MOLECULAR CHARACTERIZATION AND DETECTION OF MULTIDRUG-RESISTANT GENE IN BACTERIAL ISOLATES CAUSING LOWER RESPIRATORY TRACT INFECTIONS (LRTI) AMONG HIV/AIDS PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN UYO, SOUTH-SOUTH NIGERIA

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ABSTRACT

Background: **Antibiotic-resistant genes (ARGs) pose a significant challenge in modern medicine, rendering infections increasingly difficult to treat as bacteria acquire mechanisms to resist antibiotics. Addressing ARGs necessitates a multifaceted approach, encompassing surveillance efforts to monitor their presence and the development of strategies aimed at managing and curbing the spread of antibiotic resistance. Hence, this study characterized the genetic determinants of antibiotic resistance among isolates responsible for Lower Respiratory Tract Infections (LRTIs) in People Living with HIV/AIDS (PLWHA) in Uyo.**

Methods: **Sputum samples were collected from 61 LRTI suspects, with bacterial isolates identified using VITEK-2 technology. Polymerase chain reaction assays were employed to detect resistance genes within the isolates.**

Results: **Results revealed a bacterial etiology in 39.3% of the samples, with a majority (79.2%) originating from St. Luke Hospital, Anua (SLHA), and the remainder (20.8%) from the University of Uyo Teaching Hospital (UUTH).** ***Staphylococcus aureus*** **emerged as the predominant isolate (46.6%), while resistance was notably high against Gentamicin and Sulphamethazole/Trimethoprim. Conversely, Azithromycin, imipenem, clindamycin, erythromycin, and ceftriaxone displayed relatively lower resistance levels across all isolates. Notably, four resistance genes CTX-M, Aac, KPC, and MecA were identified, with CTX-M detected in all multidrug-resistant isolates. This underscores the predominantly community-acquired nature of resistance as conferred by CTX-M.**

Conclusion: **In conclusion, this study underscores the critical importance of continued vigilance and proactive measures in combating antibiotic resistance, particularly within vulnerable populations such as PLWHA. By elucidating the genetic mechanisms underlying antibiotic resistance, informed targeted interventions can be mitigated to curb threats posed by multidrug-resistant bacteria in clinical settings.**

Keywords: Multidrug-resistant, Bacteria, Extensively drug-resistant, Antibiotics, Enterobacteriaceae, Pathogen

BACKGROUND

The lower respiratory tract infections are the most frequent respiratory diseases among HIV infected patients, caused by diverse types of organisms including Gram positive and Gram negative bacteria which include *Streptococcus pneumoniae*, *Hemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, M. tuberculosis* and *Acinetobacter spp.*[1] Effective treatment of LRTIs in People Living with HIV/AIDS (PLWHA) hinges upon accurate diagnosis,[2] viral load reduction through increased CD4 count,[1] and appropriate antibiotic therapy. However, the emergence of antibiotic resistance poses a formidable obstacle to treatment efficacy, jeopardizing patient outcomes and exacerbating the threat of life-threatening infections.[3] This trend of antibiotic resistance is exacerbated by the widespread use of

antimicrobials in human healthcare, veterinary medicine, and agriculture, fostering the proliferation of multidrug-resistant pathogens.[4,5,6]

Some of the causes of HIV and LRTI co-infection include a weakened immune system from HIV's attack on CD4 T cells, opportunistic infections from pathogens, environmental factors such as poor living conditions, and behavioral factors like smoking, drug use, and poor dental hygiene.[7] The escalation of antibiotic resistance represents a complex interplay between bacterial adaptation and selective pressures imposed by antimicrobial agents. Bacteria exhibit remarkable resilience through genetic changes driven by horizontal gene transfer mechanisms, enabling the acquisition of antibiotic resistance genes (ARGs) hosted on mobile genetic elements (MGEs) like transposons and plasmids.[8] These MGEs serve as vehicles for the dissemination of ARGs not only within pathogenic bacterial populations but also across diverse microbial communities, encompassing both benign and commensal species.[9] Consequently, this horizontal transmission extends the spread of resistance traits to more virulent pathogens, exacerbating the challenge of antibiotic resistance propagation.[10]

