

ISOLATION, IDENTIFICATION AND SENSITIVITY PATTERN OF MICROORGANISMS ISOLATED FROM TILE URINE OF PREGNANT WOMEN

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ABSTRACT:

The present studies were conducted to detect and identify the microorganism from the urine of pregnant women having urinary tract infection. The antibiotic susceptibility of these isolated microorganisms was also determined.

The microorganisms found responsible for the infection were bacteria, fungi, yeast and protozoa. Among the bacteria two were identified as Gram-positive cocci i.e. *Staphylococcus aureus* and *S. epidennidis*, the remaining two were Gram-negative bacilli which were *Escherichia coli* and *Pseudomonas aentginosa*. The fungus was identified as *Aspetgillus niger* and the yeast like fungus *Candida albican*. The only protozoan found in some of the urine samples was *Trichomonas vaginalis*. These isolated and identified microorganisms were more susceptible to Norfloxacin, Velosef, Minocin, Nitrofurantoin, Malidixic acid and Metronidazole whereas antibiotics Penbritin and Cefaloridine were least effective against these microorganisms.

Introduction

Urinary tract infection in pregnant women is a very pervasive clinical problem. In the past scientists were aware of this infection and Pasteur (1883) noted that urine was an excellent growth medium for bacteria. Kass (1957) by relating bacterial counts to clinical history in pregnant women, developed concept of significant bacteriuria by which it meant that counts of 100,000 or more bacteria per ml indicated multiplication of organisms in the urinary tract, whereas lower counts were usually associated with contamination from urethra.

Bacteriuria is frequently found during the first trimester of pregnancy in a study of 5,000 pregnant women at their first prenatal visit. Little in 1966 found that cases of bacteriuria were 5.3 percent. The renal infection among pregnant women may range from mild to severe degree. In milder cases babies born of low birth weight than those having no infection and in severe cases intrauterine fetal death and premature birth are major

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risks (Baired, 1936; Handerson *et al.*, 1962; Wren, 1968). The chances of developing pyelitis during pregnancy are doubled in those who have previous history of infection (Dodds, 1931; Gabe, 1945).

Microorganisms causing urinary tract infection are bacteria, fungi, protozoa and viruses. The main bacteria causing urinary tract infection include *Escherichia coli*, *Proteus species*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus saprophyticus* (Shannon, 1983). Among fungi yeast like species called *Candida albicans* is commonly found in urinary infection, *Candida tropicalis* and *Candida parapsilosis* are less common (Dalton and Nottebart, 1986).

The urinary tract may be infected by a few parasites. The most frequently identified parasite in urine in North America is *Trichomonas vaginalis* (Ross and Neely, 1983). It is very essential to select an antibiotic that is safe to use during pregnancy, particularly in the first trimester. Compounds that appear to be relatively safe include penicillin, ampicillin, sulfonamides and nitrofurantoin (Joseph and Kaufman, 1986). No specific research has been carried out on urinary tract infection in various hospitals of Lahore. Thus the present work has been carried out on pregnant women who come for antenatal check up. The etiological agents are isolated, identified and their possible control by using various antibiotics are reported.

Materials and Methods

Studies have been made on a total of sixty urine samples of pregnant women collected in sterilized test tubes for antenatal section of Lahore hospitals. Five urine samples of non-pregnant women were also collected as control. The samples were analysed physically for their colour, turbidity, pH and specific gravity by using combistix reagent strips. These samples were also analysed chemically by applying Benedict's qualitative test for sugar and sulphonosalicylic acid test for proteins present in the urine. Pus cells were also examined microscopically. The microorganisms were isolated by pour plate method (Cruickshank *et al.*, 1975).

The bacteria were identified on the basis of their morphological, biochemical and fermentation tests as mentioned by Breed *et al.* (1972), MacFaddin (1976) and Koneman *et al.* (1979) while fungi were identified according to the morphological characteristics as mentioned by Rapper and Fennel (1965). However, protozoa were identified according to the microscopic appearance as mentioned by Ross and Neely (1983). The microorganisms isolated from urine samples were checked for their susceptibility against certain antibiotics by Disc plate method (Pelczar and Reid, 1972).

