

Prevalence of Asymptomatic Bacteriuria in Pregnant Women, Westren Region, Taif, Sudia Arabia

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Abstract: *Asymptomatic bacteriuria represents a serious health problem in pregnant women, Taif, Sudia Arabia. Methods: A total of 1200 pregnant women attending antenatal clinic. The study was carried between January to July 2015. Standard microbiological methods were used to collect, isolate and identify GNB from urine sample. An antimicrobial susceptibility test was performed for all isolates according to the criteria of the CLSI. Results: revealed that 62% of investigated pregnant women were positive for UTI, Klebsiella pneumoniae was the most frequently isolated bacterial agent (37%). E. coli followed by (25%). The susceptibility pattern of 745 bacteria isolated against 20 antimicrobial agents. All strains were susceptible to all antibiotic, resistance was observed in some strains of Klebsiella pneumoniae against ampicillin. Conclusion, routine urine culture should be carried out on all antenatal patients in order to identify any unsuspecting infection.*

Keywords: Antenatal women, Asymptomatic bacteriuria, Pregnant women, Prevalence, Saudi Arabia, Taif, UTI

1. Introduction

Urinary tract infection (UTI) is one of the most common infections where one or more parts of the urinary system become infected usually after bacteria overcome the natural defense mechanism of the urinary tract [1]. Urinary tract infections (UTI) affect all age groups, but women, particularly pregnant women, are more susceptible than men, due to short urethra, pregnancy, easy contamination of the urinary tract with fecal flora and various other reasons; physiological changes, extended abdomen and difficulty of personal hygiene [2].

Clinically, UTI have two principal presentations; symptomatic and asymptomatic bacteriuria [3]. Symptomatic UTI could be accompanied with a variety of clinical signs including dysuria, pyuria, strong urge to urinate frequently, even immediately after the bladder is emptied, painful burning sensation, discomfortable pressure and bloody urine, which may have a strong smell [4,5]. In asymptomatic bacteriuria (ASB), urine culture reveals significant growth of pathogen (greater than 10^5 bacteria/ml) without showing clinical manifestation [6]. Asymptomatic bacteriuria could be found in pregnant and non-pregnant women but pregnancy enhances the progression from asymptomatic to symptomatic form which could lead to pyelonephritis and adverse obstruction [7].

Asymptomatic bacteriuria (ASB) are found in 2 to 10% of pregnant women and are likely to develop acute pyelonephritis, postpartum UTI, hypertensive disease, anemia, prematurity, low birth weight babies and prenatal death if untreated [8,9]. Asymptomatic bacteriuria is a microbial diagnosis based on the isolation of a specified quantitative count of bacteria in a properly collected specimen of urine from pregnant women without signs or symptoms of UTI. Thus urine culture is the gold standard

screening technique for asymptomatic bacteriuria during pregnancy [10, 11].

ASB is defined as the presence of more than 100,000 colony forming units (CFU) per milliliter in 2 consecutive midstream clean-catch urine samples, or a single specimen with one bacterial species isolated in a quantitative count of at least 100 CFUs per mL from a catheterized urine specimen [12].

The most common infecting organism is *Escherichia coli*, which is responsible for 75-90% of bacteriuria during pregnancy, other common organisms include *Klebsiella pneumoniae* [13]. 40% of the asymptomatic bacteriuria cases develop into acute symptomatic UTI [14].

UTI increases the risk of low-birth-weight infants (weight less than 2,500 g [5 lb, 8 oz]), prematurity (less than 37 weeks of gestation at delivery) and preterm, low-birth-weight infants; weight less than 2,500 g and less than 37 weeks of gestation at delivery [15].

Hence early detection and treatment is of considerable importance not only to forestall acute pyelonephritis and chronic renal failure in the mother, but also to reduce prematurity and fetal mortality in the offspring [16]. By screening for and aggressively treating pregnant women with asymptomatic bacteriuria, it is possible to significantly decrease the annual incidence of pyelonephritis during pregnancy [17]. In randomized controlled trials, treatment of pregnant women with asymptomatic bacteriuria has been shown to decrease the incidence of preterm birth and low-birth-weight infants [18].

