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RESISTANT BACTERIAL ISOLATES AMONG HIV-SEROPOSITIVE PREGNANT WOMEN IN AKURE METROPOLIS, ONDO STATE, NIGERIA

Ako-Nai Kwashie Ajibade*[1], Makun Catherine Ifeoluwa[2] and Omo-Omorodion Blessing Itohan[3]

[1,2,3]Department of Microbiology, Obafemi Awolowo University, Ile- Ife, Nigeria.

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***Corresponding Author**
Prof. Ako-Nai Kwashie Ajibade
Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

ABSTRACT

HIV serostatus of pregnant patients recruited was determined by HIV-1/2 strip and confirmed by Abbott enzyme-linked immunosorbent assay. Quantification of CD4 and viral load for each patient was done using flow cytometry and COBAS® AmpliPrepTaqMan HIV-1 Qual test version 3.2 series 3.3, respectively at risk of Mother-to-child transmission, a major killer of the neonate early in life. Some studies have reported bacterial isolates in high vaginal swab (HVS) of HIV pregnant that are resistant to antimicrobials. Our study characterised these bacterial isolates between May 2016-November 2016 at the antenatal clinics of the State Specialist hospital in Akure metropolis, South-western Nigeria. **Methods:** Thirty- five (35) gram negative bacterial isolates were randomly selected from cultured high vaginal swabs of HIV seropositive pregnant women. Samples were collected using a sterile cotton-tipped applicator and characterised using standard microbiological methods. Antibiotics susceptibility tests of each isolate was analysed employing the Kirby-Bauer method while minimum inhibitory concentration (MIC) was carried out using the broth microdilution method. Extracellular enzymes were also evaluated using standard microbiological techniques. **Results:** All bacterial isolates tested displayed high antibiotic resistance to sulphamethoxazole/trimethoprim, amoxicillin, ampicillin, chloramphenicol and nitrofurantoin. However, 94.3% of the isolates were sensitive to ciprofloxacin. MIC concentration of the antibiotics employed ranged from 0.125mg/mL-32mg/mL underscoring the effectiveness of these antimicrobials. The results also showed exudation of varying degree of extracellular enzymes activities; on the contrary none of the isolates exhibited

DNase activity. **Conclusion:** Resistant bacteria isolates to commonly employed antimicrobials were cultured from HVS of pregnant mothers that could compromise their health. The observation could depress already immunocompromised such HIV patients thus exposing them to opportunists infections.

KEYWORDS: HIV, Pathogenic bacteria, antimicrobials, extracellular enzymes, HVS.

INTRODUCTION

The human immunodefficiency virus/ Acquired immune deficiency syndrome (HIV/AIDS) epidemic intersects with the problem of maternal mortality in many circumstancies which has ravaged humanity for the last 3-4 decades causing untold deaths to millions of humans, including the unborn, the young and the aged, (Ebhodaghe, 2017).[1] The global experience is most feasible in developing countries of Southeast Asia, sub-Saharan Africa (SSA) and the Indian sub-continent where millions died of ignorance, poverty, and lack of care, (Becquet et. al.; 2009).[2] The highest rate are still in Africa, altough prevelence in some Asian countries have risen considerably, (UNAIDS, 2012).[3] The microbiota of the female vagina is complex ecosystem harbouring diverse microbial communities. HIV/SIV infection has been shown to be associated with microbiome shift and immune activation that may affect the outcome of disease progression. It has also been reported that altered microbiome and inflammation are associated with increased risk of HIV acquisition suggesting the role of microbiome in HIV transmission and mother-to-child transmission (MTCT). Therefore, MTCT is a major concern in Nigeria which ranks highest in children acquiring HIV on the globe.

