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Bacteriological profile and antimicrobial susceptibility pattern of urinary tract infection in pregnant women during routine antenatal visit in a tribal district of West Bengal, India

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ABSTRACT

Background: Urinary tract infection (UTI) is a relatively serious infection in pregnant women as it puts the health of both mother and fetus at risk. UTI encompasses a wide spectrum ranging from asymptomatic bacteriuria to symptomatic cystitis to the most dreaded complication, pyelonephritis. As the frequency of UTI in pregnancy is much more as compared to non-pregnant women, early diagnosis and treatment are very essential for the well-being of both mother and fetus. **Aims and Objectives:** The study was aimed to assess the prevalence of UTI, establish the etiology, and determine the antimicrobial susceptibility pattern in this tribal belt of West Bengal to create awareness regarding the same. **Materials and Methods:** A cross-sectional study was carried out in Jhargram Government Medical College and Hospital situated in West Bengal, India, from June 2024 to August 2024. Randomly selected 250 pregnant women were enrolled in the study with their consent. Midstream clean catch urine samples from these women were processed in the laboratory by routine culture methods to know about the etiology and antimicrobial susceptibility pattern of the isolated pathogens. **Results:** The prevalence of UTI among pregnant women was 21.2%. 64.1% of the isolated pathogens were Gram-negative and 35.8% of the pathogens were Gram-positive bacteria. The most common isolated pathogen was *Escherichia coli* (39.62%) followed by other bacteria. Majority of culture-positive pregnant women were <20 years of age. Certain parameters such as gestational age, history of UTI, and symptoms such as dysuria and fever were found to be significantly associated with UTI ($P<0.05$). Most of the Gram-negative and Gram-positive isolates were sensitive to doxycycline, gentamicin, and nitrofurantoin. **Conclusion:** Early treatment can alter the adverse pregnancy outcome associated with UTI. Thus, routine screening of all pregnant women irrespective of symptoms is important.

Key words: Pregnant women; Urinary tract infection; *Escherichia coli*; Antimicrobial susceptibility

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections affecting pregnant women worldwide. UTI refers to a pathological condition in which microorganisms invade, multiply, and colonize any part of the urinary system. Clinically, it encompasses a wide spectrum of symptoms ranging from mild irritation during voiding to bacteremia, sepsis, or even death.[1] Global prevalence of UTI ranges from 3% to 35% during pregnancy.[2] Progesterone is the hormone associated with changes in pregnancy. During pregnancy, the risk increases due to

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enlargement of the uterus anteriorly thereby narrowing the bladder, thus limiting the complete evacuation of urine.[3] It invariably increases perinatal morbidity and mortality.[4] Pregnant females have 4 times more risk as compared to non-pregnant women for UTI.[5] Pathogens of the lower gastrointestinal tract are most commonly responsible for UTI in pregnancy followed by the pathogens from vagina and rectum, as they can easily gain access in the urethra.[6] The most common causative agent is *Escherichia coli*, and others include *Proteus* species, *Klebsiella* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus* species.[7] The pathogenesis of UTI may be due to certain virulence factors such as adhesins, the aerobactin system, hemolysin, K capsule, and resistance to serum killing.[8]

UTI in females includes both asymptomatic microbial colonization and symptomatic infection of urine out of which asymptomatic bacteriuria is most common, affecting 2–10% of pregnancies.[7] Diagnosis and treatment of such asymptomatic cases become a necessity as 40% of untreated cases lead to symptomatic UTI and 30% may lead to pyelonephritis.[9] As the treatment requires prescribing antibiotics, it is a matter of concern because of the global rise in antimicrobial resistance. Besides, factors such as age, gravida, parity, history of urine infections, history of sexual activity, and urinary tract abnormalities can increase the risk of UTI as previous research reports.[2,7] This study was initiated with the objective to reduce the morbidity related to UTI in pregnant women by screening for uropathogens by early diagnosis and treatment. The etiology and sensitivity pattern of antibiotics varies between regions and so knowledge about the local area is very important to advocate antimicrobial stewardship programs and to bring about favorable maternal and fetal outcomes.

Aims and objectives

To assess the prevalence of UTI, establish the etiology and determine the antimicrobial susceptibility pattern in a tribal belt of West Bengal to create awareness regarding the same.

