Asymptomatic Bacteriuria in Pregnant Women - Study at a Tertiary Maternity care Hospital in Hyderabad, India

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ABSTRACT

Urinary Tract Infections (UTIs) commonly occurs in pregnancy, due to the morphological and physiological changes that take place in the genitourinary tract. Asymptomatic bacteriuria refers to the presence of bacteria in urine, and is a condition in which urine culture reveals a significant growth of pathogens that is greater than 10³ bacteria/ml, but without the patient showing symptoms (Gilbert et al., 2005). The apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and non-commensal microorganisms (Scott et al., 1990). The most untoward consequence of Asymptomatic bacteriuria in pregnancy is severe renal damage. Asymptomatic bacteriuria in some woman may lead to pyelonephritis or cystitis during pregnancy, while in others there may be no symptoms of urinary tract infection through-out gestation, but may develop urinary tract infection during puerperium. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low birth weight (Connolly and Thorp, 1999) and higher fetal mortality rates (Nicolle, 1994; Delzell and Lefever, 2000). This study therefore was carried out to determine the prevalence of Asymptomatic bacteriuria (ASB) in pregnant women and to isolate, identify the causative organisms; and to test the antimicrobial susceptibility of isolated pathogens. A total of 200 pregnant women who came for ante-natal checkup in outpatient department at Govt. Maternity Hospital, Nayapool, Hyderabad were studied over a period of one year. Clean catch midstream urine sample was collected into a sterile container and then subjected to culture method. Out of 200 patients studied, significant bacteriuria was noted in 36 (18%) cases and 8(4%) patients had insignificant bacteriuria. Highest incidence of 22 cases (61.11%) were reported in the age group of 26-35 years. It was found that Asymptomatic bacteriuria showed significant increase with respect to parity, higher incidence was seen in multi gravidae 58.9% (3 and 4th parity). Incidence of Asymptomatic bacteriuria was found to decrease with the increase in gestation time, maximum number were noted in first trimester 19 (52.78%) followed by second trimester 13 (36.11%) and in third trimester 4 (11.11%). E. coli 20 (55.56%),was the most common etiological agent followed by Klebsiella spp in 9 cases (25%) Coagulase negative Staphylococcus in 2 cases (5.56%) and Pseudomonas spp in 2 cases (5.56%), Proteus mirabilis and Enterobacter, Staphylococcus aureus each in one case (2.78%) all the strains were sensitive to imipenem and meropenem. As asymptomatic bacteriuria is associated with complications in pregnancy, it is therefore imperative that pregnant women be screened for bacteriuria, periodically in every trimester of the gestational period. Routine urine culture tests should be carried out for all antenatal women to detect asymptomatic bacteriuria, and every positive case should be treated with appropriate antibiotic therapy, to prevent any obstetric complication which is associated with pregnancy. In view of changing patterns of bacterial resistance to common drugs, the importance of educating physicians on use of antibiotics accordingly to provide empirical therapy is important.

Keywords
UTI, Asymptomatic bacteriuria, E. coli

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**Introduction**

Prevalence of bacteriuria is common in women due to short urethra, and easy contamination of urinary tract with fecal flora (Girishbabu *et al*., 2011; Kerure *et al*., 2013) and it increases with age and/or sexual activity.

Urinary Tract Infections (UTIs) commonly occurs in pregnancy, due to the morphological and physiological change that take place in genito urinary tract.

Urinary tract infection is of two types, symptomatic and asymptomatic. Asymptomatic Bacteriuria (ASB) is defined as the presence of actively multiplying & persistently appearing bacteria, which is greater than 10^5/ml of urine within the urinary tract, excluding the distal urethra, at a time when the patient has no symptoms of a UTI (Jayalakshmi and Jayaram, 2008). ASB can be found in both pregnant and non–pregnant women.

When comparing the same age group females the pregnant women are more commonly affected than non-pregnant females (Chandel *et al*., 2012; Enayat *et al*., 2008). This is due to urinary stasis, progesterone effect in pregnancy, decrease in urine concentration and development of glucosuria in about 70% of pregnant women which encourages bacterial growth in the urine (Patterson and Andrriole, 1987; Lucas and Cunningham, 1993) and also due to apparent reduction in immunity, different morphological and physiological changes occurring during pregnancy. All of which contribute to the development of UTI.

