Original Article

Preponderance of bacterial isolates in urine of HIV-positive malaria-infected pregnant women with urinary tract infection

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Abstract

Introduction: This study examined HIV and malaria co-infection as a risk factor for urinary tract infections (UTIs) in pregnancy. The study group included 74 pregnant women, 20 to 42 years of age, who attended the antenatal clinic at the Specialist Hospital at Akure, Ondo State, Nigeria.

Methodology: Forty-four of the pregnant women were either HIV seropositive with malaria infection (HIV+Mal+) or HIV seropositive without malaria (HIV+Mal-). The remaining thirty pregnant women served as controls and included women HIV seronegative but with malaria (HIV-Mal+) and women HIV seronegative without malaria. UTI was indicated by a bacterial colony count of greater than 10^5/mL of urine, using cysteine lactose electrolyte deficient medium (CLED) as the primary isolation medium. Bacterial isolates were characterized using convectional bacteriological methods, and antibiotics sensitivity tests were carried out using the disk diffusion method.

Results: A total of 246 bacterial isolates were recovered from the cultures, with a mean of 3.53 isolates per subject. Women who were HIV+Mal+ had the most diverse group of bacterial isolates and the highest frequency of UTIs. The bacterial isolates from the HIV+Mal+ women also showed the highest degree of antibiotic resistance.

Conclusions: While pregnancy and HIV infection may each represent a risk factor for UTI, HIV and malaria co-infection may increase its frequency in pregnancy. The higher frequency of multiple antibiotic resistance observed among the isolates, particularly isolates from HIV+Mal+ subjects, poses a serious public health concern as these strains may aggravate the prognosis of both UTI and HIV infection.

Key words: HIV and malaria co-infection; bacteria; antibiotic resistance; UTI.

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Introduction

Urinary tract infection (UTI) occurs in all age groups and in both sexes, but it is more common in females. Bacteriuria with a colony count of 1×10^5/mL of mid-stream urine with pus cells is indicative of a UTI. Studies have shown that UTIs are common in pregnancy and that women with symptomatic bacteriuria may be at risk of developing cystitis or pyelonephritis, which may lead to septicemia and fetal complications [1-3]. The human immunodeficiency virus (HIV) and malaria have overlapping distributions in sub-Saharan Africa (SSA), the Indian subcontinent, and Southeast Asia, where about 500 million people are infected annually [4-8]. The World Health Organization (WHO) reported that severe malaria complications, mostly from *Plasmodium falciparum* infections, are responsible for over one million deaths annually in children and pregnant women [4]. Malaria is also a risk for 97% of Nigeria's population, while the remaining 3% of the population lives in malaria-free highlands [9]. Another report estimated that 169 million Nigerians were infected with malaria in 2013, indicating the endemicity of malaria in the country [10].

HIV infection in pregnancy has become the most common medical complication of pregnancy in some countries in sub-Saharan Africa [11]. It has been reported that over 70% of all HIV infections are a result of heterosexual contacts and that over 90% of infections in children come from mother-to-child

transmission [5]. The report also estimated that the prevalence of HIV in pregnant women is over 30% in some countries of southern Africa. Approximately 2%–8% of pregnant women die of pregnancy-related illnesses in developing countries, partly because of HIV and malaria complications [11] and also due to lack of access to antiretroviral drugs (ARVs) [12]. Studies have reported that HIV patients with malaria have a higher frequency of symptomatic malaria and that malaria may increase HIV plasma viral load and decrease CD4+ T cells [13,14]. These studies therefore suggest that HIV and malaria may each suppress the host's immune system, resulting in a dysregulation in the production of cytokines and antibodies.

In order to reduce the incidence of maternal and child mortality in general, the Ondo State government in south-western Nigeria embarked on the provision of free medical services to pregnant women in government-run specialist hospitals. Preliminary studies had indicated a higher incidence of UTI in pregnancy and some degree of symptomatic malaria and HIV prevalence among pregnant women in Ondo State. This study was therefore designed to determine and compare the prevalence of UTI in HIV-positive, malaria-infected pregnant women and HIV-negative control pregnant women attending the antenatal clinic at the Specialist Hospital in Akure, south-western Nigeria. An additional goal of the study was to identify the causative bacterial agents of UTIs among the subjects, along with their patterns of antibiotic resistance. It was expected that the results of this study would assist clinicians to better manage UTIs in pregnant women with HIV and malaria co-infection.

