

Original Research Article

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Prevalence of Extended Spectrum of Beta Lactamase (ESBL) Producing Uropathogens from Pregnant Women in a Tertiary Care Hospital

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ABSTRACT

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Urinary tract infection (UTI) represents the most common bacterial infection in pregnancy. Urinary tract infection poses a serious health threat because of the antibiotic resistance and high recurrence rate. Extended Spectrum Beta-Lactamases (ESBLs) producing Enterobacteriaceae are among the most problematic multidrug resistant bacteria worldwide and are increasingly causing urinary tract infection both in hospitalized patients and outpatients making infections difficult to treat. This study was undertaken to identify the changing etiological trends of urinary tract infections (UTI), detect the prevalence of ESBL producing Uropathogens and study their antibiotic resistance profile. Urinary isolates from symptomatic UTI cases were identified by conventional methods. Antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method. Isolates resistant to cephalexin were tested for ESBL production by double disc synergy test method. Total 6 ESBL producing organisms were isolated out of 54-gram negative bacilli among 170 urine samples of pregnant women. The most predominant ESBL producing isolates were *Escherichia coli* (37.5%), followed by *Citrobacter freundii* (25.0%) and *Klebsiella pneumoniae* (12.5%). The antibiogram pattern of ESBL producing isolates showed maximum sensitivity to Imipenem (75.0%), Meropenem (75.0%) and Gentamicin (62.5%). This study shows that the Prevalence rate of ESBL producing *E. coli* and *Citrobacter freundii* is high in pregnant women. Most of the ESBL producing isolates were multidrug resistant. Careful detection of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

Introduction

Pregnancy causes several changes in the woman's body that boost the probability of

urinary tract infections (UTIs). It's been considered a frequent health problem among pregnant women, occurring within 2–10% of all pregnancies.¹ If not properly treated,

asymptomatic bacteriuria can lead to acute pyelonephritis in 30% of pregnant women and increase the risk for severe complication such as early delivery, hypertension, pre-eclampsia, low birth weight and postpartum endometritis.^{2,3}

Pathogens of urinary tract include Gram-negative bacilli such as *E. coli*, *Klebsiella spp.*, *Proteus spp.* and *Pseudomonas spp.* and Gram-Positive cocci such as *Staphylococcus* and *Enterococcus spp.*⁴ Extended-spectrum beta lactamases (ESBL) are a cluster of plasmid-mediated, diverse, complex and swiftly growing enzymes that are exaggerating a chief therapeutic challenge in the treatment of hospitalized and community-based patients.

The ESBL producers often also have resistance determinants to other antibiotic groups, leaving an extremely limited range of effective antibiotic agents.⁵ A delay in appropriate therapy can cause severe complications.

Detection of ESBL producers from sample such as urine may be of utmost important as there is a rapid increase of resistance to broad spectrum beta lactams among uropathogens which has recently become a major health problem globally.

It leads to antibiotic ineffectiveness, increased severity of illness and cost of treatment.⁶ The increase in the prevalence of ESBL's worldwide creates a need for effective and easy to perform screening methods for detection.⁷

This study was undertaken to identify the changing etiological trends of urinary tract infections (UTI), to detect the prevalence of ESBL producing uropathogens and to study their antibiotic resistance profile of those bacteria.

Materials and Methods

The study was carried out in the Department of Microbiology, at tertiary care hospital, Belagavi from January 2016 to December 2016. The study protocol was approved by the Institutional Ethical Committee. In the present study, 170 non-repetitive, midstream urine sample were collected in the sterile container. The samples were processed within 1 h of collection and were subjected for macroscopic and microscopic examination.

