

Identification of ESBL producing *Escherichia coli* from Urine Samples at Tertiary Care Hospital in Jhalawar

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Abstract

Introduction: Antibiotic resistance (ABR) is a serious worldwide threat to public health due to the emergence of multidrug resistant bacteria and is considered as a great problem in the treatment of bacterial infections both in hospital as well as community settings. Extended Spectrum Beta-Lactamase (ESBL) producing strains have emerged as a significant challenge to counter with present antibiotics.

Aims: Our study was aimed to know the prevalence of ESBL producing *E. coli* in our hospital setting for effective therapeutic outcome and patient care.

Materials and Method: A total of 459 urine samples of urinary tract infection (UTI) suspects were processed in the Microbiology Department at Jhalawar in Rajasthan, India. All samples were cultured on Mac Conkey Agar and Blood Agar and incubated at 37 °C for 24–48 h. The isolates were identified and confirmed using standard microbiological methods and biochemical reaction. Antibiotic sensitivity testing of all *E. coli* isolates was performed on Muller Hinton agar plates by Kirby-Bauer disk diffusion technique with guidelines established by the Clinical Laboratory Standards Institute (CLSI). Initial screening of ESBL producing *E. coli* was performed using the Cefotaxime and Ceftriaxone antimicrobial disc. Double-Disc Synergy Test (DDST) and CLSI confirmatory test i.e., Phenotypic Confirmatory Double-Disc Test (PCDDT) were performed for confirmation of ESBL-producing *E. coli*.

Results: Of the 459 samples processed, 212 isolates were found to be culture positive. Out of 212 positive isolates, 184 (86.79%) were identified as gram negative bacilli (GNB). Of the 184 GNB, 115 (62.50%) were detected as *Escherichia coli* followed by *Klebsiella* 30 (16.30%), *Pseudomonas* 21 (11.41%), *Proteus* 15 (8.15%), *Citrobacter* 8 (4.32%), and *Acinetobacter* 5 (2.71%). Out of 115 *E. coli* isolates, 86 (74.78%) were found to be ESBL-positive by screening method and 81 (70.43%) were found to be ESBL producers by PCDDT and 68 (59.13%) were found to be ESBL producers by DDST. Imipenem and Piperacillin–tazobactam were the most active and reliable agents for the treatment of infections which were caused by the ESBL-producing organisms. The ESBL-producing strains were more resistant than non-ESBL producing strains. Among ESBL producers the resistance pattern was highest for Ceftriaxone followed by Cefotaxime and Ceftazidime.

Conclusion: As results showed that there was a high prevalence of ESBL production in our setup so, it is essential to report the ESBL production along with the routine sensitivity reports, which will help the clinician in selection of proper antibiotics.

Keywords: antibiotic resistance, Extended Spectrum Beta-Lactamase (ESBL), multidrug resistant, *Escherichia coli*

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INTRODUCTION

Urinary tract infections (UTIs) having *Escherichia coli* as etiological agent are common infections with an estimated annual global incidence of at least 250 million cases [1]. The incidence of Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* has been increasing worldwide. It varies according

to geographical location and is directly linked to use and misuse of antibiotics [2]. Antibiotic resistance (ABR) is a serious worldwide threat to public health due to the emergence of multidrug resistant bacterias.

Antibiotics that were previously used to treat bacterial infections are now rendered less

effective due to drug resistance, which in turn shows not only therapeutic difficulties, but is also associated with increased societal and medical costs, and a much higher mortality rate for patients suffering with infections of this kind.

ESBLs are the enzymes, mostly encoded by plasmids in result of mutation due to which bacteria show resistance to various β -lactam antibiotics including penicillin, cephalosporins and monobactams [3]. They are inhibited *in vitro* by β -lactamase inhibitor i.e., clavulanic acid [4]. The highest risk of infection with ESBL-producing organisms has been observed in patients with prolonged hospitalization, high score of severe illness, recent surgery, instrumentations, admission to an intensive care unit and catheterization [5].

