PRELIMINARY STUDY ON ASYMPTOMATIC BACTERIURIA IN PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN THREE HOSPITALS IN KANO, A NORTH-WEST CITY IN NIGERIA

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ABSTRACT
Asymptomatic bacteriuria in Pregnancy is the significant presence of bacteria in the urine of pregnant women without clinical symptoms of urinary tract infection (UTI). In this study, samples of urine were obtained from 310 women attending antenatal clinics in 3 major hospitals, namely: Aminu Kano Teaching Hospital, Murtala Mohammed Specialist Hospital and Mohammed Abdullahi Wase Specialist Hospital Kano, Nigeria. These were subjected to microbiological and cultural tests. The bacteria isolated were characterized by standard methods. Using 10^5 cfu/ml as significant level of bacteriuria, the prevalence was found to be 47 (15.2 %). The isolated organisms include Proteus spp., 23 (49%), Escherichia coli, 17 (36%), Staphylococcus aureus, 3 (6.4%), Pseudomonas aeruginosa, 2 (4.3%), Klebsiella spp. 2 (4.3%), Proteus spp.[ Proteus mirabilis 20 (87%), Proteus vulgaris 3 (13%)]. Proteus mirabilis was found to be the most predominant bacteria isolated, followed by Escherichia coli. 198 samples had no pus cells with 24 showing significant bacteriuria. 112 samples had 1-4 pus cells/hpf(high power field) and >5pus cells/hpf, with 20 showing significant bacteriuria.202 samples had epithelial cells, with 33 showing significant bacteriuria. 26 samples had calcium oxalate crystals, 14 samples had calcium phosphate crystals and 7 samples had amorphous phosphate crystals. The mean age of study of the pregnant women is 26.7 years (S.D:5.2 years, range: 17-41 years). The age group 25-30 years had the highest prevalence (24.2%) with respect to age, while the housewives had the highest prevalence (57.4%) with respect to occupational status. Pregnant women in last trimester had the highest prevalence of asymptomatic bacteriuria (18.2%), followed by those in second trimester (13.5%), then women in the first trimester (12.6%). There is a changing pattern in the prevalence of organisms causing asymptomatic bacteriuria in pregnant women. There is need for early routine screening of all antenatal patients.

Keywords: Asymptomatic bacteriuria, Pathogenic organisms, Pregnant women, Kano Nigeria.

INTRODUCTION
Asymptomatic bacteriuria is the presence of significant bacteria titre in a voided urine sample and is caused by bacterial colonization of the urinary tract. It occurs without usual clinical symptoms such as burning during urination or frequent urination (Imade et al., 2010). It is a major risk factor in developing symptomatic urinary tract infection (UTI). It affects about 5-10% of sexually active and pregnant women. Asymptomatic bacteriuria is less prevalent in men (Dominic, 2006). Bacteria are typically introduced into the urinary tract during intercourse or wiping after a bowel movement. In non-pregnant women, asymptomatic bacteriuria rarely causes serious problem (Dominic, 2006). However, in pregnant women, this infection can progress upward causing acute urethritis, acute cystitis, and acute pyelonephritis (kidney infection). Pyelonephritis in turn can lead to adverse outcomes such as preterm labour which is the most common cause of
serious complications—including death in newborn babies. A kidney infection can also lead to sepsis (pathogenic organisms or toxins invading the blood or tissues) and adult respiratory distress syndrome (ARDS)—both can be life threatening (Dominic, 2006). Approximately 25-30% of asymptomatic bacteriuria cases in pregnancy will progress to symptomatic infection, 3-4 times as great a progression as in non-pregnant women (Dominic, 2006).

Urinary tract infection is a common problem in both community and hospital practice. Although occurring throughout life, infections are more common in pre-school girls and in women during childbearing years (Hugo and Russel, 1993). Urinary tract infections are usually classified according to the anatomical part of the urinary tract infected; kidneys (acute pyelonephritis), bladder (cystitis), Urethra (urethritis). The common aetiological agents are Escherichia coli, Pseudomonas and Enterococcus spp. (Kass, 1960). Escherichia coli is the commonest cause of urinary tract infection, followed by Staphylococcus. Others include Proteus spp. and Pseudomonas. (Gilman et al., 1996).

It is now documented that the child bearing age has the highest incidence of urinary tract infection. During pregnancy, vagina secretion is much more increased and is a medium for bacteria growth. Also due to increased progesterone activity, the glucose level of the vagina is also increased which also aids bacteria growth (Saidi et al., 2005).

