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Frequency of urinary tract infection causing agents in pregnant women and their antimicrobial susceptibility profile

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Abstract: This study was aimed to determine the frequency of microorganisms causing UTIs in pregnant women, Antimicrobial susceptibilty of UTIs causing microorganisms against Cefexime, Imipenem, Carbenicillin, Ceftriaxone, Cefoperazone and Tobramycine. The total number of patients was 63, who had pregnancy and complaints of urinary tract infections. The frequency of different microorganisms was determined with appropriate tests. 49.2% pregnant females have urinary tract infections (UTI) and 50.8% did not have urinary tract infections (UTI). Mean age of the patients was 26.22, median 26, mode 30 and standard deviation 4.312 years. Median of pregnancies was 3, mode 1 and standard deviation was 1.67 of patients having complications of UTI. 64.5 %, *S. seprophyticus* 25.8%, *proteus* 6.5% and *klebseilla* was 3.2% caused infection in urinary tract of pregnant females. Microorganisms of urinary tract infections (UTI) were highly resistant to carbencillin and ceftriaxone 90.3% and 9.7 % were sensitive. Cefexime was 51.6 resistant and 48.4% resistant. Cefoparazone was 38.7% resistant and 61.7% was sensitive and tobramycine was 67.7% resistant and 32.3% was sensitive. It was concluded that predominant organisms causing UTIs in pregnant women were *E.coli, S. saprophyticus, Proteus* and *Klebsiella.* It was also concluded that Ceftriazone, Cefexime Carbenicillin and Tobramycine were highly resistant, while Imipenem and Cefoparazone showed highest sensitivity against UTIs causing microorganisms.

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INTRODUCTION

Urinary tract infections (UTIs) are characterized by colonization of pathogens in any part of the urinary tract[1]. Urinary tract is consisted of the organs that collect, store urine and finally pass it from the bodies which are urethra, bladder, ureters and kidneys[2.] Sign and symptoms of UTI are dysuria, urgency, hematuria, bacteruria (presence of more than 10^5 CFU/ml organisms), In pyelonephritis patient presents with flank (abdominal) pain and fever[3.] Other signs of UTIs are foul-smelling and cloudy urine[4.] UTI is one the most prevalent infectious diseases seriously infecting 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global economy[5.] It is estimated that the overall costs associated with UTIs are nearly $2 billion each year[6]. The life time risk for UTI in females is greater than 50%[7.] UTIs are common during pregnancy, estimated that 1 in 3 women of childbearing age will have a UTI[8.] Most common causative organism is *E. coli*[9.] Bacteruria can lead to the development of cystitis or pyelonephritis. In cystitis the anatomical site of the infection can be the bladder and Urethra in urethritis[10]. Acute cystitis affects approximately 1% of all pregnant women. About 30% of women with asymptomatic bacteriuria will develop acute cystitis during their pregnancy[11].

In Pyelonephritis kidneys are the anatomical site of the infection associated with inflammation of the renal parenchyma, calices and pelvis[12]. Pyelonephritis is the most serious type of urinary infection in pregnancy with an incidence rate of 2%. It is estimated that 20% to 40% of pregnant women having asymptomatic bacteriuria will develop this condition later in gestation[13]. Acute pyelonephritis may proceed to severe complications for baby and mother, such as premature delivery, low birth weight infants, hypertension, renal failure and fetal death[14].If UTI is untreated it may lead to sepsis and death, Sepsis in urology remains a severe problem with a mortality rate as high as 20 to 40%[15] .

Epidemiological studies of UTIs indicate the frequency of asymptomatic bacteriuria is 4 to 10%, cystitis 1 to 4% and pyelonephritis 1 to 2%, these

rates of infections are similar in pregnant and non-pregnant women[16].

The most common factors of UTI in pregnancy are indwelling catheter, ureteric stent, or nephrostomy tube, ureteral dilatation, increased bladder volume and decreased bladder tone along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux[17.] Recurrent UTIs in sexually active premenopausal women are due to sexual intercourse and use of spermicidal which leads to 75 to 90% of bladder infections[3]. Ability of urinary tract to resist bacteria is decreased due to decreased uretheral tone and increase progesterone and estrogen level that allows some bacteria to grow and cause UTI in pregnancy Up to 70% of pregnant women develop glycosuria, which encourages bacterial growth in the urine. [18, 19] During pregnancy bacteriuria can occur when bacteria from a fecal route gets an opportunity to the bladder by ascending the relatively short female urethra[18].

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Sheikh Zayed Medical Hospital/College, Rahim Yar Khan, Pakistan. A total of 63 samples were collected from women visiting OPD and Gynecology ward. This study was only on pregnant women having symptoms of UTI attending OPD and Gynecology ward. A questionnaire was designed containing demographic variables like name age, sex, OPD/Ward No.

