ESBL DETECTION: PREVALENCE & COMPARISON WITH NEW CRITERIA

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ABSTRACT: AIM: To study the prevalence of ESBL producing organisms in our area both by phenotypic method and latest CLSI breakpoints. Effect of change in break points on predicting ESBL was also studied. **MATERIAL & METHODS:** Successive clinical isolates were tested by phenotypic assay and zone diameters compared by Kirby Bauer's disc diffusion method.

RESULTS: Total 231 clinical isolates were studied of which 81.8% were ESBL producers. Cefotaxime had high sensitivity (97.91%), specificity (97.43%) and positive predictive value (99.47%). Cefpodoxime had strikingly 100% specificity and positive predictive value but a very poor negative predictive value. Aztreonam had a very high sensitivity (99.4%) and negative predictive value (97.61%) **CONCLUSION:** Cefotaxime was found to be the most appropriate antibiotic when used as single agent.

KEYWORDS: ESBL, Double Disc Synergy Test, Drug Resistance

INTRODUCTION: Since their first description more than twenty years ago, pathogens producing extended-spectrum β -lactamases (ESBLs) have become an increasing cause of clinical concern for several reasons¹⁻³. First, systemic infections due to ESBL-producing Enterobacteriaceae are associated with adverse clinical outcomes. Second, initially restricted to certain, these enzymes have spread globally and their prevalence varies geographically. Third, primarily characterized in limited bacteria such as *Escherichia coli* and *Klebsiella* spp., ESBLs have been spreading and reaching other genera, principally *Enterobacter* and *Proteus* spp. Finally, besides the growing species diversity, ESBL phenotypes have become more complex due to the production of multiple enzymes including inhibitor-resistant ESBL variants, plasmid-borne AmpC, production of ESBLs in AmpC - producing bacteria, production of ESBLs in KPC-producing bacteria, enzyme hyperproduction and porin loss¹⁻⁴.

A strong relationship exists between third-generation cephalosporin use and acquisition of an ESBL producing strain⁵. Other antibiotic classes associated with infections due to ESBL-producing organisms include quinolones, trimethoprim-sulphamethoxazole, aminoglycosides and metronidazole.⁶ In some centers from India as many as 86 percent of *Klebsiellae* have been found to be ESBL producers⁷.

Despite Clinical and Laboratory Standard Institute's (CLSI) recommendations^{6,8} that clinical microbiology laboratories perform specialized tests for detection of ESBLs, many of them make no effort to detect ESBL production or are ineffective at doing so. In India there is no regulation regarding performance and reporting of ESBL isolates from various hospitals and

clinical laboratories. Many laboratories lack sufficient expertise to identify and report ESBLs. There is a need for simple yet effective methodologies which can be routinely used for monitoring the prevalence of ESBL producing organisms.

The most common method of testing for extended spectrum β -lactamases (ESBLs) is screening for reduced susceptibility to cefpodoxime/ cefotaxime/ ceftriaxone/ ceftazidime/ aztreonam followed by phenotypic confirmatory testing by demonstrating a synergistic effect between an indicator cephalosporin and a β -lactamase inhibitor i.e., clavulanic acid ^{9,10}. The sensitivity of screening for ESBLs can vary depending on the type of antimicrobial agent tested.^{11, 12} According to Clinical Laboratory Standards Institute (CLSI) M100-S13 guidelines,¹⁰ use of more than one of the five indicator cephalosporins suggested for screening improves the sensitivity of ESBL detection. Some studies have reported that if it should be necessary to rely on a single screening antibiotic, cefpodoxime or ceftazidime is the recommended antibiotic for ESBL detection in *Klebsiella* spp.^{11, 12} Phenotypic methods are easier to perform as compared to genotypic and at the same time they are indicative of gene expression. If criteria for ESBL detection could be incorporated in routine susceptibility testing the detection and interpretation would become simpler.

So a study was planned to estimate the prevalence of ESBL producing organisms in our area both by phenotypic method and latest CLSI breakpoints. The isolates studied belonged to family Enterobacteriaceae.

MATERIALS & METHODS: The study was conducted in Giansagar Medical College and Hospital which is a 600 bedded tertiary care hospital. A prospective study on successive clinical isolates of family enterobacteriaceae was undertaken. All isolates were screened for ESBL production by Kirby- Bauer's disc diffusion method demonstrating reduced susceptibility to cefpodoxime (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), and aztreonam (30 μ g). Cut-off zone sizes as an indicator of potential ESBL producer for *K. pneumoniae*, *K. oxytoca* & *E. coli* were ≤ 27 mm for cefotaxime, ≤ 22 mm for ceftazidime, ≤ 27 mm for aztreonam and ≤ 17 mm for cefpodoxime. For *P. mirabilis* the zone size cut offs were cefotaxime ≤ 27 mm, ceftazidime ≤ 22 mm, aztreonam ≤ 27 mm and cefpodoxime ≤ 22 mm. *Escherichia coli* ATCC 25922 & *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used as quality control for ESBL detection.