The aim of the present study was to determine the prevalence of UTI and to investigate the most frequent causative agents and drug resistance profiles associated with

such infections among pregnant women from Western province, Taif, Saudi Arabia, attending antenatal clinic at Al-Hada Military Hospital, KSA. In the Kingdom of Saudi Arabia, there is paucity of information regarding asymptomatic bacteriuria in pregnant women. To date, only few reports are in the literature. This paper was the first data on incidence of asymptomatic bacteriuria in pregnant women at Western province, Taif, Saudi Arabia.

2. Materials and Methods

2.1 Study Population

A total of 1200 pregnant women attending the routine antenatal clinic of the Al-Hada Armed Forces Hospital were screened for asymptomatic bacteriuria. The samples were collected during 7 months, starting from January 2015 to July 2015. The approach was based on universal screening of all pregnant women for asymptomatic bacteriuria between 35 and 37 weeks of gestation. The age of the women is 15 - 44 years.

2.2 Sample Collection

According to guidelines of the Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults [19]. A total 1200 samples of urine were collected from asymptomatic pregnant women between the ages of (15-44) years attending ante-natal clinic at Al-Hada Military Hospital. Patients with history of fever ($\geq 38^{\circ}\text{C}$), genitourinary complaints and patients on antibiotics for any indication during the current pregnancy were excluded. Demographic data recorded included maternal age, gravidity, parity and age of gestation, pertinent history with emphasis on past medical and obstetric history, previous antibiotic intake, previous history of urinary tract infection, personal and family history of hypertension and diabetes. Baseline investigations such as hemoglobin levels, urine-albumin and sugar, random blood sugar, blood urea were done. Exclusion criteria included:

- 1) History of UTI symptoms (dysuria, frequency and urgency, etc).
- 2) Pregnancy induced diabetes mellitus/ hypertension.
- 3) History of antibiotic therapy in the previous two weeks.
- 4) Pyrexia.
- 5) Known congenital anomalies of the urinary tract.

Urine samples were collected by standard mid-stream "clean catch" method from all the pregnant women, after giving proper instructions to subjects in a sterile, wide-mouthed container that can be covered with a tightly fitted lid. [20]. Each sample of urine was divided into two parts and were properly labeled and immediately transferred and processed within 1-2 h of collection to the Microbiology laboratory at Al-Hada Military Hospital, with a request for complete urinalysis and urine culture.

2.3 Urine Examination

The first part of urine sample was examined by dipstick tests using Comber 10 reagent test strips (Analyticon, Germany) that have panels to detect protein, blood, nitrite and

leukocyte esterase in urine [21]. In addition, wet preparations were made from sediment of each urine sample after centrifugation and were microscopically examined at X40 for detection of white blood cells as an indicator of pyuria. Samples with ≥ 10 WBC/field were regarded as pyuric.

The second part of urine sample were subjected to culture on blood agar and MacConkey's agar plates (Saudi Prepared Media Laboratory, Saudi Arabia, Riyadh (SPML)) for semi-quantitative analysis by inoculation with standard calibrated loop delivering 0.01 mL of urine. After streaking, plates were incubated at 37°C for 24 to 48 hours, and bacterial colony counts were done. Counts $> 10^5$ organisms/ml were considered as significant bacteriuria. The plates were then examined macroscopically and microscopically for bacterial growth. Urinary tract infection was positive diagnosed by growth of "100,000" colony forming unit (CFU) of urinary tract pathogen per ml in culture of midstream urine sample.

