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Polymicrobial Agents and Antibiotic Profile of Urinary Tract Infections among Pregnant Women in Anambra State, Nigeria

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Abstract
Microbiological studies were carried out on midstream urine collected from 200 pregnant women(test) and 100 non pregnant women (control) attending antenatal clinics at General Hospital Onitsha, Enugwu-Ukwu and Chizoba Hospital Awada, Obosi. All the subjects were between 18-37years. The urine samples were cultured on to freshly prepared Eosine methylene blue (Oxoid), blood and MacConkey (Difco) agar plates and incubated at 37^{oc} for 24hours. Urinalysis using Combi-9 urine test strip and microscopic examination of the deposit were done. Organisms were identified using cultural characteristics, Gram staining reactions and some biochemical tests. Antibiotic sensitivity tests were carried out on isolates using agar diffusion methods. The result showed a prevalent rate of UTI in pregnancy 23(11.5%) and control 10(10%). There was no significant difference among pregnant and non- pregnant women $p>0.05$. *Echerichia coli* were the predominant organism isolated 78.26%, Group B *streptococcus*, *Klebsiella spp* and *Staphylococuus saprophyticus* were isolated 1 (4.3%) each, *Proteus mirabilis* were isolated 2(8.7%). In the control group *E. coli* were isolated 8 (80%) while *Proteus* and *klebsiella* were isolated 1(10%) each. All organisms isolated were 100% sensitive to gentamicin and nalidixic acid and 100% resistant to ampicillin.
Keywords: Pregnancy, urinary tract infection, polymicrobial agents antibiotic profile

INTRODUCTION

The urinary tract consists of the various organs of the body (kidney, ureters, urethra and bladder) that produce, store and get rid of urine National Kidney and Urologic Disease Centre (2003). Normal urine is sterile- that is when cultured it is free of microbial agents National Kidney and Urologic Disease Centre (2003). The bladder has an excellent mechanism to keep it free of microbial agents via the hydrokinetic aspects which includes the periodic voiding of urine, constant dilution of urine in the bladder (Sobel *et* al., 1997) and microbial factors which includes effects of secretory immunoglobulin A (IgA), prostatic and periurethral glands secretions (Teodosio *et* al., 1999). The vagina is colonized by *lactobacilli* which maintain high acid environment and produces hydrogen peroxide that eliminates bacteria and reduces the ability of *E. coli* to adhere to the vaginal cells. The female urinary tract also produces a natural antibiotic called human beta- defensin 1 (HBD-1) which fights *E. coli* within the female urinary tract (Teodosio et al., 1999). It is when these processes fail that UTI develops (Teodosio *et* al., 1999). UTI may be expressed at a single site- urethra- urethritis, bladder- cystitis. This the most common form of UTI. Pyelonephritis - infection of the kidney. UTI could be asymptomatic when the patient lacks symptoms such as pain when you want to urinate, urgency, fever, heaviness in the lower abdomen suggestive of UTI (Lucas and Cunighan, 1993) or symptomatic. Asymptomatic UTI is common in pregnancy (Robert and Edward, 1999). Complicated UTI occur at a site other than the bladder and are nosocomial in origin (Mikenzie *et* al., 1994). An estimated 40% of women report having had a UTI some point in their lives Kunin, (1994). Pregnant women are at increased risk of developing UTI. Begining in week 6 and peaking during week 22 to 26 approximately 19% of pregnant women develop urethral dilatation and 70% develop glycosuria, increase in urinary progestin and estrogen. All these lead to decrease ability of the lower urinary tract to resist invading bacteria (Sobel *et* al., 1997). In USA, the prevalent rate of UTI in pregnancy was found to be 2-4% (National Kidney and Urologic Disease Centre, USA 2003). Oslen, *et al;* (2000) found a prevalent rate of UTI in rural Tanzania 16.4% while in Jos, Nigeria Nnatu, *et al;* (1989) in their study got 8% prevalent rate of UTI. The organisms that cause UTI in pregnancy are the same as in non- pregnant women with *E. coli* being the predominant 80-90% (Mikenzie *et* al., 1994). Untreated UTI in pregnancy may lead to the development of pyelonephritis in up to 50% of the cases, increased rate of prematurity, miscarriage, low birth weight, mental retardation, fetal death, maternal anaemia and end point renal failure (Robert and Edward, 1999). It is widely recognized that many problems could be prevented by the application of knowledge available. However, many practical issues are yet to be addressed; When should urine culture be obtained in pregnancy?, What diagnostic threshold should be used to define infection?, what are the drugs of choice for UTI bearing in mind the re-emerging multi-drug resistant of initial therapy of choice for UTI. This study attempts to clarify these issues in Anambra State, Nigeria.

