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# ASYMPTOMATIC MICROBIAL URINARY TRACT INFECTION IN HIV POSITIVE PREGNANT WOMEN IN NDJAMENA (CHAD)

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## ABSTRACT

Asymptomatic bacteriuria in pregnancy is associated with an increased risk of pyelonephritis leading adverse effects as pre-term delivery and fetal loss. The study aims to determine the prevalence of asymptotic bacteriuria among HIV-positive pregnant women followed up in the "Centre de l'Appui Psycho-Médico-social (APMS)" of Ndjamena (Chad), and to identify the sensitive antibiotics against their urinary isolates. Seventy six followed up HIV-positive pregnant women who did not show any symptoms considered as urinary infection were involved after informed consents were obtained. Their socioeconomic conditions were determined using a semi-structured questionnaire. Having been taught the art of urine collection, a mid-stream urine specimen was taken and delivered onto agar Uriselect 4. Plated dishes were then incubated at 37°C overnight. After incubation, density of colonies was compared to a diagram to determine the number of cells per milliliter. Antibiotic sensitivity test were carried out using NCCLS discs diffusion method. The investigation revealed that 59.21% of subjects of the study population were in the age range of 25-34 years. The majority of them were married (69.74%), and 50% were multiparous. Regarding the occupation, 56.58% among participants were housewife. Asymptotic bacteriuria prevalence was 32.89% in the study population. *E. coli* had the highest value (56%) while *Klebsiella pneumoniae, Enterobacter cloacae* and *Staphylococcus aureus* had lowest value of (8%) each respectively. Cefoperazone, Cefsulodine and Cefixime were recorded as the most effective antimicrobial against the urinary isolates.

**Keywords:** HIV-infected, Pregnant Women, Asymptomatic Bacteriuria, Urine Culture, Antibiogram.

## INTRODUCTION

Urinary tract infections (UTI) is a serious health problem that affects millions of people each year (Jain *et al.*, 2010). They are most common infection caused by bacterial pathogens seen mostly in developing countries (John *et al.,* 2015). It may be of two types, symptomatic or asymptomatic (Kerure and Umashanker, 2013). Quantitative criteria to establish the diagnosis of significant bacteriuria in an asymptomatic person are: at least $10^5$ CFU/mL of urine in a voided midstream, so called clean-catch specimen; and at least 100 CFU/mL of urine obtained by bladder catheterization (Colgan *et al.*, 2006; Sharma *et al.*, 2011). It is estimated that > 20% of females get UTI in their lifetime (Beytyr *et al.*, 2015). They are more susceptible for these infections because of short urethra (Chandel *et al.*, 2012), closer proximity of the anus with vagina, and pathogen entry facilitated by sexual activity (Tadesse *et al.*, 2014). Asymptomatic bacteriuria (ASB) is the most common bacterial infection requiring medical treatment in pregnancy (Girishbabu *et al.*, 2011; Jain *et al.*, 2013), and it can occur at all stages. The pregnant women are two times more commonly affected than age matched non pregnant females (Kerure *et al.*, 2013), and the prevalence during