MATERIALS AND METHODS

Study setting

The study was carried out in Jhargram Government Medical College and Hospital for a period of 3 months from June to August 2024. Jhargram is situated in the Southern part of West Bengal, India, and is home to various tribal communities. Randomly selected 250 pregnant women attending outpatient department (OPD) for routine antenatal care with or without any clinical symptoms of UTI were included in the study. Pregnant women currently not on antibiotic therapy, having no known congenital anomaly of the urinary tract, and willing to participate were only included. Informed written consent was obtained from willing participants. Exclusion criteria included pregnant women who had undergone catheterization or hospitalization within the past 2 weeks. Clinical data such as trimester of pregnancy, gravida, parity, and symptoms were obtained by questionnaire-based interview. The identity of pregnant women was kept confidential.

Study design

A cross-sectional study was carried out on antenatal women during their routine visits to OPD.

Ethical considerations

Ethical approval for the study was obtained from the Institutional Ethics Committee as per memo no. IEC/JGMCH/2024/03.

Sample collection and storage

Antenatal mothers were advised to collect clean catch mid-stream urine samples in a sterile wide-mouthed screw-capped container and deliver immediately to the Department of Microbiology. Samples that could not be inoculated within 2 h of collection were stored at 4°C till further processing.

Sample processing

Standard calibrated loop (0.001 mL) was used to inoculate the samples in blood agar and MacConkey's agar media. Plates were incubated for 24 h at 37°C and inspected next day to look for any bacterial growth. Bacterial growth of $\geq 10^5$ colony-forming unit/mL, consisting of only one type of colony morphology, was considered as significant bacteriuria as per Kass concept. Three or more bacterial growths obtained on culture were considered as mixed flora and discarded as contamination. Identification of bacterial growth was done by colony morphology, Gram stain, and standard biochemical tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed following Kirby–Bauer disc diffusion method as per clinical and laboratory standard institute (CLSI) guidelines.[10] Mainly oral antibiotics appropriate for routine primary testing were included with few injectables. For antimicrobial susceptibility test, antibiotic discs used were ampicillin (10 μg), gentamicin (10 μg), cefuroxime (30 μg), cotrimoxazole (1.25/23.75 μg), amoxicillin/clavulanic acid (20/10 μg), nitrofurantoin (300 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), doxycycline (30 μg), linezolid (30 μg), ceftriaxone (30 μg), and piperacillin-tazobactam (100/10 μg). Antibiotic discs were procured from HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra. Cefoxitin (30 μg) disc was used to detect methicillin resistance in *S. aureus*. Mueller–Hinton agar was used for antimicrobial

susceptibility testing. Bacterial suspensions of the individual strains were prepared and compared to 0.5 Mc Farland standard turbidity. The suspension was then inoculated in the Mueller–Hinton agar plates, antibiotic discs were inserted as per the bacterial strains, and the plate was then incubated at 37°C for 18–24 h. At the end of incubation period, the zone sizes were interpreted as per CLSI, 2024 standardized zone diameters.[10]

Statistical analysis

Data were entered and computed onto Microsoft Excel. Standard statistical package for descriptive statistics and Chi-square test were used to determine the level of significance. P<0.05 was considered statistically significant.

RESULTS

Out of 250 antenatal mothers enrolled in the study, 173 were clinically asymptomatic and the rest 77 were clinically symptomatic as shown in Figure 1. Out of this, 173 asymptomatic cases, 23 (13.2%) had laboratory-confirmed UTI. On the other hand, 30 (38.9%) showed laboratory-confirmed UTI in 77 cases of clinically symptomatic UTI as shown in Figure 1. Thus, 53 (21.2%) yielded significant bacterial growth out of 250 urine samples processed from pregnant women.

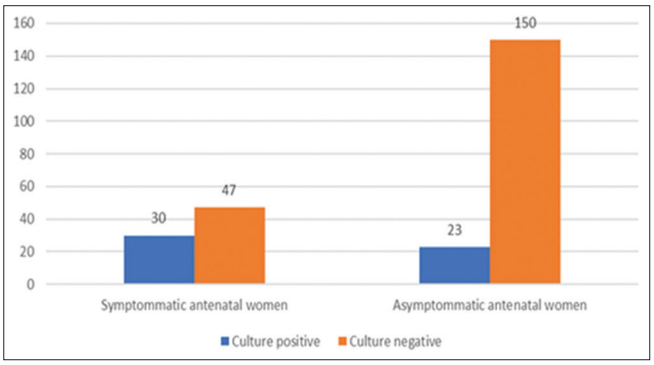

Figure 1: Culture-positive urinary tract infection in symptomatic and asymptomatic antenatal mothers Chi-square: 21.0116, P<0.00001 (significant)

As shown in Table 1, majority of the pregnant women with significant bacteriuria were <20 years, i.e., 28 (52.83%) which was followed by women of 21–25 years of age, i.e., 17 (32.07%). Based on parity, the incidence of culture-positive UTI was more in multiparous women, i.e., 35 (66.03%) as compared to primiparous, i.e., 18 (33.96%). Regarding gestational age, 38 (71.6%) women from the third trimester of pregnancy had laboratory-confirmed UTI. Age and gestational age were found to have a significant association (P<0.05) while parity and educational status did not show any statistical significance (P>0.05) with UTI. The presence of past history of UTI was significantly associated with laboratory-confirmed-positive UTI (P<0.013).

