e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(1); 1037-1043
Original Research Article

Phenotypic Detection of Extended- Spectrum Beta- Lactamases in Enterobacteriaceae from Clinical Samples at a Tertiary Care Hospital.

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Received: 25-11-2022 / Revised: 25-12-2022 / Accepted: 30-01-2023
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Conflict of interest: Nil

Introduction: Due to rise in ESBL strains, there is a need to detect this phenotypically. Hence, present study was undertaken to detect ESBL producers by Double disc synergy test (DSST), Combination disk method (CDM), Epsilometer test (*E- test*).

Methods: It was a prospective study conducted in the department of Microbiology, MediCiti Institute of Medical Sciences. Study protocol was approved by the Institutional Ethics Committee. Enterobacteriaceae isolates obtained according to the standard guidelines were included in this research. Isolates which were resistant to third generation Cephalosporins and Aztreonam were considered for ESBL detection. To consider resistance, zone diameter ≤22mm was considered for Ceftazidime, ≤27mm for Cefotaxime, ≤25mm for Ceftriaxone and ≤27mm for Aztreonam. Double disk diffusion synergy test (DDST), combination disk method (CDM) and E-test were used for ESBL. Commercially available ESBL E-test strip was used. Chi square test was used to find the statistical analysis; $P<0.005$ was to be statistically significant.

Results: Total 100 enterobacteriaceae strains were included, *Escherichia coli* was the leading (38%). Total 44% isolates were resistant to 3rd generation cephalosporins. Klebsiella species was the leading (22; 70.96%) ESBL producer. All ESBL isolates were susceptible to Piperacillin/ tazobactam and Imipenem. Among 44 isolates, all were detected as positive by E- test, 38 were positive by DDST and 43 by CDM. Six isolates which were negative by DDST, were positive by E-test and CDM. One isolate which was negative by CDM was positive by E-test and DDST. DDST was 86.3% sensitive and CDM was 97.7%.

Conclusion: Considering the challenging nature of the isolates and resource-limited settings, the phenotypic methods showed high sensitivity and specificity for ESBL detection. However, large sample size is recommended.

Introduction

Resistant bacteria are rising globally as a threat to the favorable outcome of common infections in community and healthcare settings. [1] β-lactamase production particularly gram-negative bacteria is the most important single mechanism of resistance to Penicillins and Cephalosporins; third-generation cephalosporins is the alternative. [2]

Cephalosporins were developed in response to the increased prevalence of β-lactamases in organisms like *Escherichia coli, Klebsiella pneumoniae;* this is spread to new hosts like *Hemophilus influenzae* and *Neisseria gonorrhoeae*. [3] There is an increased incidence and prevalence of Extended-spectrum β-lactamases (ESBLs), enzymes that hydrolyze and cause resistance to oxyimino-cephalosporins and aztreonam. [4] They are currently being identified worldwide in large numbers in Enterobacterales and also in *Pseudomonas aeruginosa*. Today ≥ 200 different ESBLs are described. [4]

ESBLs have been first discovered in 1983 in Klebsiella ozaenae isolates in Germany. The prevalence of extended spectrum beta lactamase generating strains amongst clinical specimens has been gradually growing during the last few years ensuing in boundaries of healing options. [5]

Major risk elements for colonization or contamination with ESBL producing organisms are prolonged utilization of antibiotics, extended period of health facility stay, extreme illness and resistance in healthcare institutions with high percentages of third generation cephalosporin utilization, instrumentation or catheterization. [1, 6]

In developing countries, the elevated prevalence of Enterobacteriaceae producing ESBLs creates an exceptional want for laboratory testing techniques phenotypically so as to accurately identify the presence of these enzymes in clinical isolates thereby supporting the appropriate use of antibiotics and guiding the empirical therapy of excessive risk units. [5] Hence, the present study was undertaken to detect ESBL producers by Double disc synergy test, Combination disk method, Epsilometer test (*E- test*) and evaluate their efficiency for suitable and well-timed management.

