

Molecular Detection and Resistance Profile of *Pseudomonas aeruginosa* and *Escherichia coli* Isolated from Pediatric Patients in Selected Hospitals in Sokoto Metropolis

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Abstract

This study investigated the molecular detection and antimicrobial resistance profiles of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from pediatric patients in two selected hospitals in Sokoto metropolis, Nigeria. A total of 193 blood samples were collected from children aged 2–12 years and analyzed using standard microbiological, biochemical, and molecular techniques. Of these, 80 samples (41.45%) yielded positive microbial growth, with *E. coli* and *P. aeruginosa* each detected in 7.5% (n = 15) of total isolates. Morphological identification was confirmed using selective media, and species identity was validated by PCR amplification of uidA (503 bp), oprL (249 bp), and 16S rRNA genes. Antimicrobial susceptibility testing showed that *E. coli* isolates exhibited complete resistance to ampicillin (100%), moderate resistance to ciprofloxacin (66.7%) and kanamycin (66.7%), but were fully susceptible to tetracycline and ceftazidime (0% resistance). *P. aeruginosa* displayed variable resistance patterns, with a maximum multiple antibiotic resistance (MAR) index of 0.7, suggesting prior exposure to high-risk antibiotic environments. These findings reveal a considerable burden of multidrug-resistant bloodstream pathogens among pediatric patients in Sokoto, underscoring the urgent need for routine molecular surveillance, strict antibiotic stewardship, and targeted infection control strategies to safeguard child health in the region.

Keywords: Antibiotics, Polymerase Chain Reaction, Pediatric Bloodstream Infections, Pathogens, Antimicrobial Resistance, Nigeria.

Introduction

Pseudomonas aeruginosa and *Escherichia coli* are among the most common bacterial pathogens implicated in pediatric infections, ranging from urinary tract infections (UTIs) and pneumonia to bloodstream infections (BSIs) and wound infections (Tamma et al., 2021). These opportunistic bacteria exhibit intrinsic resistance mechanisms and acquire additional resistance genes, making their treatment challenging, particularly in hospital settings (Lerminiaux and Cameron, 2019). In Nigeria, and particularly in Sokoto State, antimicrobial resistance (AMR) poses a significant public health concern due to the

widespread misuse and overuse of antibiotics, inadequate infection control measures, and the lack of routine molecular surveillance (Odetoyin et al., 2020).

Pseudomonas aeruginosa, a Gram-negative, non-fermenting bacillus, is a leading cause of healthcare-associated infections (HAIs) in pediatric patients, particularly among immunocompromised individuals, those in intensive care units (ICUs), and those undergoing invasive procedures (Tacconelli et al., 2018). It possesses a robust arsenal of resistance determinants, including β -lactamases, efflux pumps, and porin modifications, rendering many standard treatments ineffective (Li et al., 2022). Similarly, *Escherichia coli*, a facultative anaerobic Gram-negative bacillus, is responsible for a wide range of pediatric infections, particularly UTIs, neonatal sepsis, and gastroenteritis (Muggeo et al., 2021). The emergence of extended-spectrum β -lactamase (ESBL)-producing and carbapenem-resistant *E. coli* has further exacerbated treatment difficulties in pediatric patients (Eke et al., 2023).

Advances in molecular biology have facilitated the detection of resistance genes, providing crucial insights into the epidemiology of antimicrobial resistance (Jia et al., 2017). These molecular techniques are critical for tracking the spread of resistant pathogens and guiding antimicrobial stewardship programs (Ghafourian et al., 2019).

The resistance profile of *P. aeruginosa* and *E. coli* varies based on geographical location, antibiotic prescribing practices, and infection control measures. A study in Nigeria reported high resistance rates of *P. aeruginosa* to ceftazidime (85.4%) and meropenem (67.2%), while ESBL-producing *E. coli* exhibited resistance to third-generation cephalosporins exceeding 80% (Olonitola et al., 2022). These alarming resistance patterns pose a significant risk to pediatric patients, leading to prolonged hospital stays, increased healthcare costs, and higher mortality rates (Labi et al., 2021). Sokoto State, like many regions in Nigeria, faces a growing burden of antibiotic resistance, particularly among pediatric patients admitted to tertiary hospitals (Aliyu et al., 2023).

