
SCREENING, BIOFILM FORMATION AND SENSITIVITY TESTING OF UROPATHOGENIC ISOLATES FROM PREGNANT WOMEN

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Abstract: Asymptomatic bacteriuria is a common problem in pregnancy and is associated with risk of preterm birth and pyelonephritis if untreated. The diagnosis is based on urine culture. This study was carried out to determine the prevalence or frequency of asymptomatic bacteriuria in pregnant women and also to isolate, identify and establish antimicrobial susceptibility pattern of the pathogens responsible for asymptomatic bacteriuria. Uropathogens have an ability to produce biofilm in the bladder epithelium which forms dormant reservoir inside the bladder. In this study detection of biofilm productions by uropathogens was done by Tube method (TM). The study includes 250 pregnant women examined for asymptomatic bacteriuria. Isolates were identified by conventional methods and their antibiotic susceptibility pattern was established. A total of 19 (7.6%) were positive for significant bacteriuria.

Escherichia coli was the most predominant organism followed by *Klebsiella pneumoniae*. Cefazolin, Norfloxacin, Ciprofloxacin, Co-trimoxazole, Levofloxacin and Cefotaxime were found to be most effective antibiotics against the urinary isolates. Asymptomatic bacteriuria is not uncommon among antenatal patients in the population studied. Routine urine cultural tests should be carried out on all antenatal patients in order to identify any unsuspecting infection. This measure will go a long way in reducing maternal and obstetric complications associated with pregnancy.

Keywords: Urinary tract Infection, urine, pregnancy, asymptomatic bacteriuria, screening, antibiotics.

Introduction: Urinary tract infections (UTI) refer to the presence of clinical signs and symptoms arising from the genitourinary tract associated with the presence of one or more micro-organisms [1, 4]. Pregnancy is a predisposing factor to urinary tract infections and pregnant women suffering from this condition are at risk of various complications of pregnancy including low birth weight and preterm birth [3]. The prevalence of asymptomatic UTI has been reported to be 2% to 11% in pregnant women (6% to 8% in average) [2].

Uropathogens have an ability to produce biofilm in the bladder epithelium which serve as

reservoirs inside the bladder. Re-emergence of bacteria from biofilm might be a source of recurrent infection [7, 8]. Inability of antimicrobials to penetrate the biofilm results in the development of resistant strains. Asymptomatic bacteriuria is defined as a significant bacterial count ($\geq 10^5$ CFU/mL) in the urine of a person without symptoms and is present approximately in 5 to 10% of the pregnant women and if untreated, could lead to the development of symptomatic cystitis and pyelonephritis in upto 50% of patients [5, 6].

Due to the increase in sex hormones and the anatomic and physiologic changes during pregnancy, bladder and kidney infection is more

likely and may result in hypertension, preeclampsia, low birth weight, prematurity, septicaemia and maternal death [5]. We designed this study to evaluate the prevalence of bacteriuria and also include biofilm formation and antibiotic resistance pattern of uropathogens in pregnant women associated with urinary tract infection [3, 6].

Materials And Methods:

Location of study: This study on asymptomatic bacteriuria in pregnancy was carried out in the Department of Microbiology, K.J.Somaiya College of Science and Commerce. A total of 250 pregnant women with asymptomatic bacteriuria attending Somaiya hospital, were enrolled for the study.

Collection and Processing of specimen: Urine samples were collected by standard mid-stream "clean catch" method from all the pregnant women, in a sterile, wide-mouthed container that can be covered with a tightly fitted lid and transported to laboratory without delay.

Processing of specimen and isolation: The samples were processed using standard microbiological procedures^[3, 4]. The sample was processed immediately in the laboratory. After noting macroscopic appearance, uncentrifuged urine sample was subjected to wet mount examination, Gram stain, Catalase and Triphenyl Tetrazolium Chloride test. Culture was put-up by standard loop method on 5% sheep blood agar and Mac-conkey agar and by pour plate method on nutrient agar. The inoculated plates were incubated at 37°C for 24 to 48 hrs [3,5].

Culture results were interpreted as being significant and insignificant, according to the standard criteria. In positive cultures, colonies were counted using hand lens. Count of $\geq 10^5$ CFU/mL was taken as significant bacteriuria. Positive cultures with colony count less than 10^5 CFU/mL was considered as not significant and if

no growth was observed after 48 Hrs of incubation; cultures were reported as no growth [9, 10]. Identification of isolates was done by colony morphology, gram staining and standard biochemical tests [1, 3, 4].

Detection of biofilm: Tube method: The bacterial strains from overnight growth were inoculated in 10 mL BHI broth and incubated for 24hrs at 37° C. The tubes were decanted and washed with phosphate buffer saline and stained with 0.1% crystal violet. The excess stain was removed by washing with distilled water. Tubes were dried in inverted position and observed for biofilm production. A uniform violet film lining the wall and bottom of the tube was considered positive for biofilm production [5]. No film or ring formation at the interface of liquid was considered as negative. The test was performed in triplicate and repeated twice to avoid observer bias and read as strong, moderate, weak and absent.

Antibiotic susceptibility test: Bacterial susceptibility to antimicrobial agents was determined by the Kirby Bauer disk diffusion method on Mueller-Hinton agar. Isolates were categorised as susceptible and resistant based upon interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI). Antibiotic discs (Hi-Media) Ampicillin (10mcg), Cefazolin (30mcg), Nalidixic acid (30mcg), Norfloxacin (10), Ciprofloxacin (5mcg), Co-Trimoxazole (25mcg), Levofloxacin (5mcg), Nitrofurantoin (300mcg) were used for antimicrobial susceptibility tests.

