Detection of Bacterial Uropathogens and Their Antibiotic Susceptibility Pattern Among Pregnant Women Attending Rajshahi Medical College Hospital

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Abstract

Background: A urinary tract infection (UTI) is an infection that can affect any part of the urinary system and is a major cause for concern for pregnant women. Untreated urinary tract infections during pregnancy have been linked to adverse outcomes for both mother and child, including pyelonephritis, sepsis, septic shock, hypertensive illness of pregnancy, anemia, acute and chronic renal failure, intrauterine growth restriction, premature delivery, foetal mortality, and an increase in the rate at which cesarean sections are performed. Uropathogens may acquire resistance to antibiotics if therapy is not guided by culture and susceptibility testing of the patient's urine. **Objective:** The purpose of this study was to identify uropathogenic bacteria causing urinary tract infection among pregnant women attending at Rajshahi Medical College Hospital and to determine their antibiotic susceptibility pattern. **Method:** This cross-sectional descriptive study was conducted among pregnant women with clinically suspected UTI, attending the Gynae & Obstetrics department at Rajshahi Medical College Hospital. The sample size of this study was 294. Urine samples were collected in the early morning and cultured on MacConkey's agar, blood agar, nutrition agar, and chromogenic UTI agar. Overnight incubation at 37 degrees Celsius resulted in a significant bacterial growth when the colony count was at least 10^5 per milliliter. Then, the modified Kirby Bauer disc diffusion method was used to test for antimicrobial susceptibility, as recommended by the Clinical and Laboratory Standards Institute. **Results:** A total of 294 pregnant mothers,114(38.77%) pregnant women were culture positive for UTI. Among the 114 culture positive isolates, single growth of bacteria were 97.36% and multiple growth of bacteria were 2.94%. Gram-negative bacteria were more prevalent (80.34%): *Escherichia coli* 57(48.71%), *Klebsiella spp.* 21(17.94%), *Pseudomonas spp.* 9 (7.69%), *Proteus spp.* 5 (4.27%) where Gram positive bacteria were 19.65%: *Enterococcus spp.*11(9.4%), CoNS 7 (5.98%) followed by *Staphylococcus aureus* 5 (4.27%) respectively. Majority of the Gram negative bacterial isolates were sensitive to Meropenem, Nitrofurantoin and Gram positive bacterial isolates were sensitive to Vancomycin and Linezolid respectively. **Conclusion:** Antimicrobial resistance is on the rise, which poses a problem for treating UTIs. The problem has become more challenging

as there are fewer safe antimicrobials available. Regular antenatal checkups should include a urine culture to ensure the health of pregnant women.

Key words: urinary tract infection, uropathogen, antimicrobial susceptibility pattern, *E.coli, Klebsiella spp.,* pregnant women.

Introduction

An infection in the urinary tract causes inflammation every where in the system due to the presence and proliferation of bacteria. UTIs are more common in pregnant women because of the many ways in which their bodies change throughout pregnancy. The urethra becomes more open, the bladder swells, and the tone of the urethra weakens, all of which contribute to an increase in urine retention and vesicoureteric reflux.[1] These modifications result from the combined actions of the expanding uterus and the higher levels of progesterone and estrogen. Glycosuria, the presence of glucose in the urine, is a common pregnancy complication, affecting up to 70% of expecting mothers.[2] Pyelonephritis, sepsis, severe sepsis, septic shock, hypertensive illness of pregnancy, anemia, acute and chronic renal failure, intrauterine growth restriction, early delivery, foetal mortality, and increased caesarean section are all related with untreated UTIs in pregnancy.[1]

Urinary tract infection (UTI) is the most common bacterial infection in pregnancy with 5-10% of women experiencing a symptomatic UTI during pregnancy. Asymptomatic bacteriuria occurs in 2% to 10% of all pregnancies. In a recent review, the global prevalence of UTI and/or asymptomatic bacteriuria in pregnancy ranged from 3 to 35% in different parts of the world in which increased prevalence is predominantly seen in developing countries including Africa and Asia.[3] Pregnant women are more susceptible to develop UTI and according to WHO one in five women will encounter the UTI and pregnant women have 4 times higher rate of developing UTI.