Despite the national concern, there is limited molecular epidemiological data specifically from Sokoto metropolis where pediatric patients face high risks due to overcrowded hospital wards, limited laboratory capacity, and inconsistent antibiotic regulation. Without localized data, treatment decisions rely on generalized national statistics that may not reflect the true resistance patterns in this setting. This study aims to detect *P. aeruginosa* and *E. coli*, while also profiling their antimicrobial susceptibility patterns among pediatric patients attending selected hospitals in Sokoto metropolis. The objectives of the study were:

- i. To isolate and identify *Pseudomonas aeruginosa* and *Escherichia coli* from clinical samples obtained from pediatric patients in selected hospitals in Sokoto State.
- ii. To determine the antimicrobial susceptibility patterns of the isolated *P. aeruginosa* and *E. coli*
- iii. To conduct molecular characterization of the isolated *E. coli* and *P. aeruginosa*

Methodology

Study Area

The study were conducted in two major Hospitals in Sokoto metropolis was purposively selected which include; State Specialist Hospital and Maryam Abacha Women and Children Hospital. The State Specialist Hospital Sokoto is located along Sultan Abubakar Road in the heart of Sokoto city, Sokoto State, Nigeria. Geographically, the hospital lies at approximately 13.0151° N latitude and 5.2423° E longitude. It is a central healthcare facility that serves the wider Sokoto metropolis and neighboring areas. Its location makes it accessible for patients from various parts of the state, as it is well-connected by major roads in the city. Maryam Abacha Women and Children Hospital is situated along Kalam Baina Road, a prominent area within Sokoto city, Sokoto State, Nigeria. The hospital is geographically positioned at 13.0579° N latitude and 5.2477° E longitude. It specializes in maternal and pediatric care and is one of the primary healthcare centers in Sokoto dedicated to women and children's health.

Study Design

The cross-sectional observational study was used. The sample population was grouped into two with a total of 193 clinical blood samples of pediatric patients between the age of (2-12 years old). Group (1) comprises 100 blood samples from Specialist Hospital Sokoto and group (2) comprise 93 blood samples from Maryam Abacha Women and Children Hospital. The blood samples were collected from diagnosed pediatric patients with *P. aeruginosa* and *E. coli* attending the two Hospitals. The study was conducted between 18th October, 2024 to 24th December, 2024.

Ethical Consideration

The study was approved by the Ministry of Health Sokoto, Sokoto State (Reference numbers SMH/1580/V. IV). Ethical approval from both hospitals were obtained. The field sampling protocols, samples collected from patient and the research were conducted in full compliance with Section 20 of the human Diseases Act of 1984 (Act No 35 of 1984) and were approved by the Sokoto State Government.

Sample Size

The sample size for the study was determined using a single population proportion formula. The Prevalence of *P. aeruginosa* and *E. coli* Infections in Northwest Nigeria, was 13.1%. It used as a reference and at a 95% level of confidence and a 10% non-response rate, the total of 193 children between the age of (2-12) years diagnosed with *P. aeruginosa* and *E. coli* targeted to be included in the study as;

$$n = \frac{Z^2 \times p (1 - P)}{d^2}$$

Where: n = sample size, Z^2 = level of confidence= 1.96, P = prevalence from previous study = 13.1%. =0.131, d = margin of error (0.05).

$$n = \frac{(0.106)^2 \times 0.131 (1 - 0.131)}{(0.05)^2}$$

$$n = \frac{3.8416 \times 0.113839}{0.0025} = 145.61 + 29.122 = 174.9$$

We therefore round up which gives 175, therefore our sample size =175

With 10% attrition, the sample size is approximately 193 (Garga et al., 2024).

Inclusion Criteria and Exclusion Criteria

This comprised only children between 2-12yrs diagnosed with *P. aeruginosa* and *E. coli* infections attending Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital. Children that are < 2 years and >12 years, not on chemotherapy and those not attending the stated hospitals were excluded from the study.

Sample Collection and Processing

Fresh blood samples were collected from pediatric patients using sterile, leak-proof containers to prevent contamination and ensure sample integrity, following established guidelines (Garcia, 2010). Each sample was meticulously labeled with the patient's identification number, age, hospital, and the date of collection to maintain accurate records and facilitate traceability (Clinical and Laboratory Standards Institute (CLSI), 2022; Murray et al., 2007). All collected samples were maintained at 4°C until processing to inhibit bacterial overgrowth and preserve pathogen viability, as recommended by standard microbiological protocols (CLSI), 2022; Murray et al., 2007).

Isolation of *E. coli* and *Pseudomonas aeruginosa*

Isolation of *Escherichia coli*:

Following pre-enrichment, aliquots from each sample were streaked onto MacConkey Agar and Eosin Methylene Blue (EMB) Agar plates. The inoculated MacConkey Agar plates were incubated aerobically at 35–37°C for 18–24 hours. *E. coli* colonies manifested as pink to red due to lactose fermentation. Concurrently, the EMB Agar plates were incubated under similar conditions. On EMB Agar, *E. coli* colonies exhibited a characteristic metallic green sheen, indicative of vigorous lactose fermentation (Garga et al., 2024).

