

A Hospital Based Prospective Study Assessment of UTI among Pregnant Women Due to E. Coli: An Observational StudyRizwan Ahmad¹, Sanjay Nag²¹Tutor, Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India²Assistant professor and HOD, Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India

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Conflict of interest: Nil

Abstract**Aim:** The aim of the present study was to investigate UTI among pregnant women due to E. coli, to know its antimicrobial drug susceptibility pattern and correlate antibiogram.**Methods:** This prospective study was conducted at the Department of Microbiology for one year, Participants were recruited from among pregnant women. Mid-stream urine samples were obtained in a sterile wide-mouth container. A total of 100 samples were collected.**Results:** Among 100 samples tested, E. coli could be isolated from 43 samples with colony count of 10⁵ CFU/ml of urine and a prevalence rate of 43%. The majority of pregnant women were in their 2nd and 3rd trimester. The mean age of the participants was 24 years. Most of the urine samples were obtained from pregnant women in the age range of 18 to 23 years. Pregnant women in the age range of 27 to 29 years contributed to the least number of samples. The rate of infection was high among the participants in the age group of 18-23 years. Of the 43 isolates, biofilm formation was detected in 28 isolates by all the three methods for biofilm detection. All isolates showed the highest resistance to the antibiotic Ampicillin (93.34%), followed by Amoxicillin- clavulanic acid (53.34%), Cefuroxime (33.34%) and Ceftriaxone (33.34%). E. coli isolates which formed biofilm displayed a significant increase in the resistance pattern to all the antibiotics and proved to be statistically significant.**Conclusion:** The association between virulence factors and antibiogram was perceived in our study. Hence, screening for virulence factors and antimicrobial sensitivity must be scheduled along with the other standard tests for pregnant women. In addition, routine urine cultures would help in timely detection of UTI.**Keywords:** Antibiotic resistance, ANC, Biofilm, UTI, Uropathogenic E. coli, Virulence factors.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Urinary tract infections (UTIs) are diseases that affect the urinary tract from the bowel to the kidney, urethra and bladder. [1] These infections affect both sexes although occur 50 times more in women than men due to their short urethra and moist peri-urethral environment. [2] In pregnant women UTIs occurrence has been attributed to decreased abdominal strength in urine voiding and lack of oestrogen that causes introital colonization with E. coli and UTI recurrence. [3] Because of reduced immunity, HIV-infected pregnant women and those with diabetes tend to suffer from UTIs more often than non-infected ones. [4] These UTIs are caused majorly by members of the Enterobacteriaceae family which includes E coli, Proteus mirabilis, Klebsiella species and Pseudomonas aeruginosa. [5] According to many studies, E. coli is the leading amongst the major UTI causing bacteria and can be found at the

community level and in hospital environments. [6] Some of its strains can survive for a long time in the outside environment and has several pathogenic strains including Enterotoxigenic E. coli, Enteropathogenic E. coli and Uropathogenic E. coli. [7] Despite this bacterium existing as a commensal, it's the main reservoir for spreading antibiotic resistance to other enteric pathogenic bacteria via mobile genetic elements. [8]

The severity of an UTI is usually influenced by the virulence of the bacteria and the susceptibility of the host. Antibiotics such as ceftazidime, amoxicillin, cefoxitin, penicillin, and norfloxacin have been widely used to empirically treat clinically examined individuals with UTI. However, over time, inappropriate prescriptions for the treatment of UTIs along with their widespread use have caused bacteria to mutate and develop

