BACTERIAL ANTIBIOTIC SENSITIVITY PATTERN FROM URINE OF ASYMPTOMATIC HIV POSITIVE PREGNANT WOMEN

OA Awolude*, OA Adesina*, WB Mutiu+ and IF Adewole*

Departments of Obstetrics and Gynaecology* and Medical Microbiology+,
University College Hospital, Ibadan, Nigeria.
e-mail : tosinawolude@yahoo.com

Correspondence: Dr. O.A. Awolude

Abstract

Introduction: Asymptomatic bacteriuria (ASB) in pregnancy with adverse pregnancy outcome has been well documented with *Escherichia coli* and other gram-negative rods being the common organisms associated. However, most of these studies were done in patients without additional immunosuppressant except for pregnancy. However, the additional risk of HIV infection in pregnant women necessitates the evaluation of the microbial isolates and antibiotic sensitivity pattern from the urine samples of these patients without any symptom.

Methods: A prospective cohort study was undertaken among HIV positive pregnant women attending our Antiretroviral clinic between 1st May and 30th September 2007. Information recorded from the patient questionnaire included age, parity, marital status, occupation, and level of education. Culture and antibiotic sensitivity was carried out on aseptically collected urines from the patients.

Results: Twenty-five (15.5%) of the 161 urine samples cultured significantly grew organisms. Four of the urine specimen had, in addition, candida isolated. The mean age of participants was 30.6 ± 4.3 years and the modal parity was 2. The mean gestational age at presentation was 27.3± 3.2 weeks. *Eshcherichia coli* was isolated in 48%, *Proteus* in 16.0%, *Klebsiella in* 8.0%, and *Staphylococcus aureus* in 28.0%. The antibiotic sensitivity test showed that the organisms were 100% sensitive to ciprofloxacin, 80% sensitive to cefuroxime, 60% sensitive to ofloxacin and augmentin but only 36%, 20%, 8.0% and 4.0% sensitive to co-trimoxazole, amoxycillin, chloramphenicol, erythromycin, nitrofurantoin and gentamycin, respectively. The organisms were 100% resistant to tetracycline and ampicillin..

Conclusion: This study shows that the microbial isolates of the ASB among HIV positive pregnant women are similar to those of HIV-negative counterparts but with varying drug sensitivities. .

Keywords:

INTRODUCTION

The relationship between asymptomatic bacteriuria in pregnancy with symptomatic urinary tract infection and adverse pregnancy outcome has been well documented [1-4]. These adverse outcomes include abortions, preterm birth, maternal anaemia, intrauterine growth restriction chronic pyelonephritis, abruption placentae and maternal hypertension [5,6].

In (most) studies of asymptomatic bacteriuria, *Escherichia coli* is the most common organism associated with bacteriuria, representing at least 80% of isolates [4,7]. Other gram-negative rods like *Proteus*

mirabilis and Klebsiella species are also, commonly isolated with gram-positive organisms including *Staphylococcus saprophyticus* and Enterococci spp occasionally seen [4,7-9]. However, most of these studies were in pregnant patients without any order additional immunosuppressive condition [4,7,10-14].

Human immunodeficiency virus (HIV) is a chronic infection associated with progressive immune dysfunction. HIV appears to increase risk for developing significant bacteriuria in pregnant patients [15]. Also, in HIV infection, co-morbidity with other organisms is common and this may impact on the pregnancy outcome in these patients. Such organisms may include those of asymptomatic bacteriuria.

In view of the risk of opportunistic bacterial infections due to HIV infection and, possibly, pregnant HIV infected patients, it becomes necessary to evaluate the antibiotic sensitivity pattern of microbial isolates from the urine of these patients without urinary symptoms.

METHODOLOGY

The study was conducted at the Prevention of Mother to Child Transmission unit of Antiretroviral Therapy (ART) clinic of the University College Hospital (UCH), Ibadan, Nigeria. The participants were consenting HIV positive pregnant women participating in a research protocol on Safety, Adherence and Effectiveness of Antiretroviral Therapy in pregnant HIV-Infected Nigerians between 1st May and 30th September 2007. This protocol, which was approved by the Institutional Review Committee of the UCH and University of Ibadan, Nigeria, included screening for genitourinary infections as part of the pre-assessment evaluations prior to enrolment into treatment or care arm of the program.

