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Research article

COMPARISON OF DOUBLE DISC SYNERGY TEST AND PHENOTYPIC CONFIRMATORY DISC DIFFUSION TEST FOR DETECTION OF ESBL PRODUCTION AND THEIR ANTIMICROBIAL RESISTANCE PATTERN.

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ABSTRACT: Extended spectrum beta- lactamases (ESBLs) continue to be a major problem in clinical setup worldwide. An attempt is made to detect ESBL production among *Enterobacteriaceae* members by phenotypic methods, which is easier to perform in all laboratories. A total of 138 multi-drug resistant strains from pyogenic infection were tested for ESBL production by Double disc synergy test (DDST) and Phenotypic confirmatory disc diffusion test (PCDDT). Of the 84 ESBL producer identified, PCDDT detects 71 (84.5%) whereas DDST detects 52 (61.9%) as ESBL producers. Continued detection of ESBL is essential for proper disease management. PCDDT is better and easy test for screening than DDST. Confirmation has to be done by molecular methods.
Key words: ESBL, DDST. PCDDT, *Enterobacteriacea.*

INTRODUCTION

Monitoring the prevalence of ESBL is important to define the magnitude of the problem and may help to implement appropriate control measures. In in-vitro susceptibility testing, ESBL mediated resistance is not always observed to all Cephalosporins. Many ESBL producers are multi-resistant to non-beta lactam antibiotics such as Quinalones, Aminoglycosides and Trimethoprim, narrowing treatment options. Some producers achieve outbreak status, spreading among patients and locals, perhaps owing to particular pathogenicity traits. Therefore it is very important to detect the ESBL producing species and take steps to minimize their spread (Asma M, Al Jasser 2006).

Clinical Microbiology laboratory plays a vital role in the control of ESBL producing organisms. It is recommended that the implementation of appropriate ESBL detection methods and reporting of their detection to infection control practitioners and clinicians serves to increase awareness, guide therapy and to institute appropriate infection control precautions. Identifying these ESBL producers continues to be a major challenge for the clinical microbiology laboratory. Although most ESBLs confer resistance to one or more of the oxy-imino beta lactam antibiotics, the beta lactamase does not always increase the MICs to high enough levels to be called resistant by CLSI interpretive guidelines (Bradford PA 2001).

Hence this study was conducted to detect ESBL production and to compare the results of two phenotypic methods DDST and PCDDT for detection of ESBL production.

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MATERIALS AND METHODS

Clinical isolates

This study was conducted in the Department of Microbiology, Aarupadai Veedu Medical College, Pondicherry over a period of 18 months (October 2008 to April 2010). A total of 138 multi drug resistant clinical isolates of *Enterobacteriaceae* members obtained from pyogenic lesions were included in the study. These were identified based on colony morphology and by standard biochemical tests (Koneman EW 1997). Antibiotic susceptibility test was performed by disc diffusion technique as per standard CLSI guidelines (NCLSI, 2009).

The following phenotypic confirmation methods were used for detection of ESBL:

l. Double Disc Synergy Test (DDST): (Jarlier Y et al 1988). To demonstrate synergistic action between a third generation cephalosporin and Clavulanic acid. Isolates grown to 0.5 McFarland's standard and lawn culture of it was made on a Muller Hinton agar plate. Disc of third generation cephalosporin Ceftazidime (30 mcg) and Cefpodoxime (10 mcg) were placed 15mm apart from an Amoxicillin (20 mcg) & Clavulanic acid (10 mcg) combined disc, center to center. Incubated at 37°C for 18-24 hours. If inhibition around third generation cephalosporins showed a clear extension towards Augmentin disc, was said to be ESBL producer.

2. Phenotypic confirmatory disc diffusion test (PCDDT): (Thomson KS, Sander CC (1992). Antibiotic Susceptibility testing was done on Muller Hinton agar with 0.5 McFarland's standard of organism. Lawn culture was made and third generation cephalosporin; ceftazidime - Ca (30 mcg) disc was tested alone and along with the combination of clavulanic acid - Cac (10 mcg). Organisms with 5mm increase of zone of inhibition around Cac were confirmed as ESBL producer.

RESULTS

In the present study, more than 98.5% of *Enterobacteriaceae* isolates had showed resistance to Ampicillin. All the isolate showed resistance (100%) to Cefuroxime, whereas resistance to Cefotaxirne was 87.7% and Ceftazidime was 73.2%. Resistance to Amikacin was seen among 40.6% of isolates. Their resistance pattern is shown in Table: 1.

Table1 showing resistance pattern of Enterobacteriaceae isolates.

