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# Molecular Analysis and Detection of Antibiotic Resistance Genes in Aerobic Bacteria Isolated from Bacteremia Patients at Tertiary Healthcare Institutions

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**Abstract**

Bacteremia is the presence of bacteria in the bloodstream. Aerobic bacteria, such as *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*, are common causes of bacteremia and have developed resistance to various antibiotics. The study aims to analyze and detect antibiotic resistance genes of aerobic bacteria isolated from bacteremia patients in tertiary healthcare institutions. A total of 110 blood specimens were collected from patients attending Federal Medical Centre Yenagoa and Gloryland INRI Medical Centre Yenagoa. The specimens were analyzed by culture, biochemical test, and molecular methods. Out of the 110 blood cultures, 4.5% were positive. Organisms isolated were *Staphylococcus aureus* (60%) and *Klebsiella pneumoniae* (40%). The isolates were more common in males (80%) than females (20%). The prevalence of bacteremia between children and adults was 5% and 4.4% respectively. Antibiotic susceptibility test the gram positive isolates showed maximum resistance to ampiclox (100%), zinnacef (100%), erythromycin (100%), and 66.7% resistance to amoxacillin and rocephin respectively. However, high susceptibility was shown to gentamicin (100%), levofloxacin (100%), and ciprofloxacin (66.7%). The gram negative isolates showed maximum resistance to ciprofloxacin (100%) and


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50% resistance to cefotaxime, gentamicin, chloramphenicol, ceftriaxone, levofloxacin ampicillin/sulbactam and co-trimoxazole respectively. Nevertheless, maximum susceptibility was shown to ofloxacin, tetracycline, and azithromycin with 100% respectively. The molecular detection of antibiotics resistance genes revealed the presence of *CTX-M* (100%), and *QnRA* (33.3%) in the *S. aureus* strains. *CTX-M* (100%), *QnRA* (100%), and *QnRB* (50%) were seen in the *K. pneumoniae* strains. However, the *aaC* gene was found neither in the *S. aureus* strains nor *K. pneumoniae* strains. Phylogenetic analysis using 16s rRNA sequences identified bacterial isolates; *S. aureus* and *K. pneumoniae*. These results underscore the complexity of resistance patterns and the molecular mechanisms driving them in the studied pathogens. Crucial effort should be put in place to enhance surveillance and regularly update resistance profiles to guide treatment.

**Keywords:** Bacteremia, Aerobic bacteria, Antibiogram, Antibiotics resistance genes, 16SrRNA Sequencing

## INTRODUCTION

Bacteremia, characterized by the presence of pathogenic bacteria in the bloodstream, has emerged as an escalating global public health threat, constituting a critical infectious condition associated with substantial morbidity and mortality, particularly among pediatric and geriatric populations. (Duan et al., 2021). Bacteremia can result from various sources, including infections, medical procedures, and underlying health conditions (Skiada et al., 2017). Understanding its etiology, diagnostic strategies, and management is essential for improving patient outcomes. The risk factors for bacteremia include immunocompromised states, chronic diseases, and invasive procedures (Kallen et al., 2019). It presents symptoms such as fever (95-100% of cases), chills (50-70% of cases), hypotension (20-50% of cases), tachycardia (50-70% of cases), tachypnea (40-60% of cases), confusion or altered mental status (20-40% of cases), nausea and vomiting (20-30% of cases), abdominal pain (10-20% of cases) and diarrhea (10-20% of cases) (Seymour and Wagener., 2017; Kumar et al., 2018). Bacteremia can be diagnosed through blood cultures and molecular techniques (Seymour et al., 2016; Lodise et al., 2021). Treat options include broad-spectrum antibiotics such as piperacillin-tazobactam, ceftriaxone, or meropenem, depending on the clinical scenario and severity (Kollef, 2019). However, there has been a concerning increase in antibiotic resistance among aerobic bacteria, making treatment more difficult and resulting in higher rates of illness, death, and healthcare expenses(World Health Organization, 2017).

The Centers for Disease Control and Prevention (CDC) has reported that antibiotic-resistant bacteria cause over 2 million illnesses and 23,000 deaths annually in the United States alone (CDC, 2019).

Aerobic bacteria, such as *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus*, are common causes of bacteremia and have developed resistance to various antibiotics (Kumar et al., 2018; Sader et al., 2019). The molecular mechanisms underlying antibiotic resistance involve acquiring resistance genes, which can be spread horizontally through mobile genetic elements (Wright et al., 2019). Detecting these resistance genes is crucial for understanding the epidemiology of antibiotic resistance and developing effective treatment strategies.

