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Original Research Article

Bacterial profile and antibiotic susceptibility pattern of uropathogens in patients attended Obstetrics and Gynecology outpatient clinic in a tertiary care hospital Assam

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Abstract

Background: Urinary tract infection (UTI) is one of the most common bacterial infection during pregnancy that lead to significant perinatal and maternal morbidity. In hospital it is the second most common infection after respiratory tract infection. Bacteria associated with urinary tract infection are common worldwide but spectrum varies with individual population. Many multidrug resistant bacteria have been reported and a significant increase in isolation rates makes them difficult-to-treat pathogen. The aim of the study was to detect the antibiotic susceptibility pattern of different urinary isolates from patients attending Obstetrics and Gynecology outpatient clinic.

Materials and Methods: It is a retrospective study in Hind Lab Laboratory, Tertiary care Hospital, Assam. Urinary samples collected or received in the microbiology laboratory were processed following standard procedure. Organism isolated were tested for different antimicrobials and sensitivity pattern were observed by phenotypic and Vitek 2 compact system (bioMerieux).

Results: Out of a total of 1847 urinary samples, 689 yielded pathogens of which 239 (34.6%) were *Escherichia coli*, 221 (32%) were *Klebsiella pneumoniae,* 105 (15%) were *Enterococcus spp*. Twenty-nine (52.7%) isolates were multi-drug resistant;. The other organisms isolated were, *Enterococcus spp., Staphylococcus aureus, Acinetobacter baumanii, Candida spp, Enterobacter spp., Proteus spp*. and *Pseudomonas aeruginosa* respectively. All the isolates shown varying degree of susceptibility to commonly tested antimicrobials.

Conclusion: Thirty five (35%) per cent of urinary isolates were multidrug resistant bacteria. All of them shown varying degree of resistance pattern to different classes of antimicrobials. Cephalosporin and flouroquinolones group of drug had shown maximum resistance pattern. It is also important to limit antibiotic consumption and exposure. Hence, along with routine antimicrobial testing, tests for detection of drug resistance should be routinely incorporated for uropathogens.

Keywords: Antibiotic susceptibility testing; Multidrug resistance, Obstetrics and Gynecology clinic, Urinary tract infection.

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1. Introduction

Urinary tract infection (UTI) is one of the most common infections mostly caused by Gram-negative bacteria.[1] Almost 150 million cases of UTIs per year are reported worldwide.[2] Different microorganisms can invade urinary tract which lead to pathogenesis of urinary tract infection.[3,4] Since UTI is one of the most common recurrent acquired infection, which lead to increase in number of stillbirth cases.[5,6] It is the second most common complication in pregnant women after anemia which can adversely affect the health of pregnant mother and infant.[7,8] UTIs in pregnancy is basically classified into two categories, mainly symptomatic and asymptomatic. Involvement of lower urinary tract lead to asymptomatic bacteriuria and involvement of upper urinary tract lead to upper urinary tract infection which is characterized by pyelonephritis.[9,10] Recent published data says that,

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prevalence of symptomatic bacteriuria is account for 17.9% and asymptomatic bacteriuria is around 13%. There are various factors that can lead to UTIs, such as increased age, number of childbirth, anemia, abnormality in urinary tract and previous history of urinary tract infection.[10-12] Bacteria are one of the most common cause of UTIs among the pregnant women. Various bacterial pathogens include, *Escherichia coli, Klebsiella pneumoniae, Proteus spp. Acinetobacter spp. Enterococcus spp. Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus* and *group B Streptococcus*.[13,14] Various studies have shown that 25%-40% untreated pregnant women with asymptomatic bacteriuria develop acute pyelonephritis that lead to hospitalization before delivery.[15] Other risk factors include, low birth weight, anemia, preeclampsia, premature rupture of membrane, respiratory failure and risk of septicemia and shock. It has also been reported that infant born to mother with pyelonephritis are prone to develop mental and motor abnormality.[16] However, uropathogens have been becoming more resistant to antibiotics, particularly to routinely used antimicrobials, and the pattern of resistance varies across the group of pathogens based on the location and environmental conditions.[17] Pregnant women should not have routine urine cultures performed in most hospitals in developing nations; instead, the strip analysis approach is used to evaluate the infection, which results in an inadequate study.

