ANTIBIOGRAM PATTERN OF *Staphylococcus Saprophytics* ASSOCIATED WITH ASYMPTOMATIC BACTERIURIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN BAUCHI METROPOLIS

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Abstract: A study to isolate and determine the antibiogram pattern of *Staphylococcus saprophytics* associated with asymptomatic bacteriuria in pregnant women was carried out. A total of ninety one (91) clinical urine samples were collected from antenatal women attending some hospitals in Bauchi metropolis. Out of these samples 32.9% (30 of 91) were positive with S. *saprophytics* for significant bacteriuria (CFU > 10^5/mL). The highest prevalence of 43.8% (14 of 32) was found among the age group of 26-30 years, with the least (0.0%) in 41-50 years. Most of the isolates were highly sensitive to Amikacin (73.3%), Gentamicin (63.3%), Cefotaxime (56.7%), Imipenem (86.7%), Ciprofloxacin (83.3%) and Nitrofurantoin (70.0%). Multidrug resistance to some beta-lactam drugs (Ampicillin and Amoxycillin) was observed. This study found low socioeconomic status, decreased level of education, increased maternal age and parity as risk factors that could raised the rate of ASB. The relatively high prevalence of the infection in pregnancy is alarming, and require urgent attention of clinicians as the organism remain significant among sexually active women.

Key words: Asymptomatic bacteriuria(ASB), Antenatal, Beta-lactam drugs, Multidrug resistance, Pregnant women. *Staphylococcus saprophytics,*

INTRODUCTION

Urinary tract infection (UTI) is defined as significant bacteriuria in the presence of a constellation of symptoms such as dysuria (painful urination), increased urinary frequency and urgency, suprapubic and discomfort. Asymptomatic bacteriuria is the presence of a positive urine culture in the absence of specific symptoms of acute urinary tract infection (UTI) (Ojide *et al.*, 2014). It is also described as a presence of a significant quantity of bacteria in a properly collected urine specimen from a person without symptoms or signs of UTI (Tadesse *et al.*, 2014).This condition occurs more often in women due to the short nature of their urethra which is colonized by normal flora that are easily pushed into the bladder during sexual activity (Abdullah and Al-Moslih, 2005; Ugbogu *et al.*, 2010). It is associated with such complications, for the mother and the foetus as acute pyelonephritis, premature deliveries, low birth weight, still birth, pre-eclampsia, hypertension, anaemia, and postpartum endometritis (Al-Sabiani, 2010; Ojide *et al.*, 2014).

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Asymptomatic bacteriuria in which urine culture reveals a significant growth of pathogens that is greater than 10^5 bacteria/ml (Turpin *et al.*, 2007), but without the patient showing symptoms of UTI, can be found in both pregnant and non pregnant women. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low-birth weight, and higher foetal mortality rates. Although UTI may not always lead to complications in the mother, it is still a cause of significant morbidity (Nicolle, 1994; Connolly and Thorp, 1999).

Urinary tract infection (UTI) is a common health problem among women compared with men. It is estimated that one in three women of childbearing age contracts UTI, which may manifest symptoms or remain asymptomatic. Pregnant women are more susceptible to UTI, owing to altered anatomical and physiological state during pregnancy (Abdullah and Al-Moslih, 2005).

Urinary tract infections represent the most common bacterial infection in pregnancy. Asymptomatic *Bacteriuria* occurs in 2-10% of all pregnancies (Whalley, 2006). The prevalence has remained constant and most of the recent observational studies, including those from developing countries report similar rates (Daniyan *et al.*, 2010; Ajayi *et al.*, 2012; Tedesse *et al.*, 2014). The prevalence of *Bacteriuria* in pregnancy is closely related to socio-economic status (Tugruls *et al.*, 2005). *Staphylococcus saprophyticus*, a Gram positive coagulase negative staphylococcus, causes about 10% of infections among young, sexually active women (Hovelius *et al.*, 2009). It is often implicated in urinary tract infection. The strains have been isolated from human and animal gastro-intestinal tracts, meat, cheese products and vegetables (Taiwo *et al.*, 2009).

Pregnant women are at increased risk for UTIs. Beginning in week 6 and peaking during weeks 22 to 24, approximately 90 percent of pregnant women develop urethral dilatation, which will remain until delivery (hydronephrosis of pregnancy). Increased bladder volume and decreased bladder tone, along with decreased urethral tone, contribute to increased urinary stasis and ureterovesical reflux (Haider *et al.*, 2010). Up to 70 percent of pregnant women develop glycosuria, which encourages bacterial growth in the urine. Increases in urinary progestin and oestrogens may lead to a decreased ability of the lower urinary tract to resist invading bacteria. This decreased ability may be caused by decreased urethral tone or possibly by allowing some strains of bacteria to selectively grow (Haider *et al.*, 2010; Oli *et al.*, 2010). These factors may all contribute to the development of UTIs during pregnancy.

