

Study and Evaluation of Extended Spectrum Beta Lactamase in Isolates of Patients Suffering from Urinary Tract Infection in Tertiary Care

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ABSTRACT

Introduction: Extended-spectrum β -lactamases (ESBLs) is an enzyme that is produced by bacteria that is able to hydrolyse extended spectrum cephalosporin that causes major therapeutic challenge in the treatment of hospitalized and outdoor patients. Infections because of ESBL producers ranges from uncomplicated urinary tract infection to severe septicemia. TEM type of ESBL is derived from Temoniera, a patient from whom the strain was first isolated in Greece. Beta-lactamases, enzymes has the ability to hydrolyze third-generation cephalosporins and aztreonam and inhibited by clavulanic acid. ESBL-producing bacteria exhibit co-resistance to other classes of antibiotics and causing limitation in treatment. Study aimed at early detection of ESBL isolated in urinary tract infection and its prompt treatment.

Material and Methods: A prospective study was done in department of microbiology, PMCH, Patna. Urine sample was collected from suspected cases of UTI patients and culture and antibiotic sensitivity was done to find out the ESBL cases.

Result: 200 samples of suspected UTI patients from various department of PMCH, Patna was taken. 42 ESBL isolates was detected from total sample. 24.70% cases were ESBL.

Conclusion: ESBL detection rate in our hospital was 24.70%. Double disc diffusion method using 3rd generation cephalosporin around the amoxicillin plus clavulanic acid sensitivity disc. Then phenotypic confirmation test was done using Cephalosporin / clavulanate combination discs. Treatment of choice for infections against to ESBL-producing organisms is Carbapenems, but carbapenem-resistant have been reported.

Key words: ESBLs, TEM, clavulanic acid, Carbapenems, *E.coli*

INTRODUCTION

Urinary tract infection is one of the most common disease worldwide. It is the most common infections next to respiratory tract infection faced in day to day practice by the clinician. It has been considered as a frequent cause of morbidity especially in women since a long time.

Despite the availability of wide spectrum of antibiotics, UTI remains the challenge for the clinician to treat.

Escherichia coli and *Klebsiella* spp. responsible for urinary tract infection, it have the ability to produce extended-spectrum β -lactamases (ESBL) in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat.¹ Beta-lactamases are uses two classification systems one is Ambler molecular classification^{2a} and the other Bush-Jacoby-Medeiros functional classification.^{2b}

Ambler scheme^{2a} divides β -lactamases into four major classes from A to D. The classes A, C and D are serine β -lactamases the class B enzymes are metallo- β -lactamases. The basis of Ambler classification scheme is protein homology (i.e amino

acid similarity) and not phenotypic characteristics. The ESBLs are of molecular class A except OXA-type enzymes (in class D enzymes).

The Bush-Jacoby-Medeiros classification^{2b} β -lactamases according to functional similarities that are substrate and inhibitor profile. Bush-Jacoby-Medeiros classification have four main groups and multiple subgroups. This classification system is more important to the physician or microbiologist in a diagnostic laboratory because it considers β -lactamase and β -lactam substrates. In this ESBLs comes in group 2be or group 2d (OXA-type). Group 2d (OXA-type) shares most of the fundamental properties of group 2be enzymes but differs in being inhibitor resistant.

ESBLs are plasmid mediated, TEM- and SHV-derived enzymes, most commonly in *Klebsiella* spp., followed by *E. coli*.³

ESBLs are enzymes that hydrolyses antibiotics and causing resistance to the penicillins, cephalosporins (first, second- and third-generation), and aztreonam (but not the cephamycins or carbapenems).⁴ There are several phenotypic tests for detection of ESBL-producing organisms. All these methods utilize the two characteristics of ESBLs:

1. Reduction of susceptibility to Extended-Spectrum Cephalosporins and
2. Clavulanate Inhibition

Screening of *E. coli*, *K. pneumonia* and *Proteus* for production of ESBL recommended by CLSI (Clinical and Laboratory Standards Institute). Initial Screen Test and Phenotypic Confirmatory Test according to CSLI for ESBL detection. Study aimed at early detection of ESBL isolated in urinary tract infection and its prompt treatment.

