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Molecular Characterization and Antibiotic Resistance Profiles of Bacterial Isolates Cultured From HIV Seropositive Patients

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Abstract

Background: The aim of this study was to characterize the resistance patterns and resistance gene profiles of selected *Escherichia coli, Staphylococcus aureus* and *Pseudomonas* isolates from HIV seropositive patients in Akure, Southwestern Nigeria between April 2012 to October 2013.

Methods: Isolates from throat, skin and rectal swab cultures from HIV seropositive patients at the State Specialist Hospital, Akure were identified using standard microbiological procedures. A total of 96 isolates of the four organisms were subjected to antibiotic susceptibility testing by the disk diffusion method. Isolates from HIV seronegative individuals were also included as controls. A total of 43 isolates were subjected to plasmid profiling and screened for the presence of virulence and resistance genes by PCR.

Findings: The bacterial isolates cultured from HIV seropositive patients exhibited higher levels of multiple antibiotic resistance as compared to the isolates obtained from HIV seronegative individuals. Twenty eight (60.9%) of the 43 isolates were found to be resistant to at least six different classes of antibiotics. None of the 43 isolates had any plasmid. Results of the ERIC-PCR showed ten (10) gene band patterns for the two *Pseudomonas* species, four band patterns for *S. aureus* and two patterns for *E. coli,* suggesting a diversity of strains among the isolates. Four of the 18 *Pseudomonas* isolates carried *aadA* gene while eight had the bla_{PSE} gene. Only two isolates out of the eight of *S. aureus* isolates that were tested carried the *mecA* gene. The MAR index of the tested isolates revealed that 93.5% of the isolates from HIV seropositive individuals were above 0.2, suggesting an antibiotic pressurized community.

Conclusion: The bacterial isolates that were cultured from our HIV seropositive study cohort did not reveal presence of any plasmid, but showed high levels of multi-drug resistance and multiple gene band patterns. The presence of multi-resistant bacterial strains on the body surface of immune-compromised HIV sero-positive subjects would significantly increase the risk of superimposed opportunistic infections which may be less susceptible to antibiotic treatment. These results suggest the need for further investigation into the mechanisms of drug resistance among immune-compromised individuals.

Keywords: HIV seropositive; Multidrug resistance; Plasmid profile; ERIC-PCR; MAR

Introduction

Studies have shown that bacterial infections represent an important cause of morbidity and mortality in HIV infected patients [1-3]. The predominant causative organisms are the encapsulated bacteria, notably *Streptococcus pneumoniae* and *Haemophilus influenza*; but non-typhoidal *Salmonella*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been implicated [4-6].

S. aureus has been reported to colonize the anterior nares of HIV-infected patients with a higher frequency than the nares of HIV negative individuals, suggesting the possibility that high colonization burden may lead to a higher incidence of infection [6-8]. *P.aeruginosa* is notable for its multiple antibiotic resistance and is recognized as one of the pathogens that cause a variety of health care-associated infections [9]. It has been associated with infections of the urinary tract, respiratory system, soft tissue infection, gastrointestinal infection, dermatitis, cystic fibrosis and burns. The organism is also considered an opportunistic pathogen primarily because of its ability to undermine the immune mechanisms of immunocompromised HIV patients and those with impaired homeostasis mechanisms [10-13]. *E. coli* is a member of the normal flora of the digestive tract and its various serotypes are associated with different infections in humans [14]. One of these serotypes is the Shiga toxin producing and highly virulent *E. coli* O157: H7 which causes hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura [15,16]. In this study, we report the patterns of colonization and the characterization of the antibiotic resistance gene profiles of *E.coli, S. aureus*, *P. aeruginosa* and *P.flourescens* that were isolated from HIV seropositive and seronegative individuals in Akure, Southwestern Nigeria. It is expected that the results obtained in this study would elucidate bacterial colonization patterns among HIV seropositive patients and improve the antibiotic management of opportunistic infections in such individuals.

Materials and Methods

Study area and sample collection

The study was undertaken at the State Specialist Hospital, Akure (Ondo State, Southwestern Nigeria) with a population of 387,100 inhabitants. A total of 121 participants from the HIV clinic of the hospital were recruited for this study. They included 70 HIV seropositive patients and 51 HIV seronegative individuals. The HIV seropositive patients consisted of 52 females [mean age 36.8 years] and 18 males [mean age 45 years], while the HIV seronegative individuals comprised of 36 females [mean age 31.6 years] and 15 males [mean age 32.5 years]. Ethical clearance was obtained from both the State Specialist Hospital Management Board and the Ethical Review Board. Throat, rectal and skin swabs were collected using sterile cotton-tipped applicators (Evepon, Nigeria) dipped in sterile physiological saline. The skin swabs were collected from the elbow skin of the left hand (5 cm radius) from each patient for the sake of uniformity. Each swab was inoculated into fluid thioglycolate broth (Oxoid, England) for 24 hour growth at 37°C. Thereafter the cultures were processed and the bacterial isolates identified using Gram stain and biochemical procedures [17-19].