The advent of sophisticated molecular methodologies, including polymerase chain reaction (PCR), whole-genome sequencing, and microarray analysis, has precipitated a paradigm shift in the identification and characterization of antibiotic resistance genes. These cutting-edge techniques enable researchers to discern the intricate molecular mechanisms underlying resistance development, thereby facilitating the formulation of efficacious countermeasures(Liu et al., 2018; Zhang et al., 2020; Chen et al., 2019). These techniques offer high sensitivity, specificity, and speed, enabling the rapid identification of resistance genes and informing timely treatment decisions (Tang et al., 2020).

The emergence of antibiotic resistance genes in aerobic bacteria isolated from bacteremia patients has been reported globally (Pitout et al., 2019; Su et al., 2020). The most common resistance genes detected include *blaCTX-M, blaSHV, blaTEM*, and *vanA* (Huang et al., 2019; Li et al., 2020). The dissemination of these genes has been linked to horizontal gene transfer, clonal spread, and selection pressure imposed by antibiotic use (Martinez et al., 2019). Bacteremia poses a significant threat to public health globally. The growing prevalence of antibiotic resistance in aerobic bacteria has made treatment increasingly difficult, contributing to higher rates of illness, death, and escalating healthcare expenses. Despite the severity of this issue, a significant gap remains in comprehensive molecular investigations aimed at identifying genes responsible for causing resistance to antibiotics in aerobic bacteria recovered from haematological specimens of bacteremic individuals. Thus, this study aims to analyze and detect antibiotic resistance genes of aerobic bacteria isolated from bacteremia patients in tertiary healthcare institutions. This will provide valuable insights into the molecular mechanisms of antibiotic resistance in aerobic bacteria,

ultimately informing the development of effective treatment strategies and improving patient outcomes.

## MATERIALS AND METHODS

### Study Area and Population

The study was carried out at Federal Medical Centre (FMC), Yenagoa, Bayelsa State, Nigeria, which provides quality health services to patients within Bayelsa State, Nigeria. A cross-sectional study involving 110 consenting consecutive patients from both sexes and all age groups; including pediatric and adult attending Federal Medical Centre and Glory land INRI Yenagoa, Bayelsa State, Nigeria were eligible to participate in the study.

### Ethical Approval

This study was conducted following ethical standards and received approval from the Research Ethics Committee with application number FMCY/REC/ECC/2024/MARCH/726 at Federal Medical Centre-Yenagoa. Informed consent was obtained from all participants involved in the study. Confidentiality was strictly maintained, with all data anonymized to protect participant privacy.

### Specimen Collection and Processing

A total of 110 blood specimens were collected and inoculated directly into sterile brain heart infusion (BHI) broth bottles, which were pre-prepared under aseptic conditions to support optimal microbial growth and facilitate accurate subsequent microbiological analysis. The inoculated bottles were incubated for up to seven days to facilitate microbial growth. After the incubation period, bottles exhibiting turbidity—indicative of microbial proliferation—were subjected to subculturing. The subculturing process involved transferring aliquots from the turbid BHI broth into sterile agar plates, including Bismuth Sulphite agar, Blood agar, and MacConkey agar. These plates were then incubated at 37°C for 24 hours to enable the growth of distinct microbial colonies. Following the initial incubation, individual colonies from these plates were further subcultured onto nutrient agar plates. This step was repeated until pure cultures of the isolates were achieved, ensuring the isolation of distinct and uncontaminated bacterial colonies. Bacterial colonies were identified using morphological characteristics, gram staining, biochemical tests (such

as catalase test, oxidase test, citrate test, coagulase test, and haemolysis test), and molecular techniques.

**Antibiotics Susceptibility Testing**

The antibiotics susceptibility testing of the isolates was done using the Kirby Bauer disc diffusion method. A bacterial suspension of each isolate was prepared to match the 0.5 McFarland standard. Using a sterile swab, the bacterial suspension was even spread over the surface of Mueller Hinton agar plates to create a bacterial lawn. Using sterile forceps, the antibiotic discs were placed on the surface of the inoculated agar plates. The plates were incubated at $37^0$C for 24 hours and the zones of inhibition were measured and compared to the standard reference values provided by the Clinical and Laboratory Standards Institute (CLSI) to determine susceptibility (resistant, intermediate, or susceptible). The zone diameters were then compared to the standard interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI), and the results were classified as susceptible, intermediate, or resistant based on the zone sizes and the specific antibiotic (CLSI, 2023).

**Molecular Identification of Isolates**

**Bacterial DNA extraction**

Extraction was done using a ZR fungi/ bacteria DNA mini-prep extraction kit supplied by Inqaba South Africa. Pure colonies of the bacterial isolate were transferred into 200 microliters of isotonic buffer in a ZR Bashing Bead Lysis tube, and 750 microliters of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube was centrifuged at 10,000xg for 1 minute. Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 xg for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the

DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000xg for 1 minute. The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, and 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degrees 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 microlitres of the extracted DNA were 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.

