Original Research Article

Molecular Profiling of Antibiotic-Resistant *Pseudomonas aeruginosa* Isolated from Patients with Urinary Tract Infections in Rivers State University Teaching Hospital

ABSTRACT

Background: Antibiotic-resistant *Pseudomonas aeruginosa* poses a significant challenge in urinary tract infections (UTIs), particularly in hospital settings. **Aim**: The aim of this study was to analyze the molecular profile of antibiotic-resistant *P. aeruginosa* strains isolated from patients with urinary tract infections at the Rivers State University Teaching Hospital, Port Harcourt. **Methods**: As part of a cross-sectional study, 199 patients were interviewed, and urine samples were collected from subjects with symptoms of a urinary tract infection. A semiquantitative urine culture method was used to detect significant bacteriuria. Suspected uropathogenic*P. aeruginosa* isolates were then identified using conventional and molecular techniques. The data generated from this study was presented as frequencies and percentages using Microsoft Excel 365. **Results**: Of the 199 urine samples analyzed, *P. aeruginosa* was isolated in 17 cases, corresponding to a prevalence rate of 8.5%. Among the antimicrobial classes tested, aminoglycosides showed different resistance rates: amikacin (23.5%) and gentamicin (35.3%). Fluoroquinolones, including ofloxacin and ciprofloxacin, had resistance rates of 52.9%. Cephalosporins showed high resistance, particularly cefuroxime sodium (88.2%) and cefpodoxime (100%). Amoxicillin + clavulanic acid showed a resistance rate of 94.1%, while resistance to cefotaxime was 47.1%. Imipenem remained effective without resistance being observed. Several antimicrobial resistance indices (MAR) indices revealed complex resistance patterns across isolates, with the highest MAR index at 0.92. Genotypic analysis identified CTX-M as the most prevalent resistance gene (77.8%), followed by TEM (44.4%) and SHV (33.3%). Notably, no isolates harbored NDM or VIM genes, indicating the absence of carbapenem resistance genes among the studied isolates.**Conclusion**: This study highlights the prevalence of antibiotic-resistant *P. aeruginosa* in UTIs at Rivers State University Teaching Hospital.

Keywords: Pseudomonas aeruginosa, antibiotic resistance, urinary tract infection, antibiotic resistance genes.

1. INTRODUCTION

Urinary tract infections (UTI) are one of the most common causes of infection worldwide. While *E. coli* is primarily associated with the etiology of urinary tract infections, other organisms such as *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus* and especially *Pseudomonas aeruginosa* are becoming increasingly prevalent [1]. *P. aeruginosa* is an opportunistic pathogen responsible for 7–10% of healthcare-associated urinary tract

infections (UTIs) [2]. *P. aeruginosa* urinary tract infections often affect people with previous illnesses such as urinary tract abnormalities [3] and are particularly favored by the presence of indwelling urinary catheters. Multidrug resistance (MDR) is commonly observed in *P. aeruginosa* urinary strains as they can develop resistance to different classes of antibiotics [4]. This phenomenon, related to the ability of *P. aeruginosa* to form biofilms, leads to infections that are difficult to manage [5].

Multidrug-resistant (MDR) bacteria are characterized by being resistant to at least one active ingredient in three or more antibiotic classes [6]. Unfortunately, *P. aeruginosa* has shown resistance in several studies, making its treatment ineffective. It is a gram-negative bacillus that can thrive in various environments, including aquatic and terrestrial environments [7]. It contributes significantly to nosocomial infections and affects immuno-compromised patients [7]. The intrinsic antimicrobial resistance mechanisms of *P. aeruginosa*, which encompass reduced outer membrane permeability, heightened expression of efflux pumps with varied specificity, and the presence of AmpC beta-lactamase, confer resistance to commonly utilised antibiotics such as Penicillin G, first and second-generation cephalosporins, and quinolones [8].

Antibiotic resistance is a widespread natural phenomenon, and resistance mechanisms can adapt and evolve as quickly as bacterial cells proliferate [9]. Numerous studies suggest that humans were colonized by resistant bacterial clones that persisted for months after antibiotic treatment [10]. The emergence of resistance in *P. aeruginosa* limits therapeutic options and correlates with increased morbidity and mortality rates, increased costs and longer hospital stays compared to antibiotic-susceptible bacteria [11]. *P. aeruginosa* has emerged as a significant pathogen in the hospital setting [12], with an increasing prevalence of drug-resistant strains, particularly those isolated from UTIs. Due to the evolution of resistance mechanisms in these pathogens, we are faced with limited therapeutic alternatives, primarily due to the indiscriminate use of antibiotics, incomplete therapeutic regimens, inconsistent dosages, and over-the-counter availability of these drugs [13]. Approximately 150 million cases of UTIs are reported annually, making it the third most diagnosed infection [14, 15]. Overall, this leads to increased morbidity and mortality rates. This contributes to the significant financial burden on our healthcare system. Consequently, there is an augmented necessity for novel antibiotics to effectively treat urinary tract infections.

The results of this study will shed light on the epidemiology and phylogenetic characteristics of uropathogenic*Pseudomonas* species in Port Harcourt, Rivers State, as *P. aeruginosa* is a major opportunistic and nosocomial pathogen commonly responsible for severe UTIs and fatal infections, particularly in the immunocompromised persons. The results of this study will improve the clinical and scientific community's and health authorities' understanding of the prevalence and molecular characteristics of *Pseudomonas* associated with urinary tract infections in the study population. This knowledge will support management protocols and improve therapeutic outcomes for affected patients, while providing guidelines for mitigating associated risk factors within this population. The results of the study will benefit both researchers and students by serving as a reference for developing and conducting further research on these topics. Hence, the aim of this study was to analyze the molecular profile of antibiotic-resistant *P. aeruginosa* strains associated with urinary tract infections in Rivers State University Teaching Hospital, Port Harcourt.

2. MATERIAL AND METHODS

2.1 Study Area

The research was conducted at the Rivers State University Teaching Hospital in Port Harcourt. Port Harcourt serves as the capital of Rivers State in Nigeria. The Rivers State University Hospital is a government-owned facility situated at 5-8 Harley Street, Old GRA Port Harcourt, Rivers State, Nigeria. The facility has 375 licensed beds and employs 731

medical personnel. The departments encompass Medicine, Paediatrics, Laboratory Services, Radiology, Family Medicine, Pathology, Obstetrics and Gynaecology, Anaesthesia, Surgery, Emergency Care, Pharmacy, Medical Emergency, Finance, Maintenance, and General Administration.

2.2 Study Design
This study employed a cross-sectional study design with randomized sampling.

2.3 Study Population
The study population comprised of subjects indicating urinary tract infection attending the Rivers State University Teaching Hospital. The subjects were recruited at the reception of the medical laboratory services department of the hospital when they present for urine microscopy, culture and sensitivity (M/C/S).