Antibiotics sensitivity tests

Selected isolates were tested for their susceptibility to commonly prescribed antibiotics by the Kirby Bauer disc diffusion method [20-22]. The list of antibiotics included beta-lactams, aminoglycosides, macrolides, hydroquinolones, trimethoprim and vancomycin. Multi-drug resistance among the isolates was defined as resistance to ≥ 1 agent in ≥ 3 antibiotic classes. *S.aureus* ATCC 25923 and *Enterobacter* aero-genes (American Type Culture Collection, Rockville, USA) were used as control organisms.

Table 1 The PCR primers sequences used for virulence and resistance factors

Organisms	Target genes	Primer sequence	Amplicon size (bp)	Annealing temp (°C)
Pseudomonas sp Virulence genes	phzH phzH	F/ -5 GGGTTGGGTGGATTACAC-3 R/ -5 CTCACCTGGGTGTTGAAG-3	1,752	52
	apr apr	F/ -5 TGTCCAGCAATTCTCTTGC -3 R/ -5 CGTTTTCCACGGTGACC -3	1,017	52
	pvd pvd	F/ -5 GACTCAGGCAACTGCAAC -3 R/ -5 TTCAGGTGCTGGTACAGG -3	1,281	59
	lasB lasB	F/ -5 ACAGGTAGAACGCACGGTTG-3 R/ -5 GATCGACGTGTCCAAACTCC-3	1,220	55
Pseudomonas sp Resistance genes	bla_{PSE}	F/ -5 ACCGTATTGAGCCTGATTTA -3 R/ -5 ATTGAAGCCTGTGTTTGAGC-3	321	60
	$bla_{AMP}C$	F/ -5 GGTATGGCTGTGGGTGTTA -3 R/ -5 TCCGAAACGGTTAGTTGAG -3	882	53
	bla_{IMP}	F/ -5 CTACCGCAGCAGAGTCTTGG -3 R/ -5 AACCAGTTCTGCCTTACCAT -3	Variable	55
	aadA aadA	F/ -5 CTTGATGAAACAAGGCGG -3 R/ -5 TACCAAATGCGGGACAAC -3	Variable	55
Staphylococcus aureus	mecA1	F/ -5 AATATCGATGGTAAAGGTTGGC -3 R/ -5 AGTTCTGCAGTACCGGATTTGC-3	528	55
	Luk-pv	F/ -5 ATCATTAGGTAAAATGTCTGGACATGATCCA -3 R/ -5 GCATCAAGTGTATTGGATAGCAAAAGC-3		55

Legend: F/ Forward primer: R/ Reverse primer

Molecular Characterization of the Bacteria Isolates

DNA extraction and plasmid profiling

DNA extraction for each bacterial culture was carried out by two cycles of boiling, homogenization, vortexing and centrifugation [23]. The purity and concentration of the DNA-containing supernatant were determined using a Nanodrop spectrophotometer. The extraction of plasmid DNA from an overnight culture of each bacterial isolate was carried out by the TENS – Mini preparation method with Tris 25mM, EDTA 10mM,NaOH 0.1N and SDS 0.5% [24] in 5 ml of nutrient broth, followed by centrifugation, vortexing and pellet extraction in broth. About 300 µl of TENS solution was added, mixed by inversion 3-5 times until the solution became slimy after which 150 µl of 3.0M sodium acetate (pH 5.2) was added, vortexed for about 10 seconds and centrifuged at 13,000 rpm for 5 minutes. The supernatant was transferred into another eppendorf tube, and 900 µl of ice cold absolute ethanol was added, vortexed, and centrifuged for 10 minutes at 13,000 rpm. The supernatant was discarded, and the plasmid DNA pellet was rinsed twice with 1000 µl of 70% ethanol by centrifuging at 13,000 rpm for 5 minutes, and discarding the supernatant. The pellet was then air-dried, and 40 µl of TE buffer (Tris 10mM; 1mM Na_2 EDTA) was added to re-suspend the pellet. Each bacterial isolate was run on 0.8% agarose gel electrophoresis at 80V for 90 minutes, using HIND III digest of Lambda DNA (Fermentas, USA) as the molecular weight marker.

Enterobacterial Repetitive Intergenic Consensus -Polymerase Chain Reaction Assay (ERIC-PCR)

ERIC-PCR was used to assess the clonal relatedness and diversity of the isolates. Two primers; forward primer ERIC 1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') and reverse primer ERIC 2 (5'AAGTAAGTGACTGGGGTGAGCG-3') (Solis Biodyne) [25-27] were used to amplify repetitive sequences present in the chromosomal DNA of the isolates. ERIC-PCR was carried out in 25 µl volume containing 2.5 unit of *Taq* DNA polymerase, 200 µM deoxynucleoside triphosphates (dNTPs), 2.5 mM $MgCl_2$, 1X PCR Buffer, 100 ng of DNA and 20 pmol of each forward and reverse primer (Solis Biodyne) in sterile deionized water. Sample was amplified in an Eppendorf Nexus Thermal cycler [23] with an initial denaturation at 95°C for 3 minutes, then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds. This was followed by a final extension step of 10 minutes at 72°C. The digests were then run on 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining under UV trans-illuminator. Approximately 1kb DNA ladder (Solis Biodyne) was used as DNA molecular weight standard.