Nigeria accounts for 10% of global HIV/AIDS[4] and ranks the second country with largest burden of AIDS after South Africa[5], with about 1.72 millions of whom are women within the ages of 35-49 years mostly in their reproductive years.[5,6] This has led to a rise in the total number of children living with HIV in Nigeria.[6] Women are essentially at more risk of being infected with the virus because of biological, cultural and societal factors that remove women's control over their own bodies.[7] With over 2 million HIV-infected pregnant women each year, over 90% of them are in developing countries, while close to 600,000 women die each year from complications of pregnancy and childbirth, the majority of them also live in resource-constrained settings.[6] Studies have shown that women with HIV have a high burden of microbial load.[8] Similarly, they also have the propensity of bacterial and viral complication as a result of co-infection.[9] Studies showed that HIV pregnant women also suffer from co-infection with other viruses and bacteria that exacerbate their condition[9] and

such co-infections may significantly increase HIV plasma viral load.[8] It has been estimated that a 10-fold increase of plasma HIV RNA resulted in an 81% increased rate of HIV transmission.[11,12] Our study was initiated because of the presence of high number of pathogens from previous study recovered from high vaginal swab (HVS). We decided to characterise these bacterial isolates in order to better understand their contribution to pathogenesis. We believe that data obtained from this study will enable clinicians to evaluate the effect of pathogens in these patients thereby providing reliable that database in the system.

MATERIALS AND METHODS

Study area

Samples were obtained from HIV seropositive pregnant women attended the antenatal clinic at the Ondo State Specialist Hospital in Akure, the capital city with an estimated population of 387,087 inhabitants with 15′0″N 5 11′42″E/7.25000 N latitude 5.19500°E longitude. The study was carried out between May 2016-November 2016.

Criteria for study inclusion

Pregnant women were recruited based on attending the antenatal clinic of the hospital and were persuaded to participate in the study by the clinicians and nurses. 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.

Screening for HIV in participants

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).

Sample collection

A high vaginal swab sample was collected from the posterior fornix from each pregnant subject by the attending physician using sterile bivalve speculum (Changzhou Huankang Medical Devices Co. Ltd, Changzhou City 213116, Jiangsu Province, China) and sterile cotton-tipped applicator (Evepon, Industrial Limited, Onitsha, Anambra State, Nigeria) into freshly prepared sterile thioglycollate medium and incubated at 37^{o}C for 24 h for growth. After growth was observed, a loopful of the sample was streaked initially with the aid of

heat- flamed standard aluminum wire loop on to freshly prepared agar media plates and identified as lactose or non-lactose fermenters using eosin methylene blue (EMB) and MacConkey agar. Further speciation of isolates were based on their activities on convectional media such as triple sugar iron agar (TSI), Koser's citrate medium, sulphide indole motility agar (SIM) and urea agar (Oxoid, Basingstoke, Hampshire, England, UK) and according to methods described by Barrow and Feltham.[13] Antibiotic sensitivity tests for each bacterial isolate was carried out employing the Kirby-Bauer method. Antibiotics employed were obtained from Oxoid (Basingstoke, UK) and included amoxicillin/clavulanic acid AMC (30 µg), ampicillin AMP (10 µg), ceftriaxone CRO (30 µg), cefuroxime CXM (30 µg), chloramphenicol C (30 µg), tetracycline TE (30 µg), erythromycin ERY (15 µg), gentamycin CN (10 µg), streptomycin S (10 µg), ciprofloxacin CIP (5 µg), nitrofurantoin F (300 µg), sulphamethoxazole/ trimethoprim SXT (25 µg) and Amoxicillin AMX (10 µg). *E. coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418) *Pseudomonas fluorescens* (NCIB 3756) and *Pseudomonas aeruginosa* (NCIB 950) were used as control organisms. Minimum inhibitory concentration of each bacterial isolate was determined using the broth microdilution method in a 96 –well microwell plate (Dynatech Immulon ^{TM}, 14340 Sullyfield circle Chantilly, VA, USA) as described by CLSI.[14] One hundred microliter (100 µL) of final concentration of each antibiotic and 100 µL of standardized inoculum of each test sample was added into each well. The plates were thereafter incubated at 37^{o}C and readings were taken after 24 hrs of incubation. Wells containing only test organism without antibiotics and also antibiotics without test organism were used as controls. Results were read based on the degree of turbidity (i.e) from complete clear well to slightly turbid well while intermediate and prominent turbidity were disregarded. Determination of the minimum bactericidal concentration was conducted by introducing a loopful of clear to slightly turbid well onto the plates of freshly prepared Muller Hinton agar (MHA).

