TO CORRELATE DOUBLE DISC SYNERGY TEST (DDST) AND ETEST RESULTS FOR ESBL DETECTION IN ECOLI & KLEBSIELLA PNEUMONIAE ISOLATES

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ABSTRACT: Resistant to antimicrobial agents in microbes is a growing phenomenon worldwide.¹ β lactamase production is the most common mechanism of bacterial resistance to β lactam antibiotics.² Extended spectrum beta lactamases (ESBL) that mediate resistance to oxyimino cephalosporins such as cefotaxime, ceftazidime and aztreonam are now observed in all species of Enterobacteriaceae. ESBL are capable of efficiently hydrolyzing penicillins, narrow spectrum cephalosporins, many extended spectrum cephalosporins, the oxyimino group containing cephalosporins (Cefotaxime, ceftazidime) and monobactams (Aztreonam), but not carbapenems and cephamycins.³ ESBL producing Ecoli and Klebsiella pneumoniae are important pathogen in nosocomial infections and multidrug resistant out breaks. This study was conducted to correlate results of Double Disc Synergy Test (DDST) and E test for ESBL detection in E coli and Klebsiella pneumoniae isolate by doing the double disc synergy test (DDST) by using cefotaxime and augmemtin discs. E test was used to determine the MIC for cefotaxime and ceftazidime of these isolates. Out of 98 ESBL isolates detected by DDST, 96 isolates were positive by E test. 02 isolates were indeterminable by E test. About 95% ESBL producing E coli and Klebsiella pneumoniae had MIC >1ug/ml for cefotaxime. The MIC of about 85% ESBL producing E coli and Klebsiella pneumonia was >4ug/ml for ceftazidime.

KEYWORDS: ESBL, DDST, E- TEST.

INTRODUCTION: Resistant bacteria are emerging worldwide as a threat to the favorable outcome of common infections in community and hospital setting. Detection of these resistant bacteria is equally important for the Microbiology laboratory. In this study we have compared results of two ESBL detection tests in E.coli and klebsiella pneumoniae – Double disc synergy test (DDST) and E-test.

MATERIAL AND METHODS: The study was conducted in the Microbiology department of a Tertiary care hospital in Mumbai over a period of one and a half years from November 2004 – April 2006. Various clinical specimen viz pus & wound swab, blood, urine, ascitic and pleural fluids, CSF, sputum and other respiratory samples, intravascular catheters etc. from patients of different clinical specialties were inducted in the study.

E.coli & klebsiella pneumoniae were identified as per the standard bacteriological techniques follows by antimicrobial susceptibility testing.

Screening for ESBL production by CLSI screening test for ESBL was done. Zone of inhibition ≤ 27 mm for Cefotaxime & ≤ 25 mm for Ceftriaxone was consider a potential ESBL producing organism.⁴

ESBL production was detected by DDST (Double disc synergy test) Cefotaxime [30ug] & Ceftriaxone [30ug] disc were placed on either side at a distance of 20 mm centre to centre from Augmentin [Amoxicillin (20ug) + Clavulanic acid (10ug)].

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Enhancement of zone of inhibition of Cefotaxime and Ceftriaxone towards Augmentin [Clavulanic Acid] or ghost zone between the 2 discs was considered ESBL producer.

ESBL production was confirmed using 200 E test of AB bio disk, Sweden.

Cefotaxime (CT)/Cefotaxime + Clavulanic acid (CTL) E test strips (100 strips) and Ceftazidime (TZ)/Ceftazidime + Clavulanic acid (TZL) E test strips (100 strips) were used for each isolate:

- MIC range of Cefotaxime (CT) on E test was 0.25–16ug/ml.
- MIC range of Cefotaxime + Clavulanic acid (CTL) was 0.016–1ug/ml+4ug/ml CA.
- MIC range of Ceftazidime (TZ) was 0.5 – 32ug/ml.
- MIC range of Ceftazidime (TZ)/+ Clavulanic acid (TZL) was 0.064–4ug/ml+4ug/ml CA.
- MIC values were calculated according to the inhibition ellipses intersecting the strips.

Ratio of the MICs of CT & Ct/CTL and ratio of the MIC of TZ and TZ/TZL was calculated. Ratio of >8 was considered positive for ESBL production. Presence of phantom zone or Ellipse deformation also indicates ESBL production.

RESULT:

Gram negative bacteria	Total (8999)	%
E.coli	2253	25.04
Klebsiella	1363	15.15
E.coli + Klebsiella	3616	40.18

Table 1: Distribution of E.coli & Klebsiella pneumoniae among gram negative bacilli isolates (n=8999)

E.coli and Klebsiella pneumoniae comprises approximately 40% of total gram negative bacilli.

Organism	Total No. of Isolates	DDST Positive [%]	Resistance to 3GC [%]	
			Cefotaxime	Ceftriaxone
Ecoli	2253	463 [20.55]	463 [100 %]	447 [96.54]
Klebsiella	1363	337 [24.72]	337 [99.41 %]	331 [98.22]

Table 2: Comparison of DDST result and resistance to third generation cephalosporins among ESBL producing Ecoli and Klebsiella pneumoniae

Almost 100% ESBL producing E.coli and Klebsiella pneumoniae were resistance to Cefotaxime. Approximate 97%. E.coli and 98% Klebsiella pneumoniae were found resistance to Ceftriaxone.