Molecular identification of isolates

The isolates were subjected to PCR assay using specie specific primers to confirm phenotypic identification of the isolates. *Pseudomonas aeruginosa* specie-specific primer (forward primer 5-GGCGTGGGTGTGGAAGTC-3 and reverse primer 5- TGGTGGCGATCTTGAACTTCTT-3), *Staphylococcus aureus* specie-specific primer (Staph 756 forward primer 5-AACTCTGTTATTAGGGAAGAACA-3 and Staph 750 reverse primer 5-CCACCTTCCTCCGGTTTGTCACC-3), and *Escherichia coli* specie-specific primer (forward primer 5-AGAGCGCGAGATTATCAAGG-3 and reverse primer 5-TGCAGAGGCGAAGAAGTAAG-3) were used to amplify 16S rRNA segments of each respective bacterial specie. The cycling parameters for each specie was as follows: for *Pseudomonas* sp., initial denaturation was carried out at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, with a final extension step of 72°C for 7 minutes. For *S. aureus,* initial denaturation was also carried out at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 minute, extension at 72°C for 90 seconds and a final extension of 4 minutes at 72°C. For *E. coli,* initial denaturation at 95°C for 3 minutes, 30 cycles of denaturation at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, then final extension at 72°C for 5 minutes. The digests were then run on 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining under UV trans-illuminator. 1kb DNA ladder (Solis Biodyne) was used as DNA molecular weight standard.

Detection of virulence and resistance genes by Polymerase Chain Reaction

Specific primers were also used to amplify sequences of the virulence and resistance genes. Details of primer sequences, predicted size of the amplified product and specific annealing temperature are given in **Table 1.**

Data analysis

Multiple antibiotic resistance (MAR) index for each test isolate was calculated as proposed by Krumperman *et al.* [28]. The MAR index was determined by dividing the number of antibiotics to which test isolate displayed resistance by the total number of antibiotics against which the test organism has been evaluated for sensitivity. Data generated for age, weight, height and gender were also evaluated using independent t-test with $P \leq 0.05$ as indicator of statistical significance. Windows SPSS version 16.0 was used to perform the analyses.

Table 2 The distribution of bacterial isolates obtained from HIV + and HIV- individuals in Akure, Southwestern Nigeria

	Pseudomonas sp.			*Staphylococcus aureus*			*Escherichia coli*			Total
	Skin	*Throat*	*Rectal swab*	*Skin*	*Throat*	*Rectal swab*	*Skin*	*Throat*	*Rectal swab*	
HIV +	9	8	5	2	9	4	4	1	4	46
HIV-	-	-	-	4	15	6	5	2	18	50
TOTAL	9	8	5	6	24	10	9	3	22	96
HIV: human immunodeficiency virus										

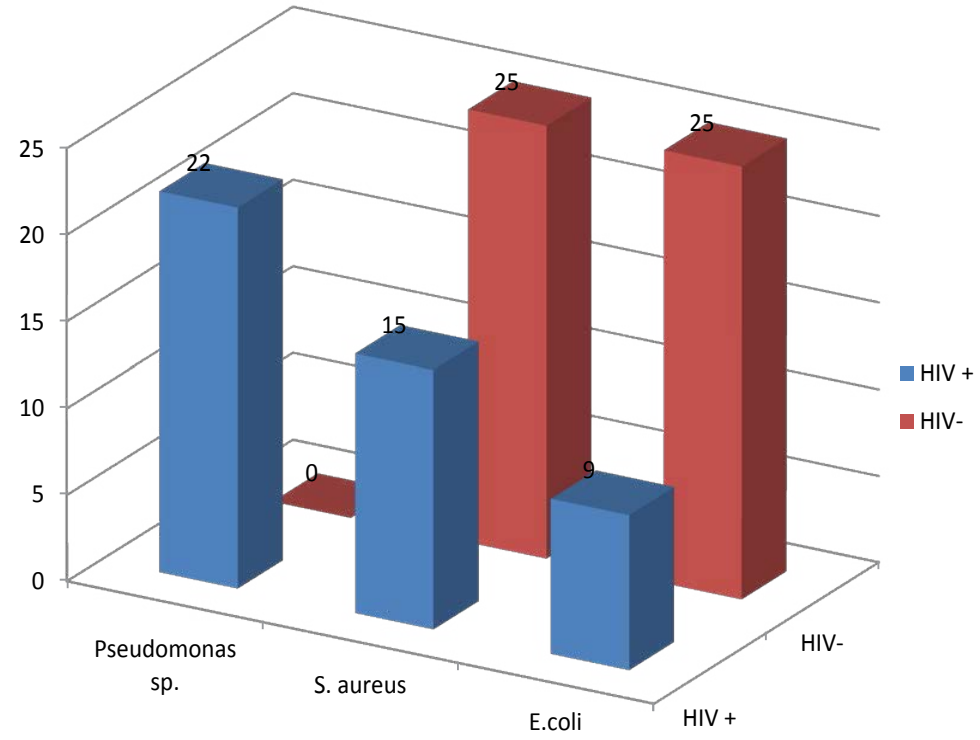

Figure 1 Distribution of bacterial isolates cultured from HIV + and HIV – individuals

Table 3 The Antibiotic Resistance profile of bacterial isolates obtained from HIV + and HIV- individuals in Akure, Southwestern Nigeria.

