

# Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363 Cross Ref DOI: 10.30574/msabp Journal homepage: https://magnascientiapub.com/journals/msabp/



(RESEARCH ARTICLE)

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# Magnitude of antibiotic resistant bacterial colonization of women attending HIV clinic in Lagos, Nigeria

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Magna Scientia Advanced Biology and Pharmacy, 2022, 05(02), 001-008

Publication history: Received on 17 February 2022; revised on 20 March 2022; accepted on 22 March 2022

Article DOI: https://doi.org/10.30574/msabp.2022.5.2.0055

## Abstract

Opportunistic infections due microorganisms particularly bacteria have posed a threat and thus an addition burden on the management of human immunodeficiency virus (HIV) infections worldwide. The high prevalence of bacterial pathogens and the emergence of multiple drug resistance among such bacteria call for serious concern. Bacteriological examination of 320 women attending HIV clinics was carried out using standard microbiological techniques. The antimicrobial susceptibility testing was carried out by the disc diffusion technique by Bauer and CLSI. Of the 320 women, 200 (62.5%) had bacteria, 120 women were confirmed as HIV positive. Ninety (75%) of these women had both HIV-1 and HIV-2, twenty (16.7%) had only HIV-1 and 10 (8.3%) had only HIV-2. *Staphylococcus aureus* and *Escherichia coli* were the predominant bacteria. Antibiogram of the bacterial isolates determined by using the Kirby Bauer disk diffusion showed that most of them were susceptible to augmentin, gentamicin, peflacin, ciprofloxacin, ofloxacin and amoxicillin but were highly resistant to commonly used, over-the-counter drugs like chloramphenicol, tetracycline and streptomycin. This thus poses additional burden on the healthcare system in its attempt to manage HIV using anti-retroviral drugs among other methods, along with immunity enhancing drugs while the search for HIV vaccines still continues.

Keywords: Bacteriological; Staphylococcus aureus; Escherichia coli; HIV-1 &-2; Speculum examination

### 1. Introduction

Human immunodeficiency virus/Acquired Immuno-deficiency Syndrome (HIV/AIDS) is a major public health challenge facing the whole world with about 33 million adults and children living with the virus worldwide [1, 2]. Most studies on HIV/AIDS infections in sub-saharan Africa have been concentrated in the eastern and central Africa perhaps due to the preponderance of the infection in these parts of Africa [2].

HIV continues to be a major global health issue, having claimed 36.3 million lives, and as at the end of 2020, 37.7 million people were living with HIV, over two thirds of whom live in Africa [3].

Microbial infections in less favoured areas account for more than 90% of infections or communicable diseases and they are the second leading cause of over 17 million death worldwide [2, 3]. Most microbial infections are caused by bacteria

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generally but particularly in HIV positive individuals in all age groups but particularly in HIV positive individuals in all groups but more in such persons because they are already immuno-compromised. The weakened immune system inadvertently subject such individuals vulnerable and victims of opportunistic infections, thereby leading to medical abuse [4]. Two serovars of HIV (HIV-1 and HIV-2) have been reported in people from over ten West African countries as well as in Europe, the United States and Brazil [5]. The epidemiology and pathogenicity of HIV infection by these serovars is well understood due to recent advances in molecular biology [6].

The females are more vulnerable than males to many genital microbial infections due to the moist and warm vaginal environment which provides a favorable and hospitable location for microorganisms [7].

The vaginal opening which is unprotected by a sphincter, leaves the vagina vulnerable to the introduction of pathogens and potentially pathogenic microorganisms especially by the insertion of an infected penis. In sexually active women, similarly the close proximity of the anus to the vagina encourages accidental transfer of rectal flora the vulva and in the vagina [8].

Individuals with HIV infection and AIDS are susceptible to infections and malignancies that are called "opportunistic infections" because of the opportunity offered by a weakened immune system [9].