Aseptically collected midstream urine samples (MSUs) were obtained from the symptom free attendees of clinic and sent on the same day for microbiological culture. Samples were immediately processed. Routine microscopy of centrifuged urine sediments was done and cultured on Cysteine-Lactose-Electrolyte-Deficient (CLED) and blood agar plate. Antibiotic sensitivity testing was by disc diffusion technique based on modified Kirby Bauer's method with maximum of six antibiotic discs on each Mueller Hinton agar plate.

A proforma was designed to obtain information about the age, parity, marital status, occupation, level of education, religion and tribe of the participants. Data entry, cleaning, validation and analysis were done using SPSS version 10.0. Descriptive statistics with student t-test for comparison of means were used to describe the characteristics of study subjects and statistical significance determined at level of $P<0.05$. The antibiotics sensitivity is by simple tabulation with results expressed in percentages.

RESULTS

A total of 165 urine samples were collected. However four samples were excluded from analysis – two from patients who were on antibiotic treatment, one was wrongly labeled and another found to be contaminated. This left one hundred and sixty one (161) analyzable samples. A total of 25 cases of asymptomatic bacteriuria was seen out of the 161 samples from the retroviral positive obstetric patients screened during the study period giving a prevalence rate of 15.5%. However, four of the urine specimens had both bacteria and Candida isolated.

The mean age of participants was 30.6 ± 4.3 years (range 19-43years) and the modal parity was 2 with a range of 0-3. The mean age and gestational age at presentation of participants was 30.49 ± 4.3 years and 27.3± 3.2 weeks (30.52 years and 30.46 years ; 27.4 weeks and 29.1 weeks, respectively, for patients with and without significant bacteriuria respectively(Table 1).. One hundred and fifteen, (71.4%) of the participants, had at least secondary education 35 (21.8%) had tertiary education while 11 (6.8%) had no formal educationTable 2 shows the bacteria and antibiotic sensitivity pattern of the isolates . *Eshcherichia coli* was isolated in 48%(into first digit) of the urine specimens. Other gram-negative rods such as Klebsiella spp (8.0%), Proteus)spp (16.0%) while the gram-positive

Table 1: Socio-demographic characteristics of study participants

Characteristics	Culture positive	Culture negative	P value
Mean age (years)	30.52	30.46	0.9823
Type of marriages (n/%)			
Monogamy	17 (68)	100 (73.5)	0.9523
Polygamy	8 (32)	36 (26.5)	0.8469
Mean gestational age at presentation (weeks)	27.4	29.1	0.9972
Modal parity (n)	2.0	2.0	0.2474
Mean packed cell volume (%)	28.16	27.96	0.8526

4

organism isolated was *Staphylococcus aureus* (28.0%).

All the isolated organisms were 100% sensitive to ciprofloxacin while only Klebsiella species and *Proteus mirabilis* were 100% sensitive to cefuroxime with an overall sensitivity of 80% to all the isolated organisms. The organisms were 60% sensitive to Ofloxacin and clavunated amoxicillin on the aggregate while they were poorly sensitive to other antibiotics like cotrimoxazole (36%), chloramphenicol (20%), amoxicillin (20%), erythromycin (8%), nitrofurantoin (4%) and genticin (4%). The organisms were 100% resistant to tetracycline and ampicillin. (Using 10 antibiotic discs on a single plate for DST is not acceptable).

prone to. The possibility of nosocomial infection as shown in a similar study in non-pregnant women [19] can, also, explain this finding.

The theory of contamination of the urinary system by organisms of the lower gastrointestinal tract [5,9,20] is again supported by the findings from this study. The lower gastrointestinal tract is believed to serve as reservoir for the organisms associated with ASB and (UTIs) [8,9,21].