At present, bacteriological examination of the urine is done mainly when signs and symptoms point to urinary tract infection, renal insufficiency, or hypertension. Urine microscopy, culture and sensitivity should always be done in persons with suspected systemic infection or fever of unknown origin. This is desirable for every woman in the first trimester of pregnancy (Jawetz et al., 1982).

Epidemiologic studies have revealed possible association of asymptomatic bacteriuria with prematurity (Kass, 1959). According to some reports, the incidence of asymptomatic bacteriuria in pregnancy ranges from 3-14.3% (Monzon et al., 1963). Kass (1959), in the course of investigating the role of asymptomatic bacteriuria in the pathogenesis of pyelonephritis, observed that the incidence of asymptomatic bacteriuria in pregnant women is approximately 6-8%. Untreated asymptomatic bacteriuria during pregnancy is followed by active pyelonephritis in about 40% of cases, and is associated with an increased perinatal mortality. When asymptomatic bacteriuria is treated during ante partum period, not only is pyelonephritis prevented, but prematurity and perinatal mortality reduced as well (Kass, 1962).

Recent epidemiologic studies in Nigeria have shown the prevalence of asymptomatic bacteriuria among pregnant women to be as high as 45.3% (Imade et al., 2010), 78.7% (Amadi et al., 2007), and 86.6% (Akerele et al., 2001).

The present study was designed to enumerate the prevalence of asymptomatic bacteriuria in pregnant women attending antenatal clinics in three (3) major hospitals namely Aminu Kano Teaching Hospital, Mohammed Abdullahi Wase Specialist Hospital, and Murtala Mohammed Specialist Hospital, all in Kano, a City in the North-Western region of Nigeria. Significant isolate i.e. those yielding >10^5cfu/ml of a pure bacteria growth were counted as positive samples, while mixed growths, scanty or insignificant growths and cultures
with no growths were counted as negative samples.

MATERIALS AND METHOD
A total of three hundred and ten (310) urine samples of pregnant women (not showing clinical symptoms of urinary tract infection) attending antenatal clinic in three major hospitals (Aminu Kano Teaching Hospital, Mohammed Abdullahi Wase Specialist Hospital, and Murtala Mohammed Specialist Hospital) in Kano, Nigeria were randomly collected and used in this study.

Collection of specimens
On each routine antenatal visit, a clean-catch mid-stream urine specimen was collected from each patient into sterile universal bottles. These were transported over an ice bath and refrigerated before analysis was carried out within 24 hours (Hooten et al, 2000).

Specimen processing
From each specimen, a loopful was inoculated on blood agar (Oxoid) plates and cystein lactose electrolyte deficient (CLED) agar plates. The plates were allowed to dry and then incubated aerobically at 37°C for 24 hours (Cheesbrough, 2002) and observed for formed colonies. The isolates were then sub-cultured accordingly using the streak plate method.

Identification of isolates
Each of the sub-cultured formed colonies were then streaked on the surface of already prepared selective media i.e. Mac Conkey agar, Cetrimide agar, Mannitol salt agar, Eosin methylene blue agar, Urea agar and Saubroud dextrose agar respectively. The streaked plates were then incubated at 37°C for 24 hours. The colonies that developed were observed, noting their characteristics. The isolates were also identified morphologically and biochemically (Cowan, 1974; Collee and Miles, 1989). The Ornithine decarboxylase and Indole production tests were used to differentiate Proteus (the most prevalent uropathogen isolated) specie type. Proteus mirabilis were ornithine decarboxylase positive, and Indole negative while Proteus vulgaris were ornithine decarboxylase negative and indole positive.

RESULT
Forty seven (47) out of the three hundred and ten (310) urine samples analyzed were positive for significant bacteriuria, with a prevalence of 15.2% (Table 1).

<table>
<thead>
<tr>
<th>Urine Sample(s)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With significant bacteria growth</td>
<td>47(15.2%)</td>
</tr>
<tr>
<td>Without significant bacteria growth</td>
<td>263(84.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>310</td>
</tr>
</tbody>
</table>

*significant level of bacteriuria=10^5 cfu/ml

These forty seven (47) positive culture yielded single bacterial isolates. Proteus spp. was isolated from twenty three (23) urine samples: twenty (20) were Proteus mirabilis, and three (3) were Proteus
Escherichia coli was isolated from seventeen (17) samples, Staphylococcus aureus was isolated from three (3) samples. Pseudomonas aeruginosa and Klebsiella spp. were both isolated from two (2) samples each (Table 2). Yeast cells were observed in thirty three (33) samples, while Candida spp. (fungi) was isolated from five (5) samples.

