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Original Article

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Antibiotic sensitivity pattern of uropathogens among diabetic and non-diabetic pregnant women in Dhaka, Bangladesh

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ABSTRACT

Objective: To determine the urinary tract infections (UTIs)-causing bacterial profile and antibiotic susceptibility pattern for the proper and complete treatment of UTIs in pregnant women with diabetes mellitus (DM).

Methods: We collected 300 urine samples from diabetic and non-diabetic pregnant women at various gestational ages. After isolating and identifying uropathogens, we conducted an antibiotic sensitivity assay against fourteen commonly used antibiotics: amikacin, amoxicillin, ampicillin, azithromycin, cefixime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, co-trimoxazole, gentamicin, levofloxacin, netilmicin, nitrofurantoin.

Results: Among the participants, 70% had DM but only 37.3% had positive uropathogen growth. Five gram-negative bacteria (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Pseudomonas* spp.) and 3 gram-positive bacteria (*Streptococcus* spp., *Staphylococcus* spp. and *Enterococcus* spp.) were indentified. *Escherichia coli* was the most prevalent bacteria (57.1%), followed by *Klebsiella* spp. (19.6%). In the antibiotic susceptibility assay, we found 96.5% of the isolated organisms were highly susceptible to levofloxacin, 94.2% to netilmicin, 88.2% to nitrofurantoin, 85.1% to amikacin, 79.8% to gentamicin, respectively. On the other hand, 64.8%, 63.6%, 61.8% of the isolates demonstrated high-level resistance to ampicillin, ceftazidime and amoxicillin whereas 38.0%, 37.1%, 33.6%, 30.9% and 30.0% of the organisms were resistant to co-trimoxazole, azithromycin, ciprofloxacin, cefuroxime, cefixime, respectivey.

Conclusions: Our results suggest that amikacin, levofloxacin, netilmicin, nitrofurantoin, and gentamycin can be used as first-line treatments for UTIs, whether the patient has DM or not. Contrarily, amoxicillin, ampicillin, ceftazidime, azithromycin, and co-trimoxazole should be avoided in treating UTIs.

KEYWORDS: Antibiotic resistance; Urinary tract infection; Pregnancy; Diabetes; Bangladesh

1. Introduction

Urinary tract infections (UTIs) are the most common bacterial infections worldwide. Among all infections, nearly 25% are UTIs, and about 50%-60% of women were affected with UTIs in their lifetime[1]. Additionally, one in three women reported having at least one, and about 8% of women have asymptomatic UTI by age 24.

Significance

Urinary tract infection (UTI) affects both diabetics and non-diabetics. The most common isolate responsible for UTI in both diabetic and non-diabetic pregnant women is *Escherichia coli*, followed by *Klebsiella* spp. and *Enterobacter* spp. More than 75% of the organisms were found sensitive to amikacin, levofloxacin, netilmicin, nitrofurantoin, and gentamycin. In order to ensure the rational use of antibiotics for the definitive treatment of UTIs, this study identified the causative uropathogens and their antibiotic susceptibility pattern in pregnant women with or without diabetes.

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Women's body structures, such as the shorter urethra close to the vagina and anal orifice, make them more prone to UTIs[2, 3]. Notably, pregnant women have a twofold increased chance of developing symptoms of UTIs compared to non-pregnant women. Common anatomical and physiological changes in the urinary tract include enlargement of the ureters, urethral dilation, the pressing effect of the gravid uterus on the ureters, and the relaxing action of progesterone on the urinary tract muscle during pregnancy[4]. These changes increase the risk of symptomatic UTIs in pregnant women, and about 20% of pregnant women reported developing UTIs[5]. Moreover, the prevalence of symptomatic UTIs during pregnancy ranges from 3%-10.1% in non-diabetic women, while it can reach 27.6% in diabetic pregnant women[6, 7].

