ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF URINARY ISOLATES FROM PREGNANT AND NON-PREGNANT WOMEN VISITING PADMA NURSING HOME (P) LTD., POKHARA

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Key Words
Antibiotics, pregnant, non-pregnant, resistance, sensitive, urine, UTI

ABSTRACT

Background:
Urinary tract infections (UTIs) are the most common infections in women. It constitutes one of the major causes of morbidity and mortality. Pregnant and sexually active women are more prone to UTI.

Aims:
The study was designed to find the prevalence of UTI and the profile of antibiotic resistance in pregnant and non-pregnant women.

Materials and methods:
The study involved 636 females who were clinically suspected for UTI attending Padma Nursing Home (P) Ltd., New Road, Pokhara. The mid-stream urine samples were collected from female patients of different age groups followed by examination with semi-quantitative culture method and determinants of antibacterial sensitivity patterns using Kirby-Bauer disc diffusion technique. Chi-square test was used to analyze data.

Results:
The overall prevalence of UTI was found to be 23.27%(148/636). The most predominant were Escherichia coli (37.83%) and Proteus (21.62%). The most effective antibiotics were Amikacin, Gentamicin and Imipenem.

Conclusion:
Pregnant and sexually active women should be aware about UTI and antibiotic sensitivity test should be done time and again to find out the effective drug for treatment of UTI.

INTRODUCTION

Urinary tract infection (UTI) simply means the presence of bacteria undergoing multiplication in human urine within the urinary drainage system[1]. UTIs are one of the most common bacterial infections encountered by both general practitioners and hospital doctors[2]. UTI is the most common cause of nosocomial infection[3]. Urine is a sterile ultra-filtrate of blood[4]. The bladder and urinary tract are normally sterile. The urethra however may contain a few commensals and also perineum which can contaminate urine when it is being collected. With female patients, the urine may become contaminated with organisms from the vagina. Vaginal contamination is often indicated by the presence of epithelial cells and mixed bacterial flora[5].

Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars[6]. According to the annual report of fiscal year (2055/2056) published by Department of Health Services, 0.46%
of total outdoor patients suffered from UTI in Nepal [7]. UTIs constitute one of the major causes of morbidity and mortality. Incidence of infection is higher in women [2]. It was reported that up to 15% of women will have one episode of UTI at some time during their life. It is a common health problem among pregnant women accounting for about 10% of primary care consultation [4].

In most instances, growth of more than 10⁵ organisms per milliliter from properly collected midstream urine sample indicates infection [5] in an asymptomatic patient, or as more than 100 organisms/ml of urine with accompanying pyuria (>5 WBCs/ml) in symptomatic patient. Particularly in asymptomatic patients, a diagnosis of UTI should be supported by a positive culture for a uropathogen [9]. In contrast to men, women are more susceptible to UTI, [10] and this is due to short urethra, promoting ascending infection to bladder and sometimes in kidney, [11] absence of prostate secretions, [12] pregnancy, and easy contamination of urinary tract with fecal flora [13].

Sexual activity increases the chances of bacterial contamination of female urethra. Sexual intercourse may also cause bacteria to be pushed into urethra. This anatomical relationship of femal urethra to vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up from urethra into bladder during pregnancy or child birth [14].

UTI has been reported among 20% of the pregnant women and it is the most common cause of admission in obstetrical wards. Both progesterone and androgens levels increase during pregnancy and these will lead to decreased ureteral and bladder tone [15]. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state; then enlarging uterus affects all the tissues of the urinary tract at various times [14]. It usually begins to occur in early pregnancy at 6 weeks, peaks during 22–24 weeks due to number of factors including uterine dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux [16].

UTI accounts for a significant part of the workload in clinical microbiology laboratories and enteric bacteria (in particular, Escherichia coli) remained the most frequent cause of UTI, although the distribution of pathogens that cause UTI is changing [17]. E. coli is the commonest urinary pathogen causing 60–90% of infections. Some strains are more invasive, e.g. capsulated strains are able to resist phagocytosis while other strains are more adhesive. UTIs caused by Pseudomonas, Proteus, Klebsiella species and S. aureus, are associated with hospital-acquired infections, often following catheterization or gynaecological surgery [18].

Microbial virulence factors like adherence (bacterial adhesions), calcification (kidney stones), toxin production, lipopolysaccharides, capsular polysaccharides, hemolysins and biofilms facilitate UTIs [19]. Defense mechanisms, which protect against UTI, include (1) hydrodynamic forces: the flow of urine removes microorganisms from the bladder and urethra; (2) phagocytosis of microorganisms by polymorphs on the bladdersurface; (3) the presence of IgA antibody on bladder wall; a mucin layer on bladder wall prevents bacterial adherence; (4) urinary pH [20].

