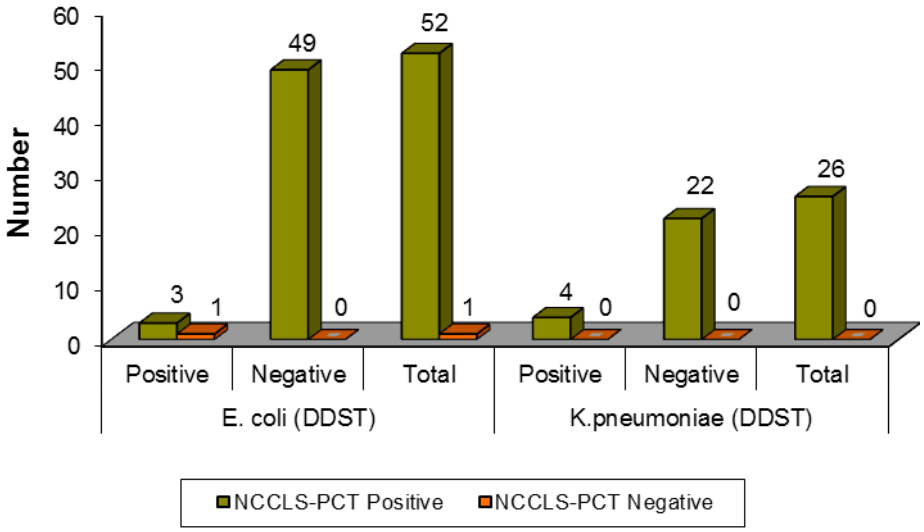

Figure 1: Bar chart showing the comparison between DDST and NCCLS methods in detecting the ESBL production in E. coli and K. pneumoniae strains.

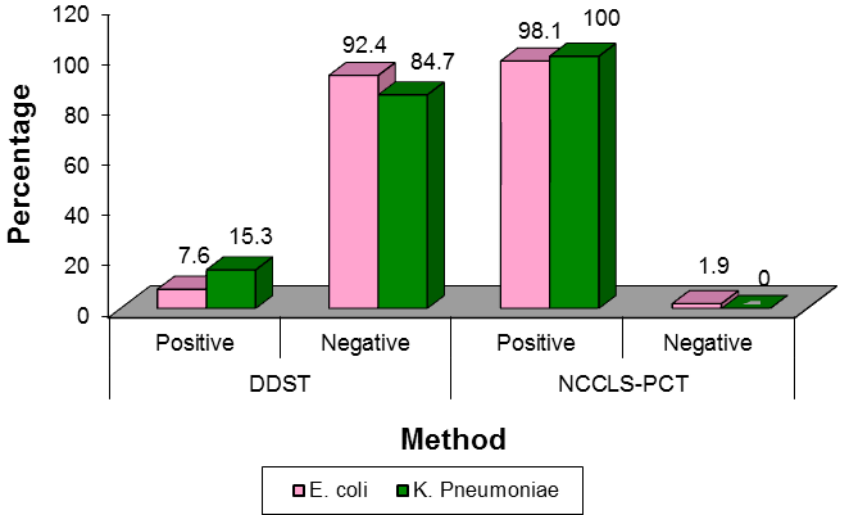

Figure 2: Bar chart showing the frequency of positive and negative results in DDST and NCCLS methods in detecting the ESBL production in E. coli and K. pneumoniae strains.

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DISCUSSION

The DDST method is not a standardized test and the results of this test are subjected to considerable variation in the interdisk distance. There are several DDST studies reported with the interdisk distance measuring 30 mm [6, 7], 25 mm [8] and 15 mm [9-11]. In our previous DDST study [4], there was an interdisk distance of 15 mm which is similar to Coudron et al. [12]. Amoxyclav and cefotaxime antibiotics were used by Babypadmini et al. [10] and Ananthakrishnan et al. [9] to study the ESBL production in their study by using the DDST method. Subha et al. [6] and Nath et al. [7] also used the amoxyclav disk in their study by DDST method. Aztreonam, Cefotaxime, Ceftazidime and Ceftriaxone were used in the study by Menon et al. [11]. Our DDST study [4] has correlated with that of Menon et al. [11] study. The frequency of ESBL positive by DDST method was 14.2% in study by Menon et al. [11] and 27.3% in the study by Shukla et al. [13]. The NCCLS has suggested the usage of the cephalosporin, ceftazidime alone and ceftazidime along with clavulanic acid in combination, for their phenotypic confirmatory test. The NCCLS test is positive if there is a difference of 5mm or more of inhibition zone around the ceftazidime and the combination of ceftazidime-clavulanic acid. The ESBL production was studied by Babypadmini et al. [10], by NCCLS method after using the ceftazidime and ceftazidime-clavulanic acid. The same test was performed by Nath et al. [7] by using ceftazidime, cefotaxime, ceftriaxone and cefotaxime-clavulanic acid disks. In our study [5] by NCCLS phenotypic confirmatory method, ceftazidime and ceftazidime-clavulanic acid were used and this study was correlating with Babypadmini et al. [10] study.

