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DIPSTICK URINE ANALYSIS SCREENING AMONG ASYMPTOMATIC BACTERIURIC PREGNANT WOMEN IN MAKURDI, CENTRAL NIGERIA

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Abstract: **A dipstick urinalysis screening was conducted to evaluate the prevalence of some analytes among pregnant women who presented with asymptomatic bacteriuria and to set up a more effective screening program for them. Four hundred and thirty eight (438) urine samples from pregnant women were collected from attendees of a tertiary hospital in Makurdi, the Benue State capital of Central Nigeria between September 2014 and April of 2015.The urine samples were screened for the presence of ten (10) analytes indicated on the urianalysis dipstick. Different bacteria species using standard cultural and biochemical procedures showed a total of 277 (63.2%) having levels of bacteria indicative of asymptomatic bacteriuria comprising of *Escherichia coli* 87 (31.4%), *Staphylococcus* spp 60 (21.7%), *Proteus* spp 34(12.3%), *Klebsiella*spp 31 (11.2%), *Pseudomonas* spp 28 (10.1%), *Enterobacter* spp 22 (7.9%), *Streptococcus* spp 10 (3.6%), *Corynebacteria* spp 3(1.1%) and *Serratia* spp 2(0.7%). From the urinalysis dipstick a total of 48(10.9%) of the pregnant women tested positive to any one of the ten chemical analytes studied. Fifteen, 15(31.3%) of the subjects had glycosuria, ketone was detected in 10(20.8%) and leukocytes in 5(10.4%) while those having urobilinogen and haematuria both recorded 2(4.2%). The findings affirm the fact that asymptomatic pregnant women harbor bacteria in their urinary tract which could predispose them to pyelonephritis relating to negative outcomes for both mother and child.**

Keywords: **Dipstick, Asymptomatic, Bacteriuria, analytes, Central Nigeria.**

1. INTRODUCTION

Urine analysis can reveal disease that have gone unnoticed because they do not produce striking signs or symptoms. Examples include diabetes mellitus, various forms of glomerulonephritis and chronic urinary tract infections. It serves as a simple and inexpensive test and the cornerstone in the evaluation of the kidney function. Serious renal diseases may be present without any symptoms. Proteinuria as well as hematuria may be the only early signs of renal disease (membranous nephropathy, membranoproliferative glomerulonephritis, post infectious glomerulonephritis, IgA nephropathy and others) (Kumar and Clark, 1998). Also, the presence of detectable nitrites in urine has been used to diagnose urinary tract infection. Urinary tract infection is very common in children with severe consequences on the kidney function leading to chronic kidney disease and hypertension if left untreated (Gorelick and Shaw, 1999). The basic dipstick method is the most rapid screening procedure that could be helpful in the early detection of renal or urinary tract diseases among apparently healthy or asymptomatic subjects in the hope of preventing and retarding progression to chronic renal failure (Deville *et al*., 2004). A urine test is used to assess bladder or kidney infections, diabetes, dehydration and preeclampsia by screening for high levels of glucose, proteins, ketones and bacteria. High levels of glucose may suggest gestational diabetes, which may develop around the 20th week of pregnancy. Higher levels of protein may suggest a possible urinary

tract infection, or kidney disease. Preeclampsia may be a concern, if higher levels of protein are found later in pregnancy, combined with high blood pressure. (Fischbach and Dunning, 2009).

Asymptomatic bacteriuria (ASB) in pregnancy relates to anatomic and physiologic changes in the urinary tract that alter the host environment. Compression of the ureters at the pelvic brim may predispose to upwards reflux of urine; thus, ASB more readily progresses to pyelonephritis during pregnancy. Decreased concentration of urine, glucosuria, and progesterone effects (promote ureteric dilatation) also influence infection (Hazhir 2007; Ipe *et al.*, 2013). It has been reported that pregnant women are more prone to asymptomatic bacteriuria because of urinary stasis and ureteric reflux (Hutchon *et al.*, 1982), as well as anatomical changes, which prevent easy passage of urine (Bellanti, 1978). Physiologic changes of pregnancy may predispose patient to bacteriuria, these include urinary retention from the weight of the enlarging uterus and urinary stasis due to urethral smooth muscle relaxation caused by increases in progesterone (Woodman, 2001). Approximately 90 percent of pregnant women develop ureteral dilation, which will remain until delivery (hydronephrosis of pregnancy), increased bladder volume and decreased bladder tone, along with decreased ureteral tone. These factors contribute to increased urinary stasis and ureterovesical reflux) (Patterson and Andriole, 1987). Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration; up to 70 percent of pregnant women develop glycosuria, which encourages bacterial growth in the urine (Patterson and Andriole, 1987; Lucas and Cunningham, 1993). Increases in urinary progestins and estrogens may lead to decreased ability of the lower urinary tract to resist invading bacteria; this decreased ability may be caused by decreased ureteral tone or possibly by allowing some strains of bacteria to selectively grow (Lucas and Cunningham, 1993). Women with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or lowbirth-weight infants and have a 20- to 30-fold increased risk of developing pyelonephritis during pregnancy compared with women without bacteriuria (Smaill, 2007).

