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Prevalence of Asymtomatic Bacteriuria among Pregnant Women Attending Antenatal in Port Harcourt Township, Nigeria and Antibiogram of Isolated Bacteria

Smart Enoch Amala[1], Easter Godwin Nwokah[1]

[1]Department of Medical Laboratory Science, Rivers State University of Science and Technology, Nkpolu Oroworukwo, Port Harcourt, Nigeria.
[+]**Corresponding Author**
Amala, Smart E.
Department of Medical Laboratory Science
Rivers State University of Science and Technology
Nkpolu Oroworukwo
Port Harcourt, Nigeria
E-mail: smart.amala@yahoo.com

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Abstract

The prevalence of asymptomatic bacteriuria among 330 pregnant women attending antenatal at two hospitals in Port Harcourt, Rivers State, Nigeria, was investigated by urine culture. One hundred (100) non-pregnant women were screened as control. The percentage prevalence of bacteriuria among the pregnant women was 13.6%, while the percentage prevalence among non-pregnant women was 11.1%. The prevalence among pregnant women attending antenatal hospital 1, CHC was 13.8%, whereas those attending hospital 2, BMSH was 13.5%. The prevalence of bacteriuria by trimesters were: 1st trimester 17.9%, 2nd trimester 12.5% and 3rd trimester 14.5%. The prevalence of bacteriuria among the pregnant women by age groups were, 16-20yrs 13.5%, 21-25yrs 14.4%, 26-30yrs 14.1%, 31-35yrs 12.0% and 36-40yrs 8.0%. The percentage occurrences of isolated bacteria from urines of pregnant women were: *Escherichia coli* 28.9%, *Staphylococci* 20.0%, *Streptococci* 15.6%, *Proteus spp.* 8.9%, *Klebsiella spp.* 8.9%, *Enterobacter* 6.7%, *Enterococcus* 8.9% and *Pseudomonas aeruginosa* 2.2%. The percentage occurrences of isolated bacteria from the urines non-pregnant women (control) were: *Escherichia coli* 27.2%, *Staphylococci spp.* 18.1%, *Streptococci* 9.1%, *Proteus spp.* 18.1%, *klebsiella* 18.1% *and Pseudomonas* 9.1%. From the results obtained, *Escherichia coli* were the most prevalent bacterium isolated from the urines of pregnant and non-pregnant women. Statistical analysis at $P < 0.05$ did not show age as a predisposing factor to bacteriuria in pregnancy. Asymptomatic bacteriuria is common among antenatal patients, therefore all pregnant women should screened during their first visit by urine culture to detect and treat overt UTI to avoid complications that may ensue in both mother and fetus. Antibiotics susceptibility testing against isolates showed that, both Gram negative bacilli and Gram positive cocci isolated were resistant to the first line antibiotics.

Keywords: Prevalence, Asymptomatic, Bacteriuria, Pregnancy

1. Introduction

Asymptomatic bacteriuria is common among women and increases in prevalence with sexual activity, short urethra, pregnancy and easy contamination of the urethra with faecal flora (Girishbabu *et al*., 2011).Asymptomatic bacteriuria is confirmed with positive culture of bacteria containing more than 10^5 cfu/ml of clean catch, mid stream urine (MSU) of a patient without symptoms of urinary tract infection (UTI) Jepsen *et al*.,(1982). Urine has significant bacteriuria when bacteria count from fresh, clean catch urine exceeds 10^5 cfu/ml of urine (Gammeguard *et al.,* 2004). Urinary tract infection is the second most common type of bacterial infection in man (Jepson *et al*., 2006). UTI may affect any part of urinary tract e.g. urethra (urethritis), bladder (cystitis), kidney (pylonephritis), etc. Urinary tract infections account for approximately 10% visit by women and 15% by pregnant women to the clinicians. The prevalence of urinary tract infection had been found to be 8% (Hultgreen, 2007). Kerure *et al*.,(2013) observed that the prevalence of bacteriuria in pregnancy may rise between 2 – 10%. Other studies in India had reported prevalence as high as 8% (Balamurugan *et al., (*2012). The prevalence of asymptomatic bacteriuria has previously been reported to be 2-13% (Masinde *et at.,* 2009). The reasons are, increased rate of urine formation during pregnancy as a result of increase load of secretory products, the rate of glomerular filtration which may increase up to 50% or more in pregnancy, progesterone and relaxation hormone secreted during pregnancy, these have direct effect on the relaxation of the ureter and renal pelvis with marked decrease in urethral peristalsis. Contamination of the female urethra is enhanced by sexual intercourse pressure which can introduce bacteria into the bladder consequently, coupled with honeymoon cystitis. The use of contraceptives, diaphragm and spermatocides alters the normal flora of introitus causing colonization by *Escherichia coli* and other bacteria thereby predisposing to bacteriuria (Stamm *et al*., 2007). *E. coli* is responsible for more than 80% of all community acquired UTI. Others bacteria were: *Proteus spp, Pseudomonas spp, Klesiella, Staphylococci,* etc. The relative high prevalence of asymptomatic bacteriuria during pregnancy and the consequences on women and their pregnancies justify screening of pregnant women for bacteriuria to avoid the squeals with treatment (Balamurugan *et al*., 2012, Patel *et al*., 2015). Urine culture remains the gold standard method for screening asymptomatic bacteriuria during pregnancy (Gayathree *et al*., 2010).

