Prevalence of asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Clinic at Plateau State Specialist Hospital, Jos, Nigeria

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Abstract
Asymptomatic bacteriuria (ASB), common occurrence in pregnancy usually results in maternal and fetal complications. Bacterial infection is often missed in pregnancy because urine microscopy, culture and sensitivity is not part of the approved laboratory tests for women attending antenatal clinic (ANC)s in Nigeria. This study was a cross-sectional, laboratory-based study involving 136 first-, second-, and third trimester, consecutive pregnant women attending ANC at the Plateau State Specialist Hospital Jos, who gave informed consent and submitted urine samples for the determination of prevalence of ASB using Cysteine Lactose Electrolyte Deficient (CLED) agar by the standard loop technique and microscopy. Demographic data was obtained by administering structured questionnaire to the study participants. Data obtained were analyzed using SPSS version 21 and p values <0.05 were considered statistically significant. Out of 136 urine samples examined 10.3% were positive for ASB. Of the total ASB isolates, 71.4% was Escherichia coli, the most prevalent uropathogen, followed by Staphylococcus aureus (14.3%) while others were 14.2%. Age group 24-28 years had the highest prevalence 5.1% followed by 19-23 years age group with 2.2%. The lowest prevalence was age groups 29-33 and 34-50 years with prevalence of...
1.5% each. In respect to trimesters, second and third trimester recorded the highest with 4.4% each while the first trimester had 1.5%. Based on the antibiogram, Ciprofloxacin and Sparfloxacin were found to be most effective antibiotics against *Escherichia coli* and *Staphylococcus aureus*. This study revealed high prevalence of ASB among antenatal women in the population studied. Further study with larger sample size covering the entire state is advocated to discover the possibility of making bacterial microscopy, culture and sensitivity part of antenatal care for the purpose of early diagnosis and proper management of pregnant women in plateau state.

**Keywords**: Asymptomatic bacteriuria; Pregnancy; Microscopy; Culture; Sensitivity testing

**Introduction**

Bacteriuria is the presence of bacteria in urine of human while asymptomatic bacteriuria (ASB) is defined as the presence of actively multiplying bacteria in the urine of patient without any obvious signs or symptom of urinary tract infection (Mitchelle *et al.*, 2003). Globally, prevalence of ASB in pregnancy varies from 2-10% (Alvarez *et al.*, 2010). In Nigeria the prevalence ASB ranges from 15-21% (Taiwo *et al.*, 2009).

Urinary tract infections (UTI) are mainly caused by the bacteria that grow and multiply in the urinary tract of all age groups and especially in pregnancy (Schnarr and Smaill, 2008). Report showed that high level of sexual activity, low socioeconomic status, advanced maternal age, diabetes, patients with Acquired Immunodeficiency Disease Syndrome (AIDS)/Human Immunodeficiency Virus (HIV), anomalies and defects of the urinary tract are other risk factors (Matuszkiewicz-Rowińska *et al.*, 2015). Next to anemia, Urinary tract infections is a serious common cause of complications in pregnant women, which if untreated can adversely affect the health of infant or the pregnant mother (Manjula *et al.*, 2013).

For a good obstetrics outcome, all urinary tract infections should be adequately treated following laboratory diagnosis by microscopy, culture and sensitivity (Coulthard *et al.*, 2010). Untreated urinary tract infection (UTI) s generally could lead to serious pregnancy complications both to the mother and the fetus (Stamm, and Hooton 1993). Immunological and physiological changes in pregnancy appear to encourage the growth of both commensal and non-commensal microorganisms (Nath *et al.*, 1996). The physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosuria (Asscher *et al.*, 1966; Gibble *et. al.*, 1995) which encourages bacterial growth in the urine. Pregnancy causes numerous changes in the woman’s body involving physical and hormonal which increases the risk of urinary stasis and vesicoureteral reflux (Chaliha and Stantoo, 2002). These changes result in difficulty with hygiene due to a distended belly thus increase the frequency of UTI in pregnant women (Stamm, and Hooton 1993). Urine microscopy, culture and sensitivity (M/C/S) does not form part of the standard laboratory investigations conducted in women during the antenatal visits in Nigerian women as well as in women in other developing countries probably due to cost and delay in obtaining culture result (Imade *et al.*, 2010). This lack of inclusion of M/C/S does not allow for proper management of women with ASB, thus increasing the risk of coming down with pregnancy complications. For this reason, there is therefore likely hoo of high burden of ASB in pregnant women and its associated complications in Jos and environment that is still highly underestimated. This study aims at determining the prevalence of ASB amongst pregnant women attending antenatal clinic at plateau state hospital, Jos.
Materials and Methods

Study Area
The study was conducted at the Plateau State specialist hospital, a tertiary health institution located in Jos, Plateau State with over 40 different ethnic groups (Hodder, 1959; Daniel 2002; Plateau State, People and Culture 2004).