In settings such as Nigeria, where antibiotic policies within healthcare institutions often lack rigor, individuals with compromised immune systems, such as People Living with HIV/AIDS (PLWHA), face heightened susceptibility to multidrug-resistant pathogens. This heightened vulnerability arises from limited treatment options due to the proliferation of resistant strains. Consequently, innovative strategies are imperative to combat infections effectively. Therefore, this study aimed to uncover the genetic basis of antibiotic resistance in isolates causing LRTIs in PLWHA in Uyo, with the goal of informing targeted interventions to enhance treatment efficacy in this vulnerable population.

METHODS

Study Size, Population and Area

A total of 61 people living with HIV/AIDS on antiretroviral therapy (ART) attending HIV clinics at University of Uyo Teaching Hospital (UUTH) and St Luke Hospital Anua (SLHA), within Uyo metropolis from the period of November to December, 2021, were recruited using simple random sampling technique (balloting).

Inclusion Criteria

Eligible individuals for this study were confirmed HIV-positive patients receiving antiretroviral therapy (ART) who provided informed consent to participate. Additionally, patients who underwent sputum microscopy, culture, and sensitivity (MCS) testing for suspected lower respiratory tract infections were included.

Exclusion Criteria

Exclusion criteria included HIV negative patients, HIV positive patients not on ART and those on antibiotic usage within 1 week prior to clinic visit.

Collection and Microbiological Laboratory Analysis of Samples

Sputum samples were aseptically collected and transported as described by Okon *et al.*[1] VITEK 2 system (BioMérieux, France) as described by Quesada *et al.*[11] was used for identification.

Antibiotic Sensitivity Screening

Mueller-Hinton agar plates were inoculated with 0.5 McFarland preparations of the inoculum using the spread plate technique as described by Edem *et al.*[12] after which standard antibiotic discs was placed and incubated at 37°C for 24 hours. The plates were interpreted using the Clinical and Laboratory Standard Institute guidelines.[13]

Determination of Multiple Antibiotics Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=x/y, where 'x' was the number of antibiotics to which test isolate displayed resistance and 'y' was the total number of antibiotics to which the test isolates has been evaluated for sensitivity (Akinjogunla and Enabulele.[14] Isolates that were resistant to two or more classes of antibiotics were taken to be multiple antibiotics resistant and sent to the Molecular Biology Laboratory of the Niger Delta University, Bayelsa State for plasmid profiling and resistant gene amplification.

Plasmid profiling of the Multi-Resistant Antibiotic Strains

Extraction of Plasmid

Detection and extraction of plasmid was carried out using the alkaline lysis method described by Odeyemi *et al.*[15]

Agarose Gel Electrophoresis

One percent agarose was prepared and loaded into electrophoresis chamber containing between 12-18 wells. The electrophoresis buffer that was used contained 40mM Tris, 20mM sodium acetate, 2mM EDTA, adjusted to pH 7.8 with acetic acid. The sample buffer contained 25% sucrose, 5mM sodium acetate, 0.05% bromophenol blue and 0.1% SDS. Electrophoresis was allowed to proceed at room temperature until bands become visible at the positive

end of the chamber. After electrophoresis, gels were stained with ethidium bromide (1ìl/ml) and viewed under UV trans-illumination. The molecular marker that was used is the bacteriophage Hind III digest.

DNA extraction

Five millilitres of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95^{0}C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

Amplification of Genes

Resistance genes from the isolates were amplified using specific primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4uM and 50ng of the extracted DNA as template. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4uM and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 50ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue transilluminator.

CTX-M genes Primers

The CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGC AG-3' and CTX-MR: 5'-ACCGCGATATCGTTGG T-3' primers.

KPC genes Primers

The KPC genes from the isolates were amplified using the KPCF: 5'-GCTCAGGCGCAACTGTAAG-3' and KPCR: 5'-AGCACAGCGGCAGCAAGAAAG-3' primers.