There is a strong association between the use of condoms coated with nonoxynol and the occurrence of UTI which suggests that vaginal spermicides interfere with the normal vaginal flora and promote colonization by *S. saprophyticus* (Delzeu and Lefeure, 2000). Other associations include outdoor swimming prior to colonization and occupations related to meat processing and meat products (Hedman *et al.*, 2000). The seasonal variation in the prevalence of colonization by *S. saprophyticus*, in cattle and pigs was similar to that of UTI in humans. It is also found to contaminate various food samples with prevalence of 34% in sample of raw beef and pork. It can occur in women who are vegetarians (Hedman *et al.*, 2000).

Untreated asymptomatic bacteriuria leads to the development of symptomatic cystitis in approximately 30 percent of patients and can lead to the development of pyelonephritis in up to 50 percent (Daniyan *et al.*, 2010). Asymptomatic bacteriuria is

associated with an increased risk of intra-uterine growth retardation and low-birth-weight infants (Heider *et al.*, 2010). The relatively high prevalence of asymptomatic bacteriuria during pregnancy, the significant consequences for women and for the pregnancy, plus the ability to avoid sequelae with treatment make screening pregnant women for bacteriuria essential.

In most developing countries including Nigeria, screening for asymptomatic bacteriuria (ASB) in pregnancy is not considered as an essential part of antenatal care. Knowledge on the epidemiology and prevalence of asymptomatic bacteriuria in pregnant women is scarce in Northern Nigeria. Routine urine culture for detecting ASB is not carried out for antenatal women probably due to cost and delay in obtaining culture result (Akinloye *et al.*, 2006; Ojide *et al.*, 2014). Strip urinalysis is often preferred by most clinicians to culture in accessing urine in pregnant women due to its simplicity. Despite that it can detect presence of protein, nitrite, and leucocytes esterase enzyme which may suggest bacteriuria, its sensitivity is low (Samad, 2006; Taiwo *et al.*, 2009).

The urinalysis cannot demonstrate the aetiologic agents and the antibiotic sensitivity pattern, for proper management of bacteriuria in antenatal woman, thus increasing her risk of developing complications. As a significant UTI pathogen in sexually active women, *S. saprophyticus* have not been specifically studied in this area to ascertain its role in asymptomatic bacteriuria. This study therefore aimed to isolate *S. saprophyticus* from clinical/antenatal urine samples, determine the antimicrobial susceptibility pattern of the isolates and the prevalence of asymptomatic bacteriuria in pregnant women.

MATERIALS AND METHODS

Subject

This was a descriptive cross-sectional study conducted at some health centres of Bauchi metropolis. The study population includes all pregnant women attending the antenatal clinic in the selected hospitals during the study period. Inclusion criteria were pregnancy, visit for antenatal care at Abubakar Tafawa Balewa University Teaching Hospital and Specialists hospital Bauchi, and participants consent. However, pregnant women who were on antibiotic treatment 1-2 weeks prior to data collection and those with clinical signs and symptoms of UTI were excluded for the study. Hospitals ethics committee's approval was obtained before commencing the study.

Sample/data collection and processing

A sample size of 91 pregnant women was included in the study using prevalence of 7.1 % (2 of 28) by Ojide *et al.*, 2014, as the prevalence of asymptomatic bacteriuria associated with *Staphylococcus saprophyticus* in Benin. It was estimated using the formula:

$$n = \frac{Z^2pq}{d^2}$$

Where:
n= Desired sample size,
p= Previous prevalence of the disease in a community 7.1 % (0.071) by Ojide *et al.*, 2014,
q= (1- p),
d= 0.05 (Degree of accuracy),
Z = 1.96 (The standard normal deviate, corresponds to the 95% confidence level).

$$\text{Therefore } n = \frac{(1.96)^2 \times 0.071\ (1-0.071)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.071 \times 0.929}{0.0025}$$

$$= \frac{0.2284}{0.0025}$$

$$= 91.32$$

Women demographic data including age, marital status, occupation, educational qualification, pregnancy gestational age (trimester), number of birth/children (parity), history of UTI frequency and antibiotics use was collected using administered questionnaires. Clean catch mid-stream urine samples were collected from all participants using pre-labeled wide-mouthed sterile capped universal container, after given them the instruction.