MATERIALS AND METHODS

A random study was done among pregnant women attending antenatal clinics at General hospitals Onitsha and

Enugwu- Ukwu, Chizoba hospital awada, Obosi all in Anambra State, Nigeria. Two hundred pregnant women aged 18 to 37years with gestational age 4 to 7 months and 100 non pregnant women aged 18-37years who gave their consent were told how to collect clean catch midstream urine according to Oslen, *et* al., (2004). On arrival at the laboratory within two hours of collection, the urine samples were inoculated on freshly prepared blood, MacConkey (Difco), Eosine methylene blue (Oxoid) agar plates and incubated at 37^{oc} for 24hours according to Bachman, *et* al., (1993). Initial reading of the plates was done after 24 hours. Organisms isolated were identified using cultural characteristics on the different agar plates- MacConkey agar was used to distinguish between lactose fermenters coliform type colonies which appear pink. The EMB agar was used to differentiate *E. coli* which gave metallic sheen color from *Klebsiella* and *Proteus* which gave pink colonies, *Staphylococuus saprophyticus* do not grow in this medium. The blood agar plate which is a general medium was used to detect the presence of haemolysis and also allows the swarming of *Proteus* and mucoid colonies of *Klebsiella* to be easily observed and Gram staining reactions were done. Isolates were stored on nutrient agar slants at 4^{oc} for further confirmatory tests which include IMViC test, sugar fermentation test for gas production, carbohydrate utilization, nitrate reduction urease production and motility. Confirmation of *S. saprophyticus* typical colonies on Baird- Parker agar was based on the bases of results of catalase, coagulase, phosphate reduction, and carbohydrate utilization. Urinalysis using Combi-9 urine test strip and microscopic examination of centrifuge urine deposit were done and results recorded. Antibiotic sensitivity tests were done on identified isolates using agar diffusion method Bachman *et* al., (1993). The degrees of sensitivity of the organisms to the drugs were determined by measuring the easily visible areas of inhibition of growth produced by the diffusion of the antibiotic from the disc into the surrounding medium using vernier caliper. Inhibition area greater than12mm were considered sensitive.

Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests.

RESULTS

In this study, a total of 200 urine samples from pregnant women aged 18 to 37years with gestational age 4 to 7 months, 23 (11.5%) urine samples showed growth of specific organisms while 177 (88.5) showed no bacteria growth (table 1). *Esherichia coli* were the predominant organism isolated 18 (78.3%), in control 8(80%) respectively. Group B *streptococuus*, *klebsiella* and *Staphylococuus saprophyticus* were isolated 1(4.3%) each, *Proteus* 2(8.7%), in control *E .coli* 8(80%), *Proteus* and *Klebsiella* 1(10%) each. The prevalence of UTI was more at gestational age 6 to 7 months 15(65.2%) and para 1-3 12(52.2%) table 2, 3. The prevalence of UTI was also more at mother's age 26-29years 9(39.1%) table 4. Examination of urine samples using Combi-9 urine test strip showed the presence of glucose 12(6%), protein 9(4.5%) and nitrite 17(8.5%), blood 7(3.9%). Microscopic examination of centrifuged urine deposit showed the presence of ova of schistosoma haematobium 1(1.5%) in one of the urine sample that contain blood, cast 5(2.5%), white blood cells >4-5PHF 16(8%) table 5. In the control group (non-pregnant women the prevalence of UTI was 10(10%). *E. coli* were the predominant isolate 8(80%), *Proteus* and *Klebsiella* 1(10%) each. Urinalysis showed the presence of glucose 21(21%), protein 1(10%), nitrite 19(19%), blood 1(10%). Microscopic examination of centrifuged urine showed only white blood count > 4-5PHF 5(5%). All organisms isolated were 100% sensitive to gentamicin and nalidixic acid while 100% were resistant to ampicillin.