Aac (6') genes Primers

The *Aac (6')* genes from the isolates were amplified using the *Aac (6')-IbF:* 5´-TTGCGATGCTCTAT GAGTGGCTA-3´ and *Aac (6')-IbR:* 5´-CTCGAAT GCCTGGCGTGTTT-3´ primers.

MecA genes Primers

The TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTG AG-3' primers.

Statistical Analysis

Raw data was entered into Microsoft Excel and the descriptive statistics was used for data summarization and presentation.

Ethics Consideration

This study was approved by the Ethical Review Board of the University of Uyo Teaching Hospital and letter of introduction was sent to St. Luke's Hospital, Anua, Uyo, Akwa Ibom State where the research was carried out. Informed consent form was issued to the eligible subjects and only subjects that gave informed consent were included in the study.

RESULTS

Distribution of Bacterial Isolates according to Facility

Out of the 61 samples processed, a total of 24 (39.3%) yielded seven species of bacterial isolates, four Gram-positive organisms (*Streptococcus pyogenes, Streptococcus agalactiae, Kocuria kristinae* and *Staphylococcus aureus*) and three Gram-negative organisms (*Escherichia coli, Klebsiella pneumonia, Proteus* species). *Staphylococcus aureus* 6 (46.6%) was the predominant isolate, while *Kocuria kristinae* 1 (0.4%) was the least (Table 1). Most of the isolates were from St. Luke Hospital, Anua (SLHA) 19 (79.2%) (Table 1).

Distribution of Bacterial growth according to CD4 count and Viral Load

In various CD4 cell count categories, the incidence of Lower Respiratory Tract Infections (LRTI) varied. Individuals with a CD4 cell count of less than 200 exhibited the highest LRTI rate at 45.8%, while those with CD4 cell counts between 301-500 had the lowest LRTI rate at 16.7% (Table 2). Among individuals on Antiretroviral Therapy (ART), those with a viral load

Table 1: Distribution of bacterial isolates according to facility

Isolate	Gram reactions	Growth (%)	UUTH (%)	SLHA (%)
Staphylococcus aureus	+ve	6 (25)	2 (33.3)	4 (66.7)
Proteus sp	-ve	2 (8.3)	0 (0)	2 (100)
Klebsiella pneumoniae	-ve	3 (12.5)	1 (33.3)	2 (66.7)
Kocuria kristinae	+ve	1 (4.2)	0 (0)	1 (100)
Escherichia coli	-ve	4 (16.7)	1 (2.3)	3 (75)
Streptococcus pyogenes	+ve	3 (12.5)	0 (0)	3 (100)
Streptococcus agalactiae	+ve	5 (20.8)	1 (20)	4 (80)
Total		**24 (39.3)**	**5 (20.8)**	**19 (79.2)**

Source: Okon et al. [1]
Keys: UUTH – University of Uyo Teaching Hospital
SLHA – St. Luke Hospital, Anua

exceeding 1000 displayed the highest prevalence of LRTI, accounting for 91.7% of cases, in contrast to individuals with a viral load below 1000, who represented only 8.3% of LRTI cases (Table 2).

Antibiotics Resistance Pattern

Table 3 depicts the antibiotic resistance pattern of the bacterial isolates. Gentamicin and Sulphamethazole/ Trimethoprim showed high levels of resistance across all organisms. Azithromycin, imipenem, clindamycin,

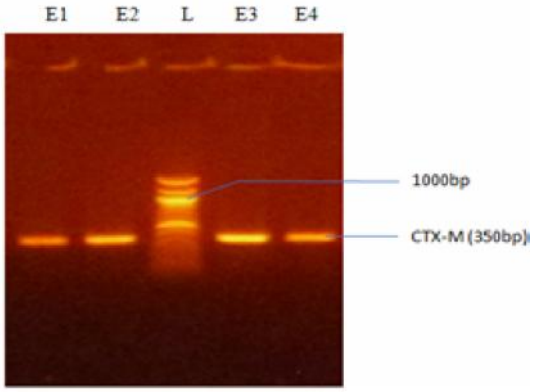

Figure 1: Agarose Gel electrophoresis showing the amplified CTX-M genes

Table 2: Distribution of growth according to immunologic staging (CD4 count) and viral load