Extracellular enzymes were determined employing standard methods.

Lipase production

A loopful of standardized test inoculum was streaked onto freshly prepared tributyrin agar and plates were incubated at 37^{o}C for 24 hrs. Development of a clear zone was termed positive and the diameter recorded.

Protease production

Protease activity was assessed according to standard methods**.** Inoculum of each isolate was streaked onto gelatin medium and incubated at 37^{o}C for 72 hrs. Plates were flooded with mercury chloride and diameter measured.

Deoxyribonuclease DNase Testing

The medium employed for DNase activity was a modified version of Jeffries *et al.*[15] for suitable bacterial growth. Inoculum of each isolate was streaked onto freshly prepared DNase medium, incubated at 37^{o}C for 24 hrs after which plates were read for by flooding each plate with 1N HCl. The diameter of the clear zone around the line of streak was measured.

Urease Test

Each bacterial isolate was streaked onto freshly prepared MacConkey plates incubated at 37^{o}C for 24 hrs. A colony each was introduced into tubes containing Christensen's urea medium, incubated at 37^{o}C for 5 days. Production of red coloration in tubes was indicative of a positive reaction.

Ribonuclease (RNase) activity

Modified medium containing 1.5 g of RNA initially dissolved in 0.1M PO_4 and sterilized using 0.22nm filter, was added to freshly prepared 50^{o} C Nutrient agar. Each isolate was inoculated onto each agar plate by point inoculation and incubated at 37^{o} C for 24 hrs. When growth was observed, each plate was flooded with 3ml perchloric acid and left to stand for 1 min. plates were visualised for transparent halos around colonies against an opaque background.

RESULTS

Altogether, randomly selected 35 gram negative bacterial isolates cultured from high vaginal swabs of HIV seropositive pregnant women were analysed for their antibiotic susceptibility pattern. The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) were also determined. The results showed 94.3% of the isolates were resistant to sulphomethoxazole/trimethoprim, 85.7% to amoxicillin, 77.14% to chloramphenicol, 74.28% to nitrofurantoin. However, 94.3% of the isolates were sensitive to ciprofloxacin Table 1.

Table 2 revealed the antibiotype profile of the bacterial isolates tested. The results showed 84.61% each of the antibiotics employed were resistant to *E. coli, K. pneumoniae and P. aeruginosa* isolates, 69.23% each were resistant to *P. fluorescens* and *Citrobacter freundii.* Similarly, 38.46% were resistant to *Enterobacter aerogenes* Table 2.

The results also showed 68.6% of the isolates tested produced protease, 51.4% expressed lipase enzymes, 42.9% produced urease while 8.6% produced RNase. However, none of the isolates exhibited DNase activities.

Fig 1 showed the distribution of gram negative bacterial isolates recovered from high vaginal swabs of HIV seropositive pregnant women. Figs 2 and 3 depict the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of bacterial isolates cultured from high vaginal swabs of HIV seropositive pregnant women.

Table i: Pattern of Antibiotic Resistant Isolates Cultured from High Vaginal Swabs of HIV Seropositive Pregnant Women.

Bacterial Isolates	Total No tested	AUG	AMX	AMP	CXM	CRO	GEN	STR	ERY	TET	CHL	NIT	CIP	SXT
P. aeruginosa	15	5	15	10	8	5	2	0	7	10	10	13	0	15
E. coli	7	2	6	3	2	5	0	8	4	2	6	4	1	6
Klebsiella pneumoniae	7	2	4	6	4	2	2	3	4	3	6	4	1	6
P. fluorescens	3	1	3	2	2	0	0	7	1	1	3	2	0	3
Citrobacter freundii	2	0	2	2	2	1	1	1	2	0	1	2	0	2
Enterobacter aerogenes	1	0	0	0	0	1	0	1	1	0	1	1	0	1
Total	35	10(28.57)	30(85.70)	23(65.71)	18(51.42)	14(40)	5(14.28)	20(57.14)	19(54.28)	16(45.7)	27(77.14)	26(74.28)	2(5.7)	33(94.3)

Legend: AUG=augmentin, AXM= amoxicillin, AMP=ampicillin ERY= erythromycin, CXM=cefuroxime, CRO=ceftriaxone, CIP=ciprofloxacin, TET=tetracycline, SXT= sulphamethoxazole/ trimethoprim, S=streptomycin, CHL=chloramphenicol, CN=gentamycin, NIT=nitrofurantoin.

