RESEARCH ARTICLE

Antibiotic Resistance Pattern in Urinary Tract Infection during Pregnancy in South Indian Population

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Abstract

Background: One of the most common complications during pregnancy is urinary tract infection (UTI) which can be either symptomatic or asymptomatic. Symptomatic bacteriuria occurs in 2%–7% of pregnant women in the first trimester and asymptomatic bacteriuria occurs in 5–10%; 20–30% of which progress to pyelonephritis if left untreated. There was a 50% increase in the risk of low birth weight and a significant increase in pre-term delivery, pre-eclampsia in pregnant women with bacteriuria. Safest antibiotics used in pregnancy are nitrofurantoin, cephalosporin, penicillin, and fosfomycin trometamol. With the emergence of drug resistance among the Gram-negative and Gram-positive bacteria, the choice of drugs for the treatment of UTI is very limited. **Objective:** The purpose of our study was to find out the antibiotic resistance pattern in UTI during pregnancy with the common causative organism, their antimicrobial sensitivity and resistance pattern. This study will help in choosing the most effective antibiotic for the treatment of UTI in pregnancy. **Materials and Methods:** Urine samples were subjected to microscopy and those found positive of UTI were inoculated in nutrient agar to confirm the presence of microorganisms. This was followed by streaking in HI chrome and McConkey agar. Identification was done using Gram staining and biochemical tests. Antibiotic resistance was evaluated using Kirby–Bauer disk diffusion method. **Results:** The most common causative organism was found to be *Escherichia coli*. Nitrofurantoin showed the highest sensitivity toward all the organisms.

Key words: Antibiotic resistance, antibiotics, bacteriuria, pregnancy, urinary tract infection

INTRODUCTION

One of the most common complications during pregnancy is urinary tract infection (UTI) which may be either symptomatic or asymptomatic. Bacteriuria occurs in 2–7% of pregnant women in the first trimester.[1] Asymptomatic bacteriuria occurs in 5–10% of pregnancies, 20–30% of which progress to pyelonephritis if left untreated.[18,22] In uncomplicated UTI, the primary urinary tract pathogens are *Escherichia coli, Klebseilla pneumonia, Protease,* and Group B *Streptococci*.[3] There was a 50% increase in the risk of low birth weight and a significant increase in pre-term delivery and pre-eclampsia in pregnant women with bacteriuria.[33] Safer antibiotics used in pregnancy are nitrofurantoin, cephalosporins, penicillin, and fosfomycin trometamol.[31-34] Both Gram-negative bacilli and Gram-positive cocci showed significantly high resistance to the β-lactam group. *Pseudomonas* isolates were resistant to cefotaxime. However, cefoperazone-sulbactam showed a better sensitivity against *Enterobacteriaceae* and *Pseudomonas* isolates.[18] All the strains of *Staphylococcus* species were resistant to oxacillin and carbapenem.[6,7] Significantly low resistance was noted for amoxicillin by

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the *Enterococcus faecalis*. Nitrofurantoin showed a good sensitivity against *Enterobacteriaceae* and *Staphylococcus aureus*, whereas resistance was shown in *Coagulase* negative *Staphylococci* isolates.[9-11] Fosfomycin trometamol had been reported to have high activity against the majority of *Enterobacteriaceae* but not toward the Gram-positive bacteria.[13]

With the emergence of drug resistance among the Gram-negative and Gram-positive bacteria, the choice of drugs for the treatment of UTI is very limited.[2] Hence, our study assesses the prevalence of the most common microorganism, antimicrobial resistance, and sensitivity in UTI in pregnant women to update the data on antibacterial activity prevalent in Sait Hospital, Government Headquarters Hospital, Ooty.

MATERIALS AND METHODS

This is a prospective, experimental, and cohort study conducted from October 2015 to March 2016 at Sait Hospital, Government Headquarters Hospital, Ooty, and the Department of Biotechnology, JSS College of Pharmacy, Ooty. The study was conducted for a total period of 6 months. Only pregnant women with a positive diagnosis of UTI were recruited for the study. Pregnant women with infections other than UTI and who are taking antibiotics for UTI and other infections were excluded from the study.

Sample collection and processing

A total of 96 urine samples were collected from pregnant women with or without symptoms of UTI who visited the Sait Hospital, Ooty, as part of their regular monthly checkup. The importance of aseptic technique of urine collection was emphasized to the patients. Simultaneously, patient data were collected, and informed consent was obtained. Urine samples were processed in the laboratory within 2 h of collection, and those samples which were not processed were kept refrigerated at 4°C until processed.