Infections are the important cause of morbidity and mortality in HIV-infected individuals in both developed and developing countries and bacterial infections especially pneumonia, occurs at high rates where there is no severe CD4 cells depletion [10]. HIV associated immunosuppression increases the vulnerability of patients to various infections, particularly enteric bacterial pathogens such as *Salmonella* sp., *Shigella* sp., *Clostridium difficile, Campylobacter* and different strains of *E.coli*. According to Lucejiko et al. [11], tuberculosis occurs in about 30% of HIV patients and causes approximately 25% deaths due to AIDS globally. There is a high rate of sexually transmitted infections (STIs) among people living with HIV, which has been associated with increased viral viral load even in patients on highly active antiretroviral therapy [12]. The most frequently observed sexually transmitted infections in HIV-infected persons includes *Chlamydia trachomatis, Treponema pallidium* and *Neiserria gonorrhoeae* [9]. Other bacterial pathogens in HIV patients include *Streptococcus pneumoniae*, methicillin resistance *Staphylococcus aureus* (MRSA), *Helicobacter gastritis, Helicobacter pylori*, and especially genital *Mycoplasma* which are often effusive or subclinical in nature [13].

The emergence of antibiotic resistant bacterial pathogens has become a major public health concern by making treatment of such infections using more expensive and less successful [14].

In this study, we isolated, identified and characterized enteric bacterial pathogens and investigated their antibiotic susceptibility profiles from HIV positive women attending HIV Clinics using standard microbiological techniques.

# 2. Material and methods

### 2.1. Study design

This is a cross-sectional study carried out from Jan 2014 to Dec 2016 at Iris Medical and Scan Centre, Ikeja on women who were confirmed of HIV.

### 2.2. Study population

This study included women diagnosed of HIV/AIDS who were on a regular consultation. Four hundred and twenty patients which included three hundred and twenty women confirmed of HIV and hundred HIV-negative women has medical laboratories and scan center were enrolled in this study after informed consent from January 2014 to December2016. Patients, whose physicians prescribed high vaginal examination and have not received any specific antibacterial therapy in the previous two weeks were included in this study. Excluded from this study were pregnant women, diabetic patients, victims of burns, patients receiving oestrogen and smokers.

#### 2.3. HIV Testing

Screening for HIV sero-status was performed using oral quick HIV (Oral Sure Technologies, USA) test kits and described by the manufacturer and as previously reported by Nkenfou et al. 2013.

#### 2.4. Blood and stool samples collection

The stool samples were collected in sterile containers by taking all precautions to avoid contamination, while the blood samples were collected by venepuncture with the aid of sterile syringes into two tubes, one containing anticoagulant (EDTA) and another without anticoagulant HVS was collected by the gynaecologist with the aid of a sterile speculum into who a sterile swab stick was inserted.

#### 2.5. Measurement of CD4 T-cell and Hs-CRP

The CD4 lymphocytes were measured using cytometry technique by a flux cytometry (Apogee Flow Systems, Hertfordshire, United Kingdom). High sensitivity C-reactive protein (hs-CRP) was measured using ELISA solid phase direct sandwhich method (Sigma Aldricti, St Louis, USA) with ELx808<sup>™</sup> Microplate reader (BioTek Instrument, Winooski, USA).

#### 2.6. White blood Cell (WBC) Count

Blood collected in EDTA tubes were greatly agitated to avoid the formation of clots. Each samples was then introduced into a cellular counter (MondayPE 6800R, PROCAN, China, Mainland). The automat calculated and automatically reported white blood cell, lymphocyte, granulocyte and monocyte counts.

#### 2.7. Bacterial isolation and identification

A single stool and high vaginal swab (HVS) samples were collected aseptically from each participant using sterile and disinfectant free containers. Stool and HVS specimens were taken to the Microbiology Laboratory in the Lagos State University, Lagos within 1 hour of collection. The stool and HVS specimen were inoculated into buffered peptone water (BPW) for 18-24 h at 37 °C, after which they were cultured on blood agar (BA), chocolate agar (CA), MacConkey agar (MCA), eosin methylenev blue (EMB) and mannitol salt aagar (MSA). All inoculated agar plates were incubated aerobically while CA plates were incubated in a carbon extinction jar anaerobically at 37 °C for 24 h. The isolated organisms were identified based on colonial morphology and Gram stain and using API 20E galleries (Biometrieux, Lyon, France) as described by the manufacturer [15].