**16S rRNA Amplification**

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 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 0.4M, and the extracted DNA as a template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, l95ºC for 30 seconds; annealing, 52ºC 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 120V for 15 minutes and visualized on a UV transilluminator.

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s, and 60°C for 4min.

**Phylogenetic Analysis**

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

**Detection of Selected Resistance Genes**

**Amplification of *aaC* gene**

AAC genes from the isolates were amplified using the AAC(6/-E). IBF: 5-TTGCGATGCTCTATGAGTGGCTA-3' and AAC(6/-E). IBR: 5'-CTCGAATGCCTGGCGTGTTT-3' primers on an 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 0.4M, 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, 58ºC for 40 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 200V for 15 minutes and visualized on a blue light imaging system for a 490bp product size.

**Amplification of *qnrA* genes**

QNRA genes from the isolates were amplified using the QNRAF: 5-AGAGGATTTCTCACGCCAGG-3' and QNRAR: 5'-GCAGCACTATKACTCCCAAGG-3' primers on an 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 0.4M, 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, 56ºC for 40 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 200V for 15 minutes and visualized on a blue light imaging system for a 455bp product size.

**Amplification of *CTX-M* genes**

CTX-M genes from the isolates were amplified using the CTX-M F: 5-CGCTTTGCGATGTGCAG -3' and CTX-M R: 5'-ACCGCGATATCGTTGGT -3' primers on an 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 0.4M, and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 94ºC for 5 minutes; denaturation, 94ºC for 40 seconds; annealing, 52C for 45 seconds; extension, 68ºC for 45 seconds for 35 cycles and final extension, 68ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light imaging system for a 550bp product size.

**Amplification of *qrnB* genes**

QNRB genes from the isolates were amplified using the QNRBF: 5-GATCGTGAAAGCCAGAAAGG-3' and QNRBR: 5'- CGATGCCTGGTAGTTGTCC-3' primers on an 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 0.4M, 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, 56ºC for 40 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 200V for 15 minutes and visualized on a blue light imaging system for a 490bp product size.

**Statistical analysis**

The data were analyzed using ANOVA IBM® SPSS® statistics version 21. One-two-way ANOVA was used to compare the mean value of the outcome variable followed by Fisher's exact test. The significance level was set at $p<0.05$.

## RESULTS

Out of the 110 specimens collected from different units in Federal Medical Centre and Glory land INRI Yenagoa, bacteremia was detected in 5 patients: 1 female and 4 from different males. Five (5) bacterial isolates identified were *S. aureus* and *K. pneumoniae*. The rate of recovery from positive samples was *S. aureus* (60%) while *K. pneumoniae* (40%). The prevalence of the isolates obtained with patients with bacteremia was *S. aureus* 3(60%) and *K. pneumoniae* 2(40%) as shown in Table 1.

**Table 1: Percentage occurrence of bacterial isolates isolated from patients with bacteremia attending tertiary health institutions.**

| **Organisms** | **Number of Isolates (%)** |
|---|---|
| *S. aureus* | 3 (60) |
| *K. pneumoniae* | 2 (40) |
| Total | 5(100) |

As shown in Table 2, the prevalence of bacteremia by gender. The prevalence rates calculated were 8% for males and 1.67% for females. These findings indicate a higher prevalence of bacteremia in males compared to females.

**Table 2: Prevalence of bacterial isolates by gender**

| **Gender** | Total | Positive | Prevalence (%) |
|---|---|---|---|
| Female | 60 | 1 | 1.67 |
| Male | 50 | 4 | 8 |

Table 3 depicts the prevalence of bacteremia between children (17 years and below) and adults (18 years and above). Out of the 20 children examined, 1 isolate was recovered giving a 5% prevalence, while out of the 90 adults examined, 4 isolates were recovered giving a prevalence of 4.4%. No statistically significant difference ($P \leq 0.05$) was observed in the prevalence of bacteremia between children and adults.

**Table 3: Prevalence of bacteremia between children and adults**

| Group | Total samples | Positive samples | Prevalence |
|---|---|---|---|
| Children (17 years and below) | 20 | 1 | 5 |
| Adults (18 years and above) | 90 | 4 | 4.4 |

Table 4 details the resistance patterns of *S. aureus* strains to various antibiotics. The isolates demonstrated complete resistance (100%) to ampiclox, erythromycin, and zinnacef. Additionally, 66.7% of the isolates were resistant to azithromycin and rocephin. Resistance to ciprofloxacin and amoxacillin was observed in 33.3% of the isolates. Conversely, the isolates showed 100% susceptibility to gentamicin and levofloxacin. A statistically significant difference ($P \leq 0.05$) was seen in the antibiotic susceptibility profile of the *S. aureus* strains.