Antimicrobial resistance (AMR) is one of the major problems throughout the world. The major mechanisms of AMR are mutations, enzymes, target alterations and efflux pump. Antibiotic therapy has played a vital role in the treatment of human infections in the 20th century, since the discovery of penicillin in 1940s [21]. In the last couple of years, there has been a lot of focus in scientific literature on inappropriate use of antimicrobials resulting in the spread of bacterial resistance. The widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance [22]. The etiological agent and their susceptibility patterns of UTIs vary in region and geographical location. Besides, the etiology and drug resistance change through time. Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made [22].

Nepal is developing county and has high illiteracy rate. In developing countries UTIs are one of the most commonly diagnosed disease among the patients seeking medical service with frequency of 180 per 1000 [23]. UTI is a common disease among Nepalese population as well as one of the commonest nosocomial infections. Today, antimicrobial drugs remain the first line therapy for conquering bacterial infection. For the successful treatment, culture and sensitivity test is essential which is lacking in many part of Nepal. Early detection and eradication of bacteriuria is very important for prevention of recurrence and complication e.g. chronic pyelonephritis, chronic renal failure etc. [24]. The emerging antimicrobial resistance has been a burden for the treatment of various infectious diseases. It has been considered as a global threat to public [25]. Therefore, the present study is aimed to determine different bacterial species causing UTI and their antimicrobial susceptibility profile among pregnant and non-pregnant patients attending Padma Nursing Home (P) Ltd.

**MATERIALS AND METHODS**

Antibiotics disc and culture media used were obtained from the manufacturer; Hi-Media Laboratories Pvt. Limited, Bombay, India.

**Sample source**

The study was carried out in Microbiology laboratory of Padma Nursing Home (P) Ltd., Newroad, Pokhara between July 04 to December 24, 2016. A total of 636 samples were collected from indoor and outdoor patients of hospital. The patients were instructed to collect 5-10 ml mid-stream urine.

**Testing urine for hCG (pregnancy test)**

The strip was dipped in the urine and the result was read after 5 minutes. A positive pregnancy test was therefore shown by colored bands (usually pinkrose color) appearing in both the control and test zones. A negative test was shown by a colored band appearing only in the control zone [18].

**Microscopic examination of samples**

Ten ml of urine sample was taken in a clean sterile centrifuge tube and was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the sediment was examined by wet mount preparation method. The mount preparation of urinary sediments was observed through microscope for the presence of
WBC, pus cells and RBC. Number of WBC and RBC was estimated as number per HPF that is 40X objective of microscope.

**Culture of urine sample**

Semi-quantitative culture technique was used to culture urine specimen and to detect the presence of significant bacteriuria by standard methods. A calibrated inoculating loop of standard dimension was used to take up approximately fixed and known volume (0.001ml) of mixed uncentrifugedurine. All the urine samples were processed on the blood agar (BA) and MacConkey agar (MA) by standard loop method and incubated at 37°Covernight. The plates were observed for bacterial growth. Culture resultswere interpreted as being significant and insignif i-cant, according to the standard criteria. A growth of ≥10^3 colony forming units/ml (cfu/ml) was considered as significant bacteriuria. Patients with significant bacteriuria and symptomatic patients with lower colony counts were also considered as having UTI. The organism was identified by standard methods from the samples showing significant bacteriuria. Cultures with more than three colonies were discarded, as contaminants and their antibiotic susceptibility were not tested.

**Identification and characterization of the isolates**

The plates were incubated at 37°C for 24 hours and examined for growth. The morphological characteristics of the colonies isolated were observed based on their pattern of growth, colour, size and shape. Gram positive organisms were identified primarily on the basis of their response to Gram’s staining, catalase, oxidase, and coagulase tests. The biochemical tests used for identification of Gram negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, VogesProskauer test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Motility test, Sulfide production test and Gas production test.

**Antibiotic susceptibility testing**

The antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by modified Kirby-Bauer disk diffusion method recommended by Clinical and Laboratory Standards Institute (CLSI) using Muller Hinton Agar (MHA). The plates were incubated at 37°C for 24 hours and examined for growth inhibition. The zone size of growth was then measured and was categorized as resistance (R) intermediate (I) and susceptible (S). The antibiotics disc used were Amikacin (30 µg), Ceftazidine (30 µg), Ciprofloxacin (5 µg), Cefixime (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Nitrofurantoin (300 µg), Ceftriazone (30 µg), Norfloxacin (10 µg) and Ofloxacin (5µg).