#### 2.8. Antibiotic Sensitivity Testing

Antibiotic susceptibility tests on the bacterial isolates were carried out by the Kirby-Bauer disc difussion method (Bauer et al., 1966), and the antibiotics tested included Penicillin (10  $\mu$ g), Amoxicillin (30  $\mu$ g), chloramphenicol (50  $\mu$ g), erythromycin (15  $\mu$ g), gentamicin (10  $\mu$ g), tetracycline (30  $\mu$ g), streptomycin (30  $\mu$ g), erythromycin (10  $\mu$ g), augmentin (30  $\mu$ g), tetracycline (30  $\mu$ g) and peflacin (30  $\mu$ g). Briefly, the test isolate was emulsified in peptone until the turbidity was similar to that 0.5% McFarland standard. A sterile cotton swab was dipped into the suspension and swabbed evenly across the entire surface of the agar plate in order to obtain a semi-confluent growth. After incubation, the zones of inhibition around the antibiotic disc were measured and interpreted based on the breakpoint criteria of the clinical and laboratory institute [16].

#### 2.9. Statistical Analysis

The mean values of data obtained were analyzed statistically, using analysis of variance (ANOVA) at 5% level of significance with the aid of the statistical package for social sciences (SPSS) v17.0.

### 3. Results

**Table 1** Incidence of bacteria in HIV Positive and negative women

Bacteria	Occurrence (%)	Positive (%)	Frequency (%)	
Staphylococcus aureus	39(37.5)	30(28.9)	9(8.7)	
Escherichia coli	22(21.2)	20(19.2)	2(1.9)	
Streptococcus pneumoniae	18(17.3)	12(11.5)	6(5.8)	
Klebsiella spp.	15(14.4)	12(11.5)	3(2.9)	
Proteus spp.	10(9.6)	7(6.7)	3(2.9)	
Total	104(100)	81(77.9)	23(22.1)	

Out of 104 bacterial isolates recovered from the HIV positive women, 39(37.5%) isolates were *Staphylococcus aureus*, 22(21.2%) were *Escherichia coli*, 18(17.33%) were *Streptococcus* species and *Proteus* species 10(9.6%) were the least frequency of occurrence. Thirty (6.9%) of the *S. aureus* were from HIV positive and nine (23%) from HIV negative women, 20(90.9%) *E. coli* were from HIV positive and 2(9.1%) from HIV negative women, twelve and 6 streptococci were HIV positive and HIV negative women respectively (Table 1).

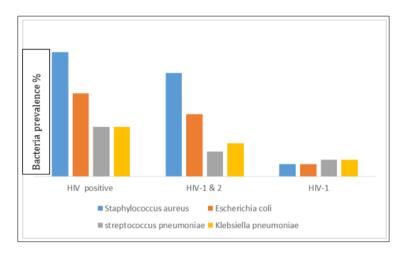


Figure 1 Distribution of Bacteria among HIV positive women

Figure 1 shows the distribution of bacteria among the HIV sero-groups (HIV-1 and HIV-2). Majority of the bacteria were recovered from women who have both HIV-1 and HIV-2 while those with HIV-1 and HIV-2 alone had fewer bacteria. Twenty five S. *aureus*, 15 *E. coli*, 8 *Klebsiella* spp. and 4 *Proteus* spp. were from those with dual HIV sero-groups and 2 *Klebsiella* spp. were recovered from those with HIV-2 and only 2 of all the bacteria but one *Proteus* spp. were recovered from those who had HIV-2 (Figure 1).

A high prevalence of co-infection between bacteria and *Trichomonas vaginalis* was observed. Of the 117 microbial pathogens recovered from the women, co-infection between bacteria, *Candida* spp. and *Trichomonas vaginalis* of 22(15.8%) was observed (Figure 2).