Methodology

Patients and study center

The study was conducted between August 2012 and June 2013 at the Ondo State Specialist Hospital in Akure, a city with an estimated population of 387,087. All study participants, including the controls, were pregnant women who attended the antenatal clinic of the hospital. Demographic information relating to the participants was obtained from interviews, questionnaire responses, and case files managed by the attending physicians. The study design was approved by the hospital management and ethical board, and informed consent was obtained from all participants after they were duly informed of the objectives of the study. Additional counsel was provided to the study participants by the counselling and nursing personnel of the HIV clinic.

Criteria for study inclusion

The HIV status of the participants was determined by blood screening at the HIV clinic of the hospital and was a requirement for inclusion in the study. The screening was done at the first, second, and third trimesters of the participants' pregnancies. Enrolment at the antenatal clinic of the hospital and strict compliance by HIV-positive participants to taking their prescribed antiretroviral drugs were additional requirements for study inclusion. This was done in accordance with the national guidelines on HIV prevention of transmission from mother-to-child (PTMTC) policy. Participants were also required to keep all physician appointments throughout the three trimesters of their pregnancy. Patients who did not meet the above-listed criteria were excluded from the study.

Screening for HIV and malaria parasitemia

A 4 mL volume of blood was collected from each participant. A small aliquot was applied onto the HIV-1/2 strip (Determine Test, Alere, London, UK) for preliminary HIV status determination. Confirmatory test for HIV infection was performed using the Abbott ELISA procedure (Abbott Labs, Chicago, USA). Thin and thick blood smears were also prepared from the collected blood for determination of malaria identification and parasitemia. The slides were fixed in 70% methanol and stained with Giemsa. Malaria parasites were speciated from the thick blood preparation, and parasite density was determined from thin blood smears.

Bacterial isolation, identification and antibiotic sensitivity tests

Each study participant was instructed to clean her vaginal area with a towelette prior to mid-stream urine collection. The urine sample was immediately streaked on cysteine lactose electrolyte deficient (CLED) medium (Biomark Laboratories, Pune, India). Sediment from urine centrifugation was also examined for pus cells. The plates were incubated overnight at 37°C and enumerated for determination of colony forming units per milliliter (CFU/mL). UTI was indicated by a bacterial count of 1×10^5/mL with > 10 pus cells/mL. The bacterial isolates were identified by Gram stain and growth characteristics on mannitol salt agar (Oxoid, Basingstoke, UK), blood agar, eosin-methylene blue agar, triple sugar iron agar, sulfide indole motility medium, and citrate agar (Oxoid). Coagulase and catalase tests and sensitivity to Taxo A (0.04 units of bacitracin) and Taxo P (5 μg)

ethylhydrocupreine hydrochloride (optochin; BD Diagnostics, Difco Laboratories, Detriot, USA) were also employed for identification. All bacterial isolates were tested for their sensitivity to commonly prescribed antibiotics using the Kirby-Bauer method. The antibiotics used were obtained from Abtek Biologicals Limited (Liverpool, UK) and included cloxacillin (5 µg), erythromycin (5 µg), gentamicin (10 µg), agumentin (30 µg), streptomycin (10 µg), tetracycline (10 µg), chloramphenicol (10 µg), ofloxacin (5 µg), nalidixic acid (30 µg), amoxicillin (25 µg), nitrofurantoin (200 µg), and cotrimoxazole (25 µg). *S. aureus* ATCC 25923 and *Enterobacter aerogenes* (American Type Culture Collection, Rockville, USA) were used as control organisms.

Statistical analysis

The resulting data was analysed by the Chi-square method and two-way *t*-test using SPSS version 16.0 software. Significant difference was taken as $p < 0.05$.

