

Detection of Extended Spectrum Beta Lactamases (ESBLs) Producing Enterobacteriaceae Family from Urinary Tract Infection (UTI) Patients

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ABSTRACT

Background: Extended spectrum beta lactamase (ESBLs) are beta-lactamase capable of conferring bacterial resistance to the first, second and third-generation Cephalosporin's but these are inhibited by lactamase inhibitors such as clavulanic acid. The ESBLs are typically plasmid-mediated enzymes, mostly produced by *E. coli* and *Klebsiella pneumoniae* Enterobacteriaceae family. ESBLs enzymes were discovered in Germany in 1983 from *Klebsiella pneumoniae*. ESBL-producing gram negative bacteria have been responsible for numerous outbreaks of infection throughout the several counties and region challenging infection control issues. Different phenotypic test for ESBL detection have been developed, which are easy to use and cost effective. **Materials and Methods:** In this study, 10 Urine samples were collected from Government Hospital, Dharmapuri, Tamil Nadu. Collected clinical samples were isolated and identified by standard microbiological method. The antibiotic susceptibility testing was performed by the disc-diffusion method. The preliminary phenotypic detection of ESBLs was carried out by combination disc-diffusion test (CDDT) Performed using Kirby-bauer disc diffusion method on muller hinton agar. **Results:** Totally 10 clinical samples were collected and investigated Among 7 clinical samples of positive of Urinary tract infection (UTI), 20 micro-organisms belonging to

3 genus were isolated. Of the patients, 7 were female and 3 were male. *E. coli* and *Klebsiella pneumoniae* were most prevalent species. *E. coli* and *Klebsiella pneumoniae* were multidrug-resistant (resistance to five or more antibiotics) among the isolates. Of the isolated in Uro pathogens such as *E. coli* and *Klebsiella pneumoniae* were ESBLs positive. **Conclusion:** Based on the results, *E. coli* and *Klebsiella pneumoniae* predominated in the samples, giving the maximum frequency of ESBLs producing followed by *E. coli* (70%) and *Klebsiella pneumoniae* (30%) from the combined disk diffusion method. Herbal medicine should be perform for UTI patients to prevent the spread of more. Only limited therapeutically options for ESBL-PE.

Key words: Extended Spectrum Beta Lactamases (ESBLs), *E. coli*, *Klebsiella pneumoniae*, Double Disk Synergy Test, Urinary tract infection.

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INTRODUCTION

ESBLs are enzymes commonly produced by Enterobacteriaceae family such as *Escherichia coli* and *Klebsiella pneumoniae* some other gram negative bacteria. An antibiotic resistance Enterobacteriaceae family bacterium is increasing emergence as a major public health problem in uropathogens. They serve as a clinical threat leading to failure of beta lactam therapy and extend hospitalization with increased morbidity and mortality.¹ Extended spectrum beta lactamases are a group of enzymes encoded by particular genes described predominantly on plasmid that are common among Enterobacteriaceae family.²

The persistent exposure of the bacterial strains to a multitude of beta-lactams has induced a dynamic and continuous production of EBLs and mutation of beta-lactamases in the bacteria, expanding their activity even against the third and fourth generation ceftazidime, cephalosporin's and cefepime, cefotaxime and also against aztreonam. Certain new beta-lactamases are called extended spectrum beta-lactamases (ESBLs).³ *Escherichia coli* and *Klebsiella pneumoniae* are the frequently causes of nosocomial and community-associated infections like urinary tract infections, extended-spectrum cephalosporin's are routinely used for treatment of many infections by these MDR species, But resistance rates have also been increasing against cephalosporin⁴.

