A STUDY OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY IN ILE - IFE, SOUTHWESTERN NIGERIA

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Asymptomatic bacteriuria presents a considerable risk to the mother and may lead to onset of acute pyelonephritis in about 5% of pregnant women and also increase the risk of fetal mortality. Apart from one previous study, no other study has been carried out in this environment hence our study. The objectives are to determine the prevalence of asymptomatic bacteriuria amongst pregnant women in the three trimesters of pregnancy, to isolate and characterize the bacteria agents involved in this condition and recommend methods of reducing incidence and possible attendant sequelae. A descriptive study with purposive sampling carried out at the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife Southwestern Nigeria between May 2000 and April 2001 examined two hundred and one consecutive pregnant women attending the antenatal clinic. This included women in the three trimesters of pregnancy. Those with urinary tract infections were excluded. Each subject was given a sterile universal bottle and requested to collect midstream urine. Each sample was plated onto Cystein-Lactose-Electrolyte-Deficient (CLED) medium and chocolate agar (CA). The major bacterial colonies were isolated and characterized employing standard bacteriologic methods. The prevalence rate was 26%. Staphylococcus aureus was predominant (43.8%), of which 68.8% were beta-lactamase producers. Forty six point six percent of total isolates were Gram-negative rods; Klebsiella pneumoniae (6.8%), Escherichia coli (4.5%), Citrobacter freundii (4.5%) and others. The study recorded a relatively high prevalence of asymptomatic bacteriuria. While the bacterial isolates were multi-resistant to drugs traditionally employed to treat uropathogens, they were relatively sensitive to nitrofuratoin in vitro. Because of the high prevalence of asymptomatic bacteriuria, we recommend routine screening for this condition in all antenatal clinics in this environment to reduce the incidence and probable attendant sequalae.

INTRODUCTION

Pregnancy is a predisposing factor to urinary tract infections and pregnant women suffering from this condition are at risk of various complications of pregnancy including low birth weight and preterm birth. Asymptomatic bacteriuria refers to significant bacteriuria in a patient without symptoms (1) while significant bacteriuria is the number of bacteria in voided urine that exceeds the number usually associated with contamination from the anterior urethra i.e. $>10^5$ bacteria/ml of urine (2). In a review by Miller and Cox (3), asymptomatic bacteriuria, cystitis and pyelonephritis are frequently encountered complications of pregnancy. In their studies, Grio et al (4, 5) noted that non-treated asymptomatic bacteriuria present a considerable risk to the mother and may lead to the onset of

acute pyelonephritis in approximately 5% of pregnant women which may increase, to some extent, the risk of fetal mortality.

The incidence of asymptomatic bacteriuria varies with study population as well as the method of collection of samples. Asymptomatic bacteriuria was reported in 5.6% of 181 black pre-natal patients in Durban, South Africa (6) while a rate of 7% was reported among 326 pregnant women in Ethiopia (7). Other investigators (8) in Ibadan, South western Nigeria have reported 12% prevalence rate. Apart from a study done by Okonofua et al (9) in Ile-Ife, there is no other study hence our study. Besides, this condition may manifest in a subtle form for the entire duration of pregnancy and complications do arise. This study addresses the incidence of asymptomatic bacteriuria in the three trimesters of pregnancy in Ile-Ife

and its environs and characterized the bacteriological agents involved. This will assist clinicians in effective management of this condition to prevent the attendant possible sequalae.

MATERIALS AND METHOD

Location of study

The study was carried out at the Obafemi Awolowo University Teaching Hospital Complex, (OAUTHC) Ile-Ife, Southwestern Nigeria between May 2000 and April 2001. The hospital is a referral centre for over half a million people within 40 km radius of the city.

Subject selection

A purposive selection consisting of pregnant women attending the ante-natal clinic was taken. This included women in the three trimesters of pregnancy. Those with overt urinary tract infection were excluded. A total of 201 pregnant women were studied. Informed consent of the women was obtained at the ante-natal clinic. A questionnaire was admitted on each patient before collection of urine specimen. Information required on the questionnaire includes the age, level of education, parity, gestational age and symptoms relating to urinary tract infection.

Collection of specimens

Each of the women was given a sterile universal bottle and was requested to collect mid-stream urine and to submit the specimen with the questionnaire.