Methods

It was a prospective study conducted in the department of Microbiology, MediCiti Institute of Medical Sciences. Study was conducted from January to June 2022. Study protocol was approved by the Institutional Ethics Committee. Various clinical samples such as urine, pus, sputum and so on were considered. Enterobacteriaceae isolates obtained according to the standard guidelines were included in this research. [7]

Kirby and *Bauer* disc diffusion technique was used to find antimicrobial susceptibility as per the CLSI guidelines. [8] Isolates which were resistant to third generation Cephalosporins and Aztreonam were considered for ESBL detection. To consider resistance, zone diameter ≤22mm was considered for Ceftazidime, ≤27mm for Cefotaxime, ≤25mm for Ceftriaxone and ≤27mm for Aztreonam. Double disk diffusion synergy test (DDST), combination disk method (CDM) and E-test were used for ESBL. [9, 10] *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as ESBL positive and negative controls, respectively. ESBL E-test was taken as a gold standard test for ESBL detection. [10]

Double disk synergy test (DDST): A lawn culture of test isolate at a concentration of 0.5 McFarland turbidity was streaked on a Mueller-Hinton agar plate. Antibiotic disks of and incubated at 35°C for 18-24 hours. Discs at a concentration of 20 µg/10 µg

amoxicillin/clavulanate and 30 µg for the rest of antibiotics. A clear development of the antibiotic zone of inhibition towards the disk containing clavulanate was indicative of a potential ESBL producing organism.

Combination disk method (CDM):

To the lawn cultured test organism plate, 30 µg of plain 3rd generation cephalosporin disc and combination of Clavulanic acid were placed in opposite. After incubation, ≥5mm zone size around the cephalosporin was considered to be ESBL. Any one the cephalosrins can be used but cefotaxime or ceftazidime discs were used in this research.

E-Test: Commercially (Himedia laboratories, Mumbai) available ESBL E-test strip was used. These were place at both the ends of the test organism lawn cultured plate and the results were read after incubation. As per the manufacturer guidelines, Ceftazidime, Ceftazidime + Clavulanic acid ratio > 8 was considered for ESBL production.

Statistical analysis: Data were analysed using SPPS version 22. Chi square test was used to find the statistical analysis; P<0.005 was to be statistically significant.

Results

Of the 1250 clinical samples, 100 enterobacteriaceae strains were isolated; maximum were isolated from urine (52; 15%) (Figure 1), Escherichia coli was the leading (38%).

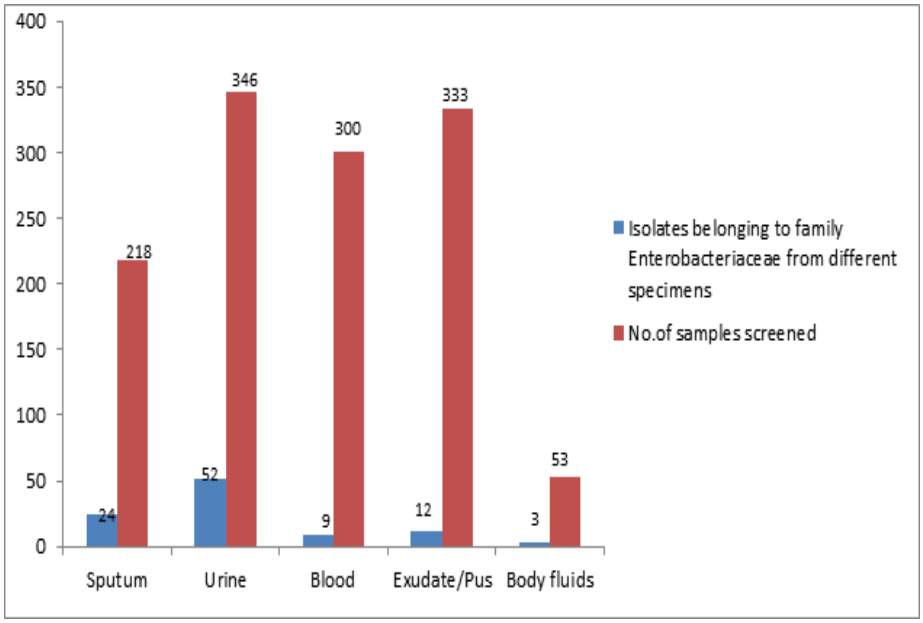

Figure 1: Number of Enterobacteriaceae isolates as per the clinical specimen

Table 1: ESBL producers among the study isolates; n (%

Isolate	ESBL producer	ESBL non producer	Total
Klebsiella species	22 (44)	9 (9)	31 (31)
Escherichia coli	16 (16)	22 (22)	38 (38)
Enterobacter species	2 (2)	4 (4)	6 (6)
Proteus species	2 (2)	8 (8)	10 (10)
Citrobacter species	2 (2)	13 (13)	15 (15)
Total	44 (44)	56 (56)	100 (100)

Total 44% isolates were resistant to 3rd generation cephalosporins. Klebsiella species was the leading (22; 70.96%) ESBL producer followed by E. coli (16; 42.1%). (Table 1). All the ESBL isolates were susceptible to Piperacillin/ tazobactam and Imipenem (Table 2).