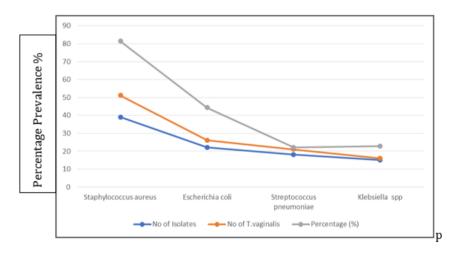


Figure 2 Incidence of co-infection, with bacteria and T. vaginalis

Table 2 shows high susceptibility to augmentin, gentamycin, peflacin, ciproflaxin, ofloxacin and amoxicillin while relatively resistance to chloramphenicol, erythromycin, tetracycline and streptomycin.

Analysis of cross-resistance results revealed that most of the bacterial isolates were multi-drug resistant and exhibited six resistance patterns to commonly used antibiotics like chloramphenicol, tetracycline, streptomycin and erythromycin (Table 3).

Bacteria Isolate	Antibiotic									
Dacteria isolate	AUG	С	Ε	TE	S	GN	PEF	СР	OFX	AMX
Staphylococcus aureus	95	0	0	0	0	70	90	85	60	60
Escherichia coli	89	0	0	0	0	94	94	94	94	78
Streptococcus pneumoniae	91	0	0	0	0	95	91	91	95	86
Klebsiella spp.	93	0	53	0	0	73	86	93	93	73
Proteus spp.	90	21	20	0	0	80	80	90	90	80

Table 2 Antibiotic Susceptibility pattern of bacterial isolates from HVS

 Table 3 Antibiotic Cross-Resistance pattern of bacteria isolated from HIV +Ve women (n=80)

Pattern	Antibiotic Resistance Patterns	Number (%)		
А	C, TE, S	22.3		
В	C, E, S	40.0		
С	C, E, TE, S	27.5		
D	ТЕ, С, Е	20.0		
E	TE, S	22.5		
F	S, E, TE	25		

Legend: C-Chloramphenicol, E-Erythromycin, S-Streptomycin, TE-Tetracycline

### 4. Discussion

The incidence of HIV in this study of 47.6% is quite high and agrees with report by UNAIDS [3], with 1.4% prevalence in 2019 and an estimated 1.9 million people living with HIV in Nigeria and that around two thirds of new HIV infections in West and Central Africa in 2019 occur in Nigeria. The high occurrence of 75% co-infection between HIV-1 and HIV-2 also agrees with WHO [2].

The predominance of *S. aureus* in the present study was consistent with a report by Adesida and Coker [18], with an incidence of 36.8% among Staphylococci recovered from HIV-infected patients in Lagos and that reported among antiretroviral naïve HIV-positive children below 5 years of age in Benin [17]. *S. aureus* has been consistently established in other studies as the predominant Gram positive agent of bacteraemic episodes and HIV-infected individuals are often at risk of various opportunistic bacterial infections, particularly the enteric bacterial pathogens [17, 18]. The occurrence of bacterial infections remains high in developing countries as a result of poverty and poor hygiene. *S. aureus* is considered as an important pathogen in HIV-infected patients with considerably high morbidity and mortality.

HIV infection is characterized by compromised immunity or immunosuppression which lowers the intestinal defense against microbes. This has thus contributed to the more likelihood of enteric bacteria as often common bacterial pathogens causing diarrhoea and some other invasive related health conditions in HIV infected patients than their healthy counterparts [19]. The rate of enteric bacterial isolates in the present study is 32%. A similar rates of 29% in India and 43.3% in South Africa were reported [19]. Lower rates had however been reported of 16% in a study in Southwest Ethiopia and 14.5% in Peru [20].

These observed differences in the rates of bacterial isolation could be due to differences in risk factors in population studies as revealed from this study where about 77.9% of the enteric bacteria were recovered from the HIV positive individuals.

Sanchez et al. (2005) 21 reported a rate as high as 80% of enteric bacterial isolates among HIV patients who had CD4<sup>+</sup> T cell count<200 cells/mm<sup>3</sup>. However, the current study showed no significant difference in the rates of bacterial

isolates among patients who had CD4<sup>+</sup>T cell count above or below 350 cells/mm<sup>3</sup> as another study in England reported 65.5% of their isolates were detected in patients with CD4<sup>+</sup>T cell count >350 cells/mm<sup>3</sup>. This may likely be due to differences in the races of the subjects for the different studies, this may be due to HIV 1&2.

