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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESBL PRODUCING *E.COLI* FROM URINARY TRACT INFECTIONS

Microbiology

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ABSTRACT

Background and Purpose: Antimicrobial resistance in *E.coli* is of particular concern because it is the most common gram negative pathogen causing infections, particularly urinary tract infections (UTIs) in humans. Due to the production of extended spectrum β-lactamases *E.coli* exhibits increasing resistance to β-lactams antibiotic

Material & Method: The study was carried out for a period of 1 year in a tertiary care hospital . 200 *E.coli* isolates from urine samples of clinically diagnosed urinary tract infection were subjected to antibiotic susceptibility test.ESBL screening test positive isolates were subjected to E test and phenotypic confirmatory test for ESBL production.

Result: : Out of 200 isolates of *E.coli* 76 (38%) were ESBL producers. ESBL *E.coli* showed maximum resistance for ampicillin sulbactum (94.7%) followed by co trimoxazole(84.3%). Resistance to imipenem was 6%.No resistance was noted for colistin .

Conclusion : Knowing the antibiotic susceptibility pattern of ESBL *E.coli* will guide the physician in treating UTI caused by ESBL *E.coli*

KEYWORDS

Urinary tract infection,Extended spectrum β lactamase,Antibiotic susceptibility,

INTRODUCTION:

Urinary tract infections (UTI) are one of the most common infections that lead to morbidity in humans with *Escherichia coli* being the most common bacteria [(1)].Resistant bacteria are increasing world wide as a threat to the favourable outcomeof common infections in community and hospital. B- lactamases production by several gram negative organisms is perhaps one of the most important single mechanism of resistance to penicillins and cephalosporins.Extensive use of third generation cephalosporins has contributed to the evolution of extended spectrum β-lactamases (ESBL,s)[(2)]. The incidence of extended spectrum β lactamase (ESBL) producing strains among clinical isolates has been steadily increasing over the past few years, resulting in the limitation of therapeutic options[(3)].

Microorganisms responsible for urinary tract infection (UTI) such as *E.coli* and *Klebsiella* spp. have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat.These enzymes are encoded by transferable conjugative plasmids, which often code resistance to other antibiotics [(4)].

The present study was undertaken to detect ESBL producing *E.coli* causing UTI and to know their antibiotic susceptibility pattern.

MATERIAL AND METHODS

This was a cross sectional study carried out for a period of 1 year in a tertiary care hospital . Study was carried out on 200 *E.coli* isolates from urine samples of clinically diagnosed urinary tract infection received in the Microbiology laboratory.

Processing of the samples:

Mid stream urine samples were processed for culture & sensitivity. Semi quantitative culture was done using a calibrated loop (0.001 ml) of well mixed uncentrifuged urine .It was inoculated onto the Blood agar & MacConkeys agar. The culture plates were incubated aerobically at 37^0 C for 24 hours. Following the appearance of pure bacterial growth, only strains of *E.coli* with significant growth (> 10^5CFU/ml) were included in the study. Standard biochemical test were used to identify *E.coli.*

Antibiotic susceptibility test:

All *E.coli* strains were subjected to antimicrobial susceptibility test by Kirby bauer's disc diffusion method .Mueller-Hinton agar plates were incubated for 24 hours after inoculation with *E.coli* and placement of the antibiotic discs.After 24 hours the inhibition zones were measured and interpreted according to CLSI guidelines [(5)].

Following antibiotic discs from HiMedia were used for antibiotic susceptibility test:

ampicillin +sulbactum (10µg/10µg), piperacillin tazobactam ((100/10µg), ceftazadime, cefotaxime, ceftriaxone (30 µg), imipenem ((10µg), meropenem ((10µg), amikacin(10µg), gentamicin (10µg), tobramycin (10µg), ciprofloxacin (5 µg), norfloxacin (5 µg), nitrofurantoin (300µg), cotrimaxozole (25µg), colistin (10µg)

Detection of ESBL:

1. Screening test for ESBL production:

Screening for ESBL production was done as per CLSI guideline [(5)] .

Isolates were tested for ceftazadime (30 µg) and cefotaxime(30µg), & ceftriaxone (30 µg) susceptibility. Strains of *E.coli* resistant to these antibiotics were further tested for ESBL production.

2. Phenotypic confirmatory test for ESBL production [(5)]

ESBL production were tested by combination disc method using ceftazidime(30 µg) & ceftazadime /clavulanic acid (combination discs.) Separate discs containing ceftazidime (30 µg) with and without clavulanic acid (10 µg) were used. An increase in the zone size of more than or equal to 5mm for ceftzadime with and without clavulanic acid were considered to indicate ESBL producing strain.

