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PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING ESCHERICHIA COLI ISOLATES AT A TERTIARY CARE INSTITUTE IN NORTH WEST REGION OF RAJASTHAN

Medical Microbiology

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ABSTRACT

Introduction: Extended-spectrum β-lactamases (ESBLs) have become an issue in community worldwide due to an increase in antibiotic resistance over the past decade. This study was aimed to investigate the phenotypic characteristics of ESBL-producing Escherichia coli in North West region of Rajasthan.

Material and methods: The current study as ESBL-producing E. coli isolates were collected from tertiary care hospitals located in various regions in North West region of Rajasthan, from Aug 2018 to Jan 2021. All participants completed a medical history form and provided informed consent. Total 213 E.coli isolates were taken irrespective of age, sex, occupation, religion and ethnicity for ESBL-producing in from various clinical samples i.e urine, pus/wound swab, blood, CSF and respiratory secretion received in microbiology laboratory. The study design was approved by the institutional ethics committee.

Results: It is observed that the total of 213 isolates of E. coli from 1050 patients were included in this study. Total 213 isolates were tested by Combined disk method with ceftazidime & Ceftazidime- Clavulanic disc. Out of which 99 (46.48%) were ESBL Producing.(Table-1) Out of 213 isolates of E.coli 197 were ceftazidime resistant, which were further tested for ESBL detection by Ceftazidime(CAZ) and Ceftazidime-Clavulanic Acid (CAC) Combined disk test method. Out of 213 E.coli isolates total 99 (46.48%) were positive for ESBL by CDT, 105 (49.3%) were ESBL positive by E strip method revealed.

Conclusion: This study provided a recent evidence of the phenotipic diversity of ESBL-producing E. coli in North West region of Rajasthan. In addition, the profile related to antimicrobial resistance pattern in this region was also demonstrated.

KEYWORDS

Antimicrobial resistance, ESBLs, *Escherichia coli*

INTRODUCTION

The rapid emergence of antibiotic resistance threatens effective prevention and treatment of an increasing range of infections. Some bacteria are naturally resistant to certain antibiotics and others can acquire resistance through mutations in some of their genes when they are exposed to an antibiotic. This acquired resistance can spread to other bacterial species. The main mechanism of antibiotic resistance mostly found is enzyme production such as β-lactamase enzymes. The β-lactamase enzymes produced by some bacteria provide resistance to β-lactam antibiotics by hydrolyzing β-lactam rings.[1]

Among antimicrobial resistant bacteria, *Escherichia coli* is one of highly concerned bacteria in the family Enterobacteriaceae. *E. coli* is a common cause of urinary tract infection and intra-abdominal infection in humans and is the second most common Gram-negative bacteria causing communityacquired bloodstream infection, accounting for 7.3% of all bloodstream infection isolates. ESBL-producing *E. Coli* isolates have become an importance in community-onset infections, as well as nosocomial infections. The prevalence of resistance to fluoroquinolones and extended spectrum cephalosporins in *E. coli* had highly increased over the past decade rendering severely limited therapeutic options for these infections [2-3].

Extended-spectrum β-lactamases (ESBLs) are extremely broad spectrum β-lactamase enzymes, which can be produced by Gram-negative bacteria. β-lactamase enzymes production is the most common mechanism of developing drug resistance to β-lactam antibiotics among Gram-negative bacteria.[4] Extended spectrum β-lactamase (ESBLs) and Amp-C β–lactamase mediated resistance are of increasing clinical concern.[5] ESBLs are group of enzymes which confers resistance to extended spectrum cephalosporins, aztreonam, and oxyimino β-lactams and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam.[6] These enzymes are commonly found in Enterobacteriaceae and other Gram-negative organisms such as Pseudomonas aeruginosa [7] and are encoded by mutated TEM1, TEM2 and SHV genes on plasmids.[8] Amp-C class of β-lactamase are cephalosporinase, belong to the molecular class 'C' of Ambler's classification[9] and are both plasmid and chromosomal mediated and are not inhibited by β-lactamase inhibitors. They hydrolyze the cephamycins and carbapenems are the only antibiotic effective against them.[10] The spread of these ESBLs β-lactamase producing strains limits the use of β-lactam class of antibiotic, causing serious therapeutic failures, compelling use of more broad spectrum and expensive drugs.[11] Therefore, this study was aimed to investigate the phenotypic characteristic related ESBL-producing E. Coli in North West Rajasthan.