Table1. **Frequency of occurrence of bacteria agents causing UTI in pregnant women**

Isolate	**Frequency**	**percentage**
E.coli	18	78.3
Proteus	2	8.7
Klebsiella	1	4.3
GBS	1	4.3
Staphylococus saprophyticus	1	4.3
Total	23	
Control		
E. coli	8	80
Proteus	1	10
Klebsiella	1	10

Table 2.**Prevalence of urinary tract infection according to duration of pregnancy**

Duration	**no. studied**	**prevalence**
4-5months	90	8(34.8%)
6-7months	110	15(65.2%)

Table 3. **Prevalence of urinary tract infection according to parity**

Parity	**no. studied**	**prevalence**
Para 0	101	8(34.8%)
Para 1-3	78	12(52.2%)
Para 4-6	21	3(13%)

Table 4.**Prevalence of urinary tract infection according to age of mother**

Mother's age (years)	**no. studied**	**prevalence**
18-21	42	6(26.1%)
22-25	54	3(13%)
26-29	62	9(39.1%)
30-33	28	2(8.7%)
34-37	12	3(13.1%)

DISCUSSION

This study showed a prevalence of 11.5% UTI in pregnant women and 10% in non- pregnant women. This is in tune with an earlier work done in Nigeria by Nnatu *et al;* (1989). They found a prevalent rate of 8% in pregnant women. Oslen *et al;* (2000) found similar prevalence of 8% in a study done in rural Tanzania while Gratacos *et al;* (1994) found 10%. In the USA the prevalence rate of UTI in pregnancy was as low as 2.4% (National Kidney and Urologic Disease Centre, USA, 2003). *E. coli* were the predominant organism isolated in both pregnant 78.3% and non-pregnant women 80%. This agrees with the findings of John and Michael (2000) of *E. coli* between 80-90%. Group B streptococcal (GBS) vaginal colonization is known to be a cause of neonatal sepsis and is associated with preterm rupture of membrane, labour and delivery (Thomson *et* al., 1997). Although it is unclear if GBS bacteriuria is equivalent to GBS vaginal colonization, it's prevalence of 4.5% in this study is worrisome. The results of this study agrees with similar works done by Thomson *et al;* (2997), Nnatu *et al;* (1989) and Mikenzie *et al;* (1994) all found a prevalence of 5% GBS in pregnant women. The prevalent rate of UTI is more 15(65.2%) between gestational age 6-7 months. Also the prevalence of 8.5% and 19% nitrite positive in pregnant and non-pregnant women respectively in this study is worthy of mention as positive nitrite has been regarded as a definite sign of UTI (Oslen *et* al., 2000). This finding did not agree with the report of 5% nitrite positive by Hall *et al*; (1991) in South Africa and higher prevalence of 40.3% found in rural Tanzania by Oslen *et al*; (2000).The prevalence of asymptomatic bacteriuria in pregnancy appears to be on the increase due the findings of this study and increased resistant of the isolates to commonly used antibiotics, the significant consequences for the woman and the pregnancy plus the ability to avoid complications with treatment justify screening pregnant women for bacteriuria using Combi-9 for positive nitrite and culture and sensitivity because of the changing patterns of antibiotic sensitivity profile so that UTI is appropriately managed in pregnancy.

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