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Full Length Research Paper

Prevalence and antimicrobial suseptibility of asymptomatic significant bacteriuria among new antenatal enrollees in Southwest Nigeria

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ABSTRACT

Asymptomatic Bacteriuria (ASB) is a common extraintestinal infection with a high prevalence in pregnancy. It portends adverse maternal and fetal outcome if unrecognised and untreated. The aim of this study was to determine the prevalence of ASB, profile and antibiotic susceptibility of isolated uropathogens. This prospective study was carried out between 1st of March 2012 and 31st of January, 2013 among newly enrolled apparently healthy pregnant women who met the inclusion criteria. Clean catch mid-stream urine samples were collected and processed using standard microbiological techniques. We found a prevalence rate of 10.5% significant bacteriuria in pregnancy. Majority (72.4%) were between 25-34 years (30.25±4.58), nulliparous (40.4%) and well educated (83%) women. 51.1% were between 26-30 weeks gestation (25.81±6.01). *Eschericia coli* (44.7%) followed by *Staphylococcus aureus*(25.5%) were the commonest bacteria. All uropathogens isolated were resistant to the commonly prescribed antibiotics; cefuroxime (0%) and ceftriazone (0.6%) but susceptible to cefotaxime (87%) and ceftaxidime (81%). Quinolones (Ofloxacine-100% and Ciprofloxacine-89%) were the most susceptible antibiotics. We found that there has been no significant change in the prevalence of ASB in the recent years. Guideline to prevent indiscriminate use of antibiotics is needed to prevent the resistance observed in this study.

Keywords: Prevalence, Asymptomatic Bacteriuria, Pregnancy.

INTRODUCTION

Urinary tract infection (UTI) is ranked among the most prevalent extra intestinal infection in the society. The disease burden of UTI is estimated to be 150 million cases annually worldwide, with different types of UTI posing serious health problem that affect millions of people (Stapleton, 2005). The financial burden is also understandably enormous with an estimated annual cost of community-acquired UTI of approximately $1.6 billion in United States of America (Barros et al., 2009; Foxman, 2002). Urinary Tract Infections is more common in women than men and in pregnant women more than non-pregnant women (Stapleton, 2005). The severity of UTI depends both on the virulence of the bacteria and the susceptibility of the host. Disease spectrum in UTI includes cystitis, pyelonephritis, urosepsis, catheter-related infection, as well as asymptomatic bacteriuria (ASB) (Nesta, 2012).

Asymptomatic bacteriuria (ASB) is a common clinical entity in pregnancy. Approximately 2-11% of community acquired asymptomatic bacteriuria occurs in pregnancy (Nicolle et al., 2005; Tolosa, 2008 and Şevki et al., 2011). Asymptomatic bacteriuria (ASB) is defined as the presence of at least 10^5 colony forming units per ml of urine, of a single uropathogen, and in a midstream clean catch specimen without urinary symptoms and signs such as dysuria, frequency, fever, loin pains, renal angle tenderness, suprapubic pain and tenderness (Smail et al., 2007; Şevki et al., 2011). There are intrinsic factors

that encourage bacteriuria in pregnancy. The reduced ability of the kidneys to adequately concentrate urine, leading to differences in the pH and osmolality of urine in pregnancy; stasis of urine due to the smooth muscle relaxation, effect of increased progesterone and pressure effect of gravid uterus on the bladder and ureters impeding the free flow of urine. Others include, pregnancy induced glycosuria and aminoaciduria resulting from reduced renal threshold and suppressed functions of the T and B lymphocytes (Şevki et al., 2011). Some closely related but not intrinsic factors include low socioeconomic status, history of recurrent urinary tract infections, diabetes and anatomical abnormalities of the urinary tract [Nicolle et al.; 2005; Tolosa, 2008; Schnarr et al 2008).

