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

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KeyWords
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 infectionsencountered 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 fewcommensals and also perineum which can contaminate urine when it is beingcollected. With female patients, the urine may become contaminated withorganisms from the vagina. Vaginal contamination is often indicated by thepresence 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 byDepartment of Health Services, 0.46%

of total outdoor patients suffered fromUTI in Nepal[7]. UTIs constitute one of the major causes ofmorbidity and mortality. Incidence of infection is higher in women[2]. It was reported that up to 15% of women will have one episode ofUTI at some time during their life. It is a common health problem amongpregnant women accounting for about 10% of primary care consultation[8].

In most instances, growth of more than 10^5 organisms per milliliter from aproperly collected midstream urine sample indicates infection[1] in an asymptomatic patient, or as more than 100 organisms/ml of urinewith accompanying pyuria (>5 WBCs/ml) in symptomatic patient.Particularly in asymptomatic patients, a diagnosis of UTI should be supportedby 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 andsometimes in kidney,[11] absence of prostatic secretions,[12] pregnancy, and easy contamination of urinary tract withfecal flora[13].

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

UTI has been reported among 20% of the pregnant women and it is the mostcommon cause of admission in obstetrical wards. Both progesterone andestrogens levels increase during pregnancy and these will lead to decreasedureteral and bladder tone[15].In the non-pregnant state, the uteruslies just behind and partly over the bladder while in the pregnant state; theenlarging uterus affects all the tissues of the urinary tract at various times[14]. It usually begins to occur in early pregnancy at 6 weeksand peaks during 22 to 24 weeks due to number of factors including ureteraldilatation, increased bladder volume and decreased bladder tone, along withdecreased ureteral tone which contributes to increased urinary stasis andureterovesicalreflux[16].

UTI accounts for a significant part of the work load in clinical microbiologylaboratories and enteric bacteria (in particular, *Escherichia coli*) remained themost frequent cause of UTI, although the distribution of pathogens that causeUTI is changing[17].*E. coli* is the commonest urinary pathogencausing 60–90% of infections. Some strains are more invasive, e.g. capsulatedstrains are able to resist phagocytosis while other strains are more adhesive. UTIscaused by *Pseudomonas, Proteus, Klebsiella* species and *S. aureus*, areassociated with hospital-acquired infections, often following catheterization orgynaecologicalsurgery[18].

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

Antimicrobial resistance (AMR) is one of the major problems throughout theworld. The major mechanisms of AMR are mutations, enzymes, targetalterations and efflux pump. Antibiotic therapy has played a vital role in thetreatment of human infections in the 20th century, since the discovery ofpenicillin in 1940s[21]. In the last couple of years, there hasbeen a lot of focus in scientific literature on inappropriate use of antimicrobialagents resulting in the spread of bacterial resistance. The widespread andinappropriate use of antibiotics is recognized as a significant contributingfactor to the spread of bacterial resistance[17]. The etiological agent and their susceptibility patterns of UTIs vary in regionand geographical location. Besides, the etiology and drug resistance changethrough time. Knowledge of the local bacterial etiology and susceptibilitypatterns is required to trace any change that might have occurred in time sothat updated recommendation for optimal empirical therapy of UTI can bemade[22].

Nepal is developing country and has high illiteracy rate. In developingcountries UTIs are one of the most commonly diagnosed disease among thepatients seeking medical service with frequency of 180 per 1000[23]. UTI is a common disease among Nepalese population as wellas one of the commonest nosocomial infections. Today, antimicrobial drugsremain the front line therapy for conquering bacterial infection. For thesuccessful treatment, culture and sensitivity test is essential which is lacking inmany part of Nepal. Early detection and eradication of bacteriuria is veryimportant for prevention of recurrence and complication e.g. chronicpyelonephritis, chronic renal failure etc.[24]. The emerging antimicrobial resistance has been a burden for the treatment ofvarious infectious diseases. It has been considered as a global threat to public[25]. Therefore, the present study is aimed to determine differentbacterial species causing UTI and their antimicrobial susceptibility profileamong pregnant and non-pregnant patients attending Padma Nursing Home(P) Ltd.

MATERIALS AND METHODS

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

Sample source

The study was carried out in Microbiology laboratory of Padma NursingHome (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 5-10ml mid-stream urine.