ANTIBIOTICS	*Pseudomonas* sp.		*Staphylococcus aureus*		*Escherichia coli*	
	HIV +	HIV-	HIV +	HIV-	HIV +	HIV-
	n= 22 (%)	n= 0 (%)	n= 15 (%)	n= 25 (%)	n= 9 (%)	n= 25 (%)
AMP	20 (90.9)	ND	14 (93.3)	21 (84.0)	9 (100.0)	13 (52.0)
OXA	21 (95.5)	ND	9 (60.0)	6 (24.0)	9 (100.0)	25 (100.0)
AMC	18 (81.8)	ND	9 (60.0)	4 (16.0)	5 (55.6)	1 (4.0)
FOX	21 (95.5)	ND	15 (100.0)	10 (40.0)	9 (100.0)	3 (12.0)
CAZ	20 (90.9)	ND	13 (86.7)	5 (20.0)	9 (100.0)	1 (4.0)
CRO	20 (90.9)	ND	11 (73.3)	8 (32.0)	8 (88.9)	3 (12.0)
CXM	22 (100.0)	ND	14 (93.3)	3 (12.0)	9 (100.0)	1 (4.0)
KF	21 (95.5)	ND	11 (73.3)	4 (16.0)	9 (100.0)	18 (72.0)
IPM	1 (4.5)	ND	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
VAN	19 (86.4)	ND	7 (46.7)	3 (12.0)	7 (77.8)	25 (100.0)
AMK	8 (36.4)	ND	6 (40.0)	1 (4.0)	5 (55.6)	0 (0.0)
GEN	1 (4.5)	ND	2 (13.3)	2 (8.0)	0 (0.0)	0 (0.0)
KAN	17 (77.3)	ND	4 (26.7)	6 (24.0)	3 (33.3)	1 (4.0)
STR	12 (54.5)	ND	4 (26.7)	1 (4.0)	6 (66.7)	13 (52.0)
ERY	18 (81.8)	ND	4 (26.7)	5 (20.0)	6 (66.7)	21 (84.0)
CIP	5 (22.7)	ND	5 (33.3)	3 (12.0)	4 (44.4)	1 (4.0)
OFX	7 (31.8)	ND	4 (26.7)	1 (4.0)	1 (11.1)	1 (4.0)
TRI	20 (90.9)	ND	10 (66.7)	20 (80.0)	9 (100.0)	17(68.0)

Legend: AMP (Ampicillin 10µg), OXA (oxacillin 1µg), AMC (Amoxicillin/clavulanic acid 20µg), FOX (Cefoxitin 30µg), CAZ (Ceftazidime 30µg), CRO ((Ceftriaxone 30µg), CXM (Cefuroxime 30µg), KF (Cephalotin 30µg), IPM (Imipenem 10µg), VAN (Vancomycin 30µg), AMK (Amikacin 30 µg), GEN (Gentamicin10µg), KAN (Kanamycin 30µg), STR (Streptomycin 10µg), ERY (Erythromycin 15µg), CIP (Ciprofloxacin 5µg), OFX (Ofloxacin5 µg), TRI (Trimethoprim5µg).

Results

A total of 96 bacterial isolates of *E. coli, S. aureus, P. aeruginosa* and *P.fluorescens* isolated from HIV seropositive individuals and seronegative controls were used in this study. The distribution of the bacterial isolates is shown on **Table 2** and is also illustrated in **Figure 1.** These included 46 isolates from 70 HIV seropositive individuals and 50 isolates from 51 seronegative controls. None of the *Pseudomonas* specie analyzed in this study was from HIV seronegative controls. The results in **Tables 3 and 4** show that 39 (84.8%) of bacterial isolates from HIV seropositive subjects were resistant to more than three classes of antibiotics as compared to 78% of isolates from seronegative individuals. Twenty (90.9%) of 22 *Pseudomonas* isolates were resistant to 3 or more classes of antibiotics and all were resistant to cefuroxime. All the *P. aeruginosa* isolates from HIV seropositive patients were resistant to ampicillin, oxacillin, cefoxitin, cefuroxime, cephalotin, vancomycin and erythromycin, while 91.7% of them were resistant to augmentin (amoxicillin with clavulanic acid), ceftriazone, kanamycin and trimethoprim. This high level of multi-drug resistance, in addition to those of penicillin derivatives, augmentin and cephalosporins, suggests that the isolates were most likely extended spectrum beta lactamase producers [29]. Both *Pseudomonas* species are an important cause of various infections and have been associated with high rates of morbidity and mortality among patients with HIV infection [30]. The *S. aureus* isolates also exhibited some degree of multiple-resistance. About 66.7% of the isolates were also resistant to 3 or more different classes of antibiotics. All the *E. coli* isolates were multi-resistant as they all had resistances to more than 3 classes of antibiotics.