This study is designed to determine the prevalence of asymtomatic bacteriuria among pregnant women, trimester prevalence of bacteriuria, prevalence among age groups, the percentage occurrences of isolated bacteria and antibiogram of the isolated bacteria.

2. Materials and Methods

2.1 Study areas

The samples were obtained from pregnant women attending antenatal clinic in Braithwaite Memorial Specialist Hospital (BMSH) which is one of the referral hospitals in Port Harcourt Metropolis and Church Hill Clinic (CHC), subsequently referred to as BMSH and CHC respectively. Both are government hospitals located in Port Harcourt Township, the capital of Rivers State, Nigeria. The hospitals are chosen to enable us obtain the required sample size from volunteers.

2.2 Collection of urine samples

Mid-stream, clean-catch urine samples were obtained from 330 pregnant women attending antenatal clinics in both BMSH and CHC. The samples were collected, irrespective of trimester of pregnancy. Sterile wide mouth containers were given to the subjects on arrival at the hospital, who were instructed on how to collect their urine specimen without contamination. The urine specimens were taken to the laboratory for immediate analysis and where delay was

envisaged, they were preserved in refrigerator at 4^0C. One hundred (100) urine samples from non-pregnant women were also collected from volunteers as control as control.

2.3 Preparation of media

All media were prepared according to the manufacturer's instructions.

2.4 Cultivation of samples

Each urine sample was inoculated and streaked out on cystine lactose electrolyte deficient agar (CLED) and Colombia blood agar using a standard wire-loop calibrated to deliver 0.002 ml of urine. CLED plates were incubated aerobically, while Colombia blood agar plates were incubated in CO_2 atmosphere; both at 37^0C for 18-24 hours. Significant bacteriuria was expressed as 10^5cfu/ml of urine. The results of urinalysis and microscopy were used to correlate with culture results.

2.5 Identification of Isolates and Susceptibility Testing

Isolates were identified following standard microbiological protocols including Gram stain, carbohydrate metabolisms, indole test, motility test, oxidase test, urease test, catalase test, citrate test, DNAase test, litmus milk decolourization test, coagulase test (Criuckshank, *et al*., 2006; Cheesbrough, 2002; Cowan & Steel, 2008). Kirby-Bauer disc diffusion method on Muller Hinton agar plate was used as recommended by (NLSI/ NCCLS, 2012). The antibiotics tested against isolated bacteria were: ofloxacin, kanamycin, carbanicillin, ciproxin, nalidixic acid, ampicillin, gentamacin, chloramphenicol. penicilliin, streptomycin, obenin, erythromycin, tetracycline.

2.6 Exclusion criteria

Women excluded were: (a) The women with history of antibiotic therapy for past two weeks, (b) Women with underlying diabetes mellitus (c) Pyrexia.

2.7 Statistical analysis

Statistical analysis was done using statistical package for social science (SPSS) version 20.

3. Results

The overall percentage prevalence of bacteriuria among pregnant women attending antenatal in BMSH and CHC was 13.6%, while the prevalence rate of bacteriuria among pregnant women attending BMSH are 13.8%, and 13.5% for CHC respectively. The prevalence of bacteriuria among non-pregnant women used as control was 11.1%. The prevalence rate of bacteriuria among pregnant women was higher by 2.5% (as shown in table 1).

Table1. Percentage Prevalence of bacteriuria among pregnant and non-pregnant women

Hospital	Number sampled	Number positive
CHC	130	18(13.9%)
BMSH	200	27(13.5%)
Total pregnant women	330	45(13.6%)
Non pregnant women	100	11(11.1%)

Number in parenthesis = percentages

The percentage of subjects by trimester were 1st trimester 28(8.84%), 2nd 192(58.18%) and 3rd 110(33.33%) respectively. The overall prevalence of bacteriuria by trimester were 1st trimester 5(17.9%), 2nd trimester 24(12.5%) and 3rd trimester 16(14.5%) respectively.