Results

The results obtained from the study show that Gram-negative enterics represented the largest group (30.5%) of the bacterial isolates, followed by Gram-positive non-spore forming bacilli (30%), Gram-positive cocci (27.2%), Gram-positive spore formers (11.2%), and coccobacilli (1.2%). *Plasmodium falciparum* was the only malaria parasite that was seen in all preparations. Forty-four of the 74 pregnant women were HIV positive, while the control group included 30 pregnant HIV-negative women. The HIV-positive and HIV-negative groups of women were each subdivided into two subgroups – those with and those without malaria parasitemia. Table 1 and Figure 1 show the four subgroups by number and percentage, as well as the distribution of bacterial isolates in each subgroup. Based on cohort size distribution, the HIV+Mal+ subgroup was expected to have 46% of the bacterial isolates. Instead, it accounted for 60.2% of the total bacterial isolates. The expected distribution of bacterial isolates in the HIV-Mal- group was 16%, compared to a significantly lower observed distribution of just 7.7%. Table 1 also shows that the HIV+Mal+ women had the highest number of bacterial isolates, with an average of 4.35 different organisms in their urine samples, compared to 3.10 for the HIV+Mal- group, 2.67 for the HIV-Mal+ group, and a mean of 1.58 different organisms for the HIV-Mal- pregnant women. These results show that the mean number of different organisms isolated in the urine samples of HIV+ pregnant women was twice the number for HIV-Mal+ women and thrice the number for the HIV-Mal- control pregnant women, thereby suggesting a role for HIV and malaria infections in selectively exacerbating the number of diverse organisms in the UTIs of pregnant women. A calculated odds ratio of 1.89 indicates that HIV and

Table 1. Distribution of bacterial isolates among the four subgroups of pregnant women in Akure, Nigeria

		No. (%) of bacterial isolates		
Study groups	No. (%)	Expected no. (%)	Observed no. (%)	Mean isolates/ subject
HIV+Mal+	34 (46)	113 (46)	148 (60.2)	4.35
HIV+Mal-	10 (14)	35 (14)	31 (12.6)	3.10
HIV-Mal+	18 (24)	59 (24)	48 (19.5)	2.67
HIV-Mal-	12 (16)	39 (16)	19 (7.7)	1.58
Total	74 (100)	246 (100)	246 (100)	3.32

HIV: human immunodeficiency virus; Mal: malaria

Table 2. Selective impact of HIV or malaria alone, HIV and malaria co-infection in relation to bacterial isolates at each trimester of pregnancy among subjects in Akure, Nigeria

	Experimental groups					Control groups				
	HIV+Mal+			HIV+Mal-		HIV-Mal+			HIV-Mal-	
Trimester of pregnancy	Total No. of subjects screened	No. of subjects involved	No. of bacterial isolates cultured	No. of subjects involved	No. of bacterial isolates cultured	Total No. of subjects screened	No. of subjects involved	No. of bacterial isolates cultured	No. of subjects involved	No. of bacterial isolates cultured
1st trimester	**4**	2	7	2	18	**2**	0	0	2	4
2nd trimester	**10**	9	40	1	6	**16**	11	28	5	13
3rd trimester	**30**	23	80	7	22	**12**	7	16	5	12
Total	**44**	**34**	**127**	**10**	**46**	**30**	**18**	**44**	**12**	**29**

malaria co-infection significantly increased the risk of UTIs. The selective impact of HIV infection or malaria alone and HIV and malaria confection in relation to the number of bacterial isolates in each trimester of pregnancy was also determined (Table 2). Altogether, 173 bacterial isolates were cultured from subjects in the two experimental subgroups, while 127 isolates came from 34 HIV+ Mal+ subjects, and 46 isolates came from 10 HIV+Mal- subjects. The resulting mean values for HIV+ Mal+ and HIV+ Mal- were 3.73 and 4.6, respectively. In contrast, a total of 73 isolates were cultured from urine of 30 subjects in the control group, while 44 different isolates came from 18 HIV-Mal+ subjects and 29 isolates came from 12 HIV-Mal+ subjects. The mean value for HIV-Mal+ was 2.44, and 2.41 for HIV-Mal- subjects. A calculated odds ratio of 1.82 also confirmed that HIV and malaria co-infection also increased the risk of UTIs (Table 2).

The distribution of Gram-positive and Gram-negative bacterial isolates from the four subgroups of pregnant women is presented in Tables 2 and 3, respectively. Table 3 shows that various species of *Staphylococcus* (35%), *Bacillus* (17%), *Corynebacterium* (17%), *Arcanobacterium* (9%), *Listeria* (8%), and *Lactobacillus* species (8%) were the major groups of Gram-positive bacteria isolated from the urine cultures of the 74 pregnant women. About 58% of *S. aureus*, 70% of *S. saprophyticus*, and 78% of *S. haemolyticus* were cultured from HIV+Mal+ women. The HIV+Mal+ women also accounted for 68% of all staphylococcal isolates, 80% of *Bacillus subtilis*, 71% of *Listeria* isolates, and 45% of *Corynebacterium urealyticum*. Table 3 also shows that 67% of *E. coli*, 60% of *P. aeruginosa*, and 100% of *P. fluorescens* also came from the HIV+Mal+ pregnant women. Coagulase-negative staphylococci made up 61.2% of the staphylococci. However, *S. aureus* alone accounted for 38.8% of all staphylococci, making it the predominant organism isolated from the urine of all the study participants. It is interesting to note that some bacterial isolates were found to colonize HIV+Mal+ subjects exclusively. These included *Staphylococcus* spp., *Streptococcus pyogenes*, *Micrococcus luteus*, *Enterococcus faecalis*, and *Lactobacillus* spp. among Gram-positive organisms. In contrast, *Pseudomonas fluorescens* was the only Gram-negative enteric found to colonize HIV+Mal+ subjects exclusively. The reason for this selective colonization is not readily apparent, but it could be associated with depression of innate immunity.