drug resistance. A UTI can be caused by bacteria resistant to common antibiotics. This makes treating UTIs more difficult and raises the risk of complications, underscoring the need for using antibiotics correctly and identifying the best drug. [9,10] The most frequent bacterial infection during pregnancy is urinary tract infection, which is connected to low birth weight, hypertension, early delivery, and intrauterine growth restriction. Urinary tract infection is classified under two types: Asymptomatic and symptomatic bacteriuria during pregnancy. Microbiologically significant bacterial growth in pregnant women's urine that does not cause UTI symptoms is referred to as asymptomatic bacteria. [11] If left untreated, it affects 2-15% of pregnant women, making it a common risk factor for pyelonephritis. Several reasons are associated with this increased rate of frequency of bacteriuria which include age and presence of genitourinary abnormalities (kidney, bladder stones, urethral, tumors, sexual activity, anemia, decreased immunity and past history of UTI) etc. *Escherichia coli* is the most frequent pathogenic microorganisms associated with both symptomatic and asymptomatic bacterial infection for 60-80% of pregnant women, which mainly causes UTI. This is the major health problem of developing countries by the surveillance data of (WHO) world health organization which reports low-income countries which high level of bacterial infection are identified with *E. coli* and *K. pneumonia* that were the most resistance pathogens. As a result, regarding the bacterial profile and antimicrobial susceptibility patterns of UTI among pregnant women is increasing in the state. [12] Some of the symptoms of UTI which includes high frequency of urination and burning sensation with pain association when urine is discharged, and rigorous urinary infection that also cause nausea, fever, chills and vomiting. The infected pregnant women are treated with particular antibiotics with the special emphasis of antibiogram results. [13]

The aim of the present study was to investigate UTI among pregnant women due to *E. coli*, to know its antimicrobial drug susceptibility pattern and correlate antibiogram with phenotypic virulence factors.

Materials and Methods

This prospective study was conducted at the Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India for one

year. Participants were recruited from among pregnant women attending ANM Medical College and Hospital, Gaya, Bihar, India after taking informed consent. Mid-stream urine samples were obtained in a sterile wide-mouth container. A total of 100 samples were collected. The following data was also gathered from the participants: maternal age, gravidity and residence. The collected samples were directly transported to the laboratory.

Bacteriological analysis of the urine samples:

The samples were processed for culture and antimicrobial drug susceptibility tests following routine microbiological techniques. Semi quantitative urine culture using a calibrated loop was performed to isolate bacterial pathogens on Blood and MacConkey's agar as per the recommendations of Kass. [14] The culture plates were incubated at 37°C for 24 hours. The negative (growth) culture plates were incubated for an additional 48 hrs. Bacterial strains were isolated from the cultures and identified using standard biochemical tests. UTI was diagnosed on the basis of pathogens being present at least 10⁵ colony forming unit (CFU)/ml of urine. However, the study dealt with only the *E. coli* isolates present.

Antibiotic sensitivity testing:

Antibiotic sensitivity testing was performed according to Kirby Bauer's disc diffusion method on Mueller Hinton agar. CLSI guidelines were followed during the experimentation.^{15,16} Sensitivity was tested against the following antibiotics: Ampicillin (AMP 10mcg), Amikacin (AK 30 mcg), Amoxicillin-clavulanic acid (AMC 30 mcg), Ceftriaxone (CTR 30 mcg), Cefuroxime (CXM 30mcg), Ciprofloxacin (CIP 5mcg), Gentamicin (GEN 10mcg), Imipenem (IPM 10mcg), Nitrofurantoin (NIT 300mcg), Norfloxacin (NX 10mcg), and Piperacillin-tazobactam (PIT 100/10 mcg) (HiMedia Laboratories, Mumbai, India).

Statistical Analysis

Statistical software package SPSS version 22 (IBM Corp. Released 2013 (IBM SPSS Statistics for Windows, Version 22.0 Armonk, NY: IBM Corp.) were used to analyse the data. Unpaired t test was applied to calculate significance differences of resistance among biofilm producing and non-biofilm producing isolates. P-value <0.05 was considered statistically significant.

Results

Table 1: Prevalence of *E. coli*

Bacteria	Source	N=100
<i>Escherichia coli</i>	Mid-stream urine samples	43 (43%)

Among 100 samples tested, *E. coli* could be isolated from 43 samples with colony count of 10⁵ CFU/ml of urine and a prevalence rate of 43%.