Laboratory detection of ESBL production can be problematic [4, 6–13] but its detection is important because its spread within the hospital may lead to endemic occurrence and repeated outbreaks from time to time. Another important implication of ESBL production is failure to treat ESBL producing organisms because of limited therapeutic choices [14]. The first report of plasmid encoded ESBL was published in Germany (1983) [15]. The first outbreak of multidrug resistant bacteria expressing ESBL was reported in France in 1987 [16].

With the spread of ESBL-producing Gram negative bacilli (GNB) in hospitals all over the world, it is necessary to know the prevalence of ESBL-producing GNB in a hospital so as to formulate an antibiotic policy in high risk units where infections due to resistant organism is higher. Therefore, the present study was undertaken to know the prevalence of ESBL-producing *E. coli* in our hospital setting for effective therapeutic outcome and patient care.

AIMS

1. Isolation and identification of ESBL producers from urinary sample in the patients.
2. To find out the prevalence of ESBL among *E. coli*.
3. To determine the antibiotic susceptibility pattern of ESBL producers to beta-lactam

antibiotics and combinations of beta-lactam + beta-lactamase inhibitors.

4. To help the clinicians to choose the right empirical treatment.

MATERIAL AND METHOD

A total of 459 urine samples of UTI suspects which were received from January 2017 to September 2017 were processed in the Microbiology Department, Jhalawar, Rajasthan, India. All samples were cultured on Mac Conkey Agar and Blood Agar and incubated at 37 °C for 24–48 h. The isolates were identified and confirmed using standard microbiological methods including Gram staining, colonial morphology on media, growth on selective media, lactose and mannitol fermentation, H₂S production, catalase, oxidase, coagulase, indole and citrate utilization, and urease test. Antibiotic sensitivity testing of all *E. coli* isolates was performed on Muller Hinton agar (MHA) plates by Kirby-Bauer disk diffusion technique with guidelines established by the Clinical Laboratory Standards Institute (CLSI). All *E. coli* isolates were included in the study.

Antibiotic Susceptibility Testing

Susceptibility to various antimicrobial agents was determined by Disc diffusion method of Kirby Bauer on MHA (Hi-media) as described by the Clinical Laboratory and Standard Institute (CLSI) guidelines. The following antibiotic discs (drug concentration in μ g) were used: Amikacin (30), Cefotaxime (30), Ceftriaxone (30), Cotrimoxazole (25), Imipenem (10), Ciprofloxacin (5), Cefoperazone–sulbactam (30), Piperacillin–tazobactam (30), Nitrofurantoin (30), Piperacillin (100), Amoxycylav (20/10), Azithromycin (15).

Test for ESBL Production

Screening Test

All *E. coli* isolates were subjected to screening tests by using Cefotaxime (30 μ g) and Ceftriaxone (30 μ g) discs. Those isolates with Cefotaxime zone \leq 27 mm and Ceftriaxone zone \leq 25 mm were considered as ESBL producer and then those isolates were subjected to confirmatory tests.

Confirmatory Test

Double Disc Synergy Test (DDST): According to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines [17] isolates which were presumed to be ESBL producers on the basis of the screening test results, were picked up and emulsified in saline to a 0.5 McFarland's turbidity standard. Discs of Ceftazidime (30 µg), Cefotaxime (30 µg) and Amoxycylav (20 µg Amoxycillin and 10 µg Clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the Amoxycylav disc in the middle on a plate of MHA being inoculated with the test strain. The plates were incubated at 37 °C aerobically overnight. Isolates which showed an enhancement of the zone of inhibition as greater than 5 mm on the Amoxycylav side of the disc as compared to that which was seen on the side without Amoxycylav, were confirmed as ESBL producers [17] (Figure 1).

