

Original Research Article

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Pregnancy associated Urinary Tract Infection: Prevalence and Screening

Y.Valentina* and S.Srirangaraj

Department of Microbiology, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Pillaiyarkuppam, Pondicherry, India

*Corresponding author

ABSTRACT

Urinary tract infections (UTI) are an important global health problem prevailing in all age groups. They are one of the commonest infective sequel during pregnancy & is of great importance due to the serious impact it has on the mother and the foetus. This study was taken up to determine the prevalence of UTI in pregnant women, to identify the common uropathogen causing UTI, comparison of various screening methods with bacteriological culture & to document the prevailing antibiogram pattern of the common uropathogens causing UTI. Materials and methods: The study involved 500 pregnant women attending the antenatal clinic of the Department of OBG at Mahatma Gandhi Medical College & Research Institute (MGMCRI), Pondicherry. A brief history(presenting illness, past history & personal history)was taken from the patient and 5ml urine sample was then collected under strict aseptic precautions, transported at the earliest to the Microbiology lab at MGMCRI for processing & identification. The sample was first subjected to a wet mount examination, a Dipstick testing: A10 REAGENT STRIPS, RAPHA(for detection of urinary leucocytes & detection of urinary nitrites) & urine culture on CLED agar & 5% sheep blood agar. Any growth if present was then identified by routine preliminary tests & biochemical tests. Antibiotic sensitivity testing was then done as per CLSI guidelines. Results & Discussion: Prevalence of UTI among pregnant women was 45 % (n=219), this high prevalence could be attributed to the low income state of the patient & anaemia. Higher prevalence of UTI in pregnancy of 44%(n=97/219)was observed in the age group of 21-25yrs,this could be due to the high sexual activity or higher prevalence of anaemia among this group. Detection of urinary nitrite was positive in 172/219 cases positive by urine culture, accounting for 78.5%. This could be due to the variability in the kit manufacturer or the time delay in the appearance of nitrites in the urine giving false negatives. Urine culture remained the gold standard for diagnosis of UTI. In our study Enterococcus emerged as the third most common isolate, following *E.coli* and Klebsiella. This may be attributed to the ablution practices following defecation in women.Gram negative bacilli showed increased sensitivity to Amikacin, Nitrofurantoin & Imipenem.Gram positive cocci showed 60% sensitivity to Amoxyclav, Gentamicin, Tetracycline, Linezolid & Teicoplanin and 80% sensitivity to Vancomycin. However, on account of teratogenicity of Amikacin, Nitrofurantoin may be a better alternative.

Keywords

Urinary Tract Infection, antibiogram pattern, Dipstick Testing

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Introduction

Urinary tract infection (UTI) is one of the commonest infective sequels associated with

pregnancy. Infection of any part of the complex renal system is considered as,

Urinary Tract Infection (UTI). Significant bacteriuria is defined as a urine sample containing more than 10^5 colonies/ml of urine in pure culture using a standard calibrated bacteriological loop.⁽¹⁾ Furthermore it leads to both maternal and neonatal adverse outcomes in terms of pyelonephritis, premature labour, preterm rupture of the membranes, low birth weight, pre-eclampsia and anaemia of pregnancy. On an average 8 to 45% of all women develop symptoms of UTI at some stage during their pregnancy^(2,3). UTI can present with a wide spectrum of symptoms ranging from mild irritative voiding to bacteraemia which could lead to sepsis and even death.⁽⁴⁾ It can be classified based on the site involved as upper UTI or lower UTI, based on association with complications as, complicated or uncomplicated and based on symptoms as symptomatic or asymptomatic.

Asymptomatic bacteriuria (ASB) is the presence of bacteria in the urine in significant number of a person without symptoms of urinary tract infection. As per recent reports, around 25% to 30% women develop symptomatic urinary tract infection while pregnant. However, the prevalence of ASB in pregnant women is 2-10%⁽²⁾. ASB is more common in pregnancy compared to non-pregnant women and this condition is often underreported as the diagnosis is challenging due to the absence of specific symptoms or signs, whereas, symptomatic bacteriuria causes no problems because of the ease in diagnosis and treatment. Acute pyelonephritis during pregnancy is a serious systemic illness that can progress to maternal sepsis, preterm labour and premature delivery.⁽⁵⁾

Early diagnosis and treatment of UTI has shown marked improvement in pregnancy outcome as well as reduction of the incidence of acute pyelonephritis, highlighting the need to screen all women in

pregnancy irrespective of symptoms. Although urine culture is considered as gold standard test, it has long turn-around time. Several rapid screening tests are currently in use, although the evidence of their efficacy is of poor quality. However, combinations of them have been reported to be a reliable alternative having sensitivity and specificity comparable to urine culture.