Randomly selected 10 isolates of *P. aeruginosa*, eight *P. fluorescens* isolates, nine *E. coli* isolates and three *S. aureus* isolates from HIV seropositive patients as well as 14 *E. coli* and 10 *S. aureus* isolates from seronegative controls were subjected to plasmid profiling and genotyping using ERIC-PCR. The results in **Table 6** revealed ten band patterns for the *Pseudomonas* isolates, two band patterns for *E. coli* isolates and four band patterns for seven of the *S. aureus* isolates. The variations in band patterns suggest the existence of strains within the four bacterial species. **Figures 2-6b** also show that eight of the 18 *Pseudomonas* isolates carried

Table 4 The Multiple Antibiotic Resistance pattern of bacterial isolates

		NO OF ANTIBIOTIC CLASS TO WHICH ISOLATES ARE RESISTANT								
HIV POSITIVE	**NO TESTED**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Staphylococcus aureus	15	0	0	5	0	2	2	4	1	1
Escherichia coli	9	0	0	0	0	1	3	3	2	0
Pseudomonas sp.	22	0	0	2	0	2	1	11	5	1
Total	46									
HIV NEGATIVE										
Staphylococcus aureus	25	1	5	6	1	4	7	1	0	0
Escherichia coli	25	0	0	0	1	14	7	3	0	0
Total	50									

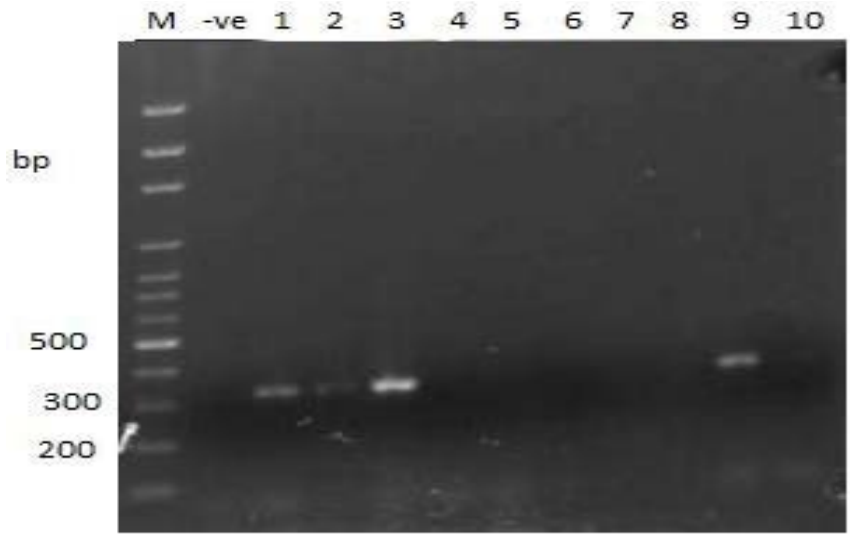

Figure 2 The gel electrophoresis PCR products using specie specific primers for the identification of *Escherichia coli*

Legend: Lane M: Molecular weight marker; Lanes 1-9*E. coli* isolates cultured from HIV seropositive patients. Lanes 11 – 24 - *E. coli* isolates cultured from HIV seronegative individuals

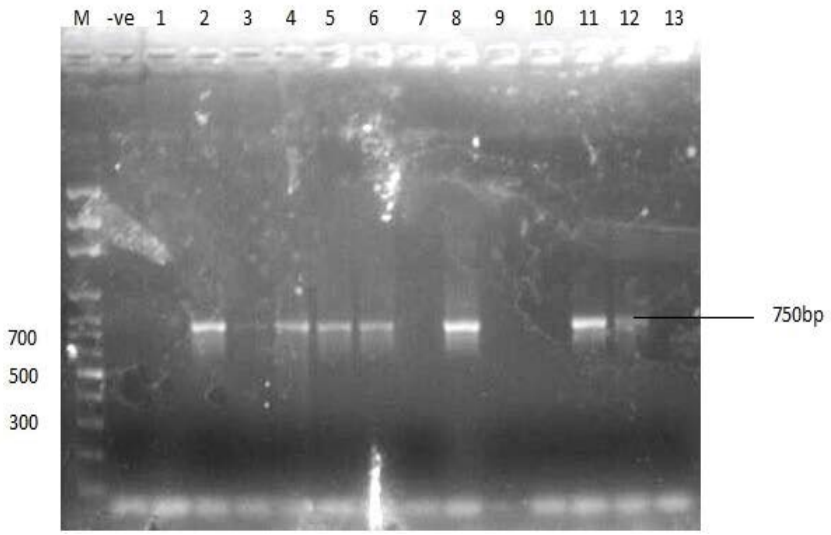

Figure 3 The gel electrophoresis of PCR products using specie specific primers for the identification of *Staphylococcus aureus* isolated from HIV positive individuals Akure, Ondo State, Nigeria

Legend: Lane M: Molecular weight marker; Lanes 1-3*S. aureus*isolates cultured from HIV seropositive patients. Lanes 4 - 13*S. aureus* isolates cultured from HIV seronegative individuals