Testing urine for hCG (pregnancy test)

The strip was dipped in the urine and the result was read after 5 minutes. Apositive pregnancy test was therefore shown by colored bands (usually pinkrose color) appearing in both the control and test zones. A negative test wasshown 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 wascentrifuged at 3000 rpm for 10 min. The supernant was discarded and thesediment was examined by wet mount preparation method. Wet mountpreparation of urinary sediments was observed through microscope for thepresence of

WBC, pus cells and RBC. Number of WBC and RBC wasestimated as number per HPF that is 40X objective of microscope.

Culture of urine sample

Semi-quantitative culture technique was used to culture urine specimen andto detect the presence of significant bacteriuria by standard methods. Acalibrated inoculating loop of standard dimension was used to take upapproximately fixed and known volume (0.001ml) of mixed uncentrifugedurine. All the urine samples were processed on the blood agar (BA) andMacConkey 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 insignificant, according to thestandard criteria. A growth of $\geq 10^5$ colony forming units/ml (cfu/ml) wasconsidered as significant bacteriuria. Patients with significant bacteriuria andsymptomatic patients with lower colony counts were also considered ashaving UTI. The organism was identified by standard methods from thesamples showing significant bacteriuria. Cultures with morethan three colonies were discarded, as contaminants and their antibioticsusceptibility 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 theirresponse 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 variousantimicrobial disks was done by modified Kirby-Bauer disk diffusion methodas recommended by Clinical and Laboratory Standards Institute (CLSI) usingMuller 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 andwas categorized as resistance (R) intermediate (I) and susceptible (S). The antibiotics disc used were Amikacin (30 µg), Ceftazidime (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 no-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 Imepenem. *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 antrimicrobial 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

	Positive	Negative	Total
Pregnant	60	100	160
Non-pregnant	88	388	476
Total	148	488	636

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

Uropathogens ⟶		*E. coli*	*Klebsiella*	*Proteus*	*Citrobacter*	*Enterobacter*	*Staphylococcus*	Total
Pregnant	**number**	28	4	8	12	4	4	60
	percentage	46.67%	6.67%	13.33%	20%	6.67%	6.67%	100%
Non-pregnant	**number**	28	12	24	12	8	4	88
	percentage	31.8%	13.63%	27.27%	13.63%	9.09%	4.5%	100%

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

Pathogens →	*E. coli*		*Proteus*		*Citrobacter*		*Klebsiella*		*Enterobacter*		*Staphylococcus*	
Antibiotics ↓	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**	**NP**	**P**	**NP**
Nitrofurantion	24 85.71%	28 100%	4 50%	20 83.33%	4 33.33%	4 33.33%	4 100%	4 33.33%	0	8 100%	4 100%	4 100%
Norfloxacin	24 85.71%	16 57.14%	8 100%	24 100%	12 100%	8 66.66%	0	12 100%	4 100%	8 100%	4 100%	4 100%
Amikacin	28 100%	28 100%	8 100%	24 100%	12 100%	12 100%	4 100%	12 100%	4 100%	8 100%	4 100%	4 100%
Ofloxacin	24 85.71%	8 28.57%	4 50%	24 100%	12 100%	8 66.66%	4 100%	8 66.66%	4 100%	8 100%	4 100%	0
Gentamicin	28 100%	28 100	8 100%	24 100%	12 100%	12 100%	4 100%	12 100%	4 100%	8 100%	4 100%	4 100%
Ciprofloxacin	24 85.71%	8 28.57%	8 100%	24 100%	12 100%	12 100%	4 100%	12 100%	4 100%	8 100%	4 100%	0
Ceftriazone	20 71.42%	8 28.57%	8 100%	20 83.33%	8 66.66%	8 66.66%	0	8 66.66%	4 100%	8 100%	4 100%	4 100%
Imepenem	28 100%	28 100%	8 100%	24 100%	12 100%	12 100%	4 100%	12 100%	4 100%	8 100%	4 100%	4 100%
Ceftazidime	0	0	0	4 14.28%	0	0	0	0	0	0	0	0
Cefixime	8 28.57%	4 14.28%	4 50%	20 83.33%	8 66.66%	4 33.33%	0	4 33.33%	4 100	8 100%	0	0