In view of the potential importance of bacteriuria in pregnancy, the present study was undertaken to devise the ideal screening method for UTI, by comparing the gold standard method of urine culture and the other rapid screening tests, and to identify the commonest isolate causing UTI in pregnancy as it may significantly improve the pregnancy outcomes.

Materials and Methods

15ml of mid-stream urine sample is collected in a disposable screw-capped, wide mouthed sterile container under aseptic conditions from patients enrolled in the study after getting the consent form signed with appropriate history.

The sample was then transported to the laboratory as early as possible without any delay.

In the lab, a wet mount was seen, a dipstick test (A10 REAGENT STRIPS, RAPHA) was done and then the routine urine culture was done using calibrated loop method on Blood agar and CLED agar and incubated at 37°C for 24 hrs.

In case of any growth, the organism was identified by basic biochemical tests and an antibiogram was done.

Sample

Urine sample was collected at the central lab microbiology collection centre.

Patient's informed consent was taken with history. Patient was instructed on how to collect the sample in the sterile urine container. The sample was then named, numbered and transported to the laboratory without any delay for further processing.⁽⁶⁾

Wet mount

The urine sample at first was subjected to a wet mount examination.

A sterile loop was used to draw a drop of urine from the sterile urine container, without touching the sides of the container, holding the loop perpendicularly.

A drop of urine was placed on a sterile microscopic slide and examined under high power of a microscope.

The sample was observed for the presence of epithelial cells, pus cells, RBC's and for organisms.

The results were taken down, and the slide was discarded into a discarding jar with disinfectant.⁽⁶⁾

Dipstick testing

The sample was then subjected to reagent strip testing. The sample was transferred into a sterile test tube. A dipstick was introduced into the test tube and the parameters were then read at the appropriate time as specified by the manufacturer. The dipstick was then discarded.

Bacteriological culture

Standardisation of Loop

Calibrated loop was used for inoculating into the specified culture media.

The loop was calibrated using the routine method of standardisation using methylene blue dye.

Procedure for Inoculation

The calibrated loop was sterilised over Bunsen flame.

The loop was then dipped into the container with the sample without touching the sides of the container.

Any extra drop of urine sample hanging from the loop was left to drop off.

The sample was then inoculated onto the specified culture media by full plate streaking.

Inoculating Media

The urine sample was inoculated onto Blood agar and CLED agar, using the calibrated loop.

Sheep Blood Agar (SBA)

Dehydrated blood agar base was reconstituted with distilled water and then pH was adjusted. It was then autoclaved at 121°C for 15mins. The prepared media was then cooled to 50°C and then sheep blood was added. The prepared sheep blood agar was then decanted into sterile petri plates and allowed to cool.⁽⁶⁾

Cystine Lactose Electrolyte Deficient Agar (CLED)

Dehydrated CLED agar was reconstituted with distilled water and then pH was adjusted. It was then autoclaved at 121°C for 15mins. The prepared CLED agar was then decanted into sterile petri plates and allowed to cool.⁽⁶⁾

Incubation Conditions

The inoculated media (SBA and CLED) were then incubated at 37°C for 24 hours and then the cultures were observed.

Interpretation of Bacterial Culture

The colony count was noted and was interpreted as: Significant bacteriuria (>10⁵CFU/ml of urine). Insignificant bacteriuria (<10⁵CFU/ml of urine). Sterile (no growth seen on the culture media). Contaminant growth (more than three types of colonies grown on culture media).⁽⁶⁾

On SBA : α / β / no hemolysis was noted.

- Size, colour, edges, surface, elevation and shape of the colonies were noted.

On CLED: Lactose fermenting / non lactose fermenting nature of the colony

Size, colour, edges, surface, elevation and the shape of the colonies were also noted.

Tests for identification

In case of growth on the culture media after 24 hours incubation, the colonies were subjected to an array of identification tests, such as:

Preliminary tests

Gram's staining; Catalase test; Oxidase test. Motility.

Basic biochemical Tests

Indole test; Citrate test; Urease test. Triple sugar iron test

Antibiogram

For all positive cultures, an antibiogram was put up.