Therefore, the good choice of antimicrobial agent to properly treat extended spectrum beta lactamases producers requires accurate identification of following methods. TEM-1, TEM-2 and SHV-1 beta-lactamases that are able of hydrolyzing mostly penicillins beta-lactamases inhibitors and most cephalosporins including cefoxithin and cefotetan. These SHV and TEM-derived extended spectrum beta lactamases were the commonly observed ESBLs production types in *E. coli* throughout the 1980s and 1990s.⁵ The initial report of plasmid-encoded beta-lactamases enzyme which are able to hydrolyzing the extended-spectrum antibiotics such as cephalosporin's was published in 1983.⁶

My whole study and aim was to detect extended spectrum beta lactamases producing *E. coli* and *K. pneumoniae* isolated from urinary tract infection patients. The ESBL producing *E. coli* and *K. pneumoniae* can be detected by phenotypic methods, it is necessary for accurate identification of such resistant strains.

MATERIALS AND METHODS

Sample collection

Totally 10 urine samples were collected from Government Hospital, Dharmapuri, Tamil Nadu.

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Isolation and identification

Collected samples were immediately transported to microbiological laboratory for isolation and identification. Bacterial identification carried out by sub-culture on Eosin methylene blue agar (EMB), CLED agar, blood agar, chocolate agar, MacConkey agar and mannitol salt agar. The colonies were identified using standard morphological examination and biochemical characterizations. Morphological test, Gram's staining, Endospore Staining, Motility test. Biochemical test Indole test, methyl red test, Voges Proskauer test, citrate utilization test, catalase test, nitrate reduction test, oxidase test and Carbohydrate fermentation test by standards methods.

Antimicrobial susceptibility test

The isolated micro-organisms were tested by the disc diffusion method (Kirby-Bauer) on Muller Hinton Agar (Hi media) the zone size should be followed by CLSI.⁷ The antibiotics which were included were cefotaxime (20mg), ceftazidime (30mg), ceftriaxone (30mg), ceftizoxime (30mg), cefepime (30mg), Amikacin (30mg), Piperacillin (100mg), Ampicillin (10mg), Amoxicillin (30mg), Gentamicin (10mg), Ciprofloxacin (50mg), Aztreonam (10mg), Imipenem (20mg) (Figure 1).

Identification of ESBL producing strains

The isolated micro-organisms were tested for their susceptibility to the third generation cephalosporins eg cefotaxime (30), ceftriaxone (30), ceftazidime (30) by using the standard disc diffusion method as recommended by the CLSI.⁷ The zone size indicating ≤ 22 mm for ceftazidime, ≤ 28 mm for cefotaxime and ≤ 29 mm for ceftriaxone were recorded, the strain was considered to be "suspicious for extended spectrum beta lactamases production."⁷ Only two isolates were resistant to 3 GCs cephalosporins were selected for the study and were processed for extended spectrum beta lactamases production.

Double disc synergy test (DDST)

Discs of, cefotaxime (30), ceftazidime (30), amoxycillin (20), clavulanic acid and amoxicillin were placed at a distance of 25 mm from center to center in a straight line, with the amoxycillin disc in the middle on Muller Hinton Agar (MHA) plates inoculated with the test strain. The inoculated plates were incubated at 37°C overnight. The selected isolates which showed an intensification of the zone of inhibition as greater than 8 mm on the clavulanic acid and amoxycillin side of the disc as compared to that which was seen on the side without clavulanic acid and amoxycillin, were confirmed as producers⁸ (Figure 2).

Phenotypic confirmatory disc diffusion test (PCDDT)

All isolates which were screened out for extended spectrum beta lactamases production were also subjected to confirmation by using the phenotypic confirmatory disc diffusion test PCDDT, as recommended by the CLSI.⁷ The ceftazidime discs alone and in combination with clavulanic acid like (clavulanic acid + ceftazidime 10/30 discs) were applied onto a plate of Muller Hinton Agar (MHA) plates which was inoculated at 37°C with the test micro-organisms. An increase of ≥ 8 mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc only considered to be a marker for extended spectrum beta lactamases production.⁷

RESULTS

The present study 10 urine samples was collected from Government Hospital, Dharmapuri, Tamil Nadu. Among the bacterial isolates *E. coli* and *Klebsiella pneumoniae*.