Moreover antibiotics are used before the urine culture report comes. To start the appropriate therapy, it is utmost important to know the causative organism and the susceptibility pattern. Hence a periodic evaluation is necessary to update information.

Therefore this study was aimed to isolate the bacterial etiologic agents and their sensitivity pattern to different antimicrobials from the patients attended obstetrics and gynecology clinics in a tertiary care hospital.

2. Materials and Methods

2.1. Place of study

This was a retrospective study of all the urine samples received from the female patients attended at the antenatal clinic of Obstetrics and Gynecology (O & G) outpatient department (OPD) in a tertiary care hospital, North Lakhimpur, Assam, over the study period October 21st, 2021 to September 21st 2022.

2.2. Data collection

Comprehensive clinical and demographic information was retrieved from records.

2.3. Culture

Urine samples submitted for culture and sensitivity testing to the Microbiology section were processed according to standard procedures. The samples were inoculated onto MacConkey agar and blood agar using a standard calibrated loop (0.001 ml). To verify notable growth, colonies were counted 24–48 hours after being incubated overnight at 37°C. Significant colony counts were defined as those that produced bacterial growth of 10^5 CFU/ml urine. Following conventional microbiological methods, the organism was identified using colony morphology, gram staining, and biochemical assays. Phenotypic and Vitek 2 compact system (bioMerieux) confirmations were also conducted.

2.4. Antibiotic susceptibility testing

To evaluate the susceptibility of isolates to various antibiotics, the Kirby Bauer's disk diffusion method was used (**Figure 1**) using a panel including ceftazidime (30µg), ceftazidime/clavulanate (30/10µg), cefoxitin (30µg), cefepime (30µg), levofloxacin (5µg), aztreonam (30µg), gentamicin (10µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), tigecycline (15µg), fosfomycin (200 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), linezolid (30 µg), vancomycin (30 µg) and co-trimoxazole (25 µg). The test organism was suspended in normal saline, adjusted to 0.5 McFarland standards, and streaked with a sterile swab stick on Mueller Hinton Agar (MHA). Antibiotic-impregnated disks were applied and incubated at 35°C ± 1°C overnight. Zone of inhibition diameters were recorded and interpreted following CLSI guidelines 2021 and 2022.

3. Results

3.1. Study population and patient characteristics

A total of 1847 urinary samples, collected from the various patients attending the antenatal clinic of O & G OPD were included. The median age was 33 years. Most of the patients were asymptomatic and few were presented with fever and burning micturition at the time of sample collection.

3.2. Study outcome

Out of a total of 1847 urinary samples, 689 yielded pathogens of which 239 (34.6%) were *Escherichia coli*, 221 (32%) were *Klebsiella pneumoniae,* 105 (15%) were *Enterococcus spp*. The other organisms isolated were, *Staphylococcus aureus, Acinetobacter baumanii, Candida spp., Enterobacter spp., Proteus spp*. and *Pseudomonas aeruginosa* respectively (**Table 1**). Among the 239 *Escherichia coli* isolates, 87 (36%) isolates showed resistance to various antimicrobials group; all were isolated in asymptomatic female. *Klebsiella pneumoniae* also showed a high degree of resistance to various group of antimicrobials. Ninety four (45%) isolates of *Klebsiella pneumoniae* have shown multidrug resistance. Eighty seven *Escherichia coli* isolates and 101 out of 221 *Klebsiella pneumoniae* isolates were positive for beta-lactamases (**Figure 2**). Fifteen (15) isolates of *Klebsiella pneuoniae* were screening positive for AmpC production by phenotypic methods.