**Table 4: Antibiotics resistance pattern of *Staphylococcus aureus* strains**

| Antibiotics | Concentration (µg/ml) | Susceptible (%) | Resistant (%) | Intermediate (%) |
|---|---|---|---|---|
| Pefloxacin | 10µg | 2(66.7) | 0(0) | 1(33.3) |
| Gentamicin | 10µg | 3(100) | 0(0) | 0(0) |
| Amiclox | 30µg | 0(0) | 3(100) | 0(0) |
| Zinnacef | 20µg | 0(0) | 3(100) | 0(0) |
| Amoxicillin | 20µg | 1(33.3) | 1(33.3) | 1(33.3) |
| Rocephin | 25µg | 1(33.3) | 2(66.7) | 0(0) |
| Azithromycin | 25µg | 1(33.3) | 2(66.7) | 0(0) |
| Levofloxacin | 20µg | 3(100) | 0(0) | 0(0) |
| Erythromycin | 10µg | 0(0) | 3(100) | 0(0) |
| Ciproflixacin | 10µg | 2(66.7) | 1(33.3) | 0(0) |

Table 5 shows the resistance profiles of *K. pneumoniae* strains. The data indicates that these isolates exhibit complete resistance (100%) to ciprofloxacin. Additionally, resistance levels of 50% were observed against several antibiotics, including cefotaxime, gentamicin, chloramphenicol, ceftriaxone, levofloxacin, ampicillin/sulbactam, and co-trimoxazole.

Conversely, *K. pneumoniae* strains demonstrated full susceptibility (100%) to ofloxacin, tetracycline, and azithromycin. This profile underscores a notable resistance to commonly used antibiotics while retaining susceptibility to specific alternative treatments. Statistically, there was no significant difference ($P \leq 0.05$) in the antibiotic susceptibility profile of the *K. pneumoniae* strains.

**Table 5: Antibiotic resistance pattern of *Klebsiella pneumoniae* strains.**

| Antibiotics | Concentration (µg/m) | Susceptible (%) | Resistant (%) | Intermediate (%) |
|---|---|---|---|---|
| Ampicillin/sulbactam | 20µg | 1(50) | 1(50) | 0(0) |
| Cotrimoxazole | 25µg | 1(50) | 1(50) | 0(0) |
| Cefotaxime | 30µg | 1(50) | 1(50) | 0(0) |
| Cefotaxime | 30µg | 1(50) | 1(50) | 0(0) |
| Chloramphenicol | 30µg | 1(50) | 1(50) | 0(0) |
| Tetracycline | 30µg | 2(100) | 0(0) | 0(0) |
| Gentamicin | 10µg | 1(50) | 1(50) | 0(0) |
| Piperacillin/tazobactam | 11µg | 0(0) | 0(0) | 2(100) |
| Azithromycin | 15µg | 2(100) | 0(0) | 0(0) |
| Ofloxacin | 5µg | 2(100) | 0(0) | 0(0) |
| Levofloxacin | 5µg | 1(50) | 1(50) | 0(0) |
| Ciprofloxacin | 30µg | 0(0) | 2(100) | 0(0) |

Figure 1 describes the agarose gel electrophoresis of *the CTX-M* gene in the bacterial isolates. Lanes 1-5 represent the *CTX-M* gene bands (550bp). Lane W represents the 100bp Molecular ladder of 1500bp. The bands appearing around the 550bp level for all five lanes depict the presence of the *CTX-M* gene in all five isolates on the agarose gel electrophoresis.

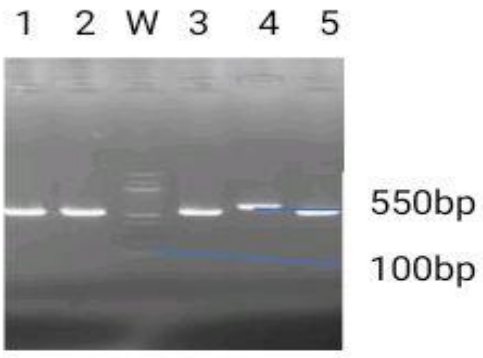



**Figure 1: CTX-M gene in the bacterial isolates**

Figure 2 describes the agarose gel electrophoresis of *the QnRA* gene in the bacterial isolates. Lanes 1,3 and 5 represent the *QnRA* gene bands (455bp). Lane W represents the 100bp Molecular ladder of 1500bp. The bands appearing at the 455bp level for the three lanes depict the presence of the *QnRA* gene in three isolates on the agarose gel electrophoresis.