Processing of specimen and isolation

The culture media used for isolation were Cystein-Lactose Electrolyte-Deficient (Difco Co, USA) and chocolate agar plates. Each urine sample was inoculated and streaked with the aid of heat-flamed standard wireloop (delivering 0.001ml urine)

on to the agar plates. The plates were incubated aerobically at 37^0C overnight and then examined. Only plates with significant growth (i.e. at least 100 colonies) 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. Those resembling staphylococci were inoculated onto Mannitol Salt agar (MSA) and colonies that fermented mannitol were presumptively identified as *Staphylococcus aureus* and confirmed by the coagulase slide and tube agglutination tests with pooled human plasma. Coagluase negative staphylococci (CONS) were noted. Gram-negative rods were identified as lactose or non-lactose fermenters using Eosin Methylene Blue (EMB) and MacConkey agar. Further speciation of the isolates was based on their activities on conventional media such as Triple Sugar Iron agar (TSI), Koser's citrate medium, Sulphide Indole Motility agar (SIM) and Urea agar and according to methods described by Cowan and Steel (10).

Detection of beta-lactamase

Each isolate was tested for β–lactamase activity by the starch paper method. Starch paper was soaked for 10 minutes in a solution of benzyl penicillin containing 10^5 units/ml and then spread smoothly in a Petri dish. Using a fine bacteriological loop (2 mm diameter), each colony of bacteria was collected from the surface of the culture plate and transferred onto surface of test paper and spread over an area of 2-3 mm. The inoculum was placed at least 1.5cm apart. Plates were

incubated for 30 minutes after which the papers were flooded with iodine solution (Gram's iodine diluted 1 in 2). Beta-lactamase producing strains were detected by the discoloration of the blue-black colour of iodine surrounding each organism with the widening of the white-halo in the course of the ensuing 5 minutes while the surface of the inoculum remained whitish. Penicillinase-negative isolate did not produce any discoloration of the surrounding area.

Antibiotic sensitivity test

The disc diffusion method of Bauer et al (11) was employed in this study. Five colonies of each strain of the isolate were suspended in a sterile bijou bottle containing 5mls of peptone water (Lab M) and incubated overnight at 37ºC. The overnight broth cultures were diluted to 10^6 colony-forming units per ml. A sterile cotton-tipped applicator was introduced into standardized inoculum and used to inoculate dried plate of sensitivity test agar (STA) for each isolate. The antibiotic discs used were ampicillin (AMP) 10 μg, ciprofloxacin (CIP) 10μg, gentamicin (GEN) 10μg, erythromycin (ERY) 10μg, nalidixic acid (NAL) 30μg, nitrofuration (NIT) 200μg, ceftriaxone (CRO) 30μg, colistin (COL) 25μg, tetracycline (TCN) 10μg, penicillin G (PEN) 1 iu, cloxacillin (CLX) 5μg, chloramphenicol (CMN) 10μg, cefuroxime (CXM) 30μg, ofloxacin (OFX) 5μg, ceftazidime (CAZ) 30μg, and cotrimozaxole (COT) 25μg,. Control organisms, Staphylococcus aureus ATCC 25923 and Enterobacter aerogenes ATCC 10342, were used.

Statistical analysis

The prevalence of bacteriuria in relation to trimester among subjects was determined using SSPS 8.0 statistical package.

RESULT

Of 27 women whose gestational age was 1-13 weeks, 14 were bacteriuric while of 87 subjects within 14-26 weeks of pregnancy, 26 were bacteriuric. Of 82 subjects in the third trimester of pregnancy, 33 had significant bacteriuria. The differences in bacteriuria in relation to gestational age were not statistically significant (p = 0.158). Although the prevalence of bacteriuria varies with parity with the highest rates occurring among nullipara (47.5%), followed by multipara (42.5%) and lowest among primipara (21.1%), the differences were not statistically significant (P=0.064). The prevalence of bacteriuria in relation to trimester among subjects also shows no statistically significant differences (p = 0.158 t = 2.206, df = 2).

Bacterial isolates

The bacteria isolates recovered from urine of the subjects are shown in Table 1. Altogether, a total of 73 isolates were recovered. Gram-positive organism constitute 53.4% made up of Staphylococcus aureus (43.8%) followed by coagulase negative staphylococci (31.5%). Gram-negative rods accounted for 46.6% of the total isolates made up of Klebsiella pneumoniae (6.8%), Escherichia coli (5.5%), Citrobacter freundii (4.1%), coliforms and Proteus mirabilis (2.7% each). Pseudomonas aeruginosa accounted for only 1.4% of the total isolates. Table 2 shows the profile of the β-lactamase producing isolates.