The prevalence of ASB was found to be 2-11% in pregnant women. As per western literature prevalence of asymptomatic bacteriuria (ASB) among pregnant women, varies from 2 to 10% (Kerure *et al*., 2013; Enayat *et al*., 2008). In Indian studies the reported prevalence rate is as high as 8% (Balamurugan *et al*., 2012; Patel *et al*., 2005; Delzell and Lefevre, 2000). Pregnancy enhances the progression from ASB to symptomatic bacteriuria and in 25% of culture positive cases, undetected and untreated ASB may lead to symptomatic infection during that pregnancy, and this may lead to acute pyelonephritis in 20-50% of cases and to adverse obstetric outcomes such as prematurity, postpartum hypertensive disease, anaemia, and higher foetal mortality rates (Connolly and Thorp, 1999; Nicolle, 1994; Delzell and Leferre, 2000). In order to prevent mother and child from any form of complication that may arise due to undiagnosed asymptomatic bacteriuria routine culture screening for all pregnant women attending antenatal clinic (Kirklam *et al*., 2005) has become mandatory Thus, urine culture is the gold standard screening technique for ASB which occurs during pregnancy (Patterson and Andrriole, 1987; Gayathree *et al*., 2010). Therefore, the most common causative agents should be investigated and communities should be made aware of their local antimicrobial resistances. The objective of this study was to identify the prevalence of ASB, its most common causative microorganisms and the antibacterial susceptibilities of the isolated microorganisms among pregnant women who attended tertiary care centre at Govt. Maternity hospital at Hyderabad, Telangana India.

**Materials and Methods**

This was a retrospective study conducted in the Department of Microbiology at Govt. Maternity hospital at Hyderabad, over a period of one year

Study Population: Study was conducted after getting clearance from the institutional ethical
committee. A total of 200 pregnant women who attended antenatal clinic were included in this study. Verbal informed consent was obtained from each patient prior to sample collection. Socio-demographic data were obtained by means of personal interviews.

**Exclusion criteria**

1) Symptoms suggestive of infections in urinary tract (dysuria, frequency and urgency)  
2) History of antibiotic therapy in previous two weeks  
3) Known congenital anomalies of urinary tract.  
4) History of fever  
5) Pregnancy induced hypertension; and  
6) Pregnancy with Diabetes Mellitus.

**Specimen collection and processing**

Clean-catch midstream urine was collected from each patient into a sterile universal container that were covered with tight-fitting lids. Urine samples of each patient was centrifuged at 3000rpm for 10-15 minutes, the supernatant was discarded and the deposit examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells and *Trichomonas vaginalis*. The samples were processed within one hour of collection, by using standard microbiological procedures. Samples were cultured on dried plates of Sheep blood agar (in 5-10% CO2 atmosphere), MacConkey’s agar and cysteine lactose electrolyte deficient agar (CLED), using a calibrated drop delivering 0.002ml of urine by standard loop method and the plates were incubated aerobically at 37°C overnight. Prolonged incubation was done for further 24hours if no growth obtained. The identification of organisms was done by Gram staining, motility test, catalase test, oxidase test, coagulase test, and routine biochemical tests as per Cowan and Steels Manual (Cowan and Steels manual for the identification of medical bacteria, 1993). The growth was interpreted as- sterile if no growth obtained. Culture results were interpreted as significant and insignificant according to the standard criteria. Colony counts yielding bacterial growth of $10^5$/CFUs ml or more of pure isolates were regarded as significant for infection Insignificant growth was reported if colony count was less than $10^5$ CFUs per ml. The standardized Kirby-Bauer disc diffusion method on Muller Hinton agar plate of the Clinical and Laboratory Standards Institute (formerly NCCLS) was used for antibiotic susceptibility testing and interpretations were carried out accordingly (Clinical and Laboratory Standards Institute, 2007).

The antibiotics which were tested were:  
amikacin (30mcg); gentamycin (30mcg);  
Cefuroxime (30mcg); ceftazidine (30mcg);  
cefotaxime (30mcg); Cefepime (30mcg) :  
ceftriaxone (30mcg); Ciprofloxacin (5mcg):  
moxifloxacine (5mcg): ofloxacine (5mcg);  
sparfloxacine (5mcg): tobramycin (10mcg);  
gatifloxacine (10mcg); Imipenem (10mcg),  
Meropenem (10mcg), nitrofurantoin;  
clindamycin (2mcg), Co-trimaxozole (25mcg);  
Lincomycin (15mcg); cefuroxil.

All the above antibiotic discs were obtained from HiMedia (Mumbai, India), and Bio-Rad, New Delhi, India.

**Results and Discussion**

Among 200 asymptomatic pregnant women who were screened, significant bacteriuria was found in only 36 (18%) cases and insignificant bacteriuria in 8 (4%) cases. 156 samples were sterile with no growth (Table 1).