Most importantly UTI in pregnancy is mainly related to poor hygiene and low socioeconomic status of developing countries.[4] The gold standard for the diagnosis of a urinary tract infection is the detection of the pathogen in urine in the presence of clinical symptoms particularly in patients with non -specific syptoms.The pathogen is detected and identified by urine culture which also allows quantitative estimation of bacteriuria.[5] The most common bacterial uropathogens in UTI are: Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis and Enterobacter cloacae.*[6]

Without the urine culture and susceptibility testing to guide therapy there is a risk for development of antimicrobial resistance among uropathogens. Else-where antimicrobial resistance is a major health problem in the treatment of UTI caused by *E.coli* and *Klebsiella pneumoniae,* the dominant uropathogens in pregnant women.[7] Therefore, it is important to determine the antibiotic susceptibility profile of the bacterial strains linked to symptomatic UTI and asymptomatic bacteriuria in pregnancy.We wanted to learn how common UTIs are among pregnant women at Rajshahi Medical College Hospital, as well as what kinds of bacteria cause them and how antibiotic-resistant they tend to be.

Methods

This cross-sectional descriptive study was carried out among pregnant women with clinically suspected UTI, attending in department of Gynae & Obstetrics Rajshahi

Medical College Hospital, Rajshahi. Microbiological works were done in department of Microbiology in Rajshahi Medical College. This study included all pregnant women based on the symptoms of lower abdominal pain, fever, dysuria, frequency, urgency. Catheterized patients in respective of symptoms and asymptomatic cases in all trimester were also included. Pregnant women who were on antibiotic therapy and who were not willing to participate in this study were excluded. sample size was 294 selected purposively. Data were collected by administered questionnaire. The urine samples from pregnant women were collected in aseptic precautions. Early morning clean-catch midstream urine was collected from each pregnant women into a wide-mouthed sterile screwcapped container. With a Calibrated micro-loop 0.001 ml. of urine was cultured on to a MacConkeys agar, blood agar, nutrient agar and chromogenic UTI agar media. Centrifuged urine deposit was examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells. Pus cells >5/HPF were also considered significant for infection. Then antimicrobial susceptibility tests were performed by modified Kirby Bauer disc diffusion method following Clinical and Laboratory Standards. Prior to the commencement of the study, the study protocol was approved by the Institutional Review Board and Ethical Review Committee in Rajshahi Medical College.

Results

Urine samples from 294 pregnant women with or without symptoms of UTI were collected so that uropathogenic bacteria could be isolated, identified, subcultured, and subjected to biochemical testing. Figure-I, showed among the total 294 cases 114 (38.77%) were culture positive and the rest 180 (61.22%) were culture negative. There were 114 cases that showed a positive culture; 111 of those were single isolates (97.36%) and 3 were multiple isolates (2.64%) (Table1). Of the 117 bacterial isolates, 94 (80.34%) were Gram-negative bacteria, while the remaining isolates 23 (19.65%) were Gram-positive bacteria (Table2). Out of total 117 isolates, *Escherichia coli* was found 57 (48.71%) followed by *Klebsiella spp.* 21 (17.94%), *Pseudomonas spp.* 9(7.69%), *Proteus spp.* 5(4.27%), *Enterobacter spp.* 2(1.7%), respectively. Among the Gram positive isolates, *Enterococcus spp.* was 11(9.4%) followed by CoNS 7(5.98%), *S.aureus* 5(4.27%) respectively (Table3). Isolated bacterial uropathogens showed a high level of multiple antimicrobial resistances against commonly prescribed drugs. Among gram negative organisms, the commonest organism *E.coli* showed the high percentage of sensitivity to towards Meropenem (75.43%), Nitrofurantoin(66.67%) and Azithromycin (61.04%). Lowest susceptibility showed towards Amoxiclav (14.03%), Cefuroxime (19.29%), Cotrimoxazole and Cefotaxime (21%) respectively. The second most prevalent pathogen. *Klebsiella spp.* showed highest sensitivity towards Meropenem (81%) followed by Nitrofurantoin and Azithromycin which were (71.42%) and (61%) respectively. Lowest susceptibility were shown against Cefuroxime and Cotrimoxazole (14.28%) followed by Ceftazidime, Cefotaxime, Amoxiclav (19.04%) respectively. Antibioticsusceptibility pattern of *Pseudomonas spp.* Pipercillin/Tazobactam showed maximum sensitivity of (88.88%) followed by Meropenem and Nitrofurantoin