The antibiotic resistance profiles of the predominant isolates are presented in Table 3. Of the 15 *S. aureus* isolates cultured from HIV+ Mal+ women, 93.3% were resistant to cloxacillin, 80% to augmentin and erythromycin each, and 60% to cotrimoxazole, suggesting the *in vitro* ineffectiveness of the penicillin, macrolide, and trimethoprim antibiotics. Of the seven isolates of *S. haemolyticus* tested, all were resistant to cloxacillin, augmentin, and erythromycin; six were resistant to streptomycin, tetracycline, and cotrimoxazole. The aminoglycoside streptomycin, but not gentamycin, was relatively effective against *S. aureus*; only 20% of the isolates showed resistance to the antibiotic. About 83% to 89% of *B. subtilis* isolates from HIV+Mal+ women were resistant to the two beta-lactam antibiotics, but were susceptible to tetracycline and the two aminoglycosides. Likewise, almost all of *C. urealyticum* and *L. monocytogenes* isolates from the same HIV-positive women were resistant to augmentin and oxacillin beta-lactam antibiotics. Table 4 also shows that all *E. coli*, *P. aeruginosa*, *P. fluorescens*, and *P. mirabilis* isolates were susceptible to gentamycin and nitrofuran, indicating the effectiveness of gentamycin in selectively treating UTIs caused by the four Gram-negative organisms.

Figure 1. Distribution of bacterial isolates cultured from urine samples of the four subgroups of pregnant women in the study

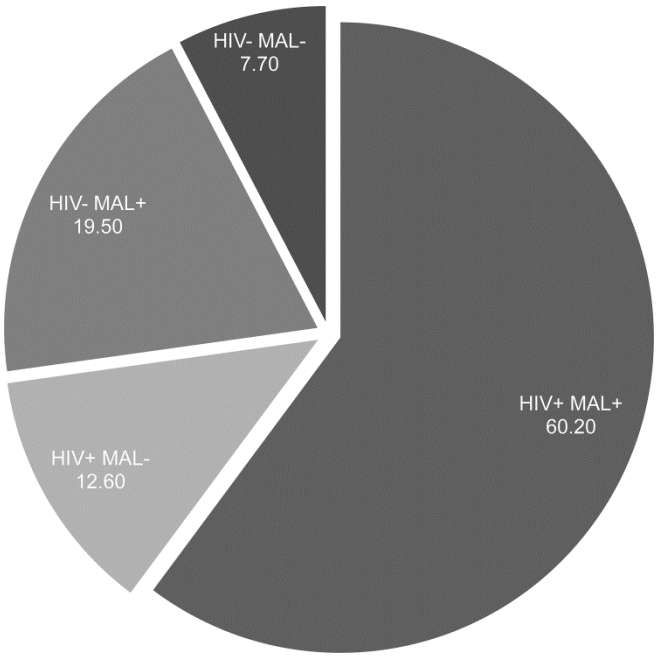

Table 3. Profile of Gram-positive bacterial isolates in urine samples of pregnant HIV seropositive and control subjects