Sridhar Rao et al. [14], in their study of detection of extended spectrum beta-lactamase from clinical isolates observed that, 26.1% of the isolates were DDST positive alone, 13.4% were NCCLS method positive and 60.3% were DDST and NCCLS methods both positive. The present comparison study is not correlating with that of from Sridhar Rao et al. [14]. According to Shukla et al. [13], 30.18% of K. pneumoniae isolates were detected as ESBL producers by NCCLS-PCT and 27.3% by DDST method. The NCCLS confirmatory test was suggested by Babypadmini et al [10], as they observed that 41% of the E.coli and 40% of K. pneumoniae were ESBL producers in NCCLS method. After the comparison of our previous reports [4, 5], it was observed that the ability of DDST method to detect ESBL producer was surprisingly low (10.12%). The ESBL production was detected by both NCCLS phenotypic confirmatory method [5] and DDST method [4] in our studies. It was observed that, 98.1% of E.coli isolates and 100% of K. pneumoniae isolates were identified as ESBL producer by NCCLS method and only 4 isolates of E.coli (7.6%) and 4 isolates of K. pneumoniae (15.3%) were identified as ESBL producer by DDST method.

From the present study of comparison between DDST and NCCLS methods in detecting ESBL production of E. coli and K. pneumoniae, it is obvious that the NCCLS-PCT test is more efficient in detecting the ESBL production than the DDST method. We also suggest that the resistant strains of E. coli and K. pneumonia should be confirmed for ESBL production by using the NCCLS-PCT phenotypic confirmatory test.

REFERENCES

[1] Livermore DM. Clin Microbiol Rev 1995; 8(4):557-84.
[2] Tenover FC, Raney PM, Williams PP, Rasheed JK, Biddle JW, et al. J Clin Microbiol 2003; 41(7):3142–6.
[3] Kaur J, Chopra S, Sheevani, Mahajan G. J Clin Diagn Res. 2013; 7(2):229-33.
[4] Singh RE, Veena M, Raghukumar KG, Vishwanath G, Rao PNS, Murlimanju BV. International Journal of Applied Biology and Pharmaceutical Technology 2011; 2(4): 415-22.
[5] Singh RE, Raghukumar KG, Veena M, Vishwanath G, Rao PNS, Murlimanju BV. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(1):559-65.
[6] Subha A, Ananthan S. Indian J Med Microbiol 2002; 20(2):92-5.
[7] Nath R, Saikia L, Mahanta J. Indian J Pathol Microbiol 2006; 49(4):623-5.
[8] Ho PL, Tsang DN, Que TL, Ho M, Yuen KY. APMIS 2000; 108(3):237-40.
[9] Ananthakrishnan AN, Kanungo R, Kumar A, Badrinath S. Indian J Med Microbiol 2000;18(4):160-5.
[10] Babypadmini S, Appalaraju B. Indian J Med Microbiol 2004; 22(3):172-4.
[11] Menon T, Bindu D, Kumar CP, Nalini S, Thirunarayan MA. Indian J Med Microbiol 2006; 24(2):117-20.
[12] Coudron PE, Moland ES, Sanders CC. J Clin Microbiol 1997; 35(10):2593-7.
[13] Shukla I, Tiwari R, Agrawal M. Indian J Med Microbiol 2004; 22(2):87-91.
[14] Sridhar Rao PN, Basavarajappa KG, Krishna GL. Indian J Pathol Microbiol 2008; 51(4):497-9.