MATERIAL AND METHODS

ESBL-producing E. coli isolates were collected from tertiary care hospitals located in various regions in North West region of Rajasthan, from Aug 2018 to Jan 2021. All participants completed a medical history form and provided informed consent. Total 213 E.coli isolates were taken irrespective of age, sex, occupation, religion and ethnicity for ESBL-producing in from various clinical samples i.e urine, pus/wound swab, blood, CSF and respiratory secretion received in microbiology laboratory. The study design was approved by the institutional ethics committee.

INCLUSION CRITERIA:

All consecutive, non-duplicate isolates of Escherichia coli collected from various specimens of patients attending various outpatient departments as well as admitted in wards at P.B.M hospital from Aug, 2018 to Jan, 2021. All kind of specimens like urine, blood, pus, wound and respiratory tract specimen, body fluid, high vaginal swab, swab from non healing ulcers and CSF were included in the study.

EXCLUSION CRITERIA:

Isolation of three organism types with no predominating organism and repeated isolate from same patients were excluded from this study.

STUDY PROTOCOL

Sample Processing:

All samples except blood were cultured on Blood agar and MacConkey's agar and Thioglycolate broth. Blood was culture on Brain heart infusion broth. Urine was cultured on Blood & MacConkey's agar. A culture plates were incubated overnight at 37ºC. Identification of organisms was done on the basis of, Gram's staining, motility, colony characteristics and biochemical confirmation tests.

Antibiotic resistance detection method in Escherichia coli:
ESBL Detection Test:- Preliminary test

Mueller Hinton Agar plates were inoculated with test isolates and antibiotic discs were placed at appropriate distance (at least 30mm apart). On incubating plates at 37ºC for 24 hour, zone of inhibition was measured. The standardized parameter for each antibiotic given by Clinical and Laboratory Standards Institute (CLSI) were used to categorize the given bacterial strain as ESBL positive or negative. The isolates with zone of inhibition ≤22mm for ceftazidime (30ug), were expected to be ESBL producers. E.coli ATCC 25922 was used for Quality control.

Combined disk Test (CDT)

Phenotypic method- double disc confirmatory test was performed on the Muller Hinton Agar (MHA) plates using sterile swabs. Ceftazidime (CAZ) disc of 30µg and Ceftazidime + Clavulanic acid (CAC) disc of 30+10µg concentration were placed at a distance of 30 mm centre to centre. The plates were incubated overnight at 37°C and readings were recorded. If difference in zone of inhibition of the antibiotic + inhibitor and antibiotic alone were ≥5mm, then the isolates were considered to be ESBL producers. E.coli ATCC 25922 were used for Negative control. Klebsiella pneumoniae ATCC 700603 were used for Postive control.[110]

ESBL Detection by Ceftazidime(0.5-32)/ Ceftazidime Clavulanic Acid (0.064-4) E Strip

Test Procedure

1. Plates were prepared with suitable make of Mueller Hinton Agar.
2. A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum and soaked swab was rotated firmly against the upper inside wall of the tube to express excess fluid. entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking.
3. Applicator was hold in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
4. Strip was placed at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator was detached from the strip.
5. Plates were transfered in the incubator under appropriate conditions. E.coli ATCC 25922 were used for Negative control. Klebsiella pneumoniae ATCC 700603 were used for Postive control.

STATISTICALANALYSIS

Detailed data of all subjects of case and control group were collected. After complete evaluation all findings were expressed in terms of Mean ± SD. Comparison between case group and control group was done using paired "t" test. All statistical analysis was done using SPSS software version 20.