Table 2: Antibiotic resistance pattern of ESBL producers; n (%)

Antibiotic	Susceptible	Resistant	Total
Ceftazidime	0	44 (100)	44 (100)
Cefotaxime	0	44 (100)	44 (100)
Ceftriaxone	0	44 (100)	44 (100)
Aztreonam	0	44 (100)	44 (100)
Cefoxitin	44 (100)	00	44 (100)
Amoxicillin-Clavulanic acid	41 (93)	03 (7)	44 (100)
Piperacillin/tazobactam	44 (100)	00	44 (100)
Imipenem	44 (100)	00	44 (100)
Amikacin	29 (66)	15 (34)	44 (100)
Cotrimoxazole	09 (20)	35 (80)	44 (100)
Tetracycline	02 (5)	42 (95)	44 (100)

Among 44 isolates, all were detected as positive by E-test, with a ceftazidime/ceftazidime-clavulanate (CAZ/CAZ+) ratio between 8 and 256. Thirty eight were positive by DDST and 43 by CDM. Six isolates which were negative by DDST, were positive by E-test and CDM. One isolate which was negative by CDM was positive by E-test and DDST. DDST was 86.3% sensitive and CDM was 97.7% (Table 3).

Table 3: Comparison of techniques used to detect ESBL

ESBL detection	Positive	Negative	Total
DDST	38	6	44
CDM	43	1	44
Statistical analysis	Chi square: 3.88; P = 0.004		
	Statistically significant		

Discussion

Globally, the incidence of ESBL amongst clinical isolates vary greatly. ESBL is rapidly increasing over the years due to the fact they are frequently undetected through routine susceptibility testing techniques which poses a prime issue for clinical practice. [11] This creates a great need for laboratory testing methods that can accurately identify the presence of these enzymes in clinical isolates. [12] Keeping this view in mind and lack of data in our region, in the present study, an attempt was made to detect the ESBL producing Enterobacteriaceae phenotypically and screen their strength.

In the present study, *Escherichia coli* (38%) was the commonest ESBL producers followed by Klebsiella sps (31%). Our findings are similar to the studies conducted by Shaikh S et al. 13 and Ampaire L et al. [14], reported 67.04% and 55.4% ESBL producers, respectively. ESBL resistance was seen against cephalosporins group (100%), tetracycline (95.4%) and cotrimoxazole (79.5%). It was of significant concern as these are the drugs of choice for most of the gram negative infections. On the other hand, susceptibility was seen for Imipenem (100%) and piperacillin/tazobactam (100%). Similar observations were published in the literature. [15] Furthermore, Paterson DL., [15] stated that ESBL producers and non ESBL producers of Enterobacteriaceae remain sensitive to carbapenems and were taken into consideration for suitable empiric therapy for Enterobacteriaceae infections.

ESBL-producing Enterobacteriaceae were detected in 44% of isolates in our study

which is in agreement with the study by Dirar M et al. [16] Whereas it was reported to be 48% by Tankhiwale SS et al. [17] The proportion of ESBL in the current research as well as reported studies were lower compared to the reports from Uganda (89%) [18] and Brazil (61.1%). [19] This high occurrence could be attributed to over usage of antibiotics, lack of antimicrobial surveillance programs in health care settings or maintenance of poor hygiene.

DDST was 86.3% sensitive and 100% specific. Antibiotic susceptibility testing sensitivity strongly depends on the correct placement of the disc. [20] Utmost care was taken and the bench work was carried by the qualified Microbiologists, authors of this. And the technical team were not allowed to do this. Our findings correlate with Singh RM et al. 9 study and in contrast to Shikha Paul et al. report. [21] In this study, CMD was 97.7% sensitive and 100% specific to detect ESBL. These findings were consistent with the available reports. 9 According to this research as well as per literature, CMD is a better technique to detect ESBL. [22]

The commercially available ESBL E-test strip is a quantitative technique and is widely used as a gold standard in many clinical laboratories [23] suggested that E-test and Vitek ESBL test are more sensitive than disc approximation test for Bush group 2be enzyme detection. 100% sensitivity of E-test in our study was in complete agreement with the previous findings. [10] Hence, it can be used for confirmation by screening the positive isolates or as a routine test as it is a sensitive and easy to use test where automated tests or molecular detection are not feasible. [24]

Conclusion:

Considering the challenging nature of the isolates and resource-limited settings, the phenotypic methods showed high sensitivity and specificity for ESBL detection, with the E-test being the most sensitive. These findings contribute the data required to choose a reliable, cost efficient and simple phenotypic test which do not need a highly skilled personnel, in order to ensure a prompt response in the management and control of these pathogens.

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