Study Design
This was a cross-sectional study conducted between the month of September and November, 2019 in Jos, North Central of Nigeria. Participants who satisfied the inclusion criteria and consented to participate in the study were recruited as they presented to the ANC at Plateau State specialist hospital.

Ethical Clearance
Ethical clearance was obtained from the Ethical Committee of the Plateau State Specialist Hospital, Jos. A written consent was obtained from the 136 study participants before the commencement of urine samples collection.

Clinical Evaluation and Selection of Participants
All pregnant women at the ANC were briefed about the nature of the study and written informed consent was taken from each person. Apparently health pregnant women (as examined by the gynecologist) who fulfilled the entry criteria were enrolled into the study. Participants’ personal data such as maternal age, marital status, educational level, socio-economic status and other information were sourced from each participant in addition to the data resulting from the gynecological and laboratory investigations and entered into the study.

Exclusion Criteria
All pregnant women with symptoms of urinary tract infection or already on treatment with antibiotics were excluded from the study.

Laboratory Methodologies

Clean-Catch Midstream Urine Sample Collection
Urine samples, 136 were collected from all consenting participants as the present at the ANCs. Recruits were instructed on how to obtain a clean catch of mid-stream urine samples in the morning into a labeled sterile, screw-capped universal container and transported (by placing the urine samples in a cold box away from sunlight) within one hour of collection to the Medical Microbiology laboratory of the Department of Medical laboratory science, University of Jos for processing.

Sample Processing (Culture Test)
Urine culture was performed using standard wire loop method. A loop-full (0.001 ml) of well mixed freshly voided mid-stream urine was streak-inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar plates. The plates were incubated aerobically at 37°C for 24 hours under aerobic conditions. Isolates were considered significant if there were ≥10^5 colony forming unit/ml (CFU/ml) with 2 or less isolates (Washington, 2006). Mixed growth of more than two organisms was considered to be contaminants. Significant isolates were identified by colonial appearance, microscopy, culture and biochemical techniques (Washington, 2006).

Antibiotic Susceptibility Testing
Antibiotics susceptibility was carried out by the Kirby-Bauer Disc diffusion method (CLSI, 2011). The following antibiotics discs (Oxoid, Basingstoke, UK) were tested on the isolates: Ciprofloxacin (10µg), Gentamicin (10µg), Streptomycin (30µg), Reflacine (10µg), Nalidixic acid (30µg), Ceporex(5µg), Seprin (25µg), Ampicillin (25µg), Augmentin (30µg), and Tarivid (10µg). Antibiotics selections for testing and results determination were based on the Clinical Laboratory Standards Institute (CLSI) protocols (CLSI 20011).
Results
Out of 136 samples collected and analyzed, 14 samples showed significant bacterial growth, giving a prevalence of 10.3% ASB among the participants.

Table 1 showed the distribution of ASB based on age group in years. It revealed that 3 (2.2%) of the age group 19-23 years of participants were positive for ASB, while age groups 24-28 and 29-33 years, showed 7 (5.1%) and 2 (1.5%) respectively. Similarly for age group 34-36 years, it was 2 (1.5%) positive for the ASB.

Table 1: Distribution of ASB based on age group in years

<table>
<thead>
<tr>
<th>Age</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-23</td>
<td>28</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td>24-28</td>
<td>55</td>
<td>7</td>
<td>5.1</td>
</tr>
<tr>
<td>29-33</td>
<td>35</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>34-50</td>
<td>18</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>14</td>
<td>10.3</td>
</tr>
</tbody>
</table>

X² = 1.166; P value = 0.761

Table 2 showed that out of the 5 single (unmarried) pregnant women screened, none was positive for ASB. While married pregnant women 14 (10.3%), were positive for ASB.