Table ii: Antibiotyping of Bacterial Isolates Cultured from High Vaginal Swabs of HIV Seropositive Pregnant Women.

Bacterial isolates	Total No tested	No of antibiotics used	Antibiogram
P. aeruginosa	15	13	CN, S, AUG, AMX, AMP, ERY, CXM, CRO, CIP, CHL, NIT
E. coli	7	13	AUG, AMX, AMP, ERY, CXM, CRO, CIP, CN, CHL, NIT, SXT
Klebsiella pneumoniae	7	13	CN, AUG, AMX, AMP, ERY, CXM, CRO, CIP, CHL, NIT, SXT
P. fluorescens	3	13	AUG, AMX, AMP, ERY, CXM, CRO, CHL, NIT, SXT
Citrobacter freundii	2	13	AMX, AMP, ERY, CXM, CRO, CN, CHL, NIT, SXT
Enterobacter aerogenes	1	13	ERY, CRO, CHL, NIT, SXT

Legend: AUG=augmentin, AXM= amoxicillin, AMP=ampicillin ERY= erythromycin, CXM=cefuroxime, CRO=ceftriaxone, CIP=ciprofloxacin, TET=tetracycline, SXT= sulphamethoxazole/ trimethoprim, S=streptomycin, CHL=chloramphenicol, CN=gentamycin, NIT=nitrofurantoin.

Table iii: Profile of Extracellular Enzymes among Bacterial Isolates Recovered from High Vaginal Swabs of HIV Seropositive Pregnant Women.

Bacterial isolates	Total No tested	Extracellular enzymes				
		Protease No(%)	Lipase No(%)	DNase No(%)	RNase No(%)	Urease No(%)
P. aeruginosa	15	10(66.7)	15(100)	0	0	8(53.3)
E. coli	7	7(100)	0	0	2(28.6)	0
Klebsiella pneumoniae	7	7(100)	0	0	1(14.3)	7(100)
P. fluorescens	3	0	3(100)	0	0	0
Citrobacter freundii	2	0	0	0	0	0
Enterobacter aerogenes	1	0	0	0	0	0
		24(68.6%)	18(51.4%)	0(00)	3(8.6%)	15(42.9%)