Sample collection, handling and transport

Only midstream urine (MSU) employed to minimize contamination with normal flora of genitourinary tract. It was made sure that women did not take any antibiotic. Freshly voided MSU samples (10-20ml) were collected from non-catheterized patients in a wide mouth, leak proof and sterile container after cleansing the genitals with soap and water. The urine container was labelled with demographic information of patient and the time of collection. The urine specimens were then delivered to the laboratory immediately and processed within one hour.

Culture of samples

Freshly collected clean-catch Urine was mixed by rotating the container. With the help of a sterilized calibrated wire loop, 0.002ml urine was inoculated on a quarter plate of CLED agar (OXOID Company) provided by Microbiology Department of Sheikh Zayed Hospital, Rahim Yar Khan. The detailed procedure of CLED agar is given in appendix B. Plate was incubated aerobically at 35-37°C. A positive urine culture was defined as colony count $>10^5$cfu/ml for MSU. All positive cultures were further identified by their characteristics appearance on the media and confirmed by the detailed panel of biochemical reactions.

Identification of isolates

Identification of all significant isolates is performed by following procedures. *E. coli* and *Klebsiella spp* were identified by Lactose fermentation on CLED agar. *E. coli* produced yellow (lactose-fermenting) opaque colonies often with slightly deeper coloured centre, whereas *Klebsiella* produced large mucoid yellow or yellow-white colonies. *Salmonella, Proteus spp,* and *Pseudomonas spp* were non-fermenters. *Proteus spp* produced transluscent blue-grey colonies, whereas *Pseudomonas spp* produced green colonies with rough periphery (characteristic color). *E. faecalis* formed Small yellow colonies whereas *S. saprophyticus* formed yellow to white colonies. Gram staining was performed to differentiate between Gram positive and Gram negative bacteria. Catalase test was performed to identify *Staphylococcus saprophyticus,* which is catalase positive. Coagulase test was performed to identify *Staphylococcus saprophyticus,* which is coagulase negative. Novobiocin disc was applied on plates to identify *Staphylococcus saprophyticus, which* is novobiocin resistant. The detailed procedure of novobiocin resistance is given in appendix F. Oxidase test was performed to identify *Pseudomonas spp* which are oxidase positive. The detailed procedure of oxidase test is given in appendix G. Motility test was performed by Hanging drop method. *E. coli, Salmonella* and *Proteus spp* were motile. The detailed procedure of hanging drop method is given in appendix H. Indole test was performed for identification of *E. coli and Proteus spp, they* were indole positive. Urease test was performed for identification of *Proteus spp*, which is urease positive. Citrate test was performed to identify *Proteus spp* and *Klebsiella spp, which* are citrate positive.

Antimicrobial susceptibility testing

The antimicrobial susceptibility profile of all isolates was done by the standard disk diffusion method using commercial disks (Oxoid) of Cefexime (5ug), Carbenicilln (100 ug),Imipenem (10g), Ceftriaxone (30µg), Cefoperazone (75µg) and Tobramycin (10µg) provided by Microbiology Department of Sheikh Zayed Hospital, Rahim Yar Khan .When pure culture was obtained, a loopful bacteria was taken from a colony and was transferred to a tube containing 5ml of normal saline and mixed gently until it formed a homogenous

suspension. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 in order to standardize the inoculums. A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle distribute the bacteria evenly over the entire surface of Nutrient agar (Oxoid). The inoculated plates were left at room temperature to dry for 3-5 minutes. With the aid of sterile needle discs of Cefexime (5µg), Carbenicilln (100µg), Imipenem (10µg), Ceftriaxone (30µg), Cefoperazone (75µg) and Tobramycin (10µg) were put on the surface of Nutrient agar (Oxoid). The plates were incubated at 37ºC for 24 hours. After overnight incubation plates were examined to read the zones of inhibition by Vernier calipers and results were interpreted according to CLSI 2010 i.e Cefexime was sensitive at ≥19mm and resistant at ≤15mm, Imipenem was sensitive at ≥16mm and resistant at ≤13mm,Carbenicillin was sensitive at ≥23mm and resistant at ≤19mm, Ceftriaxone was sensitive at≥23mm and resistant at ≤19mm,cefoperazone was sensitive at ≥21mm and resistant at ≤15mm,Tobramycin was sensitive at ≥15mm and resistant at ≤12mm.

Statistical analysis

All the data was entered and analyzed in computer based programme SPSS and mean, median, mode and standard deviation were calculated for quantitative variables whereas qualitative variables were described in rates and frequencies.