The cause of the higher prevalence of UTIs in diabetic pregnant women is not precisely reported. However, some studies suggested that several factors, including immunocompromised health conditions, an increase in the adhesion of microorganisms to the uroepithelial cells, impaired bladder emptying as a result of static pools of urine remaining in the bladder, and high glucose concentration in the urine may contribute to the development of UTIs in diabetic pregnant women[8]. It was also reported that untreated UTIs in pregnant women lead to low birth weight, premature delivery, maternal and perinatal morbidity, and mortality[9, 10]. Antibiotics are generally used to treat UTIs, but their indiscriminate use, self-medication, and insufficient dosage have led to increased UTIs caused by drug-resistant bacteria. As a result, selecting antibiotics for empirical and rational treatment becomes difficult[11, 12]. Therefore, current knowledge of the UTIs causing organisms and their antibiotic susceptibility is necessary to ensure effective therapy.

Among UTIs-causing organisms, about 80%-85% are gram-negative bacteria. *Escherichia* (*E.*) *coli* (75.5%–87% of UTI cases) was the primary organism, followed by *Klebsiella* spp. in diabetic and non-diabetic women. In addition, *Citrobacter*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Proteus* species were reported. On the other hand, *Enterococcus* and *Staphylococcus* species are the main gram-positive bacteria for UTIs[13, 14]. However, it was reported that bacterial species sensitive to commonly used antibiotics vary in time and place[15]. In developing countries like Bangladesh, there is a lack of reports about regular surveillance of UTIs causing organisms and antibiotic resistance patterns. Therefore, this study investigated the prevalence of UTIs in diabetic and non-diabetic pregnant women to identify the most-common UTIs causing bacterial species and their sensitivity patterns toward commonly used antibiotics. The outcome should guide the physicians' choice of antibiotic for treatment of pregnant DM patients.

2. Materials and methods

2.1. Study area and population

This study was carried out in the microbiology laboratory of the Women and Children Hospital Uttara, NHN, Dhaka, Bangladesh. The hospital provides health care services to the country's inhabitants. From July 2018 to June 2019, we collected 300 urine samples from pregnant women of various gestational ages who had UTI symptoms. The studied women have one of the following conditions: DM, gestational diabetes mellitus (GDM), DM with anemia, or no diabetes. The pregnant women in the study ranged in age from 18 to 40.

2.2. Inclusive and exclusive criteria

Pregnant women aged between 18 and 40 years with symptomatic UTI symptoms including frequency, urgency, and dysuria were selected for the study. On the other hand, pregnant women who have taken antibiotics within the last two weeks prior to the study and those who did not agreed to participate in the study were excluded.

2.3. Sample collection and processing

We gave sterile screw-capped universal containers to participants to independently collect clean mid-stream urine samples during the process of urination. The collected samples were labelled individually[16]. After labeling the samples, 0.2 mg of boric acid was added to inhibit bacteria from growing in the urine samples. Within two hours of sample collection, samples were processed, and samples were stored at 4 ℃ for less than 24 hours in certain cases. To isolate bacterial species, a loopful of urine samples was cultured on cysteine-lactose electrolyte deficient agar and blood agar using a 4 mm platinum-wired calibrated loop and incubated at 37 ℃ overnight. The samples were considered positive if a sample contained

bacterial species at a concentration of 10^4 CFU/mL. The presumptive bacterial isolates were identified by standard biochemical tests[17].

2.4. Antibiotic sensitivity assay

Following the standard recommendation, the modified disc diffusion method was employed to perform an antibiotic sensitivity assay. In brief, isolated colonies were spread on Muller-Hinton agar media, and then antibiotic paper discs were placed on top. The zones of inhibition around the colonies were evaluated after incubation at 37 ℃ for 24 hours in order to detect their susceptibility or resistance by comparing with the clinical and laboratory standards institute guideline[18]. We selected 14 antibiotics: amikacin (30 μg), amoxicillin (25 μg), ampicillin (10 μg), azithromycin (30 μg), cefixime (5 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), co-trimoxazole (30 μg), gentamycin (10 μg), levofloxacin (5 μg), netilmicin (30 μg), and nitrofurantoin (300 μg) based on frequent prescription and use.