Phenotypic confirmatory test was performed for confirmation. The combined disk method was used to confirm the presence of ESBL on all the isolates of enterobacteriaceae family by placing a disk of ceftazidime (30 μ g) and ceftazidime (30 μ g)/ clavulanic acid (10 μ g) besides cefotaxime (30 μ g) and cefotaxime (30 μ g)/ clavulanic acid (10 μ g) on a Mueller-Hinton agar plate as recommended by CLSI. A ≥ 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs its zone when tested alone was considered ESBL producer. CLSI has reviewed the interpretation criteria as shown in Table 2.¹⁸ If new interpretation criteria are being used then the screening test need not be performed. Penicillins, cephalosporins and aztreonam should be reported as found on susceptibility testing without altering susceptibility status as recommended earlier.

RESULTS: Altogether 231 clinical isolates were studied. The most prevalent isolate was *E. coli* (78.3%) followed by *Klebsiella* spp.(12.1%), *Proteus mirabilis* (4.3%), *Citrobacter* spp. (2.1%) & others (Table 1). Overall ESBL producers were 81.8% and ESBL non producers were 18.2%. Outpatient clinics contributed to 65 isolates of total 231 isolates. Amongst them the prevalence of ESBLs was 75.38% (49/65). From inpatients 166 isolates were obtained amongst which the prevalence of ESBLs was 84.34% (140/166) (Table 3).

TABLE 1: Species distribution

Species	No. of isolates	ESBL producers
<i>Escherichia coli</i>	181 (78.3%)	150 (82.8%)
<i>Klebsiella</i> species	28(12.1%)	22 (78.5%)
<i>Proteus mirabilis</i>	10 (4.3%)	8(80%)
<i>Citrobacter</i> species	5 (2.1%)	5(100%)
<i>Serratia</i> species	4 (1.7 %)	3(75%)
<i>Enterobacter</i> species	1 (0.4%)	1 (100%)
<i>Morganella morganii</i>	1 (0.4%)	1(100%)
<i>Salmonella typhi</i>	1(0.4%)	0(0%)

TABLE 2: Revised CLSI zone diameter (in mm)

	Sensitive	Intermediate	Resistance
Cefotaxime	≥26	23-25	≤22
Ceftazidime	≥21	18-20	≤17
Aztreonam	≥21	18-20	≤17
Cefpodoxime	≥21	18-20	≤17

TABLE 3: In patient & outpatient distribution

	Isolates	ESBL producers	Non ESBL producers
Out patient	65	49 (75.38%)	16 (24.61%)
In patient	166	140 (84.34%)	26 (15.66%)
Total	231	189 (81.80%)	42(18.18%)

TABLE 4: Comparison of old and new CLSI criteria for ESBL detection

	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
	Old criteria	New criteria	Old criteria	New criteria	Old criteria	New criteria	Old criteria	New criteria
Cefotaxime	97.91%	97.91%	97.43%	97.43%	99.47%	99.47%	90.47%	90.47%
Ceftazidime	91.45%	95.72%	78.12%	77.27%	96.29%	94.7%	59.52%	80.95%
Aztreonam	89.9%	99.4%	91.3%	66.12%	98.94%	88.88%	50%	97.61%
Cefpodoxime	96.89%	82.17%	94.73%	100%	99.94%	100%	85.71%	2.38%

The species distribution of ESBLs amongst the various isolates was as shown in Table 1. Using phenotypic methods the highest number of ESBLs was contributed by *E. coli* followed by *Klebsiella* spp. and *Proteus mirabilis*. *Citrobacter* spp. though had only 5 isolates but alarmingly all of them were ESBL producers. The drugs that were tested for ESBL production were cefotaxime, aztreonam, ceftazidime & cefpodoxime. We also compared the zone diameters

according to old and current CLSI criteria to see if a single drug was better predictor of ESBL activity when used in the absence of ESBL phenotypic assay.