2. MATERIALS AND METHODS

Sample Collection

The study was carried out in a tertiary hospital in Makurdi, Middle belt region of Nigeria between September 2014 and April 2015. The hospital serves as a referral Centre for over half a million people within 40 km radius of the city. The town is divided by the River Benue into the north and south banks. Owing to its location in the Benue River, Makurdi experiences warm temperature most of the year.

A purposive selection consisting of pregnant women attending the ante-natal clinic was taken. This included women in the three trimesters of pregnancy. Patients were excluded if they had symptoms of urinary tract infection, had taken antibiotics during the previous week, or had any signs of labor.

A total of 438 pregnant women participated in this study. Information regarding each patient's medical and obstetric history was recorded on a predesigned proforma before collection of urine specimen. Information required on the proforma includes the age, parity, Trimester *i.e.* gestational age and how they wash their private parts, either from front to back or from back to front.

Collection of specimens

Written informed consent was obtained from the women for the collection of each specimen, in accordance with the ethical guidelines of the medical Institution.

Each of the women were instructed on how to collect a clean-catch midstream urine sample in a sterile container.

Processing and isolation of samples

The culture media used for isolation of bacteria from urine samples were Cystein-Lactose Electrolyte-Deficient (Difco Co, USA), Blood and chocolate agar plates. Each urine sample was inoculated and streaked with the aid of heat-flamed standard wire loop (delivering 0.001 ml urine) on to the agar plates. The plates were incubated aerobically at 37^{o}C for 24hrs and then examined. Only plates with significant growth (i.e. at least 100cfu/ml) were considered significant and further analyzed. The cultural and morphological characteristics of distinct and isolated colonies were studied. This included size, elevation, opacity and colour. Distinct and isolated colonies from each significant growth were Gram stained.

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The dipstick adopted consists of 10 reagents: Ascorbate, specific gravity, protein, blood, glucose, leucocytes, nitrites, urobilinogen, bilirubin and ketones (urine quick test; Combur-10-Test™,Roche, Mannheim, Germany). Dipstick test was performed by a laboratory attendant on duty. Each reagent strip, impregnated into a chemical, reacts with the substance present in urine and quickly changes color (60-120 seconds). The color of the strip was compared to the color chart present in the bottle label.

Urinalysis including the following findings were detected:

- >5-10 RBC/µl; hematuria (Green dots on yellow test: intact erythrocytes; Uniform green coloration of test: free hemoglobin or hemolysed erythrocytes);
- 1+ or greater proteinuria (trace, 1+, 2+, 3+, 4+ correspond to 10 mg/dl, 30 mg/dl, 100 mg/dl, 300 mg/dl, 1000 mg/dl respectively);
- 1+ or greater glycosuria (1+, 2+, 3+ corresponds to 100 mg/dl, 300 mg/dl, 1000 mg/dl respectively);
- Positive nitrites; and
- >25 WBC/µl; leukocyturia.

Data was coded, computed and analyzed using SPSS version 20.0 and p values ≤ 0.05 were considered to be statistically significant

3. RESULTS

From 438 urine samples screened for ASB, 277 were positive for significant bacteriuria (10^5cfu/ml) giving a prevalence rate of 63. 3%. The bacteria isolated from the culture of the urine samples of pregnant women with asymptomatic bacteriuria were identified based on colony morphology, Gram staining reaction and biochemical tests. The distribution of uropathogens among asymptomatic bacteriuric pregnant women attending in Makurdi are presented on Table 1. Results showed that organisms present in the urine essentially belong to nine (9) genuses, predominantly Gram-negative organisms constituting 66.6%.

