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Asymptomatic bacteriuria in pregnancy in Port Harcourt

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ABSTRACT

Objective: To determine the prevalence of asymptomatic bacteriuria in pregnancy, to consider the antimicrobial sensitivity patterns of involved uropathogens, to elucidate the safety profiles of antibacterial agents, and to evaluate the role of urinalysis in screening for asymptomatic bacteriuria. **Methods:** About 760 apparently healthy pregnant subjects attending the Antenatal Clinic of the University of Port Harcourt Teaching Hospital were randomly selected for this study. Urinalysis and microscopy, culture, and sensitivity tests were carried out on clean-catch midstream urine samples obtained from subjects. Biochemical reagent strips were used for urinalysis while the standard wire loop and agar diffusion technique were respectively employed for culture and susceptibility testing. **Results:** A total of 111 samples yielded moderate or severe growth on culture after 48 hours comprising 35, 31, 27, and 18 isolates of *Staphylococcus spp.*, *Proteus spp.*, *Klebsiella spp.*, and *Escherichia spp*, respectivehy. Urinalysis results were positive for the presence of nitrate reductase and leucocyte esterase activity in 17 urine samples of these 111 samples. The isolates showed a general sensitivity to the fluorinated quinolones and to Nitrofurantoin. **Conclusions:** The prevalence of asymptomatic bacteriuria is 14.6%, with the predominant organism being *Staphylococcus spp*. Drugs used for treatment should have excellent fetal safety profiles, and a rapid screening test with a high negative predictive value for asymptomatic bacteriuria would be ideal.

1. Introduction

Urinary tract infections (UTI) represent the most common bacterial infection in pregnancy[1] and are classed as either asymptomatic or symptomatic[2]. The original criterion for diagnosing bacteriuria was $>10^5$ CFU/mL of a single uropathogen on two consecutive clean catch samples, with a 95% probability of significant bacteriuria[3]. The detection of $>10^5$ CFU/mL of a single uropathogen in a single voided midstream urine is accepted as a more practical and adequate alternative, although there is only an 80% probability of true bacteriuria.

Though asymptomatic bacteriuria (ASB) in non-pregnant women is generally benign, pregnant women with bacteriuria have an increased susceptibility to pyelonephritis[4], and the incidence of acute pyelonephritis in pregnant women with ASB is significantly increased.

Testing for the presence of micro-organisms in the urinary tract, in order to diagnose ASB or symptomatic UTI (UTI), is very common at all levels of health care[5]. Screening for and treatment of ASB in pregnancy has become a standard of obstetric care and most antenatal guidelines include routine screening for asymptomatic bacteriuria[6]. The current recommendation is to obtain a urine culture between 12-16 weeks of gestation and pregnant women in whom ASB is detected should be treated with antibiotics targeting the cultured organism, and they should undergo follow-up monitoring[7].

The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition, and natural history of bacteriuria during pregnancy[1]. The renal pelvis and ureters begin to dilate as early as the eighth week of pregnancy[8]. Additionally, the physiologic increase in plasma volume during pregnancy decreases urine concentration and increases urinary progestins and estrogens, which may lead to a decreased ability of the lower urinary tract to resist invading bacteria[1]. Differences in urine pH and osmolality and pregnancy-induced glycosuria and aminoaciduria may facilitate bacterial growth[9].

The prevalence of ASB in pregnancy from literature is 2-11% [10], when it can progress to symptomatic UTI, postpartum UTI or pyelonephritis. The prevalence of bacteriuria in pregnancy is associated with a history of recurrent urinary tract infections, diabetes, anatomical abnormalities of the urinary tract, and host factors: race, sickle cell disease, age and parity[11]. Untreated bacteriuria

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during pregnancy has been shown to be associated with low birth-weight and premature delivery[12], and treatment for ASB reduces the incidence of symptomatic urinary tract infections, low-birth weight children, and preterm delivery. The antibiotic chosen should have a good maternal and fetal safety profile, excellent efficacy and low resistance rates in a given population.