Isolation of *Pseudomonas aeruginosa*

For isolation of *P. aeruginosa*, portions of the enriched samples were streaked onto Cetrimide Agar and MacConkey Agar plates. The Cetrimide Agar plates were incubated at 33–37°C for 24–48 hours. *P. aeruginosa* colonies appeared as greenish-blue, often with a mucoid texture, due to the production of pyocyanin and pyoverdine pigments. Simultaneously, the MacConkey Agar plates were incubated at 37°C for 24–48 hours. On this medium, *P. aeruginosa* presented as lactose non-fermenting colonies, typically colorless or pale, distinguishing them from lactose fermenters (Garga et al., 2024).

Gram Staining

A small inoculum from each bacterial culture was aseptically transferred onto a clean, grease-free glass slide. The sample was evenly spread to create a thin smear and allowed to air-dry completely. The smear was then heat-fixed by passing the slide through a flame several times, ensuring the bacteria adhered firmly to the slide surface. The heat-fixed smear was flooded with crystal violet stain, covering the entire area. The stain was left in place for 30 seconds to one minute to allow penetration into the bacterial cells. Excess stain was gently rinsed off with distilled water. Gram's iodine solution was applied to the smear, covering it completely. The iodine was allowed to act for 30 seconds to one minute, forming a crystal violet-iodine complex within the cells. The slide was rinsed gently with distilled water to remove unbound iodine. The smear was exposed to a decolorizing agent (95% ethanol solution), by allowing it to flow over the slide for approximately 10 to 20 seconds. The slide was immediately rinsed with distilled water to halt the decolonization process. The smear was then counterstained with safranin, covering the entire area. The counterstain was allowed to sit for 30 seconds to one minute, staining the decolorized Gram-negative cells. Excess safranin was rinsed off with distilled water. The slide was gently blotted dry using absorbent paper, taking care not to disturb the stained smear. Once dried, the slide was examined under a brightfield microscope at 1000x magnification using oil immersion. *E. coli* and *P. aeruginosa*, both being Gram-negative rods, appeared pink to red under the microscope due to the uptake of the safranin counterstain (Ado et al., 2019).

Biochemical Tests

Biochemical test such as Indole Test, Citrate Utilization Test, Oxidase Test, Urease Test, Catalase Test, and Triple Sugar Iron (TSI) Agar were carried-out on the isolates.

Standardization of Inoculum:

Bacterial isolates were cultured in nutrient broth and incubated at 37°C until they reached the logarithmic phase of growth. The turbidity of each bacterial suspension was then adjusted to match the 0.5 McFarland standard, corresponding to an approximate cell density of 1.5×10^8 CFU/mL. This standardization ensures consistency and reliability in the susceptibility testing results (Ado et al., 2019).

Inoculation of Mueller-Hinton Agar Plates:

A sterile cotton swab was dipped into the standardized bacterial suspension, and excess liquid was removed by pressing the swab against the inner wall of the tube. The surface of the MHA plate was then uniformly inoculated by streaking the swab across the entire agar surface in three directions, ensuring even distribution of the inoculum. The plate was allowed to stand for 3 to 5 minutes to permit absorption of excess moisture before applying antibiotic disks (Garga et al., 2024).

Application of Antibiotic Disks:

Using sterile forceps, antibiotic-impregnated disks were placed onto the inoculated agar surface, ensuring adequate spacing to prevent overlapping zones of inhibition. The antibiotics tested were selected based on their clinical relevance to each bacterial species:

For *P. aeruginosa*: Ceftazidime, Piperacillin-Tazobactam, Ciprofloxacin, Meropenem, and Gentamicin.

For *E. coli*: Amoxicillin-Clavulanate, Ciprofloxacin, Ceftriaxone, Tetracycline, and Nitrofurantoin.

The disks were gently pressed to ensure complete contact with the agar surface. The inoculated plates with antibiotic disks were incubated in an inverted position at 37°C for 16 to 18 hours. This incubation period allows sufficient time for bacterial growth and antibiotic diffusion to occur, resulting in clear zones of inhibition around effective antibiotics. Following incubation, the diameter of each zone of inhibition was measured in millimeters using a ruler. The measurements were then compared to the Clinical and Laboratory Standards Institute (CLSI) guidelines to categorize each bacterial isolate as susceptible, intermediate, or resistant to the tested antibiotics. This standardized interpretation ensures accurate determination of antimicrobial susceptibility profiles (Garga et al., 2024).