As shown in Figure 2, *E. coli* (39.62%) was found to be the predominant pathogen followed by *S. aureus* (18.86%), *Klebsiella pneumoniae* (15.09%), *Staphylococcus saprophyticus* (13.20%), *Klebsiella oxytoca* (9.43%), and Enterococcus species (3.77%).

As shown in Table 2, Gram-positive bacteria were fully sensitive to doxycycline (100%), nitrofurantoin (100%), and linezolid (100%) but highly resistant to ampicillin (89.47%) and ciprofloxacin (73.68%).

Both *S. aureus* and *S. saprophyticus* were fully sensitive to gentamicin (100%) but the susceptibility pattern of *Enterococcus* species towards gentamicin could not be reported due to unavailability of high-level gentamicin disc. Of the 10 isolates of *S. aureus*, 8 (80%) were methicillin-resistant *S. aureus* and 2 (20%) were methicillin-susceptible *S. aureus*.

Table 1: Comparison of demographic status and gestational variables with culture-positive and culture-negative UTI

Parameters	Findings	Culture positive n=53 (%)	Culture negative n=197 (%)	P-value
Parity	Primi	18 (33.96)	94 (47.71)	0.07388 (not significant)
	Multi	35 (66.03)	103 (52.2)	
Age	16–20	28 (52.83)	57 (28.9)	0.004098 (significant)
	21–25	17 (32.07)	85 (43.14)	
	≥26	8 (15.09)	55 (27.91)	
Gestational age	First trimester (≤13 weeks)	6 (11.32)	54 (27.41)	<0.00001 (significant)
	Second trimester (14–26 weeks)	9 (16.98)	93 (47.20)	
	Third trimester (≥27 weeks)	38 (71.69)	50 (25.38)	
Education	Literate	22 (41.50)	80 (40.60)	0.9057 (not significant)
	Illiterate	31 (58.49)	117 (59.39)	
History of UTI	Yes	17 (32.07)	33 (16.7)	<0.013 (significant)
	No	36 (67.92)	164 (83.2)	

UTI: Urinary tract infection

Table 2: Antimicrobial susceptibility pattern of Gram-positive cocci

Organism isolated	AST pattern	AMP	AMC	NIT	COT	DOX	CIP	LE	LZ
Staphylococcus aureus (n=10)	S	0	4	10	3	10	4	5	10
	R	10	6	0	7	0	6	5	0
Staphylococcus saprophyticus (n=7)	S	2	4	7	5	7	0	2	7
	R	5	3	0	2	0	7	5	0
Enterococci (n=2)	S	0	0	2	2	2	1	2	2
	R	2	2	0	0	0	1	0	0
Subtotal (n=19)	S (%)	2 (10.52)	8 (42.10)	19 (100)	10 (52.63)	19 (100)	5 (26.31)	9 (47.36)	19 (100)
	R (%)	17 (89.47)	11 (57.89)	0	9 (47.36)	0	14 (73.68)	10 (52.63)	0

AST: Aspartate aminotransferase, AMP: Ampicillin, AMC: Amoxicillin, NIT: Nitrofurantoin, COT: Cotrimoxazole, DOX: Doxycycline, CIP: Ciprofloxacin, LE: Levofloxacin

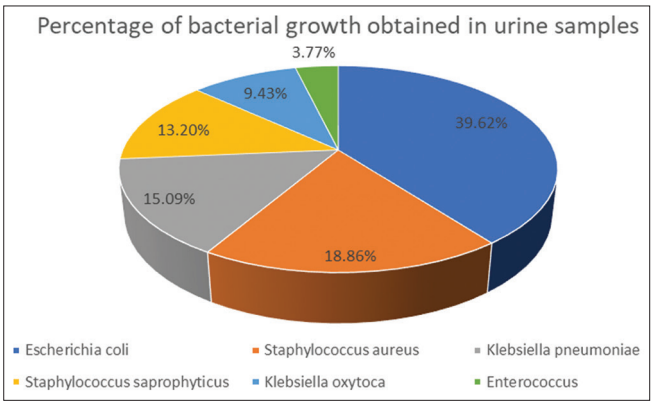

Figure 2: Bacterial profile of urinary tract infection in pregnant women

As shown in Table 3, the Gram-negative bacteria isolated showed high sensitivity to doxycycline (100%), gentamicin (100%), and nitrofurantoin (97%) but high resistance against ampicillin (97%), amoxicillin/clavulanic acid (85.29%), and cefuroxime (85.29%). *E. coli* was the predominant pathogen and was sensitive to doxycycline (100%), gentamycin (100%), and nitrofurantoin (95.23%) followed by other drugs.