Organisms	CD4 Count *(cells/mm³)*					Viral Load *(copies/ml)*	
	<200 (n=11)	201-300 (n=9)	301-400 (n=2)	401-500 (n=2)	>500 (n=0)	<1000 (n=2)	≥1000 (n=22)
Staphylococcus aureus (n=6)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	0	1 (16.7)	5 (83.3)
Proteus sp (n=2)	1 (50)	0	1 (50)	0	0	0	2 (100)
Klebsiella pneumoniae (n=3)	1 (33.3)	2 (66.7)	0	0	0	1 (33.3)	2 (66.7)
Escherichia coli (n=4)	3 (75)	1 (25)	0	0	0	0	4 (100)
Streptococcus pyogenes (n=3)	2 (66.7)	1 (33.3)	0	0	0	0	3 (100)
Streptococcus agalactiae (n=5)	2 (40)	3 (60)	0	0	0	0	5 (100)
Kocuria kristinae (n=1)	0	0	0	1 (100)	0	0	1 (100)

Source: Okon et al.[1]

Table 3: Antibiotic resistance patterns of bacterial isolates

Organisms	Antibacterial agents (µg/disc)								
	AMC (10)	CN (10)	SXT (25)	AZM (15)	IMI (10)	DA (2)	E (15)	CRO (30)	CAZ (30)
Staphylococcus aureus (n=6)	2	4	2	0	0	0	0	1	-
Proteus sp (n=2)	0	1	2	0	0	-	-	1	0
Klebsiella pneumoniae (n=3)	2	2	3	0	2	-	-	2	3
Escherichia coli (n=4)	1	2	1	0	0	-	-	0	0
Streptococcus pyogenes (n=3)	0	0	2	0	0	0	0	0	-
Streptococcus agalactiae (n=5)	0	1	5	1	0	1	1	0	-
Kocuria kristinae (n=1)	0	0	1	0	0	0	0	0	-

Source: Okon et al. [1]
Abbreviations: AMC = amoxicillin clavulanic acid, CN = gentamicin, AZM = azithromycin, IMI = imipenem, DA = clindamycin, SXT = Sulphamethazole/Trimethoprim, E = erythromycin, CRO = ceftriaxone, CAZ = ceftazidime

Table 4: Multiple Antibiotic Resistance (MAR) index of gram-negative bacterial isolate

MAR Index	*Klebsiella pneumoniae* (n =3) No (%)
0.0	0 (0.0)
0.14	0 (0.0)
0.29	0 (0.0)
0.43	0 (0.0)
0.57	1(33.3)
0.71	2 (66.6)
0.86	0 (0.0)
1.00	0 (0.0)

Source: Field data, 2021

erythromycin, and ceftriaxone showed relatively lower resistance levels across all isolates (Table 3).

Multiple Antibiotic Resistance (MAR) Indices of Bacterial Isolates

The MAR index reveals that *Klebsiella pneumoniae* with MAR indices greater than 0.71 were 2 (66.6%) and 1 (33.3%) with MAR (0.36) (Table 4). *Staphylococcus aureus* with an MAR index of 0.57 was 1 (100%) (Table 5).

Molecular Detection of Resistance Genes

Table 6 illustrates the resistance genes present in the resistant bacterial isolates. *Klebsiella pneumoniae* KP45 had resistance genes CTX-M and KPC, *Klebsiella pneumoniae* KP13 had CTX-M and aac genes, *Klebsiella pneumoniae* KP36 had CTX-M and KPC genes, while *Staphylococcus aureus* SA27 had CTX-M and MecA genes. Figure 1-4 showcases the molecular bands corresponding to these resistance genes.