The prevalence by trimesters from each hospital was CHC 1st trimester 16, 3(18.8%), 2nd trimester 74, 9(12.2%) and 3rd trimester 40, 16(14.5%) respectively.

In BMSH, 1st trimester 12, 2(16.6%); 2nd trimester, 118, 15(12.7%); and 3rd trimester 70, 10(14.3%) respectively (as shown in table 2).

The pregnant women used for this study were between ages 16 and 40 years. They were grouped into 5 groups at 5 years (yrs) interval. The prevalence of bacteriuria among the pregnant women by age group were:

Ages 16 - 20 yrs 62, 9(14.5%), ages 21 - 26 yrs 139, 20(14.4%) ages 26 - 30 yrs 85, 12(14.1%), 25 subjects from the ages 31- 35 yrs 25, 3(12.0%) and ages 36 - 40 yrs 19, 1(8.0%) respectively. The prevalence of bacteriuria among pregnant women was high from ages 16 - 35 yrs. (Table 3).

Table 2: **Prevalence of bacteriuria by trimester among pregnant women**

Trimester	CHC		BMSH		CHC/BMSH	
	Number sample	Number positive	Number sampled	Number Positive	Number Sampled	Number Positive
First	16	3(18.8)	12	2(16.7)	28	5(17.9)
Second	74	9(12.2)	118	15(12.7)	192	24(12.5)
Third	40	6(15.0)	70	10(14.3)	110	16(14.6)
Total	130	18(13.9)	200	27(13.5)	330	45(13.6)

Numbers in parenthesis =percentages

Table 3: **Age prevalence of bacteriuria among pregnant and non- pregnant women(control)**

Age group (years)	Pregnant women	Number positive	Non pregnant women	Number positive
16 – 20	62	9(14.5)	16	2(12.5)
21 – 25	139	20(14.4)	28	4(14.2)
26 – 30	85	12(14.1)	24	3(12.5)
31 – 35	19	3(12.0)	17	2(11.8)
36 – 40	19	1(8.0)	9	0(0.0)
Total	330	45(13.6)	100	11(11.0)

Numbers in parenthesis =percentages

Table 4: **Percentage occurrences of isolated bacteria from the urines of pregnant and non pregnant women(control)**

Isolated bacteria	BMSH % occurrences	CHC % occurrences	BMSH/CHC % occurrences	Nonpregnant women % occurrences
E.coli	8(29.6)	5(27.8)	13(28.9)	3(27.3)
Staphylococci spp.	5(18.5)	4(22.2)	9(20.0)	2(18.2)
Streptococci spp.	4(14.8)	3(16.7)	7(15.6)	1(9.1)
Proteus spp.	3(11.1)	1(5.6)	4(8.9)	2(18.2)
Klebsiella spp.	2(7.4)	2(11.1)	4(8.9)	2(18.2)
Enterobacter spp.	2(7.4)	1(5.6)	3(6.7)	0(0.00)
Enterococcus faecalis	2(.4)	2(11.1)	4(8.9)	0(0.00)
Pseudomonas spp.	1(3.70)	0(0.00)	1(2.2)	1(9.1)
Total	27	18	45	11

Numbers in parenthesis =percentages

The overall percentage occurrences of isolated bacteria from the urines of pregnant women were: *Escherichia coli* 13(28.9%), *Staphylococci spp.* 9(20.0%), *Streptococci* 7(15.6%), E*nterococcus* 4(8.89%), *Proteus spp.* 4(8.9%), *Enterobacter spp.* 3(6.7%), *klebsiella spp.* 4(8.9%) and *Pseudomonas aeruginosa* 1(2.2%) respectively.

The percentage occurrences of isolated bacteria from each hospital are: BMSH, *E. coli* 5(29.6%), *Staphylococci spp.* 4(18.5%) *Streptococci spp.* 3(14.8%), *Enterococcus faecalis* 2(7.2%) and *Pseudomonas* 0(0.00%) *Enterobacter* 2(7.4%), *Proteus* spp. 3(11.1%), K*lebsiella spp.* 2(7.4%) respectively; whereas CHC, *E. coli* 5(27.8%), *Staphylococci spp.* 4(22.2%), *Streptococci spp.* 3(16.7%), *Proteus spp.* 1(5.6%), *Klebsiella spp.* 2(11.1%), *Enterococcus faecalis* 2(11.1%) and *Pseudomonas* 1(0.00%) respectively.