Many screening tests are available for the diagnosis of bacteriuria. An ideal test requires only limited technical expertise, is cheap and has a high accuracy, enabling a quick diagnosis in high-risk patients. Although urine cultures are expensive, require laboratory expertise and take 24-48 hours for results to become available, quantitative culture remains the gold standard for diagnosis of urinary tract infection in pregnancy because it has high sensitivity and negative predictive value in this population, and the performance of rapid urine screening tests in pregnancy is poor[13].

The biochemical reagent strip test (dipstick test) operates by detection of a leucocyte esterase (LE) and a nitrate reductase(NR) activity. These tests have poor negative predictive values to detect bacteriuria in asymptomatic persons[14]. Disparities in urine collection and analysis, and patient selection may influence the presence of micro-organisms which can be detected by the dipstick, as well as the presence of substances that may give false results[15]. The dipstick test for NR had its highest accuracy and lowest sensitivity in pregnant women[5]. Sensitivity of the urine dipstick test for leukocyte-esterase was slightly higher than for the dipstick test for NR, while the specificity was slightly lower[5], and combining the results of both parts of the dipstick tests should logically increase sensitivity. Though the presence of nitrite is highly specific for bacteria, several uropathogens do not reduce nitrate to nitrite, and therefore its utility is restricted to enterobacteriaceae which reduce nitrate to nitrite and give a positive test result[3].

This study focuses on the prevalence of ASB in pregnant women attending the antenatal clinic of the University of Port Harcourt Teaching Hospital, identification of the uropathogens involved and their antimicrobial sensitivity patterns, and to evaluate the diagnostic efficacy of urinalysis in screening for ASB among pregnant women.

2. Materials and methods

2.1. Study population

A descriptive cross sectional design was adopted and a stratified sampling method was used, with a working sample size of 800. Ethical approval for this study was obtained from the relevant authorities. The sample size was obtained using Kish formula[15]. Inclusion criteria were apparently healthy pregnant females attending the antenatal clinic at UPTH between January and June 2009. Exclusion criteria was pregnant women who presented with any recent history of antibiotic therapy or any two of the following genitourinary complaints: dysuria, urinary hesitancy, urgency, slow stream, incontinence, frequency, incomplete voiding, and flank, suprapubic, or hypogastric pain. However, the symptoms of frequency, urgency and nocturia are not specific for an infectious process and are commonly described by pregnant women in the absence of a urinary tract infection[16,17].

2.2. Collection and analysis of sample

Mid stream, clean catch urine samples were collected and immediately analysed. The patients were instructed on how to collect the samples into sterile universal bottles containing 1% boric acid to stem overt multiplication of bacterial cells. Combi-9 biochemical reagent strips (dipsticks) were used to screen for the presence of NR and LE activity.

2.3. Culture and microscopy

A semi-quantitative technique was employed (standard wire loop method). A standard bacteriological loopful of urine was spread over the surface of Cystine Lactose Electrolyte Deficient (CLED) agar plate. The loop used can transfer 0.002 mL of urine. After inoculation, the plates were left on the bench for 10 to 20 minutes to allow the urine to be absorbed into the agar medium. The plates were then inverted and incubated at 37 ℃ for 18-24 hours. Using morphological and cultural features, the number of bacterial colony forming units was counted on each CLED agar medium. Plates containing 200 colony forming units (CFU) or more were considered to be significant bacteriuria because 200 CFU in 1/500 mL of urine is proportional to 10^5 organisms per ml of urine. Pure isolates of resulting growth were identified using biochemical method as described by Holt *et al*[18].

2.4. Antibiotic susceptibility testing

The agar diffusion technique as described by Bauer *et al*[19] was used. Five colonies of the test organisms were streaked on agar plates using sterile inoculating wire loop. The appropriate multi-disc depending on whether the test organism plated was a gram negative or grampositive organism was then placed firmly onto the surface of the dried plates, using sterile forceps. The plates were left at room temperature for one hour to allow diffusion of the different antibiotics from the disc into the medium. The plates were then incubated at 37 ℃ for 18-24 hours. Interpretation of results was done using the zone sizes. Zones of inhibition greater than 10 mm were considered sensitive, 5-10 mm moderate sensitive and no zone of inhibition resistant.