2.4 Sample Size Determination
The minimum sample size of subjects recruited for the study was calculated using the prevalence of P. *aeruginosa* among other clinical isolates, which is 7.39% [16].

Using the formula: $N = \frac{z^2pq}{d^2}$, where N = Minimum sample size, Z = Standard normal deviation corresponding to 95% confidence level set at 1.96, p =7.39% = 0.0739, q = 1-p = 0.9261 and d = desired precision, 5% (0.05), the sample size is 106. To cater for 50% non-response, an additional 53 subjects were added to bring the sample size to 159. However, 199 subjects were recruited for the study.

2.5 Ethical Approval
Ethical approval was obtained from the Rivers State University Teaching Hospital Research and Ethics Committee, Registration Number: RSUTH/REC/202352.

2.6 Consent Form
The research was explained to all willing subjects who passed the minimum inclusion criteria. Thereafter, all subjects were required to agree to provide oral informed consent.

2.7 Eligibility of Subjects
Subjects indicating signs and symptoms of UTI were included in the study. Only those willing to provide at least oral consent were included in the study. Individuals who did not consent to study were excluded. Terminally ill patients who could not consent to provide samples were excluded from the study. Female subjects currently in their menstrual period were excluded from the study.

2.7 Collection of Specimen
A total of one hundred ninety-nine (199) urine samples were obtained from inpatients and outpatients meeting the eligibility criteria. All urine specimens were collected either midstream clean catch, catheter, or urine bags employing aseptic methods. Subsequent to collection, the urine samples were promptly conveyed to the microbiology laboratory for culture and antibiotic susceptibility analysis. All collected samples were identified based on their physical characteristics observed on culture media and by biochemical tests.

2.9 Urine Culture
A calibrated loop that contains 0.01 mL of material, was utilised to transfer a loopful of urine to a blood agar plate. The primary inoculation consisted of a solitary streak traversing the width of the agar plate. The plate was subsequently rotated 90 degrees and streaked again, this time in a zigzag pattern across the initial line to uniformly distribute the sample across the entire plate. After a 24-48 hours of incubation period at 37°C, the resultant colonies were enumerated, and the population density, termed original cell density (OCD), was estimated.

The OCD will be documented in colony-forming units per millilitre (CFU/mL) and computed as follows

$$OCD = \frac{CFU}{Loop\ Volume}$$

2.10 Isolation and Identification Processes Culture

The specimens were cultured on cetrimide agar and incubated at 37°C for 24 hours. After 18 to 48 hours of incubation, the plates displayed isolated colonies in streaked areas and dense growth in regions of heavy injection. Colonies were provisionally identified as *Pseudomonasaeruginosa* based on their blue green to green pigmentation and fluorescence under short wavelength (254 nm) UV light.

2.11 Biochemical Analysis

Biochemical analyses of the bacterial isolates included urease, citrate utilization, catalase, oxidase, and sugar fermentation tests, in addition to assessing growth at 42°C.

2.12 Antibiotic Susceptibility test

Antimicrobial susceptibility testing was performed via the disc diffusion technique in accordance with the Clinical and Laboratory Standards Institute guidelines (2014). The following antibiotics (Oxoid, UK) were utilized: Cefpodoxime (30 µg), Tetracycline (30 µg), Ceftazidime (30 µg), Amoxicillin + Clavulanic Acid (30 µg), Imipenem (10 µg), Cefepime (30 µg), Amikacin, Gentamicin (10 µg), Cefotaxime (30 µg), Cefuroxime (30 µg), Ofloxacin (5 µg), and Ciprofloxacin (5 µg). Five milliliters (5 ml) of sterile peptone water were inoculated with a new culture of each *Pseudomonas* strain, and the turbidity of the suspension was adjusted to 0.5 McFarland's standard. A sterile cotton swab was saturated with the inoculum and uniformly distributed across the surface of the Muller-Hinton agar plate. The inoculation plates were kept at room temperature for 10 minutes to allow moisture absorption, following which the antibiotic discs were firmly placed on the plates using forceps to ensure contact with the agar, followed by incubation at 37ºC for 24 hours. After incubation, the diameters of the inhibitory zones were measured in millimeters and compared to the established standards [17].

2.13 16S rRNA Characterization of the Bacteria Isolates

Bacteria DNA was extracted using the ZR fungal/bacterial DNA mini-prep kits and the extracted DNA was measured with Nanodrop 1000 spectrophotometer. The 16s rRNA region was identified using the following primers: 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3'. The following were the parameters of the polymerase chain reaction (PCR): 35 cycles of denaturation at 95°C for 5 minutes, 40 seconds of annealing at 52°C, and 5 minutes of extension at 72°C. After 30 minutes of analysis on a 1% agarose gel at 130V, the results were visualized using a blue light transilluminator.

2.14 Gene Sequencing

The 3510 ABI sequencer was used for sequencing by Inqaba Biotechnological of Pretoria, South Africa, via the BigDye Terminator kit. A total of 10 µL was used for the sequencing process, with 0.25 µL of BigDye terminator v1.1/v3.1, 2.25 µL of 5x BigDye sequencing buffer, 10 µM PCR primer, and 2-10 ng of PCR template per 100 bp included. Here are the sequencing parameters: Twenty-two repetitions of 10 seconds at 96°C, 5 seconds at 55°C, and 4 minutes at 60°C.

2.15 Amplification of Resistance Genes

After bacterial DNA extraction, the following resistance genes were amplified: blaSHV SHVF: 5' CGCCTGTGTATTATCTCCCT-3' and SHVR: 5'-CGAGTAGTCCACCAGATCCT-3',

blaTEM TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGAG-3', blaCTX-M CTX-MF 5'-ATTGAGTATTCAACATTTCCGTG-3' and CTX-MR 5'-TTACCAATGCTTAATCAGTGAG-3', NDM NDMF: 5-GGTTTGGCGATCTGGTTTTC-3' and NDMR: 5'-CGGAATGGCTCATCACGATC-3' and VIM VIMF: 5-GTTTGGTCGCATATCGCAAC-3' and VIMR: 5'- CTACTCGGCGATTGAGCGAT-3' primers Standard PCR protocols were used to amplify the genes. This was repeated for 35 cycles. The product sizes were achieved by resolving it on a 1% agarose gel at 130V for 30 minutes and then visualizing it with blue light imaging equipment.

2.15 Data Analyses

The data generated from this study was represented as frequency and percentages with the aid of Microsoft Excel 365.To modify the obtained sequences, the Trace Edit bioinformatics technique was used. Using the Basic Local Alignment Search Tool (BLASTN), sequences that were similar were located in the NCBI database. The ClustalX program was used to align the sequences. By utilizing the Neighbor-Joining approach in MEGA 6.0, the evolutionary history was inferred. To show how the species under study have evolved through time, we used the 500-replicate bootstrap consensus tree. Geographical distances were determined using the Jukes-Cantor technique.