**TABLE 2: Prevalence of Uropathogens in urine samples of pregnant women attending antenatal clinic**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Percentage prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
<td>20</td>
<td>42.6</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>3</td>
<td>6.4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>17</td>
<td>36.0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>3</td>
<td>6.4</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

One hundred and twelve (112) samples had 1-4 pus cells/hpf and < 5 pus cells/hpf with twenty (20) showing significant bacteriuria. One hundred and ninety eight (198) samples had no pus cells with twenty four (24) showing significant bacteriuria. Two hundred and two (202) samples had epithelial cells with thirty three (33) showing significant bacteriuria. Twenty six (26) samples had calcium oxalate crystals, Fourteen (14) samples had calcium phosphate crystals, and seven (7) samples had amorphous phosphate crystals. The frequency of isolation (prevalence) of isolated micro-organisms (uropathogens) is shown in Table 2. Proteus spp. has the highest isolation frequency of 49%.

**Table 3: Prevalence of uropathogens with respect to age group of pregnant women attending antenatal clinic**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of patient</th>
<th>Number infected</th>
<th>Percentage (% infected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20</td>
<td>30</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>20-25</td>
<td>84</td>
<td>3</td>
<td>3.6</td>
</tr>
<tr>
<td>25-30</td>
<td>124</td>
<td>30</td>
<td>24.2</td>
</tr>
<tr>
<td>30-35</td>
<td>49</td>
<td>10</td>
<td>20.4</td>
</tr>
<tr>
<td>35-40</td>
<td>23</td>
<td>2</td>
<td>8.7</td>
</tr>
<tr>
<td>Total</td>
<td>310</td>
<td>47</td>
<td>15.2</td>
</tr>
</tbody>
</table>

*Escherichia coli* has an isolation frequency of 36%, *Staphylococcus aureus* has an isolation frequency of 6.4%, while *Pseudomonas aeruginosa* and *Klebsiella spp* both have the least isolation frequency of 4.3%. The age group of 25-30 years has the highest bacteriuria prevalence of 24.2% with respect to age (Table 3). Pregnant women in the last (third) trimester had the highest bacteriuria prevalence of 18.2% with respect to gestational age (Table 4), while pregnant women classified as housewives had the highest bacteriuria prevalence of 57.4% (Table 5) with respect to occupational status.

**Table 4: Prevalence of uropathogens with respect to gestational age (trimester)**

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Number of patient</th>
<th>Number infected</th>
<th>Percentage (% infected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>87</td>
<td>11</td>
<td>12.6</td>
</tr>
<tr>
<td>Second</td>
<td>104</td>
<td>14</td>
<td>13.5</td>
</tr>
<tr>
<td>Third</td>
<td>119</td>
<td>22</td>
<td>18.2</td>
</tr>
<tr>
<td>Total</td>
<td>310</td>
<td>47</td>
<td>15.2</td>
</tr>
</tbody>
</table>
**Table 5: Prevalence of uropathogens with respect to occupational status**

<table>
<thead>
<tr>
<th>Occupational status</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housewives</td>
<td>27</td>
<td>57.4</td>
</tr>
<tr>
<td>Working class</td>
<td>20</td>
<td>42.6</td>
</tr>
</tbody>
</table>

**DISCUSSION**

From the results obtained, urine is seen to be a medium in which various micro-organisms thrive, some of which are pathogenic and infectious. In this study, the prevalence of asymptomatic bacteriuria was found to be 15.2%; this is lower than 23.9% from the study in Sagamu (Olusanya et al., 1993), 45.3% reported in Benin City (Imade et al., 2010), and 78.9% in Abakaliki (Amadi et al., 2007), all in Nigeria. The lower prevalence may be associated with the antenatal teachings (which often lay emphasis on good personal hygiene practice like regular hand-washing, etc) given at each routine antenatal clinic in the three major hospitals used in this study. Also majority of the patients in this study practice the religion in which they often wash their hands and other body parts before saying their regular daily prayers. This could have helped to reduce bacteria load and transmission. The organisms isolated from the urine samples of these pregnant women were *Proteus spp.* (*Proteus mirabilis* and *Proteus vulgaris*), *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella spp.*