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pregnancy ranges between 2% to 11% (Hazhir, 2007; Schnarr and Smaill, 2008). Physiologic changes in pregnancy brought about by hormonal changes and uterine compression make the pregnant woman with asymptomatic bacteriuria particularly susceptible to the development of persistent and symptomatic urinary tract infection (Guito *et al.*, 2010; Girishbabu *et al.*, 2011). Several predisposing factors for asymptomatic bacteriuria in pregnancy have been identified. These include advanced maternal age, multiparity, low socioeconomic status and advanced gestational age. Other factors associated with an increased risk of bacteriuria include anatomical abnormalities of the urinary tract, history of recurrent urinary tract infections, diabetes mellitus, previous antibiotic intake, sickle cell disease and possibly Human Immunodeficiency Virus (HIV) infection (Ansari and Rajkumari, 2011; Girishbabu *et al.*, 2011; Titoria *et al.*, 2014; Adekunle and Adetokunbo, 2014). As HIV induces the immunitary system imbalance of the infected persons, it has been suggested that people living with this infection are likely to be predisposed to urinary tract infection (Schnarr and Smaill, 2008; Akinbami *et al.*, 2013; Adekunle and Adetokunbo, 2014). In contrast, limited studies involving HIV-infected patients have reported no association between asymptomatic bacteriuria and HIV infection in women (Nicolle *et al.*, 2005; Widmer *et al.*, 2010; Banu and Jyothi, 2013). *Escherichia coli* is the most common pathogen associated with both symptomatic and asymptomatic bacteriuria (Coetzer, 2004; Bigwan and David, 2013), representing 70–80% of isolates (Schnarr and Smaill, 2008). Other bacteria including *Staphylococcus aureus*, *Providencia* species, *Klebsiella* species, coagulase-negative staphylococci, *Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter freundii*, *Enterobacter cloacae* and *Proteus rettgeri*, were commonly isolated from pregnant women infected by ASB (Gabre-Selassie, 1998; Hancock and Klemm, 2007; Mokube *et al.*, 2013; Tadesse *et al.*, 2014). If left untreated, in 20-30% of cases, women may go on to develop serious complications such as pyelonephritis leading adverse effects as hypertensive disorders in pregnancy, pre-term delivery, intrauterine growth retardation, endometritis, fetal loss, low birth weight and preeclampsia (Khattak *et al.*, 2006; Ullah *et al.*, 2007; Ansari and Rajkumari, 2011; Marahatta *et al.*, 2011; Senthinath *et al.*, 2013). Adequate treatment needs a good knowledge of the bacteria species involved and their susceptibility to antimicrobial agents. Detection of ASB in antenatal women is important. Available data on the prevalence of asymptomatic bacteriuria among HIV- positive pregnant women are not as copious as those of the general obstetric studies. The study aims to determine the prevalence of asymptotic bacteriuria among HIV-positive pregnant women followed up in the "Centre de l'Appui Psycho-Médico-social (APMS)" of Ndjamena (Chad), and to identify the sensitive antibiotics against their urinary isolates.

**Table 1. Distribution of age group and parity**

| Age group | | |
|---|---|---|
| 15-24 years | 25-34 years | 35-45 years |
| 29 (38.16%) | 45 (59.21%) | 2 (2.63%) |

| Parity | | |
|---|---|---|
| No birth | First births | Multiple births |
| 11 (14.47%) | 27 (35.53%) | 38 (50%) |

**Table 2. Distribution of marital status and occupation**

| Marital status | | | |
|---|---|---|---|
| Married | Divorced | Single | Widow |
| 53 (69.74%) | 16 (21.05%) | 5 (6.58%) | 2 (2.63%) |

| Occupation | | | |
|---|---|---|---|
| Housewife | Trader | Seller | Student |
| 43 (56.58%) | 17 (22.36%) | 9 (11.84%) | 7 (9.22%) |

## MATERIALS AND METHODS

### *Selection of participants and survey instrument*

The research is a prospective study carried out in the " Centre de l'Appui Psycho-Médico-social (APMS)" of N'Djamena (Chad) from 1st January to 30th March 2014. Seventy six followed up HIV-positive pregnant women who did not show any symptoms considered as urinary infection were involved after informed consents were obtained. Their socioeconomic conditions were determined using a semi-structured questionnaire focused on: age, marital status, parity, occupation.

### *Sample collection and analysis*

Subjects were duly counseled regarding the correct technique of self-collection of mid-stream urine at APMS between 7:30 and 9:00 a.m. Before collecting the sample, patient must wash and dry hands. The external genitalia should be cleaned carefully using Dakin's solution. First part of the