DNA Extraction

Genomic DNA was extracted from bacterial cultures using two primary methods: commercial extraction kits and the boiling method. For the kit-based approach, the TaKaRa MiniBEST Bacterial Genomic DNA Extraction Kit (TaKaRa, Dalian, China) was employed, following the manufacturer's instructions. This method facilitated efficient and reliable isolation of genomic DNA from the bacterial suspensions (Iseghohi et al., 2020).

PCR Amplification for Species Confirmation:

Species-specific PCR assays were performed to confirm the identity of the bacterial isolates by targeting characteristic genes.

For *E. coli*, the following genes were targeted:

16S rRNA gene: Universal primers were employed to amplify the 16S rRNA gene: universal primers were employed to amplify the 16S rRNA gene, a conserved region presents in all bacterial species, providing a basis for identification. **uidA (β -glucuronidase) gene:** Primers specific to the uidA gene, encoding β -glucuronidase, were used, as this gene is commonly associated with *E. coli* (Iseghohi et al., 2020).

For *P. aeruginosa*, the following genes were targeted:

oprL (outer membrane lipoprotein) gene: Primers specific to the oprL gene were utilized, as this gene encodes an outer membrane lipoprotein unique to *P. aeruginosa*. **16S rRNA gene:** Similar to *E. coli*, universal primers were used to amplify the 16S rRNA gene for *P. aeruginosa* identification (Iseghohi et al., 2020).

PCR Conditions

The PCR reactions were prepared with the following thermal cycling conditions: Initial denaturation: 94°C for 5 minutes to denature the DNA strands. 35 amplification cycles: Denaturation: 94°C for 30 seconds to separate the DNA strands. Annealing: 55–60°C for 30 seconds to allow primers to bind to their complementary sequences. The specific annealing temperature was optimized based on the primer pair used. Extension: 72°C for 45 seconds to enable the DNA polymerase to synthesize the new DNA strand. Final extension: 72°C for 5 minutes to ensure complete synthesis of all DNA fragments. These conditions were adapted from standardized protocols to ensure specificity and efficiency in amplifying the target genes (Merza and Jubrael, 2015).

Agarose Gel Electrophoresis:

The PCR products were analyzed using 1.5% agarose gel electrophoresis. The agarose gel was prepared by dissolving agarose powder in 1× TAE buffer, followed by heating until complete dissolution. After cooling to approximately 60°C, ethidium bromide was added to a final concentration of 0.5 µg/mL to enable visualization of DNA bands under ultraviolet light. The gel was then poured into a casting tray with a comb to create wells and allowed to solidify. Subsequently, 5 µL of each PCR product was mixed with 1 µL of 6× loading dye and loaded into the wells. A DNA ladder was also loaded to serve as a molecular weight marker. Electrophoresis was performed at 100 volts for approximately 45 minutes in 1× TAE buffer. Following electrophoresis, the gel was visualized under a UV trans illuminator, and the sizes of the PCR products were determined by comparing them to the DNA ladder (Merza and Jubrael, 2015).

Method of Data Analysis

Data obtained from the antimicrobial susceptibility testing and molecular characterization were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including frequency distributions and percentages, were used to summarize the data, which were presented in tables for clarity. The antimicrobial resistance profiles of *Pseudomonas aeruginosa* and *Escherichia coli* were compared using data from phenotypic susceptibility testing (Kirby–Bauer disk diffusion method) and molecular characterization of resistance genes. Interpretation of antimicrobial susceptibility was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) breakpoints (Perovic et al., 2023), and resistance was classified as susceptible, intermediate, or resistant. Phenotypic and molecular results were tabulated side by side to identify concordance or discrepancies between the two methods.

Results

The result presented in (Table 1) summarize the prevalence of *Pseudomonas aeruginosa* and *Escherichia coli* isolates from clinical samples collected at two hospitals in Sokoto, Nigeria: Maryam Abacha Hospital and General Hospital. Out of a total of 193 samples examined, 80

(41.45%) showed positive microbial growth. The results highlight variations in both the overall prevalence of bacterial growth and the specific occurrence of *P. aeruginosa* and *E. coli* at the two locations. At Maryam Abacha Hospital, 93 samples were examined, with 44 (47.31%) showing positive microbial growth. Of these, 2 isolates (4.55%) were molecularly identified as *P. aeruginosa*, while 3 isolates (6.82%) were identified as *E. coli*. Similarly, at the General Hospital, 100 samples were analyzed, and 36 (36%) yielded positive growth. From these, 4 isolates (11.11%) were positive for *P. aeruginosa*, and 3 isolates (8.33%) were positive for *E. coli*.