As shown in Table 4, certain symptoms such as fever, dysuria, burning micturition, and hematuria were found to have significance (P<0.00001).

DISCUSSION

Pregnant women are more predisposed to developing UTI and associated pyelonephritis due to the morphological and physiological changes in the genitourinary tract during pregnancy.[11] The short female urethra and its proximity to the vagina along with poor sanitary practices predispose easy access of bacteria to the female urinary tract.[12] UTI in pregnancy may result in low birth weight of infants, premature delivery, and occasionally, stillbirth, thus imposing serious threat for the mother and fetus.[13] The prevalence of UTI among pregnant women in our study was 21.2% (53/250). This aligns with a study from Nigeria which also reported a prevalence of 21.7%.[14] A bit lower prevalence of 16.3%, 16.2%, and 16.4% was reported by Mudey et al., Sadhvi and Kose, and Olsen et al., from Wardha (India), Nagpur (India), and Tanzania, respectively.[15-17] A high culture positivity rate of 66.22% was reported by Shamim and Sathiss.[7] The difference in prevalence rate between different studies may be due to variety of factors such as category of subjects involved, their standard of living, practices, proper sample collection, and methods involved in urine examination.

In our study, 64.1% of the organisms isolated were Gram-negative bacilli and 35.8% organisms were Gram-positive cocci. This is similar to studies done by Shazia et al., and Mudey et al., which also report Gram-negative bacilli as the predominant isolate.[9,15] Gram-negative uropathogens may exhibit a high rate of isolation due to specific proteins present on its surface that helps in attachment to uroepithelial cells and thus prevent bacteria from being washed away by urinary flow. The most common uropathogen isolated in the study was *E. coli* (39.62%) followed by *S. aureus* (18.86%), *K. pneumoniae* (15.09%), *S. saprophyticus* (13.20%), *K. oxytoca* (9.43%), and *Enterococcus* species (3.77%). Previous studies from West Bengal by Mukherji and Mukherji, Saha et al., and Basu et al., also reported *E. coli* as the predominant pathogen.[8,18,19] The prevalence of *E. coli* as the predominant pathogen in UTI may be due to the fact of its presence in the rectal region as commensal and the proximity of urethra to the anal opening in female favors fecal contamination of urinary tract.[20] Our study reported *S. aureus* as the second most predominant pathogen which is in contrast to the previous studies by Shamim and Sathiss from Bangalore (India) and Basu et al., from West Bengal (India) where *K. pneumoniae* was found to be the second most isolated organism.[7,19]

Antimicrobial resistance is an alarming problem worldwide as the development of resistance is faster than the development of new antimicrobial agents.[21] The major reason is its misuse, overuse, and over-the-counter availability in our country. Both the Gram-positive and Gram-negative organisms

Table 3: Antimicrobial susceptibility pattern of Gram-negative bacilli

Organism isolated	AST pattern	AMP	AMC	PIP/TAZ	NIT	COT	DOX	GEN	CIP	LE	CXM	CTR
Escherichia coli (n=21)	S	3	5	18	20	16	21	21	9	15	5	8
	R	18	16	3	1	5	0	0	12	6	16	13
Klebsiella pneumoniae (n=8)	S	0	0	6	8	5	8	8	5	5	0	2
	R	8	8	2	0	3	0	0	3	3	8	6
Klebsiella oxytoca (n=5)	S	0	0	5	5	3	5	5	0	2	0	1
	R	5	5	0	0	2	0	0	5	3	5	4
Subtotal (n=34)	S (%)	3 (8.82)	5 (14.7)	29 (85.29)	33 (97.0)	24 (70.58)	34 (100)	34 (100)	14 (41.17)	22 (64.7)	5 (14.7)	11 (32.35)
	R (%)	31 (97.0)	29 (85.29)	5 (14.70)	1 (2.94)	10 (29.41)	0	0	20 (58.8)	12 (35.2)	29 (85.29)	5 (14.70)