The samples were subjected to urine microscopy. This was done by adding 10ml of urine sample into the test tubes which then underwent centrifugation at 2000 rpm for 2 min. The supernatant was discarded, and a drop of deposit was placed on the slide and examined under the microscope at 40× magnification. Pus cells were detected in urine samples, and those samples which checked ≥10 cells/hpf were considered as positive of UTI.

Subsequently, the urine samples were inoculated in nutrient broth to confirm the presence of microorganisms. After 24 h of incubation at 37°C, when the growth was confirmed in the nutrient agar, the samples were processed to identify the uropathogens.

MacConkey agar and HI Chrome UTI agar were used for streaking.[4] These two media were selected because of their ability to facilitate the growth of majority of organisms that cause UTI such as *E. coli, E. faecalis* and *K. pneumoniae*. Once the samples were streaked on to the medium, it was left overnight for incubation at 37°C. Identification of the bacterial pathogens was made by relating the colors of the colonies formed to their respective medium. For example, pink-red color in MacConkey agar indicates the presence of *E. coli.*

Furthermore, confirmatory tests such as Gram staining method and biochemical tests were used to confirm the identified organisms. Accordingly, the common causative organism for UTI was identified.

The antibiotic resistance pattern was obtained by performing Kirby–Bauer disk diffusion method according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2007).[21-30] The panel of antibiotic discs chosen for the isolated organisms included nitrofurantoin (300 mcg), ciprofloxacin (5 mcg), norfloxacin (10 mcg), cefotaxime (30 mcg), gentamicin (10 mcg), and ceftriaxone (30 mcg). More importantly, the antibiotics were chosen based on their usage in Sait Hospital, Ooty, as well as based on their availability in the laboratory. The results were classified into sensitive, intermediate, and resistant according to the criteria set by CLSI [Figure 1].

RESULTS

Of 96 urine samples collected 24 were found to be positive on culture [Table 1].

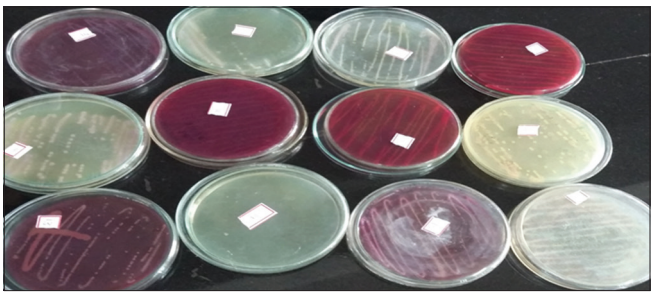

Figure 1: Microbial growth in Hi chrome urinary tract infection agar and Macconkey agar

Table 1: Prevalence of UTI among pregnant women

Total number of urine samples screened	Number of samples showed significant growth in culture	Percentage of UTI prevalence in pregnant women
96	24	25

UTI: Urinary tract infection

The age of the patients ranging from 18 to 26 years showed a high prevalence of UTI. Most of the patients with bacteriuria were in the second trimester (41.6%) followed by third (37.5%) and first trimester (20.8%) of pregnancy. Majority of patients with UTI were in gravida-1 (45.8%), and weight ranged from 30 to 50 kg [Table 2].

Among the 91 cases screened for asymptomatic UTI, 5 were found to be culture positive accounting for 21% positive asymptomatic UTI cases. On the other hand, 5 symptomatic cases which were screened showed positive culture [Table 3].

From the 24 isolates, 20 were Gram-negative while 4 were Gram-positive bacteria [Table 4]. Gram-negative isolates were *E. coli, Pseudomonas aeruginosa,* and *K. pneumoniae* whereas the only Gram-positive organism isolated was *E. faecalis.*

Table 2: Patient characteristics

Variables	Numbers (%)
Age (years)	
18–22	9 (37.5)
23–26	9 (37.5)
27–30	6 (25)
Gestational period (trimester)	
First	5 (20.8)
Second	10 (41.7)
Third	9 (37.5)
Gravida	
G1	11 (45.8)
G2	9 (37.5)
G3	4 (16.6)
Weight (kg)	
30–50	13 (54.1)
51–70	9 (37.5)
71–90	2 (8.3)

Common causative organism

The most common causative organism involved in the present study was found to be *E. coli* accounted for 12 (50%), followed by *P. aeruginosa* 6 (25%), *E. faecalis* 4 (17%), and *K. pneumoniae* 2 (8%) [Figure 2].

Antibiotic resistance pattern

The antibiotics tested for resistance were nitrofurantoin, ciprofloxacin, norfloxacin, amoxicillin-clavulanate (amoxiclav), cefotaxime, gentamicin, and ceftriaxone [Table 5]. Antibiotic resistance pattern of the isolates revealed that *E. coli* with 8.3% resistance to nitrofurantoin, 8.3% resistance to ciprofloxacin, 25% resistance to norfloxacin, 83.3.% resistance to amoxiclav, 75% resistance to gentamicin, and 8.3% resistance to ceftriaxone.