MATERIAL AND METHODS

Study was conducted in department of microbiology in Patna medical college, Patna after ethical clearance by ethical board. The study period from February 2012 to February 2013 (1 year interval). The study contains 200 randomly selected patients with suspected UTI infections from various department of PMCH, Patna.

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Inclusion criteria

1. All age group
2. Symptoms of UTI like fever, dysuria, frequency, hematuria etc.

Exclusion criteria

1. Patients who have taken antibiotics
2. Patients with any other fungal infection, diabetes, hypertension

Two hundred specimens collected from patients with suspected urinary tract infections were cultured on blood agar (Hi media) and MacConkey agar (Hi media). Strains were identified on the basis of colony morphology and biochemical reactions.⁵

The gram negative bacilli susceptibility to antimicrobial agents was performed on Muller Hinton agar (Hi media, Mumbai, India) and modified by Kirby Bauer disc diffusion method according to CLSI⁶ (NCCLS; 2004) with third generation cephalosporins (3GCs), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). The inoculated plates were incubated overnight at 37°C. Isolates found resistant or Intermediate resistant to any one of the 3GC antibiotics were selected for the presence of ESBLs.⁷

Antibiotic sensitivity of each isolate was also determined by modified Kirby Bauer disc diffusion method⁸ Amikacin (10µg), Amoxicillin +Clavulanate (10/10µg), Ampicillin (10µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75 µg), Imepenem (10µg), Levofloxacin, Nitrofurantoin, Piperacillin+Tazobactam (100µg/10 µg),

Testing for ESBL presence-

ESBL detection by two procedures

1. Screening for ESBL producers - It was done by Double disc synergy assay

The Double disc synergy assay was performed on Muller Hinton Agar (MHA) as a standard disc diffusion assay. Discs contain 30µg aztreonam and 30µg of ceftazidime, ceftriaxone and cefotaxime were placed 30 mm apart (centre to centre) around amoxicillin plus clavulanic acid (augmentin 20µg + 10µg) disc. The Muller Hinton Agar plate was incubated at 37°C for 24 hrs. Presumptive ESBL production was tested by enhancement of inhibition zone of any one of the test antibiotics towards augmentin disc and then subjected to phenotypic confirmatory test.⁹

If the screening test was found negative it was repeated after placing the discs 20mm apart.¹⁰

2. Phenotypic confirmatory test by Cephalosporin /clavulanate combination discs method: To test this CLSI recommends disc diffusion test on Muller Hinton agar. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared with the inhibition zone around the disc with the cephalosporin alone. Increase in ≥ 5 mm zone diameter is more in clavulanic than without clavulanic acid. This is called positive test.¹¹

Quality control

E. coli ATCC 25922 and *P. aeruginosa* ATCC 27853 is taken for quality control of AST (antibiotic sensitivity test). Quality control when performing screening and phenotypic confirmatory tests. Non-ESBL producing organism *E. coli* ATCC 25922 and an ESBL-producing organism *K. pneumoniae* ATCC 700603 simultaneous testing was performed.

RESULT

A total of 200 bacteria were isolated from 200 from UTI patients. Enterobacteriaceae was isolated in 170 cases. 42 was ESBL isolates. Detection rate was 24.70%. Among all enterobacteriaceae, Klebsella had maximum number of ESBL that is 40% (14 ESBL out of 30 total Klebsella). ESBL was 88.17% in hospitalized patients and in non hospitalized it was 11.90%. ESBL was 64.28% in catheterized and 35.71% in non catheterized. Antibiotic sensitivity pattern showed among ESBL isolates, 97.61% of associated resistance was observed for Ampicillin and 95.23% for Cotrimoxazole. Ciprofloxacin and Levofloxacin showed co-resistance of 73.83% and 69.04% respectively. Associated resistance for Amoxycillin + Clavulanic acid and Piperacillin + Tazobactam was 33.33% and 23.80%. But all were sensitive to Imepenam.