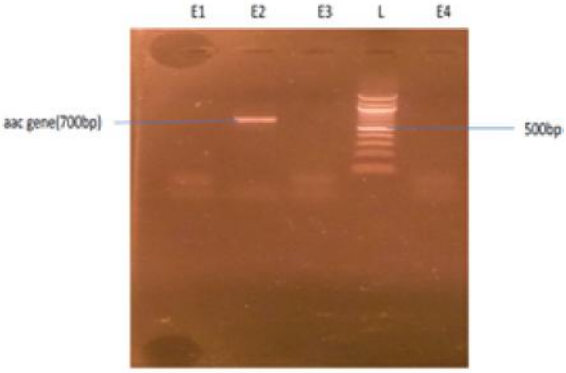

Figure 2: Agarose gel electrophoresis showing the amplified *aac* genes

Molecular detection of Resistance Genes

Table 6 shows the resistance genes present in the resistant bacterial isolates. *Klebsiella pneumoniae* KP45 had resistance genes CTX-M and KPC, *Klebsiella pneumoniae* KP13 had CTX-M and *aac* genes, *Klebsiella pneumoniae* KP36 had CTX-M and KPC genes, while *Staphylococcus aureus* SA27 had CTX-M and MecA genes.

Table 5: Multiple Antibiotic Resistance (MAR) index of gram-positive bacterial isolate

MAR Index	*Staphylococcus aureus* (n=1) No (%)
0.0	0 (0.0)
0.13	0 (0.0)
0.25	0 (0.0)
0.36	1 (100.0)
0.50	0 (0.0)
0.63	0 (0.0)
0.75	0 (0.0)
0.86	0 (0.0)
1.00	0 (0.0)

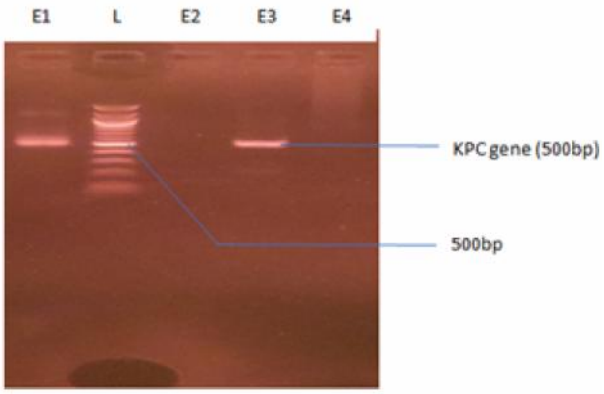

Figure 3: Agarose Gel Electrophoresis showing the amplified KPC genes

Table 6: Molecular detection of resistance genes from bacterial isolates

Bacterial strains	Antibiotic Resistant Genes CTX- M	*aac*	KPC	MecA	Total
Staphylococcus aureus SA27	+	-	-	+	2
Klebsiella pneumoniae KP45	+	-	+	-	2
Klebsiella pneumoniae KP13	+	+	-	-	2
Klebsiella pneumoniae KP36	+	-	+	-	2
Total	4	1	2	1	**8**