Results

The urinary tract infection in pregnant women is an extremely common clinical problem. The present studies, carried out on the urinary tract infection of pregnant women, were channelised in three different directions i.e. collection and study of urine samples, isolation and identification of microorganisms from the infected urine samples and finally ascertaining the susceptibility of the isolated microorganisms to various antibiotics. From a total of sixty cases only 16 showed significant bacteriuria. An average pH of infected urine sample was in the range of 6.4 to 7.6 (Table 1). The protein was present in almost all the infected samples, ranging from 0.3g/L to 1.0g/L. Three patients were diabetic as the sugar in their urine was ranging from 5 mmol/L to 30 mmol/L. In microscopic examination, pus cells were present in all the infected samples, ranging from 8 to 22/microscopic field. Four types of microorganisms were isolated and identified. They were bacteria, fungi, yeast and protozoan. Their viable counts per ml were noted (Table 2). The morphological characteristics and biochemical tests of Gram positive and Gram negative bacteria were studied. The ability of Gram positive and Gram negative bacteria to ferment different carbohydrates was also noted. During microbiological studies four types of bacteria were isolated and identified. These include *E. coli*, *P. aemginosa*, *S. aureus* and *S. epidemlids*.

Table 1
Average pH of urine samples collected from different hospitals

Hospitals and Group No.	Average pH of Control and Infected Samples*
Control	5 ($\pm$ 0.11)
Fatima Memorial	
1	6.1 ($\pm$ 0.28)
2	6.0 ($\pm$ 0.28)
3	6.0 ($\pm$ 0.33)
4	7.1 ($\pm$ 0.21)
5	6.0 ($\pm$ 0.28)
Lady Willingdon	
6	7.6 ($\pm$ 0.19)
7	7.6 ($\pm$ 0.33)
8	5.2 ($\pm$ 0.11)
9	6.1 ($\pm$ 0.28)
Sir Ganga Ram	
10	7.1 ($\pm$ 0.21)
11	6.2 ($\pm$ 0.28)
12	6.0 ($\pm$ 0.28)

*Standard Errors are in parenthesis.

Table 2
Viable count of microorganisms isolated from infected urine
samples of pregnant women

Microorganisms Identified	Viable count/ML of the Urine		
	Fatima Memorial Hospital	Lady Willingdon Hospital	Sir Ganga RAM Hospital
1. <i>Escherichia coli</i>	106* x 10 ³	108* x 10 ³	105 x 10 ³
2. <i>Pseudomonas aeruginosa</i>	107 x 10 ³	—	—
3. <i>Staphylococcus aureus</i>	—	106* x 10 ³	—
4. <i>Staphylococcus epidermidis</i>	—	—	*104 x 10 ³
Average total bacterial count	106* x 10 ³	107* x 10 ³	*104 x 10 ³
5. <i>Candida albicans</i>	18* x 10 ³	106* x 10 ³	—
6. <i>Aspergillus niger</i>	21* x 10 ³	—	—

*Average viable count.

There was only one type of yeast like fungi (*Candida albican*) present in the three infected samples. The fungus *Aspergillus niger* was present in two infected samples, whereas the protozoan *Trichommmnas vaginalis* was found in four cases. The percentage of these isolated organisms are given in Table 3. The microorganism isolated from the infected urine samples were tested for their susceptibility against various antibiotics by Disc plate method (Pelczar and Reid, 1972). Fifteen types of antibiotic discs were used in the present studies. Fourteen were effective against bacteria (Table 4). One was effective against protozoa and none was effective against the fungi. The results were divided into three categories. The zone of inhibition was considered as "maxi-mum" when it was more than 22 mm. It was "moderate" when it ranged from 15 mm to 21.9 mm and "minimum" when it was less than 15 mm (Table 4). The antibiotic Norfloxacin showed maximum zone of inhibition (24-25 mm) against *S. mucus*, *E. coil*. and *P. aenginosa* and it showed moderate effect on *S. epidermidis*. The maximum activity was observed by Minocin and Velosef against *S. mucus*, *S. epidemudis* and *E. coll*. The Cegamendol was effective against the Gram-positive bacteria isolated in the present studies. Pyopen showed moderate zone of inhibition (about 18 mm to 20 mm) against *E. coli* and it showed moderate activity against *S. aureus* and *S. epidermidis*. A moderate zone of inhibition was obtained by Tobramycin against *S. aureus* and *E. coli* and minimum effect on the remaining tested bacteria. Cefatoxime showed moderate zone of inhibition and is

effective against *S. epidemidis* and *E. coil*. Augmentin showed maximum inhibition against *P. epidermidis*. Penbritin antibiotic showed only minimum effect against *P. aeruginosa*, *E. coli* and *S. epidentidis*. The Erythrocine showed maximum zone of inhibition against *E. coli* and minimum against *S. aureus* and *S. epidermtidis* (Fig.1). Minimum inhibitory effect was observed by Cefaloridine against *S. epidemridis*. Moreover, the Nitrofurantoin was effective against all the bacteria so far tested in the present studies. This indicates that the majority of the antibiotic used showed inhibitory effect against the bacteria isolated from urine samples of pregnant women. However, it was noted that *Aspergillus niger* and *Candida albican* were not inhibited by any antibiotic. *Trichomonas vaginalis* isolated from urine samples was inhibited by Metronidazole.