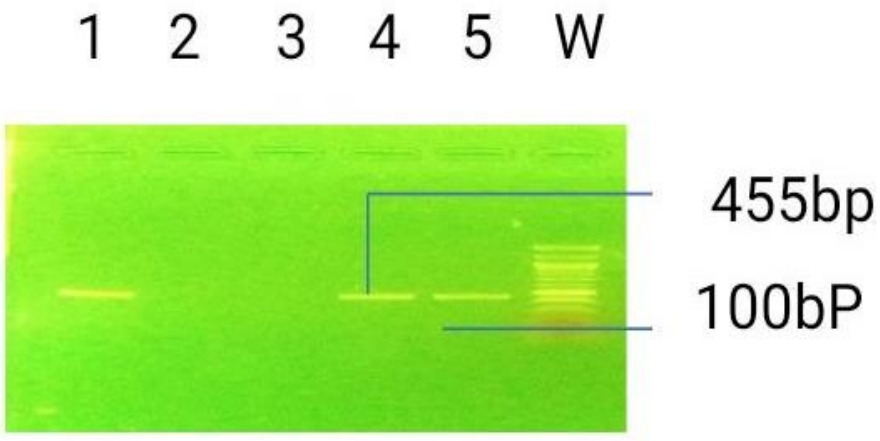



**Figure 2: *QnRA* gene in the bacterial isolates**

Figure 3 highlights the agarose gel electrophoresis of the bacterial isolates tested for *QnRB* genes. Lane 5 represents the positive *QnRB* gene bands(490bp). Lane W represents the 100bp Molecular ladder. However, the *QnRB* gene was absent at the 490bp level of the ladder.

