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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM B-LACTAMASE PRODUCING ESCHERICHIA COLI.

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ABSTRACT

Extended Spectrum β-Lactamase producing *Escherichia coli* have been demonstrated to increase resistance to antimicrobial agents. ESBL enzymes are capable of hydrolyzing oxyimino β-lactam compounds and contribute to high resistance to β-lactam antibiotics. The aim of this present study is to isolate and to identify the ESBL producing *E. coli* strains from UTI and Wound Infection. 67.3%, 17.3% of Urine and Pus samples were positive for *E.coli* strains. Out of 72 *E.coli* isolates 66.6%, 68%, 62.2% were resistant for Cefotaxime, Ceftriaxone, Ceftazidime respectively. The positive isolates from Primary screening test were further confirmed with phenotypic tests by performing Double Disc Diffusion and E-Test. 63.8% of clinical isolates were positive for Double Disc Diffusion and E-test. Current investigation reveals that E. coli isolates are multi-drug resistance and produce ESBL in large proportions.

KEYWORDS: Extended Spectrum β-Lactamse, Escherichia coli, Double Disc Diffusion Test, ESBL E-Test, Urinary tract Infection.

1. INTRODUCTION

Antimicrobial resistance has been identified as an emerging worldwide problem both in developed and underdeveloped countries.[1] In Gram negative pathogens, multidrug resistance are frequently detected in family Enterobacteriaceae against all major B-lactams, fluoroquinolonnes and amino glycosides.[2,3] In Enterobacteriaceae antimicrobial resistance in

E. coli is of great concern because it is the most common pathogen causing infections in humans.[4] Additionally, resistant *E. coli* strains have the potential to transfer antibiotic resistant genes to other strains within the gastrointestinal tract by means of gene transfer mechanisms. The Extended spectrum β-lactamase enzymes are plasmid mediated and capable of hydrolyzing first, second and third generation cephalosporin's, Aztreonam and are inhibited by clavulanic acid and sulbactum.[5]

Clinical Laboratory Standards Institute (CLSI) recommends screening for ESBL production among *E. coli* and *K.* pneumoniae.[6] Screening ESBLs accelerate an interpretation of its development, deciding pragmatic antibiotic therapy, establish measures to reduce increasing resistance among pathogens.[7] The aim of the present study is to isolate and to identify the ESBL producing *E.coli* strains from clinical samples by performing Double Disc Diffusion and E-test.

2. MATERIALS AND METHODS

2.1. Sample collection and handling

The present study includes 141 clinical samples (46 pus and 95 urine samples), collected from hospitals in and around Chennai. All the clinical samples were collected in sterile container and vortexed before processing.

2.2. Isolation and identification

The clinical samples were processed as per the guidelines mentioned by Isenberg (1998) and WHO manuals (1980).[8,9] Pure isolates were further submitted to BD Phoenix™ Automated Microbiology System for *in vitro* identification of clinical isolates.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility tests for the *E. coli* isolates were carried out by Kirby- Bauer Disc diffusion technique on Mueller Hinton agar (Hi-Media), by following the Standard guidelines.[10] The diameters of the zones of inhibition were measured by Antibiotic Zonescale (Hi-Media). The antimicrobials included in this study were Amoxyclav (20/10µg), Piperacillin-tazobactum (100/10µg), Cefoxitin (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Imipenem (10µ), Gentamycin (10µg), Amikacin (30µ), Naldixic acid (30µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75µg).

2.4. Detection of ESBLs

2.4.1. ESBLs screening test

The isolates were analysed for their susceptibility to the third generation cephalosporins namely Ceftriaxone (CTR), Cefotaxime (CTX) and Ceftazidime (CAZ) by performing standard disc diffusion method as recommended by CLSI guidelines. If a zone diameter of >27mm for Ceftriaxone,>25 mm for Cefotaxime and > 22 mm for Ceftazidime was considered positive for ESBL screen test and further employed for Double Disc Diffusion and E-Test.

2.4.2. Double-disc diffusion test (Phenotypic confirmatory test)

ESBL screen positive isolates were subjected to Double Disc Diffusion Test (DDDT), as recommended by the CLSI.[11] In this study four disc namely Cefotaxime (CTX-30µg), Ceftazidime (CAZ-30µg), Cefotaxime +Clavulanic acid (CEC-30/10µg) and Ceftazidime + Clavulanic acid (CAC-30/10µg) were used. An increase of ≥5mm in the zone of inhibition of the combination disc in comparison to that of the antibiotic alone was considered to be positive for ESBL production.[12]

2.4.3. ESBL E-test

The ESBL E-Test strips included in this study were purchased from Hi-Media (Ezy MIC^{TM} strip, HiMedia). The Detection of ESBL by E-Test strips is more sensitive in comparison to Double Disc Diffusion Test.[13,14] The gradient of Cefotaxime (CTX) at one end and Cefotaxime plus Clavulanic acid (CTX +) at the other end are tested in parallel. A decrease in MIC of 3 doubling dilutions in the presence of Clavulanic acid is interpreted positive for ESBL production.

3. RESULTS AND DISCUSSION

A total of 141 (95 urine, 46 pus) clinical samples were collected in this study period of one year, of which 64(67.3%) urine and 8(17.3%) pus samples were positive for *E.coli* strains. Sex wise distribution of UTI and wound infection is displayed in **Table 1** and **Table 2**.