Table 1: Distribution of Uropathogens isolated from pregnant women in Makurdi.

Gram reaction	Organisms Isolated	Number (%)
Gram negative	*Pseudomonas aeruginosa*	28 (10.1)
Gram negative	*Escherichia Coli*	87(31.4)
Gram negative	*Klebsiella*spp	31(11.2)
Gram negative	*Proteus* spp	34(12.3)
Gram negative	*Serratia*spp	2(0.7)
Gram positive	*Staphylococcus* spp	60(21.7)
Gram positive	*Streptococcus* spp	10(3.6)
Gram positive	*Corynebacteria*spp	3(1.1)
Gram negative	*Enterobacter* spp	22(7.9)

A total of 48(10.9%) of the subjects tested positive to any one of the ten (10) chemical parameters studied.(see Figure 1) The subjects having Glycosuria *i.e* glucose in urine recorded 15(31.3%) being the most positive tested chemical analyte as against those with urobilinogen and haematuria recording 2(4.2%) both being the least tested chemical parameter.

Figure 2 shows the distribution of chemical analysis obtained from urinalysis dipstick in relation to age, the 26-30 and the 31-35 age group recorded the highest total values of the tested parameters, both having 13(27.1%) while the least total value of the tested parameter was recorded in the ≤20 age group with 4(8.3%).

The distribution of chemical analytes obtained from urinalysis dipstick in relation to parity is displayed in Figure 3. Subjects carrying their first pregnancies recorded the highest total values of the tested parameters having 15(31.3%).

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Figure 4 and 5 shows the distribution of chemical analytes obtained from urinalysis dipstick in relation to trimester (age of pregnancy) and washing habit. The women in their second trimester and those who wash from back to front recorded 22(45.8%) and 35(72.9%) of the total chemical parameters tested respectively. However, these differences were not statistically different.

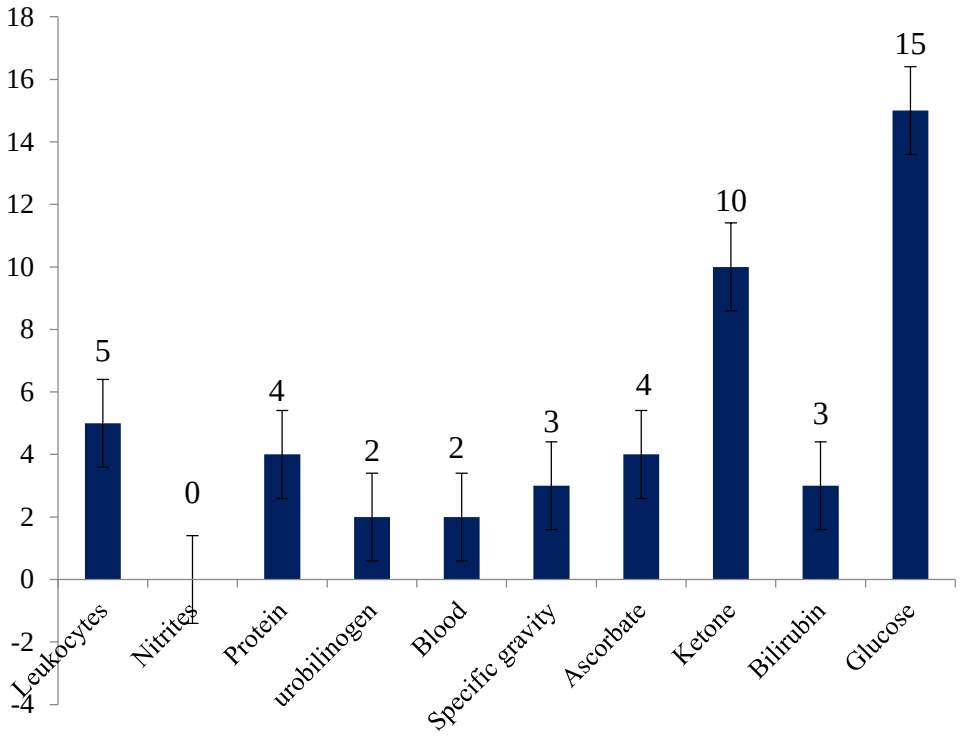

Figure 1: Distribution of chemical analytes obtained from Urinalysis dipstick from ASB pregnant women in Makurdi.