((77.77%), Azithromycin (66.66%) respectively. Lowest susceptability were shown against Cefuroxime, Cefepime and Cotrimoxazole (11.11%) respectively. However, all others gram negative isolates were similarly resistant to most of the antibiotics as that of E. coli and Klebsiella pneumonia (Table-4).

Table-5 showed that, Gram positive bacterial isolates were 100% sensitive to Vancomycin and Linezolid respectively. Lowest susceptibility was shown against Ciprofloxacin, Ceftriaxone and Ceftazidime respectively.

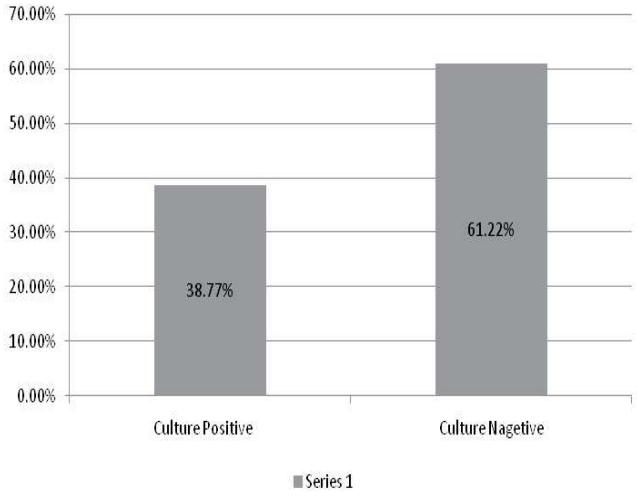

Figure 1: Frequency of culture positive and culture negative cases (N=294)

Table1: Proportion of single and multiple bacterial isolates in culture positive cases (N=114)

Bacterial isolates	Frequency	Percentages (%)
Single growth	111	97.36
Multiple growth	3	2.64

Table 2: Gram staining characteristics of bacteria among the bacterial growth (N=117)

Isolated Organism	Number (%)
Gram negative	94(80.34%)
Gram positive	23(19.65%)

Table 3: Pattern of bacteria isolated from urine culture (N=117)

Bacteria	Number	Percentages (%)
Escherichia coli	57	48.71
Klebsiella spp.	21	17.94
Pseudomonas spp.	9	7.69
Proteus spp.	5	4.27
Enterobacter spp.	2	1.7
CoNS	7	5.98
Enterococcus spp.	11	9.4
Stapphylococcus aureus	5	4.27

Table 4: Antimicrobial susceptibility pattern of Gram negative bacteria (n=94)

Antimicrobial Agent		*E.coli (n=57) %*	*Klebsiella spp.(n=21) %*	*Pseudomonas spp.(n=09) %*	*Proteus spp.(n=05) %*	*Enterobacter spp.(n=02) %*
Cotrimoxazole	S	21%	14%	11%	20%	0%
	R	79%	86%	89%	80%	100%
Ciprofloxacin	S	36.50%	33.33%	55.55%	40%	50%
	I	3.17%	9.52%	11.11%	20%	
	R	60%	33.33%	55.55%	20%	50%
Meropenem	S	75%	81%	78%	80%	100%
	R	25%	19%	22%	20%	0%
Cefotaxime	S	21%	19%	-	40%	50%
	I	4.76%	-	-	-	-
	R	75%	81%	-	40%	50%
Cefepime	S	24.56%	33.33%	22.22%	60%	50%
	R	76.19%	66.67%	77.77%	40%	50%
Ceftriaxone	S	39%	38%	44%	40%	50%
	I	-	-	11%	20%	-
	R	61%	62%	56%	40%	50%
Ceftazidime	S	30%	19%	33%	20%	0%
	I	-	5%	-	-	-
	R	70%	76%	66.66%	80%	100%
Cefuroxime	S	19.29%	14.28%	11.11%	20%	0%
	I		4.76%	-	-	-
	R	80.70%	76.16%	88.88%	80%	100%
Nitrofurantoin	S	65.32%	71.42%	77.77%	60%	100%
	I	6.34%	-			
	R	28.57%	28.58%	22.22%	40%	100%
Azithromycin	S	60.31%	61.90%	66.66%	60%	50%
	R	39%	38%	33%	40%	50%
Amoxiclav	S	14%	19%	22%	20%	0%
	I	3.17%	-	-	-	-
	R	83%	81%	78%	80%	100%
Piperacilline/ Tazobactam	S	-	-	89%	-	-
	R			11%		