Table 1: Sex wise distribution of urinary tract infection.

S. No.	Sex	No. of samples tested	Percentage
1.	Male	28	29.4%
2.	Female	67	70.5%

Table 2: sex wise distribution of wound infection.

S.No.	Sex	No. of samples tested	Percentage
1.	Male	24	52.1%
2.	Female	22	47.8%

The antimicrobial potency of 12 selected antimicrobial agents against **E. coli** isolates are summarized in **Table-3**. In antibiotic susceptibility test 16 (22.2%) isolates were resistant to Amoxyclav, 16 (22.2%) were resitstant to Piperacillin-tazobactum, 32 (44.4 %) were resistant to cefoxitin, 48 (66.6%), 49 (68%) and 47 (65.2%) were resistant to Cefotaxime, Ceftriaxone and Ceftazidime respectively. 1(1.3%) were resistant to Imipenem, 39 (54.1%), 9 (12.5%) and 56 (77.7%), were resistant to Gentamycin, Amikacin and Nalidixic acid respectively.

Table 3: Antibiogram pattern of E.coli (n=72).

S.No.	Name of the antibiotic	Susceptible Isolates (%)	Intermediate Isolates (%)	Resistant Isolates (%)
1.	Amoxyclav	56(77.7%)	0	16(22.2%)
2.	Piperacillin-tazobactum	56(77.7%)	0	16(22.2%)
3.	Cefoxitin	40(55.5%)	0	32(44.4%)
4.	Cefotaxime	24(33.3%)	0	48(66.6%)
5.	Ceftriaxone	23(31.9%)	0	49(68%)
6.	Ceftazidime	25(34.7%)	0	47(65.2%)
7.	Imipenem	71(98.6%)	0	1(1.3%)
8.	Gentamycin	31(43%)	2.7%	39(54.1%)
9.	Amikacin	61(84.7%)	2.7%	9(12.5%)
10.	Nalidixic acid	16(22.2%)	0	56(77.7%)
11.	Ciprofloxacin	23(31.9%)	1.3%	48(66.6%)
12.	Co-Trimoxazole	15(20.8%)	0	57(79.1%)

Out of 72 isolates tested 49(68%) were resistant to third generation cephalosporins namely Ceftriaxone (CTR), Cefotaxime (CTX) and Ceftazidime (CAZ) by ESBL screening test (**Table-4**) and all these isolates except one are sensitive to Imipenem. Thus 68% of *E.coli* strains were confirmed to as ESBL producing strains by phenotypic confirmatory double disc diffusion and E-test.

Table 4: ESBL Screen test for E. coli (n=72).

S. No.	Antibiotics	Sensitive	Resistant
1.	Cefotaxime	24 (33.3%)	48 (66.6%)
2.	Cefotriaxone	23 (31.9%)	49 (68%)
3.	Ceftazidime	25 (34.7%)	47 (65.2%)

DDDT method is technically simple, reliable and inexpensive for the detection of ESBLs.[15] In the present study 46(63.8%) were found to be ESBL producers when tested with Cefotaxime/Clavulanic acid (CEC-30/10µg) and Ceftazidime/Clavulanic acid (CAC-30/10µg) in combination with Cefotaxime (CTX-30µg) and Ceftazidime (CAZ-30µg) respectively (**Fig-1**). These results are found to be in agreement with a study conducted by Naik and Desai (2012).[16]

The Detection of ESBL by E-Test strips is more sensitive in comparison to Double Disc Diffusion Test.[13,14] The gradient of Cefotaxime (CTX) at one end and Cefotaxime plus Clavulanic acid (CTX +) at the other end are tested in parallel. In this study out of 49 isolates tested 46(63.8%) were positive for E-test. A decrease in MIC of 3 doubling dilutions in the presence of Clavulanic acid is interpreted positive for ESBL production as shown in **Fig-2**.

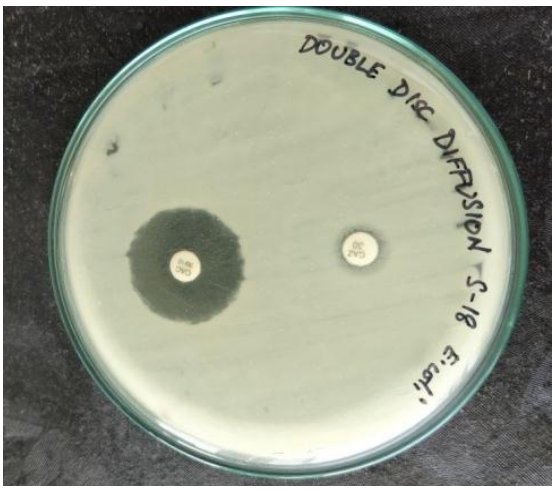

Fig. 1: Double disc diffusion test.

Fig. 2: E-test for *E.coli* isolate.

4. CONCLUSION

In present study, 63.8% of *E. coli* isolates were positive for ESBL production. ESBL production is the chief mechanism for the spread of multidrug resistance among isolates. The prevalence of ESBL producing *E.coli* was high consequently, it is mandatory to monitor ESBL production by standard protocol recommended by CLSI to reduce increasing resistance among pathogens.

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