3. RESULTS

3.1 Sociodemographic Information

The study involved a total of 199 subjects whose sociodemographic characteristics were analyzed. The age distribution of the subjects indicated a diverse range with the highest representation of the 21-30 age group. Specifically, 33 participants (16.6%) were aged 20 years or younger, 67 participants (33.7%) fell within the 21-30 age range, 49 participants (24.6%) were between 31-40 years old, 34 participants (17.1%) were aged 41-50, and the smallest group, consisting of 16 participants (8.0%), was 51 years or older. In terms of gender distribution, the majority of the subjects were female, comprising 121 individuals, which accounted for 60.8% of the total sample. Males, on the other hand, constituted 39.2% of the sample with 78 participants as shown in table 1.

Table 1: Sociodemographic Information of the Subjects

Variable	Frequency (N)	Percentage (%)
Age		
≤ 20	33	16.6
21 - 30	67	33.7
31 - 40	49	24.6
41 - 50	34	17.1
≥ 51	16	8
Total	199	100
Gender		
Male	78	39.2
Female	121	60.8
Total	199	100

3.2 Prevalence of Uropathogenic*P. aeruginosa* species

The prevalence of uropathogenic*Pseudomonas* species among the subjects was determined through microbiological analysis. Out of the 199 subjects studied, significant growth of *Pseudomonas* species was observed in 17 cases, indicating a prevalence rate of 8.5%. In

contrast, most of the subjects, totaling 182 individuals, showed no growth of *Pseudomonas* species, representing 91.5% of the sample as shown in Table 2 below.

Table 2. Prevalence of uropathogenic*Pseudomonas species* among subjects

Pseudomonas species	**Positive**	**Prevalence (%)**
Significant growth	17	8.5
No growth	182	91.5
Total	199	100

3.3 Antimicrobial Susceptibility Pattern of Uropathogenic*Pseudomonas* Isolates

The antimicrobial susceptibility of *Pseudomonas* isolates from 17 subjects was evaluated against various antibiotics from different classes as shown in Table 3. Among the aminoglycosides, imipenem and amikacin showed the least resistance rate of 0% and 23.5% being resistant respectively. Cefpodoxime showed complete resistance with 100% cases of resistance in the 17 isolates. Amoxicillin + Clavulanic Acid demonstrated 94.1% cases of resistance.

The study evaluated the Multiple Antimicrobial Resistance (MAR) index of uropathogenic*Pseudomonas* species, identifying various resistance patterns across different antibiotic classes. The MAR index is a crucial indicator of the extent of antibiotic resistance within bacterial isolates.

The first resistance pattern observed included resistance to 11 antibiotics spanning five different classes. These antibiotics were Amikacin (AK), Gentamicin (CN), Cefuroxime Sodium (CXM), Cefotaxime (CTX), Cefpodoxime (CPD), Ceftazidime (CAZ), Cefepime (FEP), Amoxicillin + Clavulanic Acid (AMC), Ofloxacin (OFX), Ciprofloxacin (CIP), and Tetracycline (TE). This extensive resistance pattern resulted in a high MAR index of 0.92 and was observed in 3 isolates, accounting for 17.6% of the total resistant isolates.

The second resistance pattern included resistance to 10 antibiotics, also spanning five classes, but excluding Amikacin (AK) from the list. The antibiotics resisted in this pattern were Gentamicin (CN), Cefuroxime Sodium (CXM), Cefotaxime (CTX), Cefpodoxime (CPD), Ceftazidime (CAZ), Cefepime (FEP), Amoxicillin + Clavulanic Acid (AMC), Ofloxacin (OFX), Ciprofloxacin (CIP), and Tetracycline (TE). This pattern resulted in a MAR index of 0.83 and was the most prevalent, observed in 9 isolates, which constituted 52.9% of the resistant isolates.

The third pattern was similar to the second but included resistance to Amikacin (AK) and excluded Gentamicin (CN). The antibiotics that resisted were Amikacin (AK), Cefuroxime Sodium (CXM), Cefotaxime (CTX), Cefpodoxime (CPD), Ceftazidime (CAZ), Cefepime (FEP), Amoxicillin + Clavulanic Acid (AMC), Ofloxacin (OFX), Ciprofloxacin (CIP), and Tetracycline (TE). This pattern also resulted in a MAR index of 0.83 and was observed in 5 isolates, accounting for 29.4% of the resistant isolates.

The high MAR indices observed in the study indicate a significant level of multiple antibiotic resistance among the uropathogenic*Pseudomonas* species as shown in Table 4 below.

Table 3.Antimicrobial Susceptibility Pattern of *Pseudomonas* Isolates from the Subjects

Antibiotics (Zone of Inhibition Range in mm)	**Antibiotic Class**	**Resistant (%) N = 17**
Amikacin 30 µg (AK) (< 18 = R; ≥ 18 = S)	Aminoglycoside	4 (23.5)

Gentamicin 120 µg (CN) (< 17 = R; ≥ 17 = S)	Aminoglycoside	6 (35.3)
Ofloxacin 5 µg (OFX) (< 22 = R; ≥ 24 = S)	Fluoroquinolone	9 (52.9)
Ciprofloxacin 5 µg (CIP) (< 22 = R; ≥ 25 = S)	Fluoroquinolone	9 (52.9)
Cefepime 30 µg (FEP) (< 19 = R; ≥ 19 = S)	Cephalosporin	11 (64.7)
Cefuroxime Sodium (CXM) (< 19 = R; ≥ 19 = S)	Cephalosporin	15 (88.2)
Cefpodoxime 10 µg (CPD) (< 21 = R; ≥ 21 = S)	Cephalosporin	17 (100)
Ceftazidime 30 µg (CAZ) (< 19 = R; ≥ 22 = S)	Cephalosporin	10 (58.8)
Amoxicillin + Clavulanic Acid 30 µg (AMC) (< 19 = R; ≥ 19 = S)	β-lactam and β-lactamase inhibitor	16 (94.1)
Cefotaxime 30 µg (CTX) (< 17 = R; ≥ 20 = S)	β-lactam	8 (47.1)
Imipenem 10 µg (IPM) (< 19 = R; ≥ 22 = S)	Carbapenem	0 (0)
Tetracycline 30 µg (TE) (< 19 = R; ≥ 19 = S)	Tetracycline	13 (76.5)

***Key**: S - Sensitive; R - Resistant*

Table 4. Multiple Antimicrobial Resistance Index (MAR) of the Uropathogenic*Pseudomonas species*

S/N	Antimicrobial Resistance Patterns	Antibiotic Classes	Antibiotics Resisted	MAR Index	Frequency (%)
1	AK, CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	5	11	0.92	3 (17.6)
2	CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	5	10	0.83	9 (52.9)
3	AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	5	10	0.83	5 (29.4)

Key: AK - Amikacin 30 µg, CN - Gentamicin 120 µg, OFX - Ofloxacin 5 µg, CIP - Ciprofloxacin 5 µg, FEP Cefepime 30 µg , CXM - Cefuroxime Sodium, CPD - Cefpodoxime 10 µg, CAZ - Ceftazidime 30 µg, AMC - Amoxicillin + Clavulanic Acid 30 µg, CTX - Cefotaxime 30 µg, IPM - Imipenem 10 µg, TE - Tetracycline 30 µg.

