

To Determine Antibiotic-Resistant Patterns and Phenotypic Confirmation Of Extended-Spectrum β -lactamase (ESBL) Producing Gram-Negative Uropathogens

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ABSTRACT

Background: UTIs involves bacterial invasion and multiplication of the pathogen in the organs of the urinary tract system is classified into uncomplicated and complicated infections on the basis of organ involved. ESBLs are Gram-negative bacteria that produce an enzyme; beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillins and cephalosporins and render them ineffective for treatment. Detection of ESBL producing organisms from urine samples will be useful as this represents an epidemiologic marker of colonization.

Materials & Methods: This is a Cross sectional study which is to be carried out by the Department of Microbiology, Dr. S.N. Medical College, Jodhpur. All Gram-negative aerobic bacteria isolated from the mid-stream and catheterized urine sample of patients who will be clinically suspected of suffering from UTI. The samples will be received from different wards, intensive care units and outpatient departments of Govt. mahatma Gandhi Hospital, Jodhpur. These strains were identified on the basis of colony morphology and various biochemical reactions. Antibiotic susceptibility testing was performed on all the strains identified. All the strains identified were tested for ESBL production and MBL production as per CLSI guidelines.

Results: Out of the total 625 urinary tract specimens from patients suspected of having UTI, 100 (16%) showed significant growth of at least one uropathogen confirming the urinary tract infection. Females (67%) were the significant

subgroup of patients ($p < 0.05^*$) affected with UTI, and most of them belonged to the age group 21–30 years. Gram-negative bacteria (82%) were more common, and Escherichia coli (62%) remained the predominant pathogen associated with UTI in all age groups. Diverse pattern of antimicrobial susceptibilities was observed among the *E. coli* isolates. Nitrofurantoin (92%) and gentamycin (76%) were the most effective first-line therapeutic regimens for uropathogenic *E. coli* isolates.

Conclusion: High burden of antimicrobial resistance and increased prevalence of ESBL-producing Escherichia coli associated with UTI are the major findings of this study.

Keywords: UTI, ESBL, Uropathogens, Antimicrobials, Susceptible.

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INTRODUCTION

Urinary tract infection includes the infection of urethra, bladder, ureters, and kidneys, which comprise the urinary tract. UTI is an important cause of morbidity and mortality in both developing and developed countries of the world, affecting all age groups and both the sexes.¹⁻³ Most UTI usually occur by the ascending route after entry via the urethral meatus, this is by far the most common route of infection in the female and in association with instrumentation, in both sexes.⁴ UTIs involves bacterial invasion and multiplication of the pathogen in the organs of the urinary tract system is classified into uncomplicated and complicated infections

on the basis of organ involved.^{5,6} Infection may be expressed predominantly as pyelonephritis, pyelitis, ureteritis, cystitis, proctitis and urethritis but the entire urinary tract is always at risk of invasion by bacteria.⁷ Microorganisms belonging to Enterobacteriaceae have been documented as elementary reason of nosocomial and community acquired UTIs.⁸ The infinite majority of uncomplicated UTIs are caused by the Gram negative bacilli and with other pathogens including Enterococci, Staphylococcus saprophyticus, Klebsiella spp, Pseudomonas spp, Proteus spp, Staphylococcus aureus and Proteus mirabilis.⁹

Numerous studies have barbed towards high incidence rate of UTI associated with *E. coli* and antibiotic resistance. The emergence of Multi Drug Resistant (MDR) variant of *E. coli* has been accounted.^{10,11} MDR is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracyclines and/or erythromycin.^{12,13} MDR in many bacteria is due to the action of multi-drug efflux pumps and by the accumulation on Resistance (R) plasmids or transposons, of genes with each coding for resistance to a specific agent.¹⁴ Nowadays, in UTIs Extended Spectrum Beta-Lactamase-expressing Gram-Negative Bacilli (ESBLGNB) generally cause community-acquired infections.¹⁵ The resistance of Gram-negative bacteria is typically owed to plasmid mediated enzymes called Extended-Spectrum B-Lactamases (ESBLs).¹⁶ ESBL producing bacteria are typically associated with MDR and antibacterial choice is often complicated by multi-drug resistance.¹⁷⁻¹⁹ ESBLs are Gram-negative bacteria that produce an enzyme; beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillins and cephalosporins and render them ineffective for treatment. ESBLs are commonly spread via direct and indirect contact with colonized/infected patients and contaminated environmental surfaces, most commonly spread via unwashed hands of health care providers. Detection of ESBL producing organisms from urine samples will be useful as this represents an epidemiologic marker of colonization.