It was reported as significant if the growth obtained was confluent or the number of colonies corresponded to 10^5 colony forming units (CFU) per ml i.e., 400 colonies or more. Insignificant growth was reported if colony count obtained corresponded to less than 10^5 CFUs per ml of urine [22]. Identification of bacterial pathogens was confirmed by observation of Gram staining, biochemical analysis using, and commercial identification kits were used to identify the isolates up to species level: API 20E for identification of *Enterobacteriaceae* (Analytab product, Plainview, NY), and Vitek system (bioMérieux, Inc., Durham, NC, USA); GNBCard for identification of Gram-negative bacteria [23].

2.4 Antimicrobial Susceptibility Testing

Susceptibility testing was performed to all GNB-positive samples. All procedures for disk susceptibility were performed according to the criteria of guidelines of Clinical and Laboratory Standards Institute (CLSI, 2013) [24]. Fresh subcultures of GNB were used after overnight growth on MacConkey's agar plates (Saudi Prepared Media Laboratory, Saudi Arabia, Riyadh (SPML)). The inoculums were standardized by suspending colonies in sterile phosphate-buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standards. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess inoculums. Then the swab was inoculated on Mueller-Hinton agar plate (SPML, Riyadh, Saudi Arabia), antibiotic disks were placed and incubated at 37°C for 20 h.

The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria [24].

Amoxicillin/Clavulanic acid (AMC, 20/10 μg), Ampicillin (AMP, 10 μg), Nitrofurantoin (F, 300 μg), Gentamicin (CN, 10 μg), Oxacillin (OX, 1 μg), Sulfamethoxazole/Trimethoprim (SXT, 1.25/23.75 μg), Chloramphenicol (C, 30 μg), Ciprofloxacin (CIP, 5 μg), Tetracycline (TTC, 30 μg), Clindamycin (Cln, 2 μg), Penicillin (P, 10 units), Erythromycin (E, 15 μg), Norfloxacin (NOR, 10 μg).

Vancomycin(Van,30μ),Cefoxitin(FOX,30μg),Ceftazidime(CAZ,30μg),Cefuroxime/Sodium(CXM,30μg),Cephazoline(KZ,30μg),Cefotaxime(CTX,30μg) were used in the study with their respective concentration (Oxoid, United Kingdom).

2.5 Quality control

To maintain the quality of data, every sample was processed in triplicates and every result was cross-checked by the principal investigator. *Klebsiellapneumoniae* subsp. *Pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 25922) were used as quality control throughout the study for culture, Gram stain, and antimicrobial susceptibility testing. All the strains were obtained from the RemelMicrobiology products (Thermoscientific)

2.6 Data analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS), Version 16 for Windows. Continuous variables were summarized using descriptive statistics in terms of means± standard deviations; 95% confidence intervals (95% CI), minimums and maximums, while a Chi-square test were used to compare categorical variables between the pregnant women with different age groups. Statistical methods, descriptive statistics included statements of frequency with percentages, means, standard deviations (SD, and differences were considered significant if $p < 0.05$ with confidence intervals (95% CI). Prevalence figures were calculated for the total study population and separately by age groups. Occupation, number of antenatal clinic visit (ANC), and type of gravid also recorded.

3. Results

The study shows highest number of culture positive cases among pregnant women in the age group 25-29 years (68.3%). Prevalence of UTI and among pregnant women. Out of 1200 pregnant women, 754 women (63%) were positive for UTI. Pregnant women enrolled in this study were with the age ranges of 15-44 years with mean age of 26 years.

All pregnant women accepted, interviewed and participated for study related tests in addition to routine antenatal and baseline assessments, complete data were used for the analysis.

Table 1: Frequency of UTI-associated microbial agents:

Bacterial isolates	Frequency	Percent (%)	P value
<i>Klebsiellapneumoniae</i>	445	37	0.1
<i>E. coli</i>	300	25	
Total	745	62	

Out of the 1200 symptomatic and asymptomatic UTI cases, *Klebsiellapneumoniae* was the most frequently isolated pathogen 445(37%) followed by *E.coli* 300(25%). (Table 1).

Table 2: Socio-demographic characteristics of 1200 women attending ante-natal clinic at AL-Hada Armed Forces Hospital, Taif.