The effect of change in CLSI criteria on predicting ESBL production is shown in Table 4. For cefotaxime the sensitivity, specificity, positive predictive value and negative predictive value was 97.91%, 97.43%, 99.47% & 90.47% respectively. Upon testing with ceftazidime the sensitivity was 95.72% and positive predictive value was 94.7%. A fall in specificity (77.27%) and negative predictive value (80.95%) was observed. With ceftazidime 182 isolates were confirmed ESBL producers, 25 were ESBL non producers and 24 were doubtful. Out of these isolates 17 were later found to be non ESBL producers and 7 were ESBL producers. With aztreonam 187 isolates were confirmed ESBL producers, 21 were non ESBL producers and 23 were doubtful. Of these 23, 21 isolates were found to be non ESBL producers but 2 were ESBL producers. Aztreonam showed improved sensitivity (99.4%) and negative predictive value (97.61%) but reduced specificity (66.12%) and positive predictive value (88.88%). For cefpodoxime the current criteria resulted in 100% specificity and positive predictive value but a poorer sensitivity (82.17%) and negative predictive value (2.38%).

DISCUSSION: ESBL producing bacteria have become quite prevalent in Indian hospitals and community. Different studies from India have quoted variable ESBL detection rates. According to multicentric SMART study conducted in Asia Pacific region during the year 2007 ESBL rates in India for *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were 79.0%, 69.4%, and 100%, respectively¹³. Another study found ESBL producing *E. coli* increase from 40 per cent in 2002 to 61 per cent in 2009, with a significant ($P<0.05$) rise in resistance to cefotaxime (75 to 97%). This study also found the resistance directly related to high antibiotic usage.¹⁴ A study from central India reports the incidence of 69% and 41% ESBL producing isolates of *E. coli* and *Klebsiella pneumoniae* respectively.¹⁵ Another study from Vellore in South India found 50.29 % of inpatients and 45.86 % of outpatients harboring ESBL producers.¹⁶ A study from Northwestern India found ESBL production as 23.83% in *E. coli* strains and 8.69% in *Klebsiella* strains¹⁷. We observed that 81.8% of clinical isolates were ESBL producers. From outpatient clinics the reporting of ESBL producers was 75.38% whereas from inpatient clinics it was 84.34%.

Though ESBLs are prevalent in our hospitals and community they are not specifically reported by Microbiology labs. For certain reasons there is also a wide variability in prevalence reporting of ESBLs. The possible factors could be different geographical locations, variable proficiency levels of microbiology trained technical staff, different antibiotic cut offs being used, different guidelines being followed (as they differ between different infectious diseases unions throughout the world) and different techniques being used for ESBL detection.

In developing countries like India it is desirable to incorporate both ESBL detection and antimicrobial susceptibility testing. Different drugs have been used to detect ESBLs but no single antibiotic has been perfect at ruling -in or -out an ESBL producer. CLSI has recently changed the breakpoints of for aztreonam, cefotaxime and ceftazidime for routine testing.

Criteria for cefotaxime to be used for ESBL detection have remained unchanged. With high sensitivity, specificity and positive predictive value it remains the best drug for ESBL detection. Ceftazidime has remained almost equally sensitive and specific as old criteria with improvement in negative predictive value from 59.52% to 80.95% with almost sustained sensitivity, specificity and positive predictive value. Aztreonam had shown improved sensitivity

from 89.9% to 99.4% but reduction in specificity from 91.3% to 66.12%. Its positive predictive value drops from 98.94% to 88.88% with significant increase in negative predictive value to 97.61%. Therefore, to improve the interpretation aztreonam remains additional preferred agent besides cefotaxime. Cefpodoxime has shown reduced sensitivity from 96.89% to 82.17% with sustained improvement in specificity and positive predictive value to 100%. The most striking observation was a very poor negative predictive value. This will result in need to perform additional confirmatory tests if used as single agent.

Overall we found that cefotaxime was the single most appropriate antibiotic for predicting ESBL production. Cefpodoxime can be considered a good agent due to high specificity and positive predictive value. However, it has a very poor negative predictive value. Therefore, no single agent can be recommended for predicting ESBL production.

CLSI has so far not given any specific criteria with the use of above mentioned four antibiotics for detecting ESBLs while performing routine susceptibility testing. At the same time it recommends that with the current criteria antibiotics should be reported as observed on susceptibility testing. Earlier it recommended the reporting of all drugs as resistant when it was observed as resistant to any one drug of the four. These guidelines are based on PK/PD analysis.

More clinical studies are needed to support the current recommendation which is based on PK/PD measurements of the above mentioned drugs. Therapeutic failure or success of antibiotic therapy when one drug is sensitive and other resistant in vitro needs to be studied in parallel with clinical outcome of cases.

To conclude, cefotaxime was found to be the most appropriate drug when used as single agent with both old as well as current criteria. Adding Aztreonam to the interpretation improves the detection of ESBL. The current criteria definitely reduce the need for ESBL screening test due to increased sensitivity and negative predictive value with at least three out of four antibiotics. However, the need for using more than one drug for predicting ESBL activity continues to be there as before.

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