Source: Field data, 2021

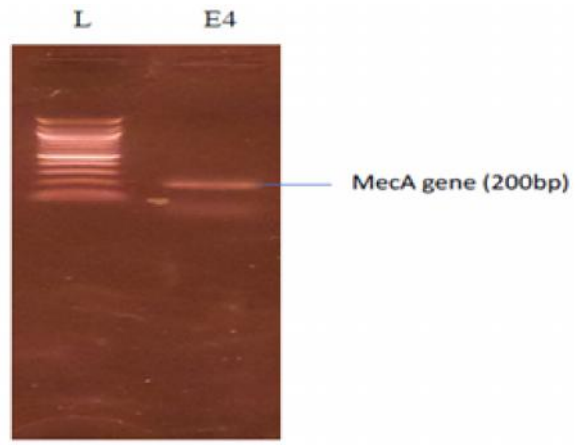

Figure 4: Agarose gel electrophoresis showing the amplified MecA gene

DISCUSSION

The study reported a prevalence of lower respiratory tract infection (LRTI) at 39.3%, which exceeds rates documented by previous studies such as Ahmed *et al.*[16] at 23.6% and Santella *et al.*[17] at 39.1%, but falls below the rate found by Atiaa *et al.*[18] at 71.1%. Of the 24 isolates examined, 62.5% were identified as Gram-positive bacteria, while 37.5% were Gram-negative. This variance could be attributed to diverse virulence factors, environmental factors, and the misuse of antibiotics. Notably, this finding aligns with Atiaa *et al.*[18], where Gram-positive bacteria accounted for 50.3% and Gram-negative bacteria for 49.7%. *Staphylococcus aureus* (46.6%) emerged as the most prevalent isolate, contrasting with studies by Akingbade *et al.*[19] and Uzoamaka *et al.*[20], where *Klebsiella pneumoniae* predominated. Although Klebsiella species are typically associated with urogenital and peritoneal infections, their involvement in pulmonary infections, while rare, can lead to significant lung function deterioration.

The immunocompromised state induced by HIV, particularly the depletion of CD4 cells, predisposes individuals to opportunistic infections like tuberculosis and bronchitis.[21] LRTI occurrences were predominantly observed in participants with CD4 counts less than or equal to 500 cells/mm3, with the highest incidence among those with CD4 counts less than 200 cells/mm3. Similarly, individuals with higher viral loads, especially those ≥1000 copies/mL, demonstrated elevated rates of LRTI.

Antimicrobial resistance analysis revealed imipenem as the least resistant antibiotic, likely due to its limited utilization and abuse. Conversely, Gentamicin and Sulphamethazole/Trimethoprim exhibited high resistance levels, consistent with their frequent prescription for people living with HIV/AIDS (PLWHA). The Multiple Antibiotic Resistant (MAR) index was determined for *Klebsiella pneumoniae* isolates, with values of 0.57 and 0.71 obtained from two (66.6%) and one (33.3%) isolates respectively, indicating a high level of antibiotic resistance. This finding is consistent with studies by Oguche *et al.*[22] and Zain *et al.*[23] Additionally, one *Staphylococcus aureus* sample exhibited a MAR index of 0.57. The emergence of MAR bacteria poses a significant threat to public health, often stemming from the indiscriminate use of antibiotics in clinical, agricultural, and aquaculture settings.[24,25]

The investigation delved into the plasmid profiles of three *Klebsiella pneumoniae* and one *Staphylococcus aureus* strains isolated from sputum samples. Notably, the resistance to antibiotics in these pathogens was traced back to plasmid-mediated mechanisms. Bacterial plasmids are known to harbor genes responsible for antibiotic resistance, suggesting that these genes might be inhibiting the effectiveness of antibiotics against these pathogens.

Using agarose gel electrophoresis, the study identified four antibiotic-resistant genes—CTX-M, aac, KPC, and MecA in the isolates. The focus was particularly on detecting CTX-M, aac, and KPC genes in *Klebsiella pneumoniae* due to its global reputation as one of the primary producers of extended-spectrum beta-lactamases (ESBLs).[26,27,28]

According to the World Health Organization (WHO), *Klebsiella pneumoniae* exhibits resistance to third-generation cephalosporins, including those conferred by ESBLs and carbapenems.[29] The blaCTX-M gene, responsible for ESBL production, has seen a marked increase in infections over the last decade.[30] This surge could be attributed to the horizontal mobilization of genes encoding CTX-M enzymes by various genetic elements, as supported by previous studies.