Prevalence of ESBL producing Ecoli and Klebsiella pneumoniae was 20.55% and 24.72% respectively.

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E test Strips	Total No. of Positive (MIC ratio >8)	Total No. of Negative (MIC ratio <8)	Non-determinable (No zone)
Ct / CTL	94	1	3
TZ / TZL	91	3	4
Interpretation	96	0	2

Table 3: Etest results of 98 ESBL positive (by DDST) isolates of E.coli (61) and Klebsiella pneumoniae (37)

All ESBL isolates positive by DDST were also positive by E test except 2 ESBL isolates which were non-determinable by E test, as the MIC for these isolates was more than the concentration of antibiotics provided in the E test strips.

MIC (ug/ml)	No. of Isolates	%
<1	4	6.55
>1	57	93

Table 4: MIC of Cefotaxime for E.coli by E test (n=61)

Approximate 93% ESBL producing E coli isolates had MIC of > 1 ug/ml for Cefotaxime.

MIC (ug/ml)	No. of Isolates	%
<4	9	14.75
>4	52	85.25

Table 5: MIC of Ceftazidime for E.coli using E test (n=61)

85 % ESBL producing E.coli had MIC >4 ug/ml.

MIC (ug /ml)	No. of Isolates	%
<1	1	3.03
>1	36	94.59

Table 6: MIC of Cefotaxime for Klebsiella pneumoniae. By E test (n=37)-

Approximately 95 % ESBL producing Klebsiella pneumoniae had MIC of >1ug/ml for cefotaxim.

MIC (ug /ml)	No. of Isolates	%
<4	4	10.81
>4	33	89.18

Table 7: MIC of Ceftazidime for Klebsiella pneumoniae by E test (n=37)

Approximately 90% ESBL producing Klebsiella pneumoniae had MIC >4 µg /ml.

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DISCUSSION: The study of prevalence of ESBL producing Ecoli and Klebsiella pneumoniae was carried out in a public hospital over a period of one and half years from Nov. 2004 to April 2006. Various clinical specimens from patients undergoing treatment at different clinical specialties were processed as per standard bacteriological techniques.

The prevalence of E.coli was found to be 25.04% and that of Klebsiella pneumoniae was 15.15%. Datta and Thakur et al found Klebsiella pneumonia (35.07%) was the most common isolate followed by Ecoli (29%).⁵

Kumar et al reported higher prevalence of E. coli i.e. 50.79% and Klebsiella pneumonia isolates were 27.31%.⁶

All ESBL producing E.coli and Klebsiella pneumoniae were resistant to Cefotaxime while 97% E.coli and 98 % Klebsiella pneumoniae were found to be resistant to Ceftriaxone.

Out of 98 ESBL and isolates detected by DDST, 96 were also positive by E test. 2 of the isolates show non-determinable result by E test (2%).

Therefore DDST has good sensitivity and specialty comparable to E test. The CLSI recommends the use of both Cefotaxime and Ceftazidime for detection of ESBL. This increases the sensitivity of the test as seen in this study. Linscott and Brown et al reported sensitivity and specificity of E test as 97% and 94% respectively in a study in 2005.⁷

Leverstein-Van Hall et al reported that E test is an accurate test but was limited by its in determinable results i.e. 4 %.⁸

Datta et al reported that DDST missed 23 ESBLs out of 38 ESBLs producers ⁴ while Ho et al reported the sensitivity of DDST as 96%.

These lacks of sensitivity result from the fact that in DDST the distance between Augmentin and cephalosporin discs varies from study to study.

Table IV shows the MIC of Cefotaxime for ESBL producing Ecoli isolates by E test strip containing Cefotaxime.

93% of isolates have MIC >1ug/ml i.e. they are resistant to Cefotaxime. Kumar et al reported 95% of ESBL E coli resistant to Cefotaxime.⁵

Table V shows the MIC of Ceftazidime for ESBL producing E coli isolates by using E test strip containing Ceftazidime.

85.25 % isolates have MIC >4 i.e. they were resistant to Ceftazidime.

Table VI shows the MIC of Cefotaxime for ESBL producing Klebsiella pneumoniae 94.59 % isolates were resistant to Cefotaxim with MIC>1ug/ml.

Table VII shows MIC of Ceftazidime for ESBL producing Klebsiella pneumoniae 98.18 % isolates are resistance to Ceftazidime with MIC >4ug/ml. All ESBL isolates show good sensitivity (100%) to Imipenem and other Carbapenems. Cefotaxime + Sulbactam also show good sensitivity.

This study shows that DDST is a good test to routinely detect ESBL as it is simple, economical with good sensitivity and specificity. It can be done along with antibiotic sensitivity test. Results of DDST correlate well with Etest.

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