Table 1: Prevalence of *P. aeruginosa* and *E. coli* Isolates at Maryam Abacha and General Hospitals Sokoto

Hospitals	No. of Samples Examined	No. of Positive Growth (%)	No. Positive for <i>P. aeruginosa</i> (%)	No. Positive for <i>E. coli</i> (%)
Maryam Abacha	93	44(47.31)	2(4.55)	3(6.82)
General	100	36(36)	4(11.11)	3(8.33)
Total	193	80(41.45)	6(7.5)	6(7.5)

Source: Authors analysis, 2025.

The morphological characteristics (Table 2) of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from blood samples, as presented in Table 4.2, provide crucial insights into the identification and differentiation of the metallic green colonies with dark centers (samples 11, 17, 51, 59, 70, 87) are pathogenic for *E. coli* on EMB agar. This morphology arises from the organism's vigorous lactose fermentation, which acidifies the medium, causing precipitation of eosin and methylene blue dyes. Co-colonization increases the risk of horizontal gene transfer (HGT) of resistance determinants, such as extended-spectrum β -lactamase (*bla*_{CTX-M}) or carbapenemase (*bla*_{NDM}) genes, between Gram-negative species.

Table 2: Morphological Characteristics of the *E. coli* and *P. aeruginosa* isolated from the blood Samples

Samples ID	Morphological Characteristics of the isolates on EMB and Cetrinide agar	Suspected Bacteria
11	Presence of metallic green colonies with a dark center.	<i>Escherichia coli</i>
17	Presence of metallic green colonies with a dark center	<i>Escherichia coli</i>
17	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
18	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
19	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
51	Presence of metallic green colonies with a dark center	<i>Escherichia coli</i>

59	Presence of metallic green colonies with a dark center	<i>Escherichia coli</i>
66	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
70	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
70	Presence of metallic green colonies with a dark center	<i>Escherichia coli</i>
87	Presence greenish-yellow and blue-green colonies.	<i>Pseudomonas aeruginosa</i>
87	Presence of metallic green colonies with a dark center	<i>Escherichia coli</i>

Source: Authors analysis, 2025.

The antibiotic susceptibility profiles (Table 3) of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from blood samples is presented in (Table 3). The results indicate that *E. coli* isolates exhibited 100% susceptibility to tetracycline (TE) and ceftazidime (CAZ), with moderate susceptibility to nalidixic acid (75%), chloramphenicol (58.3%), and trimethoprim-sulfamethoxazole (50%). Alarming, *E. coli* showed complete resistance (100%) to ampicillin (AMP) and high resistance to ciprofloxacin (66.7%), kanamycin (66.7%), and amikacin (58.3%). The total resistance to ampicillin reflects widespread production of β -lactamases among clinical *E. coli* strains. The moderate to high resistance to aminoglycosides such as gentamicin and amikacin may result from aminoglycoside-modifying enzymes or efflux mechanisms, commonly found in multidrug-resistant *E. coli* isolates. In *P. aeruginosa*, the isolates demonstrated 100% susceptibility to tetracycline and ceftazidime. However, resistance to ciprofloxacin (33.3%), kanamycin (66.7%), and amikacin (58.3%) in *P. aeruginosa* still indicates significant antimicrobial pressure and the presence of resistance genes, such as those encoding aminoglycoside-modifying enzymes and efflux systems.

Table 3: Antibiotic Susceptibility Profile of *E. coli* and *Pseudomonas aeruginosa* Isolated from the blood samples

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
CIP	6(66.67)	0(0)	6(66.67)
TE	12(100)	0(0)	0(0)
NA	9(75)	0(0)	3(25)
AMP	0(0)	0(0)	12(100)
K	4(33.33)	0(0)	8(66.66)
C	7(58.33)	3(25)	2(16.66)
SXT	6(50)	0(0)	6(50)
CAZ	12(100)	0(0)	0(0)
CN	5(41.67)	5(41.67)	2(16.67)
AK	2(16.67)	3(25)	7(58.33)

KEY: CIP= Ciprofloxacin, TE= Tetracycline, NA= Nalidixic acid, AMP= Ampicillin, K= Kanamycin, SXT= Trimetoprim, CAZ= Ceftazidime, CN= Gentimicin, AK= Amikacin

Source: Authors analysis, 2025.

The MAR index (Table 4) for *E. coli* isolates ranged between 0.3 and 0.6. Isolate *E. coli* exhibited the lowest MAR index (0.3), indicating resistance to three out of ten antibiotics tested, suggesting a relatively lower level of antibiotic resistance. On the other hand, isolates *E. coli* 2, 3, 4, and 6 had a MAR index of 0.6, indicating resistance to six out of ten antibiotics. This suggests that these strains might have originated from high-risk environments with frequent antibiotic exposure, such as hospitals, wastewater, or heavily contaminated water sources. The MAR index for *Pseudomonas aeruginosa* isolates ranged from 0.3 to 0.7. Isolates *P. aeruginosa* 1, 2, and 3 exhibited a MAR index of 0.3, indicating relatively low resistance. However, isolate *P. aeruginosa* 6 had the highest MAR index (0.7), indicating resistance to seven out of ten antibiotics tested. This suggests the presence of highly resistant strains, possibly due to intrinsic resistance mechanisms such as efflux pumps, biofilm formation, and beta-lactamase production.