All three MDR *Klebsiella pneumoniae* isolates were found to harbor the CTX-M gene, consistent with previous researches by Kang *et al.* and Shao *et al.*[31] where CTX-M was predominant among ESBL-producing *Klebsiella pneumoniae*. Additionally, two *Klebsiella pneumoniae* isolates were identified with the *Klebsiella pneumoniae* carbapenemase (KPC) gene, aligning with prior studies by El-Kholy *et al.*[32] and Gatya Al-Mayahie *et al.*[33] where the blaKPC gene was the most frequently detected carbapenemase encoding gene. The presence of KPC confers resistance to a broad spectrum of beta-lactam antibiotics, including carbapenems, and its dissemination via various plasmids poses a significant threat to global healthcare systems.[5,34]

Furthermore, one *Klebsiella pneumoniae* isolate was found to harbor the aac gene, which confers resistance to aminoglycosides such as gentamicin. This is done by modifying specific chemical groups, thereby reducing the efficacy of aminoglycosides against bacterial ribosomes.[35]

In *Staphylococcus aureus*, the presence of CTX-M and MecA genes was detected. The MecA gene, located in the staphylococcal cassette chromosome mec (SCCmec), encodes penicillin-binding protein 2a, conferring resistance to β-lactam antibiotics such as methicillin.[36] Various mechanisms contribute to resistance against beta-lactams, including changes in penicillin-binding proteins, ribosomal target sites, bacterial genome alterations, enhanced efflux, and acquisition of plasmid-encoded genes.[37]

CONCLUSION

In conclusion, this study revealed that commonly prescribed antibiotics like gentamicin and sulphamethoxazole/trimethoprim were highly resisted by isolates. Alarmingly, the prevalence of antibiotic resistance, particularly among *Klebsiella pneumoniae* isolates, underscores the imminent threat posed by multidrug-resistant pathogens. This necessitates a paradigm shift towards judicious antimicrobial stewardship and innovative treatment modalities. Unraveling plasmid-mediated resistance mechanisms, such as the presence of genes like CTX-M, aac, KPC, and MecA, highlights the complexity of microbial resistance and the critical need for sustained research to combat the growing tide of antimicrobial resistance. Moreover, the presence of multidrug-resistant organisms can significantly compromise the efficacy of HAART in HIV patients. Co-infections with resistant pathogens not only complicate treatment regimens but can also lead to weakened immune responses, higher viral loads, and diminished effectiveness of antiretroviral drugs, ultimately resulting in poorer health outcomes and increased mortality. All multidrug-resistant isolates in this study were observed to harbor the *bla* CTM-X gene, indicating community association. This further illustrates how resistant co-infections can disrupt HIV management by placing additional strain on the immune system, making it more difficult for HAART to achieve optimal results. In essence, this study not only elucidates the intricate interplay between microbial pathogens and host immunity but also serves as a clarion call for concerted global efforts to combat the pervasive menace of antimicrobial resistance, safeguarding public health and preserving therapeutic efficacy for generations to come.

List of Abbreviations

ARGs - Antibiotic-resistant genes
LRTIs - Lower Respiratory Tract Infections
PLWHA - People Living with HIV/AIDS
SLHA - St. Luke Hospital, Anua
UUTH - University of Uyo Teaching Hospital
HAART - Highly Active Antiretroviral Therapy
MGEs - Mobile genetic elements
ART - Antiretroviral therapy
MAR - Multiple Antibiotics Resistance
ESBLs - Extended-spectrum beta-lactamases
KPC - *Klebsiella pneumoniae* carbapenemase
SCCmec - staphylococcal cassette chromosome mec
aac - Aminoglycoside acetyltransferase
LB - Luria Bertani

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Review Board of the University of Uyo Teaching Hospital and letter of introduction was sent to St. Luke's Hospital, Anua, Uyo, Akwa Ibom State where the research was carried out. Informed consent form was issued to the eligible subjects and only subjects that gave informed consent were included in the study.

Consent for publication

Not applicable

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article

Competing interests

None

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Nil

Authors' contributions

R.S.O., I.A.O., E.N.E., S.B., were involved in the conceptualization, supervision, investigation, methodology, data curation, and writing original draft. R.S.O., I.A.O., E.N.E., S.B., E.N.E., N.S.U., G.M.N., A.G contributed to the visualization, investigation, methodology, and writing—review and editing. All authors have read and approved the final manuscript.

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