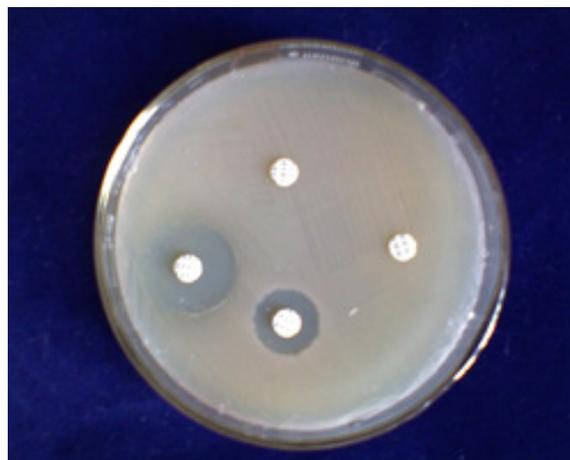


Figure 1: Antibiotic sensitivity susceptibility test.



Figure 2: Bacteria showing enhanced zone of inhibition between ceftazidime and cefotaxime and amoxicillin/ clavulanic acid containing disc indicating ESBL production.

Morphological and Biochemical Characteristics of isolates

The isolated micro-organisms were identified based on the colony morphological and biochemical characteristics result summarized (Table 1, 2).

The sensitivity pattern data of the prospective study revealed the *E. coli* and *Klebsiella pneumoniae* was highly imipenem (100 %), followed by amikacin (70.6%), piperacillin/tazobactam (76.5%), gentamicin (33.6%), ampicillin (31.6%) and piperacillin/tazobactam (94.4%), imipenem (86.2%), gentamicin (61.1%), ceftizoxime (47.2%). A high resistance rate was seen for piperacillin (91.8%), Ceftizoxime (87.7%), ceftazidime (83.6%), aztreonam (82.6%), carbenicillin (81.6%), amoxicillin (80.6%), cefotaxime (79.5%) and ceftriaxone (77.5%) and piperacillin (91.6%), carbenicillin (83.3%), ceftriaxone (77.7%), aztreonam (75%), amoxicillin (69%), ceftazidime (63.8%) (Table 3).

Figure 2 shows antibacterial resistance of ESBL and non-ESBL producing *E. coli*. ESBL producing *E. coli* showed maximum resistance to cefotaxime (100%), ceftazidime (100%), ceftizoxime (100%), Piperacillin (100%), amoxicillin (100%), aztreonam (100%) while minimum resistance was seen with amikacin (50%). The non-ESBL producing *E. coli* showed maximum resistance to piperacillin (90.2%), ceftizoxime

Table 1: Morphology and Biochemical Characterization of Isolates.

TEST	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>
Priliminary test		
Gram's stain	Negative rod	Negative rod
Capsule stain	Negative	Positive
Catalase	+	+
Oxidase	-	-
Motility test	+	-
Biochemical test		
Indole	+	-
Methyle red	+	-
Voges-proskauer	-	+
Citrate	-	+
Urease	-	+
Nitrate	+	+
H ₂ S	-	-
GAS	+	-
Gelatin	-	-
Fermentation test		
Glucose	+	+
Lactose	+	+
Sucrose	-	+

Table 2: Colony Morphology on Selective Media.

Selective media	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>
Macconkey agar	Dark pink colour colony	Pink color with large mucoid colony
Eosin methyle blue agar	Green with metallic sheen colony	Purple with mucoid colony
CLED agar	Transparent colony	Mucoid colony

(85.3%), ceftazidime (80.4%), aztreonam (79.2%), amoxicillin (76.8%), ceftriaxone (75.6%), cefotaxime (75.6%) while the minimum resistance to amikacin (25.6%) and no resistance to imipenem.