P=pregnant and NP= non-pregnant

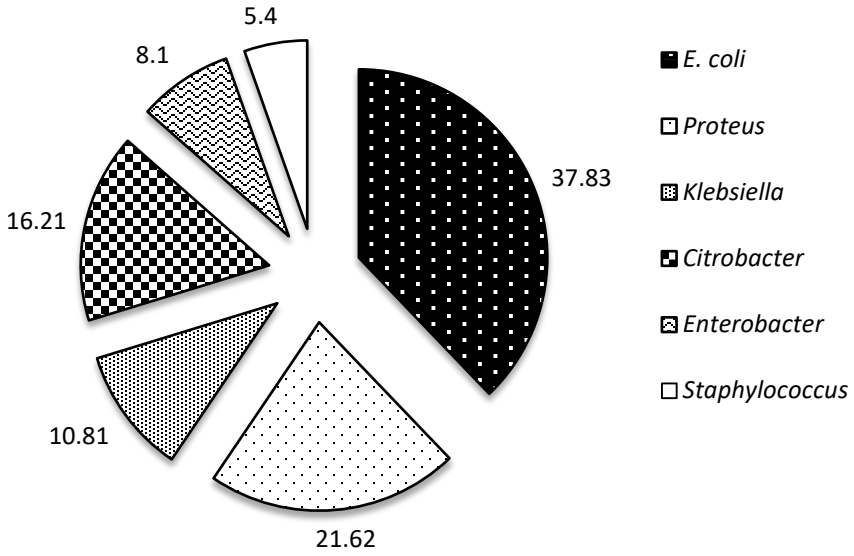

Figure 1: Species wise distribution of isolated species

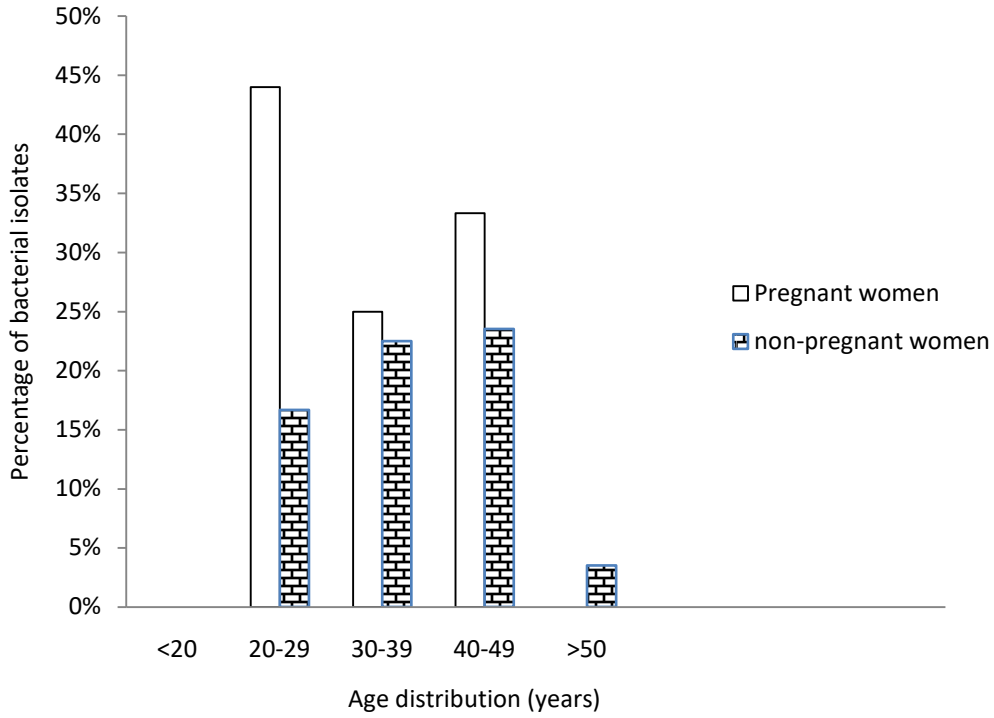

Figure 2: Bacteriuria among pregnant and non-pregnant women in different age group.