This study showed that ciprofloxacin is the best drug, among those evaluated, to which the organisms were sensitive to. Other drugs with good sensitivity spectrum were cefuroxime, clavunated amoxicillin and ofloxacin. Cefuroxime (spelling) only come in parenteral form which might make its use on outpatient

Table 2: Microbial isolates and antibiotic sensitivity pattern

Isolated microbes Bacteria	No	Antibiotic sensitivity pattern (%)											
		OFL	CXM	NIT	AUG	CIP	GENT	COT	TET	ERY	AMX	AMP	CHL
Eshcherichia coli	12 (48)	66.7	75.0	R	75.0	100.0	8.3	50.0	R	R	33.3	R	R
Klebsiella species	2(8)	100.0	100.0	R	50.0	100.0	R	R	R	R	R	R	R
Proteus mirabilis	4(16)	75.0	100.0	25.0	25.0	100.0	R	R	R	R	R	R	R
Staph aureus	7(28)	28.6	71.4	R	42.9	100.0	R	42.9	R	28.6	14.3	R	71.4
Total	25	60.0	80.0	4.0	60.0	100.0	4.0	36.0	R	8.0	20.0	R	20.0

OFL =Ofloxacin; CXM =Cefuroxime; NIT=Nitrofurantoin; AUG=Augmentin; CIP = Ciprofloxacin; GENT= Genticin ; COT= Cotrimoxazole ; TET= Tetracycline ; ERY= Erythromycin; AMX= Amoxycillin; AMP= Ampicillin; CHL = Chloramphenicol

DISCUSSION

The asymptomatic bacteriuria rate of 15.5% among these studied patients is higher than the range generally quoted for non-pregnant and pregnant women.[16] This can be associated with the fact that women included in this study were HIV positive and were recruited from a tertiary hospital which serves as a referral centre for most ill patients and, as such, may represent a population with more severe disease. However, the rate is similar to those quoted for patients with similar immunosuppressive conditions like diabetes mellitus and sickle cell diseases [17,18].

The organisms isolated from the urine samples still follow the pattern of *E.coli* being responsible for majority of the microbes implicated in Asymptomatic bacteriuria . The 28% isolated rate for *Staphylococcus aureus* might indicate the risk of opportunistic infections to which HIV positive patients are particularly

basis cumbersome;, ofloxacin and ciprofloxacin are not commonly used in pregnancy for safety reasons leaving only clavunated amoxicillin as the drug of choice for these patients. This narrow window of antibiotic choice has great implication for clinical practice especially in terms of affordability.

The isolated organisms were generally sensitive to nitrofurantoin, genticin, erythromycin, amoxycillin chloramphenicol and 100% resistant to tetracycline and ampicillin. These findings might not be surprising as most of these drugs have been in circulation for decades and their repeated exposure and inappropriate use could have led to rectal and vaginal flora alteration leading to development of resistant strains [22-24]. However, some resistant strains can develop *de novo* as part of evolutionary adaptation. There have been several studies that have documented increasing resistance to ampicillin, especially,

by *Escherichia coli* and these resistant strains may constitute over 20% of the isolates in some studies [8,24,25]. This problem of resistance is compounded by the limited choice of safe antibiotics for obstetric use. not done.

The gram negative organisms remain the leading cause of UTI [21] and the commonest isolates from the urinary tract of the HIV positive pregnant women in this study. With the safety issues surrounding ciprofloxacin use in pregnancy, augmentin will be the preferred alternative for oral use while cefuroxime will be preferred when parenteral route is indicated especially while awaiting culture results in suspected cases of (UTI) in HIV positive pregnant women. However, culture and sensitivity results, when available, should always guide our antibiotic use in our patients.

REFERENCES

1. Makuyana D, Mhlabi D, Chipfupa M, Munyombwe T and Gwanzura L: Asymptomatic bacteriuria among outpatients with diabetes mellitus in an urban black population. *Cent Afr J Med* 2002, 48:78-82.

2. Crisiane Guberman, Jeffrey Greenspon, Murphy Goodwin. Rena, Urinary tract, Gastrointestinal and Dermatologic Disorders in pregnancy. In: Current diagnosis and treatment; obsterics and gynaecology, 10th edition (edited by Allan H. Decherny, Lauren Nathan, T. Murphy Goodwin and Neri Laufer),2007. Chapter 23: 374-385.

3. Samuel P and Colombo DF. Renal Disease: In Gabbe SG, Niebyl JR and Simpson JL (eds). Obstetrics: Normal and Problem Pregnancies 4th ed. USA: Churcill Livingstone, 2002; 1067-1068.