Table 1: Organism isolated from pregnant women with urinary tract infection

Organism isolated	**No of isolates (n= 689)**	**Percentages (%)**
Escherichia coli	239	34.6
Klebsiella pneumoniae	221	32
Enterococcus spp.	105	15
Methicillin-sensitive Staphylococcus	72	10
Methicillin resistant Staphylococcus(MRSA)	25	3
Acinetobacter baumanii	8	1.1
Candida spp.	7	1
Enterobacter spp.	6	.87
Pseudomonas spp.	4	.58
Proteus spp.	2	.29

Table 2: Susceptibility profile of common gram negative urinary pathogens

Antimicrobials		**Bacterial pathogens**				
	Escherichia coli	***Klebsiella pneumoniae***	***Enterobacter spp.***	***Pseudomonas spp.***	***Acinetobacter baumanii***	***Proteus spp.***
Gentamicin	191(80)	159 (72)	5 (87)	4(100)	1(12)	2(100)
Ceftazidime	107 (45)	66 (33)	6 (100)	2(50)	2(25)	2(100)
Cefoxitin	93(39)	92 (42)	6 (100)	1(25)	2(25)	2(100)
Levofloxacin	131(55)	139 (63)	6(100)	4(100)	8(100)	2(100)
Piperacillin- Tazobactam	100(42)	119 (54)	5(87)	4(100)	4(50)	2(100)
Meropenem	210 (88)	145 (66)	6(100)	4(100)	2(25)	2(100)
Aztreonam	124(52)	121 (55)	5 (87)	4(100)	2(25)	2(100)
Cefepime	100(42)	88 (40)	2(33)	2(50)	0(0)	2(100)
Nitrofurantoin	181(76)	187 (85)	6(100)	4(100)	3(37)	0(0)
Fosfomycin	219(92)	192 (87)	NA	NA	NA	NA
Norfloxacin	155 (65)	137 (62)	5(87)	2(50)	6(75)	2(100)
Cotrimoxazole	215(90)	181 (82)	6(100)	4(100)	3(37)	2(100)
Tobramycin	NA	NA	NA	4(100)	7(87)	NA
Ciprofloxacin	124(52)	92 (42)	5(87)	2(50)	5(62)	2(100)
Tigecycline	NA	NA	NA	0(0)	NA	NA

Table 3: Susceptibility profile of common gram positive urinary pathogens.

Antimicrobials	**Bacterial pathogens**		
	Enterococcus spp.	***Staphylococcus aureus* (MSSA)**	***Staphylococcus aureus* (MRSA)**
Gentamicin	84(80)	57(80)	20(80)
Cefoxitin	NA	72(100)	0(0)
Levofloxacin	70(67)	47(67)	18(72)
Nitrofurantoin	89(85)	56(78)	23(92)
Fosfomycin	90(85)	NA	NA
Norfloxacin	75(72)	51(72)	16(67)
Cotrimoxazole	86(82)	56(78)	20(82)
Erythromycin	54(52)	41(58)	15(60)
Clindamycin	75(72)	47(66)	18(72)
Ciprofloxacin	70(67)	44(62)	15(62)
Linezolid	105(100)	72(100)	25(100)
Vancomycin	100(95)	NA	NA
Tigecycline	85(80)	NA	NA
Ampicillin	67(63)	43(60)	14(57)
High level Gentamicin	70(66)	NA	NA

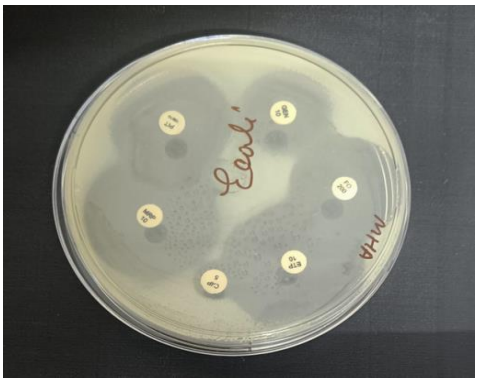

Figure 1: Antibiotic sensitivity of different antimicrobials by Kirby-Buer disc diffusion method

Figure 2: Phenotypic confirmatory test for ESBL using Kirby-Bauer disk diffusion method. Positive test if the zone of inhibition around cefotaxime+ clavulanate and ceftazidime+clavulanate is increased by ≥ 5mm as compared to that of cefotaxime or ceftazidime on MHA plate.