Further the sample is cultured on Blood agar and MacConkey agar by Standard loop technique. The culture plates were incubated at 37°C for 18-24 h under aerobic conditions. Identification of bacterial growth was confirmed by standard microbiological and biochemical techniques.⁸

Antibiotic sensitivity testing (AST) was performed by the Kirby-Bauer disc-diffusion method on Mueller-Hinton agar.⁹ The following antibiotics were tested: Gentamycin (10 mcg), Ampicillin (10 mcg), Amoxycylav (30mcg), Co-trimoxazole (25mcg), Norfloxacin (10 mcg), Nitrofurantoin (300 mcg), Ceftazidime (30 mcg), Cefuroxime (30mcg), Piperacillin-Tazobactam (100/10 mcg), Imipenem (10 mcg) and meropenem (10 mcg). The selection of antibiotics and interpretation of inhibition zone sizes was done according to Clinical Laboratory Standards Institute-2017 guidelines.¹⁰

Phenotypic confirmatory of ESBL production was done by double-disc synergy test (DDST). Ceftazidime (30µ) and Ceftazidime/clavulanic acid (30/10 µg) disc (Hi Media) were placed on the Mueller Hinton agar and incubated 37° C for 18-24 hrs. An increase in the zone diameter by greater than or equal to 5mm around the Ceftazidime/ Clavulinic acid disk over the Ceftazidime disk alone confirmed as ESBL producers.¹⁰ *Escherichia coli* 25922 and

a known in house ESBL producer were used as negative and positive controls respectively.

Statistical analysis

Data is analyzed using R i386 3.6.0 statistical software and Excel. Continuous variables are represented by mean \pm SD form and categorical variables by a frequency table. In below table p-value less than 0.05 indicates the significance of that variable.

Results and Discussion

The prevalence of UTI among the pregnant women was found to be 36.5% (62/170). Among total 62 isolates, 8 were Gram-positive cocci of which 2 were *Staphylococcus aureus* and 6 were *Enterococcus* species.

The ESBL and non-ESBL subjects (Patients) are more in age less than 20 years. There was no significant difference in the mean age between ESBL and non ESBL subjects as determined by t-test.

Gravidity is made into two categories as primigravida and multigravida. Gravidity and infection were independent by Chi-square test. However, odds ratio was 3.97 (CI: 3.77-4.17), that is, odds of having non-ESBL infection is 3.97 times more for the multigravida subjects compared to primigravida subjects (Table 1).

Parity is also made into two categories, primiparous and multiparous. Parity and infections are independent by Chi-square test. However, odds (2.91, CI: 2.77-3.065) of having non-ESBL infection is 2.91 times more for the multiparous subjects compared to primiparous subjects.

There is no significant difference in the gestational age for ESBL and non-ESBL subjects by t-test (Table 1).

Among 54 gram negative bacilli, *E. coli* 31(50%) was the predominant one followed by *K. pneumonia* 11(17.7%). ESBL producing phenotype was found most frequently among *Escherichia coli* 3(9.68%), followed by *Citrobacter freundii* 2(50%), *Klebsiella pneumonia* 1(12.5%), *Citrobacter koseri* 1(50%), *Proteus mirabilis* 1(20%) [Table 2].

Antibiotics resistance outcome on ESBL

The antibiotic resistance profile of ESBL bacteria, 77.78% resistance is observed for Cefuroxime antibiotic, followed by 62.5% for Trimethoprim-sulfamethoxazole and Gentamycin. A low degree of resistance to Meropenem (12.5%) and Imipenem (14.6%) was observed for ESBL producers and hence may be helpful in combating severe infections. *Escherichia coli* showed higher resistance to most of the antibiotics.

The prevalence of UTI in pregnant women was found to be 36.5%. The Gram-negative bacteria predominated, with *E. coli* being the most common pathogen (50%) isolated in the study. Prevalence of ESBL production in Enterobactericea in the present study is 14.8%. Imipenem and Meropenem were found to be the most effective drug in this study, indicating that Carbapenemes are the drugs of choice for treating serious infections caused by ESBL – producing bacteria.¹² Non-ESBL majority of the organism showed resistance for Amoxicillin-clavulanic acid and Ampicillin.

The ESBL producers also have resistance to other antibiotic groups and there are very few antibiotics which are effective against these agents. A delay in appropriate therapy can cause severe complications. The susceptibility patterns seen in our study seem to suggest that it is absolutely necessary to obtain sensitivity reports before initiation of antibiotic therapy in cases of suspected UTIs (Table 3).