Direct microscopy and urinalysis: The supernatant of the centrifuged urine samples were first examined microscopically. Rapid detection of blood, urobilinogen, billirubin, protein, nitrite, ketones, ascorbic acid, glucose and pH value in urine was made using Combi test strip () for presumptive diagnosis.

Culture and identification methods: The urine samples (0.002ml each) were inoculated using calibrated wire loop on Cystine lactose electrolyte deficient (CLED) (Oxoid, UK). The resulting plates was incubated aerobically at 37°C overnight and then examined for typical morphological characteristics of *S. saprophyticus*. Samples that were not cultured within two hours were stored at 4°C.The colonies were counted for each plate with growth and number of colonies per ml of urine determined by dividing the number of colonies counted by the volume of urine delivered by the calibrated wire loop (0.002 ml).Colony counts of bacterial growth of 10^5/ml or more of pure isolates were considered significant for bacteriuria. Further colony identification was made by Gram stain and microscopy (for pus cells and bacteria). The isolates were finally identified by standard biochemical tests as described by Thompson and Miller (2003) and Chessbrough (2010).

Antimicrobial Susceptibility Testing

Modified Kirby-Bauer disk diffusion method was used and results interpreted according to NCCLS guidelines (2012), as described by Motayo *et al.* (2012). The identified pure isolates were inoculated with 0.5 McFarland standards unto Muller-Hinton agar plates. The following antibiotics were tested; Ampicillin (10µg), Amoxycillin (10µg), Ciprofloxacin (30µg), Amikacin (10µg), Vancomycin (30µg), Nalidixic acid (25µg), Gentamicin (10µg) Augmentin (30µg), Ofloxacin (30µg), Nitrofurantoin (30µg), Imipenem (10µg) and Cefuroxime (30µg) (Abtek biological U.K.) (Chessbrough, 2010; Motayo *et al.*, 2012).

RESULTS AND DISCUSSION

A total of ninety one (91) clinical urine samples were collected from ante-natal women attending some hospitals in Bauchi metropolis. Out of these samples 32.9% (30 of 91) were positive with *S. saprophyticus* for significant bacteriuria (CFU ≥ 10^5/mL). The highest prevalence of 43.8% (14 of 32) was found among the age group of 26-30 years, with the least (0.0%) in 41-50 years (Table 1). High age-specific prevalence of 51.7% within similar group 21-30 years was reported in previous studies in Benin by Ojide *et al.* (2014). But they reported relatively low frequency, 7.1% (1 of 28) of *S. saprophyticus* isolates associated with ASB. A small number of these isolates 1 of 16 (6.25%) was obtained in Kumasi, Ghana by Turpin *et al.* (2007). High prevalence of staphylococci (45.6%) was reported by Tadesse *et al.* (2014).

The slightly higher rate of 32.9% in our study indicated the significance of *S. saprophyticus* in asymptomatic bacteriuria in pregnancy. This is consistent with most previous studies (Taiwo *et al.*, 2009; Daniyan *et al.*, 2010; Ajayi *et al.*, 2012), but at variance with others that reported *Staphylococcus aureus* as the most prevalent causative agent of asymptomatic bacteriria (ASB) (Akerele *et al.*, 2001; Akinloye *et al.*, 2006). The findings of this study observed that mother's age can be a risk factor for ASB among pregnant women

ially the sexually active ones as orted by the findings of Akinloye *et al.*) and Imade *et al.* (2010), but opposed by studies of Samad, (2007) in Iran who a significant relationship between maternal age and ASB.

Most of the women involved in this attended primary, secondary, tertiary Qur'anic schools. Highest frequency %) was found among those with primary level of education, followed by the Qur'anic (36.1%). The lowest rate 12.5% among those with tertiary showed the importance of educational awareness in maternal health. According to Tadesse *et al.* (2014) poor genital hygienic practices by pregnant women increases the rate of colonization by *Staphylococcus species*, leading to asymptomatic bacteriuria.

e 1: Prevalence of Asymptomatic bacteriuria associated with *Staphylococcus ophyticus* according to patients' demographic and obstetric characteristics

racteristics	No. of Samples Tested (n=91)	No. of Positive Samples (n=30)	Prevalence (%)
e (years)			
0	11	02	18.2
5	18	05	27.8
0	32	14	43.8
5	19	07	36.8
0	07	02	28.6
5	03	00	0.0
0	01	00	0.0
c. Qual.			
ary	16	11	68.8
ndary	31	05	16.1
iary	08	01	12.5
'anic	36	13	36.1
upation			
lent	14	03	21.4
	11	03	27.3
V	07	02	28.6
N	59	22	37.3
nester			
	13	02	15.4
	46	18	39.1
	32	10	31.3
ty			
	12	05	41.7
	45	30	66.7
	34	21	61.8