4. Delzell JE and Lfevre ML. Urinary tract infection in pregnancy. Am Fam Physiscian, 2000; 61: 713-721

5. Barnick CGW and Cardozo L. The lower urinary tract in pregnancy, labour and puerperium. In Studd J (ed). Progress in obstetrics and Gynaecology, vol 9. London, Churchill Livinstone. 1991; 195-204

6. Cardozo L. Urinary tract disorders in Preganncy. In Whitfield CR (ed): Dewhurst Textbook of Obstetrics and Gynaecology. 5th Edition. Oxford; Blackwell Scientific Publication; 1995; 251-261.

7. Okonofua FE, Amole F, Adediran A and Okonofua B. Incidence and pattern of asymptomatic bacteriuria of pregnancy in Nigerin women. Nig Med Pract 1989; 17: 33-38

8. Mikhail MS and Anyaegbuna MA. Lower urinary tract dysfunction in pregnancy: a review. Obstet Gynecol Surv, 1995; 50: 675-683.

9. Scieve LA, Handler A, Hershow R, Persky V and Davis F. Urinary tract infection during pregnancy: its association with maternal morbidity and perinatal outcome. Am J Pub Health, 1994; 84: 405-410.

10. McNair RD, McDonald SR, Dooley SI and Peterson LR. Evaluation of the centrifuged and Gram-stained smear, urinalysis and reagent strip testing to detect asymptomatic bacteriuri in obstetric patients. Am J Obstet Gynaecol 2000; 182: 1076.

11. Macleans AB. Urinary Tract Infection in Pregnancy. Br J Urol 1997: 80(1): 10-13

12. Ojo OA and Akinkugbe OO. The significance of asymptomatic bacteriuria in pregnancy in Ibadan. W Afr J Surg 1976; 1: 23-28.

13. Okubadejo OA, Akinkugbe OO and Ojo OA. Asymptomatic bacteriuria in pregnancy in Nigeri. E Afr Med J 1969; 46: 367-370.

14. Akah PA and Onah HE. Urinary Tract Infection in Pregnancy in a rural Nigeria Setting. W Afr J Pharmacol.Drug Res 1997; 13L1 & 2): 8-12.

15. Ojoo J, Paul J, Batchelor B, et al. Bacteriuria in a cohort of predominantly HIV-1 seropositive female commercial sex workers in Nairobi, Kenya. : J Infect. 1996 Jul;33(1):33-7

16. Oyetunji JA, Ahmed Y, Nwobodo EI, Ekele BA and Audu-Airede LR. Asymptomatic Bacteriuria in Pregnancy in Sokoto Nigeria. Trop J Obstet Gynaecol 2005; 22 (Suppl 1): pp S23.

17. Vanessa Cumming and Susanna Ali1, Terrence Forrester, Karen Roye-Green and Marvin Reid Asymptomatic bacteriuria in sickle cell disease: a cross-sectional study BMC Infectious Diseases 2006, 6:46.

18. Makuyana D, Mhlabi D, Chipfupa M, Munyombwe T and Gwanzura L: Asymptomatic bacteriuria among outpatients with diabetes mellitus in an urban black population. Cent Afr J Med 2002, 48:78-82.

19. Lucas MJ and Cunninggham FG. Urinary infection in pregnancy. Clin North m, 1987; 1: 807-822.

20. Lawrence Gugino, Jean Wactawski-Wende, Susan L. Goodnough, Debra A. Trisram, Joseph and Mylotte J. Asymptomatic bacteriuria in human immunodeficiency (HIV) –infected women. Prim Care Update Ob Gyns. 1998 Jul 1; 5(4):146.

21. OC. Ezechi, OB. Fasuba and FO. Dare ; Antibiotic Sensitivity Pattern of Microbial Isolates from Urine of Pregnant Women with Urinary Tract Infections. Trop. J Obstet Gynaecol, 2003; 20 (2): 113-115.

22. Rang HP, Dale MM and Ritter JM. Resistance to antibiotics: In Pharmacology.

23. Weller TMA and Wise R. Urinary tract infection: In Rubin P (ed). Prescribing in Pregnancy. 3rd Edition. India. Jaypee Brothers. 1995; 37-40

3rd Edition, London, Churchill Livingstone. 1995; 690-695

24. Peddie BA, Bailey RR and Wells JE. Resistance of urinary isolates of E. coli to co-trimoxazole, sulphonamide, trimethoprim and ampicillin: an 11 year survey. N Z Med J, 1987; 100: 341-342.

25. Sanders CC and Sanders WE. Beta-lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin Infect Dis, 1992; 15: 824-839