Table.1 Frequency distribution of age, gravidity and parity with ESBL and Non-ESBL isolates from patients

Variable	Sub-category	Number of isolates from patients	ESBL isolates from patients (n=8)	Non-ESBL isolates from patients (n=44)	p-value
Age	< 20	11 (6.51)	1 (9.09)	5 (45.45)	0.545
	20-25	81 (47.93)	4 (4.94)	19 (23.46)	
	25-30	56 (33.14)	3 (5.36)	14 (25)	
	30-35	16 (9.47)	0	5 (31.25)	
	35-40	5 (2.96)	0	1 (20)	
	Mean ± SD	24.69 ± 4.15	23.62±3.16	24.57±4.15	
	Age Range	(18,40)	(18,28)	(18,35)	
Gravidity	primigravida	71 (42.01)	5 (2.96)	13 (7.69)	0.1034
	multigravida	98 (57.99)	3 (1.78)	31 (18.34)	
Parity	primiparous	79 (46.75)	5 (2.96)	16 (9.47)	0.2634
	multiparous	90 (53.25)	3 (1.78)	28 (16.57)	
Gestational Age (in days)		210.22±58.4	164.62±67.6	207.86±61.56	0.07764

Table.2 ESBL producers among Gram negative bacteria

Isolates	ESBL Producers (%)	Non-ESBL Producers (%)	Total (%)
<i>Escherichia coli</i>	3 (9.68)	28 (90.32)	31 (59.61)
<i>Citrobacter freundii</i>	2 (50)	2 (50)	4 (7.69)
<i>Klebsiella pneumoniae</i>	1 (12.5)	10 (90.9)	11 (17.31)
<i>Citrobacter koseri</i>	1 (50)	1 (50)	2 (3.84)
<i>Proteus mirabilis</i>	1 (20)	4 (80)	5 (9.62)
<i>Proteus vulgaris</i>	0 (0)	1 (100)	1 (1.92)
Total	8 (15.38)	46 (85.18)	54 (100)

Table.3 Antibiotic resistance profile of ESBL bacterial isolates

Antibiotics	<i>Escherichia coli</i> (n=3)	<i>Citrobacter freundii</i> (n=2)	<i>Citrobacter koseri</i> (n=1)	<i>Klebsiella pneumoniae</i> (n=1)	<i>Proteus mirabilis</i> (n=1)	Total (%)
MRP	0	0	0	0	1 (100)	1 (12.5)
COT	2 (66.67)	1 (50.00)	0	1 (100)	1 (100)	5 (62.5)
CXM	2 (66.67)	1(50%)	1 (100%)	0	1 (100)	5 (62.5)
AMP	2 (66.67)	1 (50.00)	0	0	0	3 (37.5)
GEN	1 (33.33)	0	1 (100.00)	0	1 (100)	3 (37.5)
NX	3 (100)	0	0	0	1 (100)	4 (50)
CAZ	2 (66.67)	2 (100)	1 (100.00)	1 (100)	1 (100)	7 (77.78)
AMC	3 (100)	1 (50.00)	0	0	0	4 (50)
PTZ	1 (33.33)	0	1 (100.00)	1 (100)	1 (100)	4 (50)
IMP	1 (33.33)	0	1 (100.00)	0	0	2 (25)
NIT	1 (33.33)	2 (100)	0	1 (100)	0	4 (50)

Abbreviations: - AMP:-Ampicillin, AMC:-amoxicillin-clavulanic acid, COT: - cotrimoxazole, CXM:- Cefuroxime, GEN:- gentamicin , NX :- Norfloxacin, CAZ:- Ceftazidime, PTZ:- Piperacillin-tazobactam, IMP:- Imipenem, NIT:- Nitrofurantoin,

The study emphasizes that microbiology laboratories should adequately screen for ESBL-producing strains of the family Enterobacteriaceae as the infections caused by these organisms are difficult to treat. Limitation of this study is, only the symptomatic pregnant women were included in the study, further studies should be done to include both symptomatic and asymptomatic pregnant women with UTI over a longer period of time to get proper trend of antibiotic resistance in the hospital.

ESBL producing organisms are increasing rapidly and are a major problem among the pregnant women all over the world. This study showed that pregnant women harbour one or more urinary pathogens that are antibiotic resistant. So the routine urine culture test for pregnant women is mandatory. Further the pregnant women should be educated to maintain hygiene in pregnancy. Therefore it is also suggested that further investigations to be undertaken to study the microbiological factors that make ESBL producing bacteria.

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