In healthy non-pregnant women, asymptomatic bacteriuria may require no special attention. However in pregnancy, ASB portends adverse outcomes if not treated. Studies have shown that between 20-40% of asymptomatic bacteriuria progress to acute pyelonephritis if untreated whereas with treatment this risk reduces to 1-2% (Schnarr et al., 2008). Other maternal complications include chronic pyelonephritis, anaemia and septicaemia. The fetal complications of untreated asymptomatic bacteriuria include intrauterine growth restrictions, premature labour and preterm delivery and intrauterine fetal demise (Hill, 2005; Addo 2002).

Screening for and treatment of asymptomatic bacteriuria in pregnancy has become a standard of obstetric care in many countries. For instance, the American College of Obstetrician and Gynaecologists (ACOG, 2006), US Preventive Services Task Force (U.S.PSTF, 2008), International Disease Society of America (IDSA) (Nicolle et al., 2005), National Institute for Health and Clinical Excellence (NICE, 2008) and American Academy of Family Physicians (AAFP, 2012) strongly recommends screening for asymptomatic bacteriuria in all pregnant women at 12 to 16 weeks' gestation with urine culture or at the first prenatal visit if after that time. This is a grade A recommendation to prevent adverse pregnancy outcome.

Escherichia coli remain the single most common organism isolated from pregnant women with asymptomatic bacteriuria. *Escherichia coli* strains isolated from women with asymptomatic bacteriuria are characterized by fewer virulence characteristics than are those isolated from women with symptomatic infections (Stapleton, 2005). Other Enterobacteriaceae (such as *Klebsiella sp* and *Proteus s*p) and other organisms (including coagulase negative *Staphylococcus spp*, *Enterococcus species*, *group B Streptococci*, and *Gardnerella vaginalis*) are common as well (Schnarr, 2008). There are very few studies on the prevalence of asymptomatic bacteriuria in our environment. Therefore, no screening programmes to prevent the adverse outcome of this clinical condition. This study will fill this gap and provide a template for further research on this subject.

MATERIALS AND METHODS

Study design

A prospective study design was adopted

Study Period

The study was conducted between 1st of March 2012 and 31st January 2013

Study population

Pregnant women attending their first antenatal clinic

Study location

This study was conducted at the booking clinic of the Ekiti State University Teaching Hospital (EKSUTH), Ado- Ekiti, Southwest Nigeria. Ekiti state covers 6,353 km2 with a population of about 2.73 million people (National Population Commission, 2006). The Teaching Hospital is the only tertiary health facility in Ado-Ekiti, the capital town of Ekiti State and has a population of about 460,000 people. The Maternity Complex of the Teaching Hospital receives referred antenatal patients from Ekiti, Ondo and Osun States in South west Nigeria and also from Kogi State in North central Nigeria. The Hospital has an annual booking rate of 2600 per annum and delivery rate of about 2400 per annum.

Study Population

Four hundred and forty six pregnant women attending their first antenatal clinic during the duration of the study who were willing to participate were recruited for the study. The following categories of women were excluded from the study; pregnant women with symptoms of urinary tract infection, HIV positive, Sickle Cell Disease, other medical conditions in pregnancy such as Diabetes mellitus, hypertension, malaria in pregnancy, use of antibiotics within the last 6 weeks and refusal of consent to participate in the study.

Sample collection

Demographic and clinical information relevant to the study such as age, parity, gestational age, education status and occupation were obtained and recorded on prepared data collection forms. Mid-stream (clean catch) urine samples were collected in sterile disposable universal bottles as described by (Skobe, 2004; Cheesbrough, 2006). The samples were labelled with

the identification number for each client which is also similar with the one on the data collection form, stored in ice packs, transported to the laboratory, and were analysed within 30 minutes to 1hour of collection.