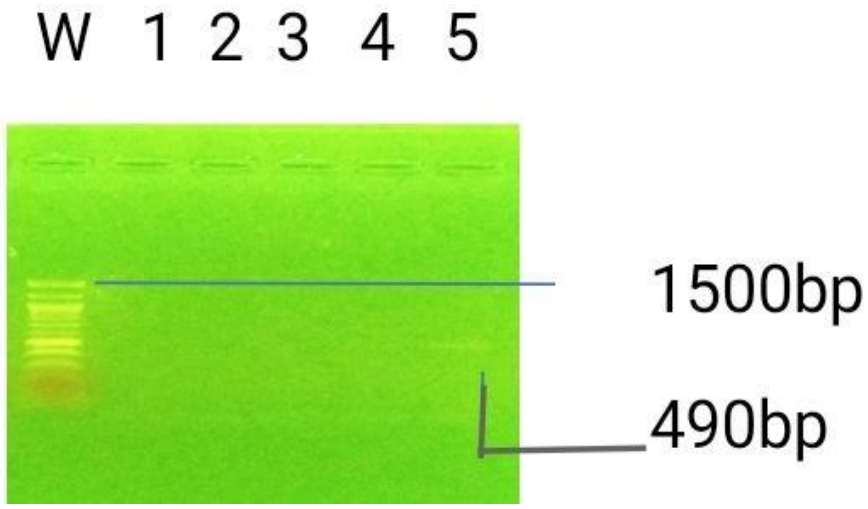



**Figure 3: *QnRB* gene in the bacterial isolates**

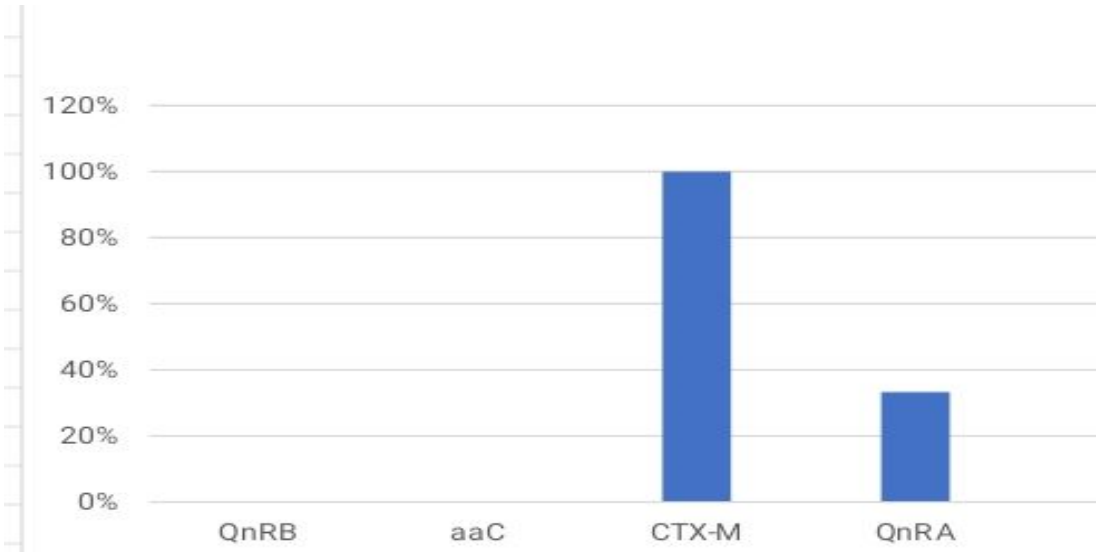



**Figure 4: Distribution of antibiotics resistance genes among *S. aureus* strains**

Figure 4 depicts the distribution of antibiotic resistance genes among *S. aureus* strains. Molecular detection revealed that the *CTX-M* gene was present in 100% of the isolates, while the *QnRA* gene was found in 33.3% of the isolates. Both the *qrnB* and *aaC* genes were absent, with a 0% detection rate.

**Figure 5** illustrates the distribution of antibiotic resistance genes in *K. pneumoniae* strains. The analysis reveals that the *CTX-M* gene is present in 100% of the isolates, while both the *QnRA* and *QnRB* genes are detected in 100% and 50% of the strains, respectively. Notably, the *aaC* gene is absent, with a detection rate of 0%.

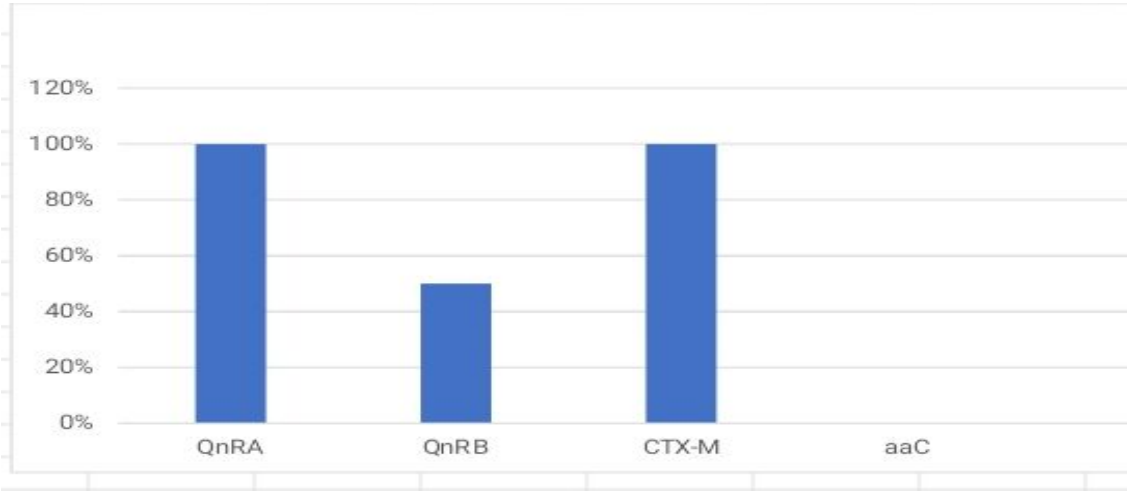



Figure 5: Distribution of antibiotics resistance genes among *K. pneumoniae* strains

**Table 6** shows the characteristics for blastin analysis and the identity of the isolates sequenced. Out of five isolates, three were identified as *S. aureus* and two were *K. pneumoniae*

**Table 6: Characteristics for Blastin Analysis and identity of Isolates sequenced.**

**Table 6: Characteristics for Blastin Analysis and identity of Isolates sequenced.**

| S/N | Specimen Code/Type | Sequence Nucleotide obtained | Gene Bank Accession Number | Identity of the Isolate obtained |
|---|---|---|---|---|
| 1 | W1 | GCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTAC GGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGT CAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTT CACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCCAATG ACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAAACCGCCTACGCGCG CTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGC ACGTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGATGTGCACAGTTACTTACAC ATATGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGC GTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA GTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTTCAGGTCGGCTATGCAT CGTTGCCTTGGTAAGCCGTTACCTTTACCAACTAGCTAATGCAGCGCGGATC | CP168499 | *Staphylococcus aureus* |
| 2 | W2 | GCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTAC GGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGT CAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTT CACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCCAATG ACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAAACCGCCTACGCGCG CTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGC ACGTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGATGTGCACAGTTACTTACAC ATATGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGC GTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA GTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTTCAGGTCGGCTATGCAT CGTTGCCTTGGTAAGCCGTTACCTTTACCAACTAGCTAATGCAGCGCGGA | CP168499 | *Staphylococcus aureus* |
| 3 | W3 | GCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTAC GGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGT CAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTT CACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCCAATG ACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAAACCGCCTACGCGCG CTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGC ACGTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGATGTGCACAGTTACTTACAC ATATGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGC GTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA GTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTTCAGGTCGGCTATGCAT CGTTGCCTTGGTAAGCCGTTACCTTTACCAACTAGCTAATGCAGCGCGG | CP168717 | *Staphylococcus aureus* |
| 4 | W4 | GTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATCGACATCGTTTACGGC GTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAG TCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCAC CGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCA GTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCT TTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCAC GGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACAAGGTTATTAACCTTATC GCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCAT GGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGT CTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGT CGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCT | PQ203360 | *Klebsiella pneumoniae* |
| 5 | W5 | ATTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATCGACATC GTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCT GAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTAC GCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTAAAGACTCTAGCCTGCCGTTTC GAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGC GTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTG CTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGATAAGGTTATTAA CCTTAACGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACAC GCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCG TAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTA GGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACCAGCTAATCCCATCTGGG | FJ22772 | *Klebsiella pneumoniae* |