3.4 Confirmation of 16S Ribosomal RNA of Bacterial Isolates

The outcome of the 16s rRNA region amplification of the rRNA gene for the molecular characterization of bacteria 16S genes. All 9 representative isolates were positive for this gene, confirming that they are all bacteria. The 16S status of the tested isolates was 100% as shown in Figure 1.

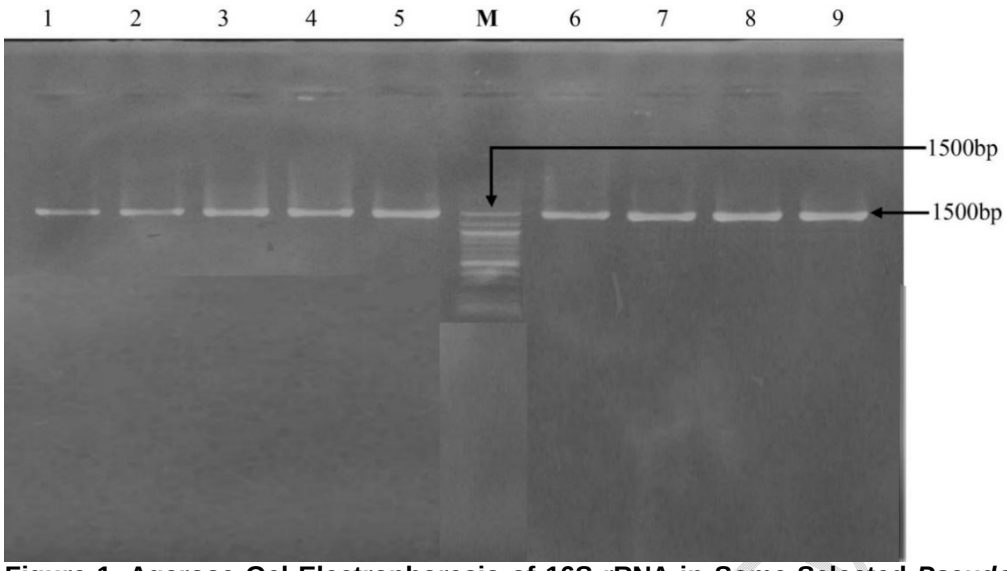

Figure 1. Agarose Gel Electrophoresis of 16S rRNA in Some Selected *Pseudomonas* isolates. Lanes 1 – 9 represent 16S rRNA gene bands (1500 bp). Lane M represents the 100 bp DNA ladder.

3.5 Prevalence of Antimicrobial Resistant Genes

The acquired 16s rRNA sequence from the isolates yielded a precise match in the megablast search against very similar sequences in the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates exhibited 100% similarity to other species. The evolutionary distances calculated by the Jukes-Cantor approach aligned with the phylogenetic positioning of the 16S rRNA of the isolates within *Pseudomonas*sp and indicated a close relationship with *Pseudomonas aeruginosa*, *Pseudomonas xiamenensis* and *Pseudomonas putida* as shown in Figure 2.

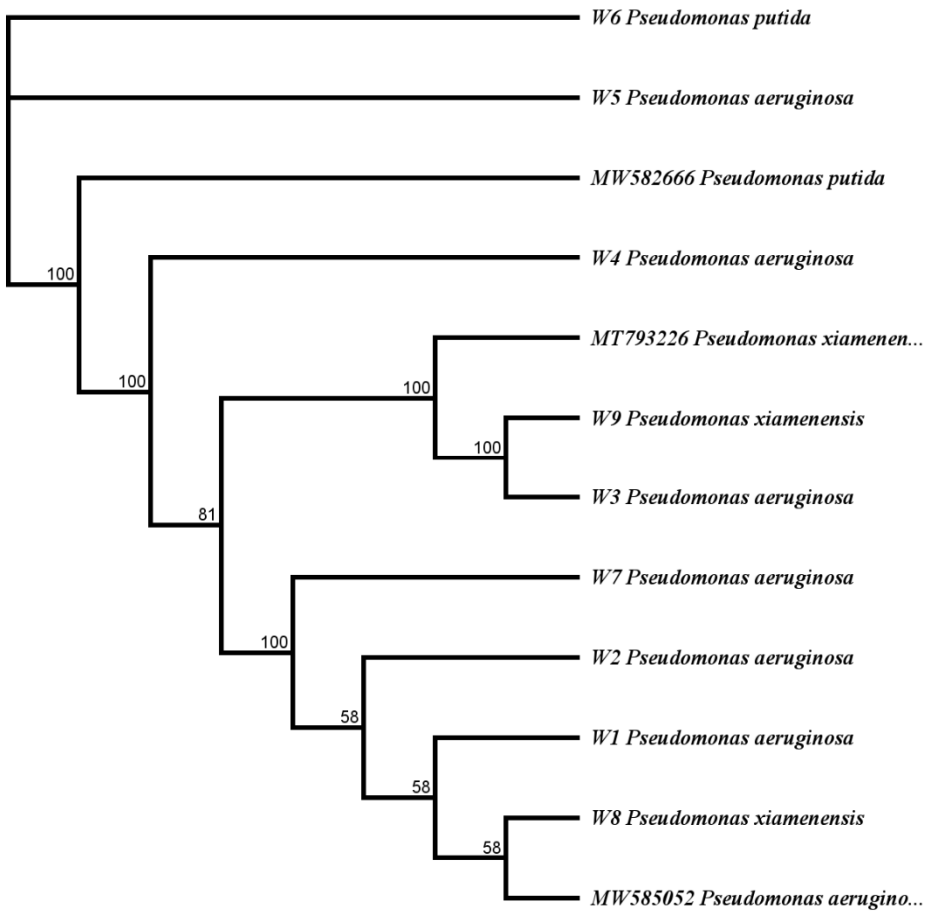

Figure 2: Phylogenetic Tree Showing the Evolutionary Distance Between the Bacterial Isolates