stream was discarded and sample was collected in a sterile container of 10 mL. Then urine was collected in the container half full and tightens securely. For plating, sterile loop of 10 µL was held vertically and submerge in the urine. Urine was delivered onto agar (Uriselect 4 from Bio-Rad, France) gently as recommended. Plated dishes were then incubated at 37°C overnight. After incubation, density of colonies was compared to a diagram to determine the number of cells per milliliter. Indole production and Tryptophane deaminase were revealed according to colonies colour, or culture was examined under microscope to identify the germ. Germs sensitivity to antibiotics was carried out using the discs method diffusion (NCCLS, 2002). Filter paper disks impregnated with a known and standardized quantity of antimicrobial quantity of antimicrobial agent were applied over Müller Hinton medium (Bio-Rad, with bacteria. After diffusion in the medium, the antimicrobial agent constitutes a concentration gradient that will inhibit or not bacterial growth. According to the inhibition zone diameter produced after incubation at 37°C overnight, the effect is considered as sensible or resistant. The following antimicrobial discs were used: Ampicillin, Amoxicillin, Amoxicillin + acid clavulanic, Carbenicillin, Imipenem, Oxacillin, Penicillin G, Piperacillin, Cephalothin, Cefazolin, Cefoxitin, Cefuroxime, Cefotaxime, Cefoperazone, Cefsulodine, Ceftriaxone, Ceftazidime, Cefixime, Erythromycin, Lincomycin, Oxytetracycline, Streptomycin, Chloramphenicol, Acid nalidixic, Ciprofloxacin, Pefloxacin, Pipemidicacid, Norfloxacine and Oflo**x**acine.

Data were analyzed using Epi info version 2007. The descriptive data were given as means.

## RESULTS AND DISCUSSION

### *Socio-demographic characteristics*

A total of 76 HIV-positive pregnant women were enrolled in this study. Socio-demographic characteristics of participants are summarized in Tables 1 and 2. There were 45 (59.21%) in the age range of 25-34 years whereas 29 (38.16%) belong to 15-24 years age range. Our finding is in accordance with report of Mokube *et al.* (2013) where 68% of subjects were aged 21-30. In contrast, in India, a similar study done by Ansari and Rajkumari (2011) lend support that more than 90% were in 15-25 years age range. These women had different marital status: the majority of them were married (69.74%) followed by those who were divorced (21.05%). Widow women were minority (2.63%). A similar trend was observed in Tanzania (Masinde *et al.*, 2009). Results indicated that 38 (50%) subjects of the study population were multiparous where 27 (35.53%) had already two or more children, and 11 (14.47%) had no birth before. Elsewhere in Nigeria, more than 83% multiparous were recorded among HIV-positive pregnant women (Adekunle and Adetokunbo, 2014). Regarding the occupation, 43 (56.58%) among participants were housewife.

### *Isolates bacteria*

Out of the total of 76 HIV-positive pregnant women enrolled for the study, 25 urine samples had significant bacteriuria giving a prevalence of 32.89% for asymptomatic bacteriuria. It is consistent with the findings of Adekunle and Adetokunbo (2014) (31.3%). Tosin *et al.* (2014) found 37.1% as prevalence rate, whereas Ullah *et al.* observed 12%. The most common bacterial isolates from midstream urine samples of asymptomatic pregnant women enrolled in this study were *Escherichia coli* (56%) followed by *Streptococcus agalactiae* (20%). *Klebsiella pneumoniae, Enterobacter cloacae* and *Staphylococcus aureus* were only 8% each (Table 3).

**Table 3: Bacteriological isolates**

| Quarantined colonies | | Number | % |
|---|---|---|---|
| Germ to GRAM negative | *Enterobacter cloacae* | 2 | 8 |
| | *Escherichia coli* | 14 | 56 |
| | *Klebsiella pneumoniae* | 2 | 8 |
| Cocci to GRAM positive | *Staphylcoccus aureus* | 2 | 8 |
| | *Streptococcus agalactiae* | 5 | 20 |
| Total | | 25 | 100 |