S=Sensitive, I=Intermediate, R=Resistant

Table 5: Antimicrobial susceptibility pattern of Gram positive bacteria (N=23)

Antimicrobial agent		*Enterococcus spp.(n=11)* %	*CoNS (n=07)* %	*Staphylococcus aureus* (n=05) %
Vancomycin	S	100.0	100.0	100.0
	R	0.0	0.0	0.0
Oxacillin	S	72.72	100.0	80.0
	R	27.0	0.0	20.0
Linezolid	S	100.0	100.0	100.0
	R	0.0	0.0	0.0
Meropenem	S	82.0	86.0	80.0
	R	18.0	14.0	20.0
Ciprofloxacin	S	18.18	28.57	20.0
	I	9.09	-	-
	R	72.72	71.42	80.0
Ceftriaxone	S	27.0	57.0	40.0
	I	-	14.28	-
	R	83.0	29.0	60.0
Ceftazidime	S	27.0	43.0	60.0
	I	9.09	-	-
	R	63.63	57.14	40.0
Cefuroxime	S	36.36	28.57	20.0
	R		71.42	
Azithromycin	S	63.63	57.14	40.0
	R	36.0	43.0	60.0
Amoxiclav	S	55.0	29.0	60.0
	R	45.0	71.0	40.0

S=Sensitive, I=Intermediate, R=Resistant

Discussion

Antimicrobial resistance to the bacterial infections can be a great barrier for the effective treatment options even in case of UTI. The antimicrobial resistance is closely related to greater mortality or morbidity and burden of total health care costs. Irrational use of antibiotics, lack of education, unhygienic condition, unauthorized sale of antibiotics, limited access of health care facilities, inadequate surveillance or regulatory systems and use of antimicrobial in animal firms, poultry and agriculture are main causative factors for resulting antimicrobial resistance in the developing country.[8]

This study was nearly similar with Ranjan, *et al.*, (2017)[9] in India; Nguefack, *et al.* (2019)[10] in Cameroon found 35.0% & 32.0% were cultures positive, and 65.0% & 68.0% were found culture negative respectively. Lower frequency of UTIs may be caused by the habit of administering antibiotics prior to the results of urine culture reports and the possibility that the majority of urine samples are obtained from patients with asymptomatic infections.[8] The current findings were dissimilar with Begum, *et al.* (2022)[11] in Pakistan; Tahir, *et al.* (2022)[12] in Iraq with culture positive 16%, 61% and 84%, 39% were culture negative. The reason behind for higher prevalence could be attributed to the low income status of the patients, anemia, sexual activity during pregnancy, lack of proper personal, environmental hygiene, population susceptibility, and poor housing, ventilation, sanitation and drainage systems.[13]

Among the culture positive total cases 111 (97.36%) were single and 3 (2.63%) were multiple. The finding corresponded with the study of Krishnamurthy, *et al*. (2022)[14] in India (single isolates 94.28%, multiple isolates 5.71%), and Alam, *et al.* (2017)[15] in Bangladesh (single isolates 89.8% and multiple isolates 10.20%). Dissimilarity showed O' Leary, *et al.* (2020)[16] in UK with single isolates 17.1%% and multiple isolates 82.84% and Kindinger, *et al.* (2023)[17] in USAwith single isolates 28.76% and multiple isolates 71.23%. Rate of polymicobial growths may vary due to different geographical locations.