3.6 Prevalence of Antimicrobial Resistant Genes

The study investigated the presence of specific antimicrobial-resistant genes in representative uropathogenic*Pseudomonas* isolates. The CTX-M gene, associated with extended-spectrum β-lactamase (ESBL) production, was detected in 7 out of 9 isolates, accounting for 77.8% of the samples. The TEM gene, another ESBL gene, was present in 4 isolates, representing 44.4% of the total. The SHV gene, also linked to ESBL production, was found in 3 isolates, which is 33.3% of the samples.The analysis did not detect the NDM (New Delhi metallo-β-lactamase) and VIM (Verona integron-encoded metallo-β-lactamase) genes in any of the isolates. These genes are known to confer resistance to carbapenem antibiotics. The results indicate that while CTX-M, TEM, and SHV genes were prevalent, NDM and VIM genes were absent in the uropathogenic*Pseudomonas species* studied as shown in Table 5, Figures 3 to 5.The study analyzed the antimicrobial resistance patterns and the presence of specific resistance genes in the representative uropathogenic*Pseudomonas* isolates. Isolate 1 exhibited resistance to a broad spectrum of antibiotics, including Amikacin (AK), Gentamicin (CN), Cefuroxime Sodium (CXM), Cefotaxime (CTX), Cefpodoxime (CPD), Ceftazidime (CAZ), Cefepime (FEP), Amoxicillin + Clavulanic Acid (AMC), Ofloxacin (OFX), Ciprofloxacin (CIP), and Tetracycline (TE), resulting in a MAR index of 0.92 and the presence of the CTX-M gene.Isolates 2 and 3, both with a MAR index of 0.83, showed resistance to CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, and TE. Isolate 2 harbored both the CTX-M and TEM genes, while isolate 3

contained only the CTX-M gene. Isolate 4 had a resistance pattern similar to that of isolate 1 but excluded Gentamicin (CN). With a MAR index of 0.83, it possessed the CTX-M, TEM, and SHV genes. Isolate 5 shared a similar extensive resistance pattern as isolate 1 and had a MAR index of 0.92, with the SHV gene detected. Isolate 6, with a MAR index of 0.83, was resistant to AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, and TE and carried the CTX-M and TEM genes. Isolate 7, showing a MAR index of 0.92, was resistant to AK, CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, and TE and contained the CTX-M gene.
Isolate 8 exhibited resistance to AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, and TE, resulting in a MAR index of 0.83 and the presence of TEM and SHV genes. Finally, isolate 9, with a MAR index of 0.83, was resistant to CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, and TE, and carried the CTX-M gene. The analysis revealed varied resistance patterns and the presence of multiple resistance genes, including CTX-M, TEM, and SHV, across the isolates, highlighting the complexity and extent of antimicrobial resistance among the uropathogenic*Pseudomonas species* as shown in Table 6 below.

Table 5. Prevalence of Antimicrobial Resistant Genes in the Uropathogenic*Pseudomonas*species

Isolates	*CTX-M*	*TEM*	*SHV*	*NDM*	*VIM*
1	+	-	-	-	-
2	+	+	-	-	-
3	+	-	-	-	-
4	+	+	+	-	-
5	-	-	+	-	-
6	+	+	-	-	-
7	+	-	-	-	-
8	-	+	+	-	-
9	+	-	-	-	-
Total (%)	7 (77.8)	4 (44.4)	3 (33.3)	0 (0)	0 (0)

Table 6. Antimicrobial resistance pattern and antimicrobial resistant genes in the uropathogenic*Pseudomonas species*

Antimicrobial Resistance Pattern	MAR Index	Antimicrobial Resistance Genes
AK, CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.92	*CTX-M*
CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*CTX-M, TEM*
CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*CTX-M*
AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*CTX-M, TEM, SHV*
AK, CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.92	*SHV*
AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*CTX-M, TEM*
AK, CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.92	*CTX-M*
AK, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*TEM, SHV*
CN, CXM, CTX, CPD, CAZ, FEP, AMC, OFX, CIP, TE	0.83	*CTX-M*

Key: AK - Amikacin 30 µg, CN - Gentamicin 120 µg, OFX - Ofloxacin 5 µg, CIP - Ciprofloxacin 5 µg, FEP - Cefepime 30 µg , CXM - Cefuroxime Sodium, CPD - Cefpodoxime 10 µg, CAZ - Ceftazidime 30 µg, AMC - Amoxicillin + Clavulanic Acid 30 µg, CTX -

Cefotaxime 30 µg, IPM - Imipenem 10 µg, TE - Tetracycline 30 µg, CTX-M – Cefotaximase-Munich, TEM - Temoneira, SHV - Sulfhydryl Variable

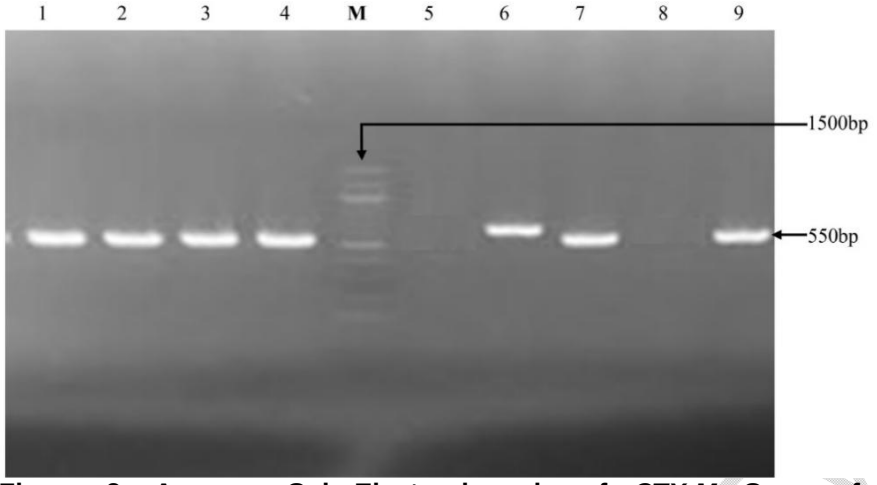

Figure 3. Agarose Gel Electrophoresis of *CTX-M* Gene of Some Selected *Pseudomonas* isolates. Lane 1-4, 6,7 and 9 represents the CTX-M gene bands (550 bp). Lane M represents the 100 bp Molecular ladder.