Results And Discussion: Table 1 show that patients were divided into five age-groups: Group I (15-20 years), Group II (21-26 years), Group III (27-32 years), Group IV (33-38 years), and Group V (39-44 years). Out of the total number of pregnant women included in this study 19 (7.6%) patients were identified by culture to have significant bacteriuria. Out of 250 pregnant women examined for

asymptomatic bacteriuria, 219 urine samples had (7.6%) cases and insignificant bacteriuria in 6 no growth when plated and were found to be (2.4%) cases. sterile. Significant bacteriuria was found in 19

Table 1: Prevalence of ASB in the study population based on age distribution.

Group	Colony forming Units (CFU/mL)				Total
	Age group	10 ⁵	10 ² -10 ⁴	<10 ²	
		Positive (%)	Suspected (Doubtful)%	Negative (%)	
I	15-20	1(5.3)	2(33.3)	2(33.3)	5(16.1)
II	21-26	5(26.3)	3(50.0)	3(50.0)	11(35.5)
III	27-32	10(10.0)	1(16.7)	1(16.7)	12(38.7)
IV	33-38	3(15.8)	0(0.0)	0(0.0)	3(9.7)
V	39-44	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Total	19(61.3)	6(19.4)	6(19.4)	31(100)

Table 2: Uropathogens isolated in the study group

Sr. No.	Isolates	No. of urine samples from which isolates were obtained	Percentage (%)
1.	<i>Escherichia coli</i>	13	41.9
2.	<i>Klebsiella pneumoniae</i>	04	12.9
3.	<i>Proteus mirabilis</i>	06	19.3
4.	<i>Pseudomonas aeruginosa</i>	02	6.4
5.	<i>Staphylococcus aureus</i>	02	6.4
6.	<i>Candida albicans</i>	01	3.2
7.	<i>Streptococcus pyogenes</i>	03	9.7
	Total	31	100

Table 2 describes the pattern of isolates out of the total 31, *E.coli* was the predominant organism isolated in 41.9% of pregnant women followed by other uropathogens such as *Klebsiella pneumonia* (12.9%), *Proteus mirabilis* (19.3%), *Pseudomonas aeruginosa* (6.4%),

Staphylococcus aureus (6.4%), *Candida albicans* (3.22%), *Streptococcus pyogenes* (9.67%). Among 19 strains subjected to biofilm production, 5 (26.3%) strains showed highly positive, 6 (31.5%) showed moderate positive and 8 (42.1%) showed weakly positive or absent for tube method. Isolates showing high and moderate biofilm formation is considered as biofilm producers and those showing weak and absent biofilm formation is considered as biofilm nonproducers.

The antibiotic sensitivity patterns observed among all the isolates including biofilm producers and biofilm non producers are shown in Table 3. This study revealed that Cefazolin, Norfloxacin, ciprofloxacin, Co-trimoxazole, Levofloxacin and Cefotaxime were effective against most of the urinary isolates. Nalidixic acid, Nitrofurantoin, Ceftazidime were moderately effective against the urinary isolates. Ampicillin, Cefpodoxime were highly resistant to the isolates.

Table 3: Antibiotic pattern of biofilm producing and nonproducing uropathogens.

Sr No.	Antibiotics	Biofilm producers (%)		Biofilm non-producers (%)	
		Resistance	Sensitive	Resistance	Sensitive
1.	Ampicillin (AMP)	36.3	64	57	43
2.	Cefazolin (CZ)	-	100	14	86
3.	Nalidixic acid (NA)	27	73	29	71
4.	Norfloxacin (NX)	-	100	14	86
5.	Ciprofloxacin (CIP)	-	100	14	86
6.	Co-trimoxazole (COT)	-	100	29	71
7.	Levofloxacin (LE)	-	100	14	86
8.	Nitofurantoin (NIT)	27	73	29	71
9.	Ceftazidime (CAZ)	18	82	29	71
10.	Cefotaxime (CTX)	-	100	14	86
11.	Cefpodoxime (CPD)	36.3	64	57	43

Conclusion: Monitoring bacteriuria in pregnant women is a critical checkpoint during pregnancy. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria, which could lead to hypertension, preeclampsia, septicaemia, maternal death pyelonephritis and adverse obstetric outcomes such as prematurity, low birth weight, and higher foetal mortality rates [1, 2]. The adverse effects of undiagnosed

asymptomatic bacteriuria on mother and child to suggest routine urine culture screening for all pregnant women attending antenatal clinic in order to prevent mother and child from any form of complication that may arise due to infection [3, 6].

Formation of microbial biofilm may pose a public health problem for pregnant women, as

the microorganisms in the biofilm are difficult to treat with antimicrobial agents. Biofilm can be composed of a single or multiple organisms on various biotic and abiotic surfaces. Hence, biofilm producing *E. coli* may promote the colonization and lead to increased rate of UTIs [7]. These studies indicate a need for continued surveillance of antimicrobial resistance among uropathogens during pregnancy, so as to increase positive outcomes of clinical interventions and prevent preterm birth and low

birth weight. As biofilm production was detected in many of our isolates, it is necessary to establish standard guidelines on the care of pregnant women suffering from urinary tract infection.

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