Socio-demographic Characteristics	Frequency	Percent (%)	P value
1- Age groups in years			
15-19	65	5.4	
20-24	80	6.6	
25-29	820	68.3	
30-34	145	12	
35-39	50	4.1	0.006
40-44	40	3.3	
Total	1200	100	
2-Occupation			
House wife	90	7.5	
Teacher	800	66.6	
Student	80	6.6	
Nurse	40	3.3	
Medical technologist	130	10.8	0.006
Doctor	60	5	
Total	1200	100	

The mean age of the participants was 25.6 years ranging 15-44 years. The majority of the participants were between the ages of 25- 29 years 820(68.3%), while most of them were teachers (800,66.6%), the remaining were medical technologist (130,10.8%), house wife (90,7.5%), students (80, 6.6%), doctor (60, 5%), and nurse (40, 3.3%). respectively. Table(2). Statistically significant association of asymptomatic bacteriuria was observed in connection with socio-demographic characteristics of the study subjects by type of age 35-39, and occupation of medical technologist $P = 0.006$

Table 3: Variables associated/not associated with *Klebsiellapneumoniae* in pregnant women attending ante-natal clinic at AL-Hada Armed Forces Hospital, Taif.

Variables	<i>Klebsiellapneumoniae</i>	Percentages (%)	P value
1- Age (in years)			
15-19	35	11.6	
20-24	40	13.3	
25-29	95	31.6	
30-34	55	18.3	
35-39	45	15	0.005
40-44	30	10	
Total	300	100	
2-Occupation			
House wife	50	16.6	
Teacher	105	3.5	
Student	55	18.3	
Nurses	35	11.6	
Medical technologist	49	16.3	0.01
Doctors	6	2	
Total	300	100	
3-Number of ANC Visit			
One time	100	22.7	
Two times	199	45.2	
Three times	80	18.18	0.03
Four times	61	13.8	
Total	300	100	
4-Type of Gravida			
Primigravida	205	46	0.04
Multigravida	235	53	
Total	300	100	

Out of the 745 (62%) asymptomatic UTI cases, *Klebsiellapneumonia* was the most frequently isolated pathogen 445 (37%) followed by *E. coli* 300(25%). The majority of the participants were between the ages of 25- 29 years 95(31.6%). In contrast most of them were teachers 105(3.5%), while the remaining were medical technologist 49(16.3%), students (55, 18.5%), house wife (50,16.6%), nurse (35, 11.6%).doctor (6, 2%), and respectively. Most of the pregnant women were visit ANC two times 199(45.2%) while the remaining were one time 100(22.7%), three times for students 80 (18.18%),and four times 61(13.8%),respectively. Most of them were multigravida 235(53%) while the remaining primigravida 205(46%), Table(3). Statistically significant association of asymptomatic bacteriuria duo to *Klebsiellapneumoniae* infection was observed in connection with socio-demographic characteristics of the study subjects by type of age 35-39 P 0.005, occupation of medical technologist P 0.01, number of ANC Visit at three times P 0.03, and type of gravida, Primigravida P 0.04.

Table 4: Variables associated/not associated with *E. coli* in pregnant women attending ante-natal clinic, at AL-Hada Armed Forces Hospital, Taif.

Variables	<i>E. coli</i>	Percentages (%)	P value
1- Age (in years)			
15-19	40	8.9	
20-24	50	11.2	
25-29	215	48.3	
30-34	60	13.4	
35-39	50	11.2	
40-44	30	6.7	0.007
Total	445	100	
2-Occupation			
House wife	50	11.2	
Teacher	200	44.9	
Student	65	14.6	
Nurses	40	9.2	0.04
Medical technologist	80	17.9	
Doctors	10	2.2	
Total		100	
3-Number of ANC Visit			
One time	100	22.4	
Two times	199	44.7	
Three times	80	17.9	0.03
Four times	66	14.8	
Total	445	100	
4-Type of Gravida			
Primigravida	205	46	
Multigravida	240	53.9	0.05
Total	445	100	