Isolation and Identification of isolates

Samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED) (Oxoid, Basingstoke, England) using a calibrated wires loop capable of delivering 0.002ml of urine. Plates were incubated aerobically of 37◦ C for 24 hours. Culture plates without visible growth were further incubated for additional 24 hours before being discarded. Colony counts yielding bacterial growth of 10^5cfu/ml or more of pure isolates on (CLED) medium were regarded as significant bacteriuria.

Identification of bacterial isolates was based on the combination of cultural, morphological and biochemical characteristics as described by (Cheesbrough, 2006).

Antimicrobial Susceptibility Testing

The antimicrobial in-vitro susceptibility testing was done using agar disc diffusion method. The Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards M2-A9 and M7-A7 were used (CLSI, 2007). The following commercially prepared antibiotics were used: Cefotaxime (30µg), Ceftazidime (30µg), Astreonam (30µg), Amoxicillin-clavulanic acid (20/10µg), Nitrofurantoin (300µg), Ampicillin (30µg), Cefuroxime Sodium (30µg), Ceftriaxone (30µg), Gentamicin (10µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg%), Ciprofloxacin (5µg), Ofloxacin (5µg).

Statistical analysis

This was performed using Statistical Package for Social Sciences (SPSS) version 20. Frequency tables were made and results tested using the student t-test for continuous variable and chi-square for categorical variable with the level of significance (α) set at 0.05.

Ethical consideration

Ethical clearance was obtained for this study from the ethics and research committee of the Ekiti State University Teaching Hospital, Ado-Ekiti. Informed consents were obtained from all the clients and those who refused were excluded without any penalty.

RESULTS

Forty-Seven (47) out of the 446 pregnant women whose urine were tested yielded significant bacteriuria giving a prevalence rate of 10.5% asymptomatic bacteriuria. Three hundred and Twenty two (72.2%) showed no or insignificant growth while 77 (17.3%) yielded mixed growth (Table 2).

The summary of prevalence of asymptomatic bacteriuria relating to age group, gestational age, parity, religion, educational status and occupation is presented on Table 1. Out of the 47 asymptomatic bacteriuria, 34 (72.4%) were between the age range of 25-34 years. The majority of the patients (19 out of 47 representing 40.4%) with asymptomatic bacteriuria were nullipara while 13 (27.7%) and 12 (25.5%) were primipara and secundipara respectively. Women of low parity (para 0, 1 and 2) account for 93.6% with asymptomatic bacteriuria. Precisely 80.9% of asymptomatic bacteriuria occurred between gestational ages 21-30 weeks out of which 51.1% were between 26-30 weeks. The mean gestational age was 25.81±6.01. Majority (97.9%) of significant growth were Christians while only 2.1% were Muslims. Most of the asymptomatic bacteriuria were found among the well educated population as 83.0% of the significant culture has tertiary education and almost half 48.9% were civil servants.

Escherichia coli was the most frequent bacteria (44.7%), followed by *Staphylococcus aureus* (25.5%). Other isolates found in this study included *Klebsiella aerogenes* (12.8*%), Proteus mirabilis* (8.5%) *Enterococcus faecalis* (6.4%) and *Enterobacter sp* (2.1%) (Table 3).

The antimicrobial susceptibility pattern of the bacteria isolated in this study revealed that Ofloxacin was the most effective antibiotic followed by Ceftriazone, Ceftaxidime, Cotrimoxazole and ciprofloxacin. The least effective antimicrobial agents were Cefuroxime Sodium, Amoxycillin, Amoxicillin-clavulanic acid and Nitrofurantoin (Table 4).