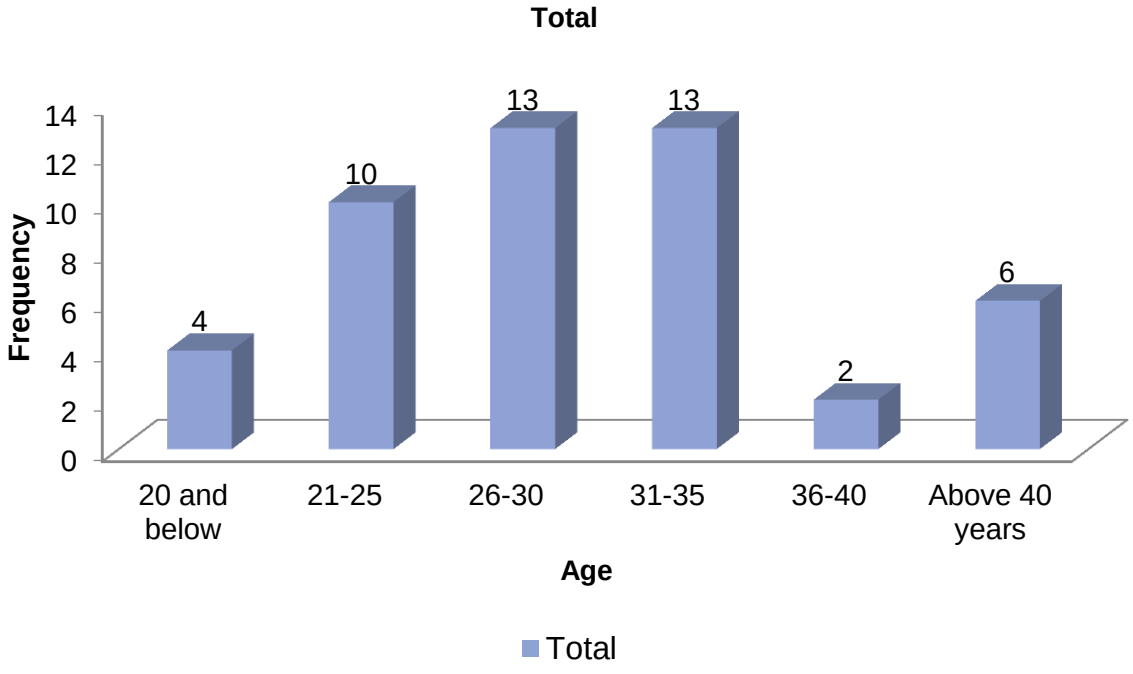

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Figure 2: Distribution of chemical analytes obtained from Urinalysis dipstick in relation to age.

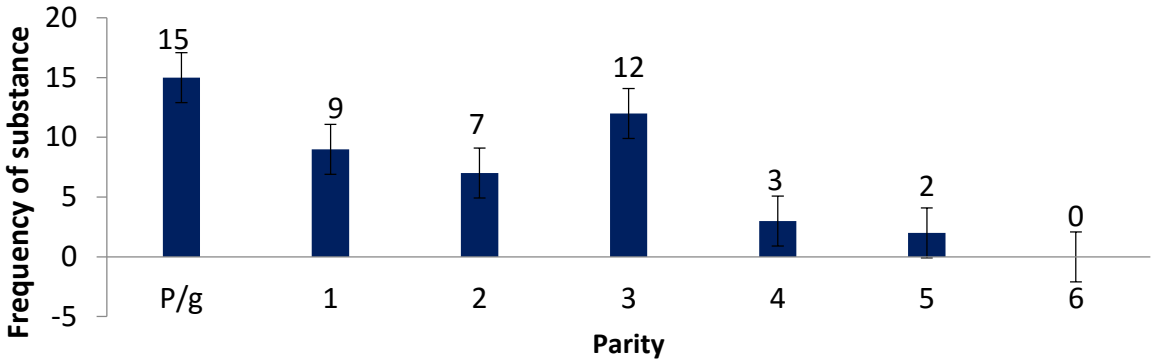

Figure 3: Distribution of chemical analytes obtained from Urinalysis dipstick in relation To Parity (Number of pregnancies).