The colonies were inoculated into peptone water and incubated at 37°C for 2 hours, and matched with 0.5 McFarland standards for turbidity. Muller Hilton Agar (MHA) was used for testing antibiotic sensitivity. The peptone broth with the organism was inoculated into the MHA plate and the routine antibiotic discs were put up. Then the plate was incubated at 37°C, overnight. The zone size of each drug was noted and compared with the standard CLSI drug chart for interpretation.⁽⁷⁾ Appropriate differential discs like Cefoxitin for differentiating MRSA, Novobiocin for differentiating *S.saprophyticus* and Bacitracin for differentiating Streptococci were added.

Quality Control for Antibiotic Sensitivity Testing

The gram positive panel of drugs comprising of Amoxyclav, Ciprofloxacin, Tetracycline, Erythromycin, Vancomycin, Teicoplanin, Linezolid & igh level gentamicin and gram negative panel of drugs comprising of Amikacin, Gentamicin, Ciprofloxacin, Cotrimoxazole, Imipenem, Nalidixic acid (as per CLSI 2013)⁽⁷⁾, Norfloxacin and Nitrofurantoin were tested with ATCC *S.aureus* 25923 and ATCC *E.coli* 25922 respectively. Quality control testing was said to have been passed, when the zone sizes of these drugs were as per the CLSI criteria.⁽⁷⁾

The remaining sample and plates (SBA,CLED and MHA) with the colonies were discarded into autoclavable plastic bags and autoclaved at 121°C for 15 mins before disposal.⁽⁶⁾

Statistical Methods

Percentage and ratio calculation. Sensitivity and specificity.

Results and Discussion

A total of 500 pregnant women were screened for UTI irrespective of their symptoms. The prevalence of UTI among pregnant women is shown in *Figure 1*. The age group-wise distribution of UTI among 219 positive UTI cases, irrespective of their trimesters is shown in *Figure 2*. Among the 219 positive UTI cases, there was a higher incidence of UTI among primigravida, when compared to multigravida and this distribution is shown in *Table 1*.

Table 2: shows the comparison of various screening tests with the gold standard of urine culture. Various combinations were made to estimate the most accurate screening tests to detect UTI in pregnancy. A combination of Urinary nitrites detection and Urinary leucocytes detection was found to be the most sensitive among the tests studied. In this study *E.coli* was the most predominant uropathogen accounting for 123 isolates(56%), followed by *K.pneumonia* (31 isolates-14%) and Enterococci(19 isolates-8%). Gram negative bacilli showed increased sensitivity to Amikacin, Nitrofurantoin & Imipenem. Gram positive cocci showed increased sensitivity to Vancomycin

Adabara *et al*⁽⁸⁾, Little P *et al*⁽⁹⁾, Mokube MN⁽¹⁰⁾*et al* and August *et al*⁽¹¹⁾, estimated the prevalence of UTI as 75%, 66%, 23.5% and 21.15% respectively. Turpin *et al*⁽¹²⁾, Demilieet *al*⁽¹³⁾ and Thakre *et al*⁽¹⁴⁾, estimate the prevalence of UTI as 7-10%. Whereas, in our study, the prevalence was 45%. This high prevalence could be attributed to the low income state of the patients, anaemia and sexual activity during pregnancy.⁽¹⁵⁾

A higher prevalence of UTI in pregnancy (44%-n=97/219) in the age group of 21-25yrs, is seen in our study, which is in

contrast to other studies in which the prevalence is high in the age group of 26-30yrs. This could be attributed to the high sexual activity or higher prevalence of anaemia among these groups.⁽¹⁵⁾

Majority of the studies, both Indian and western studies show a higher prevalence of UTI in multigravida and stress on the fact that the prevalence of UTI in pregnancy increases with parity. In contrast to this, it was found that, the prevalence of UTI associated with pregnancy was higher among the primigravida by Marahatta *et al*⁽¹⁶⁾ and Lavanya *et al*⁽¹⁷⁾ which is similar to the findings in our study.

Urinary nitrite detection has been reported by many researchers as an ideal screening method for detecting pregnancy associated UTI. This is in contrast to our study. In our study urinary leucocyte detection has been an ideal screening method for detecting pregnancy associated UTI, which has already been quoted by Jido TA⁽¹⁸⁾ in a similar study in Nigeria. The number of positive cases by the detection of leucocyte was 215 of 219, accounting for 98%. Whereas the number of positives by detection of urinary nitrite was 172 of 219 cases, accounting for 78.5%. This could be due to the variability in the kit manufacturer, the timing used to read the results may vary between manufacturers, the time delay in the appearance of nitrites in the urine giving false negatives, delay in sample processing and technical errors. Urine for bacterial culture continues to remain as the gold standard for confirming UTI and a combination of urinary leucocytes estimation and detection of urinary nitrites was an ideal screening method.

