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Original Article

Comparison of Different Phenotypic Tests for Detection of ESBL (Extended Spectrum Beta Lactamases) Producing Pseudomonas Aeruginosa

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Abstract

Background: P. aeruginosa is the most common nosocomial pathogen encountered and a known organism causing high mortality and morbidity. Due to indiscriminate antibiotic use, resistance is very commonly known in them and ESBL enzyme production being the most predominant one. *Material and Methods*: A total of Ninty isolates of P. aeruginosa were tested for the presence of ESBL enzyme by both disc diffusion and double disc synergy test. Antibiotic sensitivity pattern of ESBL-positive P. aeruginosa was determined. *Results*: Of the 1200 pus samples screened, 90 isolates of pseudomonas were tested for ESBL production. Of the 90 P.aeruginosa isolates, 57 (63%) were sensitive to 3GC and 33 (37%) were resistant.Of the 33 P.aeruginosa resistant to ceftazidime, DDT detected 9 (27%) of ESBL producers and DDST detected 17 (51%). And 17 (51%) did not show ESBL production by either of the methods used in the study. All the ESBL-positive P. aeruginosa were multi-drug resistant, with 100% sensitivity to imipenem; followed by ofloxacin (70%). *Conclusion*: From this study, we conclude that DDST proved to be better method than DDT to detect ESBL producing P. aeruginosa. However in the absence of any CLSI guidelines for detection of ESBL in Non-fermenters, we reframe from commenting on specificities of either of tests. There is a strong need for standardization/ CLSI guidelines for detection.

Keywords: Third Generation Cephalosporins; Double Disk Synergy Test; Disc Diffusion Test; P. Aeruginosa.

Introduction

Pseudomonas aeruginosa is one of the most prevalent opportunistic human pathogen and the most common gram-negative bacteria causing nosocomial infections belonging to ESKAPE group of pathogens (Enterococcus faecium, Staphylococcus aureus, Klebseilla pneumonia, acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species). A major challenge has aroused in treatment of Pseudomonas aeruginosa infections exhibiting high level resistance to all antibiotic classes, not only due to innate nature but due to their additional acquiring through plasmids [1]. ESBL mediated resistance is one of the important emerging resistance mechanisms. ESBL enzyme encoding genes SHV-2a and TEM-42 are responsible for the same [2,3].

At present Clinical Laboratory Standards Institute (CLSI) guidelines do not describe any methods for detection of these enzymes in P. aeruginosa [4].

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Hence this study was conducted to know the prevalence and to compare the different phenotypic tests for detection of ESBL (Extended Spectrum Beta lactamases) producing P. aeruginosa isolates from pus samples

Objective/Aim

Present study was conducted to compare the different phenotypic tests for early and accurate detection of ESBL (Extended Spectrum Beta lactamases) producing P. aeruginosa isolates from pus samples.

Material and Methods

A total of 1200 pus samples were screened in one year which were received at the Department of Microbiology, J. N. Medical College, KLE University, from hospitalized patients of K.L.E.'S DR. Prabhakar Kore's Charitable Hospital and MRC, Belagavi.

Only those isolates of P. aeruginosa obtained from pus samples as pure and predominant growth were included in the study.

Based on colony morphology and biochemical tests, organisms were identified. Using disc diffusion method, sensitivity of the isolates to the third-generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, 30μg each) was determined using P. aeruginosa ATCC 27853 as control strain. Results were interpreted according to the CLSI guidelines, which suggest a diameter of inhibition zone > 22 mm for ceftazidime, > 27 mm for cefotaxime and >25 mm for ceftriaxone as susceptible [5].

Only those isolates showing resistance to third generation cephalosporins were tested for ESBL production by the following two methods .

a) Disc diffusion test

b) Double Disc Synergy Test

- *Disc Diffusion Test* [6]:

Ceftazidime (30mcg) & with Ceftazidime/ Clavulanic acid (30mcg/10mcg) discs were placed on Mueller Hinton agar (MHA, Hi-Media) inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture and was incubated overnight at 37^{0}C.

