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Research article

Extended-spectrum beta-lactamases and antibiotic co-resistance in *E. coli* isolated from patients in a tertiary care hospital

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Abstract

Extended-spectrum β-lactamases (ESBL) producing organisms pose a major problem for clinical therapeutics. The incidence of ESBL producing strains of *E .coli* among clinical isolates have been steadily increasing over the past few years resulting in limitation of therapeutic options.
Aim: To detect the ESBL producers in *E .coli* and determine the antibiotic co resistance of ESBL isolates.
Method: The ESBL phenotype was determined by both screening and phenotypic confirmatory methods among different strains of *E.coli* isolated from clinical samples. The ESBL producing isolates were also studied for the presence of co-resistance with other antibiotics.
Result: A total of 130 strains of *E. coli* were isolated from 953 samples. Phenotypic confirmed ESBL production was detected in 82 (63.08%) of the isolates. The maximum number 56 (66.70%) of *E. coli* isolates from urine were found to be ESBL producers. All the ESBL producing isolates were sensitive to Cefaperazonesulbactam and Imipenem. Among the 82 ESBL producers, 92.68% were resistant to ciprofloxacin, followed by 79.27% to cotrimoxazole, 63.41% to Gentamicin and 20.73% to Amikacin.
Conclusion: All ESBL producers were found to be sensitive to Cefaperazonesulbactam and Imipenem. Highest co resistance was found to be with ciprofloxacin and least to Amikacin.

Keywords: Extended-spectrum β-lactamases, *E. coli*, Screening, Phenotypic confirmatory method, Clinical samples.

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1. Introduction

In Gram-negative bacteria, production of beta-lactamases is one of the most common mechanisms resulting in resistance to beta-lactam antibiotics. Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. These enzymes are usually plasmid-encoded and have the capacity to hydrolyze many antibiotics including penicillin, cephalosporin, and aztreonam and are inhibited by clavulanic acid [1, 2]. These phenotypic characteristics facilitate the identification of ESBL-producing organisms using routine laboratory tests such as double disk diffusion test or E-test. The detection of specific genes by PCR and sequencing are commonly used for final confirmation of ESBL producers. Since ESBLs are frequently encoded by genes located on different transferable genetic elements, a variety of epidemiological situations have been documented, ranging from sporadic cases to large outbreaks [3]. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis. Being plasmid mediated, they are easily transmitted among members of Enterobacteriaceae thus facilitating the dissemination of resistance not only to β-lactams but to other commonly used antibiotics such as quinolones and amino glycosides which complicates the treatment strategies in many hospitalized patients [4-7]. Although ESBLs have been detected in many different Gram-negative bacteria, *Klebsiellapneumoniae* and *Escherichia coli* remain the major ESBL-producing organisms worldwide [8-10].

Aim

1. To detect the ESBL producers among *E .coli* isolated

from various clinical samples by screening and phenotypic confirmatory methods.

2. To determine the co resistance of ESBL isolates with other antibiotics.

2. Materials and methods

The study was carried out over a period of one year at the Institute of Microbiology, Madras Medical College and Government General Hospital, Chennai. Clinical Samples like Urine, Blood, Sputum, Pus, Tracheal swab, Body fluids, Bronchoalveolar lavage and Devices (Shunt tube, catheter tips etc) received from symptomatic patients were included in the study. Ethical clearance was obtained from the Institutional Ethical Committee, Government General Hospital and Madras Medical College, Chennai, India.

Sample processing for *Escherichia coli* isolation

Samples from symptomatic patients were collected and transported according to the specific specimen collection and transport techniques [11]. They were processed immediately using standard procedures [12, 13]. *E. coli* isolates were identified based on colony morphology on Mac Conkey's agar, blood agar, Gram staining and by standard biochemical tests [13]. Only typical *E. coli* strains isolated from the above mentioned samples were included in the study.

Tests for extended spectrum beta lactamases

Screening by standard disc diffusion method

Screening for ESBL production was done according to criteria recommended by CLSI.
Two discs, Ceftazidime (30µg) and Cefotaxime (30µg), were used for in –vitro sensitivity testing by Kirby Bauer disc diffusion method. Zone diameters were read using CLSI criteria. An inhibition zone of ≤ 22 mm for Ceftazidimeand ≤ 27 mm for Cefotaxime indicated a probable ESBL producing strain requiring phenotypic confirmatory testing [14].

Phenotypic confirmatory disc diffusion test

Disc diffusion method was used to confirm ESBL production by *E.coli*strains. Ceftazidime (30µg) vs. Ceftazidime/Clavulanic acid (30µg/10µg) and Cefotaxime (30 µg) vs Cefotaxime/Clavulanic acid (30µg/10µg) were placed onto Mueller Hinton Agar plate lawned with the test organisms and incubated overnight at 35 °C. Regardless of zone diameters, a > 5 mm increase in a zone diameter of an antimicrobial agent tested in combination with Clavulanic acid vs. its zone size when tested alone, indicated ESBL production.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the ESBL isolates was done using Kirby Bauer disc diffusion method. The antibiotic discs used were Ampicillin, Amikacin, Cefotaxime, Ciprofloxacin, Gentamicin, Ceftazidime, Cefoperazone–sulbactam, Cotrimoxazole and Imipenem.
Mueller Hinton agar plate was inoculated with 0.5 McFarland standard inoculums to obtain a lawn culture. Using a sterile forceps, discs were placed over the agar surface, incubated at 37 °C for overnight. The results were interpreted as per Clinical Laboratory Standards Institute (CLSI) standards [15]. The *E. coli* strain ATCC 25922 was included as a quality control.