RESULTS

A total of 213 isolates of E. coli from 1050 patients were included in this study and this was screened for ESBL production by phenotypic method. Bacterial identification of these clinical samples was done by culture on Blood agar and Mac Conkey's Agar. Biochemical Identification was done using Indole test, Methyl red test, Citrate Utilization test, Urease Hydrolysis test, and Triple sugar iron test (TSI). Total 213 isolates were tested by Combined disk method with ceftazidime & Ceftazidime- Clavulanic disc. Out of which 99 (46.48%) were ESBL Producing.(Table-1) Out of 213 isolates of *E.coli* 197 were ceftazidime resistant, which were further tested for ESBL detection by Ceftazidime(CAZ) and Ceftazidime-Clavulanic Acid (CAC) Combined disk test method. Out of 213 E.coli isolates total 99 (46.48%) were positive for ESBL by CDT, 105 (49.3%) were ESBL positive by E strip method.(Table-2) Out of 105 samples positive for ESBL production by E Strip, CDT detects only 99 isolates of E.coli. In case of ESBL non producer by E strip none of method showed false positive result. (Table-3). Results of table no 4 Showing E strip test is found to be most sensitive and specific test for ESBL detection. Although in our study, Sensitivity of all three methods -Disc diffusion and E Strip were compared in which E Strip were found most sensitive method for the detection of ESBL.(Table-4) The current study revealed that antibiogram of *E.coli* isolated from various patient. Tigecycline 99.53% found most sensitive drug of choice followed by Cefoparazone sulbactum 96.24%, Colistin 94.83%, polymyxin B 92.01%. In Urine isolates fosfomycin was 100% sensitive. (Table-5)

Table-1 Detection of ESBL producing *E.coli* by Combined disk test (CAZ-CAC)

Combined disk test	No. of E.coli Isolated (N=213) (Frequency)
ESBL Producer	99 (46.48%)
ESBL Non Producer	114 (53.52%)

Table-2 Comparison of Combined disk Test (CDT) & E Strip for ESBL detection in *E.coli isolates*

ESBL Detection Method	No. of E.coli Isolated (N=213) (Frequency)
ESBL Detection by CDT	99 (46.48%)
ESBL Detection by E Strip	105 (49.3%)

Table-3 Comparison of ESBL methods with E strip confirmative test for ESBL detection E.coli isolates

Test Method for ESBL	ESBL E- Strip Positive n=105		ESBL E- Strip Negative n=108	
	Positive(A)	Negative (C)	Positive (B)	Negative (D)
ESBL Double Disc Diffusion Test	99	6	0	108

Table-4 Performance parameters of all ESBL Detection methods for drug resistance in *E.coli* cases isolated.

Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
DDDT ESBL	94.29%	100%	100%	94.74%	97.18%
E – Strip Test	100%	100%	100%	100%	100%

Table-5 Antibiotic sensitivity pattern of *E.coli isolates*

Antibiotic	E.coli Isolates (n=213)	Percentage
Amoxyclave	58	40.0 %
Amikacin	145	68.07 %
Aztreonam	100	46.94 %
Cefepime	104	48.82 %
Cefoxitin	148	69.25%
Ceftazidime	16	7.51%
Cefotaxime	90	42.25 %
Cefoparazone-sulbactum	205	96.24 %
Ciprofloxacin	58	27.23 %
Colistin	202	94.83 %
Doxycycline	98	46.00 %
Imipenem	148	69.48 %
Levofloxacin	71	33.33 %
Meropenem	139	65.25%
Piperacillin- Tazobactum	134	62.91%
Tigecycline	212	99.53%
Fosfomycin (Urine only n-114)	114	100%
Nitrofuraintoin (Urine only n-114)	62	54.38%
Polymyxin B	196	92.01%