An increase in the zone diameter by > 5mm of Ceftazidime versus its zone when tested in combination with Clavulanic acid was considered as an ESBL producer.

- *Double Disc Synergy Test [7]:*

30mcg disc of each third generation cephalosporin antibiotics: Cefotaxime, Ceftriazone and Ceftazidime, are placed on Mueller Hinton agar (MHA, Hi-Media) inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture at distance of 15mm center to center from Augmentin disc (Amoxicillin/Clavulanic acid- 20mcg/10mcg) and was incubated overnight at 37^{0}C.

Increase in the inhibition zone of any one of the three third generation antibiotic disc towards augment disc was considered as an ESBL producer.

In both the methods, increase in zone size occurs because the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism.

Results

Out of 1200 pus sample screened, 90 P. aeruginosa isolates were isolated.

Of the 90 P.aeruginosa isolates, 57 (63%) were sensitive to 3GC and 33 (37%) were resistant.

Of the 33 P.aeruginosa resistant to ceftazidime, DDT detected 9 (27%) of ESBL producers and DDST detected 17 (51%). And 17 (51%) did not show ESBL production by either of the methods used in the study.

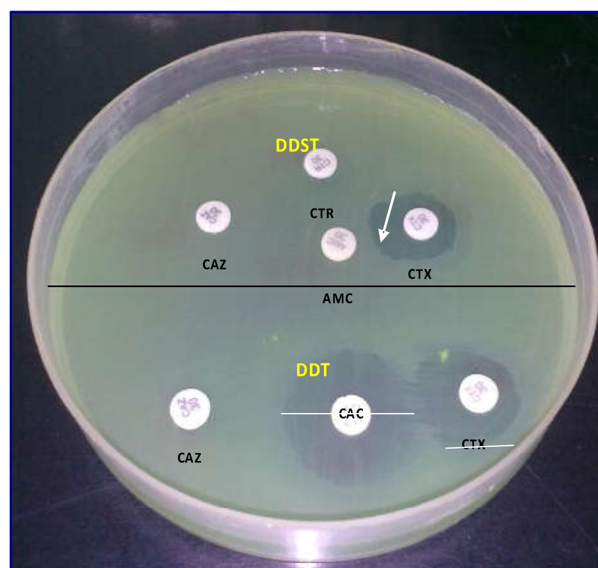
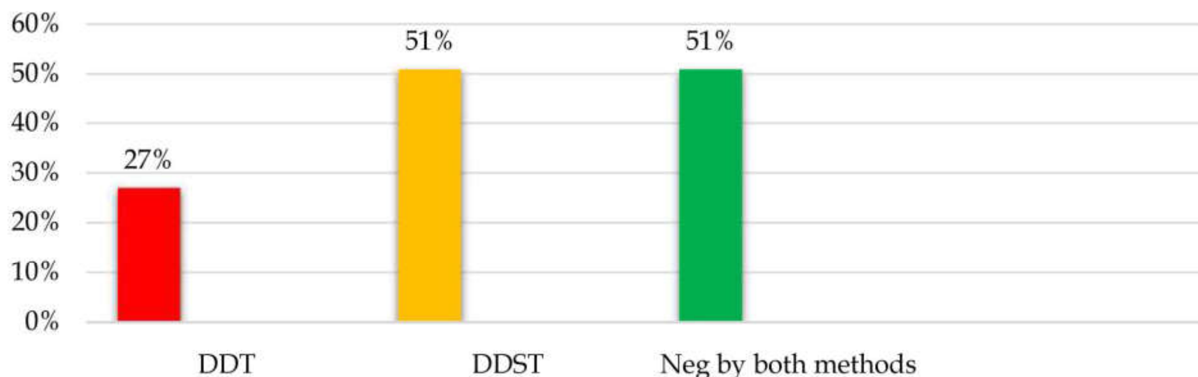


Fig. 1: Extended-spectrum-â-lactamase(ESBL) producing *P.aeruginosa* detection by Double disk synergy test(DDST)& Disc diffusion test (DDT):
Interpretation of DDST: Increase in inhibitory zone around CTX antibiotic disc towards Augmentin disc.
Interpretation of DDT: Increase in zone diameter of Ceftazidime by > 5mm.