2.5. Analysis of results

Statistical analysis was done using Microsoft Excel and SPSS software for windows version 21.0 (SPSS Inc., Chicago IL., USA).

2.6. Ethical approval

Verbal informed consent from the target population was obtained before the beginning of the experimental procedure. The institutional ethics committee also gave its approval to the study (Ref No: IRB/18/73).

3. Results

Total 300 cases were enrolled in the study, 90 pregnant women (30%) were from 25-29 years age group. Mean age of cases was 26.5±5.6 years. Approximately 10%, 55%, and 35% of the study participants were experiencing the 1st, 2nd, and 3rd trimesters of pregnancy, respectively. Among the individuals, approximately 30%, 35%, and 5% had a history of DM, GDM, and DM with anemia, respectively, while about 30% of pregnant women did not have a history of diabetes (Table 1).

The overall prevalence of UTIs among pregnant women was 37.3%. In this study, eight different bacterial species were isolated and identified. Most bacterial species were gram-negative organisms. The most prevalent bacterial species were identified as *E. coli* (57.1%) followed by *Klebsiella* spp. (19.6%), *Enterobacter* spp. (6.2%), *Streptococcus* spp. (5.4%), *Staphylococcus aureus* (4.5%), *Citrobacter* spp. (3.6%), *Enterococcus* spp. (1.8%), *Pseudomonas aeruginosa* (1.8%) (Table 2).

Antibiotic sensitivity assay showed that single and multiple antibiotic resistance levels against commonly prescribed drugs were found (Table 3 and Figure 1). Levofloxacin showed the highest overall sensitivity (n=83; 96.5%) against 86 bacterial isolates. Netilmicin (n=97; 94.2%), nitrofurantoin (n=97; 88.2%), and amikacin (n=80; 85.1%) also exhibited overall sensitivity above 85% against multiple uropathogens. In addition, gentamycin, ceftriaxone, cefixime, cefuroxime, ciprofloxacin, azithromycin, and co-trimoxazole were showed 79.8%, 76.4%, 70.0%, 69.1%, 66.4%, 62.9% and 62.0% sensitivity against different isolates, respectively. However, other tested antibiotics showed lesser than 40% of the sensitivity, such as amoxicillin (n=42; 38.2%), ceftazidime (n=36; 36.4%), and ampicillin (n=38; 35.2%) against different uropathogens.

Table 1. Characteristics of UTI in pregnant women.

Variables		*n*=300	Percentage (%)
Age	18-29 years	225	75.0
	30-34 years	71	23.7
	35-40 years	4	1.3
Gestational period	First trimester	30	10.0
	Second trimester	165	55.0
	Third trimester	105	35.0
Co-morbidities	DM*	90	30.0
	GDM#	105	35.0
	DM with anemia	15	5.0
	No DM	90	30.0

*Diabetic mellitus; #Gestational diabetic mellitus.

Table 2. Percentage distribution of the isolated bacteria species.

Growth	Frequency	Percentage (%)
Positive	112	37.3
Escherichia coli	64	57.1
Klebsiella spp.	22	19.6
Enterobacter spp.	7	6.3
Streptococcus spp.	6	5.4
Staphylococcus aureus	5	4.5
Citrobacter spp.	4	3.6
Enterococcus spp.	2	1.8
Pseudomonas aeruginosa	2	1.8

Table 3. Antibiotics sensitivity and resistance pattern of different organisms isolated in the study.