As shown in table 1, Total 200 patients suffering from UTI, Enterobacteriaceae was detected in 170 isolates. 42 of these isolates produced ESBL. So the detection rate of ESBL in the study was 24.70%. Total number of *E. coli* detected was 120 of which 18 produced ESBL (15%). Klebsiella detected was 35 of which 14 produced ESBL (40%). 2 isolates out of 4 (71.42%) of *Proteus mirabilis* produced the enzyme. Maximum ESBL producers were detected in *Proteus vulgaris*, *Enterobacter* spp., *Providencia* and *Acinetobacter* spp. (100%). *Pseudomonas aeruginosa* detected 3 out of 5 (60%) produced ESBL. 1 isolates out of 2 isolates (50%) of *Citrobacter* spp. produced Extended Spectrum Beta Lactamase.

| Organisms detected | Number detected | No. of ESBL detected | Percentage (%) |
|-------------------------------|-----------------|----------------------|----------------|
| <i>Acinetobacter</i> spp | 1 | 1 | 100 |
| <i>Citerobacter</i> spp | 2 | 1 | 50 |
| <i>Enterobacter</i> spp | 1 | 1 | 100 |
| <i>Escherichia coli</i> | 120 | 18 | 15 |
| <i>Klebsiella</i> | 35 | 14 | 40 |
| <i>Proteus mirabilis</i> | 4 | 2 | 50 |
| <i>Proteus vulgaris</i> | 1 | 1 | 100 |
| <i>Providencia</i> spp | 1 | 1 | 100 |
| <i>Pseudomonas aeruginosa</i> | 5 | 3 | 60 |
| Total | 170 | 42 | 24.70 |

Table-1: Distribution of Extended Spectrum Beta Lactamase strains among the isolated uropathogen

| Patients characteristic | ESBL detected | Percentage (%) |
|-------------------------|---------------|----------------|
| No. of non-hospitalized | 5 | 11.9 |
| No. of hospitalized | 37 | 88.17 |
| Total | 42 | 100 |

Table-2: Distributions of patients attending Outpatient Departments (Non Hospitalized patients) and admitted in the Wards in Hospital (Hospitalized patients) (n=42)

| Patients characteristic | ESBL detected | Percentage (%) |
|-------------------------|---------------|----------------|
| Catheterized | 27 | 64.28 |
| Non-catheterized | 15 | 35.71 |
| Total | 42 | 100 |

Table-3: Distribution of patients having in dwelling catheter (catheterized patients) and non-catheterized patients among ESBL isolates

| Antimicrobial Agent | No. of isolates | | | | | | | | | |
|--------------------------|-----------------|-----------------------|---------------------|--------------------|-------------------------|-----------------------|-----------------------------|--------------------------|--------------------------|-------|
| | 18 | 14 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 42 |
| | <i>E.coli</i> | <i>Klebsiella Spp</i> | <i>P. mirabilis</i> | <i>P. vulgaris</i> | <i>Providencia spp.</i> | <i>Ps. Aeruginosa</i> | <i>Enterobacter spp spp</i> | <i>Citerobacter spp.</i> | <i>Acinetobacter spp</i> | Total |
| Amikacin | 13 | 12 | 2 | - | 1 | 3 | - | 1 | 1 | 33 |
| Amoxicillin +Clavulanate | 6 | 3 | 1 | - | 1 | 2 | - | 1 | - | 14 |
| Ampicillin | 17 | 14 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 41 |
| Ciprofloxacin | 13 | 9 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 31 |
| Cotrimoxazole | 16 | 14 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 40 |
| Imepenem | - | - | - | - | - | - | - | - | - | - |
| Levofloxacin | 12 | 9 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 29 |
| Nitrofurantoin | 10 | 9 | - | - | - | 3 | - | - | - | 23 |
| Piperacillin+ Tazobactam | 6 | 3 | - | - | - | 1 | - | - | - | 10 |