DISCUSSION

The prevalence of asymptomatic significant bacterial found in this study among 446 pregnant women was 10.5%. (Nicolle et al., 2005) reported that the prevalence of asymptomatic bacteriuria varies from 4-7 %. (Oyagade et al., 2004) reported a slightly higher prevalence of 12.2% from this same location. This is also similar to 12% reported in Ibadan (Okubanjo et al., 1969) and 14.1% in Ile-Ife (Okonofua et al., 1989) from Southwest Nigeria. A lower prevalence of 7.0% (Gabre-Selassie, 1998) and 7.3% (Turpin et al., 2007) were reported from Ethiopia and Ghana respectively. The results of this study further affirm that certain biosocial variables affect the frequency of UTIs in pregnancy. This study showed that 72.4% of significant bacteriuria occurred among 25 – 34 year age group with a mean age of 25.81±6.01. It is believed that subjects within this age group are more sexually active and are therefore more prone to UTI. This also reflects the peak reproductive period in this environment. The finding of an increased prevalence among women of low

Table 1. Socio-demographic characteristics of the population

PARAMETER	FREQUENCY N=446	PERCENT (%)	NUMBER WITH SIGNIFICANT GROWTH N=47	PERCENT (%)	MEAN
Age					
≤19	1	0.2	0	0	
20-24	37	8.3	3	6.4	
25-29	164	36.8	17	36.2	30.25±
30-34	169	37.9	17	36.2	4.58
34-39	61	13.7	10	21.3	
≥40	14	3.1	0	0	
Parity					
0	204	45.7	19	40.4	
1	138	30.9	13	27.7	
2	62	13.9	12	25.5	
3	37	8.3	3	6.4	
4	4	0.9	0	0	
5	1	0.2	0	0	
Gestational age					
≤20	69	15.5	2	4.3	
21-25	97	21.7	14	29.8	
26-30	187	41.9	24	51.1	
31-35	83	18.6	7	14.9	25.81±
35-40	10	2.2	0	0	6.01
≥41	0	0	0	0	
Religion					
Christianity	417	93.5	46	97.9	
Islam	29	6.5	1	2.1	
Educational status					
None					
Primary	3	0.7	0	0	
Secondary	3	0.7	0	0	
Tertiary	67	15.0	8	17.0	
	373	83.6	39	83.0	

Table 2. Growth pattern of urine culture among pregnant women in EKSUTH

GROWTH	NUMBER	PERCENT (%)
Significant growth	47	10.5%
Insignificant growth/No growth	322	72.2%
Mixed growth	77	17.3
Total	446	100

parity (para 0, 1, 2) accounting for majority (93.6%) of cases contradicted some earlier researchers, who found increased prevalence with advanced maternal age and parity (Oyagade et al., 2004; Oli et al., 2010). The highest prevalence was found in the second trimester (80.9% between gestational ages 21-30 weeks). Although most guidelines recommend screening at 16 weeks or on booking, this finding showed that majority of our patients book after 20 weeks of gestation. Literacy level was high among our patients with significant bacteriuria with 83.0% having tertiary education and almost half (48.9%) were civil servants. However, some studies had reported high prevalence among women with low socioeconomic and educational status. This discrepancy might be due to high level of literacy in Ekiti State, Southwest Nigeria.

The pattern of bacterial isolates found in this study was similar to what had been previously reported. The result of this study revealed that *Escherichia coli* (44.6%) was the

Table 3. The profile of bacteria in cases of asymptomatic significant bacteriuria in pregnancy

Organism	Number	Percent (%)
Escherichia coli	21	44.7
Staphylococcus aureus	12	25.5
Klebsiella species	6	12.8
Proteus species	4	8.5
Enterococcus faecalis	3	6.4
Enterobacter species	1	2.1
Total	47	100

Table 4. Antimicrobial susceptibility pattern of bacteria from asymptomatic bacteriuria in pregnancy