Table 4: Antibiotic Resistant Pattern of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from bloods samples

Isolate	Number of Resistant Antibiotics	Total Antibiotics Tested	MAR Index
<i>E. coli</i> 1	3	10	0.3
<i>E. coli</i> 2	6	10	0.6
<i>E. coli</i> 3	6	10	0.6
<i>E. coli</i> 4	6	10	0.6
<i>E. coli</i> 5	5	10	0.5
<i>E. coli</i> 6	6	10	0.6
<i>Pseudomonas aeruginosa</i> 1	3	10	0.3
<i>Pseudomonas aeruginosa</i> 2	3	10	0.3
<i>Pseudomonas aeruginosa</i> 3	3	10	0.3
<i>Pseudomonas aeruginosa</i> 4	5	10	0.5
<i>Pseudomonas aeruginosa</i> 5	6	10	0.6
<i>Pseudomonas aeruginosa</i> 6	7	10	0.7

Source: Authors analysis, 2025.

The gel electrophoresis image represents the PCR amplification of two bacterial species: *Escherichia coli* and *Pseudomonas spp.* The expected fragment sizes for *E. coli* and *Pseudomonas* are 503 bp and 249 bp, respectively. The image also includes a 100 bp molecular ladder (M) for size estimation. Lanes 1-6 (*E. coli*: 11, 15, 17, 59, 70, 87): The bands are all at approximately 503 bp, indicating successful amplification of *E. coli*. Lane M

(Marker/Ladder): The 100 bp DNA ladder provides reference points for band size determination. Lanes 7-12 (*Pseudomonas*: 17, 18, 19, 66, 70, 87): The bands appear at approximately 249 bp, confirming the presence of *Pseudomonas spp.* No visible non-specific bands or smears are observed, indicating that the PCR reaction was specific and efficient.

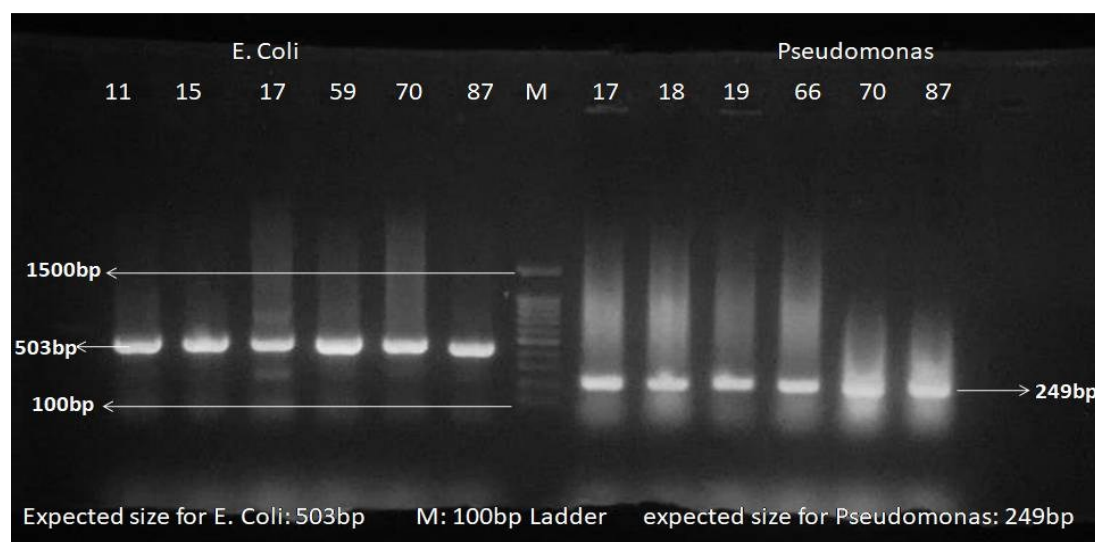


Figure 1: Showing the Gel Electrophoresis of *P.aeruginosa* and *E.coli*

Source: Authors analysis, 2025.