Methicillin –resistant Staphylococcus aureus (*MRSA*) was isolated in 25(3%) number of females. Among the 105 *Enterococcus* isolates, Vancomycin resistant were seen in 10 isolates by disc diffusion method. Further confirmation cannot be done by microbroth dilution method.

4. Discussion

Pregnancy-related hormonal changes and changes in the urinary tract's posture increase the incidence of UTIs in pregnant women. These alterations allow bacteria to more easily reach the kidneys, resulting in both symptomatic and asymptomatic bacteriuria. Both the mother and the fetus may be impacted by a urinary tract infection if prompt action is not taken. Antimicrobial therapy and at least initial screening should be addressed to avoid the negative outcome.

E. coli was the most often found bacterium in our investigation, which is consistent with the Tandan et al.[18] study. With a mean age of 25, Gessese et al.[19] discovered that *E. coli* was responsible for 46.4% of UTIs. According to their research, the relative rates of infection by *Proteus species*, *Coagulase-negative Staphylococcus*, and *Staphylococcus aureus* were 10.6%, 14.3%, and 14.3%. Due to several virulence characteristics that facilitate invasion and colonization of the urinary epithelium, *E. coli* is regarded as the most common uropathogenic bacteria. As it is also a common contaminant of the fecal matter so it can ascent from per urethral region to bladder very easily. Another study by Souza et al.[20] found that *E.coli* and *S. aureus* were the most commonly identified organism and 89% of the isolates were sensitive to fosfomycin. In our study 92% of the isolates were sensitive to fosfomycin (**Table 2**). A study conducted by Assefa et al.[21] also found high sensitivity to amoxicillin/clavulanic acid (70%), chloramphenicol (83.3%), gentamicin (93.3%), nitrofurantoin (87.7%), and cotrimoxazole (73.3%). According to Kibret et al.[22] there was a high sensitivity to ciprofloxacin (79.6%), gentamicin (90.6%), and nitrofurantoin (96.4%), as well as a high resistance to amoxicillin (86%) and tetracycline (72.6%). High levels of sensitivity to nitrofurantoin (76%), gentamicin (80%), cotrimoxazole (90%), fosfomycin (92%), and meropenem (88%), were also demonstrated by our investigation. About 65% of the *E. Coli* isolates in our investigation were norfloxacin-sensitive. During pregnancy, it is a reasonably safe medication.[23 -25] Because they are teratogenic during pregnancy, cotrimoxazole, tetracycline, and chloramphenicol should be avoided.[26] However, nitrofurantoin is safe in pregnancy as it doesn't associated with any teratogenic effect, but risk of hypo plastic left syndrome has been documented.[27] Munda et al.[28] found that exposure to clindamycin, doxycycline, quinolones and macrolides can lead to organ specific malformation whereas there is no any major congenital malformation after exposure to amoxicillin, cephalosporin and nitrofurantoin. A study conducted by Bookstaver et al. highlighted that beta lactams, vancomycin, nitrofurantoin, metronidazole, clindamycin, and fosfomycin were considered safe in pregnancy and these are effective as well whereas fluoroquinolones and tetracycline should generally be avoided in pregnancy. In our study among the gram positive bacteria *Staphylococcus aureus* showed higher resistance rate to ciprofloxacin, erythromycin, and gentamicin. *Klebsiella pneumoniae* was the second most often isolated organism. The majority of the isolates exhibited notable cefotaxime and ceftazidime resistance. AmpC and ESBL beta lactamases have been discovered to be highly prevalent in *Klebsiella pneumoniae*. Along with antibiotic sensitivity testing to aid in the treatment of drug resistance cases, regular diagnostic laboratories should prioritize the phenotypic identification of extended-spectrum beta-lactamases (ESBL) for improved management. The majority of the *Enterococcus* isolates in our investigation were sensitive to ampicillin, vancomycin, tigecycline, nitrofurantoin, and linezolid.