The phylogenetic tree showing the evolutionary relationship between the isolates is shown in **Figure 6.** The phylogenetic analysis, based on evolutionary distances calculated using

the Jukes-Cantor method (Jukes & Cantor, 1969), placed the 16S rRNA sequences within the genera *Staphylococcus* and *Klebsiella,* with a high degree of relatedness to *Staphylococcus aureus* and *Klebsiella pneumoniae.* This finding supports the close phylogenetic relationship between the isolates and these two species.

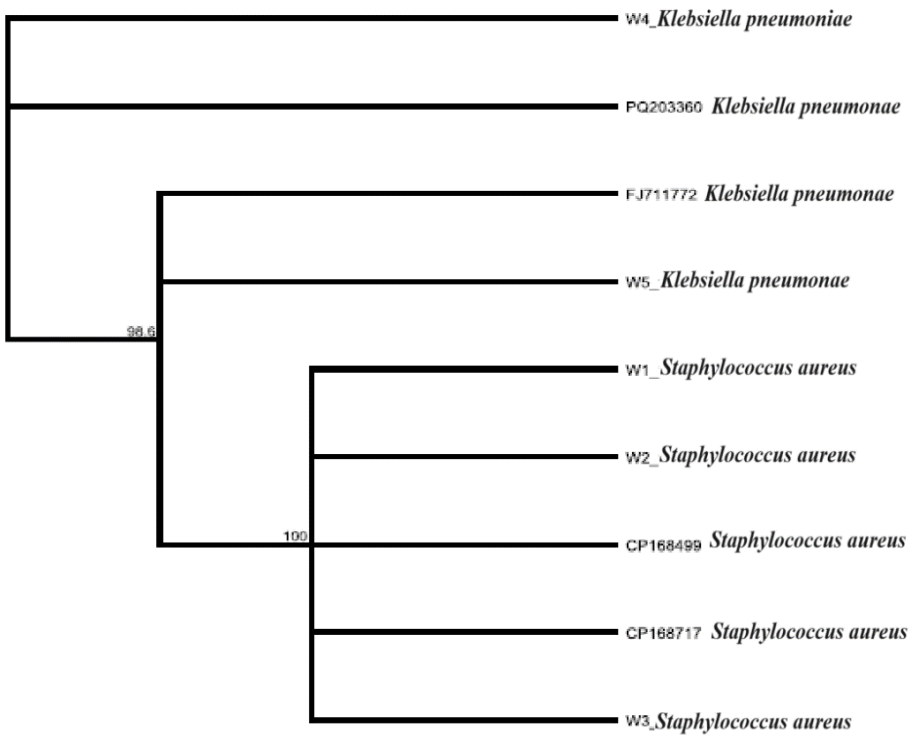



**Figure 6: Phylogenetic Tree with the evolutionary relationship between the bacterial isolates.**

## DISCUSSION

In our findings, the detected positivity rate for blood cultures was 4.5%, indicating that a small fraction of the population studied suffered from bacteremia. This is in line with reports from other studies in similar settings, where the prevalence of positive blood cultures typically ranges from 2% to 6% (Kumar et al., 2019). The identification of *S. aureus* (60%) and *K. pneumoniae* (40%) as the predominant pathogens is consistent with the commonality of these organisms in bloodstream infections. *S. aureus* is frequently

implicated in both hospital-acquired and community-acquired infections, and its prevalence in this study reflects its clinical significance, corroborating the findings by Liu et al. (2021). Similarly, *K. pneumoniae* is known for its role in serious infections and is a major concern due to its potential for multidrug resistance (Murray et al., 2022).