Antibiotic/ Organism		*Escherichia coli*	*Klebsiella* spp.	*Enterobacter* spp.	*Streptococcus* spp.	*Staphylococcus aureus*	*Citrobacter* spp.	*Enterococcus* spp.	*Pseudomonas aeruginosa*	Total (%)
Amikacin	S	58	20	0	0	0	0	2	0	80 (85.1)
	R	6	2	0	6	0	0	0	0	14 (14.9)
Amoxicillin	S	24	6	3	6	2	1	0	0	42 (38.2)
	R	40	16	4	0	3	3	0	2	68 (61.8)
Ampicillin	S	22	1	7	6	2	0	0	0	38 (35.2)
	R	42	21	0	0	3	4	0	0	70 (64.8)
Azithromycin	S	51	1	0	6	3	0	0	0	61 (62.9)
	R	13	21	0	0	2	0	0	0	36 (37.1)
Cefixime	S	43	20	5	0	0	0	0	2	70 (70.0)
	R	21	2	2	0	5	0	0	0	30 (30.0)
Ceftazidime	S	24	12	0	0	0	0	0	0	36 (36.4)
	R	40	10	0	6	5	0	0	2	63 (63.6)
Ceftriaxone	S	45	20	3	6	5	3	0	2	84 (76.4)
	R	19	2	4	0	0	1	0	0	26 (23.6)
Cefuroxime	S	38	21	0	0	5	3	0	0	67 (69.1)
	R	26	1	0	0	0	1	0	2	30 (30.9)
Ciprofloxacin	S	44	8	4	6	5	4	2	0	73 (66.4)
	R	20	14	3	0	0	0	0	0	37 (33.6)
Co-trimoxazole	S	32	18	4	6	5	0	0	2	67 (62.0)
	R	32	4	3	0	0	0	2	0	41 (38.0)
Gentamycin	S	50	18	5	0	5	3	2	0	83 (79.8)
	R	14	4	2	0	0	1	0	0	21 (20.2)
Levofioxacin	S	64	0	5	6	5	3	0	0	83 (96.5)
	R	0	0	2	0	0	1	0	0	3 (3.5)
Netilmicin	S	58	22	0	6	5	4	2	0	97 (94.2)
	R	6	0	0	0	0	0	0	0	6 (5.8)
Nitrofurantoin	S	60	16	4	6	5	4	2	0	97 (88.2)
	R	4	6	3	0	0	0	0	0	13 (11.8)

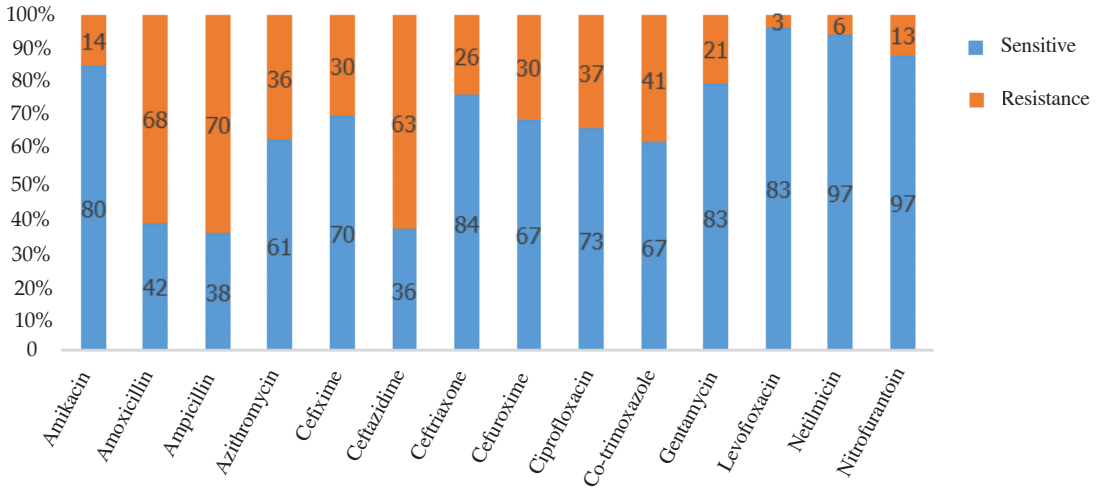

Figure 1. Overall antibiotics sensitivity and resistance pattern of different uropathogens.

4. Discussion

Overall prevalence of UTI in pregnant patients found 37.3% is much lower than a recent study conducted in Dhaka, Bangladesh, where an overall prevalence of 76.0% is achieved in pregnant women[15] and also lower than in India (46.6%)[16]. However, this finding is higher than reported in Sudan (14.0%)[19], Libya (30.0%)[20], and Tanzania (14.6%)[21]. The different geographic locations, sample sizes, sensitivity of the test protocol, effectiveness of the health systems, level of personal hygiene of the pregnant women in the different countries, prevalence, and level of microbial contaminations in the environment etc. could cause this discrepancy in prevalence[22].