AST: Aspartate Aminotransferase, AMP: Ampicillin, AMC: Amoxicillin, PIP/TAZ: Piperacillin-Tazobactam, NIT: Nitrofurantoin, COT: Cotrimoxazole, DOX: Doxycycline, GEN: Gentamicin, CIP: Ciprofloxacin, LE: Levofloxacin

showed excellent sensitivity patterns toward nitrofurantoin, doxycycline, and gentamicin which concur with a study from Ethiopia.[22] Although nitrofurantoin and gentamicin can be prescribed in pregnancy, doxycycline is not considered safe during pregnancy. All the Gram-positive bacteria were 100% sensitive to linezolid, but safety efficacy of the drug is questionable in antenatal mothers. Resistance was observed mainly against ampicillin, amoxicillin-clavulanic acid, and oral cephalosporins in Gram-negative bacteria like most other studies.[23] Our study reported high resistance to ampicillin (97%), cefuroxime (85.29%), and amoxicillin/clavulanic acid (85.29%). Ampicillin was considered a safe drug in pregnancy but the rampant spread of beta-lactamase-producing strains of bacteria and its indiscriminate use has resulted in the emergence of resistance.

In this study, 13.2% had culture-confirmed asymptomatic bacteriuria and 38.9% had symptomatic bacteriuria. Previous study by Gowda and Rajini, Balamurugan et al., from India, reported a prevalence of 10.8% and 10.25%, respectively, among asymptomatic patients.[24,25] Asymptomatic bacteriuria and its early diagnosis and treatment are very important in antenatal mothers as it reduces chances of developing cystitis and pyelonephritis.[26] Our study indicated symptomatic UTI in 38.9% of pregnant women which echoes with a study done by Taye et al., (35.3%).[27] Our findings were higher than other studies from Saudi Arabia (12.1%) and Tanzania (17.9%).[28,29] This emphasizes the importance of screening of asymptomatic bacteriuria and treatment of symptomatic bacteriuria to reduce UTI-related complications in pregnancy.

In our study, 52.83% of pregnant women diagnosed with UTI were below 20 years followed by the age group 21–25 years (32.07%). The higher prevalence of UTI in lower age groups may be due to early marriage, early sexual activity, and early childbearing.[30] Our study found a significant association between maternal age and UTI (P=0.004) unlike the study done by Mohamed et al.[31]

Our study assessed that maximum number of urine culture positivity was in the third trimester (57.14%) followed by the second trimester (28.5%) and first trimester (14.2%). This corroborates with the finding by Basu et al., who also reported higher proportion of culture-positive UTI with increased gestational age.[19] The increased size of the uterus in the last trimester puts pressure on the bladder resulting in vesicoureteric reflex and retention of urine which serves as good growth medium for bacteria. Multiparous women are at a higher risk of UTI during pregnancy. Our study also suggested increased culture positivity in multiparous (66.66%) to primiparous (33.33%) as reported by other studies.[32] However, it was not significantly associated with UTI (P=0.0738). We found previous history of UTI

Table 4: Symptoms of antenatal mothers and its correlation with culture-positive UTI

Symptoms	Culture positive n=53 (%)	Culture negative n=197 (%)	P-value	OR at 95% CI
Dysuria				
Present	24 (45.28)	1 (0.5)	<0.00001 (significant)	162.2069 (21.13–1245.066)
Absent	29 (54.71)	196 (99.49)		
Fever				
Present	16 (30.18)	2 (1.01)	<0.00001 (significant)	42.1622 (9.300–191.13)
Absent	37 (69.81)	195 (98.98)		
Burning micturition				
Present	48 (90.56)	2 (1.01)	<0.00001 (significant)	936.000 (176.20–4972.12)
Absent	5 (9.43)	195 (98.98)		
Hematuria				
Present	7 (13.20)	1 (0.50)	<0.00001 (significant)	29.826 (3.58–248.43)
Absent	46 (86.79)	196 (99.49)		
Loin pain				
Present	5 (9.43)	10 (5.07)	0.243 (not significant)	1.947 (0.63–5.96)
Absent	48 (90.56)	187 (94.92)		

OR: Odds ratio, CI: Confidence interval

(P<0.013) and certain symptoms such as dysuria, fever, burning micturition, and hematuria to be significantly associated with UTI (P<0.00001).

Limitations of the study

Minimum inhibitory concentration (MIC) of antibiotics could not be determined and commented upon and follow-up of pregnant women could not be done.

CONCLUSION

A hospital-based knowledge of the prevalent pathogens of UTI among pregnant women and an antibiotic policy aimed at providing empiric treatment before the culture report arrives will help in reducing adverse pregnancy outcomes in antenatal women. Regular surveillance of antimicrobial susceptibility patterns, record keeping to look for any change in the pattern, and routine screening of asymptomatic patients by urine culture examination will serve as a boon for the antenatal mothers. Furthermore, health education for pregnant women will help prevent a lot of infections.

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