The demographic distribution shows a higher prevalence of isolates in males (8%) compared to females (1.67%). This finding is consistent with studies indicating that males may be at a greater risk for certain infections, potentially due to differences in health-seeking behavior or underlying conditions (Hedrich et al., 2020). The observation that children (5.0%) had a higher prevalence compared to adults (4.4%) is intriguing and suggests that children may be at an increased risk of certain infections, possibly due to factors like a developing immune system or increased susceptibility to infections (Kumar et al., 2019).

The antibiotics resistance profiles of *S. aureus* isolates showed complete resistance to ampiclox, zinnacef, and erythromycin (100%). This high level of resistance is concerning and aligns with global trends where resistance to beta-lactam antibiotics and macrolides is increasing (Liu et al., 2021). Resistance to azithromycin and rocephin (66.7%) also highlights significant concerns. The observed susceptibility to gentamicin and levofloxacin (100%) suggests these antibiotics remain effective for treating *S. aureus* infections, which is supported by their inclusion in empirical therapy guidelines (NICE, 2023). *K. pneumoniae* isolates exhibited maximum resistance to ciprofloxacin (100%). This level of resistance is particularly concerning as ciprofloxacin is a commonly used fluoroquinolone (CDC, 2021). Resistance to cefotaxime, gentamicin, chloramphenicol, ceftriaxone, levofloxacin, ampicillin/sulbactam, and co-trimoxazole (50%) underscores the growing problem of multidrug resistance in *K. pneumoniae* (Paterson & Bonomo, 2023). Despite this, the full susceptibility to ofloxacin, tetracycline, and azithromycin (100%) suggests that these antibiotics could still be effective treatment options.

The presence of the *CTX-M* gene in 100% of *S. aureus* and *K. pneumoniae* strains indicates a widespread issue with extended-spectrum beta-lactamase (ESBL) production. The *CTX-M* family of ESBLs is known to confer resistance to a wide range of beta-lactam antibiotics, a concern highlighted in numerous studies (Woodford et al., 2022). The presence of *CTX-M* in both pathogens emphasizes the need for effective monitoring and updated treatment guidelines.

The presence of the QnRA gene was detected in 33.3% of *S. aureus* strains and 100% of *K. pneumoniae* strains. This gene is known to be associated with resistance to quinolones, a class of antibiotics commonly used as second-line treatments for severe infections (Zhang et al., 2021). Additionally, the *QnRB* gene was found in 50% o*f K. pneumoniae* strains, which further corroborates the observed resistance patterns in this study. These findings are consistent with other research that highlights the increasing prevalence of quinolone-resistance genes in Gram-negative bacteria (Chen et al., 2022). This suggests a notable spread of resistance mechanisms within these bacterial populations, which could have significant implications for treatment strategies and antibiotic stewardship.

The absence of the *aaC* gene in both *S. aureus* and *K. pneumoniae* strains is consistent with its limited role in resistance patterns in these pathogens. The gene is less commonly associated with the types of resistance observed in this study, aligning with other findings that report variable prevalence across different regions (Chen et al., 2022).

The use of culture and biochemical techniques in this study identified organisms showing their morphology and physical characteristics but has a limitation because it did not identify some of the organisms up to the species level. All the five isolates recovered were identified by PCR. The 16S rRNA sequence obtained from the isolates exhibited a perfect match (100% similarity) when compared to sequences in the NCBI non-redundant nucleotide (nr/nt) database using a megablast search. Phylogenetic analysis, based on evolutionary distances calculated using the Jukes-Cantor method (Jukes & Cantor, 1969), placed the 16S rRNA sequences within the genera *Staphylococcus* and *Klebsiella*, with a high degree of relatedness to *Staphylococcus aureus* and *Klebsiella pneumoniae.* This finding supports the close phylogenetic relationship between the isolates and these two species.

## CONCLUSION

The study of 110 blood specimens from patients at Federal Medical Centre-Yenagoa and Gloryland INRI Medical Centre-Yenagoa revealed a 4.5% positivity rate, with *Staphylococcus aureus* and *Klebsiella pneumoniae* as the predominant isolates. The data indicated a higher prevalence of infections in males and children. Antimicrobial susceptibility testing showed significant resistance in both gram-positive and gram-negative isolates, with *Staphylococcus aureus* exhibiting 100% resistance to several antibiotics, though it was susceptible to gentamicin, levofloxacin, and ciprofloxacin. *Klebsiella pneumoniae* demonstrated maximum

resistance to ciprofloxacin and piperacillin/tazobactam while showing high susceptibility to ofloxacin, tetracycline, and azithromycin. Molecular analysis identified the presence of *CTX-M* and *QnRA* genes in both bacterial species, with varying prevalence, but the *aaC* gene was absent in all isolates. These results underscore the complexity of resistance patterns and the molecular mechanisms driving them in the studied pathogens.

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