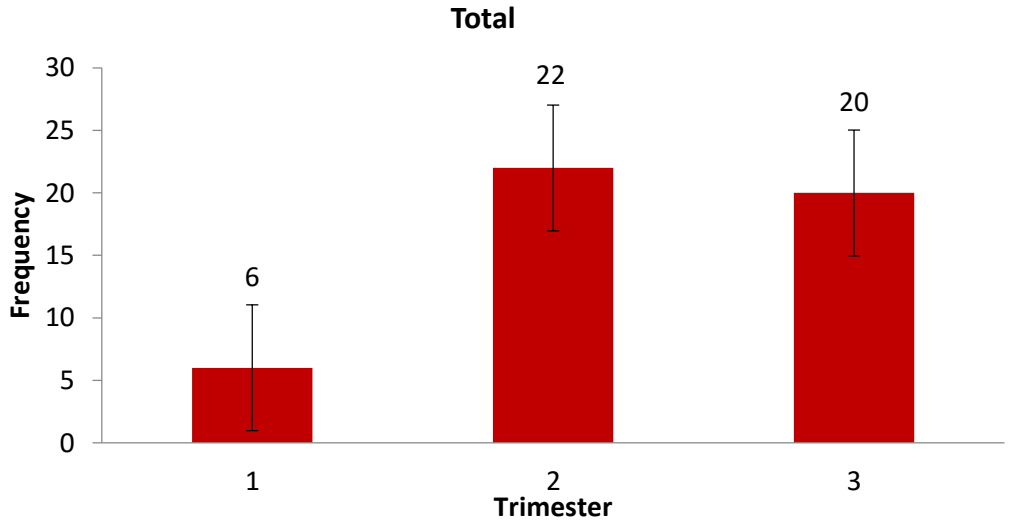

Figure 4: Distribution of chemical analytes obtained from Urinalysis dipstick in relation to Trimester (Age of pregnancy)

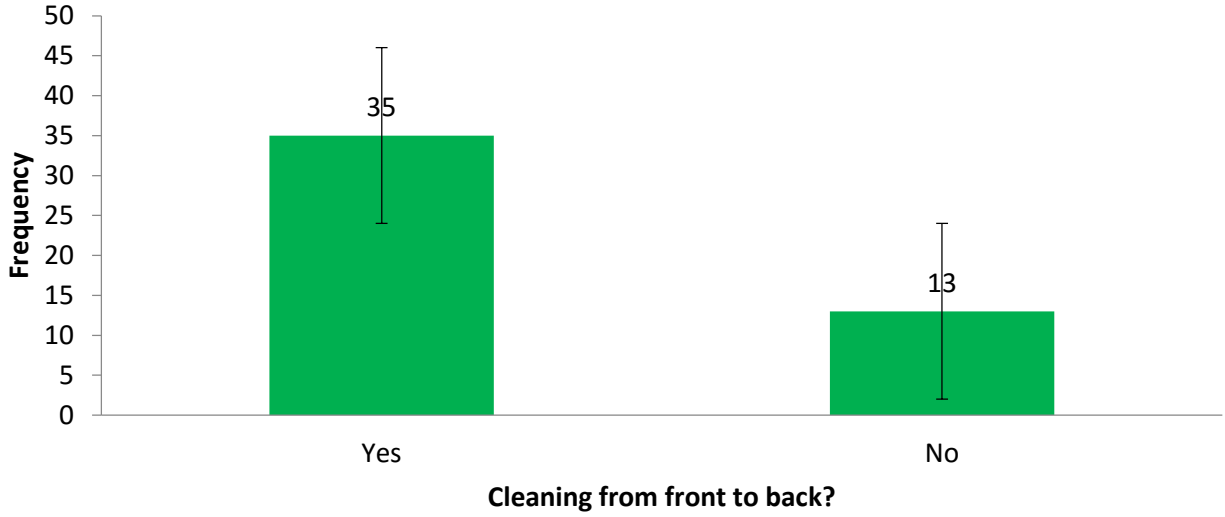

Figure 5: Distribution of chemical analytes obtained from Urinalysis dipstick in relation to toilet washing practice.