Table-4: Associated resistance to other antibiotics among ESBL isolates. (Figure showing total no. of isolates, number of isolates sensitive to the drugs)

Isolates producing Extended Spectrum Beta Lactamase detected in hospitalized patients presenting with Urinary Tract Infection was 37 (88.17%). In the non-hospitalized patients with UTI ESBL isolates detected in Enterobacteriaceae was 5 (11.9%). Enterobacteriaceae producing Extended Spectrum Beta Lactamase in catheterized patients were 27 (64.28%). Isolates producing ESBL in non-catheterized patients were 15 (35.71%). Among the 42 isolates of Enterobacteriaceae producing ESBL 97.61% of associated resistance was observed for Ampicillin and 95.23% for Cotrimoxazole. Ciprofloxacin and Levofloxacin showed co-resistance of 73.83% and 69.04% respectively. Associated resistance for Amoxycillin + Clavulanic acid and Piperacillin + Tazobactam was 33.33% and 23.80%. All the isolates of Enterobacteriaceae producing Extended Spectrum Beta Lactamase were 100% sensitive to Imipenem. The ESBL producing isolates of *E.coli* showed co-resistance of 94.4% against Ampicillin and 88.88% to Co-trimoxazole. The isolates of *Klebsiella* producing ESBL showed 100% co-resistance against Ampicillin and Cotrimoxazole.

DISCUSSION

This study demonstrates the presence of ESBL-mediated resistance in gram-negative bacilli causing infections in various wards of a in PMCH.

Identification of ESBL production is important in terms of treatment and infection control in our hospitals. Infections caused by ESBL producers, there is delay in the initiation of appropriate therapy compared with patients with non-ESBL infections. Infection with ESBL-producing bacteria raises mortality, it prolongs hospital stay and also increases the cost of treatment. ESBL-producing bacteria is more frequent in patients with contact to the health care system (recent hospitalization, residence in a long-term care facility, recent surgery, and bladder catheterization. The carbapenems group (that includes Imipenem, meropenem, ertapenem, doripenem) is first choice of treatment for ESBL-producing *E. coli* and *K. pneumoniae* infection. But carbapenems have also developed resistance. It has been noted that >98% of the ESBL-producing *E. coli*, *K.*

pneumonia and *P. mirabilis* are still susceptible to carbapenam drugs.

In our study, ESBL producing isolates were significantly more resistant to ampicillin, cotrimoxazole, tetracycline, nitrofurantoin, ciprofloxacin and Piperacillin+ Tazobactam as compared to non-ESBL producing gram-negative isolates. In our study, resistance to 3rd Generation Cephalosporin was also with resistance to other antibiotics such as ampicillin, cotrimoxazole, ciprofloxacin, nitrofurantoin, piperacillin + Tazobactam.^{12,13} that shows multidrug resistance pattern. The mechanisms of co-resistance is not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids.¹⁴

Carbapenam resistance has developed, but still susceptible to *E.coli*, *K.pneumoniae*.¹⁵ The main strong point of the study is that, the screening test is as effective as the phenotypic confirmatory test. The weak point of study is that we haven't used any advanced molecular methods because of lack of infrastructure.

CONCLUSION

To conclude the study prevalence was 42 of these isolates produced ESBL. So the detection rate of ESBL in the study was 24.70% in our hospital which cannot be ignored. Since ESBL producers were detected with equal efficacy by screening test DDST and phenotypic confirmatory test; and the sensitivity of screening test improved with the use of more than one antibiotic, addition of one or two antibiotics would not increase the cost and labor, we recommend DDST to be used routinely as a screening test using multiple antibiotics in all microbiology units.

Resistance have developed against Carbapenems but still it is considered as treatment of choice for ESBL. The presence of ESBL producing organisms affects the course and outcome of an infection and management of these are challenging.

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