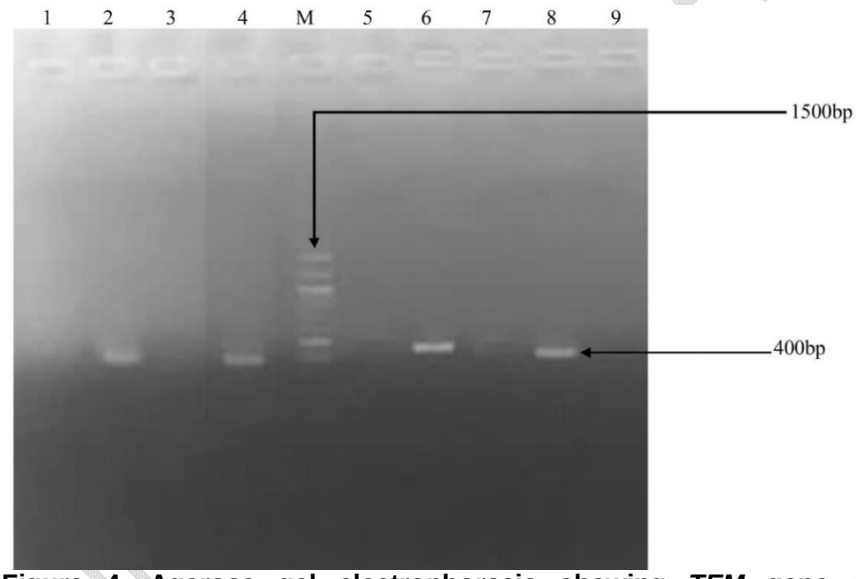

Figure 4. Agarose gel electrophoresis showing *TEM* gene of some selected *Pseudomonas* isolates. Lanes 2, 4, 6, and 8 represent positive *TEM* gene at 400bp. Lane M represents the 100 bp Molecular ladder.

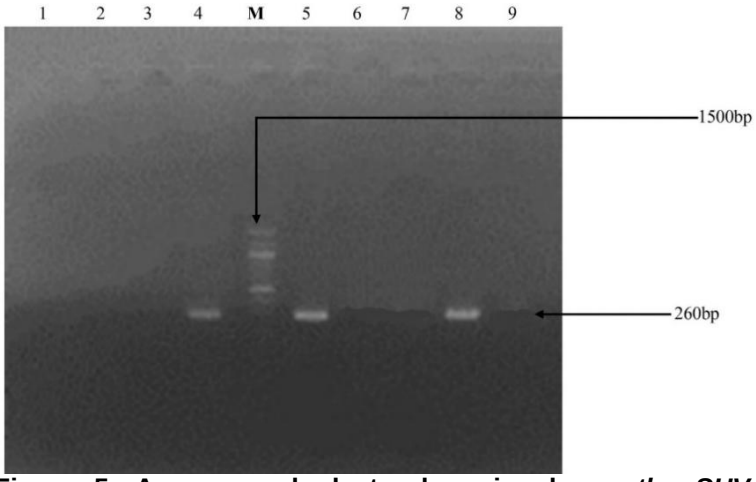

Figure 5. Agarose gel electrophoresis shows *the SHV* gene of some selected *Pseudomonas* isolates. Lanes 4, 5 and 8 are the isolates tested positive *SHV* genes at 260 bp. Lane M represents the 100 bp Molecular ladder.

4. DISCUSSION

The study determined the prevalence of uropathogenic*Pseudomonas* species in 199 subjects using microbiological analysis. Significant growth of *Pseudomonas* species was observed in 17 cases, indicating a prevalence rate of 8.5%. This is higher than the prevalence found in women's hostels in the same state [18]. In contrast, 182 subjects, representing 91.5% of the sample, showed no growth of *Pseudomonas* species. The observed low prevalence rate could be due to several factors. One possible reason is the effectiveness of local infection control practices and hygiene standards that could limit the spread of *Pseudomonas* species. Additionally, the study setting, patient demographics, and underlying health conditions could also have an impact on prevalence. The low prevalence rate of uropathogenic*Pseudomonas* species suggests that *Pseudomonas* infections are not the primary cause of UTIs in this population. This finding has important clinical implications, particularly as a guide to empirical antibiotic therapy. This suggests that broad-spectrum antibiotics specifically targeting *Pseudomonas* may not be necessary as initial treatment in this context, potentially reducing the risk of promoting antibiotic resistance. This finding differs from the study done in Ethiopia, where the prevalence of *Pseudomonas* species was also noted in catheterized patients, although the prevalence rate was significantly higher (49.32%) [19]. The higher prevalence in their study could be due to the focus on catheterized patients, a group at higher risk for *Pseudomonas* infections.

The study examined the antimicrobial susceptibility of *Pseudomonas* isolates from 17 subjects to a range of antibiotics from different classes. An important finding is the high resistance rates of *Pseudomonas* isolates to multiple antibiotics, particularly within the fluoroquinolone and cephalosporin classes, while maintaining susceptibility to imipenem. The study also identified three distinct patterns of multiple antimicrobial resistance (MAR) with high MAR indices, indicating a significant level of multidrug resistance (MDR) among the isolates. The high resistance rates observed in this study can be attributed to several factors. Excessive or misuse of antibiotics in clinical settings often leads to the selection of resistant strains. Resistance to aminoglycosides, fluoroquinolones and cephalosporins suggests that these antibiotics are commonly used to treat urinary tract infections, leading to increased selective pressure on *P. aeruginosa* to develop resistance mechanisms.

Resistance to aminoglycosides such as amikacin (23.5%) and gentamicin (35.3%) could be due to the presence of aminoglycoside-modifying enzymes, efflux pumps, or changes in the permeability of the bacterial cell wall. The significant resistance to fluoroquinolones (52.9% for both ofloxacin and ciprofloxacin) suggests that mutations in DNA gyrase and topoisomerase IV, target modifications and efflux mechanisms may play a significant role. Cephalosporin resistance rates varied, with cefpodoxime showing the highest resistance (100%), possibly due to the production of extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamase enzymes. The combination antibiotic amoxicillin + clavulanic acid showed a high resistance rate of 94.1%, probably because β-lactamase inhibitors such as clavulanic acid are less effective against the β-lactamase enzymes they produce in Pseudomonas aeruginosa. The complete susceptibility to imipenem (0% resistance) is encouraging because carbapenems are often reserved for the treatment of serious infections caused by resistant bacteria. This susceptibility suggests that carbapenemase-producing strains are not widespread in this population.

The high resistance rates among *Pseudomonas* isolates have significant clinical implications. These highlight the need for routine antimicrobial susceptibility testing to guide appropriate antibiotic therapy and avoid the use of ineffective medications. The high efficacy of imipenem suggests that it should be considered for empirical therapy, particularly in severe cases or when resistance to other antibiotics is suspected. The observed high MAR indices, with some isolates, indicate a significant public health challenge. This suggests that these isolates can survive exposure to a variety of antibiotics, complicating treatment options and increasing the risk of treatment failure. The results are consistent with a study where high levels of resistance to a wide range of antibiotics were observed [20]. Similarly, in Iraq, it has been reported of a significant resistance to doxycycline and regional differences in resistance patterns [21]. In contrast, another study found a high prevalence of metallo-β-lactamase-producing *Pseudomonas* in Japan with a notable presence of IMP-1 resistance genes, highlighting the genetic diversity and different resistance mechanisms in different regions [22]. Ogbolu et al. [23] reported a high prevalence of ESBL-producing *Pseudomonas* species, particularly the CTX-M-15 gene, in Nigeria, consistent with the high rates of resistance to cephalosporins in the population.