Table 2: Percentage distribution of E. coli isolated from pregnant women according to age

Age	Number of samples (n=100)	E. coli (n=43)
≤18-23	48	24
24-26	28	8
27-29	5	2
≥30	19	9
Total	100	43

The majority of pregnant women were in their 2nd and 3rd trimester. The mean age of the participants was 24 years. Most of the urine samples were obtained from pregnant women in the age range of 18 to 23 years. Pregnant women in the age range of 27 to 29 years contributed to the least number of samples. The rate of infection was high among the participants in the age group of 18-23 years.

Table 3: Antibiotic susceptibility pattern of the biofilm producing and non-producing Uropathogenic E. coli

Antibiotics	Non-biofilm producer (n=15)		Biofilm producer (n=28)		P Value
	R %	S %	R %	S %	
Ampicillin	14 (93.34%)	1 (6.66%)	27 (96.42%)	1 (3.57%)	p < 0.032
Amikacin	3 (20%)	11 (73.34%)	4 (14.28%)	24 (87.71%)	
Amoxicillin-clavulanic	8 (53.34%)	6 (40%)	24 (85.71%)	3 (10.71%)	
Ceftriaxone	5 (33.34%)	10 (68.18%)	17 (60.71%)	11 (39.28%)	
Cefuroxime	5 (33.34%)	15 (66.66%)	20 (71.42%)	8 (28.57%)	
Ciprofloxacin	2 (13.34%)	12 (80%)	16 (57.14%)	11 (39.28%)	
Gentamicin	0 (0%)	15 (100%)	5 (17.85%)	24 (85.71%)	
Imipenem	0 (0%)	15 (100%)	0 (0.00%)	28 (100%)	
Nitrofurantoin	0 (0.00%)	15 (100%)	1 (3.57%)	27 (96.42%)	
Norfloxacin	2 (13.34%)	12 (80%)	12 (42.85%)	15 (53.57%)	

Of the 43 isolates, biofilm formation was detected in 28 isolates by all the three methods for biofilm detection. All isolates showed the highest resistance to the antibiotic Ampicillin (93.34%), followed by Amoxicillin- clavulanic acid (53.34%), Cefuroxime (33.34%) and Ceftriaxone (33.34%). E. coli isolates which formed biofilm displayed a significant increase in the resistance pattern to all the antibiotics and proved to be statistically significant. The data clearly shows similarity in the sensitivity pattern of all the E. coli isolates. The isolates were sensitive to broad spectrum antibiotics like Imipenem, Piperacillin-tazobactam, Nitrofurantoin and to the drugs Chloramphenicol and Gentamicin (83.60%). Further, unpaired t test analysis indicated that the difference in the resistance pattern of biofilm and non-biofilm forming isolates against the 11 different antibiotics which were tested was statistically significant (p < 0.032).

Discussion

Urinary tract infection (UTI) is one of the most common infections in pregnant women and it occurs approximately in 5%-10% of all pregnancies. This study aimed to determine bacteria associated in pregnant women and their antibiotic susceptibility test in developing countries like India. The overall magnitude of UTI organism are Escherichia coli which found to be the most frequent organisms and also Klebsiella pneumonia,

Citrobacter, salmonella group A, and Enterbacterclocae. Gram negative bacteria were sensitive to ciprofloxacin, gentamicin, and nitrofurantain and fully resistant cefuroxime. The frequent appearance of isolates from urine sample are mainly growing the resistance capability. The above antibiotics can be prescribed based on side effect to prevent pregnant women in case of empirical treatment. The highest prevalence rate was observed in infected women were in the age group of 28-38yrs, with 3rd trimester of pregnant women also highest infection rate observed in the 7th month of pregnancy increased parity prone for UTI apart from individual hygiene and economic status. The condition in which bacteria are established and multiplied within the urinary tract is called Urinary Tract Infection (UTI) and mainly causes by pregnant women. It can be easily preventable and it is prevalent in women in pregnancy which shows a great challenge by physicians, pregnancy is a case associated with structural, functional and physiological changes in the urinary tract which elevate infections through urethra. [17] The majority of cases, UTI's are produced by asymptomatic bacteriuria. The risk factors such as low birth weight, preterm birth, still birth, preeclampsia, maternal anemia, sepsis, andamnionitis even when the infection is asymptomatic. [18] Other study reported that Escherichia coli, Klebsiella spp, and enterococcus spp, are the most causative agents of

UTI's. The most causative agent of urinary tract infection is *Escherichia coli* bacteria belongs to the family Enterobacteriaceae, which are rod-shaped bacteria. [19] The most common bacteria isolated in pregnant women were *E. coli*. [20] The increase in resistance of antibiotics in the progress rate of pragmatic treatments and hence it is essential to carry out susceptibility tests. UTI can either be symptomatic or asymptomatic.