Ullah *et al.* (2007) and John *et al.* (2015) in separate studies also reported *E. coli* as being the commonest pathogen responsible for bacteriuria. In Nepal, Marahatta *et al.* (2011) pointed out that the cautative organism for ASB was *E. coli* in 78% of cases. The anatomical proximity of the anal and urogenital opening in females makes it possible for faecal contamination of the urinary tract from commensals of the bowel of which *E. coli* is a typical example (Akram *et al.,* 2007). Contrary to our results, studies carried out in India by Ansari and Rajkumari (2011) indicated that *Klebsiella pneumonia* and *Staphylococcus aureus* were common isolates germs, whereas, Tedesse *et al.* (2014) found that the most frequently isolated bacteria were coagulase negative Staphylococcus. Mohammad *et al.* (2002) suggested that the high risk of acquiring *E. coli* UTI is because of the

**Table 4. Antibiotic sensitivity profile of the bacterial strains isolated in HIV positive pregnant women with ASB**

| Antibiotics family | Antibiotics tested | Sensitivity (%) | | | | |
|---|---|---|---|---|---|---|
| | | ***E. Coli*** **(n=11)** | ***K. pneumoniae*** **(n=2)** | ***E. cloacea*** **(n=2)** | ***S. aureus*** **(n=2)** | ***S. agalactiae*** **(n=5)** |
| **Beta-lactam (Penicillin + Cephalosporins)** | Ampicillin | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| | Amoxicillin | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 3 (60.00) |
| | Amoxicillin + acid clavulanic | 6 (54.55) | 1 (50.00) | 2 (100) | 2 (100) | 3 (60.00) |
| | Carbenicillin | 11 (100) | 1 (50.00) | 2 (100) | 1 (50.00) | 2 (40.00) |
| | Imipenem | 11 (100) | 2 (100) | 2 (100) | 1 (50.00) | 2 (40.00) |
| | Oxacillin | 0 (0.00) | 2 (100) | 2 (100) | 0 (0.00) | 3 (60.00) |
| | Penicillin G | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| | Piperacillin | 6 (54,55) | 2 (100) | 2 (100) | 1 (50.00) | 2 (40.00) |
| | Cephalothin | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 5 (100) |
| | Cefazolin | 11 (100) | 2 (100) | 2 (100) | 1 (50.00) | 3 (60.00) |
| | Cefoxitin | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 5 (100) |
| | Cefuroxime | 11 (100) | 2 (100) | 2 (100) | 2 (100) | 5 (100) |
| | Cefotaxime | 11 (100) | 2 (100) | 2 (100) | 1 (50.00) | 3 (60.00) |
| | Cefoperazone | 11 (100) | 2 (100) | 2 (100) | 2 (100) | 5 (100) |
| | Cefsulodine | 11 (100) | 2 (100) | 2 (100) | 2 (100) | 5 (100) |
| | Ceftriaxone | 11 (100) | 1 (50.00) | 2 (100) | 2 (100) | 5 (100) |
| | Ceftazidime | 6 (54.55) | 2 (100) | 2 (100) | 2 (100) | 2 (40.00) |
| | Cefixime | 11 (100) | 2 (100) | 2 (100) | 2 (100) | 4 (80.00) |
| **Lincosamides** | Erythromycin | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 3 (60.00) |
| | Lincomycin | 6 (54.55) | 1 (50.00) | 2 (100) | 0 (0.00) | 3 (60.00) |
| | Oxytetracycline | 4 (36.36) | 1 (50.00) | 2 (100) | 1 (50.00) | 2 (40.00) |
| | Streptomycin | 11 (100) | 2 (100) | 2 (100) | 1 (50.00) | 3 (60.00) |
| **Polypeptides** | Chloramphenicol | 6 (54.55) | 1 (50.00) | 2 (100) | 0 (0.00) | 3 (60.00) |
| **Quinolones** | Acid nalidixic | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 5 (100) |
| | Ciprofloxacin | 11 (100) | 2 (100) | 2 (100) | 1 (50.00) | 3 (60.00) |
| | Pefloxacin | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 2 (40.00) |
| | Pipemidicacid | 5 (45.45) | 2 (100) | 1 (50.00) | 2 (100) | 3 (60.00) |
| | Norfloxacine | 11 (100) | 2 (100) | 1 (50.00) | 0 (0.00) | 5 (100) |
| | Oflo**x**acine | 11 (100) | 2 (100) | 2 (100) | 0 (0.00) | 5 (100) |

anatomical and the functional changes that occur during pregnancy and the fact that *E. coli* is the most common micro-organism in the vaginal and rectal area.