*Proteus spp.* (a major soil contaminant) was observed to be the most prevalent micro-organism (49%), this may have been introduced into the urinary tract of these women (especially the house-wives who are more busy with house chores exposing them to soil contact like sweeping, farming, etc) while they clean up using their hands after defecating or urinating. In time past *Proteus spp.* was observed to be among the least organism likely to cause asymptomatic bacteriuria, this study has shown that *Proteus spp.* (especially *Proteus mirabilis*) could be a major uropathogen causing urinary tract infection; this is at variance with other studies in the country, and it suggests a changing pattern in the prevalence of organisms causing infection in the population. This is followed by *Escherichia coli* (36%); this organism is observed to thrive well in the urinary stasis condition found in most pregnant women, and also poor hygiene practice after defecating and urinating.

Women in the third trimester were observed to have the highest prevalence 18.2% than those in their second and first trimesters. This agrees with the findings of Lindsay (2003), that asymptomatic bacteriuria increases with increase in gestational period. In terms of occupational status, the housewives were observed to have a higher bacteriuria prevalence of 57.4% than women of the working class, which could be as a result of reduced social exposure and poor hygiene. The working class women are more educated, and enlightened about precautionary measures (which includes personal hygiene, etc) to take to avoid been infected. The age group 25-30 years had the highest prevalence of 24.2%, women in this age group are more sexually active and multiparity is also a risk factor in acquiring asymptomatic bacteriuria.

Kidney stones was also observed to be a major risk factor in developing bacteriuria in pregnancy, forty seven (47) urine samples were observed in this study to have crystals which could aid the progression of bacteriuria. In this study, *Proteus mirabilis* was observed to be the most predominant isolate, and some of the virulence (degree of pathogenicity) factors of *Proteus spp*.
include: urease, hemolysin, hemagglutinin, adherence factors e.g fimbriae (pili), flagella/swarming phenomenon, etc. Urease production has been observed to result in the elevation of pH in the surrounding of bacterial growth resulting in stone formation and cytotoxicity (Antoni et al., 1997).

The occurrence and apparent prevalence of these microorganisms (some of which are pathogenic) in a vast number of women, especially those pregnant, gives cause for concern as they are prone to several complications as well as foetal risks. Due to the increase in sex hormones, the anatomic and physiologic changes in during pregnancy, bladder and kidney infection is more likely and may result in hypertension, preeclampsia, low birth weight, prematurity, septicemia, and maternal death. (Klein and Gibbs, 2004; Dalziel and Lefevre, 2000; Christensen, 2000). Some of the complications of bacteriuria in pregnancy include maternal aneamia (some of these uropathogens like Proteus, Escherichia coli, Klebsiella etc are known to possess pili/fimbriae which hemagglutinate human erythrocytes resulting in anaemia), increase in mid trimester abortion, low neonatal birth weight, hypertension, growth retardation and preterm delivery (Mc Grady et al., 1985). Most of the anatomic and physiologic changes in pregnancy influence the urinary tract and can result in urologic diseases and changes in kidney function which are serious threats for both the mother and fetus. Asymptomatic bacteriuria must be treated in pregnancy in order to prevent complications such as pyelonephritis, premature labour, still birth, hypertension, preeclampsia and septicemia (Raz, 2003).

Forty-seven (47) of the pregnant women were observed to have crystals (Calcium Oxalate, Calcium Phosphate and Amorphous Phosphate) in their urine samples. This signifies an underlying renal abnormally (kidney stones), which may be associated with repeated infections caused by Proteus species (Hugo and Russel, 1993). The pus cells, epithelial cells, and yeast cells observed also signifies an infection. A kidney infection can also lead to sepsis-pathogenic organism or toxin invading the blood or tissues and adult respiratory distress syndrome (ARDS) both of which can be life threatening (Dominic, 2006). Some of these pathogens isolated have been observed to bring about miscarriages, prevent future conception (infertility), blindness in newborn and several other damages to the foetus (Dominic, 2006).

**CONCLUSION**

In this study, the prevalence of asymptomatic bacteriuria was found to be 15.2% (and since approximately 25-30% of asymptomatic bacteriuria in pregnancy will progress to symptomatic infection, 3-4 times as great progression as in non-pregnant women), there is need for early routine screening of all antenatal patients presenting or not presenting with clinical symptoms of urinary tract infection, in order to prevent adverse outcome both to the mother and child.

There is also a changing pattern in the prevalence of the organisms causing asymptomatic bacteriuria; Proteus spp., is seen to be the most prevalent uropathogen isolated.

**REFERENCES**