Majority (71.6%) of the bacteria were isolated from patients who had both HIV-1 and HIV-2 while only 17.3% and 11.1% of the bacteria were recovered from those with only HIV-1 and HIV-2 respectively. A very high incidence of co-infection between HIV-1 and HIV-2 was observed, thus may have predisposed such women to opportunistic infections as reflected by the preponderance of *S. aureus* and enteric bacterial pathogens. High prevalence rates of *S. aureus* with *Trichomonas vaginalis* in HIV positive women reported in Africa include South Africa (9.9%), Tanzanian (24.7%) [22]. Nigeria, (27%), Kenya (34%) [23]. Our findings of 30.8% *S. aureus* with *T. vaginalis* infection is consistent with these earlier studies.

The frequency of *Streptococcus pneumoniae* among the HIV positive women was 6.0% which was relatively lower compared to earlier reports, Nigeria (10.5%), Ivory Coast (19.0%), Malawi (16.5%), Gambia (20%), Zimbabwe (20-32%) [24, 25, 26].

The low incidence of *Streptococcus* spp. corresponds with 7.0% in Korea and 5.5% in Mozambique and Togo respectively [24]. *Klebsiella* spp. (4.4%) and *Proteus* spp. (9.5%) were also isolated in this study and the incidence of these bacteria in HIV patients has been generally reported to be low [23, 24].

Increased bacterial resistance to antimicrobial agents is raising a significant public health concern worldwide. This study showed that most bacterial isolates were susceptible to augumentin, gentamicin, peflacin ciprofloxacin, ofloxacin and amoxicillin. It was observed that most bacterial isolates were resistant to chloramphenicol, erythromycin, tetracycline and streptomycin. Resistance is likely to have developed due to unrestricted, frequent and quite inappropriate usage of antimicrobials in the treatment among the study population. Most of these drugs are commonly used for empirical treatment as prophylactics before and after surgery and often through patients' own self-medication [27, 28].

Seven cross-resistance drug resistance patterns were observed. The multi-resistance among human isolates which is consistent with previous findings [19]. The challenge of antibiotic resistance among enteric bacterial pathogens typifies the growing concern among health care personnel on the continued effectiveness of antibiotics in the managements of infections. This study underscores the essence of monitoring antibiotic resistance profiles among enteric pathogens in order to provide updated data for clinicians so as to facilitate the use of appropriate and much more effective regimens in treatment.

The antimicrobial susceptibility of *S. aureus* was however, not particularly different from those in the general population in Nigeria, as revealed in this study [18, 19, 28].

# 5. Conclusion

Vaginal bacterial colonization is often concomitant with fungal infections in HIV positive women. A high level of coinfection with both HIV-1 and HIV-2 among the women resulted in very high bacterial colonization, perhaps due to the attendant immune-suppression. A high in vitro efficacy was shown by the antimicrobial agents tested, hence the bacterial isolates may likely to be opportunistic pathogens. Routine bacteriological investigation of HIV patients is therefore recommended as a compulsory test requirement for patients. The management of HIV by using appropriate anti-retroviral treatment along with immune enhancers or immune modulators and effective antimicrobial treatment would surely enhance longevity among people living with HIV.

# Compliance with ethical standards

### Acknowledgments

The authors acknowledge all the staff of Microbiology Laboratory, Lagos State University, Ojo, Lagos, Nigeria and Dr. K. A Akinsinde of Molecular Microbiology Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. We also acknowledge Mr. Chinedu Emmannuel Usuah for typing and Mr. Baite Fukpene for final corrections of the manuscript.

### Disclosure of conflict of interest.

The authors declare no conflict of interest, financial or otherwise.

#### Statement of informed consent

Verbal and written consents were taken from all the study population through the assistance of the physician in charge in accordance with the Helsinki Declaration. Patient's names were replaced with codes, age and sex were recorded. Issues of confidentiality and anonymity were also maintained.

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