Types of bacteria	Number of bacteria isolates	No. (%) of isolates in subgroups of study participants			
		HIV+Mal+ No. (%)	HIV+Mal- No. (%)	HIV-Mal+ No. (%)	HIV-Mal- No. (%)
Staphylococcus aureus	26	15 (57.7)	2 (7.7)	7 (26.9)	2 (7.7)
Staphylococcus saprophyticus	10	7 (70)	3 (30)	0	0
Staphylococcus haemolyticus	9	7 (77.7)	1 (11.1)	1 (11.1)	0
Staphylococcus epidermidis	5	4 (80)	1 (20)	0	0
Staphylococcus lentus	3	1 (33.3)	1 (33.3)	1 (33.3)	0
Staphylococcus sp.	8	8 (100)	0	0	0
Streptococcus pyogenes	1	1 (100)	0	0	0
Micrococcus luteus	3	3 (100)	0	0	0
Enterococcus faecalis	4	4 (100)	0	0	0
Rhodococcus equi	3	2 (66.7)	1 (33.3)	0	0
Bacillus subtilis	24	18 (80)	5 (13.3)	1 (6.7)	0
Bacillus cereus	4	2 (50)	1 (25)	1 (25)	0
Corynebacterium urealyticum	24	11 (45.8)	1 (4.2)	9 (37.5)	3 (12.5)
Arcanobacterium heamolyticum	15	8 (53.3)	2 (13.3)	4 (26.7)	1 (6.7)
Corynebacterium riegelii	5	2 (40)	0	2 (40)	1 (20)
Listeria monocytogenes	14	10 (71.2)	3 (21.4)	1 (7.1)	0
Lactobacillus acidophilus	10	3 (30)	2 (20)	4 (40)	1 (10)
Lactobacillus sp.	3	3 (100)	0	0	0

HIV: human immunodeficiency virus; Mal: malaria

Table 4. Profile of Gram-negative bacterial isolates in urine samples of pregnant HIV-seropositive and control subjects

Types of bacteria	Number of bacteria isolates	Subgroups of pregnant women			
		HIV+ Mal+ No. (%)	HIV+Mal- No. (%)	HIV-Mal+ No. (%)	HIV-Mal- No. (%)
Eschericia coli	15	10 (66.7)	3 (20)	1 (6.7)	1 (6.7)
Klebsiella pneumoniae	5	2 (40)	0	1 (20)	2 (40)
Klebsiella oxytoca	3	1 (33.3)	1 (33.3)	0	1 (33.3)
Citrobacter freundii	3	1 (33.3)	0	0	2 (66.6)
Proteus mirabilis	11	4 (36.4)	1 (9.1)	4 (36.4)	2 (18.2)
Proteus vulgaris	3	1 (33.3)	0	1 (33.3)	1 (33.3)
Salmonella typhi	4	1 (25)	0	1 (25)	2 (50)
Salmonella typhimurium	3	0	0	3 (100)	0
Pseudomonas aeruginosa	15	9 (60)	2 (13.3)	4 (26.7)	0
Pseudomonas fluorescens	8	8 (100)	0	0	0
Providencia rettgeri	3	2 (66.7)	0	1 (33.3)	0
Serratia marcescens	2	1 (50)	1 (50)	0	0
Total	75	40 (53)	8 (11)	16 (21)	11 (15)