The antibiotic which showed high sensitivity against *E. coli* was nitrofurantoin.

P. aeruginosa and *K. pneumoniae* showed high resistance to gentamicin (83.3 and 100%, respectively). *E. faecalis* showed similar resistance pattern to nitrofurantoin, ciprofloxacin, norfloxacin, and amoxiclav (25%). Overall, the resistance was highest for gentamicin (66.6%). A complete resistance to gentamicin was observed with *K. pneumonia. E. faecalis* showed 25% resistance to nitrofurantoin, ciprofloxacin, norfloxacin, and amoxiclav 83.3 and 66.6% of *P. aeruginosa* were resistant to gentamicin and ciprofloxacin, respectively.

When evaluating the overall sensitivity patterns of the antibiotics used, nitrofurantoin showed the highest

Table 3: Prevalence of asymptomatic and symptomatic UTI among pregnant women

Total cases	Number of cases screened	Culture positive	Prevalence %
Asymptomatic	91	19	21
Symptomatic	5	5	100

UTI: Urinary tract infection

Table 4: Frequency of Gram-positive and Gram-negative uro pathogens isolated

Bacterial Isolates	Gram-positive uro pathogens	Gram-negative uro pathogens
Total no of isolates	4	20
% age of isolates	17%	83%

sensitivity toward all the organisms. Ciprofloxacin stands at an intermediate position with their sensitivities varying from 25 to 50%. In the case of norfloxacin, they showed high sensitivity toward all the four organisms *(E. coli* - 75%, *K. pneumonia* - 100%, *E. faecalis* - 75%, and *P. aeruginosa* - 83.3%). The sensitivity of *E. coli* toward amoxiclav is low (16.6%) as compared to other organisms such as *K. pneumonia* and *E. faecalis*. Cefotaxime and ceftriaxone remain at a safe position in relation to susceptibility pattern. Gentamicin is at a stake position with minimum sensitivity toward all the organisms. The sensitive antibiotics to *E. coli* isolates were nitrofurantoin (91.6%), norfloxacin (75%), ceftriaxone (75%), gentamicin (75%), and cefotaxime (66.6%). 100% sensitivity was observed with nitrofurantoin, norfloxacin, cefotaxime, and ceftriaxone against *K. pneumoniae*. Norfloxacin and amoxiclav (both 75%) were effective against *E. faecalis*. *P. aeruginosa* showed 66.6% sensitivity to cefotaxime.

Table 5: Overall resistance pattern of antibiotics in UTI

Antibiotic/Organism	PA	EF	KP	*E. coli*
NF	NA	I	S	S
CF	R	I	S	I
NFX	S	S	S	S
AMX-CL	NA	S	I	R
CEF	S	NA	S	S
CFM	I	NA	S	S
GENTA	R	NA	R	R

S: Sensitive, I: Intermediate, R: Resistance, NA: No activity, -: Nil, n: Sample size, PA: *Pseudomonas aeruginosa*, EF: *Enterococcus faecalis*, KP: *Klebseilla pneumonia*, *E. coli*: *Escherichia coli*, NF: Nitrofurantoin, CF: Ciprofloxacin, NFX: Norfloxacin, AMX-CL: Amoxicillin clavulanate, CEF: Ceftriaxone, CFM: Cefotaxime, GENTA: Gentamicin