3. E-test for ESBL:

Ezy MIC ™stripsbyHiMeida containing ceftazadime and Ceftazadime /Clavulanic acid was used. Manufacturer's instructions were followed. In this E-test predefined quantitative gradient is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of the antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Quality control:

1. *E. coli* ATCC 25922 was used as ESBL negative control
2. *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control.

RESULTS AND OBSERVATION

A total of 200 *E.coli* isolated from urine samples of clinically diagnosed cases of UTI were studied.Maximum number of patients were in the age group of above 60 years (35%), Out of 200 patients studied, 91(45.5%) patient were males, while females constituted 109 (54.5%)

Out of total 200 *E.coli* ,124 (62%) were positive for ESBL production by screening test.Out of 124 screening test positive *E.coli* isolates 76 (61%) were positive for ESBL by phenotypic confirmatory test (Double Disc diffusion test –CLSI) as well as E test. So the prevalence of ESBL *E.coli* was 38%(76/200)

Table-1 : Distribution of ESBL producing *E. coli* isolates in various disciplines.

Discipline	Total No of E.coli	No of ESBL producers (%)
ICU	48	24(31.5%)

Medicine	60	21(27.6%)
Surgery	38	11(14.7%)
Obst &Gynaecology	23	9 (11.8%)
Paediatrics	17	9(11.8%)
OPD	14	2 (2.6%)
Total	200	76(100%)

Maximum ESBL producers were reported from ICU (31.5%) and minimum (2.6%) ESBL producers were reported from OPD patients

Table No.2: Antimicrobial susceptibility pattern of ESBL producer E.coli

Antimicrobial agent	***Esch.coli n=76***	
	No. Resistance (%)	**No. Sensitive (%)**
Ampicillin+sulbactam	72 (94.7%)	4 (5.3%)
piperacillin+Tazobactam	36 (47.3%)	40 (52.7%)
Imipenem	3 (4%)	73 (96%)
Meropenem	3 (4%)	73 (96%)
Cotrimaxazole	64 (84.3%)	12 (15.7%)
Gentamicin	35 (46%)	41 (54%)
Tobramycin	31 (40.8%)	45 (59.2%)
Amikacin	12 (15.8%)	64 (84.2%)
Norfloxacin	60 (79%)	16 (21%)
Ciprofloxacin	70 (92.1%)	6 (7.9%)
Nitrofurantion	17 (22.4%)	59 (77.6%)
Colistin	0 (0%)	76 (100%)

ESBL *E.coli* showed maximum resistance for ampicillin sulbactum (94.7%) followed by co trimoxazole(84.3%).Resistance to imipenem was 6%. No resistance was noted for colistin (table 2)

Table 3: Antimicrobial susceptibility pattern of non ESBL producer *E.coli*

Antimicrobial agent	***Esch.coli* n=124**	
	No. Resistance (%)	**No. Sensitive (%)**
Ampicillin+sulbactam	98 (79%)	26 (21%)
Amoxicillin.clavulonic acid	98 (79%)	26 (21%)
piperacillin+Tazobactam	22 (18%)	102 (82%)
Imipenem	0 (0%)	124 (100%)
Meropenem	0 (0%)	124 (100%)
Cotrimoxazole	99 (80%)	25 (20%)
Gentamicin	33 (27%)	91 (73%)
Tobramycin	30 (24%)	94 (76%)
Amikacin	18 (15%)	106 (85%)
Norfloxacin	104 (84%)	20 (16%)
Ciprofloxacin	114 (92%)	10 (8%)
Nitrofurantion	22(18%)	102 (82%)
Colistin	0 (0%)	124 (100%)

DISCUSSION:

In this study total 200 *E.coli* isolates from urine sample of clinically diagnosed cases of UTI were studied.There was female preponderance (54%) in our study. It is stated that UTI is predominantly a disease of females due to a short urethra and proximity to anal opening. Present findings were also in agreement with the findings of R Prabha et al [6].
Out of total *E.coli* isolates tested 62 % were screening test positive for ESBL,while in a similar study by B. Sasirekha et al 71.42% of *E.coli* were screening test positive (2013) [7].

While Anil Chander reported 34.9% screening test positive isolates in their study on 444 urinary *E.coli* (2013)[8].The World Health Organization and the EuropeanCommission have recognized the importance of studying the emergence and determinants of acquired anti-microbial resistance and the need to devise appropriate strategies for their control [9,10,11]. In particular, the Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* are emerging worldwide.The ESBL-producing strains are particularly feared as they are resistant to all penicillins, to cepahlosporins (including third and fourth generation agents), and to aztreonam. Furthermore, they are often cross-resistant to trimethoprim/sulfamethoxazole and quinolones. This combination of properties can significantly affect the course and outcomes of infections, both in the community and in the hospital setting [12,13]. Our study showed equal sensitivity of both phenotypic confirmatory disc diffusion test & E-test .Phenotypic confirmatory disc diffusion test recommended by CLSI is simple, cost effective & easy to perform, therefore it can be used as a routine test for ESBL detection. The E-test is limited by its indeterminate results, difficulties in recognizing subtle zone deformities & cost.