MATERIALS & METHODS

This is a Cross sectional study which is to be carried out by the Department of Microbiology, Dr. S.N. Medical College, Jodhpur. All Gram-negative aerobic bacteria isolated from the mid stream and catheterized urine sample of patients who will be clinically suspected of suffering from UTI. The samples will be received from different wards, intensive care units and outpatient departments of Govt. mahatma Gandhi Hospital in the department of Microbiology, Dr. S.N. Medical College, Jodhpur.

Bacterial Isolates: A total of 100 urine samples were collected from suspected patients of UTI in the Department of Microbiology, Dr. S.N. Medical College, Jodhpur. Gram-negative bacteria will be isolated from the samples and they will examine phenotypically for ESBLs production.

Inclusion Criteria

- Gram negative bacteria such as Enterobacteriaceae family, *Pseudomonas* spp and *Acinetobacter* species are included in the study.
- Only patients who had significant bacteriuria ($>10^5$ CFU/mL) were included in the microbiological analysis.

Exclusion Criteria

- Isolation of three types of organisms with no predominating organism will be considered as contaminants and repeated isolates from same patient will be excluded from the study.

These strains were identified on the basis of colony morphology and various biochemical reactions. Antibiotic susceptibility testing were performed on all the strains identified. All the strains identified were tested for ESBL production and MBL production as per CLSI guidelines.²⁰

Isolation of Gram-negative Bacteria: All the samples were inoculated on blood agar and MacConkey agar media and incubated at 37°C aerobically for 24 hours. The incubated plates were examined for bacterial growth and the organisms will

identified by colony morphology, hemolytic criteria, staining character, pigment production and biochemical tests such as oxidase test, reaction in MIU and simmon's citrate media and different sugar fermentation tests.²¹

Colony Morphology:

On Mac-Conkey Agar
On Blood agar
On Nutrient agar

Grams staining: Gram-negative bacilli will be identified on gram-staining.

Hanging Drop for Motility:

A small fraction of the colony will then emulsified in sterile peptone-water and incubated at 37°C for two to three hours. Place a small drop of liquid culture on the cover-slip. Then invert the slide with plasticine, over the cover-slip. Then quickly turn round the slide so that the cover-slip is upper-most and the drop is hanging. Observed in the low power and high power of the microscope for motility of the test organism, motile strains suggested the colonies to be of *Escherichia coli*; *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas* spp. etc. and non-motile strains suggested to be the colonies of *Klebsiella* species. They will be next subjected to various biochemical reactions to confirm and identify the various species.

Biochemical Tests:

- Catalase Test
- Oxidase test
- Indole Test
- Methyl-Red (MR) Test
- Vogues-Proskauer (VP) Test
- Citrate Test
- Urease Test
- Triple-Sugar-Iron (TSI) Test
- Phenyl alanine agar

ANTIBIOTIC SENSITIVITY TESTING

Antibiotic susceptibility was performed by the Kirby-Bauer disc diffusion method on Mueller Hinton agar.²² The following antibiotics will be tested:

- Amoxyclav (20/10 mcg)
- Gentamicin (30 mcg)
- Cefoxitin (30 mcg)
- Meropenam (10 mcg)
- Trimethoprim/sulphamethoxazole(co-trimoxazole)(25/ 23.75 mcg)
- Nalidixic acid (30 mcg)
- Nitrofurantoin (300 mcg)
- Norfloxacin (10 mcg)
- Fosfomycin (200 mcg)
- Cefixime (30 mcg)

For *Pseudomonas* species –

- Aztreonam (30 mcg)
- Ceftazidime (30 mcg)
- Ceftazidime + Clavulanic acid (30/10 mcg)
- Cefepime (30 mcg)
- Amikacin (30 mcg)
- Polymyxin B (300 unit)
- Imipenam (10 mcg)
- Colistin (10 mcg)

Dehydrated media and antibiotic discs were procured from Himedia, India. The controls strains used were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* 25922.