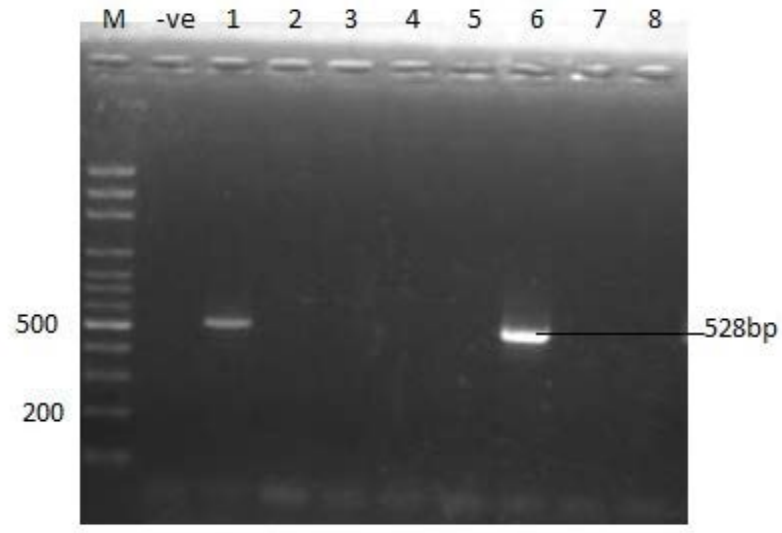

Figure 4 The gel electrophoresis of PCR products using *mecA*primers for the detection of *mecA* gene in *S. aureus.*

Legend: Lane M: Molecular weight marker; Lanes 1-2 *S. aureus*isolates cultured from HIV seropositive patients. Lanes 3 – 8 *S. aureus* isolates cultured from HIV seronegative individuals

the bla_{PSE} gene, while four had the *aadA* gene, although none of them carried a single plasmid. One of the isolates also carried both bla_{PSE} and *aadA* genes. In contrast, only two of the eight *S. aureus* isolates carried the *mecA* gene for methicillin resistance. Each of the two isolates came from a seropositive HIV patient and a seronegative HIV control. The results also show that the MAR indices in this study were >0.2 in 95.5% of *Pseudomonas* species, 86.7% of *S. aureus* and 100% for *E. coli* isolated from HIV seropositive patients **(Table 5).** The MAR indices were also > 0.2 in 40% and 64% for *S. aureus* and *E. coli* from HIV seronegative individuals respectively. These suggest that the environment from which the isolates were obtained is an antibiotic pressurized one.

Discussion

We have shown in this study that HIV seropositive patients have a higher degree of colonization with multiple antibiotic resistant *E.coli, S. aureus, P. aeruginosa* and *P. flourescens*. While we did not isolate a single plasmid from any of the 96 bacterial isolates,

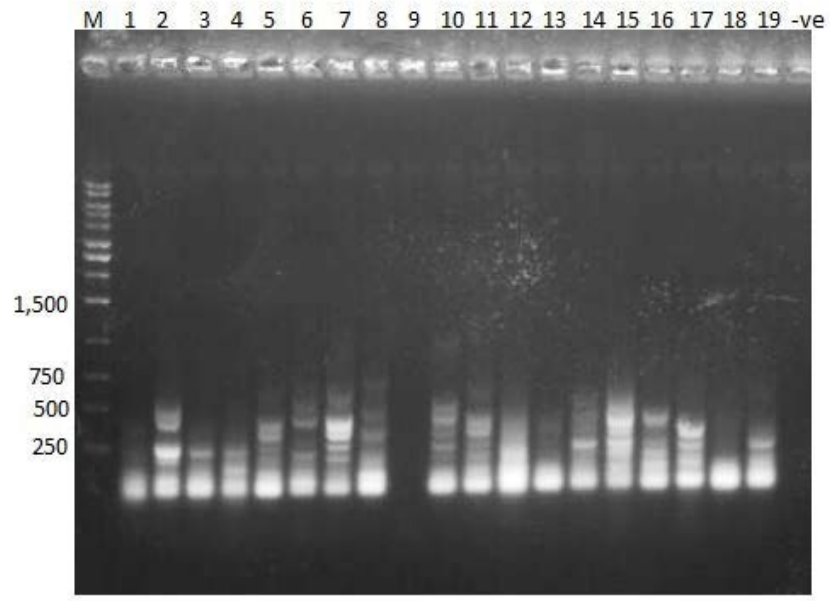

Figure 5 The gel electrophoresis of ERIC-PCR using ERIC 1 and 2 primers for the characterization of *Pseudomonas*sp, isolated from HIV positive individuals Akure, Ondo State, Nigeria

Legend Lane M: Molecular weight marker; Lane 1- 8, 10-19 *Pseudomonas* sp.