In our study 38% of the *E.coli* isolates were ESBL producers .Similar results were noted by Babak Pourakbari et.al in 2012 in Tehran [14] .While Shfaq Aiyaz has reported 54% ESBL in *E.coli* from urine in 2011[15], which is much higher than our study . Few studies have shown much lower rate of ESBL producers in *E.coli*. In a study by Abhijit Awari et al 13.26 % of the *E.coli* were ESBL producers[2] , which is much lower than our study.

Maximum isolation of ESBL were from ICU patients (31.5%),while minimum isolation was from OPD patients(2.6%). In ICU critically ill patients are admitted .Higher prevalence in ICU could be because of more use of 3rd generation cephalosporins

In this study resistance to 3rd generation cephalosporins was found to coexist with resistance to other antibiotics ,as also reported M Chatterjee, et al in (2012). [16]. The ESBL producing *E.coli* showed a marked increase in the overall resistance pattern when compared with the resistance pattern of the Non ESBL producing *E.coli* . This observation coincided with the observation made by M Chatterjee et al [16]. ESBLs are encoded by plasmids ,which also carry resistance genes for other antibiotics. Hence multidrug resistance is expected to be more common in ESBL producing organisms. But resistance for Amikacin in ESBL producers was 15.5% almost same (15%) resistance was noted in non ESBL isolates. Both ESBL producers and non ESBL producers did not show any resistance for colistin.

Carbapenems are regarded as the antibiotic of choice and mainstay against severe infections caused by ESBL producing organisms . The increasing usage of carbapenems, the first treatment option in ESBL producing *E. coli* infections, has brought out the problem of carbapenem resistance [17].With increasing resistance to carbapenem treatment options are limited for ESBL producers. 4% of our isolates showed resistance to imipenem & meropenem. Ritu Aggarwal et al [18] (2009) have reported no resistance to imipenem in their ESBL *E.coli* isolates.Amikacin and nitrofurantoin were the other two drugs to which low resistant was noted. The resistance to amikacin and nitrofurantoin was 15.8% and 22.4% respectively. Similar observations were noted by Nair T et al in 2011[19]. In their study resistance to amikacin and nitrofurantoin was 16 % and 9% respectively. Nitrofurantoin is used for treating uncomplicated UTI. Good sensitivity to this drug in our study may be due underuse of this drug for treating UTI.

Our isolates showed 46 % resistance to Gentamicin. Much higher results were noted by Shafaq Hassan et al in 2011 [15] .They reported 65 % resistance to gentamicin. But a lower resistance (23.33%) to gentamicin was reported by Anil Chander et al in 2013 [8].

ESBLs are usually inhibited by ß-lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam. Therefore, use of beta lactam/b-lactamase inhibitor combinations has been considered for the treatment of infections due to ESBL-producing organisms. In our study isolates were highly resistant (94.7%) to amoxicillin/clavulanic acid. Our results corroborate with the results of Taneja et al in 2008 .They reported 93% resistance among ESBLs[20]. while Shafaq et al reported 85% resistance[15]..Resistance to piperacillin/tazobacam was 47.4% in our study.

In our study the ESBLs showed a very high resistance against commonly used antibiotics , ciprofloxacin (92%), Norfloxacin (79%) and co-trimoxazole (84.3%). These results are consistent with the study by other authors [7].Norfloxacin is an oral antibiotic commonly prescribed for the treatment of UTI in India and other countries. High resistance noted for this drug in our isolates indicates a rise in quinolone resistance in our area.

In our study significantly high resistance was noted amongst ESBL producing strains to non ß-lactam antibiotics. This observation may be explained by the fact that ESBL are plasmid mediated enzymes which are transferrable between one bacterium to another and such transferrable plasmids also code for resistance determinate to antimicrobial agents other than ß-lactams. In these cases amikacin and nitrofurantoins with good sensitivity are alternative for treating UTI.

ESBL producing strains are resistant to a wide variety of commonly

used antimicrobials. Their proliferation possesses a serious global health concern that has complicated stratergies for a growing number of hospitalized patients. Hence routine ESBL testing for uropathogen along with conventional antibiogram would be useful for all cases of UTI.

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