Discussion

The total prevalence of *P. aeruginosa* and *E. coli* across both hospitals (Table 1) was 7.5% for each organism, despite differences in individual hospital distributions. These results may indicate slight differences in infection control practices, patient demographics, or antibiotic usage policies between the hospitals (Olayemi et al., 2022; Yusuf et al., 2023). *P. aeruginosa* is known for its opportunistic pathogenicity and intrinsic resistance to many antibiotics, often posing challenges in hospital settings (Reyes et al., 2021). Its higher occurrence in the Specialist Hospital could reflect environmental or procedural factors that favor its survival and transmission. Meanwhile, the consistent prevalence of *E. coli*, a common cause of urinary tract and gastrointestinal infections across both hospitals suggests it remains a prevalent nosocomial pathogen in the region (Ibrahim et al., 2023). Olayemi et al. (2022) reported a higher prevalence of *E. coli* at 18.3% among isolates from clinical and environmental sources in a tertiary health facility in Kwara State.

The lower *E. coli* prevalence in the Sokoto hospitals (7.5%) could be attributed to differences in infection control practices, diagnostic methods, or patient case mix. Similarly, Yusuf et al. (2023) found *E. coli* to be the most frequently isolated pathogen (25.4%) in a study involving secondary and tertiary hospitals in northern Nigeria, further highlighting regional variability and possible underreporting in Sokoto's settings. In contrast, the prevalence of *P. aeruginosa* found in the Sokoto hospitals (7.5%) is relatively consistent with that reported by Ibrahim et al. (2023), who noted a prevalence of 9.2% in a study of nosocomial infections

in Kano State. This organism is often associated with healthcare-associated infections (HAIs), especially in intensive care units, due to its resistance to multiple antibiotics and ability to survive in moist hospital environments (Reyes et al., 2021). Interestingly, the slightly higher prevalence of *P. aeruginosa* (11.11%) in Specialist Hospital compared to Maryam Abacha (4.55%) may indicate differences in hygiene protocols, availability of disinfection infrastructure, or frequency of device-related infections such as those involving catheters or ventilators.

Globally, the World Health Organization (WHO, 2023) has identified both *E. coli* and *P. aeruginosa* as critical priority pathogens due to their increasing resistance to commonly used antibiotics. In sub-Saharan Africa, the median prevalence of *E. coli* in clinical isolates ranges from 15% to 25%, while *P. aeruginosa* generally accounts for 5–10% of isolates (WHO, 2023; Oduyebo et al., 2022).

EMB agar selectively inhibits Gram-positive bacteria while differentiating lactose fermenters (e.g., *E. coli*) from non-fermenters (Murray et al., 2021). The isolation of *E. coli* from blood samples reflects its status as a dominant commensal in the human gut, though pathogenic strains can cause infections (Russo and Johnson, 2023). The greenish-yellow and blue-green colonies (samples 17, 18, 19, 66, 70, 87) on Cetrimide agar are characteristic of *P. aeruginosa*. Cetrimide (a quaternary ammonium compound) suppresses non-*Pseudomonas* species, while pyocyanin (blue-green) and pyoverdine (yellow-green) pigments produced by *P. aeruginosa* confirm its identity (Khan et al., 2022). The isolation of *P. aeruginosa* from blood is clinically significant; its presence suggests colonization, often linked to prior antibiotic exposure or hospitalization (Tacconelli et al., 2023). Notably, samples 17, 70, and 87 harbored both *E. coli* and *P. aeruginosa* (Table 2), indicating polymicrobial colonization.

The antibiotic susceptibility profiles of *Escherichia coli* and *Pseudomonas aeruginosa* isolates (Table 3) from Maryam Abacha and General Hospitals in Sokoto display patterns reflective of both regional and broader antimicrobial resistance trends. The presence of *E. coli* in blood samples is a significant public health concern as certain strains, particularly Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enterohemorrhagic *E. coli* (EHEC), are associated with severe diarrheal diseases (Croxen et al., 2013). Resistance to ampicillin (100 %) by *E. coli* isolates aligns with high rates of β -lactam resistance observed in clinical isolates; for example, 88.9 % resistance was documented among isolates in Nasarawa hospital, Nigeria (Tama et al., 2021). Conversely, 100 % susceptibility to tetracycline is surprising, given tetracycline resistance between 81 % and 94 % reported in Southwest Nigeria (Adelowo et al., 2014; Ayandiran et al., 2018). This discrepancy may suggest differing antibiotic use environments or isolate sources. Moderate ciprofloxacin resistance (50 %–66.7 %) in this study is consistent with reported Nigerian resistance levels ranging from 42 % to 41 % (Ayandiran et al., 2018; Adelowo et al., 2014).