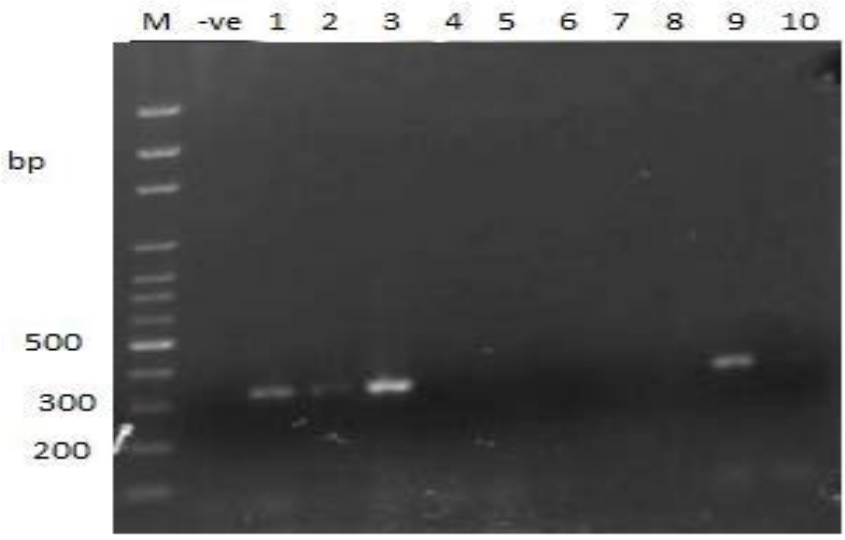

Figure 6a The gel electrophoresis PCR products using blaPSEprimers for the detection of blaPSE gene in Pseudomonas sp.

Legend: Lane M: molecular weight marker; Lane 1-10 Pseudomonas sp.isolates

we showed that several of the isolates carried resistance and virulence genes [31-36]. Twenty eight (60.9%) of the isolates were found to be resistant to at least six different classes of antibiotics. Results of the ERIC-PCR showed ten (10) gene band patterns for the two *Pseudomonas* species, four band patterns for *S. aureus* and two patterns for *E. coli,* suggesting a diversity of strains among the isolates. Four of the 18 *Pseudomonas* isolates carried *aadA* gene while eight had the bla_{PSE} gene **(Table 6).** Only two isolates out of the eight of *S. aureus* isolates that were tested carried the *mecA* gene. The MAR index of the tested isolates revealed that 93.5% of the isolates from HIV seropositive individuals were above 0.2, suggesting an antibiotic pressurized community. Colonization with *S. aureus* has been reported to be a risk factor for subsequent clinical infections in HIV positive patients [37,38]; and the site of colonization may also play a key risk factor [39]. The anterior nares as well as the skin, throat, ear and feet are considered a major reservoir of *S. aureus* in this environment [40]. The presence of such multi-resistant strains on the body surface of immune-compromised individuals portends serious implications in view of the fact that these organisms

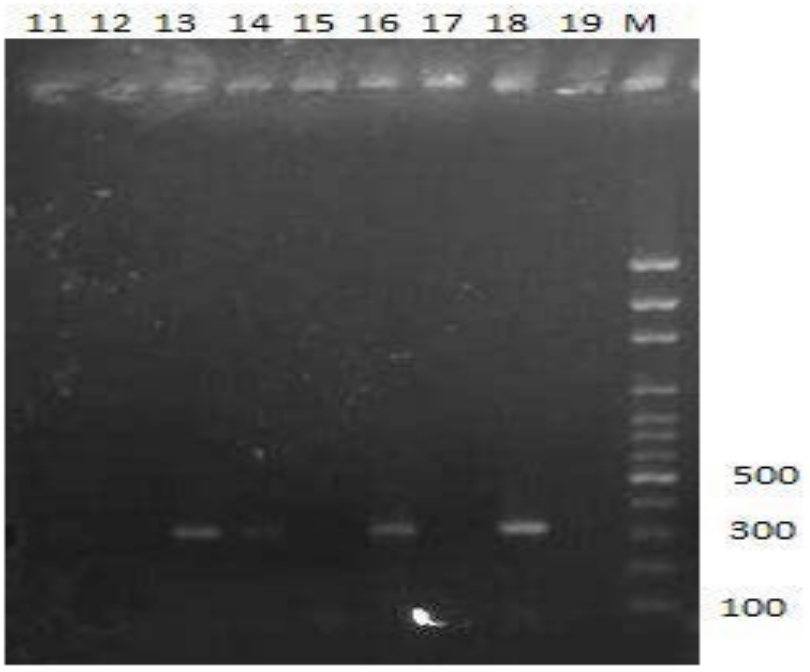

Figure 6b The gel electrophoresis PCR products using blaPSEprimers for the detection of blaPSE gene in Pseudomonas sp.