4. DISCUSSION

In this study, glycosuria among pregnant women recorded 31.3% prevalence. High levels of glucose may suggest gestational diabetes, which may develop around the 20th week of pregnancy. In this study hematuria, proteinuria, and nitrituria records 4.2%, 8.3% and 0.0% respectively as compared to studies of Farah *et al* 2011 who reported hematuria, proteinuria and nitrituria in school children as nearly 2.1% of the studied group and compared them to similar results recording 2.5% and the 2.3% reported in Northern Iran and Malaysia respectively. In their studies prevalence were higher than the 0.6% and the 0.72% reported in Tokyo and Egypt respectively and lower than the 7.2% and the 9.6% reported in Bolivian and Nigerian studies respectively. The studies of Akor *et al* 2009 who reported 9.6% from Nigeria closely resembles findings from this current study which recorded 8.3%. Glycosuria was the most common abnormality found in the studied group of this study standing at 31.3%. This was contrasted to other studies as in Egypt and Nigeria where proteinuria was the most common positive findings. (Bakr *et al* 2007; Akor *et al* 2009). Farah *et al* 2011 reports that the development of asymptomatic microscopic hematuria is relatively common in children. Its prevalence in school aged children has been estimated at 0.5% to 2.0% depending on the population screened and compared this standard to their results that showed a prevalence of 1.5% at the second screening (1.0% for isolated hematuria IH; 0.45% for combined hematuria and nitrituria CHN). Nigerian and Xiamen cities reported a prevalence of 1.5% and 1.21% respectively. On the other hand, hematuria had a lower prevalence in Malaysia, Egypt and Shanghai (0.21%, 0.36% and 0.46% respectively) comparable to the 4.2% of hematuria recorded in this study.

The current study records the prevalence of gram negative rods (66.6%) as higher than gram positives (33.4%). Similar results were obtained in a previous study (Tuladhar et al., 1990) who suggested that the presence of a unique structure in gram negative bacteria which helps for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion – resulting in invasive infection and pyelonephritis could be involved in it. Among the isolates, E. coli was found to be the most predominant organism followed by *Staphylococcus* spp. E. coli is the most common microorganism in the rectal area (Jazayeri and Irajian, 2009), the close proximity of the region might be the reason behind this. It is suggested that E. coli is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. Pathogenic E. coli expresses specific adhesions such as P fimbriae and produce alpha and beta hemolysins. Strains of E. coli appear well adapted to invade urinary tract which forms the majority of isolates of UTI (Chakraborty, 2001). This ability of E. coli may be the reason to be the most frequent organism to cause UTI. Several studies have also demonstrated that the geographical variability of pathogens occurrence in case of UTI is limited by the predominance of Gram negative, usually Enterobacteriaceae and particularly (Staphylococcus) were the second most common urine isolate and are similar to the findings of Enayat *et.al*. (2008).

This study shows that of the 438 women examined, 277 *(63.2%)* showed significant bacteriuria. In a similar study carried out by Olusanya*et al., (1984)* among 510 pregnant women and 304 non-pregnant women at Ogun State University Teaching Hospital, Sagamu South-WestNigeria, 23.9% of the population examinedshowed significant bacteriuria. The value obtained in our study is higher than the 23.9% in their study, Differences in the location, and different cultural habits of the people living within these geographic zones may account for variation in findings. In this study nurses had the highest colonization with ASB standing at 75.0%, this may be justified by the fact that some organisms are nosocomial and since these women work in a hospital setting, they may have acquired it from the hospital environment being more exposed to the risk. The risk of acquiring bacteriuria increases with the duration of pregnancy from 0.8% of women with bacteriuria in the 12th gestational week to 2% at the end of pregnancy.(Nicolle, 2003) agreeing with findings in this study that showed highest prevalence, 70% of ASB women in their third trimester (gestational age of 6-9 months). There was statistical significance of 0.049; $p<0.05$ among women across the three trimesters. About 69.1% of the women who had more than five pregnancies recorded the highest colonization with ASB, though there was no significant difference among parity. The prevalence of bacteriuria not only increases with age but also with sexual activity and parity (Khatun and Mahmood, 1998). ASB is found with an incidence of about 2% in primigravidae (women carrying their first pregnancy) under the age of 21 and in 8-10 in multiparae over the age of 35 (Reddy and Campbell, 1986). Women who washed their private parts from front to back recorded the highest colonization with ASB than those who washed from back to front. This association may be due to poor knowledge and practice of personal hygiene in pregnancy. Another reason could be as a result of poor genital hygiene practices by antenatal women who may find it difficult to clean their anus and genitals properly after defecating or passing urine.

5. CONCLUSION

This study helped to assess the prevalence of urinary abnormalities in pregnant women attending antenatal in Makurdi, Central Nigeria. Glycosuria was found to be the most prevalent abnormality. Also, the prevalence of asymptomatic bacteriuria was found to be 63.2% (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.

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