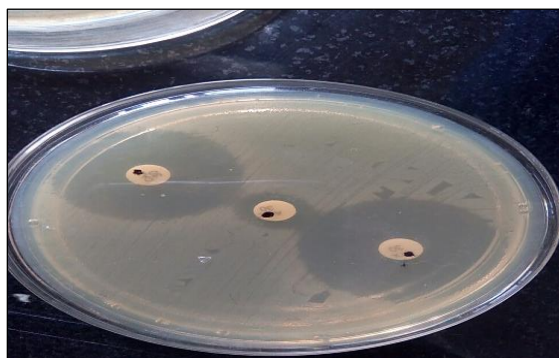


Fig. 1: Double disc Synergy Test (DDST).

CLSI Confirmatory Test (PCDDT-Phenotypic Confirmatory Double Disc Test)

For this test disc of Ceftazidime (30 µg) and Ceftazidime plus Clavulanic acid (30/10 µg) were placed on MHA and incubated. An increase of > 5 mm in the zone of inhibition of the combination discs in comparison to the Ceftazidime disc alone was considered to be a marker for ESBL production. *E. coli* ATCC 25922 and *K. pneumonia* ATCC 700603 were used as negative and positive controls, respectively (Figure 2).

RESULT

Of the 459 samples processed, 212 isolates were found to be culture positive. Out of 212 positive isolates, 184 (86.79%) were identified as GNB and 28 (13.20%) as gram positive

cocci (GPC). Of the 184 GNB, 115 (62.50%) were detected as *E. coli* followed by *Klebsiella* 30 (16.30%), *Pseudomonas* 21 (11.41%), *Proteus* 15 (8.15%), *Citrobacter* 8 (4.32%), and *Acinetobacter* 5 (2.71%). Out of 115 *E. coli*, 86 (74.78%) were found to be ESBL positive by screening method and 81 (70.43%) were found to be ESBL producers by PCDDT and 68 (59.13%) were found to be ESBL producers by DDST (Table 1).



Fig. 2: PCDDT-Phenotypic Confirmatory Double Disc Test.

Table 1: ESBL Producing *E. coli* by Various Test.

Organism (N)	PCDDT test N (%)	DDST test N (%)
<i>Escherichia coli</i> (115)	81 (70.43%)	68 (59.13%)

Imipenem and Piperacillin-tazobactam were the most active and reliable agents for the treatment of infections which were caused by the ESBL-producing organisms. The ESBL strains were more resistant than non-ESBL producing strains. Among ESBL producers the resistance pattern was highest for Ceftriaxone followed by Cefotaxime and Ceftazidime.