= Civil Servant, B/W= Business Woman, H/W= House Wife

In our area married women with oor educational background and socio-conomic status were occupationally egarded as just house wives. This study bserved 64.8% (59 of 91) housewives with ighest rate (37.3%) of asymptomatic acteriuria associated with *S. saprophyticus*. licolle (1994) stated that the prevalence of SB will be higher among individuals with wer socioeconomic status. This study und that decreased level of education of e subject increase the rate of ASB.This is agreement with similar study in Pakistan Haider *et al.* (2010) and in Nigeria by Oli *al.* (2010) where they observed level of lucation as a risk factor for ASB and the ast educated women had higher equency. Awusi *et al.* (2009) and Dairo *et* . (2010) confirmed that higher educational ualification of both parent is one of the ctors that have a positive influence on the ilization of antenatal service in Nigeria.

It was found that majority of the egnant women studied were in their 2nd mester 50.5% (46 of 91) and 3rd trimester .6% (36 of 91) with ASB prevalence of .1% and 31.3% respectively. This dicated that most of the pregnant women ort at the antenatal clinic for booking ring these periods in this area. Although colle (1994) recommended that the best ne of screening for asymptomatic cteriuria and urine culture is during early egnancy (12 to 16 weeks). This study also served that increase in number of children (parity) can raised the prevalence of ASB, where 66.7% and 61.8% was found among those women with 2 to 3 and ≥ 4 children. This could be due to changes in the urinary tract, such as ureteral dilatation and decrease in bladder tone resulting in increased urinary stasis, occurring during each pregnancy and often not perfectly returning to normal even after delivery (Heider *et al.*, 2006; Ojide *et al.*, 2014).

Antimicrobial susceptibility testing in this study indicated that most of the *S. saprophyticus* isolates were highly sensitive to the antibiotics tested, especially Amikacin (73.3%), Gentamicin (63.3%), Cefotaxime (56.7%), Imipenem (86.7%), Ciprofloxacin (83.3%), Nitrofurantoin (70.0%), and Ofloxacin (76.7) (Table 2). Multi-drug resistance (resistance to two or more drugs) was observed in some beta-lactam drugs (ampicillin and amoxycillin). This is consistent with previous study in Nigeria by Daniyan *et al.* (2009).Similar findings have been reported from a study in Tanzania by Masinde *et al.* (2009) and Ethiopia by Assefa *et al.* (2008). The sensitivity of these antibiotics is due to the fact that they are comparatively less frequently used in this area, unlike the other drugs like ampicillin, commonly and indiscriminately used. The multidrug resistance observed in this study, may be due to emergence of beta-lactamase-producing strains of staphylococcus.

ble 2: Antimicrobial susceptibility pattern of *Staphylococcus saprophyticus* isolates sociated with asymptomatic bacteriuria

ntimicrobial ents	No. of susceptible isolates (n=30)	% susceptibility
npicillin	02	6.7
noxycillin	04	13.3
gmentin	11	36.7
nikacin	22	73.3
fotaxime	17	56.7
profloxacin	25	83.3
ntamicin	19	63.3

Imipenem	**26**	86.7
Nitrofurantoin	**21**	70.0
Nalidixic acid	**14**	46.6
Ofloxacin	**23**	76.7
Vancomycin	**13**	43.3

CONCLUSION AND RECOMMENDATIONS

The findings of this study observed that bacteriuria associated with *S. saprophyticus* was relatively high (32.9%), compared to the low rates (7.1% and 6.25%) obtained in the previous studies. This was attributed to asymptomatic nature of the infection, low level of awareness, maternal age, parity and the mode of transmission. Drug susceptibility pattern of *S. saprophyticus* associated with asymptomatic bacteriuria shows multidrug resistance to some commonly recommended antibiotics during pregnancy, but appreciably sensitive to the newer generation antibiotics like Amikacin, cefotaxime, ciprofloxacin, imipenem, and nitrofurantoin. Therefore appropriate antimicrobial use during pregnancy is only possible after culture and sensitivity test. Physicians should emphasize on routine urinalysis and urine MCS for all pregnant women at each antenatal visit. There is need to improve the health services during pregnancy. High level of hygiene should be maintained in our homes and general standard infection control measures such as hand washing, and other practices should be strictly maintained.

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