**RESULTS**

Out of 636 samples, among pregnant 60 showed significant bacterial growth out of 100 samples and among non-pregnant 88 showed significant growth out of 476 samples (table 1). Table 2 shows the distribution of isolates in pregnant and non-pregnant women. E. coli was the most prevalent uropathogens among both pregnant and non-pregnant cases. Table 3 depicts the antibiotic sensitivity pattern of uropathogens among pregnant and non-pregnant cases. E. coli were highly sensitive to Amikacin, Gentamicin and Imipenem. Proteus was highly sensitive to Norfloxacin, Amikacin, Gentamicin and Imipenem. Citrobacter was highly sensitive to Amikacin, Gentamicin, Ciprofloxacin and Imipenem. Klebsiella was highly sensitive to Amikacin, Gentamicin, Ciprofloxacin, Ceftriazone, Imipenem and Cefixime. Staphylococcus was highly sensitive to Nitrofurantoin, Norfloxacin, Amikacin, Gentamicin and Imipenem.

Figure 1 explains the species wise distribution of isolates. E. coli were isolated predominantly 56 (37.83%) out of 148 isolates and Staphylococcus was the least 8 (5.4%) out of 148. Figure 2 demonstrates the prevalence of bacteriuria among pregnant and non-pregnant females in different age group. The age group 20-30 has highest number of isolates. Also, pregnant women of same age group showed more positive growth than non-pregnant woman. The antimicrobial susceptibility pattern of total urinary isolates is shown in figure 3. The most effective drugs were Amikacin, Gentamicin and Imipenem for all isolates and the least sensitive was Cefixime.

**Table 1: Growth pattern of uropathogens among pregnant and non-pregnant**

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**Table 2: Patterns of uropathogens in pregnant and non-pregnant cases**

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Table 3: Antibiotic sensitivity pattern of uropathogens among pregnant and non-pregnant cases

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P=pregnant and NP= non-pregnant
Figure 1: Species wise distribution of isolated species

- **E. coli**: 37.83%
- **Proteus**: 16.21%
- **Klebsiella**: 10.81%
- **Citrobacter**: 8.1%
- **Enterobacter**: 5.4%
- **Staphylococcus**: 21.62%

Figure 2: Bacteriuria among pregnant and non-pregnant women in different age group.

- Pregnant women
- Non-pregnant women

Age distribution (years)
- <20
- 20-29
- 30-39
- 40-49
- >50

Percentage of bacterial isolates
- 0%
- 5%
- 10%
- 15%
- 20%
- 25%
- 30%
- 35%
- 40%
- 45%
- 50%
DISCUSSION

The main findings of this study were the prevalence of UTI among pregnant women in Padma Nursing Home (P), Ltd., Pokhara, Nepal which was 37.5% compared to the non-pregnant that gave 18.48%. Similar high prevalence of UTI among pregnant women was obtained in a previous study[5]. Prolonged stasis of urine in urinary bladder favors growth of microorganism, relaxation of vesico-ureteric junction leads to reflux of urine from bladder to ureter and later up to renal pelvis and later can affect the renal parenchyma affecting the function of kidneys. In addition, some maternal defense mechanisms are less effective during pregnancy[26].

In this study, age group 20 -30 years had got high prevalence of UTI. A total of 68 (45.94%) patients of total UTI positive cases were found in this age group. Previous studies[9] [27] [28] also found similar results. A recent study[25] found that women in the age group 21-30 years were more prone to UTI. This result tells that sexually active women and pregnant women are more prone to UTI.

Many previous studies also have found similar results which suggested that sexual intercourse is one of the significant factors of UTI. Similar study[2] reported the highest prevalence of UTI among the women of age 21-30. It could be due to these set of age being the most sexually active age of life when most women tend to give birth to their children before menopause sets in[14].

Among total pregnant 62.5% cases were between 20-30 years which correlates with a study among pregnant women[29] in which 20-30 years showed highest growth rate (65%) and similar study[30] showed 61% growth rate. This is explained by the fact that highest incidence of pregnancy in Nepalese women is usually between 20-30 years. Altogether, there were 6 different species of bacteria were isolated, in which 5 species were gram negative and 1 species was Gram positive. Among Gram negative, E. coli (37.83%) was the most prevalent uropathogen followed by Proteus species (21.62%), Citrobacter (16.21%), Klebsiella (10.81%), Enterobacter (8.1%) and Staphylococcus (5.4%). In this study higher number of E. coli was observed which is similar to the result obtained by previous studies[2][20][27][28][31]. Similar findings were reported in previous study[17] in which E. coli (47.5%) was one of the most predominant organisms as the major cause of urinary tract infection among the pregnant and non-pregnant women, which was followed by S. aureus (27.2%), Proteus (23.7%) and Klebsiella (1.7%). However lower percentage of E. coli (18.14%) were shown in previous study[33].