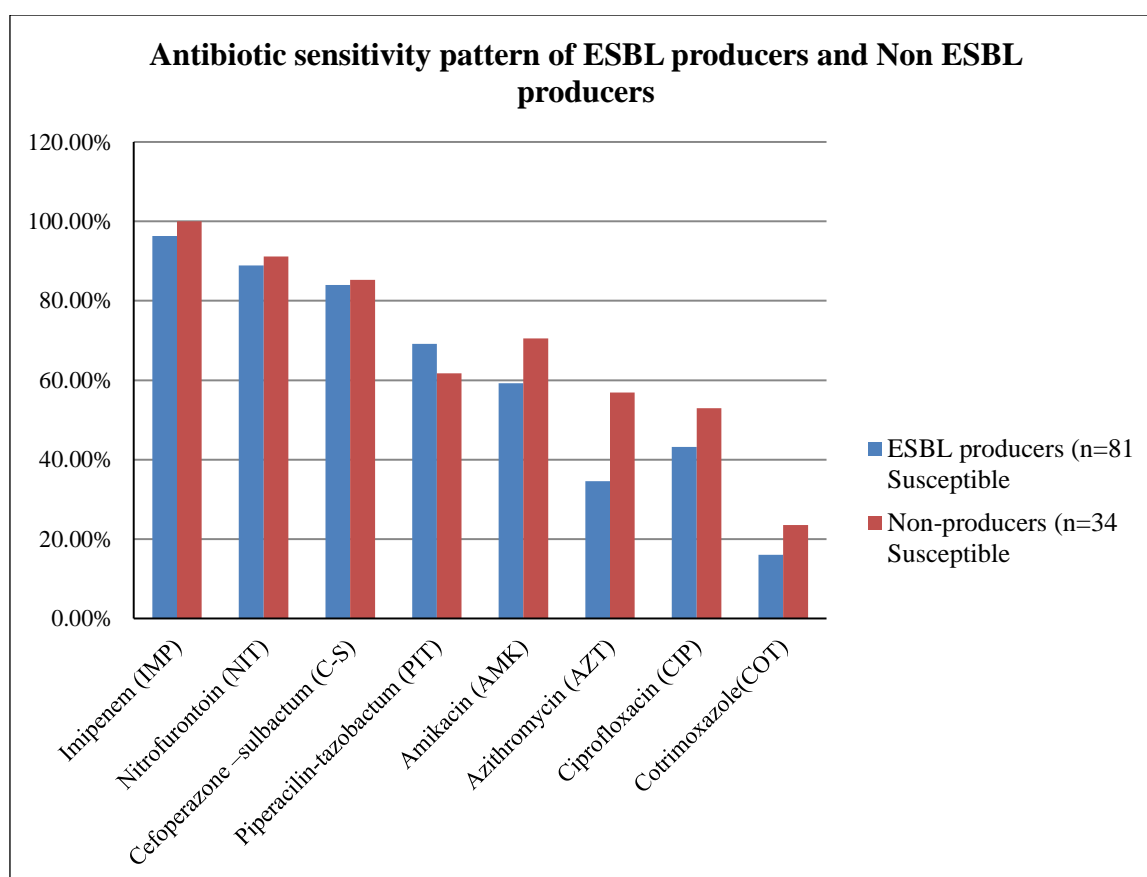
The antibiotic sensitivity pattern for ESBL producer revealed that maximum sensitivity was seen for Imipenem (96.29%), followed by Piperacillin-tazobactam (69.13%), Amikacin (59.25%), Azithromycin (34.56%), Ciprofloxacin (43.20%), and Cefepime (33.33%) (Table 2).

A high resistance rate was seen for Cotrimoxazole (83.95%), Ceftriaxone (80.24%), Ceftazidime (79.01%), Amoxycylav (80.2%), Piperacillin (83.95%), and Cefotaxime (77.77%).

Table 2: Antibiotic Sensitivity Pattern of ESBL Producers and Non-ESBL Producers.

Antibiotics	ESBL producers (n=81) susceptible	Nonproducers (n=34) susceptible
Imipenem (IMP)	78 (96.29%)	34 (100%)
Nitrofurantoin (NIT)	72 (88.88%)	31 (91.17%)
Cefoperazone–sulbactam (C-S)	68 (83.95%)	29 (85.29%)
Piperacillin–tazobactam (PIT)	56 (69.13%)	21 (61.76%)
Amikacin (AMK)	48 (59.25%)	24 (70.51%)
Azithromycin (AZT)	28 (34.56%)	19 (56.88%)
Ciprofloxacin (CIP)	35 (43.20%)	18 (52.94%)
Cotrimoxazole(COT)	13 (16.04%)	8 (23.52%)

Chi sq 3.325 p value =0.8534

**Fig. 3:** Antibiotic Sensitivity Pattern of ESBL Producers and Non ESBL Producers.

Imipenem and Piperacillin–tazobactam were the most active and reliable agents for the treatment of infections which were caused by the ESBL producing organisms. The ESBL strains were more resistant than non-ESBL producing strains. Among non-ESBL producers the resistance pattern was highest for Ceftriaxone followed by Cefotaxime and Ceftazidime (Figure 3).

The antibiotic sensitivity pattern for ESBL producer revealed that the maximum

sensitivity was seen for Imipenem (96.29%), followed by Nitrofurantoin (88.88%), Piperacillin–tazobactam (69.13%), Amikacin (59.25%), Azithromycin (34.56%) and Ciprofloxacin (43.20%).