ESBL production was observed in 7 of three isolates. Seventy percent (70%) of *E. coli* (30%) and *Klebsiella pneumoniae* was identified as ESBL producers and was found to be resistant to multiple antibiotics Table 4.

DISCUSSION

Our study discussed that, the ESBL producing enterobacteriaceae bacteria isolates in India only moderate not very high. ESBL associated with increase in morbidity and mortality rate due to infections them patients. These multidrug-resistant pathogens are a serious concern in worldwide. The most common bacteria responsible for urinary tract infection in the present study are *E. coli* and *Klebsiella pneumoniae*. The deducting of the present study were supported by ESBL and another study where *E. coli* and *Klebsiella pneumoniae* were found in human beings as dominant species only responsible for the Urinary tract infection (UTI) among the children in worldwide particularly Pakistan.⁹ *E. coli* and *Klebsiella pneumoniae*, is a main species of family enterobacteriaceae,

Table 3: Antibiotic resistance pattern of *E. coli* and *Klebsiella Pneumoniae*.

TEST	<i>E. coli</i>		<i>Klebsiella pneumoniae</i>	
	R(%)	S(%)	R(%)	S(%)
Cefotaxime (20mg)	78(79.5)	20(20.4)	22(61.4)	14(39.4)
Ceftazidime(30mg)	82(83.6)	16(16.3)	23(63.8)	13(36.3)
Ceftriaxone(30mg)	76(77.5)	22(22.4)	28(77.7)	8(22.2)
Ceftizoxime(30mg)	86(87.7)	12(12.2)	19(52.7)	17(47.2)
Cefepime(30mg)	70(71.4)	28(28.5)	20(55.5)	16(44.4)
Carbenicillin(100mg)	80(81.6)	18(18.3)	30(83.3)	6(16.6)
Amikacin(30mg)	29(29.5)	69(70)	20(55.5)	16(44.4)
Piperacillin(100mg)	90(91.8)	8(8.1)	33(91.6)	3(8.3)
Ampicillin(10mg)	67(68.3)	31(31.6)	15(41.6)	11(30.5)
Amoxicillin(30mg)	79(80.6)	19(19.3)	25(69)	11(30.5)
Piperacillin/ Tazobactam(110mg)	75(76.5)	23(23.4)	34(94.4)	2(5.5)
Gentamicin(10mg)	65(66.3)	33(33.6)	14(38.8)	22(61.1)
Ciprofloxacin(5mg)	73(74.4)	25(25.5)	21(58.3)	15(41.6)
Aztreonam(10mg)	81(82.6)	17(17.3)	27(75)	9(25)
Imipenem(20mg)	0	98(100)	5(13.8)	31(86)

Table 4: ESBL Positive Isolates from UTI Patients.

Name of the organisms	No of isolates	ESBL Positive	ESBL Negative (%)
<i>E. coli</i>	7	16(16.3)	82(83.6)
<i>Klebsiella pneumoniae</i>	3	2(5.5)	34(94.4)

is most common cause of urinary tract infection and nosocomial infection.¹⁰ Broad spectrum antibiotics give more side effects compared with Beta lactam antibiotics are considered for treatment of bacterial infection.^{11,12} Antibiotic resistance is a major problem in worldwide, Risk factors for antibiotic resistance are long term and inappropriate use of antibiotics, severe illness, comorbidities, long term hospital staying patients dose not follow personal hygiene's, particularly poor sanitation and instrumentation or catheterization^{13,14} etc., Carbapenems generally exhibited maximum activity of anaerobic organisms such as Bacteriodes thus antibiotic choice and mainstay of treatment used against infections caused by ESBLs producing bacteria.^{15,16} This is agreeing with decting this study which gives a 100% susceptibility to imipenem and some antibiotics which is similar to results from other studies.^{17,18} *In vitro* studied by carbapenems (including ertapenem, meropenem and imipenem) have the most common compatible activity against ESBL-producing micro-organisms, give their stability to hydrolysis by ESBLs.¹⁹ Comparable results were reported in another study of meropenem, imipenem, ampicillin-sulbactam, piperacillin/tazobactam and amikacin were found as effective drugs against EBLs.²⁰ So, the results correlate in our study showed a maximum currency of ESBL producing micro-organisms particularly *E. coli* and *Klebsiella pneumoniae* among the hospitalized patients. These results are different from those of a study