From the results, *E. coli* was the most predominant bacteria isolated from the urines of pregnant and non-pregnant women (Table 4).

The results of antibiotic susceptibility testing of isolated bacteria were: Ofloxacin 100%, Ciprofloxacin 100%, Gentamacin 81.2%, Nalidixic acid 75.0%, Kanamycin 56.3%, Chloramphenicol 37.5%, Carbenicillin 0.00% and

Ampicillin 0.00%; for the Gram positive bacteria, whereas, for the Gram positive cocci, Gentamacin 81.87%, Eryrhromycin 72.7%, Chloraphenicol 36.4%, Streptomycin 36.4%, Tetracycline 36.4%, Obenin 0.00% and Penicillin 0.00% respectively.

4. Discussion

From the results, 8.84% pregnant women attended antenatal in the 1st, trimester, this delay may have aided the establishment of some overt bacteriuria in the 2nd and 3rd trimesters. If they had reported early, most asymptomatic cases may have been detected and treated in 1st trimester and the prevalence observed in 2nd and 3rd trimesters would have dropped. Various workers examining the prevalence of bacteriuria among pregnant women had reported varing percentage prevalence rates of bacteriuria. It had been observed that the prevalence of bacteriuria among non-pregnant women was from 8-10% and the percentage prevalence rate among pregnant women could be as high as 15% (Kass, 1998) In this study, the percentage prevalence of bacteriuria among pregnant women was 13.6%. Asymptomatic bacteriuria quoted in literatures varies from 2-10% (Enayat *et al*., 2008, Kerure *et al*., 2013). Masinde *et al*., (2009) observed prevalence of 2-13% in Tanzania. In a study conducted in West Bengal, a prevalence of 11% among pregnant women was recorded (Rajshkehar & Umashanker, 2013). In Bangladeshi, prevalence of 12% was obtained (Ullah, *et al*., 2007) and 7.3% in Kumasi, Ghana (Turpin *et al*., 2007). In Yobe State, Nigeria, (Musbau & Muhammed, 2013) observed a prevalence of 43.3% and 55.0% by (Bankole *et al*., 2015). The prevalence rate observed in this study was within ranges obtained by other workers. The difference in prevalences could be attributed to difference in socioeconomic status and healthcare development (Taiwo *et al*., 2009). Kass, (1998) observed that pregnancy may not predispose to bacteriuria but to the bacteriuric women, it is an added burden. Kunin, (1970), also noted that about 50% of women with previously diagnosed bacteriuria develop UTI within three months of marriage or regular sexual intercourse. Abbey, (1987) also noted that previous experience of urinary tract infection predisposes to bacteriuria. In this study, the difference in the prevalence of bacteriuria among pregnant and non-pregnant women (control) was 2.5%. Factors that may predispose to bacteriuria in pregnancy includes: increased rate of urine formation during pregnancy as a result of increased load of secretory products, 50% increase in glomerular filtration, progesterone and relaxing hormones secreted during pregnancy which have direct effects on the relaxation of the ureter and pelvis, coupled with marked decrease in urethral peristalsis and stasis (Girishbabu *et al.*, 2011, Kenure *et al*., 2013, Imade *et al*., 2010, Turpin *et al*., 2007). Statistical analysis at $p<0.05$ did not show significant difference in the prevalence rate of bacteriuria among pregnant and non-pregnant women. The percentage prevalence of bacteriuria by age groups among pregnant women from the age 16-35 years were high. This was in line with the findings of (Rajshekhar & Umashenkar, 2013) that most positive cases of bacteriuria in pregnancy were within the above age groups. The prevalence rate of bacteriuria in women rises with age and sexual activities (Santiago *et al*; 1980; Kunin & Degroot, 1975). The association between overt bacteriuria and sexual activities in young married women is well known, that the term "honey moon cystitis" is used to describe the entity; however, infection does not subside at this stage; but rather becomes an important complication in pregnancy (Kunin, 1972; Ellis, 2007). Sexual activities may aid increase prevalence of bacteriuria observed among women from ages 16-35 years, since sexual pressure introduces bacteria from vagina into the urethra, aided by the burden of pregnancy.

The pregnant women screened were 330, 16(4.84%) were in the first trimester and the prevalence was 17.9%. Registration of pregnant women to antenatal clinic early in 1st trimester is necessary to detect and treat underlying asymptomatic bacteriuria to avoid complications later in pregnancy. High prevalence of bacteriuria observed in the first trimester could be as a result of underling asymptomatic urinary infection before pregnancy ensued or at unset of pregnancy

due to changes associated with it. Asymptomatic bacteriuria in pregnancy may cause complications such as pylonephritis, hypertensive disease of pregnancy, anemia, chronic renal failure, premature delivery and foetal mortality (Foxman ,2002, Msbau and Muhammad, 2013).