In our study, 22 cases (61.11%) were reported in the age group of 26-35 years, which is the highest incidence, next highest of 10 (27.78%) cases were seen in 18-25 years group & 04 cases (11.11%) were seen in the age group of more than 36 years (Table 2).
The prevalence of asymptomatic bacteriuria showed significant increase with respect to parity, higher incidence was seen in multigravidae 58.3% (Table 3).

In our study, it was found that among positive cultures incidence of significant bacteriuria has a relation with gestational period; maximum number were noted in first trimester 19 (52.78%) followed next by second trimester 13 (36.11%) and third trimester 4 (11.11%) (Table 4).

The uropathogens which were isolated in culture were *Escherichia coli* in 20 patients (55.56%) the most predominant organism, followed by *Klebsiella pneumonia* in 09 cases (25%) Coagulase negative *Staphylococcus* in 2 cases (5.56%), and *Pseudomonas* spp 02 cases (5.56). *Staphylococcus aureus*, *Proteus mirabilis* and *Enterobacter* spp. each in one case (2.78%) (Table 5).

*Escherichia coli*, the most common isolate, was found to be almost 100% sensitive to the Carbapenems - Imipenem, and Meropenem, 4th generation cephalosporin - Cefepime showed 95% sensitivity (Table 6).

Similar sensitivity (90%) was also recorded against Aminoglycosides like Amikacin and Gentamycin, cefuroxime 2nd generation cephalosporin showed 80%, sensitivity and Furantoin - Nitrofurantoin documented 75% sensitivity, while other cephalosporins like Cefatazidine (20%), Cefotaxime (10%), Quinolines like Gatifloxacin (40%), ciprofloxacin (30%), exhibited varied sensitivity.

*Klebsiella pneumoniae*, the second most frequent organism, showed almost 100% sensitivity to carbapenems - imipenem, meropenem, and cephalosporins like: cefepime and cefuroxime. Sensitivity to amikacin and ofloxacin was 77.8% while for ceftriaxone it was 66.7%. *Pseudomonas* spp. showed 100% sensitivity to cefoperazone, ceftazidime and cefipime.

Gram positive cocci showed good sensitivity to imipenem, meropenem, clindamycin, Lincomycin and cefuroxime. The overall antimicrobial susceptibility pattern showed broad spectrum carbapenems - imipenem, and meropenem to be the most effective antibiotics.

In females urinary tract infections are common and in pregnancy due to the morphological and physiological changes that takes place in the genitourinary tract, infection is much more common (Kerure et al., 2013; Patel et al., 2005) One of the important causative factors for premature or low birth infants, postpartum urinary tract infections and higher fetal mortality rates in pregnant women is Asymptomatic bacteriuria. Women who have bacteriuria have a 20-50 fold increased risk of developing pyelonephritis as compared to women who do not have bacteriuria (Celen et al., 2011; Kacmaz et al., 2006) Asymptomatic bacteriuria of pregnancy needs special attention, due to lack of symptoms and its adverse consequences in pregnancy.

In this study, the prevalence of asymptomatic bacteriuria was 18%, which was similar to those seen in various other studies by (Girishbabu et al., 2011; Kerure et al., 2013; Balamurugan et al., 2012; Patel et al., 2005; Celen et al., 2011; Kass, 1960).

The incidence in various Indian studies (Yashodhara et al., 1987) was found to be between 5 and 12%, and in Western studies (Kerure et al., 2013), the incidence ranges from 2-7%. The incidence of ASB was 8.4% in a south Indian population by a study by (Lavanya and Jogalokshmi, 2002; Neupane et al., 2012) (26%) and (Imade et al., 2010) (45.3%) reported a higher prevalence.
Table 1 Showing culture interpretation of total cases screened (200)

| Culture interpretation       | Number of cases | Percentage |
|------------------------------|-----------------|------------|
| Significant bacteriuria      | 36              | 18%        |
| Insignificant bacteriuria    | 8               | 4%         |
| Sterile with no growth.      | 156             | 78%        |

Table 2 Showing age distribution of culture positive cases

| Age Group         | Number of cases | Percentage |
|-------------------|-----------------|------------|
| 18-25 years       | 10              | 27.78%     |
| 26-35 years       | 22              | 61.11%     |
| More than 36 yrs  | 04              | 11.11%     |

Table 3 Showing distribution of parity of culture positive case

| PARITY      | Number of cases | Percentage |
|-------------|-----------------|------------|
| Multigravida| 21              | 58.33%     |
| Primie      | 15              | 41.64%     |

Table 4 Showing Gestational distribution of culture positive cases

| Trimester        | Number of cases | Percentage |
|------------------|-----------------|------------|
| first trimester  | 19              | 52.78%     |
| second trimester | 13              | 36.11%     |
| third trimester  | 4               | 11.11%     |