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Table 1: Distribution of bacterial isolates

Microbe	No of isolate (%)
Staphylococcus aureus	32 (43.8)
Coagulate negative staphylococci	23 (31.5)
Klebsiella pneumoniae	5 (6.8)
Escherichia coli	4 (5.5)
Citrobacter freundii	3 (4.1)
Coliforms	2 (2.7)
Proteus mirabilis	2 (2.7)
Streptococcus faecalis	1 (1.4)
Pseudomonas aeruginosa	1 (1.4)
Total	**73 (99.9)**

Table2: Profile of beta-lactamase production amongst isolates

Microbe	No tested	β-lactamase positive (%)
Staphylococcus aureus	32	22(68.8)
Coagulate negative staphylococci	23	10(43.5)
Klebsiella pneumoniae	5	2(40.0)
Escherichia coli	4	2(50.0)
Citrobacter freundii	3	3(100)
Proteus mirabilis	2	0(0)
Coliforms	2	0(0)
Streptococcus faecalis	1	0(0)
Pseudomonas aeruginosa	1	0(0)

DISCUSSION

This study shows that of the 196 women examined, only 73 (37.2%) showed significant bacteriuria at the first collection. According to Kincaid-Smith and Bullen (12), only 70% of women who have positive culture at the first examination displayed this symptom in the second collection. This suggests that in reality the prevalence rate in this study can be adjudged as 26%. The prevalence of asymptomatic bacteriuria varies from one study to another. Little in 1996 (13) found an incidence of 5.3% in 5,000 women which was similar to that of Sleigh et al (14) who reported an incidence of 6.6% in a survey of 4,349 patients. In a study carried out by Olusanya et al (15) among 510 pregnant women and 304 non-pregnant women at Ogun State University Teaching Hospital, Sagamu South-West

Nigeria, 23.9% of the population examined showed significant bacteriuria. The value obtained in our study is a little higher than the 23.9% in their study, which is within the same geographic zone. Investigators in Trinidad (16) recorded a prevalence rate of 16.7% among Trinidadian women, which is similar to that reported by Reddy and Campbell (17) in a racially mixed community in Gisborne, New Zealand. However, Al-Sibai (18) reported 14.2% among Saudi-Arabian women, which was about the rate reported by Okubadejo et al (8) in Ibadan, South-Western Nigeria, underscoring the variation of prevalence of bacteriuria from one locality to another.

Our result also showed that the incidence of symptomatic bacteriuria in the three trimesters of pregnancy was not significantly different. In a study carried out

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by Nnatu et al (19), the incidence of bacteriuria was highest in first trimester of pregnancy in which 3.3% of women screened displayed this symptom compared with 4.1% and 2.8% in the second and third trimester respectively.

The aetiologic agents of asymptomatic bacteriuria also vary (20, 8, 15). In our study both Gram-positive and Gram-negative organisms were cultured from the urine. Out of the total 73 isolates encountered, 56 (76.7%) were Gram-positive cocci of which *Staphylococcus aureus* accounted for 32 (57.1%), coagulase negative staphylococci 23 (41.1%) and *Streptococcus faecalis* 1(1.8%). The predominance of *Staphylococcus aureus* in the urine sample examined in this study corroborates a study done in Sagamu Southwestern Nigeria by Olusanya et al (20), who reported *Staphylococcus aureus* as the predominant organism in their study. Most studies done in Nigeria have reported Gram-negative rods as the major organisms in bacteriuria in pregnant women (8, 21). Isolation of enteric organisms in the urine may be due to the proximity of the perineum to the vulva and urethra (the organism being normal flora in the bowels readily colonize the perineum and then the vulva). Nnatu et al (19) in Lagos recorded *Escherichia coli* in 45% of bacteriuric patients while Okubadejo et al (8) reported *Escherichia coli* in 41% followed by *Klebsiella pneumoniae* 19.4% and *Proteus mirabilis* 16.1%. The present study shows *Klebsiella pneumoniae* (23.5%), *Citrobacter freundii* (17.6%), *Proteus mirabilis* and coliforms (11.7% each) and *Pseudomonas* aeruginosa (5.7%).