Graph 1: Percentage of ESBL producing *p.Aeruginosa* Detected by different methods

All the ESBL-positive P. aeruginosa were multi-drug resistant (drug resistance to more than three drugs). Maximum sensitivity (100%) was seen with imipenem; followed by ofloxacin, which showed good sensitivity (70%). The least effective drugs were cephalothin, cefamandole, azlocillin, ticarcillin, ticarcillin/clavulanate.

Conclusion

In the present study 36.6% (33/90) P.aeruginosa were resistant to Ceftazidime which is similar to the study done by Aggarwal et al [7], at Haryana showing 20.27% of Cefazidime resistant P.aeruginosa. Another study done by Singh et al [8], at Mysore also showed 27.2% P.aeruginosa resistant to Cefazidime, 20.9% by a study done by Zahra et al [9], West Bengal and 20.27% by a study by Wayne et al [10].

Studies in some places like in Nagpur, the figures of ESBL producers were 50% [11] and another comparatively recent study in 2005, from New Delhi, showed 68.78% of the strains of gram negative bacteria to be ESBL producers [12], which is high compared to our study. And studies in few other places like in Varanasi, Upadhyay S et al showed the prevalence of ESBL producing P.aeruginosa was 3.3%[13] and Rodrigues C et al, in their study showed 5.9% of P.aeruginosa isolates harbored ESBLs in Mumbai [14], which is less in comparison to our study.

This variation in the prevalence of ESBL producing P.aeruginosa in different places/studies could be due to the variation in sample size studied or due to their differences in hygienic practices.

In our study of the 33 P.aeruginosa resistant to Ceftazidime processed for ESBL detection, Double Disc synergy test detected 17(51%) compared to Disc diffusion test which detected 9(27%) of ESBL producing P.aeruginosa and thus DDST proved to be better method than DDT to detect ESBL producers.

A similar study by Umadevi S et al [15], in which the the two conventional methods DDST and DDT were compared. They found, no significant differences between the ESBL detection rates by two conventional methods in P. aeruginosa. Their failure to detect the better performance of the double disk synergy test as compared to the disk diffusion test for the detection of ESBL production among the Pseudomonas aeruginosa isolates could be due to the relatively small number of isolates which were tested in their study (27 isolates).

A study done by Jiang X et al, to detect ESBL producing P.aeruginosa from 75 isolates showed that there were no ESBL false positive detected in the ESBL-screening methods like DDST and Combined Disc test when compared to IEF (isoelectric focusing electrophoresis), PCR, and PCR product sequencing. And hence found the conventional methods to be more cost effective, easy to perform in routine clinical laboratory and are as sensitive as molecular techniques like IEF and PCR [16].

In a study by Shukla et al, they found DDT to be more sensitive for detecting ESBL producers than the DDST. And the reason coated was the problem of optimal disc space and correct storage of the Clavulanate containing disc [17].

The 17 (51%) Ceftazidime resistant P.aeruginosa which gave negative results both methods used to detect ESBL producing may have other mechanism of resistance such as impermeability of outer membrane and or active efflux mechanism or may be due to masking effect of presence of AmpC or due to MBL production by the organism.

The main limitation of our studies were, due to the absence of any standard methods to detect ESBL in non-fermenters, it is difficult to comment on true or false ESBL producers, MIC reduction would be a better method to know the drug susceptibility, but it is a cumbersome, laborious method and PCR could have been an additional investigation to detect the genes responsible for resistant, but has the disadvantage of its high cost.

This early and accurate detection of ESBL producing P.aeruginosa has helped the doctors to treat the patients early with appropriate antibiotics, thereby improving the patient outcome and decreased the morbidity and mortality.

However in the absence of any CLSI guidelines for detection of ESBL in Non-fermenters, we reframe from commenting on specificities of either of tests. There is a strong need for standardization/ CLSI guidelines for detection.

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