Several groups of bacteria have been implicated in UTI, the most common group were the Enterobacteriaceae and most commonly reported bacteria among this group is *E. coli,* accounting for over 80% of isolated bacteria (Kerure et al., 2013; Chandel *et al.,* 2012, Kass, 2002). Sujatha & Manju, (2014) found *E. coli* was 61% of isolated bacteria among pregnant women in Kupur, India.

Abu-bakarer & Oyaide (1982), reported that *E. coli* accounted for 25% of isolated bacteria, whereas (Akinkugbe *et al.,* 1973) found *E. coli* was 23%, (Turpin *et al*., 2007) recorded *E, coli* as 37%, (Musbau & Muhammad, 2013) recorded *E. coli* 36.9% of isolated bacteria among pregnant women and (Oyeyipo & Onorlode 2014) observed *E. coli* to be 53%. In this study, *E. coli* accounted for 28.8% of isolated bacteria among pregnant women and 27.2% among the non-pregnant women (control group).

This finding is in agreement with the findings of other workers that *E. coli* is the most predominant bacteria isolated from bacteriuric women. *E. coli* is the most predominant aerobic bacteria in human intestine, the proximity of the female genital tract and anal region aids easy transfer of *E. coli* to the vagina and easy transport to the urethra by sexual pressure during sexual intercourse. Engaging in both anal and vaginal sex in tandem is likely to aid contamination of the vagina with *E. coli* for onward transmission to the urethra. Urinary stasis is common in pregnancy and since *E. coli* prefers such environment, they utilize the opportunity to cause UTI. *Staphylococci* species are the second most prevalent bacteria 20%. *Staphylococci* are carried by humans as normal microbiota on the skin, hands, nostril etc. Conditions .that causes depletion of *Lactobacillus acidophilus* in the vagina usually encourages the establishment of *Staphylococci* and other bacteria. The percentage occurrence of *Staphylococci* reported by (Ekwempu *et al;* 1981) was 20%, whereas (Musbau & Muhammad, 2013) reported 22% occurrence. The percentage *Streptococci* from this was 15.5% of isolated bacteria.

Islam, (1981) quantitatively cultured specimen from vulva and vagina swabs of antenatal women, the isolates from the sites were similar with overall carriage rate of 21% *Streptococci*. Wood and Dillon, (1981) in a study of bacteriuria in pregnancy reported 14 isolates of group B-*Streptococci* which was (30.4%) of isolated bacteria. In Benin City, Nigeria, (Bankole *et al*., 2015), had 22.5% of *Streptococci.* The shows that the percentage occurrence of *Streptococci* might vary in different population. The frequent implication of *Streptococci* in bacteriuria may be due to their persistent presence in female genital tract, since the anatomy favours their existence in the environment (Victoria & Mark, 2012). The common source of the recalcitrant and opportunistic bacterium *P. aeruginosa* in UTI is the environments, the organism is widely distributed in soil, water, sewage, plants and animal surfaces. It is associated with biofilm formation (catheter) which are resistant to antimicrobial agents as well as host defense mechanism (Ravi *et al.,* 2009). Due to this reason, *Pseudomonas aeruginosa* is of concern to treating physicians. Most bacteria associated with asymptomatic bacteriuria in this study, were bacteria suspected to cause female infertilities in cases where they had been isolated from the cervix of females suffering from unexplained infertility (Vijay *et al*., 2011). The antibiotics effective against urinary pathogens (Gram negative bacilli) were: ofloxacin, ciproxacin, gentamacin and nalidixic acid. Gentamacin is known to be nephrotoxic, but can be used when necessary. The Gram negative positive are susceptible to gentamicin and erythromycin. This result was similar to that of (Oyeyipo and Onorlode, 2013) in UPTH, Port Harcourt. Both groups of bacteria are resistant to the first line antibiotics.

5. Conclusion

The prevalence of bacteriuria among pregnant women obtained and the trimester

prevalence are within the rage observed by other workers. The pregnant women within age groups from 16-35 yrs had high prevalence of bacteriuria (the ages of active sexual activities), this may be coupled with the influence of pregnancy. *E. coli* was the most predominant bacteria isolated from urines of pregnant and non-pregnant women. Isolated bacteria were resistant to first line antibiotics.

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