Table 2: Distribution of ASB according to Marital Status

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Married</td>
<td>131</td>
<td>14</td>
<td>10.3</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>14</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Table 3 shows the distribution of ASB among the participants in relation to occupation. It showed that the highest prevalence 5 (3.7%) of ASB, was the unemployed pregnant women followed by self-employed and civil servants; 4 (2.9%) and 3 (2.2%) respectively. While the least was the students 2 (1.5%).

Table 3: Distribution of ASB in Relation to Occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unemployed</td>
<td>50</td>
<td>5</td>
<td>3.7</td>
</tr>
<tr>
<td>Civil servants</td>
<td>22</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td>Students</td>
<td>21</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Self-employed</td>
<td>43</td>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>14</td>
<td>10.3</td>
</tr>
</tbody>
</table>

X² = 0.330; P value = 0.95
Table 4 showed the distribution of ASB among pregnant women in relation to educational qualification. Women who attended only secondary school (5.2%), recorded the highest prevalence, followed by tertiary institutions while the least was (4.4%), among those who attended only primary school (0.7%).

**Table 4:** Distribution of ASB among Pregnant Women in Relation to Educational Qualification

<table>
<thead>
<tr>
<th>Educational qualification</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary school</td>
<td>21</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Secondary school</td>
<td>60</td>
<td>7</td>
<td>5.2</td>
</tr>
<tr>
<td>Tertiary institution</td>
<td>55</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>136</strong></td>
<td><strong>14</strong></td>
<td><strong>10.3</strong></td>
</tr>
</tbody>
</table>

$X^2 = 0.841; P$ value = 0.657

Table 5 shows the percentage occurrence and distribution of bacteria isolated in the urine sample of all participants. Four (4) different bacteria were isolated in this study, namely; *Escherichia coli* 10 (71.4%), *Proteus mirabilis* 1 (7.1%), *Klebsiella spp* 1 (7.1%) and *Staphylococcus aureus* 2 (14.3%).

**Table 5:** Percentage Occurrence and Distribution of Bacteria Isolates in Urine of Pregnant women in the ASB study

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number isolated</th>
<th>Percentage Occurance(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>10</td>
<td>71.4</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td><em>Klebsiella species</em></td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2</td>
<td>14.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>14</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 6 shows the distribution of ASB in relation to trimesters. Women in their second and third trimesters recorded the prevalence of 4.4% each, while those women in their first trimester had a prevalence of 1.5%.

**Table 6:** Distribution of ASB with respect to trimesters of the women

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Number Examined</th>
<th>Number Positive</th>
<th>Prevalence(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>21</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Second</td>
<td>56</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td>Third</td>
<td>59</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>136</strong></td>
<td><strong>14</strong></td>
<td><strong>10.3</strong></td>
</tr>
</tbody>
</table>

$X^2 = 0.025; P$ value = 0.987
Table 7 shows the antibiotics sensitivity pattern of bacterial isolates. Ten different antibiotics were used in this study, these include; Streptomycin, Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin and Ofloxacin.

Sparfloxacin (85.7%) and Ciprofloxacin (85.7%) were the most sensitive, while Augmentin (28.6%) and gentamycin (28.6) were the least sensitive antibiotics to ASB.

Table 3: Antibiotic Susceptibility Pattern of Isolates among Asymptomatic Bacteriuria Pregnant Women Attending Plateau State Hospital.

<table>
<thead>
<tr>
<th>Bacterial Isolate</th>
<th>Number Isolated</th>
<th>S (%)</th>
<th>SXT (%)</th>
<th>CH (%)</th>
<th>SP (%)</th>
<th>CPX (%)</th>
<th>AM (%)</th>
<th>AU (%)</th>
<th>CN (%)</th>
<th>PEF (%)</th>
<th>OFX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>10</td>
<td>50</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>P. Mirabilis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K. Species</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. Aureus</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

KEY: E=Escherichia, P= proteus, K=klebsiella, S= Staphylococcus

S - Streptomycin      AMX- Amoxicillin
SXT - Septrin         AU- Augmentin
CH - Chloramphenicol  CN- Gentamycin
SP - Sparfloxacin     PEF- Pefloxacin
CPX - Ciprofloxacin   OFX- Ofloxacin

Organism
E. coli= Escherichia coli
S. aureus= Staphylococcus aureus

Discussion
From the results obtained in this study, 10.3% of the participants were positive for ASB while 89.7% were negative.