This study showed that gram negative bacteria were 94 (80.34%) and gram positive bacteria were 23 (19.65%). In contrast to this study, similar findings were found with 89.79%

gram negative and 10.29% gram positive bacteria of Rahiman, *et al.* (2015)[18] in Indiaand 79.2% gram negative and 20.7% gram positive were found in a study of Ullah, *et al.* (2018)[19] in Pakistan. This may be due to the existence of unique structure in Gram negative bacteria which assist for attachment to the uroepithelium and prevent pathogens from urinary lavage, allow for growth and tissue invasion resulting in invasive infection and pyelonephritis during pregnancy.[18] Dissimilar with Nkwelle, *et al.* (2020)[20] in Cameroon and Rosana, *et al.* (2020)[21] in Indonesia which were found 22.4% and 68% as gram negative bacteria and 77.6% and 32% as gram positive bacteria.

Regarding 117 culture positive cases *E.coli* was the most predominant organism 57(48.71%). Beksac, *et al.* (2019)[22] in Turkey and Krishnamurthy, *et al.* (2017)[14] in India also observed similar findings. This finding was dissimilar with the study of Rahiman, *et al.* (2015)[18] in India and Subedi, *et al.* (2017)[23] in Nepal who found 34.33% and 86% E.coli respectively. The most common microorganism found in vaginal and rectal area is *E.Coli.* During pregnancy, it is very difficult to maintain personal hygiene due to anatomical and functional changes in them. This may increases the chance of *E.coli* infection during pregnancy.[18] In this study Klebsiella spp. 21 (17.94%) was the second highest bacteria recorded causing UTI. This finding was nearly similar to Ullah, *et al.* (2018)[19] in Pakistan and Tahir, *et al.* (2020)[12] in Iraq which was 26.7% and 15.5% respectively. Dissimilarity was observed Hines, *et al.* (2014)[24] in China and Krishnamurthy, *et al.* (2017)[14] in India 7.9% and 30% respectively. Increased prevalence of *Klebsiella spp.* has been attibuted to its emerging and inherent virulent factors such ascapsular polysaccharides, LPS and siderophores, types 1 and 3 fiimriae, biofilm formation and antibiotic resistance. The other gram negative bacteria (Table-3) were Pseudomonas spp. 9 (7.69%), Proteus spp. 5 (4.27%), Enterobacter spp. 2 (1.7%). These similar findings were Ullah, *et al.* (2018)[19] in Pakistan (Pseudomonas spp. 8.62%, Proteus spp. 12.3%) Gebremariam, *et al.* (2019)[25] in Ethiopia (Proteus spp. 2.24%, Enterobacter spp. 1.37 %). Dissimilar was observed with Simon-Oke, *et al.* (2019)[26] in Nigeria *(Pseudomonas spp.* 2%, *Proteus spp.* 14.34%, *Enterobacter spp.* 22.31%); Yadav, *et al.* (2019)[13] in Nepal *(Pseudomonas spp.*15.2%, *Proteus spp.* 12.6%). *Pseudomonas spp.* is a highly opportunistic bacteria that produces biofilm on catheters and easily gains access to the bladder in patients with repeated hospital stays. Proteus is normally found in the human intestine along with other organisms, and highly motile which facilitates its movement up the urethra and they are metabolically involve in urease production which act as one of the reasons the pathogen is successful in colonizing the urinary tract and causes infection in humans.[27]

Among the Gram positive isolates the predominant bacateria was *Enterococcus spp.* (11, 9.4%), followed by CoNS (7, 5.98%), S.aureus (5, 4.27%) in (Table-3). Yadav, *et al.* (2019)[13] in Nepal *(S.aureus* 8.9%, *Enterococcus* 11.8%), Rahiman, *et al.* (2015)[18] in India (*S.aureus* 4.48%, CoNS 2.28%, *Enterococcus spp.* 2.98%) were similar to this study. Dissimilar study were with Nkwelle, *et al.* (2020)[20] in Cameroon (*S.aureus* 17.4%, CoNS 26.2%) and Kanji, *et al.* (2012)[28] in Argentina (*Enterococcus spp.*1.80%, S. aureus 12.5%, CoNSspp. 15.32%). The capacity of CoNS to cause UTIs in young women is due to capacity to adhere to uroepithelial cells by 160-kDa hemagglutinin / adhesin. *Enterococcus spp.* is one of the hardest bacteria and most commonly found

in complicated UTI associated with urological device. The presence of S. aureus in the urine generally suggests hematogenous dissemination. Ascending S.aureus infections occasionally result from instrumentation of genitourinary tract.