In a study by Fallah et al.[29] mentioned that nitrofurantoin and linezolid are the most effective drug against *Enterococcus* species. Hussain et al.[30] found that 90% of *Enterococcus faecalis* isolates were resistance to gentamicin, 86.95% to norfloxacin and 85.71% exhibit multidrug resistance.[31,32] Our study showed 14% of *Staphylococcus aureus* out of which 3% were methicillin resistant *Staphylococcus aureus* (MRSA). A few number of *Candida spp.*, *Enterobacter spp.*, *Proteus spp*, and *Pseudomonas*

aeruginosa were also isolated in our study (**Table 1**). According to a study done in the US, the total recurrence rate for non-pregnant women with uncomplicated UTIs was 102/1,00000[33] and *Candida* was responsible for just 7.8% of the infections. It is known that the pattern of antibiotic sensitivity in uropathogenic bacteria varies by area and changes with time.[34] The effects of the best antibiotics with a high sensitivity rate against uropathogens with a low resistance rate were detailed in our study. For gram negative organism nirtofurantoin (76%), fosfomycin (92%), meropenem (88%), and cotrimoxazole (90%) showed high sensitivity pattern however high resistance seen among pipercillin /tazobactam (44%), cefepime (49%), and ciprofloxacin (52%).

In contrast sensitivity pattern against gram positive organisms was nitrofurantoin (85%), erythromycin (52%), cotrimoxazole (82%), levofloxacin (67%), gentamicin (80%), tetracycline (70%) and oxacillin (76%) (**Table 3**).

Urinary tract infection is the most common bacterial infection in pregnancy and are classified as asymptomatic bacteriuria, cystitis and acute pyelonephritis. Evidence proved that untreated asymptomatic bacteriuria can lead to acute pyelonephritis which is associated with higher maternal and neonatal morbidity. It can be because of routine use of antibiotics for asymptomatic bacteriuria. On the recent evidence it is reasonable to suggest a single urine screen for asymptomatic bacteriuria in first trimester of pregnancy to reduce the risk of acute pyelonephritis and its complications. It should be mentioned that MDR is steadily rising globally, which is concerning because it means we have less treatment options for bacterial illnesses. Clinicians need to be made aware of the significant likelihood of multidrug resistance. In the current day, where antibiotic resistance is increasing at an alarming rate, additional research is urgently needed to develop newer quick diagnostic tests

Countries where women have reduced access to care, a screen and treat approach is recommended so that it reduces the need for medical visit and maternal and neonatal complications. Moreover education programs should be conducted among the community to reduce the prevalence of disease in the community as well as enlighten the quality of life for patients especially living in low and middle income region. Prophylactic measures, both non-pharmacological and pharmacological and more cost effective diagnostic modalities also require further study.

5. Conclusion

Urinary tract infection is the most common bacterial infection in pregnancy.

Gram negative bacilli are the more common causative agents of UTI in comparison to Gram positive cocci. Increasing trend of drug resistance like ESBLs, AmpC beta lactamases, and MRSA has limited the antibiotic options available to treat such cases. Application of routine testing of urine for culture & sensitivity in pregnancy can prevent unfavorable outcome and complication during pregnancy. Moreover developing hospital antibiogram can help in selection of appropriate antimicrobial agents to treat various clinical spectrum of urinary tract infections.

6. Conflicts of Interest

No conflicts of interest.

7. Sources of Funding

None.

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