S.No	Antibiotics	Resistance	Percentage (%)
1	Ampicillin (A)	136	98.5
2	Amoxyclav (Ac)	127	92.02
3	Amikacin (Ak)	56	40.6
4	Cefuroxime (Cu)	138	100
5	Ciprofloxacin (Cf)	103	74.6
6	Cefotaxime (Ce)	121	87.7
7	Ceftazidime (Ca)	105	73.2
8	Ceftriaxone (Ci)	121	87.7
9	Cotrimoxazole (Co)	119	86.2
10	Gentamycin (G)	104	75.4
11	Tetracycline (T)	120	91.3
12	Imipenam (I)	1	0.7

Out of 138 isolates tested for ESBL production by both methods, 84 (60.9%) were ESBL producers. Both DDST & PCDDT were positive in 39 (46.4%) isolates, DDST alone in 13 (15.5%) isolates and PCDDT alone in 32 (38.1%) isolates. PCDDT detects 71 (84.5%) whereas DDST detects 52 (61.9%) as ESBL producers.

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Table 2 Showing comparison of ESBL producers by two methods.

S.No	Method	Number Positive (n)	Percentage (%)
1	Only DDST	13	15.5
2	Only PCDDT	32	38.1
3	Both 1 and 2	39	46.4
		84	100

Figure 1: DDST showing extension sensitive of zone around Augmentin disc.

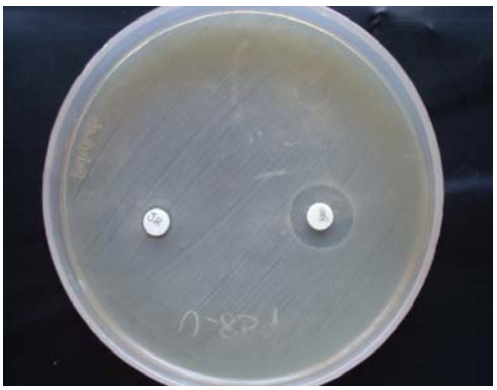

Figure 2: PCDDT showing >5mm increase in the zone of inhibition around Ceftazidime/Clavulanic acid Disc.

DISCUSSION

Extended spectrum beta- lactamases (ESBLs) continue to be a major problem in clinical setup worldwide. A survey in Connecticut found that 21% of laboratories fail to detect ESBL producing isolates. A proficiency testing project for clinical laboratories participating in the National Nosocomial infections surveillance system indicates that as many as 58% laboratories failed to detect and report ESBL isolates correctly. These data suggest that improvement in the ability of clinical laboratory to detect ESBL is needed (Chaudary U, Aggarwal R 2004).

A study by Reema et al found PCDDT to be more effective in detecting the ESBLs than DDST. Shukla et al also reported similar findings. DDST lacks sensitivity because of problem of optimal disc space and correct storage of clavulanate containing discs. Comparison of methods employed for detection of ESBLs showed that DDST was less sensitive than PCDDT. NCCLS guidelines also state that PCDDT is more effective in detecting the ESBL than DDST (David L et al 2005).

The sensitivity and specificity of a susceptibility test to detect ESBLs vary with the cephalosporins tested. A number of investigations have suggested that Cefpodoxime detected more ESBLs than cephalosporins such as Ceftazidime, Cefotaxime and Ceftriaxone. However, more recent data suggests that testing with Cefpodoxime can lead to a high number of false positive if the current CLSI interpretive criteria are used. In the present study 84 isolates were reported as ESBL producers and hence should be resistant to third generation Cephalosporins. But 17.2% were susceptible to Ceftazidime and 16.7% susceptible to Cefotaxime, indicating false susceptibility to Ceftazidime and Cefotaxime. Similarly (Khurana et al 2002 and Vinod Kumar et al 2004) reported approximately 30% of ESBL producers showing false susceptibility to third generation cephalosporins. (Ananthakrishnan et al 2000) reported 53% of ESBL producers sensitive to Cefotaxime. (Padmini Baby et al 2004) reported 14% and 12% of ESBL strains showing false susceptibility to Ceftazidime and Cefotaxime.

ESBL producers also showed wide resistance to the non beta lactam antibiotics. In the present study, 78.6% of strains showed resistance to the Gentamicin, 42.9% to Amikacin and 75.0% to Ciprofloxacin. (Radosz Komoniewsk Halina et al 2004) reported 84% resistance to Gentamicin and 79% to Amikacin. (Ananthakrishnan et al 2000) reported 81.25% resistance to Gentamicin and 90.62% to Ciprofloxacin. Above findings can be comparable with our present study. Our study confirms that these isolates were resistant to different classes of antibiotics.

All the above findings suggest the importance of detecting ESBLs in the laboratory, which helps in proper selection of antibiotics.

CONCLUSION

Routinely used disc diffusion susceptibility methods fails to detect ESBLs because these enzymes exhibit wide spectrum of substrate specificity. Sometimes they show false susceptibility zone of inhibition in Kirby Bauer disc diffusion test. Quick detection of ESBL strains is important since they become resistant to available antibiotics and they also pass the gene to other strains. Use of only one disc combination might fail to detect ESBL production resulting in under reporting of prevalence. Hence inclusion of more than one indicator drugs in the screening tests is recommended. Phenotypic confirmatory disc diffusion test is a simple and cost effective method for the detection of ESBLs.

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