In this study *E.coli* showed 75.43%, 66.67%, sensitivity towards Meropenemand Nitrofurantoin respectively. In accordance with present study, Jonson, *et al.* (2021)[5] in Uganda observed 83.3%, 75.6% sensitivity to Meropenemand Nitrofurantoin respectively and Meropenem showed 82.40% susceptibility by Kumar, *et al.* (2017)[29] in India which was similar with this study. The high susceptible rate detected for these antimicrobials may be due to rare routinely use for the empirical treatment of UTI in pregnancy, kept as reserve for critical situation and the use of these antimicrobials only in hospitalized patients (Siddiqua *et al.* (2017).[30] Dissimilarity was observed Tula, *et al.* (2020)[31] in Ehiopia with 100% sensitivity to Nitrofurantoin and Ullah, *et al.* (2018)[19] in Pakistan with 50% sensitivity to Meropenem.

The second prevalent organism, *Klebsiella spp.* showed the highest sensitivity towards Meropenem (81%) followed by Nitro furantoin (71.42%) respectively. These findings were nearly comparable with other studies by Johnson, *et al.* (2021)[5] in Uganda (Meropenem 83.3%, Nitrofurantoin 63.25%) and Akila, *et al.* (2016)[32] in India (Nitrofurantoin 68.75%). The higher rate of sensitivity is probably due to comparatively more judicious use of this antibiotic as a whole. Dissimilarity was found Ejaz, *et al.* (2011)[32] in Saudi Arabia (Meropenem 96.4%) and Manjula, *et al.* (2014)[34] in India (Nitrofurantoin 30.7%).
The highest sensitivity of *Pseudomonas spp.* showed that Pipercillin/Tazobactam (88.88%) followed by Meropenem and Nitrofurantoin (77.77%) respectively.Piperacillin + tazobactam showed 88.88% susceptibility in this study which corresponds to the findings of Sharmin, *et al.* (2021)[35] in Bangladesh (81.81%). Dissimilarity found by Rahiman, *et al.* (2015)[18] in India (Nitrofurantoin 33.33%, Meropenem 100%) and Johnson, *et al.* (2021)[5] in Uganda (Nitrofurantoin 0%, Meropenem 57.1%). The reason of lower rate of susceptibility compared to others may be due to increased resistance rate of the isolated bacteria in this patient group who are often catheterized which in turn introduces more virulent strains of bacteria inside the patient. In this study, *Proteus spp.* showed the 80% susceptibility towards Meropenem and Nitrofurantoin and Azithromycin (60%) respectively. This study was nearly similar to Al-Jumaily, *et al.* (2016)[27] in Iraq (97.4% Meropenem, 68.4% Azithromycin) and dissimilar to Biswas, *et al.* (2014)[36] in Bangladesh (100% Nitrofurantoin, 0% Meropenem). Proteus isolates present an elevation in the resistance level to Meropenem due to many reasons: the loss of outer membrane porins, decreased expression of PBP1a or reduced binding of Meropenem by PBP2.

in this study, most effective drugs for Gram positive bacteria showed 100% sensitivity to Vancomycin and Linezolid respectively and Meropenem 82% . This findings were similar to Talukdar, *et al.* (2020)[37] and Multani, *et al.* (2021)[38] in India 100% respectively. Dissimilarity was shown by Biswas, *et al.* (2014)[36] in Bangladesh.

Conclusion

In this study it is observed that pregnancy safe antibiotics like Meropenam and Nitrofurantoin showed more than 75% sensitivity towards Gram negative bacteriuria. Vancomycin and Linezolid showed sensitivity towards Gram positive bacteriuria. Pregnant women should be regularly monitored and urine culture should be included in antenatal check-up.

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