Out of the 476, urine samples collected from non-pregnant women attending the nursing homes, 88 (18.48%) were positive for various species of bacteria which includes E. coli 28 (31.81%), Citrobacter 12 (13.63%), Proteus species 24 (27.27%), Klebsiella species 12 (13.63%), Enterobacter 8 (9.09%), and Staphylococcus 4 (4.5%). Similar results were obtained by previous studies[17].

Among the ten drugs used, the mean susceptibility was high for Amikacin(100%), Gentamicin (100%), Imipenem (100%), Norfloxacin (100%) and Ciprofloxacin (81.08%) but low for Nitrofurantoin (72.97%), Ofloxacin(72.97%), Ceftriazone (67.56%), Cefixime (43.24%) and Ceftazidime (2.7%) which is similar to previous study[17]. In a similar study[3] the organisms isolated were sensitive to Ofloxacin, Nalidixic acid, Gentamicin, and Norfloxacin. Similarly in a previous study[16] high sensitivity pattern was seen towards Amikacin (95%), Gentamicin (87%), Nitrofurantoin (87%), Ciprofloxacin (91%) which was similar to this study. In a previous study[6] majority of Gram negative bacteria showed high susceptibility towards Amikacin, Nitrofurantoin, Gentamicin and Norfloxacin. In a study[29] it was found that Nitrofurantoin was most effective drug followed by Ofloxacin similar to this study.

The most dominant pathogen E. coli was 100% sensitive to Amikacin, Gentamicin, Imipenem where as 100% resistance to Ceftazidime in both pregant and non-pregnant cases. E. coli was 85.71% sensitive to Nitrofurantoin among pregnant and 100% sensitive among non-pregnant cases. E. coli was 85.71% sensitive...
to Norfloxacin among pregnant and 57.14% sensitive among non-pregnant cases. E. coli was 85.71% sensitive to both Oxofloxin and Ciprofloxacin among pregnant and 28.57% sensitive among non-pregnant cases. However, previous study found E. coli 49% sensitive to Ciprofloxacin. E. coli was 71.42% sensitive to Ceftriazone among pregnant and 28.57% sensitive among non-pregnant cases. 71.42% sensitive was 28.57% sensitive to Cefixime among pregnant and 14.28% sensitive among non-pregnant cases. In a study done among pregnant women 73.7% of E. coli were sensitive to Nitrofurantoin. In another study E. coli was most sensitive to Amikacin, Gentamicin, Norfloxacin which is similar to this study.

This study shows that the emergence of drug resistance strains is common in the UTI patients visiting Padma Nursing Homes (P) Ltd. and this may be due to empirical treatment of UTI. The main cause of drug resistance in developing countries like Nepal is due to less facility for the health care, taking antibiotics without laboratory investigation and not taking appropriate dose of drugs.

**Conclusion**

UTI is more prevalent among pregnant women than in non-pregnant women. Escherichia coli were the most prevalent causative organism in this research. The women of age group of 20-30 are more prone to UTI because they are sexually active and it is the appropriate age for pregnancy also.

Resistance of the isolates to some of the antibiotics is not only due to drug abuse, it could be also due to their vulnerable cell wall that is protected by an outer membrane that prevents permeation of the antibiotics and also due to inappropriate use. Antibiotics such as Amikacin, Gentamicin, Imipinem, Norfloxacin and Ciprofloxacin play a great role in the treatment of urinary tract infection. Antimicrobial sensitivity test helps to find out the appropriate drug for UTI and to identify the resistance antibiotics.

All pregnant women should be screened for UTI with a urine culture, treated with antibiotics if the culture is positive and then retested for cure. The goal of early diagnosis and treatment of UTI during pregnancy is to prevent complications with all the added benefits to the mother and the fetus. Regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with nosocomial UTIs. Drug abuse which has been a bane of our society and enabling factor to antibiotic resistance should be a target source to save these newly developed antibiotics from suffering ineffectiveness. Finally, the Ministry of Health of Nepal should enlighten women about UTI through antenatal lectures, radio and television health programs by qualified nurses and doctors.

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