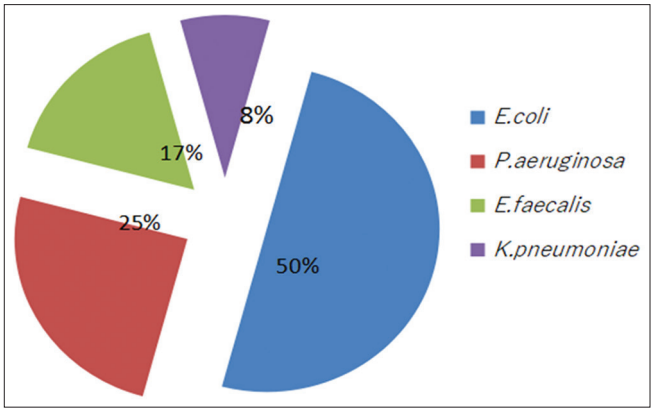

Figure 2: Common causative organism

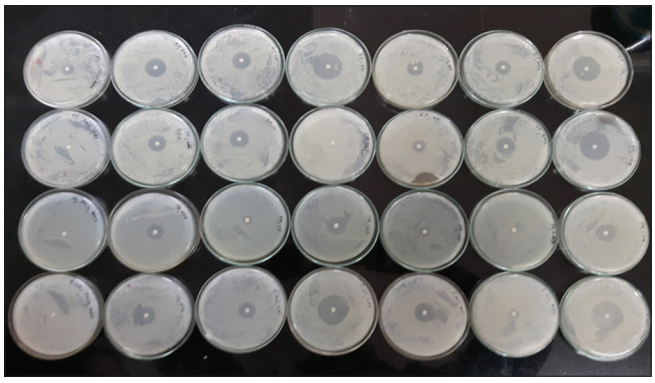

Figure 3: Results of Kirby–Bauer disc diffusion method

Cefotaxime, gentamicin, and ceftriaxone do not possess activity toward *E. faecalis;* as a result, making it naturally resistant to these antibiotics. Similarly, *P. aeruginosa* species does not hold the targets required for nitrofurantoin and amoxiclav thereby these antibiotics do not show activity against this organism [Figure 3].

DISCUSSION

UTI leads to serious complications to both mother and fetus if left untreated.[34] Hence, to prevent these complications early detection and appropriate antibiotic treatment is needed.[5]The present study was conducted to evaluate the common causative organism in UTI among pregnant women in our locality and to aid in appropriate management based on antibiotic resistance pattern. The overall prevalence rate of UTI (25%) in pregnant women in our study was higher than previous studies which were reported in Northwest Ethiopia (10.4%), Northern Tanzania (16.4%), and Chennai (14.9%) but lower than study in Bengaluru (46.6%) and UP (84.12%). The prevalence of UTI was high in the age group of the patients ranging from 18 to 26 years which are comparable to study conducted by Moghadas *et al*.[26] (age range from 20 to 30 years). In the present study, most of the patients with bacteriuria were in the second trimester (41.6%) followed by third (37.5%) and first (20.8%) trimester of pregnancy. On the other hand, studies conducted by Rizvi *et al*.[19] (53.5 and 44.5%) and Sabharwal[16] (59 and 38%) showed a higher prevalence of UTI in the first and third trimester of pregnancy, respectively.

In our study, the prevalence of asymptomatic bacteriuria was higher (79.1%) than symptomatic UTI (20.9%). This is similar to the results obtained from Tazebew *et al*.[14] (80 vs. 20%) and Nithyalakshmi and Vijayalakshmi[12] (14.77 vs.10.25%). The Gram-negative bacteria predominated, with *E. coli* (50%) as the most common pathogen isolated in the present study. Comparable findings have been reported in Tazebew *et al*.[14] (45.7%), Alemu *et al*.[15] (47.5%), and Nithyalakshmi and Vijayalakshmi[12] (65.1%). In our study, the second predominant isolate was *P. aeruginosa* followed by *K. pneumoniae*. On the contrary, the study in Rizvi *et al*.[3] and Nithyalakshmi and Vijayalakshmi[12] recognized the second common isolate as *K. pneumoniae*.

In our study, nitrofurantoin was sensitive to *E. coli* and *K. pneumoniae* (91.66% and 100%) and resistant (25%)

toward *E. faecalis*. On the other hand, a study reported that nitrofurantoin developed resistance toward Gram-negative bacteria.

Pseudomonas species showed a significantly high resistance toward ciprofloxacin (66.6%) which is completely opposed in other studies.[14,20] More than 70% of isolates were sensitive to norfloxacin. A comparable result was obtained from the study conducted by Shalini *et al*.[20] Amoxicillin clavulanate showed high resistance against *E. coli* (83.3%) this is similar to the observation made by Shalini *et al*.[20] All the four organisms had a good susceptibility pattern toward cephalosporin drugs which include ceftriaxone and cefotaxime. A similar susceptibility pattern was obtained by Sibi *et al*.[8] The resistance pattern observed for gentamicin during this study stands at an exceptional point as compared to other studies. More than 70% resistance was developed by all the isolates in the study toward gentamicin. This is very unlike the results obtained by Agersew *et al*. who came up with the conclusion that gentamicin is 92.6% sensitive to Gram-negative bacteria.

CONCLUSION

This study emphasizes the importance of regular screening of symptomatic and asymptomatic bacteriuria in pregnancy. Similar to findings from other studies this study also concluded *E. coli* as the common causative organism for UTI in pregnancy. *E. coli* showed high resistance against amoxiclav (83.3%) and high sensitivity toward nitrofurantoin. With the emergence of antibiotic resistance, the choice of antibiotics turns out to be narrow in pregnancy. This further strengthens the importance of choosing appropriate antibiotics for treating UTI in pregnancy with the help of culture test. Gentamicin with high resistance to almost all organisms causing UTI in pregnancy and being a pregnancy category D drug becomes the last choice of drug. Nitrofurantoin having a good safety profile would be a rational choice of antibiotic to treat UTI in pregnancy.

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