Legend: Lane M: molecular weight marker; Lane 11-19Pseudomonas sp.isolates

Table 5 The Multiple Antibiotic Resistance Index pattern of bacterial isolates

MAR index	*Pseudomonas* sp.		*S. aureus*		*E. coli*	
	HIV +	HIV-	HIV +	HIV-	HIV +	HIV-
	n= 22	n= 0	n= 15	n= 25	n= 9	n= 25
0	0 (0.0)	ND	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
0.1	0(0.0)	ND	0(0.0)	11 (44.0)	0(0.0)	0(0.0)
0.2	1 (4.5)	ND	2 (13.3)	3 (12.0)	0(0.0)	9 (36.0)
0.3	1 (4.5)	ND	4 (26.7)	2 (8.0)	0(0.0)	6 (24.0)
0.4	1 (4.5)	ND	0(0.0)	5 (20.0)	0(0.0)	8 (32.0)
0.5	1 (4.5)	ND	1 (6.7)	3 (12.0)	1 (11.1)	1 (4.0)
0.6	1 (4.5)	ND	3 (20.0)	0(0.0)	2 (22.2)	1 (4.0)
0.7	11 (50.0)	ND	2 (13.3)	0(0.0)	4 (44.4)	0(0.0)
0.8	2 (9.1)	ND	0(0.0)	0(0.0)	2 (22.2)	0(0.0)
0.9	3 (13.6)	ND	2 (13.3)	0(0.0)	0(0.0)	0(0.0)
1.0	1 (4.5)	ND	1 (6.7)	0(0.0)	0(0.0)	0(0.0)

Legend: MAR-Multi-antibiotic resistance index

have also been known to cause recurrent infections [3]. Globally, *Staphylococcus aureus* infections have been reported to be important causes of morbidity and mortality [41]. HIV positive individuals are at increased risk of opportunistic and common bacterial infections, and *S. aureus* ranks as one of the most common causes of bacterial infections [42-44]. Furthermore, HIV infection is associated with a higher risk of recurrent infection [45]. Interestingly, despite the high degree of resistance among isolates from HIV seropositive patients, some of the isolates were still sensitive to imipenem as well as gentamycin. This corroborates previous studies in which investigators reported antibiotic resistance patterns of *Pseudomonas aeruginosa* to imipenem. Resistance patterns to imipenem has been found to be lower than to other antibiotics; 16.3% in Brazil [46], 2.9% in Iran [47], 21% in Greece [48] and 13% in Spain [49]. All these studies reported low resistance values to imipenem. The low level of resistance to gentamycin has also been recorded in other studies [50-53], and it has been suggested in all cases that the efficacy of gentamycin may be related to the mode of its administration which has limited its abuse and misuse.

The study also revealed the presence of resistance genes (*mecA, bla*$_{PSE}$ and *aadA*) in some of the bacterial isolates analyzed from the HIV seropositive as well as HIV seronegative

Table 6 Probable variants of bacterial isolates subjected to ERIC-PCR using ERIC 1 and ERIC 2 primers

Bacteria specie	Variants	Base pairs of the bands	No of isolates
Pseudomonas sp.	1	280, 300, 350, 400, 500	1
	2	280, 300, 480, 500	2
	3	280, 300, 350, 480	1
	4	280, 300, 350, 400	3
	5	300, 350, 400, 500	1
	6	280, 320, 480	1
	7	150, 300, 400	1
	8	150, 300	1
	9	350	2
	10	300	2
Escherichia coli	1	220, 280	6
	2	150, 280	1
Staphylococcus aureus	1	100, 150, 250, 290	7
	2	100, 250, 290	1
	3	150, 250, 290	1
	4	100, 250	4

individuals. About 90.9% of the two *Pseudomonas* species were multi-resistant to more than 3 classes of antibiotics among seropositive patients. The expression of the efflux pump has been implicated in resistance of *Pseudomonas* sp. to beta lactam antibiotics. These proteins transport the antibiotics from within the cell to the external environment. This multi- drug system has been shown to provide resistance to a very wide range of compounds in Gram negative bacilli [31,32], coupled with a low permeability of the outer membrane [33], and a remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents, including **β**-lactams, aminoglycosides and fluoroquinolones [34]. It is notable that many of the resistant mechanisms are often present simultaneously, thereby conferring multi-resistant properties to the organism [35]. Studies have shown that *P. aeruginosa* is the second most common cause of nosocomial pneumonia (17%), the third most common cause of urinary tract infection (7%), the fourth most common cause of surgical site infection (8%), the seventh most frequently isolated pathogen from the bloodstream (2%) and the fifth most common isolate (9%) overall from all sites [36].

MAR values calculated from this study show that the indices for the HIV positive population were higher than those of their HIV negative controls. MAR indexing is an effective as well as a useful tool for evaluating the spread of bacterial resistance in a given population [52]. The high MAR indices of organisms isolated from our HIV seropositive cohort poses a challenge to the clinical management of these immunocompromised individuals.

Antimicrobial resistance is an increasing problem worldwide. In Nigeria where health institutions have little-to-none antibiotic policy, widespread antibiotic resistance is common and seems higher in immune-compromised subjects than in immune-competent individuals as revealed by our data. Therefore, there is still an urgent need for continuous and constant monitoring of resistance patterns in bacteria in view of the increasing emergence of multidrug resistance among different bacterial species. Our findings have therefore provided baseline data to institute effective therapeutic strategies in combating the challenges of antibiotic resistance among healthcare providers in Nigeria. Hence, surveillance data, continuous antibiotic susceptibility testing in hospitals and treatment regimens based on history of previous antibiotics use as well as education of the population may lower the incidence of multidrug resistant organisms.

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