2.5. Statistical analysis

The data obtained were analysed using the Statistical Package for Social Sciences, version 17 (SPSS-17).

3. Results

A total of 760 urine samples were collected and analysed for bacteriuria using urinalysis, then culture, microscopy, and sensitivity testing. Subjects were aged <18 (9), 18-22 (141), 23-27 (194), 28-32 (183), 33-37 (165), 38-42 (64) and >42 (4). The parity of subjects were nullipara (178), primipara (141), P2 (161), P3 (131), P4 (77), and grandmultipara (72).

A total of 111 samples yielded moderate or severe growth on culture after 48 hours, of which 62 samples were from nulliparous subjects. 649 samples yielded no growth or mixed growth of doubtful significance after culture for 48 hours. Urinalysis results were positive for the presence of

NR and LE activity in 17 urine samples of the 111 samples that yielded moderate or severe growth on culture after 48 hours. Urinalysis results were positive for the presence of NR and LE activity in 9 urine samples of the 649 samples that yielded no growth or mixed growth of doubtful significance after culture for 48 hours.

The isolates identified on microscopy of the 111 samples which yielded moderate or severe growth on culture were *Staphylococcus spp*. (35), *Proteus spp*. (31), *Klebsiella spp*. (27), and *Escherichia spp*. (18). The microorganisms identified on microscopy of the 17 samples positive for the presence of NR and LE activity on urinalysis were *Staphylococcus spp*. (4), *Proteus spp*. (7), *Klebsiella spp*. (6), and *Escherichia spp*. (1). The sensitivity patterns of the various isolates are presented in Table 1.

Table 1
Sensitivity patterns of isolates.

Drugs/Isolates	Flouroquinolones	β lactam antibiotics	Nitrofurantoin	Cotrimoxazole	Tetracycline
Staph spp (35)	31	18	30	6	7
Proteus spp (31)	12	12	13	5	8
Klebsiella spp (27)	12	3	18	4	0
Escherichia spp (18)	9	9	13	0	0

4. Discussion

The prevalence of ASB in pregnancy in this study is 14.6%. The prevalence of ASB varies between the studies even within the same country. For instance, ASB in Nigerian studies ranges from 4% to 21%, depending on the population studied in different Nigerian provinces[20]. In Ethiopia and Ghana, the incidence of ASB was 9.3% and 7.3% respectively[21]. The prevalence of ASB is 6.1 % and 4.8% among pregnant women in Iran and United Arab Emirates respectively[22], and 12% in rural areas in Bangladesh[23]. This variation can be attributed to several factors such as the geographical variation, ethnicity of the subjects, setting of the study (primary care, community based, or hospitals), and the variation in the screening tests (urine dipstick, microscopy, culture).

Escherichia coli is the most common pathogen associated with both symptomatic and asymptomatic bacteriuria[24], accounting for as much as 79%[21] of isolated uropathogens in reports. The predominance of *Escherichia coli* could be attributed to urinary stasis, which is common in pregnancy and since most (*Escherichia coli*) strains prefer that environment, to cause UTI. However, we found *Escherichia coli* to be the least common uropathogen isolated, compared with *Staphylococcus spp.*, *Proteus spp.*, and *Klebsiella spp*. The isolates showed a general sensitivity to the fluorinated quinolones and to nitrofurantoin; and poor antibacterial effects of the sequential anti folate, cotrimoxazole and the protein synthesis inhibitor, tetracycline.

The penicillins have been shown to be safe in all trimesters of pregnancy, and have not been associated with increase in the rate of malformations of major birth defects[25], though high resistance rates limit its use as a single agent. The oral second generation cephalosporins (which are inactive against *Enterococcus spp*.) have also been shown to be relatively safe and non toxic in pregnancy[25]. The β-lactams are sometimes associated with allergic or anaphylactic reactions and the pharmacokinetic changes of pregnancy decrease plasma concentrations of β-lactams by up to 50%.