Variables associated/not associated with *E. coli* in pregnant women attending ante-natal clinic, at AL-Hada Armed Forces Hospital. The majority of the participants were between the ages of 25- 29 years 215(48.3%), while most of them were teachers 200(44.9%),and the remaining were medical technologist 80(17.9%), students 65(14.6%), house wife 50(11.2%), nurse 40(9.2%).doctor 10(2.2%), and respectively. Most of the pregnant women were ANC Visit two times 199(44.7%) while the remaining one time 100(22.4%), three times for students 80 (17.9%),and four times 66(14.8%),respectively. In contrast most of them were multigravida 240(53.9%) while the remaining primigravida

205(46%), Table(4). Statistically significant association of asymptomatic bacteriuria duo to *E. coli* infection was observed in connection with socio-demographic characteristics of the study subjects by type of age 40-44 P 0.007, occupation of nurses P 0.04, number of ANC Visit at three times P 0.03, and type of gravida, multigravida P 0.05.

Overall Prevalence

Out of 1200 asymptomatic pregnant females screened, 455(38%) of the samples came out to be insignificant growth by semiquantitative culture method. 745 (62%) were found to be asymptomatic bacteriuria in pregnant women. Variables associated/not associated with *Klebsiellapneumoniae* in pregnant women attending ante-natal clinic was 445(37%), while with *E. coli* was 300(25%).

Risk Factors Analysis

In the present data, a statistically significant association of asymptomatic bacteriuria was observed in connection with any of the sociodemographic characteristics of the study subjects by age groups: 35-39 years (P 0.006), as shown in Table 2. A statistically significant association of asymptomatic bacteriuria was observed in connection with any of the socio-demographic characteristics of the study subjects by occupation, where medical technologist gave high incidence 130(10.8%) (P 0.006).

In contrast, there is also statistically significant association of asymptomatic bacteriuria due to (*Klebsiellapneumoniae*) by age groups: 35-39 years (P 0.005), whereas by occupation, where medical technologist gave high incidence 49(16.3%) (P 0.01). In contrast, was observed in number of ANC visit three times was gave a high significant value 80(18.18%) P 0.03. Statistically significant was observed in connection with gravida, primigravida 205 (46%) P 0.04. The susceptibility pattern of 745 bacteria isolated from pregnant women against 20 antimicrobial agents. All strains were susceptible to all antibiotic used in study(100%), resistance was observed in some strains of *Klebsiellapneumoniae* against ampicillin

4. Discussion

Asymptomatic bacteriuria is common during pregnancy. It gives a clear predisposition to the development of symptomatic UTI, which in turn pose risk to mother and fetus. In the present study, it is observed that pregnant women in the age group 25-29 years had highest percentage of infection 820(68.3%). This results correlates with Imade et al [25]. Advanced maternal age was reported as risk factor for asymptomatic bacteriuria in pregnancy and also could be due to the fact that many women within this age bracket are likely to have had many children before the present pregnancy and it has been reported that multiparity is a risk factor for acquiring asymptomatic bacteriuria in pregnancy[26, 27]. Most cases of asymptomatic bacteriuria were found during second times of visiting the ANC(40.7%) in case *Klebsiellapneumoniae* (45.2%), and *E.coli* infection of pregnancy respectively. This results correlates with other studies [28].

In our study significant growth was found in (63.3%) cases and (37%) samples were sterile. These results were consistent with reports of the recent studies. The presence of significant bacteriuria indicates the significance of microbiological culture to settle the diagnosis of urinary tract infection. Bacterial isolates have been changing from time to time from place to place. In our study organisms isolated, correlated with various others studies [29, 30, 31]. This pattern could be due to the fact that urinary stasis is common in pregnancy and since most *Escherichia coli* strains prefer that environment, they cause UTI. Another reason could be as a result of poor genital hygiene practices by pregnant women who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine [32].