Discussion

The above results show that the invested urine specimens indicated an average pH from 6 to 7.6, whereas the pH of the control samples was only 5. This increase in pH towards neutral facilitates the growth of bacteria (Ganong, 1985).

On the other hand the specific gravity was normal (ranged from 1.025 to 1.030) in both pregnant and non-pregnant women's urine. The presence of protein in the urine was due to various pathological disorders (Friedman, 1983). Its presence in the collected urine samples was due to urinary tract infection as the pus cells were present in these samples. Sugar is a good culture medium for the growth of fungi and usually diabetic patients suffer more by fungal infections (Sheldon and Cherry, 1979). The results also indicate that fungal infection was found in two out of three diabetic patients.

The results showed that *E. coli* was the most common etiological agent accounting for 75% in the total infected urine samples. The *Candida albican* was found in only three of the infected urine samples, whereas *Trichomonas vaginalis* was present in four infected urine samples.

The antibiotic susceptibility pattern of microorganisms isolated from infected urine samples was studied which showed that the most effective antibiotic against bacteria was Norfloxacin. The reason is that it is one of the most effective antibiotic against conform bacteria, *Pseudomonas aeruginosa* and aerobic Gram positive cocci inhibiting the lower urinary tract (Shah, 1976; Ronald et al., 1983).

Ampliclox is a safe drug during pregnancy, but it is observed that many microorganisms have become resistant to this antibiotic in the present studies. The isolated bacteria were found moderately sensitive to Minocin, but this antibiotic cannot be used during pregnancy due to its hazards on the developing fetus (Girdwood, 1984).

Antibiotic, Velosef and Nalidixic acid were also found to be effective against the *E. coli*, *S. epidermidis* and *S. aureus*. The drug Metronidazole was very effective for *Trichomonas vaginalis*.

In the light of this study we will be able to know that which organism is most frequently responsible for urinary tract infection and to which antibiotic they are usually sensitive. This will help the clinicians to select the proper antibiotic for the management of urinary tract infection.

Table 3
Microorganism present in the infected urine samples
and their percentage occurrence

Name of organisms	No. of patients	Percentage (%)
1. <i>Escherichia coli</i>	12	75%
2. <i>Pseudomonas aeruginosa</i>	1	6.25%
3. <i>Staphylococcus aureus</i>	1	6.25%
4. <i>Staphylococcus epidermidis</i>	1	6.25%
5. <i>Candida albicans</i>	3	18.75%
6. <i>Aspergillus niger</i>	2	12.50%
7. <i>Trichomonas vaginalis</i>	4	25%

Table 4
Zone of inhibition (in mm) of various antibiotics against difference bacteria isolated
from urine samples of pregnant women

S.No.	Antibiotics	<i>S. aureus</i> mm	<i>S. epidermidis</i> mm	<i>E. coli</i> mm	<i>P. aeruginosa</i> mm
1.	Norfloxacin	24.60	16.33	24.00	27.66
2.	Minocin	17.9	24.00	25.00	N.E.
3.	Cefamandol	17.00	22.50	N.E.	N.E.
4.	Velosef	24.00	22.66	30.33	N.E.
5.	Pyopen	10.33	8.66	17.92	20.30
6.	Nalidixic acid	15.33	15.55	29.88	N.E.
7.	Tobramycin	16.00	13.33	19.22	N.E.
8.	Ampiclox	12.44	12.66	16.00	10.33
9.	Cefotaxime	N.E.	18.44	16.00	N.E.

Table continue . . .

S.No.	Antibiotics	<i>S. aureus</i> mm	<i>S. epidermidis</i> mm	<i>E. coli</i> mm	<i>P. aeruginosa</i> mm
10.	Augmentin	10.88	34.77	13.33	10.00
11.	Penbritin	N.E.	10.00	8.22	10.00
12.	Erythrocine	10.00	8.33	16.00	N.E.
13.	Cefaloridine	N.E.	12.00	N.E.	N.E.
14.	Nitrofurantoin	17.98	15.33	19.22	16.33

N.E. means: No effect.

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