3. Results

The study undertaken at the Institute of Microbiology, Madras Medical College, Chennai among clinical Samples received from patients admitted in the Government General Hospital, Chennai, showed the following results.

130 (13.64%) *E. coli* was isolated from total 953 clinical samples received from patients.

Table No 1: *E.coli* isolates from different clinical samples (n =130)

Samples	no	%
Urine	84	64.62
Pus	32	24.62
Sputum	07	05.38
Blood	05	03.85
Tracheal swab	01	0.77
Shunt tube	01	0.77
Total	130	100

Maximum number of *E.coli* was isolated from urine samples 84 (64.62%).

Table No 2: ESBL isolates detected by screening method (n=130)

Samples(No of isolates)	No of ESBL producers	% of ESBL producers
Urine (84)	81	96.43
Pus (32)	31	96.88
Sputum(7)	7	100
Blood (5)	5	100
Tracheal swab (1)	-	-
Shunt tube (1)	-	-
Total	124	95.39

Out of 130 samples screened for ESBL, 124 (95.39%) were found to be ESBL producers.

Table No-3: Phenotypically confirmed ESBL isolates among ESBL producers detected by screening test (n =124)

Samples (No of isolates)	No of confirmed ESBL producers	% of confirmed ESBL producers
Urine (81)	56	69.14
Pus (31)	20	64.52
Sputum (7)	3	42.86
Blood (5)	3	60
Total	82	66.13

Out of 124 samples, 82(66.13%) samples were phenotypically confirmed for ESBL production.

Table No 4: Sensitivity pattern of (phenotypically confirmed) ESBL positive isolates (n =82)

Antibiotic	No. sensitive	% sensitive
Amikacin	65	79.27
Cefoperazone - sulbactam	82	100
Ciprofloxacin	6	7.32
Cotrimoxazole	17	20.73
Gentamicin	30	36.59
Imipenem	82	100

All the isolates were sensitive to Cefaperazonesulbactam and Imipenem, followed by 79.27 % sensitive to Amikacin.

Table No 5: ESBL and co –resistance (n=82)

Antibiotic	No. of Co-Resistance	% of Co-resistance
Amikacin	17	20.73
Gentamicin	52	63.41
Ciprofloxacin	76	92.68
Cotrimoxazole	65	79.27

Maximum Co resistance was found to be with Ciprofloxacin - 92.68%

Minimum Co resistance was found to be with Amikacin – 20.73%.

3. Discussion

E. coli is able to cause a variety of infections such as urinary tract infection, soft tissue infections, bacteraemia and neonatal meningitis. ESBLs have emerged as a major problem in hospitalized patients worldwide and have been involved in epidemic outbreaks in many institutions in Europe and USA and constitute a serious threat to the current β-lactam therapy as these enzymes cause resistance to most penicillin, cephalosporin and aztreonam [16]. In the present study 130 strains of *E. coli* was isolated from 953 clinical samples received from patients. Maximum number of *E. coli* was isolated from urine samples - 84 (64.62%) (Table1). On screening all 130 isolates for ESBL, 124 isolates were found to be ESBL producers (Table 2). Out of these 124 isolates, 82 isolates were confirmed as ESBL producers by phenotypic confirmatory method (Table 3). ESBL production (phenotypically confirmed) was detected in 63.08% (82/130) of the isolates. The maximum number 56 (66.7%) of *E.coli* isolates from urine were found to be ESBL producers. A high rate of ESBL production by *E. coli* was observed which may be due to the selective pressure imposed by extensive use of antimicrobials. The indiscriminate use of cephalosporin is responsible for the high rate of selection of ESBL producing microorganisms [1].

Antimicrobial sensitivity pattern of ESBL isolates showed, 100 % sensitive to Cefaperazonesulbactam and Imipenem, 79.27 % (65) were sensitive to Amikacin, followed by 36.59% (30) to Gentamicin, 20.73% (17) to Cotrimoxazole and lowest 7.32% (6) to Ciprofloxacin (Table 4).

ESBL and co resistance were studied which showed that maximum co resistance 92.68% (76) was found to be with ciprofloxacin, followed by 79.27% (65) with co-trimoxazole, 63.41% (52) with Gentamicin and a minimum of 20.73% (17) with Amikacin (Table 5).

Conclusion

Drug resistance is on the rise among *E. coli* strains that cause human infections. This study demonstrates the occurrence of ESBL producers in *E. coli* and their antibiotic susceptibility pattern. It shows that, Imipenem and Cefaperazonesulbactam remains most effective drug against ESBL-producing E.coli followed by Amikacin. ESBL detection and its drug susceptibility pattern should be done routinely to help the clinician to select appropriate drug regimen for better management of infections.

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