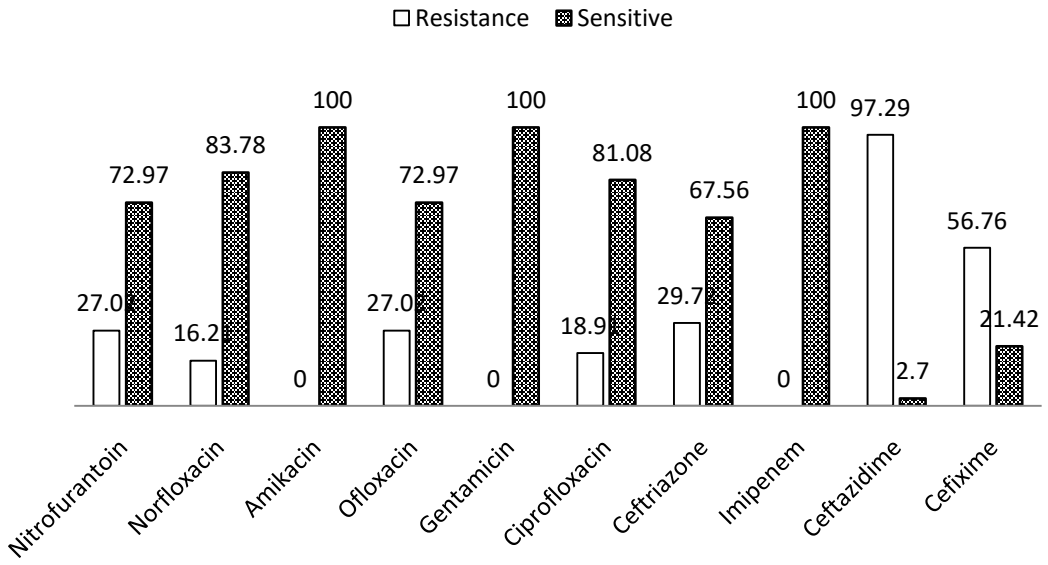

Figure 3: Antimicrobial sensitivity patterns of different antibiotics

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% compred 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 ofmicroorganism, relaxation of vesico-ureteric junction leads to reflux of urinefrom bladder to ureter and later up to renal pelvis and later can affect the renalparenchyma affecting the function of kidneys. In addition, some maternaldefense mechanisms are less effective during pregnancy[26].

In this study, age group 20-30 years had got high prevalence of UTI. A total of68 (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 tellsthat sexually active women and pregnant women are more prone to UTI.

Many previous studies also have found similar results which suggested thatsexual intercourse is one of the significant factors of UTI. Similar study[2] reported the highest prevalence of UTI among thewomen of age 21-30. It could be due to these set of age being the mostsexually active age of life when most women tend to give birth to theirchildren before menopause sets in[5].

Among total pregnant 62.5% cases were between 20-30 years which correlateswith a study among pregnant women[29] in which20-30 years showed highest growth rate (65%) and similar study[30] showed 61% growth rate. This is explained by the fact that highestincidence of pregnancy in Nepalese women is usually between 20-30 years.Altogether, there were 6 different species of bacteria were isolated, in which 5species were gram negative and 1 species was Gram positive. Among Gramnegative, *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 numberof *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 themajor causes of urinary tract infection, among the pregnant and non-pregnantwomen, 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 attendingthe nursing homes, 88 (18.48%) were positive for various species of bacteriawhich includes *E. coli* 28 (31.81%), *Citrobacter* 12 (13.63%), *Proteus* species24 (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) andCiprofloxacin (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[3]. In a similar study[5] the organisms isolated weresensitive to Ofloxacin, Nalidixic acid, Gentamicin, and Norfloxacin. Similarlyin a previous study[16] high sensitivity pattern was seentowards 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 towardsAmikacin, Nitrofurantoin, Gentamicin and Norfloxacin. In a study[25]it was found that Nitrofurantoinwas 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 bothpregnant and non-pregnant cases. *E. coli* was 85.71% sensitive toNitrofurantoin among pregnant and 100% sensitive among non-pregnantcases. *E. coli* was 85.71% sensitive

to Norfloxacin among pregnant and57.14% sensitive among non-pregnant cases. *E. coli* was 85.71% sensitive toboth Ofloxacin and Ciprofloxacin among pregnant and 28.57% sensitiveamong non-pregnant cases. However, previous study[25]found *E. coli* 49%sensitive to Ciprofloxacin. *E. coli* was 71.42% sensitive to Ceftriazone amongpregnant and 28.57% sensitive among non-pregnant cases. 71.42% sensitivewas 28.57% sensitive to Cefixime among pregnant and 14.28% sensitiveamong non-pregnant cases. In a study[15] done among pregnant women 73.7% of *E. coli* were sensitive to Nitrofurantoin. In astudy[3]*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 inthe UTI patients visiting Padma Nursing Homes (P) Ltd. and this may be dueto empirical treatment of UTI. The main cause of drug resistance indeveloping countries like Nepal is due to less facility for the health care,taking antibiotics without laboratory investigation and not taking appropriatedose 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, Imipenem, 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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