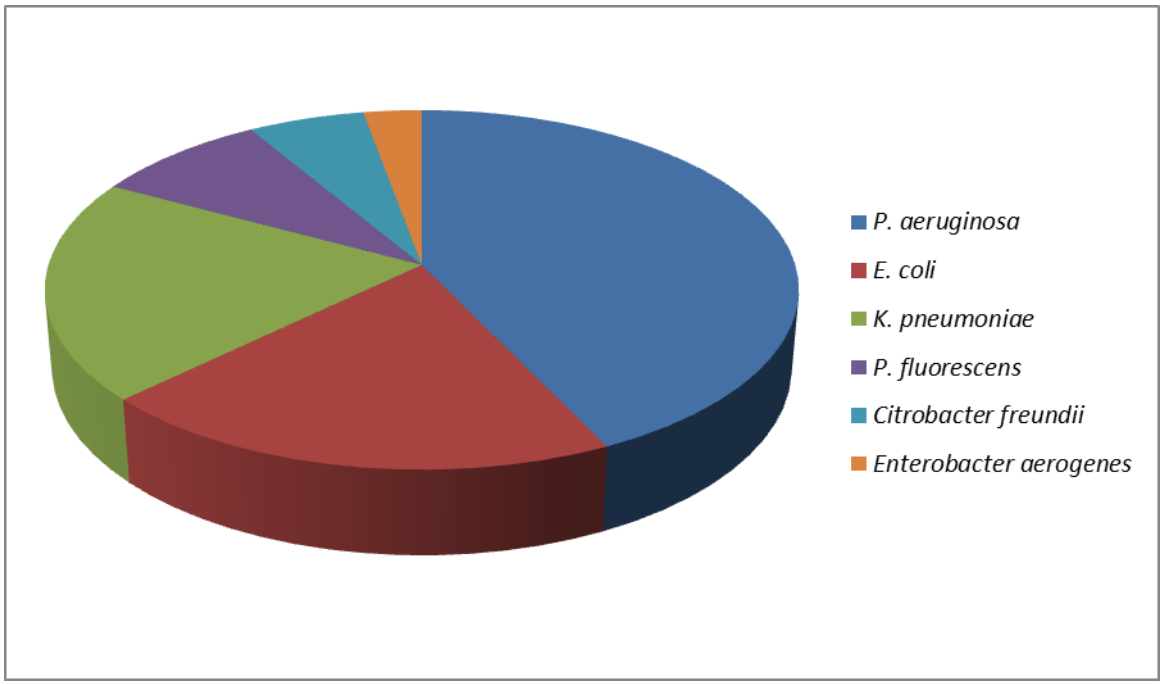

Fig. 1: Distribution of bacterial isolates recovered from high vaginal swabs of HIV seropositive pregnant women.

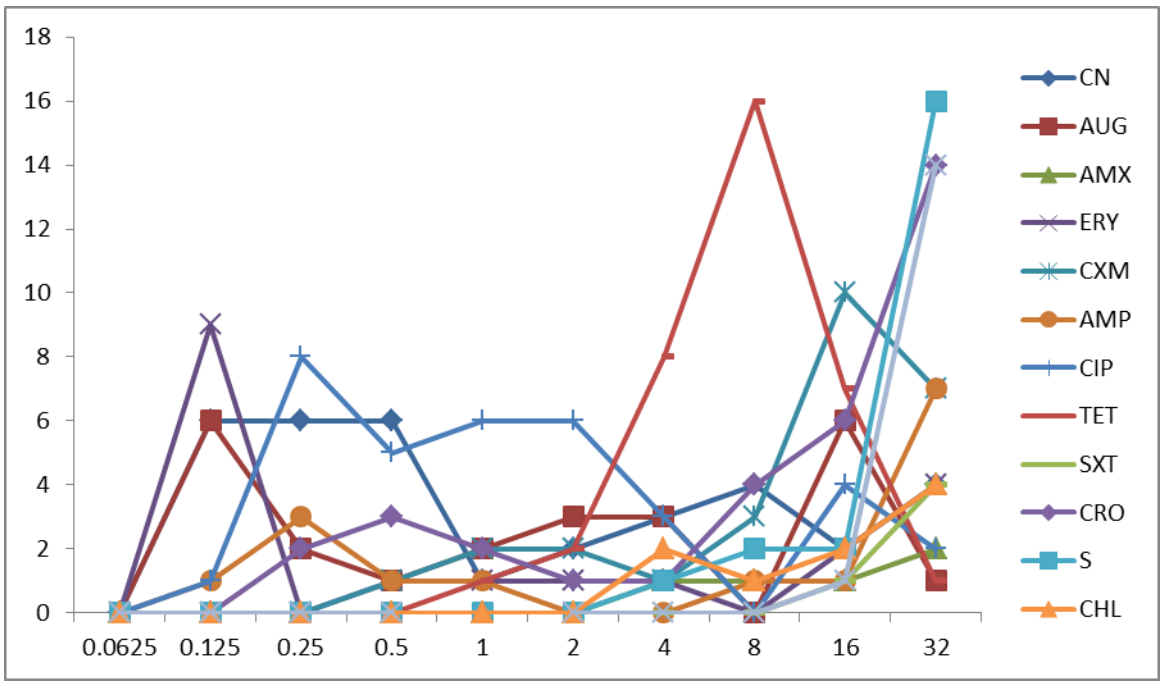

Fig. 2: Profile of Minimum inhibitory concentration of bacterial isolates cultured from high vaginal swabs of HIV seropositive pregnant women.