In both Indian as well as in Western studies *E.coli* has been reported as the most predominant uropathogen causing UTI in pregnancy followed by *Staphylococcus*,

Klebsiella and *Enterococcus*. Rarely *Staphylococcus* is the most predominant pathogen to cause UTI, which may be because of improper sample collection. In our study *Enterococcus* emerged as the third

most common isolate, following *E.coli* and *Klebsiella*. This may be attributed to the ablution practices following defecation in women.

Table.1 Prevalence Among Primi and Multi Gravida

Primigravida	68
Multigravida	32

Table.2 Comparison Between Different Screening Tests and Culture

Tests	Positives (%)
Wet mount, Detection of urinary nitrites, Detection of Urinary leucocytes	41
Wet mount, Detection of Urinary nitrites	46
Detection of Urinary nitrites, Detection of Urinary Leucocytes	75
Urine Culture	100

Table.3 Percentage of Uropathogens Causing UTI

Organism	Prevalence (%)
<i>Escherichia coli</i>	56
<i>Klebsiella pneumonia</i>	14
<i>Enterococcus</i>	8
<i>Other NFGNB</i>	8
<i>Citrobacter</i>	4
<i>Staphylococcus</i>	3
<i>Streptococcus</i>	2
<i>Pseudomonas</i>	2
<i>Proteus</i>	1
<i>Coliforms</i>	1
<i>Acinetobacter</i>	1

Table.4 Antibiogram of Gram Negative Bacilli

Antibiotic	Sensitivity %
Amikacin	76
Gentamycin	53
Ciprofloxacin	61
Cotrimoxazole	57
Norfloxacin	54
Nitrofurantoin	75
Nalidixic acid	68
Imipenem	72

Table.5 Antibiogram of Gram Positive Cocci

Antibiotic	Sensitivity %
Amoxyclav	69
Ciprofloxacin	54
HLG	51
Erythromycin	61
Linezolid	68
Tetracycline	70
Teicoplanin	65
Vancomycin	71

Figure.1 Prevalence of UTI

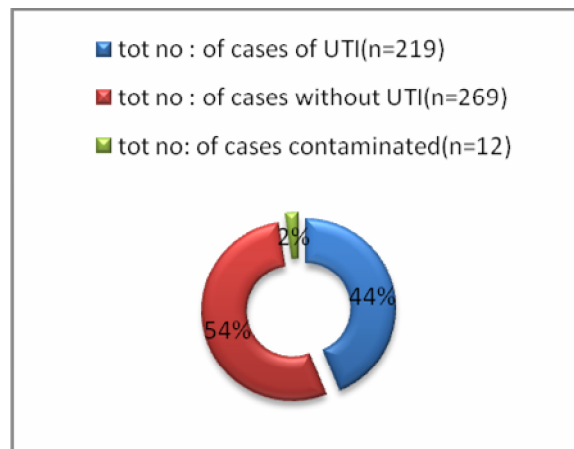
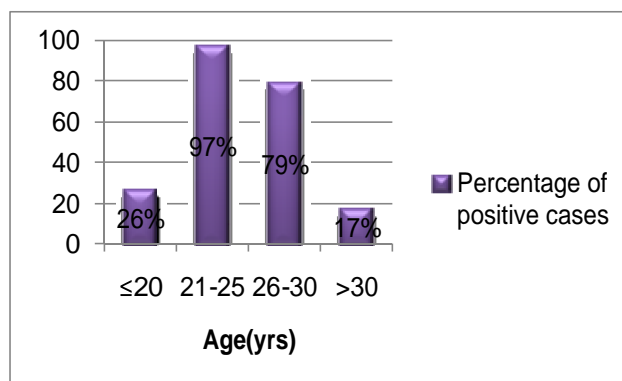


Figure.2 Age Group Wise Distribution



Among the Gram negative bacilli, higher degree of sensitivity was observed with Nitrofurantoin, Amikacin and Imipenem and among the Gram positive cocci, higher degree of sensitivity was observed with Vancomycin, Amoxycyclav and Tetracycline. Owing to the teratogenicity of Amikacin and tetracycline, we recommend empirical therapy with, Nitrofurantoin for Gram negative bacilli and Ampicillin for Gram positive cocci.

Based on the results and the methodology employed, we have concluded that:

The prevalence of UTI in pregnancy was 45%. The commonest uropathogen causing UTI in pregnancy was *Escherichia coli*. Urine for bacterial culture continues to remain as the gold standard for confirming UTI and a combination of urinary leucocytes estimation and detection of urinary nitrites was an ideal screening method.

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