HIV: human immunodeficiency virus; Mal: malaria

Discussion

The goals of this study were to determine the frequency of urinary tract infections in a study cohort of HIV-positive and HIV-negative pregnant women with malaria co-infection, to identify the causative organisms associated with their UTIs, and to determine the antibiotic resistance patterns of these organisms. An additional goal was to determine the selective impact of HIV, malaria, and HIV/malaria co-infection on the pathogenesis of UTIs in pregnant women. The study cohort of 74 women came from 3,225 pregnant women who attended the antenatal clinic of the Ondo State Specialist Hospital in Akure, south-western Nigeria, between August 2012 and May 2013. Testing revealed 114 women were HIV positive, indicating a prevalence rate of 3.5%, which was significantly higher than the rate of 2.3% reported for the population of Ondo State in 2011 by the Ondo State Agency for the Control of AIDS. Our study comprised 74 pregnant women who met the criteria for participation, 44 of whom were HIV positive and 30 who were HIV-negative control subjects. Among the 44 HIV-positive women with UTIs, 34 (77.3%) were co-infected with malaria and the remaining 10 (22.7%) were free of malaria. Among the 30 pregnant control women, 18 (60%) were HIV negative but infected with malaria, while 12 (40%) were HIV negative and uninfected with malaria. UTIs were diagnosed in all 44 HIV-positive pregnant women, but only in 89% and 92%, respectively, of the HIV-Mal+ and HIV-Mal- control groups. Although *Bacillus* spp. are considered non-pathogenic, a few studies have isolated them in episodes of UTIs and fatal septicemia [15-17]. These findings suggest that isolation of these organisms in immunocompromised hosts may not always be assumed as contaminants. This may be the case in our study, where 93.3% of the total number of *B. substilis* isolates were cultured from HIV+Mal+ (80%) and HIV+ Mal- (13.3%) subjects. In addition, three of the four *B. cereus* isolates were similarly cultured from these subgroups, respectively (Table 3), most probably due to depression of both innate and cell-mediated immunity as a result of HIV and malaria co-infection. Our study also shows that HIV-positive pregnant women had a higher frequency of UTI infections than did the HIV-negative pregnant women, and that co-infection with malaria also increased the number and diversity of organisms that were responsible for their UTIs. (Tables 3 and 4). While the knowledge of complications of HIV and malaria co-infection and the consequences of these interactions are still poorly understood, many studies have associated pathogenesis in the patient with the physiological impact of HIV infection, including depletion of CD4+ T lymphocytes and the aggravation of immune complexes that put the subject at risk for opportunistic infections and malignancy [18]. Some pro-inflammatory cytokines have been shown to play an important role both in control and pathogenesis of HIV infection. During HIV infection, it has been shown that viral particles are taken up by antigen presenting cells, specifically macrophages and, to a lesser extent, dendritic cells and B lymphocytes, which are recognized by CD4+ T lymphocytes, causing activation and release of IL-2 and IFN-gamma [19]. These pro-inflammatory cytokines in turn stimulate CD8+ lymphocytes, which control viremia. In our companion study carried in the same institution, the mean CD4+ T lymphocyte count decreased significantly, while HIV plasma load increased in HIV+Mal+ pregnant subjects [20]. HIV also has been shown to up-regulate adhesion molecules on endothelial cells, which may compound the adherence and sequestration seen in malaria [21]. In addition, HIV may also put more pregnancies at risk of complications associated with malaria given the lack of gravidity-specific protection against malaria seen with HIV infection in pregnant women. Sera analyzed by a flow cytometer contained few antibodies to VSAs in both placental and pediatric isolates of malaria than the sera obtained from HIV-uninfected mothers, underscoring the combined immunosuppressive effect of HIV and malaria co-infection [22]. Studies from Cameroon in West Africa [23,24] reported that malaria infection during pregnancy increased the risk of mother-to-child transmission of HIV. The findings of these studies suggest that the increased risk of HIV may be due to a mechanism whereby *P. falciparum* adhesion to chondroitin sulphate A on human placenta cells increases HIV-1 replication in those cells, possibly by TNF-alpha stimulation.

It is therefore plausible that the diversity and preponderance of pathogens and opportunists displayed in this study (Tables 3 and 4) could also be due to elaboration of virulence factors such as beta-lactamases, DNase, RNase lipase, and protease by *S. aureus* strains and other bacteria, which enabled these organisms to proliferate alongside opportunists such as *Staphylococcus* spp. and *Micrococcus luteus* and to thrive and flourish exclusively in HIV+Mal+ subjects and not in control HIV-Mal- subjects.

Table 5. Pattern of antibiotic resistance of bacterial isolates cultured from urine samples of HIV-positive malaria-infected pregnant women