### *Antibiotic resistance of isolates bacteria*

The antibiotic sensitivity test is recorded in Table 4. Results of antibiotic sensitivity revealed that Cefoperazone, Cefsulodine and Cefixime were very effective against all the isolates. All the strains of *E. coli*, *K. pneumonia* and *E. cloaceae* were sensitive to fifteen antibiotics following Imipenem, Cephalothin, Cefazolin, Cefoxitin, Cefuroxime, Cefotaxime, Cefoperazone, Cefsulodine, Cefixime, Erythromycin, Streptomycin, Acid nalidixic, Ciprofloxacin, Pefloxacin and Oflo**x**acine. *Enterobacter cloacae* revealed sensitivity to more antibiotics 24 (> 82%). In similar studies, Ophori *et al.* (2010) pointed out that cotrimoxazole and ciprofloxacin where very effective against most of the isolates from UTI patients. Regarding all isolated bacteria, antibiotic resistance was observed totally for ampicillin and penicillin G. The same pattern was recorded for amoxicillin except the case of *S. agalactiae* with sensitivity 60%. Isolates which displayed resistance to more antibiotics were *Staphylococcus aureus*: total resistance to 13 antibiotics (Ampicillin, Amoxicillin, Oxacillin, Penicillin G, Cephalothin, Cefoxitin, Erythromycin, Lincomycin, Chloramphenicol, Acid

nalidixic, Pefloxacin, Norfloxacine and Oflo**x**acine) and 50% to 8 antibiotics (Carbenicillin, Imipenem, Piperacillin, Cefazolin, Cefotaxime, Oxytetracycline, Streptomycin and Ciprofloxacin). Other studies showed ampicillin, tetracycline, chloramphenicol and erythromycin resistance to isolated germs in UTI cases (Ophori *et al.*, 2010; Girishbabu *et al.*, 2011). The antibiotics used against ASB differ according to preference in different countries. The choice should however be based on urine culture, stage of gestation, clinical data and the characteristics of the antibiotic. β-lactam antibiotics (particularly pivmecillinan) and nitrofurantoin are first choice drugs in northern Europe; in the USA, amoxycillin use is common, whereas in Canada, trimethoprim and nitrofurantoin are preferred; in the UK, they advocate the use of penicillins and cephalosporins (Guinto *et al.*, 2010). In Africa area, the following antibiotics are used frequently as remedies against ASB: tetracycline, chloramphenicol, ciprofloxacin, cotrimoxazole, nalidixic acid, nitrofurantoin (Okonko *et al.*, 2009; Ophori *et al.*, 2010). Although aggressive antibiotic treatment may be necessary to reduce the risk of pyelonephritis and other complications of asymptomatic bacteriuria in pregnancy, this should be done with caution as it known that urinary pathogens are becoming resistant to commonly used antibiotics which could be attributed to wide spread and indiscriminate use of the drugs (Okonko *et al.*, 2009). Antibiotic use in nursing homes is a strong driver for the emergence of multi-drug resistant organisms such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and fluoroquinolone-resistant Gram negative bacilli (Phillips *et al.*, 2012). In addition, the majority of the treatments begins or is done completely empirically, the knowledge of the organisms, their epidemiological characteristics and their susceptibility is therefore mandatory (Okonko *et al.*, 2009; Vukmanich, 2012).

## CONCLUSION

This study showed that approximately 32.89% of the pregnant women recruited had asymptomatic bacteriuria. Among bacteria isolated from patients urine samples, *Escherichia coli* had the highest value (56%). Cefoperazone, Cefsulodine and Cefixime were recorded as the most effective antimicrobial against the urinary isolates. It is therefore imperative that pregnant women are urinary tract infection among pregnant women screened for bacteriuria periodically in every trimester of the gestational period. Talks on personal hygiene and cleanliness around the urogenital and anal area to prevent faecal contamination of the urinary tract should be emphasized during antenatal visits.

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