Table 5 Showing percentage distribution of isolated organism among culture positive cases

| Organism isolated            | Isolated Number | Percentage |
|------------------------------|-----------------|------------|
| Escherichia coli             | 20              | 55.56%     |
| Klebsiella                   | 09              | 25%        |
| Pseudomonas spp              | 02              | 5.56%      |
| Coagulase negative           | 02              | 5.56%      |
| Staphylococcus               |                 |            |
| Staphylococcus aureus        | 01              | 2.78%      |
| Enterobacter spp             | 01              | 2.78%      |
| Proteus mirabilis            | 01              | 2.78%      |
Table 6 Showing antibiotic sensitivity pattern of isolated organism among culture positive cases

| Anti | E. coli (20) | Kleb (09) | Pseudo (02) | St.epi (02) | Enterobacter (01) | Prote (01) | St. aureus (01) |
|------|-------------|-----------|-------------|-------------|-----------------|------------|----------------|
| AMK  | 18(90%)     | 7(77.8%)  | 0           | NT          | 1(100%)         | 1(100%)    | NT             |
| GEN  | 18(90%)     | 5(55.5%)  | 02(100%)    | NT          | NT              | NT         | NT             |
| CXM  | 16(80%)     | 09 (100%) | NT          | NT          | 0               | 1(100%)    | NT             |
| CTZ  | 4(20%)      | 1(11%)    | 2(100%)     | NT          | 0               | 0          | NT             |
| CTR  | 2(10%)      | 6(66.7%)  | 1(50%)      | NT          | 0               | 0          | NT             |
| CPM  | 19(95%)     | 09(100%)  | 2(100%)     | NT          | 1(100%)         | 1(100%)    | NT             |
| CTR  | 9(45%)      | 0         | 0           | NT          | 1(100%)         | 1(100%)    | NT             |
| CIP  | 6(30%)      | 2(22%)    | 1(50%)      | NT          | 1(100%)         | 1(100%)    | NT             |
| MOX  | 8(40%)      | 4(44%)    | NT          | NT          | 0               | 0          | NT             |
| OFL  | 10(50%)     | 7(77.8%)  | 1(50%)      | NT          | 0               | 0          | NT             |
| SPA  | 10(50%)     | 5(55.5%)  | 0           | NT          | 0               | 0          | NT             |
| TOB  | 1(05)       | 2(22%)    | 0           | NT          | 0               | 0          | NT             |
| GTX  | 8(40%)      | 6(66.7%)  | NT          | NT          | 0               | 0          | NT             |
| IPM  | 20(100)     | 09(100)   | 2(100)      | NT          | 1(100%)         | 1(100%)    | NT             |
| MEM  | 20(100)     | 08(88.8%) | 2(100)      | NT          | 1(100%)         | 1(100%)    | NT             |
| NTF  | 15(75%)     | 4(44.4%)  | 1(50%)      | NT          | 1(100%)         | 1(100%)    | NT             |
| CD   | NT          | NT        | NT          | 2(100%)     | NT              | NT         | 1(100%)        |
| COT  | NT          | NT        | NT          | 2(100%)     | NT              | NT         | 1(100%)        |
| LM   | NT          | NT        | NT          | 2(100%)     | NT              | NT         | 1(100%)        |
| CFD  | NT          | NT        | NT          | 2(100%)     | NT              | NT         | 1(100%)        |

AMK: amikacin; GEN: gentamycin CXM: Cefuroxime CTZ: ceftazidine; CTX: cefotaxime; CPM: Cefepime CTR: ceftriaxone; CIP: Ciprofloxacin MOX: moxifloxacin OFL: ofloxacin SPA: sparflaxin TOB: tobramycin; GTX: gatifloxin; IPM: Imipenem, MEM - Meropenem, NTF: nitrofurantoin; CD: clindamycin COT: Co-trim LM: Lincomycin CFD: cefuroxil

In studies conducted by Maryam Kasraeian et al., (2009) and Ansari and Rajkumari (2001) incidence rate among pregnant women correlates with the present study (Verma et al., 2016; Jain et al., 2013) This high prevalence is attributed to their socio-economic status, lack of personal and environmental hygiene. There was significant association between presence of bacteriuria and certain risk factors like, low socioeconomic class, lower education level and multiparty (Tahir, 2015).

This variation may be explained by the fact that there were differences in the environment, social habits of the community, socio-economic status, the standards of personal hygiene and education among the patients who were studied. As the prevalence of ASB is18% in our study screening of all antenatal women for ASB, especially in early pregnancy by quantitative urine culture is recommended.