Bacteria	Bacteria species	Total no. of strain	β-lactams			Aminoglycosides		Tetracycline	Nitrofurantoin	Macrolides	Quinolones		Chloramphenicol	Trimethroprim
			AUG	CXC	AMX	STR	GEN	TET	NIT	ERY	NAL	OFL	CHL	COT
Gram-positive cocci														
	Staphylococcus aureus	15	12 (80)	14 (93.3)	ND	3 (20)	5(33.3)	8 (53.3)	ND	12 (80)	ND	ND	4 (26.7)	9 (60)
Coagulase negative Staphylococci														
	Staphylococcus saprophyticus	7	2 (28.6)	5 (71.4)	ND	2 (28.6)	0	1 (14.3)	ND	3 (42.9)	ND	ND	3 (42.9)	6 (85.7)
	Staphylococcus haemolyticus	7	7 (100)	7 (100)	ND	6 (85.7)	5 (71.4)	6 (85.7)	ND	7 (100)	ND	ND	4 (57.1)	6 (85.7)
	Staphylococcus epidermidis	4	4 (100)	4 (100)	ND	3 (75)	2 (50)	3 (75)	ND	4 (100)	ND	ND	1 (25)	3 (75)
	Staphylococcus cohnii	2	2 (100)	2 (100)	ND	0	0	2 (100)	ND	1 (50)	ND	ND	0	2 (100)
	Staphylococcus lentus	1	1 (100)	1 (100)	ND	1 (100)	0	1 (100)	ND	1 (100)	ND	ND	1 (100)	0
	Staphylococcus sciuri	1	1 (100)	1 (100)	ND	1 (100)	0	0	ND	1 (100)	ND	ND	1 (100)	1 (100)
	Staphylococcus xylosus	1	1 (100)	1 (100)	ND	1 (100)	0	1 (100)	ND	1 (100)	ND	ND	1 (100)	1 (100)
	Staphylococcus capitis	1	1 (100)	1 (100)	ND	1 (100)	1 (100)	1 (100)	ND	1 (100)	ND	ND	1 (100)	1 (100)
	Staphylococcus schleiferi	1	1 (100)	1 (100)	ND	-	0	0	ND	0	ND	ND	1(100)	1 (100)
Streptococci														
	Streptococcus pyogenes	1	1 (100)	1 (100)	ND	0	1 (100)	1 (100)	ND	1 (100)	ND	ND	1 (100)	1 (100)
Enterococci														
	Enterococcus faecalis	4	1 (25)	4 (100)	ND	2 (50)	0	3 (75)	ND	3 (75)	ND	ND	3 (75)	
Micrococci														
	Micrococcus luteus	3	2 (66.7)	3 (100)	ND	0	0	2 (66.7)	ND	2 (66.7)	ND	ND	1 (33.3)	1 (33.3)
coccobacilli														
	Rhodococcus equi	3	1 (33.3)	1 (33.3)	ND	0	0	0	ND	1 (33.3)	ND	ND	0	0
Bacilli														
	Bacillus subtilis	12	10 (83.3)	10 (83.3)	ND	0	0	2 (16.7)	ND	5 (41.7)	ND	ND	0	6 (50)
	Bacillus anthracis	1	1 (100)	1 (100)	ND	1 (100)	0	1 (100)	ND	1 (100)	ND	ND	1 (100)	1 (00)
	Bacillus cereus	6	6 (100)	6 (100)	ND	3 (50)	0	3 (50)	ND	3 (50)	ND	ND	0	3 (50)

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Table 5. ***(continued)*** Pattern of antibiotic resistance of bacterial isolates cultured from urine samples of HIV-positive malaria-infected pregnant women

Bacteria	Bacteria species	Total no. of strain	β-lactams			Aminoglycosides		Tetracycline	Nitrofurantoin	Macrolides	Quinolones		Chloramphenicol	Trimethroprim
			AUG	CXC	AMX	STR	GEN	TET	NIT	ERY	NAL	OFL	CHL	COT
Gram-positive rods														
	**Corynebacterium urealyticum*	11	9 (900)	10 (100)	ND	5 (50)	1 (10)	6 (60)	ND	8 (80)	ND	ND	4 (40)	4 (40)
	**Arcanobacterium haemolyticum*	8	6 (85.7)	6 (85.7)	ND	0	0	2 (28.6)	ND	4 (57.1)	ND	ND	2 (28.6)	4 (57.1)
	Corynebacterium reigelii				ND				ND		ND	ND		
	Listeria monocytogenes	10	10 (100)	10 (100)	ND	1 (10)	1 (10)	6 (60)	ND	4 (40)	ND	ND	0	1 (10)
	Lactobacillus acidophilus	3	2 (66.7)	2 (66.7)	ND	0	9 (66.7)	1 (33.3)	ND	1 (33.3)	ND	ND	1 (33.3)	1 (33.3)
	Lactobacillus spp.	3	19 (33.3)	1 (33.3)	ND	1 (33.3)	0	1 (33.3)	ND	1 (33.3)	ND	ND	0	1 (33.3)
Gram-negative rods (enterics)														
Lactose fermenters														
	Escherichia coli	10	6 (60)	ND	7 (70)	ND	1 (10)	5 (50)	0	ND	6 (60)	1 (10)	ND	4 (40)
	Klebsiella pneumonia	2	1 (50)	ND	1 (50)	ND	0	1 (50)	0	ND	0	0	ND	1 (50)
	Klebsiella oxytoca	1	1 (100)	ND	1 (100)	ND	0	1 (100)	0	ND	1 (100)	0	ND	1 (100)
	Citrobacter freundii	1	0	ND	0	ND	0	0	0	ND	1 (100)	0	ND	0
Non-lactose fermenters														
	Pseudomonas aeruginosa	9	6 (66.7)	ND	6 (66.7)	ND	0	5 (55.6)	2 (22.2)	ND	4 (44.4)	0	ND	6 (66.7)
	Pseudomonas fluorescens	8	5 (62.5)	ND	6 (75)	ND	1 (12.5)	2 (25)	2 (25)	ND	8 (100)	2 (25)	ND	4 (50)
	Proteus mirabilis	4	4 (100)	ND	4 (100)	ND	0	4 (100)	0	ND	1 (25)	0	ND	4 (100)
	Providencia rettegeri	2	2 (100)	ND	2 (100)	ND	1 (50)	2 (100)	1 (50)	ND	1 (50)	1 (50)	ND	2 (100)
	Proteus vulgaris	1	1 (100)	ND	1 (100)	ND	0	1 (100)	1 (100)	ND	0	0	ND	1 (100)
	Salmonella typhi	1	1 (100)	ND	1 (100)	ND	0	0	0	ND	0	0	ND	0
	Serratia marcescens	1	1 (100)	ND	1 (100)	ND	0	1 (100)	0	ND	0	0	ND	0