Tests for extended spectrum beta lactamases production

Double Disc Approximation Test

The organism will be swab be on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 mcg) and a cephalosporin disc (cefotaxime (30 mcg), ceftriaxone (30 mcg), and ceftazidime (30 mcg)) were placed at a distance of 15 mm apart and incubated. Organism that showed a clear extension of any of the cephalosporin inhibition zone towards the disc containing clavulanate will be considered as ESBL producer.²³

Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL-

All the strains screened out for ESBL production were subjected to confirmation using the PCDDT Test as given by the CLSI.³⁸ This test requires use of both cefotaxime (30 µg) and ceftazidime (30 mcg) discs alone and in combination with clavulanic Acid (30 µg/10 µg) applied onto a plate of Mueller Hinton Agar (MHA) inoculated with the test strain. An increase of ≥ 5mm in the zone diameter of the combination discs (ceftazidime + clavulanic Acid & cefotaxime + clavulanic Acid), in comparison to that seen around individual test antibiotic will be considered to be a marker for ESBL production.

Table 1: Patients with urinary tract infection.

Age group (yrs)	Male (%)	Female (%)	p-value	Outpatients (%)	Inpatients (%)	p-value
0-10	2	4	>0.05	4	2	>0.05
11-20	1	7	<0.05*	8	0	<0.05*
21-30	2	19	<0.05*	20	1	<0.05*
31-40	2	14	<0.05*	14	2	>0.05
41-50	4	5	>0.05	7	2	>0.05
51-60	6	6	1.00	10	2	>0.05
61-70	4	5	>0.05	7	2	>0.05
71-80	10	4	<0.05*	10	4	>0.05
>80	2	3	>0.05	4	1	>0.05

Table 2: Antibiotic susceptibilities of ESBL-producing and nonproducing uropathogenic *E. coli* isolates.

Antimicrobials	Total Susceptible (%)	<i>Escherichia coli</i> urinary isolates		
		ESBL producers (n = 25)	ESBL nonproducers (n = 37)	p-value
Ampicillin	7 (11.29%)	0	7	<0.05*
Piperacillin	19 (30.64%)	3	16	<0.05*
Cefixime	31(50%)	0	31	<0.05*
Cefotaxime	33 (53.22%)	2	31	<0.05*
Ceftazidime	34 (54.83%)	2	32	<0.05*
Gentamycin	47 (76%)	20	27	>0.05
Cotrimoxazole	32 (51.61%)	13	19	>0.05
Nitrofurantoin	57 (92%)	22	35	>0.05
Ofloxacin	36 (58.06%)	9	27	<0.05*
Imipenem	59 (95.16%)	22	37	>0.05
Meropenem	55 (88.70%)	21	34	>0.05

RESULTS

Out of the total 625 urinary tract specimens from patients suspected of having UTI, 100 (16%) showed significant growth of at least one uropathogen confirming the urinary tract infection. Females (67%) were the significant subgroup of patients (p<0.05*) affected with UTI, and most of them belonged to the age group 21–30 years. Incidence of UTI varied with different age group, gender, and type of patients (inpatients or outpatients) (Table 1). One hundred bacterial uropathogens were recovered from a total of 625 patients with suspected UTI. Gram-negative bacteria (82%) were more common, and *Escherichia coli* (62%) remained the predominant pathogen associated with UTI in all age groups. Other pathogens isolated from our UTI cases

were *S. aureus* (10%), *Klebsiella pneumoniae* (9%), *Enterococcus faecalis* (7%), *Pseudomonas aeruginosa* (4.0%), and *Candida albicans* (3%).