A high resistance rate was seen in ESBL producers for Amoxyclav (80.2%), Cotrimoxazole (83.95%), Ceftriaxone (86.41%), Piperacillin (87.65%), Cefotaxime (90.12%), and Ceftazidime (92.59%).

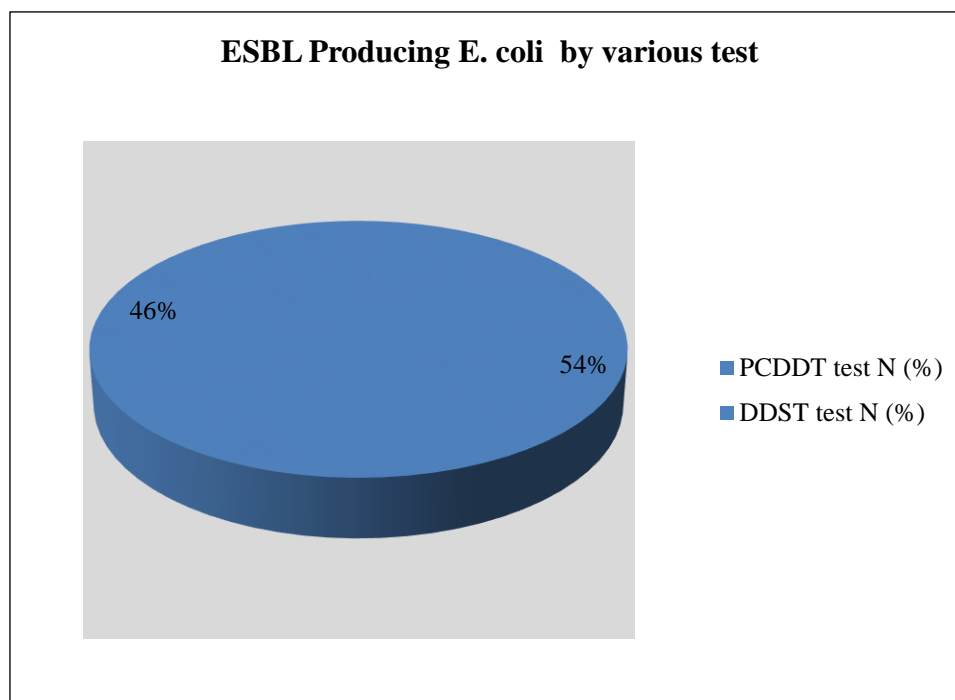


Fig. 4: ESBL Producing *E. coli* by Various Test.

DISCUSSION

The emergence and rapid spread of ESBL producing bacteria has become a worldwide problem indicating that continuous monitoring systems and effective infection control measures are absolutely required.

Prevalence of ESBL varies across continents, countries and hospitals as demonstrated by large scale studies such as SENTRY, SMART [18], MYSTIC [19]. As per the SMART study conducted in Asian-Pacific in 2007, the prevalence of ESBL production in Enterobacteriaceae was reported to be highest from India. ESBL production among *E. coli* was 79.0% [18]. Rodrigues *et al.* [20] reported 65.8% ESBL positivity among *E. coli*. This finding correlated well with those of our study. The occurrence of ESBL producers among *E. coli* in the current study was 81/115 (70.43%). In another study from Dehradun, India Sapna *et al.* [21] reported 53% ESBL-positive *E. coli* which shows less prevalence as compared to our results. Similarly in a study from Nepal, Chander and Shrestha [22] reported only 13.51% ESBL-positive *E. coli* which indicates great variation in ESBL positivity throughout India (Table 3).

Carbapenems (including Imipenem, Meropenem, and Ertapenem) have the most consistent activity against ESBL-producing organisms, showing their stability to hydrolysis by ESBLs. In our study also Carbapenems are the most effective with 96.29% ESBL producers sensitive to Imipenem.