The lower prevalence of *Pseudomonas aeruginosa* in blood samples aligns with existing literature, which indicates that this bacterium, while found in the gastrointestinal tract, is not a dominant enteric pathogen in healthy individuals. Rather, it is frequently associated

with nosocomial infections, particularly in patients with compromised immunity or underlying conditions such as cystic fibrosis, chronic obstructive pulmonary disease, and cancer (Klockgether and Tümmler, 2017; Pang et al., 2019). Complete resistance to ampicillin by *P. aeruginosa* is intrinsic and widely expected due to AmpC β -lactamase activity, as noted globally. The low ciprofloxacin susceptibility (33.3 %) parallels moderate-to-high resistance trends in Middle Eastern and North African clinical isolates (MENA region: 0–44 %) (Al-Orphaly et al., 2021). The 100 % susceptibility to ceftazidime is promising, contrasting with other resistance rates (29–66 %) in tertiary hospitals in Saudi Arabia (~29 %) and Ethiopia (~35–66 %) (Al-Orphaly et al., 2021). However, low amikacin efficacy (16.7 % susceptibility) mirrors generally low aminoglycoside resistance in MENA ICU isolates, reported often under 6 %, though higher in some countries (up to ~33 %) (Al-Orphaly et al., 2021).

MAR index values above 0.2 (Table 4) indicate bacterial strains originating from high-risk environments with significant antibiotic exposure. The MAR values observed in this study suggest that both *E. coli* and *Pseudomonas aeruginosa* isolates might have been exposed to environments where antibiotic use is prevalent. The high MAR index values in some isolates highlight the need for stringent antibiotic stewardship programs to prevent the spread of multi-drug-resistant (MDR) bacteria (Zhang et al., 2023).

Gel electrophoresis is a crucial molecular technique for identifying bacterial species through DNA amplification. The successful amplification of *E. coli* and *Pseudomonas* in this experiment highlights the effectiveness of species-specific primers in PCR (Arora et al., 2023). The presence of clear bands at the expected sizes confirms the accuracy of the PCR assay and suggests the absence of contamination. PCR-based molecular identification is widely used for distinguishing bacterial species based on conserved genetic markers such as 16S rRNA, rpoB, and specific virulence genes (Tenaillon et al., 2022). In this gel, the 503 bp amplicon for *E. coli* likely corresponds to genes such as *uidA* or 16S rRNA, which are commonly targeted for bacterial identification (Clermont et al., 2019). Similarly, the 249 bp amplicon in *Pseudomonas spp.* may represent amplification of the *oprL* gene, a specific marker used for the identification of *Pseudomonas aeruginosa* (De Vos et al., 2022). The absence of non-specific bands or primer-dimers suggests that the primers used were highly specific for the target bacteria, and the PCR conditions (annealing temperature, Mg²⁺ concentration, and extension time) were optimal. This specificity is essential for clinical and environmental microbiology studies, where accurate bacterial identification is crucial (Ahmed et al., 2021).

The identification of *E. coli* and *Pseudomonas* has significant clinical and environmental implications. *E. coli* is a common indicator of fecal contamination in water and food and is also associated with gastrointestinal and urinary tract infections (Gomi et al., 2017). *Pseudomonas spp.*, particularly *Pseudomonas aeruginosa*, is an opportunistic pathogen responsible for infections in immunocompromised patients and is known for its antibiotic resistance mechanisms (Pang et al., 2019). Studies have shown that PCR-based detection

methods are superior to traditional culturing techniques in terms of sensitivity and specificity, making them valuable tools for microbial diagnostics (Oliveira et al., 2023).

Conclusion and Recommendations

Conclusively, the prevalence of *E. coli* and *Pseudomonas aeruginosa* in blood samples is relatively low, but their presence in some patients highlights the need for routine surveillance, infection control measures, and public health interventions to prevent gastrointestinal and opportunistic infections. The isolation of *E. coli* and *P. aeruginosa* from blood highlights emerging risks of gut colonization by resistant pathogens, driven by antibiotic misuse and hospital-acquired transmission. Based on the present study it will be recommended that:

1. Regular microbial screening of blood samples in hospital settings, along with stringent hygiene and sanitation measures, should be implemented to prevent the spread of *E. coli* and *Pseudomonas aeruginosa*, especially among immunocompromised patients.
2. Implement hospital-wide genomic surveillance and antibiotic stewardship programs to detect and mitigate resistance gene transfer between co-colonizing pathogens like *E. coli* and *P. aeruginosa*.
3. Prioritize region-specific antibiotic stewardship programs and environmental surveillance to disrupt resistance gene transmission pathways.
4. Integrate PCR-based diagnostics into routine surveillance programs to enhance early detection of antibiotic-resistant *E. coli* and *P. aeruginosa* in healthcare and environmental settings.

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