Ayoyi et al., (2017) reported a higher prevalence (21.5%) in Kenya compared to this study. In comparison, reports in Ghana, Turpin et al., (2007) revealed a prevalence of 7.3% while Hazhir (2007) reported a prevalence rate of 6.1% in Teheran. In Mexico, Hernandez et al. (2007) reported a prevalence of 8.4% while Tadesse et al., (2007) reported a prevalence of 9.8% in Ethiopia. Far lower prevalence (3.3%) was reported by Irajian and Moghadas, (2009). Prevalence rate as high as 40% (Akinola et al., 2012) have also been reported. In contrast to this study, these differences in prevalence rates may have been due to the difference in study population, sample sizes, sampling technique or selection criteria used in the studies.
Considering the effect of maternal age of participants on pregnancy in ASB, this study did not establish any significant relationship on ASB in pregnancy and maternal age \((P=0.761; P \leq 0.05)\) as the highest frequency of the bacteriuria occur in patients of age group 24-28 years (5.1%); this is lower compared to the report of Turpin et al., (2007) and Amadi et al., (2007) who reported a high prevalence of asymptomatic bacteriuria in pregnant women aged 35 to 39 years. In this study age group 24-28 years with the highest frequency of ASB is a sexually active age group, and therefore are at higher risk of contacting urinary tract infection. This has been reported in other studies (Stamm and Hooton, 1993; Ronald, 2002; Sescon et al, 2003; Colgan et al., 2006).

In comparison between married women and singles (unmarried) women in this study, married women 14 (10.3%) had ASB while singles (unmarried) had none. This finding is lower compared to study by Essien et al., (2015) who reported 10.7% among the singles and 13.6% among married women. The reduce prevalence may be due to increased awareness on UTI amongst women.

In this study, the finding of high prevalence of ASB in second (4.4%) and third trimester (4.4%) women agrees with the findings of Alghalibi et al., (2007) who worked on bacterial urinary tract infection among second and third trimesters pregnant women in Yemen. This is however did not agree to the findings of Turpin et al., (2007) who reported a high percentage of asymptomatic bacteriuria in the first and early second trimesters of pregnancy. In this study, the high frequency in the second and third trimester may be as a result of the pressure effect of a bigger uterus on the ureter at the second and third trimester, and also the increasing smooth muscle relaxing effect of pregnancy hormones on the pressure of the bladder from descending this may lead to stasis of urine, which will encourage bacterial multiplication (Lawani et al., 2005). However, the relationship of trimester and urinary tract infection in this study is not statistically significant \((P=0.987; P \leq 0.05)\).

In this study, the most common bacterial isolates from midstream urine samples of asymptomatic pregnant women were E. coli (71.4%) followed by staphylococcus aureus (14.3%), and lastly Klebsiella species and Proteus mirabilis which accounted for (7.1%). In studies done elsewhere, Amala and Nwokah, (2015) and Obirikorang et al., (2012) in separate studies also reported E. coli as being the commonest pathogen responsible for bacteriuria which is consistent with the findings of this study. Different studies done by Delzell and Lefevre (2000), Colgan et al., (2006), Turpin et al., (2007), Hernandez et al., (2007) and Hazhir (2007) have all shown E. coli as the most common isolate. High risk of acquiring E. coli urinary tract infection could be due to the anatomical and the physiological changes that occur during pregnancy and the fact anatomical proximity of the anal and urogenital opening in females makes it possible for fecal contamination of the urinary tract from commensals of the bowel of which E. coli is a typical example and since most E. coli strains and other bacteria prefer that environment, they are able to persist and cause UTI.

In term of sensitivity pattern, Sparfloxacin and ciprofloxacin (85.7%) had the highest sensitivity while Augmentin and Gentamycin (28.6%) had the least sensitivity. The variation in the sensitivity pattern of isolates might be due to antibiotic abuse and self-medication.

**Conclusion**

The study revealed 10.3% prevalence of ASB among pregnant women attending antenatal clinic at Plateau State Specialist hospital Jos. The most predominant organism was *Escherichia coli*. Antimicrobial susceptibility pattern of test organism revealed that
Sparfloxacin and Ciprofloxacin are the antibiotics of choice for the treatment of ASB in this study. Early diagnosis and treatment of urinary tract infection during pregnancy can ensure the safety of the mother and fetus and also prevent complication during delivery.

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