In our study incidence of ASB was more in multigravida 58.33% this is similar to study by Girishbabu et al., (2011) and Qudsia (2011).

In this study, age group of 26-35 years had the highest percentage of infection (61.11%), followed by age group of 18-25 years.
(27.78%) and this was closely followed by age group more than 36 years (11.11%). This is similar to the study done by Al Senani (2011) and Sudha Biradar et al., (2013) also reported majority of the women in the age group of 26-35 years. The observed trend of bacteriuria in this study and reports from other studies showed that the age range of 21-40 years served as the high risk group for development of UTIs in pregnant women. This high incidence of ASB in the young reproductive age group is due to early marriage and childbearing in our country, especially in the rural sector. Turpin et al., (2007) reported a higher prevalence of ASB in pregnant women who were aged 35-39 years. Many studies show advancing age as a risk factor for acquiring ASB in pregnancy because there is decrease in glycogen deposition and reduction in the lactobacillus as a part of ageing process which enhances bacterial adherence and invasion by pathogens and make them more susceptible (Sudha Biradar et al., 2013).

In this study, incidence of asymptomatic bacteriuria was higher in multigravidae (58.33%), which was similar to study done by Okonko et al., (2010), Roy et al., (1974), Verma et al., (2016) and Sujatha and Manju Nawani (2013). The higher incidence of ASB in the multigravida is due to increased colonization of urinary tract by pathogens due to repeated exposure to urinary stasis or previous infections.

In our study the prevalence of asymptomatic bacteriuria showed variation with gestational age, a higher rate of infection was detected in first trimester of pregnancy, which was similar to that seen in the study of Yashodara et al., (1987).

The higher incidence in first trimester could be caused by hormonal changes occurring prior to occurrence of anatomical changes Turpin et al., (2007) reported a high percentage of asymptomatic bacteriuria in the first and early second trimesters of pregnancy. Nath et al., (1996) and Roy et al., (1974) detected higher rate of infection in second trimester. This may be attributed to pregnant women reporting at the antenatal clinic usually during this period.

The most prevalence organism observed in this study was Escherichia coli (55.56%), followed by Klebsiella species (25%). The other organisms isolated included, Pseudomonas aeruginosa (5.56%). Coagulase negative Staphylococcus (5.56%), Proteus species (2.78%), Staphylococcus aureus (2.78%), and Enterobacter spp (2.78%). The bacteria which are responsible for asymptomatic bacteriuria are of faecal origin, which colonize the periurethral area. The same predominant trend of Escherichia coli infection pattern was also shown by different studies done by Girishbabu et al., (2011), Chandel et al., (2012), Enayat et al., (2008), Imade et al., (2010), Jain et al., (2013) and Senthinath et al., (2013). This could be due to the fact that most Escherichia coli strains prefer the environment of urinary stasis seen commonly in pregnancy, and another reason could be as a result of poor genital hygienic practices during defecation and micturition by pregnant women.

In this study, most of the isolates showed 100% sensitivity to imipenem and meropenem. Among the aminoglycosides, amikacin showed good sensitivity. While some of the cephalosporins also showed good sensitivity. This is in line with the study done by Sujatha and Manju Nawani (2013).

Gram positive microorganisms which were isolated showed good sensitivity to imipenem, meropenem, clindamycin, Lincomycin, and ceftriaxil Antibigram in this study correlated with that of study by Enayat et al., (2008). The antimicrobial sensitivity and resistance pattern vary from community to community.
and from hospital to hospital, this upsurge in antibiotic resistance patterns could have been caused by emergence of resistant strains, which may be due to indiscriminate use of antibiotics. Its abuse, self-medication, also low costs and availability of drugs over the counter could be other factors contributing to antibiotic resistance.

As asymptomatic bacteriuria is associated with complications in pregnancy, it is therefore imperative that pregnant women be screened for bacteriuria, periodically in every trimester of the gestational period. Early screening and appropriate treatment with susceptible antibiotic during antenatal period helps in reducing the incidence of pyelonephritis. It is important also to educate treating doctors, regarding empirical use of antibiotics in view of changing patterns of bacterial resistance to common drug. Age, parity, medical or obstetrical high risk factors all play a role in causing urinary tract infection and adverse perinatal outcome and maternal morbidity as seen in our study. Further studies are needed to conclude that asymptomatic bacteriuria can lead to adverse perinatal or maternal outcomes. Thus, we conclude that early prenatal screening of women for urinary tract infection and treatment of women prevents pyelonephritis and helps in reducing adverse maternal outcomes.

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