We observed that pregnant women aged 18-40 years were more susceptible compared to >40 years old women to developing UTIs (90%). These results are supported by previous reports stating that young females show high susceptibility than older women to develop UTIs[16]. Reasons might be the higher level of sexual activity or the recent use of contraception methods[23]. About 70% of study participants are diabetic positive. Complications in diabetic conditions such as high glucose levels in blood and urine,

immunocompromised health condition, and retention of urine in the bladder of diabetic patients favor bacterial growth. In this study, the high prevalence rate of UTIs was found in the 2nd and 3rd trimesters of pregnant women echoing previous studies stating that UTI levels were very high in the 3rd trimester of pregnancy. Physical and hormonal changes such as enlargement of ureters, gravid pressure on the ureter, and high level of urine remaining in the bladder might be the reasons for high bacteriuria.

In the first three trimesters, pregnant women with UTIs are most likely to infect with gram-negative bacteria such as *E. coli* and *Klebsiella* spp.[24]. Indeed, it was reported that gram-negative bacteria are predominant in UTIs. Consistent with this statement, the most prevalent organism found in this study was *E. coli*, followed by *Klebsiella* spp. Interestingly, gram-negative bacteria were predominantly found here. Among the gram-positive organism, *Enterococcus* spp. and *Staphylococcus* spp. are reported as frequent causes of UTIs[16], and similar kind of results was found in this study. Amikacin, levofloxacin, and nitrofurantoin are effective against most *E. coli* strains, and similar outcomes were reported in previous studies[16, 25]. Ampicillin was found 100% ineffective against multiple organisms such as *Citrobacter* spp., *Pseudomonas* spp., and most *Klebsiella* spp. Amoxicillin was found ineffective against *Citrobacter* spp. and *Pseudomonas* spp. *Streptococcus* spp. was found to be resistant to ceftazidime, *Klebsiella* spp. to azithromycin, ciprofloxacin, *Pseudomonas* spp. to ceftazidime and cefuroxime, which was supported by the previous report[15].

The antibiotic susceptibility assay also revealed that uropathogens such as *E. coli*, *Klebsiella* spp., and others showed high resistance to ampicillin, amoxicillin and azithromycin (beta-lactam group) antibiotics, as observed in previous studies[26, 27]. This might be due to the widespread use of these antibiotics and the emergence of bacteria that produce beta-lactamase. Despite being generally regarded as a safe conventional medication during pregnancy, this result limits the usage of the beta-lactam category of antibiotics. It was found that third-generation antibiotics such as ceftazidime were found poor in effectiveness against uropathogens. Trimethoprim, formerly reported as an effective drug against uropathogens[28], is losing its effectiveness. On the other hand, amikacin, levofloxacin, netilmicin, nitrofurantoin and gentamycin were shown to have minimal levels of resistance, which suggests that these medications may be used as first-line treatments for UTIs in pregnant women. The growth of more resistant bacteria may result from the empirical use of antibiotics, complicating the management of UTIs[29]. The findings showed that amoxicillin, ampicillin, azithromycin and ceftazidime were less effective against uropathogens, limiting their usage in treating UTIs.

The limitation of the study is that it only involves women who attended the healthcare system, and the results do not reflect the entire community. Therefore, a further study including women from the community will be required to validate these findings.

5. Conclusions

Our results suggest that antibiotic susceptibility should be done before antibiotic therapy initiation for pregnant women with UTIs. These findings would also help to develop rules for prescribing antibiotics to diabetic and nob-diabetic pregnant women.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

Islam MA and Shamin F designed the study. Sharmin F carried out the experiments. Hasan M and Azad AK analyzed the data and drafted the initial manuscript. Sharmin F and Islam MA reviewed the article critically for intellectual content. All authors reviewed subsequent drafts of the manuscript and approved the final version.

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