Ethical consideration

This research project was approved by the Department of Microbiology and was ethically cleared. Written informed consent was obtained from all patients participating in the study. Patient directly or indirectly was not affected.

RESULTS

The total number of patients was 63, who had pregnancy and complaints of urinary tract infections. The frequency of different microorganisms was determined with appropriate tests. Detail about results is present in below tables.

Out of 63 numbers of patients 49.2% pregnant females have urinary tract infections (UTI) and 50.8% did not have urinary tract infections (UTI). Mean age of the patients was 26.22, median 26, mode 30 and standard deviation 4.312 years. Median of pregnancies was 3, mode 1 and standard deviation was 1.67 of patients having complications of UTI. 64.5 %, *S .saprophyticus* 25.8%, *Proteus* 6.5% and *Klebseilla* was 3.2% caused infection in urinary tract of pregnant females. Microorganisms of urinary tract infections (UTI) were highly resistant to carbencillin and ceftriaxone 90.3% and 9.7 % were sensitive. Cefexime was 51.6 resistant and 48.4% resistant. Cefoparazone was 38.7% resistant and 61.7% was sensitive and tobramycine was 67.7% resistant and 32.3% was sensitive.

Table 1: General statistics of age and number of pregnancies in patients having complaints of UTIs (n=63)

Statistics	Age (years)	No. of Pregnancies
Mean	26.22	3.07
Standard Deviation	4.312	1.676
Median	26	3
Mode	30	1

Table 2: Distribution of Urinary tract infections among patients.

Observations	Frequency	%age
Growth	31	49.2
No growth	32	50.8

Table 3: General statistics of age and no of pregnancies in patients having UTI (n=31).

Statistics		Age(year)	No of pregnancies
Mean		26.68	3.40
Standard Deviation		3.331	1.429
Median		26.00	3.00
Mode		25	3

Table 4: Frequency of microorganisms causing UTI in pregnant women (n=31).

Microorganisms	Frequency	%age
E.coli	20	64.5
S.saprophyticus	8	25.8
Proteus	2	6.5
Klebsiella	1	3.2

Table 5: Sensitivity or resistance of antimicrobial drugs.

Antimicrobial Drugs	Frequency		Susceptibility	
	Sensitive	Resistance	Sensitive %age	Resistance %age
Cefexime	15	16	48.4%	51.6%
Imipenem	28	3	90.3%	9.7%
Carbencillin.	3	28	9.7%	90.3%
Ceftriaxone	3	28	9.7%	90.3%
cefoperazone	19	12	61.3%	38.7%
Tobramycin	10	21	32.3%	67.7%

DISCUSSION

Urinary tract infections (UTIs) are the most commonly encountered infectious diseases in developing countries with an estimated annual global incidence of at least 250 million[1,2.] It affects all age groups, but women are more susceptible than men, due to absence of prostatic secretion, short urethra, pregnancy and easy contamination of the

urinary tract with faecal flora[20.] Pregnant women are more susceptible to UTI due to a number of factors including ureteral dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux. Glycosuria in pregnancy encourages bacterial growth in the urine[21]. Microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and from time to time. In our study a total of 63 urine samples were collected from Gynaecology ward and OPD. According to this study mean age of patients was 26.22, median 26, mode 30 and standard deviation 4.312 years. It shows that 49.2% symptomatic patients have UTIs and 50.8% asymptomatic patients don't have UTIs. The mean age of patients having UTIs was 26.68, median 26, mode 25 and standard deviation 3.331 years. In our study *E.coli* (64.5%) was predominant isolate followed by S.saprophyticus (25.8%), *Proteus spp.* (6.5%) and *Klebseilla*(3.2%) respectively. Similar findings were observed by many researchers around the world. Our study results matched with the study results of Gomal Medical College, Dera Ismail Khan Pakistan. Aziz Marjan Khattak et *al* showed that *E.Coli* was the most common pathogen (38.89%) followed by *S. saprophyticus*(16.68%) and *Proteus mirabilis* (05.55%). The most useful antibiotics in our study were Cefixime (5µg), Imipenem (10µg), Carbenicillin(100µg), Ceftriaxone (30µg), Cefoperazone (75 µg) and Tobramycin (10 µg). Our study shows highest sensitivity (90.3%) to imipenem. Our study revealed that bacterial resistance in uropathogens continues to be a great problem that should be properly evaluated.

CONCLUSION

It was concluded that predominant organisms causing UTIs in pregnant women were *E.coli, S.saprophyticus, Proteus* and *Klebsiella.* It was also concluded that Ceftriazone, Cefexime Carbenicillin and Tobramycine were highly resistant, while Imipenem and Cefoparazone showed highest sensitivity against Urinary tract infections causing by microorganisms.

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