AUG: augmentin; GEN: gentamicin; NAL: nalidixic acid; TET: tetracycline; COT: cotrimoxazole; CXC: cloxacillin; AMX; amoxicillin; NIT: nitrofurantoin; STR: streptomycin; ERY: erythromycin; CHL: chloramphenicol; OFL: ofloxacin; Number of strain not tested: *1; **2; ND: not determined

The ineffectiveness of several of the antibiotics employed against the bacterial agents as well as the high rates of resistance recorded in the study (Table 5) were therefore not unexpected because of the destruction of the molecular structure of the antibiotics by extracellular enzymes (Table 5).

We note with interest that 148 (60.2%) of the bacterial isolates were cultured from HIV-positive malaria-infected subjects, compared to 31 isolates (12.6 %) in HIV-negative malaria-uninfected subjects. Studies have shown that HIV infects and decimates CD4+ cells, thus increasing the probability of reactivation of dormant and opportunistic infections such as cytomegalovirus and *Toxoplasma gondii.* HIV causes apoptosis of immune cells and decreases expression of pro-inflammatory cytokines such as IL-12 and IFN-gamma. On the other hand, HIV infection increases expression of IL-10, which impairs CD4+ T lymphocytes [25]. Such impairment of adaptive immunity contributes to susceptibility to malaria infection and UTIs. In a study of UTIs and antibiotic sensitivity in Khartoum, Sudan, Hamdan *et al.* [26] found that *E. coli* and *S. aureus* were the most prevalent bacterial isolates from 235 pregnant women, with the two organisms also displaying resistance to several antibiotics, a phenomenon replicated in our study. Another study by Alemu *et al*. (2012) [27] in Ghondar, Ethiopia, similarly found that *E. coli* and *S. aureus* were the predominant isolates in pregnant women with UTIs. Additional studies in Ibadan, Nigeria, showed *E. coli*, *Proteus*, *Klebsiella*, and *S. aureus* as the predominant associated organisms with UTIs in HIV-positive pregnant women [28]. The investigators found that the CD4+ cell counts were significantly lower, but that the HIV plasma viral loads were higher in HIV-positive pregnant women with bacteriuria than in those without bacteriuria. Similar results were reported by Brown *et al*. [29] in a study of HIV-positive pregnant women in Kenya. In Ghana, Boaitey *et al*. (2012) [30] reported that 35% of 500 HIV-positive individuals carried gastrointestinal parasites *Giardia lamblia* and *Cryptosporidium*, compared to 4.3% of HIV-negative individuals, underscoring the vulnerability of HIV-positive pregnant women to opportunistic parasites in general. Their study also demonstrated that of the 258 HIV-positive patients who presented with diarrhea and fever, 60 (23.3%) had bacteriuria with predominantly *E. coli* and *S. aureus*. These studies collectively suggest that HIV is a significant risk factor for susceptibility to UTIs.

The major strengths of our study include the demonstration of the selective individual impact of HIV and falciparum malaria on the increased development of UTIs in pregnancy, the isolation of increased numbers of UTI-associated bacterial organisms, and the increased resistance of these organisms to antibiotics. It is expected that the results of this study would lead to better management of UTIs in pregnancy and provide appreciation for increased level of UTI pathogenesis in HIV/malaria co-infection, because UTIs can lead to adverse pregnancy outcomes such as low birth weight, premature birth, and other serious complications [31].

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