		% Susceptibility											
Uropathogens	No Tested	CFT	CFZ	AST	AUG	CFX	CRO	NIT	GEN	COT	OFL	AMX	CPX
Escherichia Coli	21	19 (91)	19 (91)	16 (76)	1 (5)	0	0	0	11 (52)	15 (71)	21 (100)	0	18 (86)
Staph aureus	12	9 (75)	10 (83)	0	0	0	0	0	0	10 (83)	12 (100)	0	12 (100)
***Klebsiella* species**	6	5 (83)	5 (83)	5 (83)	1 (17)	0	0	0	4 (67)	4 (67)	6 (100)	0	5 (83)
***Proteus* species**	4	1 (25)	3 (75)	2 (50)	1 (25)	0	0	0	0	4 (100)	4 (100)	0	4 (100)
Enterococcus faecalis	3	3 (100)	3 (100)	3 (100)	1 (33)	0	2 (67)	0	2 (67)	0	3 (100)	0	3 (100)
Enterobacter aerogenes	1	1 (100)	1 (100)	1 (100)	0	0	1 (100)	0	0	0	0	0	0
Total (Sensitivity)	47 (100%)	38 (81)	41 (87)	27 (57)	4 (.08)	0 (0)	3 (.06)	0 (0)	17 (36)	33 (70)	47 (100)	0 (0)	42 (89)

CFT- Cefotaxime **CFZ**- Ceftazidime **AST**-Astreonam **AUG-** Amoxyclav **CFX-** Cefuroxime **CRO-**Ceftriaxone **NIT-** Nitrofurantoin **GEN-** Gentamicin **COT-** Cotrimoxazole **OFL-** Ofloxacin **AMX-** Amoxicillin **CPX-** Ciprofloxacin.

most predominantly isolated uropathogen followed by *Staphyloccocus aureus* (25.5%). Others include; *Klebsiella aerogenes* (12.8%), *Proteus mirabilis* (8.5%), *Enterococcus faecalis* (6.4%) and *Enterobacter* sp (2.1%). However, this result revealed a changing trend in the bacterial profile found in asymptomatic bacteriuria. It is important to note that although earlier studies had shown that *E. coli* was implicated in greater than 85% of cases of asymptomatic bacteriuria, more recent studies are showing a reduction in this prevalence and increased prevalence of other uropathgens such as coagulative negative Staphylococci. Infact, (Oyagade et al., 2004) reported that *Staphylococcus aureus* was the predominant bacterium (21.3%).

The in vitro antimicrobial susceptibility pattern in this study revealed that most of the common antibiotics used in most hospital in the treatment of UTI in pregnancy exhibited a high degree of resistance. Cefuroxime, a 2nd generation cephalosporin and most commonly used antibiotics in UTIs and other related infection was 100% resistant. Amoxycillin, Augmentin and Nitrofurantoin which are equally widely used were highly resistant. The use of these antibiotics should be discouraged as much as possible in the empirical treatment of UTI. The 3rd generation cephalosporins and the quinolones showed excellent in vitro activities against all the uropathogens isolated in this study. All the uropathogens isolated in this study showed close to 100% susceptibility to Ceftriazone, Ceftaxidime and Ciprofloxacin. However, the quinolones are not safe in pregnancy.

An important finding in this study is that Cotrimoxazole (Trimethoprim-sulfamethoxazole) showed excellent antimicrobial susceptibility to all the uropathogens found in this study. It had been observed in earlier studies that there is high resistant to this agent in Nigeria due to the widespread of its usage. However, the loss of confidence in its use by most clinicians in the last one decade could have contributed to this resurgence of activity shown in this -study. This is particularly informative in HIV positive patients where this agent is used empirically against opportunistic infections.

CONCLUSION

This study showed that there has not been a significant change in the prevalence of asymptomatic bacteriuria in the recent years. There seems to be a paradigm shift in biosocial risk factors and profile of uropathogens isolated from UTIs. This might have influenced the change in the antibiogram of UTIs in this environment. Meta-analysis of properly conducted studies from our environment should be carried out to determine antibiogram of uropathogens in order to ascertain both the empirical and curative antibiotic treatment of UTIs. Antibiotic use policy should also be formulated to prevent indiscriminate use of antibiotics to prevent the current high level of antibiotic resistance which is a monumental threat to the present and future antibiotics.

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