DISCUSSION

Emergence of ESBLs producing Gram-negative organisms presents significant diagnostic and therapeutic challenge in the management of infection.[12] The risk factor for the colonization or infection with these organisms is due to prolonged hospital stay, intensive care unit admission, urinary and arterial catheterization and exposure to antibiotics including extended spectrum cephalosporins.[13] The emergence of antibiotic resistance is a matter of great concern, particularly in hospitals. Antibiotic resistant bacteria appear to be biologically fit and capable of causing serious life threatening infections. The increase in antibiotic resistance among gram-negative bacteria, such as Enterobacteriaceae group is a notable example of how bacteria can procure, maintain and express new genetic information that can confer resistance to one or several antibiotics. Resistance in gram-negative bacteria is a serious problem and calls for an effective infection control measures to curb their dissemination.[14]

In the current study, total 213 isolates were tested for ESBL production. Out of which 99 (46.48%) were ESBL Producer. A similar study done by Navin chandra et al. on 303 E.coli clinical isolates at Department of Microbiology, NKP Salve Institute of Medical Science & RC, Nagpur, Maharashtra. 78.22% of them were ESBL producer. In the study of A Malini et al, at puducherry, India observed in their study that 77.02%, were ESBL producers of E.coli isolates. In the study of Mohammed Nasir Khan et al. at Jaipur showed 44.5% ESBL producing E.coli while in the another study conducted by Parul Sinha et al in SMS medical College Jaipur 2016, 34% were ESBL producer

were observed in E.coli isolates. In Nepal a study was conducted by Krishus Nepal et al. 34.5 % were ESBL was 18.9% among the E.coli isolates were reported. In 2018, Uma chaudhary et al. observed 42.5% ESBL among E.coli isolates.which was in agreement with present study. While in the study of Rituparna Tewari et al. in Karnataka, they had observed ESBL rate-79%, among E.coli isolates which was comparatively high from the present study. In 2021, a study was published by Ruchi Jain et al., they have reported ESBL 45.51%, which is in an agreement with the present study.[15-21]

Out of 105 samples positive for ESBL production by E Strip, 99 isolates were detected by combined disk test isolates of E.coli. In case of ESBL non producer by E strip none of method showed false positive result. E strip test is found to be the most sensitive and specific test for ESBL detection. Although in our study, specificity of all three methods -Disc diffusion and E Strip 0% while E Strip was found to be the most sensitive method for the detection of ESB Limitation of our study was that we have taken in the study of Deepti Rawat et al. found E strip was 87% sensitive and 100% specificity. E strip sensitivity was 83.6% and specificity was 100%, which was lower sensitivity than present study.[22-25]

The study further suggests that there is a need to emphasize on the rational and judicious use of antimicrobials, and avoid the misuse and overuse of available antimicrobial agents for the treatment of ESBLs producing E.coli. Use of antimicrobial agents as per the reports of sensitivity studies, restricted use of antimicrobial agents, and avoidance of unwarranted and unnecessary use of antimicrobial agents may lead to withdrawal of selective pressure on bacteria prone to develop resistance and thereby reduce the survival of resistant strains.

CONCLUSIONS

The prevalence of ESBL production varies periodically in different regions, which limits the clinical use of β-lactams. Early detection of ESBL is of paramount importance for surveillance and control of antibiotic resistance and must be routinely evaluated in all hospital settings. Our study identifies the multiple mechanisms involved in drug resistance among Gram-negative bacteria and supports use of phenotypic method to detect types of ESBL production. The implementation of simple and accurate laboratory method to detect ESBL producing E.coli production is useful; our study high lights the ESBL among E.coli isolated from OPD & IPD units and especially from intensive care unit, indicates that widespread use of those antibiotics that leads to selection of resistant organisms. ESBL pose serious problem choosing in the right antibiotic for treatment, various strategies such as applying strict infection control measures, judicious prescribing of antibiotics, implementation of antibiotics resistance surveillance programs and antibiotic cycling must be done. Regular monitoring and documentation of ESBL should be done by all microbiology laboratories.

DISCLOSURE STATEMENT

This study is investigator initiated. The authors declare that they have no competing/conflict of interests in relation to this article.

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CONSENT FOR PUBLICATION

- Not applicable.
- Ethics approval and consent to participate.
- The study protocol was approved by the medical ethics committee of the Rajasthan University of Health Sciences, Jaipur, Rajasthan (State), India.

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