conducted in Iran, where lower numbers of ESBLs producing bacteria such as *E. coli* and *Klebsiella pneumonia* (60% each) have been isolated from the hospitalized patients.²¹ ESBL producing *E. coli* showed lower resistance to amikacin (50%), imipenem (100%) and higher resistance was seen against amoxicillin (100%), cefotaxime (100%), ceftazidime (100%), piperacillin (100%), aztreonam (100%) and ceftizoxime (100%). A similar study conducted in Australia, revealed that ertapenem, meropenem and imipenem has the similar susceptibility pattern against ESBL producing *E. coli* and *Klebsiella pneumonia* isolates and ESBL non-producing isolates.²² ESBL producing *E. coli* and *Klebsiella pneumonia* were 26.3% and 8.5%, respectively. In the another study conducted in Pakistan, 56 % isolates of *E. coli* were ESBL positive²³ and in a study from India, nearly 40% urinary pathogens like *E. coli* and *Klebsiella pneumonia* were positive.²⁴ ESBL producing *Klebsiella pneumonia* were 54% in a study from America.²⁵ ESBL producing 53% *E. coli* and *Klebsiella pneumonia* from the patients suffering from urinary tract infections.²⁶ During the past few years, ESBL producing *E. coli* and *Klebsiella pneumonia* have emerged as serious pathogens both in hospital and community acquired infections such as nosocomial infection. Recent studies revealed that outpatients with ESBL producing organisms had been significantly higher fatality rate than those with non-ESBL isolates (*E. coli* and *Klebsiella pneumonia*).²⁷ ESBL production was detected in up to 75% isolates from the emergency patients of intensive care units (ICU) in Brazil.²⁸ Our similar study done in tertiary care facility in Turkey showed drug resistance rates of 6.6% and 23.2% in ESBL-negative and ESBL-producing *E. coli*.²⁹ This is illness increasing concern over the sensible use of carbapenems antibiotic in our health facilities. The dominant strains of Enterobacteriaceae family particularly *E. coli* and *Klebsiella pneumonia* of ESBL-producing enzymes have become a concern in the treatment of infections and infection control programs in hospital environment. Mostly *E. coli*, *K. pneumonia* and *Klebsiella oxytoca*, are resistant to main antibiotic like cotrimazole, amoxicillin, ciprofloxacin and ampicillin because of the production of β -lactamase enzymes. Most of the isolates are susceptible to later generation aztreonam and cephalosporins however, spontaneous mutations occurrence may result in novel β -lactamases which can inactivate extended-spectrum penicillins, aztreonam and cephalosporins. These β -lactamases are known as extended-spectrum β -lactamases (ESBLs) against *E. coli* and *Klebsiella pneumonia* and some gram negative bacteria. Recently, extended-spectrum β -lactamases enzymes were found in other genera and species, including *Enterobacter* spp., *Proteus mirabilis* and *Salmonella* spp.³⁰

CONCLUSION

In the present study, ESBL producing *E. coli* and *Klebsiella pneumonia* are considered to be multidrug resistant strains. The majority of ESBL producing *E. coli* and *Klebsiella pneumonia* were resistant to the common antibiotics used in the treatment of UTI. These findings suggest that imipenem may be a valuable treatment option such as herbal medicine for ESBL-producing uropathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ESBLs: Extended Spectrum Beta Lactamases; **UTI:** Urinary Tract infection.

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