Diverse pattern of antimicrobial susceptibilities was observed among the *E. coli* isolates. Nitrofurantoin (92%) and gentamycin (76%) were the most effective first-line therapeutic regimens for uropathogenic *E. coli* isolates. Almost half of the isolates were resistant to cephalosporins and fluoroquinolones. Moreover, 65% (65/100) of *Escherichia coli* were found multidrug resistant.

About 40.32% (25/62) of our *Escherichia coli* isolates were confirmed as ESBL producers. ESBL-producing *Escherichia coli* isolates were significantly more resistant to antibiotics as compared to nonproducers of ESBL (Table 2).

DISCUSSION

Overall incidence of UTI in our study was quite low (16%) when compared to the previous reports from similar studies.²⁴⁻²⁶ The lower incidence in this study might be due to the prior use of antibiotics and infection due to slow-growing organisms or due to those organisms that were not able to grow on our routine culture media. In addition, more outpatients were found with UTI than inpatients. Concomitantly, significantly more females (67%) were found with UTI, as previously described elsewhere.²⁵⁻²⁷ The higher occurrence of UTI in females of the reproductive age group in this study has been well supported by other studies.^{27,28} Furthermore, elderly males were found more affected by UTI in this study, as they might have bladder outflow obstruction and other chronic comorbid conditions.

We observed that Gram-negative bacteria were the most predominant (82%) organisms associated with our cases of UTI, and *Escherichia coli* (62.0%) was the major pathogen. Members of Enterobacteriaceae have been well described as the primary agents for UTI than other organisms in several studies. Higher incidence of *E. coli* seen in our study also resembled the results of previous studies.^{25,26,29} Although very low number of Gram-positive bacteria and yeasts were isolated in this study, they are also responsible for UTI in various studies.^{24,30}

Antimicrobial resistance among uropathogenic bacterial species is one of the major findings of this study. *Escherichia coli*, the major uropathogen, was highly resistant to commonly used therapeutic drugs (beta-lactams, sulphonamides, quinolones, and aminoglycosides). Out of 62 *E. coli* isolates, 11.29% were resistant to ampicillin, 30.64% resistant to piperacillin, 50% to cefixime, 51.61% to cotrimoxazole, and 58.06% to ofloxacin. This finding is similar to the previous reports by Baral et al.²⁹, Neupane et al.³¹, and Rijal et al.²⁸. Ampicillin and other oral cephalosporins were ineffective in our study, hence should be assessed before using as an empirical therapy. In addition to this, susceptibility findings of isolates against cephalosporins and quinolones show a substantial increase in their resistance, as reported by others.^{25,32} However, nitrofurantoin (92%) and gentamycin (76%) were effective against uropathogenic *E. coli* strains. As stated by others too, these can be considered as the first-line therapeutic regimen for UTI cases in our settings.^{28,33} Carbapenems including imipenem and meropenem would be useful as secondary therapy for multidrug-resistant and complicated UTIs.³⁴ However, in the recent years, the emergence of urinary isolates with carbapenem resistance is further complicating the treatment of UTIs.³⁵

In this study, 40.32% of the *E. coli* isolates were found as ESBL producers, and the patients over age of 50 years were found with higher incidence of ESBL *E. coli*. The rate of ESBL in this study is very high when compared to the reported rates from previous studies.^{29,32} From international perspectives, similar rates of ESBL-producing *E. coli* are also reported by Jena et al (41.07%) from India³⁶, Masud et al (40.9%) from Bangladesh³⁷, Moore et al (44%) from Cambodia³⁸, and Kizilca et al (41.4%) from Turkey³⁹. However, the rates of ESBL-producing uropathogenic *E. coli* from developed countries are very low as reported elsewhere.^{34,40} The reason behind the variation in ESBL-producing strains among studies might be attributable to the local antibiotic prescribing practices, extensive use of broad-spectrum antibiotics especially third-generation cephalosporins, and endemicity of drug-resistant pathogens in the locality.

CONCLUSION

High burden of antimicrobial resistance and increased prevalence of ESBL-producing *Escherichia coli* associated with UTI are the major findings of this study. Nitrofurantoin and aminoglycosides were found as the most useful first-line drugs to be used in the cases of UTI in our setting.

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