Legend: AUG=augmentin, AXM= amoxicillin, AMP=ampicillin ERY= erythromycin, CXM=cefuroxime, CRO=ceftriaxone, CIP=ciprofloxacin, TET=tetracycline, SXT= sulphamethoxazole/ trimethoprim, S=streptomycin, CHL=chloramphenicol, CN=gentamycin, NIT=nitrofurantoin.

DISCUSSION

The study was undertaken to investigate the antibiotic resistant profile and elaboration of extracellular enzymes of bacterial isolates cultured from high vaginal swabs of HIV seropositive pregnant women. Resistant bacteria from already compromised host may exacerbate the patients' illness by slowing the healing process. Our results also showed all the bacteria screened elaborated extracellular enzymes. The MIC and MBC of each test isolate was also determined (fig. 2). Our results showed of the 35 selected bacterial isolates examined, all displayed high resistance to antibiotics employed at least *in-vitro* tests ranging from a high 94.3% to a low 5.7%, sulphamethoxazole/trimethoprim being the least effective while ciprofloxacin recorded the most effective. Furthermore, 84.6% of the pathogens displayed multiple resistance. (Table i & ii) Studies reported by Adeyemi *et al.*,[16], in a similar environment, reported similar findings among HIV patients. Their study revealed all gram negative rods tested *P. aeruginosa, E. coli and Klebsiella pneumoniae* isolates were multiply resistant to cefuroxime, ampicillin and sulphamethoxazole/trimethoprim which corroborates with our present findings.

When extracellular enzymes were evaluated, the selected 35 isolates tested, elaborated varying enzymes such as protease, lipase, urease, and RNase activities while none of the 35 isolates produced DNase. It is not apparently clear why DNase was not produced in these isolates, (Table iii). It is interesting to note that all the 35 bacterial isolates cultured from the HVS were lactose fermenters except *P. aeruginosa* and *P. fluorescens*. Elaboration of enzymes activities is a hallmark of pathogenesis which suggests destruction of host factors, and slows the healing process. The presence of lactose fermenters in the HVS increased contamination of the vagina and exposed the patients' vulnerability to opportunists.[18] The result of the MIC showed 0.125 mg/mL for gentamycin, augmentin and erythromycin indicating their effectiveness. However ciprofloxacin displayed a higher MIC at 0.25 mg/mL indicating less abuse at this concentration (fig 2). However, streptomycin and cefuroxime showed high MIC at 32 mg/mL indicative of poor effectiveness of these antimicrobials.

CONCLUSION

Bacteria resistance in pregnant HIV women has been reported by Ebhodaghe *et al.*[17] Pregnant HIV seropositive women are at risk of contaminating their foetus if adequate therapy is not administered early in the course of infection. Highly active antiretroviral therapy (HAART) is used. HAART prevents the HIV-1 from making copies of itself thus

limiting how many virus is made. (17) Antiretroviral drugs used early in pregnancy are zidovudine, lamivudine plus either efavirenz or nevirapine that have been found effective (17) Previous studies have shown high viral load in HIV pregnant mothers which exacerbated the disease state and encourage high load of opportunists.[18,19,20] Therefore HIV pregnant women are at risk of MTCT if they fail to employ the use of HAART early in their pregnancy which make the foetus/ neonate vulnerable to HIV. Pathogenic bacteria cultured from HVS have been reported to be resistant to commonly employed antimicrobials in this environment.[21] This can slow wound healing among women and constitute late recovery thus affecting the mother and foetus. Resistant bacterial isolates are also major causes of nosocomial and community acquired infections if not treated early. The high rate of resistance and virulence factors carried by bacterial agents in this study is worrisome and portends abuse of these antimicrobials. We believe their existence can be controlled with proper hygiene and early monitoring by clinicians (gynaecologists and obstetricians.) The need to reduce sale of antibiotics on over-the- counter basis by the public should be encouraged. Purchase of antimicrobials without proper prescription from practitioners must also be discouraged. HIV infection is immunosuppressive and contributes to the depression of the immune system which further exacerbates other diseases in the host.[22]

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