Among 100 samples tested, *E. coli* could be isolated from 43 samples with colony count of 105 CFU/ml of urine and a prevalence rate of 43%. The majority of pregnant women were in their 2nd and 3rd trimester. The mean age of the participants was 24 years. Most of the urine samples were obtained from pregnant women in the age range of 18 to 23 years. Pregnant women in the age range of 27 to 29 years contributed to the least number of samples. The rate of infection was high among the participants in the age group of 18-23 years. Pregnant women are more prone to UTI than non-pregnant women. The fundamental differences in the prevalence of UTI among the pregnant population are based on the following factors: age, parity, gestation age and level of education. [21] The primary causative organisms for UTI in pregnant women are gram-positive and gram-negative bacteria, as well as yeast. In addition, the virulence characteristics and antibiotic profile were investigated. Our study revealed a higher incidence rate of UTI among females in the second and third trimester of pregnancy. According to our results, the prevalence of UTI is high in north Karnataka region as compared to other parts of Karnataka with commonest isolated pathogen as *E. coli*. [22] The effectiveness of antibiotic treatment depends on the analysis of virulence factors and antimicrobial resistance pattern of uropathogens responsible for UTI. Antimicrobial prophylaxis for women with recurrent UTI includes β -lactam drugs and Cephalosporins. Since there is emergence of drug resistance in UTI, we aimed to study the sensitivity pattern of *E. coli*, which is the predominant pathogen causing UTI. When the microbes were tested against various antimicrobials to determine their susceptibility, isolates showed high resistance to the β -lactam group of antimicrobials. This fact is a matter of concern because this group of drugs is traditionally used in UTI therapy. Similar accounts of resistance to the extended spectrum of β -lactamases among the general population infected with urinary pathogens have been reported. [23,24]

Of the 43 isolates, biofilm formation was detected in 28 isolates by all the three methods for biofilm detection. All isolates showed the highest resistance to the antibiotic Ampicillin (93.34%), followed by Amoxicillin-clavulanic acid (53.34%), Cefuroxime (33.34%) and Ceftriaxone (33.34%).

E. coli isolates which formed biofilm displayed a significant increase in the resistance pattern to all the antibiotics and proved to be statistically significant. The data clearly shows similarity in the sensitivity pattern of all the *E. coli* isolates. The isolates were sensitive to broad spectrum antibiotics like Imipenem, Piperacillin-tazobactam, Nitrofurantoin and to the drugs Chloramphenicol and Gentamicin (83.60%). Further, unpaired t test analysis indicated that the difference in the resistance pattern of biofilm and non-biofilm forming isolates against the 11 different antibiotics which were tested was statistically significant ($p < 0.032$). Studies by ME Terlizzi et al [25] and Chakraborty et al [26] concur to these findings. Their work consented to a significant correlation between virulence factors and antimicrobial resistance. In addition, they showed a high resistance of the isolates to the antibiotics generally used in UTI therapy.

Conclusion

The association between virulence factors and antibiogram was perceived in our study. Hence, screening for virulence factors and antimicrobial sensitivity must be scheduled along with the other standard tests for pregnant women. In addition, routine urine cultures would help in timely detection of UTI. Such measures could help prescribe safe and effective drugs so that appropriate therapy may be initiated. Further, periodic studies are recommended, especially among the pregnant population, to screen changes in the susceptibility pattern of UPEC. Our study assists in understanding the local antibiotic resistance rate and virulence pattern of *E. coli*, which a clinician needs take into consideration when deciding on therapy.

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