Tsering *et al.* [31] in a similar study have reported that 97.53% ESBL producers were sensitive to Imipenem which is almost same as in our study and 48.11% of isolates were sensitive to Piperacillin-tazobactam. In the present study, sensitivity to Piperacillin-tazobactam was 69.13% and sensitivity to Cefoperazone-sulbactam was 83.95%. In another study by Mohanty *et al.* [34] sensitivity of ESBL-positive isolates was reported to be 81.37% to Piperacillin-tazobactam, 76.06% to Cefoperazone-sulbactam and 45.48% to Ticarcillin-clavulanic. Manoharan *et al.* [26] reported 89.7% sensitivity to Amikacin and 85.3% to Piperacillin-tazobactam. Sarma *et al.* [35] reported sensitivities to Piperacillin-tazobactam 89%, Amikacin 22%, Gentamicin 56%, and Tobramycin 78%. Similar kind of resistance pattern was also reported in SMART [18] study (Asia-Pacific) and MYSTIC [19] study (Table 4).

Table 3: ESBL Producing *E. coli* Detection Rates in Different Indian Studies.

Authors	Year	ESBL positive <i>E. coli</i> (%)
Trupti Bajpai <i>et al.</i> [23]	2014	41.6%
Meet Sharma <i>et al.</i> [24]	2013	52.49%
Mohamudha <i>et al.</i> [25]	2012	87.1%
Manoharan <i>et al.</i> [26]	2011	78%
Shoorashetty <i>et al.</i> [27]	2011	41%
Sridhar Rao <i>et al.</i> [28]	2008	62.91%
Wani <i>et al.</i> [29]	2009	52.94%
Goyal <i>et al.</i> [30]	2009	63.6%
Tesring <i>et al.</i> [31]	2009	26.15%
Agarwal <i>et al.</i> [32]	2008	30%
SMART study [18]	2007	79%
Kumar <i>et al.</i> [33]	2006	24.8%
Rodrigues <i>et al.</i> [20]	2004	65.8%
Sapna <i>et al.</i> [21]	2015	53%
Chander <i>et al.</i> [22]	2013	13.51%
Current study	2017	70.43%

Table 4: Sensitivity Pattern in International Studies.

Organism	Antibiotics	Our study	SMART study	MYSTIC study
<i>E. coli</i>	Imipenem	96.29%	98%	98.2%
<i>E. coli</i>	Piperacillin–tazobactam	69.13%	98.5%	94.2%

Nitrofurantoin has also shown good sensitivity among urinary isolates and is a good choice for UTI, being available orally and cheaper than its alternatives.

Many workers have found that resistance to third generation cephalosporins coexists with resistance to other antibiotics such as, Cotrimoxazole, Ciprofloxacin, Amikacin etc. indicating multidrug resistance pattern. We found such associated resistance with Cotrimoxazole, Amikacin and fluroquinolones.

CONCLUSION

In the current study, a high prevalence of ESBL-producing *E. coli* was detected. Most of these isolates were from hospitalized patients indicating that these were important nosocomial pathogens. The outpatient presence of ESBL is of concern as it shows that ESBL is spreading fast in the community and responsible for community-acquired ESBLs. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporin has been

associated with increased morbidity. In antibiotic sensitivity pattern many ESBL producers were found resistant to non β -lactam antibiotics such as quinolones and to lesser extent to aminoglycosides. Nitrofurantoin was found as a good choice in the form of oral drug for treatment.

It is clear that reporting of ESBL production along with the routine susceptibility testing is essential, which will help the clinician in prescribing proper antibiotics. To reduce the prevalence of antimicrobial resistant pathogens, including ESBL-producing *E. coli*, effective infection control measures such as hand washing and proper hospital hygiene are required.

There must be some guidelines for the judicious use of antibiotics and policies should be formulated which will help in minimizing the emergence of resistant bacteria among the patients. In the last it has been felt that there is a need to formulate some strategies to detect and prevent the emergence of ESBL-producing strains for the effective treatment.

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