The bacteria isolated from pregnant women in this study are remarkably similar to those reported by Olusanya et al (20). In their study, coagulase positive staphylococci were recorded in 27.9% of pregnant women compared with our study of 57.1% which doubled their value, followed by coagulase negative staphylococci 19.1% compared 41.1% in our study. The variation in their value and that of our study may be due to their relatively large sample size of 510, which is more than double the sample size of 196 in our study. The significance of *Staphylococcus aureus* as predominant organism in bacteriuric pregnant women in this study is not apparently clear. However, in a study of urinary tract infection in Ile-Ife carried out in the same hospital in 1993, *Staphylococcus aureus* was responsible for 15.4% of the total bacteria isolated from females. In addition, about 20-40% of individuals in the environment are known to be carriers of *Staphylococcus aureus* (22, 23) and might therefore acquired the organism by auto-infection. Similarly, like in Olusanya et al study (20), coagulase negative staphylococci were rated second among Gram-positive cocci encountered. The isolation of coagulase negative staphylococci from urine may be significant only when *Staphylococcus saprophyticus* is involved. Other species may be seen as contaminants.

Klebsiella pneumoniae and *Escherichia coli* were also encountered in this study. The isolation of Gram-negative organisms as in pyelonephritis may suggest ascending infection (8). Our study showed that more than half (39) of the bacteria isolated produced beta-lactamase. This enzyme hydrolyzes the beta-lactam drugs such as the penicillins and cephaloporins, though the cephaloporins are reported to be more stable. This finding may in part

explain the rate of resistance to beta-lactam drugs in this study. Sixty percent of the *Staphylococcus aureus* isolates were resistant to ampicillin, 87.5% to penicillin G and 89.3% resistant to cloxacillin respectively (Table not shown). Sensitivity to gentamicin was only moderate being 44.5% among *Staphylococcus aureus* isolates, 31.8% for coagulase negative staphylococci, 40% for *Klebsiella pneumoniae*, and 50% each for *Citrobacter freundii*, *Proteus mirabilis* and *Escherichia coli*. Susceptibility to augmentin was similar, 38.1% of *Staphylococcus aureus*, 58.3% of coagulase negative staphylococci, 25% of *Klebsiella pneumoniae* and 75% of *Escherichia coli* strains were sensitive to the drug. These data suggest that resistance to beta-lactam drugs such as penicillin may also be co-transferred with resistance to other antibiotics such as gentamicin, tetracycline and even chloramphenicol. Such findings have been reported among bacterial isolates from cases of acute otitis media in Ile-Ife, Southwestern Nigeria (26).

It is also noteworthy the high frequency of resistance to cotrimozaxole by all the isolates. All the *Staphylococcus aureus*, coagulase negative staphylococci, *Klebsiella pneumoniae* and *Escherichia coli* isolates tested against cotrimozaxole were resistant. Other workers (25, 26, 27) have reported similar findings. Ako-Nai *et al* (26) reported only 45%, 43%, 40% and 40% susceptibility for *Escherichia coli*, *Klebsiella spp*, coliforms and *Proteus spp* respectively. This finding suggests possible abuse of cotrimozaxole in this environment based on its over-the counter availability.

It is interesting to note that virtually all the organisms tested against nitrofuration were susceptible. The susceptibility value ranged from 50% amongst coagulase negative staphylococci, to 80% amongst *Klebsiella pneumoniae* and 100% among *Staphylococcus aureus*, *Citrobacter freundii* and *Pseudomonas aeruginosa* isolates. Dempsey *et al* (28) reporting the characteristics of bacteriuria in a homogenous maternity hospital population noted that the most effective antibiotic in their study was nitrofuration, with over 90% of isolates sensitive to it. The implications of this finding, is that nitrofuratoin, a traditional urinary antiseptic, is still effective in the treatment of urinary tract infection in this environment.

Finally, there are conflicting views as to the rationale of routine screening pregnant women for significant bacteriuria. Al-Sibai *et al* (18) advocated screening on a selective basis (i.e. for young teenage parous women, those coming from disadvantaged socio-economic conditions and those with a past history of urinary tract infection). Olusanya *et al* (20) on the other hand advocated routine screening for all pregnant women at least during the first visit to the ante-natal clinic. Our results which shows that about one in every four (26%) pregnant women in this environment have asymptomatic bacteriuria, is rather high. It may be a worthwhile exercise therefore, if routine screening for bacteriuria of pregnant women is part of antenatal clinic facilities in this environment. This is even more important since early detection of asymptomatic significant bacteriuria has been reported to prevent eclampsia in pregnancy and reduce the incidence of prematurity and pyelonephritis later in life among women with such problem (29).

Similarly, untreated pregnant women with significant bacteriuria have been reported to have higher complication in pregnancy than the ones treated (21).

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