This study identified a high prevalence of specific antimicrobial-resistant (AMR) genes among nine representative uropathogenic*Pseudomonas* isolates. Notably, the CTX-M gene was detected in 77.8% of the isolates, the TEM gene in 44.4%, and the SHV gene in 33.3%. In contrast, the study found no evidence of the NDM and VIM genes, which are commonly associated with carbapenem resistance. These findings highlight the significant role of CTX-M, TEM, and SHV genes in shaping the antimicrobial resistance profiles of these isolates [24]. The detection of the CTX-M gene in the majority of isolates suggests that this gene is a primary contributor to extended-spectrum β-lactamase (ESBL) production in uropathogenic*Pseudomonas* species. ESBLs are enzymes capable of hydrolyzing a wide range of β-lactam antibiotics, including penicillins and cephalosporins, rendering them ineffective [25]. The high prevalence of the CTX-M gene in this study is consistent with findings from Ogbolu et al. [23] in Nigeria, who reported a dominant presence of the CTX-M-15 gene among uropathogenic*Pseudomonas* isolates. This suggests a widespread distribution of CTX-M-type ESBLs in different regions, contributing to high resistance levels to third-generation cephalosporins. The predominance of the CTX-M gene underscores its pivotal role in conferring resistance, particularly as this gene has been implicated in various outbreaks of multidrug-resistant infections globally [26].

Although less prevalent, the TEM and SHV genes also contribute to ESBL production. These genes, often coexisting with CTX-M, can further enhance resistance, increasing the difficulty of clinical management of infections caused by these isolates [27]. The coexistence of multiple ESBL genes in some isolates reflects the potential for these bacteria to exhibit resistance to a broad spectrum of β-lactam antibiotics, compounding the therapeutic challenge.

The absence of NDM and VIM genes is a notable finding. These carbapenemase genes are associated with resistance to carbapenem antibiotics, often considered a last line of defense for treating multidrug-resistant bacterial infections [28]. The lack of these genes in the isolates aligns with the observation that imipenem demonstrated the highest efficacy, with no resistance detected. This suggests that carbapenem resistance has not yet become a significant issue among the uropathogenic*Pseudomonas* isolates in this population. However, it is essential to note that the global spread of NDM and VIM genes has been reported, underscoring the need for vigilant surveillance [29].

The high prevalence of ESBL genes (CTX-M, TEM, and SHV) poses significant challenges for clinical treatment, as infections caused by such isolates are likely to be resistant to β-lactam antibiotics. This underscores the necessity of considering alternative treatment strategies, such as using carbapenems or other non-β-lactam antibiotics, guided by antimicrobial susceptibility testing. Routine screening for resistance genes in clinical isolates is crucial to inform effective therapeutic decisions and limit the spread of resistance [30]. While the absence of carbapenemase genes is encouraging, continuous monitoring is imperative. The emergence and dissemination of these genes could rapidly compromise the efficacy of carbapenems, leading to more limited treatment options. The findings of this study reinforce the importance of proactive surveillance programs and strict antimicrobial stewardship practices to mitigate the impact of AMR.

Osawa et al. [22] in Japan found a significant presence of metallo-β-lactamase (MBL) genes, such as IMP-1, in *Pseudomonas* isolates, indicating a regional variation in the prevalence of specific resistance genes. While the current study did not find NDM or VIM genes, the Japanese study highlights the importance of monitoring for these carbapenemase genes, which could emerge and spread in different populations. Abbas et al. [31] in Egypt identified multiple resistance mechanisms, including efflux pumps and AmpC β-lactamase, contributing to high levels of multidrug resistance (MDR). The current study's findings on the prevalence of ESBL genes align with the complexity of resistance mechanisms previously reported emphasizing the need for comprehensive diagnostic approaches to guide therapy [31]

A limitation of this study is that it did not explore the presence of the resistance genes on the plasmid of the bacterial isolates. Studies have shown that bacteria encode betalactamase enzymes on the plasmids within the bacteria. This makes the gene to be present on both the plasmid and chromosome of the bacteria [32].

5. CONCLUSION

The study determined that the prevalence of uropathogenic*Pseudomonas species* was 8.5% among the subjects. Resistance rates were 52.9% for fluoroquinolones (ofloxacin and ciprofloxacin) and 64.7% for cefepime. Cefuroxime sodium and cefpodoxime showed even higher resistance rates at 88.2% and 100%, respectively. The combination antibiotic amoxicillin + clavulanic acid faced a resistance rate of 94.1%. The study found that the isolates exhibited extensive resistance patterns across multiple antibiotic classes, resulting in high MAR indices. This indicates a significant level of multiple antibiotic resistance among the uropathogenic*Pseudomonas species*, complicating treatment strategies. The CTX-M gene was detected in 77.8% of the isolates, TEM in 44.4%, and SHV in 33.3%. The absence of carbapenemase genes (NDM and VIM) suggests that carbapenems remain effective against these isolates, although continuous monitoring is necessary to prevent the emergence of these resistance mechanisms.

CONSENT

All authors declare that written informed consent was obtained from the patient. A copy of the written consent is available for review by the Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCES

1. Amala, S.E., Karibi-Botoye, R., Nwokah, E.G. and Pius, M.T., 2021. Prevalence of Asymptomatic Bacteriuria in Pregnancy and Urinary Tract Infection in Non-pregnant Symptomatic Women. *American Journal of Biomedical Sciences*, *13*(4).
2. Lamas Ferreiro, J. L., Álvarez Otero, J., González González, L., NovoaLamazares, L., Arca Blanco, A., Bermúdez Sanjurjo, J. R., ... & de la Fuente Aguado, J. (2017). *Pseudomonas aeruginosa* urinary tract infections in hospitalized patients: Mortality and prognostic factors. *PloS one*, *12*(5), e0178178.
3. Gomila, A., Shaw, E., Carratalà, J., Leibovici, L., Tebé, C., Wiegand, I., Vallejo-Torres, L., Vigo, J.M., Morris, S., Stoddart, M. and Grier, S., 2018. Predictive factors for multidrug-resistant gram-negative bacteria among hospitalised patients with complicated urinary tract infections. *Antimicrobial Resistance & Infection Control*, *7*, pp.1-11.
4. Jiménez-Guerra, G., Heras-Cañas, V., Gutiérrez-Soto, M., del Pilar Aznarte-Padial, M., Expósito-Ruiz, M., Navarro-Marí, J. M., & Gutiérrez-Fernández, J. (2018). Urinary tract infection by Acinetobacter baumannii and *Pseudomonas aeruginosa*: evolution of antimicrobial resistance and therapeutic alternatives. *Journal of medical microbiology*, *67*(6), 790-797.
5. Monsi, T.P., Egeolu, M.N., Amala, S.E., Giami, L.K. and Abbey, S.D., 2021. Phenotypic Response of *Pseudomonasaeruginosa* to Different Hydrogen Ion Concentrations of a Herbal Drug. *Clinical Microbiology: Open Access*, *10*(10), p.225.
6. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.
7. Eguchi, H., Miyamoto, T., Kuwahara, T., Mitamura, S., &Mitamura, Y. (2013). Infectious conjunctivitis caused by *Pseudomonas aeruginosa* isolated from a bathroom. *BMC research notes*, *6*(1), 1-3.
8. Strateva, T., & Yordanov, D. (2009). *Pseudomonas aeruginosa*–a phenomenon of bacterial resistance. *Journal of medical microbiology*, *58*(9), 1133-1148.
9. Monsi, T.P., Amala, S.E. and Ugoaru, N.F., 2017. Acquisition of antibiotic resistance in Escherichia coli exposed to a locally produced herbal drug. *Microbiology Research Journal International*, *22*(2), pp.1-7.
10. Osterblad, M., Hakanen, A., Manninen, R., Leistevuo, T., Peltonen, R., Meurman, O., ... & Kotilainen, P. (2000). A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *Antimicrobial agents and chemotherapy*, *44*(6), 1479-1484.

11. Dimatatac, E. L., Alejandria, M. M., Montalban, C., Pineda, C., Ang, C., & Delino, R. (2003). Clinical outcomes and costs of care of antibiotic resistant *Pseudomonas aeruginosa* infections. *Philipp. J. Microbiol. Infect. Dis*, *32*, 159-167.
12. Gailienė, G., Pavilonis, A., &Kareivienė, V. (2007). The peculiarities of *Pseudomonas aeruginosa* resistance to antibiotics and prevalence of serogroups. *Medicina*, *43*(1), 36.
13. Bashir, M. F., Qazi, J. I., Ahmad, N., & Riaz, S. (2008). Diversity of urinary tract pathogens and drug resistant isolates of Escherichia coli in different age and gender groups of Pakistanis. *Tropical journal of pharmaceutical research*, *7*(3), 1025-1031.
14. Najar, M. S., Saldanha, C. L., & Banday, K. A. (2009). Approach to urinary tract infections. *Indian journal of nephrology*, *19*(4), 129.
15. Manjunath, G. N., Prakash, R., & Vamseedhar Annam, K. S. (2011). Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. *Int J Biol Med Res.*, *2*(2), 504-507.
16. Abdulfatai, K., Sanusi, S. B., Usman, A., Lawal, S. M., & Idris, H. (2023). Prevalence and antimicrobial susceptibility pattern of Klebsiella pneumoniae and *Pseudomonas aeruginosa* among women with urinary tract infections attending antenatal care in Kaduna, Nigeria. *Science World Journal*, *18*(1), 114-119.
17. Clinical and Laboratory Standards Institute, 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
18. Mbata, C., Aleru, C., Azike, C., Adewoye, M., Emeka, E. and Ugwueze, V., 2016. Incidence of asymptomatic bacteriuria amongst female students residing in school hostels of Rivers state University of Science and Technology, Port Harcourt. *Int J Curr Res Med Sci*, *2*(10), pp.55-9.
19. Bekele, T., Tesfaye, A., Sewunet, T., &Waktola, H. D. (2015). *Pseudomonas aeruginosa* isolates and their antimicrobial susceptibility pattern among catheterized patients at Jimma University Teaching Hospital, Jimma, Ethiopia. *BMC research notes*, *8*, 1-4.
20. Shah, D. A., Wasim, S., & Abdullah, F. E. (2015). Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from urine samples of Urinary Tract Infections patients in Karachi, Pakistan. *Pakistan journal of medical sciences*, *31*(2), 341
21. Salih, H. A., Abdulbary, M., &Abdulrida, A. S. (2011). Susceptibility of *Pseudomonas aeruginosa* isolated from urine to some antibiotics. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, *10*(2).
22. Osawa, K., Shigemura, K., Kitagawa, K., Fukuda, T., Takasaka, A., Wakabayashi, S., ... & Fujisawa, M. (2019). Molecular characteristics of carbapenem-resistant *Pseudomonas aeruginosa* isolated from urine in Hyogo, Japan. *International Journal of Urology*, *26*(1), 127-133.
23. Ogbolu, D. O., Terry Alli, O. A., Webber, M. A., Oluremi, A. S., & Oloyede, O. M. (2018). CTX-M-15 is established in most multidrug-resistant uropathogenic Enterobacteriaceae and Pseudomonaceae from hospitals in Nigeria. *European Journal of Microbiology and Immunology*, *8*(1), 20-24.
24. Okogeri, E.I., Amala, S.E., Nwokah, E.G. and Monsi, T.P., 2020. Cross-sectional study of extended spectrum β-lactamase genes (SHV, TEM, CTX-M and OXA) in Klebsiella species from clinical specimens in Port Hacourt, Nigeria. *Am. J. Med. Sci. Med*, *8*, pp.180-186.
25. Paterson, D.L. and Bonomo, R.A., 2005. Extended-spectrum β-lactamases: a clinical update. *Clinical microbiology reviews*, *18*(4), pp.657-686.
26. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. 2018. Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and

Carbapenemase-Producing Enterobacteriaceae. Clin Microbiol Rev 31:10.1128/cmr.00079-17. https://doi.org/10.1128/cmr.00079-17
27. Bradford, P.A., 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical microbiology reviews*, *14*(4), pp.933-951.
28. Nordmann, P., Naas, T. and Poirel, L., 2011. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerging infectious diseases*, *17*(10), p.1791.
29. Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S. and Krishnan, P., 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet infectious diseases*, *10*(9), pp.597-602.
30. Livermore, D.M., 2008. Defining an extended-spectrum β-lactamase. *Clinical Microbiology and Infection*, *14*, pp.3-10.
31. Abbas, H. A., El-Ganiny, A. M., & Kamel, H. A. (2018). Phenotypic and genotypic detection of antibiotic resistance of *Pseudomonas aeruginosa* isolated from urinary tract infections. *African health sciences*, *18*(1), 11-21.
32. Monsi, T.P., Abbey, S.D., Wachukwu, C.K. &Wokem, G.N. 2019. Plasmid-mediated resistance in extended spectrum beta–lactamase–producing Klebsiella pneumoniae isolates at Rivers State University Teaching Hospital. European Journal of Pharmaceutical and Medical Research 6 (2), 75-82.