Fluoroquinolones are uncommonly prescribed for the treatment of UTI due to concerns regarding the safety of this class of drugs which originated from reports of arthropathy in animal studies; such reports are rare in human cases. The safety of these drugs in pregnancy has been explored [26]. Based on existing data, fluoroquinolone exposure during human gestation is not associated with increased risk of major malformations, adverse effects in the fetal musculoskeletal system, spontaneous abortions, prematurity, intrauterine growth retardation, or postnatal disorders. However, because of the concern about the emergence of antibiotic-resistant pathogens with frequent use, fluoroquinolones should not routinely be employed as first-line agents in uncomplicated UTIs.

Nitrofurantoin can theoretically induce hemolytic anemia in the fetus or newborn, particularly in those with glucose-6-phosphate dehydrogenase deficiency; however, cases of this toxicity are rare[27]. Case-control, case series and meta-analysis studies shows that Nitrofurantoin is safe in all trimesters of pregnancy[28]. There is a low level of resistance to Nitrofurantoin among uropathogens (only a rate of 1%). The drawback that Nitrofurantoin only achieves therapeutic levels in the urine (so it cannot be used to treat pyelonephritis) makes its use in bacteriuria perfect. Nitrofurantoin is poorly active against *Proteus spp*. It may cause severe nausea and reduce compliance.

Sulfamethoxazole can persist in neonatal circulation for several days after delivery if taken near term and there is a theoretical risk of sulfonamides increasing unbound bilirubin owing to competitive protein binding[29]. This displacement of bilirubin from albumin-binding sites and could cause severe jaundice leading to kernicterus Trimethoprim is a folic acid antagonist and its use during the first trimester has been associated with structural defects, such as neural tube and cardiovascular defects[30]. Trimethoprim-sulfamethoxazole (cotrimoxazole) should be avoided in pregnancy.

As a result of chelation with Ca^{2+}, tetracyclines bind to, and damage growing bones and teeth. Tetracyclines are deposited in newly formed teeth or bone in young children. If administered after 5 months gestation, they can be deposited in foetal teeth leading to fluorescence (discoloration of deciduous teeth), discoloration, and enamel dysplasia. They can impair liver function especially during pregnancy.

Our results revealed that urinalysis could only identify bacteriuria in 17 of the 111 samples identified by culture. Neither the NR nor LE test showed appreciable sensitivity with poor negative predictive value (NPV). Theoretically the combined NR and LE tests should have better sensitivity and NPV values, but however still gave poor values. Urinalysis results were positive for the presence of NR and LE activity in 9 urine samples of the 649 samples that yielded no growth

or mixed growth of doubtful significance after culture for 48 hours. Thus, the positive predictive value (PPV) and specificity of the screening test isn't accurate. This isn't in keeping with reports that the combined NR and LE dipstick test may provide an acceptable alternative to urine culture [31]. The NR test is an indirect measure of nitrate reducing bacteria, which includes all enterobacteriaceae, most non-fermenters and gram-negative cocci, provided the urine contains sufficient dietary nitrates and has been retained in the bladder for longer than 4 hours. The poor sensitivity of the NR test (false negatives) may be due to infections caused by gram-positive cocci like staphylococci.

Although urine culture remains the gold standard for screening for and diagnosis of ASB in pregnant women, it is time, cost, and labour-intensive and patients may have difficulty providing uncontaminated samples for testing. An ideal screening test should be simple, rapid and accurate and must identify all positive cases, thus a sensitive test with a high NPV and specificity is desirable. Thus screening methods will find increased usefulness as full bacteriological analysis could be reserved for those patients who are symptomatic or have a positive screening test results.

The prevalence of ASB in pregnant women attending the University of Port Harcourt Teaching Hospital antenatal clinic is 14.6%, with the predominant organism being *Staphylococcus spp*. Close attention should be given to the safety profiles of drugs used in treatment of ASB, and though urine culture remains the gold standard for screening for and diagnosis of ASB in pregnant women, it would be ideal to identify a rapid test with a high negative predictive value for ASB that could replace urine culture as a screening test.

Conflict of interest statement

We declare that we have no conflict of interest.

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