The pioneer in reporting incidence of asymptomatic bacteriuria was Kass, who reported it to be 6–7% [33]. Overall the incidence in various Indian studies was found to be between 5 and 12% and in western studies the incidence ranges from 2 to 7% [34].

In the present study the incidence of asymptomatic bacteriuria came out to be 62% which is higher than many foreign and different studies. In the present study the percentage of positive cases in age groups wise distribution, the incidence reported was 820 (68.3%) in age groups 25–29 years. Roy et al. found the incidence to be 15.9, 11.7 and (11.6%), respectively in these age groups [35]. In a study by Lavnya et al., showed very high incidence of asymptomatic bacteriuria in women 20 years of age [36].

In the present study, out of positive culture results, 46% were primigravida and 53.9% were multigravida $P < 0.05$. The higher incidence in primigravida can be explained by the fact that physiological and anatomical changes are more marked in primigravida [34], and could be because of hormonal changes occurring prior to anatomical changes. Moreover, earliest study by Kass explains that there is rare acquisition of bacteriuria after the second month of pregnancy [33]. The higher incidence to some extent in primigravida showed positive correlation with the studies by Lavnya et al. and Nath et al. [36, 37]. The bacteria responsible for asymptomatic bacteriuria are of fecal origin which colonize the periurethral area. The gram negative bacteria are the main culprit. In the analysis of different studies [38–41]. Interestingly, the overall prevalence of UTI among pregnant women was (20%), which is relatively higher in Saudi Arabia [42]. Moreover, lower rates (4.8% and 6.1%) were recorded in neighbor countries as UAE and Iran, respectively [43, 44]. Regarding the causative agent of UTI, the current study revealed that *Klebsiella pneumoniae* (37%) was the most frequently *E. coli* (25%) isolated pathogen from all UTI cases followed by the current findings is in general contrary to expectations with the majority of the reported studies, which reported *E. coli* as the most common UTI-associated pathogen with a rates of 41.5%, 44%, 47.2%, 63%, 37.5% and 42.1% in Yemen, Ethiopia, Tanzania, Philippines, Ghana and Nigeria, respectively [45–50]. Few studies were reported about asymptomatic bacteriuria in other city in Saudi Arabia where the prevalence in Abha (19.6%), Hail (50%), Makkah (8%), and Jeddah (1.7%) [51–54].

The susceptibility pattern of 745 bacteria isolated from pregnant women against 20 antimicrobial agents. All strains were susceptible to all antibiotic (20 antimicrobial agents) used in study (100%), resistance was observed in some strains of *Klebsiella pneumoniae* against ampicillin. All patients with significant bacteriuria were advised to take appropriate antibiotics as per the sensitivity report to avoid complications.

5. Conclusion

The current results show that urinary tract infection in pregnancy is a very frequent medical problem in Taif (62%), which indicate fecal contamination and low personal hygiene. Although symptoms are good markers of UTI during pregnancy, antenatal care should include direct questioning and urine examination to discover asymptomatic cases. Pregnancy associated bacteriuria is a common entity. The common pathogens involved are of fecal origin. Because of complications associated with asymptomatic bacteriuria in pregnancy, it should be made mandatory to screen every antenatal women in early pregnancy for it. The most sensitive test for its detection is urine culture with clean-catch mid stream urine. Treatment with appropriate antibiotic therapy should be done in every positive case. The current findings highlighted the need for constant monitoring of susceptibility of specific pathogens in different populations to commonly used anti-microbial which will significantly assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse of antibiotics. Women with ASB may have serious consequences on both mother and fetus. Therefore, it is important to screen all antenatal women for asymptomatic bacteriuria at their first prenatal visit, preferably in first trimester, and those who are positive should be followed up closely after treatment because about 1/3rd will experience a recurrence. It's time that we have a look at this strategy for improving the healthcare and for reducing the maternal and fetal morbidity and mortality.

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