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Antibiotic Resistance Profiles of *Pseudomonas aeruginosa* Isolated from Tertiary Hospitals in Dar es Salaam, Tanzania

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Abstract

Background: Healthcare-associated infections (HAIs) are a significant global concern due to their contribution in mortality and morbidity. Individuals with recent antibiotic exposure or indwelling medical devices are particularly vulnerable to these infections, especially those caused by drug-resistant bacteria like *Pseudomonas aeruginosa*. This bacterium presents a formidable challenge to treatment due to its resistance to multiple antibiotics.

Materials and methods: 129 *Pseudomonas aeruginosa* isolates were isolated from hospital environmental swabs and water samples. Antimicrobial susceptibility testing was performed by disk diffusion and five resistant genes corresponding to each antibiotic class were screened by PCR.

Results: The antibiotic susceptibility testing results indicated a significant low level of resistance among the isolated *Pseudomonas aeruginosa* as they were highly resistant to aztreonam with only 11.6% and less resistant to norfloxacin, amikacin and ciprofloxacin with 2.3%, 3.1% and 3.1% respectively. The strong positive correlation (r=0.83) was found between phenotypic and genotypic agreement, while the most common resistance gene was *blaVIM* (100%). The study also found a presence of 3 multidrug-resistant isolates that resisted fluoroquinolones, aminoglycosides, third-generation cephalosporin and Monobactam.

Conclusion: This study highlights the presence of *Pseudomonas aeruginosa* in hospital environments and its alarming resistance to certain antibiotics thus emphasizing the necessity of cautious antibiotic use and management.

Keywords; Antibiotic Resistance, *Pseudomonas aeruginosa*, Tertiary hospitals, Infections

Introduction

Infections acquired at hospitals or other healthcare facility are referred to as nosocomial infections or hospital-acquired infection (Haque et al. 2018). It is likely that during the patient's hospital stay, these illnesses spread beyond boundaries. Bloodstream infections, urinary tract infections (UTIs), surgical site infections (SSIs), and pneumonia infections account for 80% of all nosocomial infections (Nimer 2022). *Pseudomonas aeruginosa* and other multidrug resistant bacteria are the main sources of these infection (Spagnolo et al. 2021).

Pseudomonas aeruginosa exhibit a rod-shaped morphology, typically measuring between 0.5 to 0.8 µm in width and 1.5 to 3.0 µm in length, and are Gram-negative, often appearing singly or in pairs (Aryal 2021). This bacterium is commonly found in soil, water, and vegetation, and it thrives in moist environments (Crone et al. 2019). It is an opportunistic pathogen primarily affecting

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immunocompromised individuals, such as those with cystic fibrosis (CF), burns, or patients undergoing prolonged hospital stays (Spagnolo et al. 2021). In healthcare settings, *P. aeruginosa* is a leading cause of nosocomial infections, particularly in intensive care units (ICUs) and among patients with indwelling medical devices (Spagnolo et al. 2021).

Because of its resistance to several antibiotics, this bacterium poses a serious therapeutic problem. *P. aeruginosa* resistance mechanisms are divided into acquired, intrinsic and adaptive (Pang et al. 2019, Langendonk et al. 2021, Kunz Coyne et al. 2022a). The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability (12- to 100- fold lower than that of *Escherichia coli*), expression of efflux pumps that expel antibiotics out of the cell (Pang et al. 2019, Langendonk et al. 2021). The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational changes (Langendonk et al. 2021, Kunz Coyne et al. 2022b). The adaptive resistance of *P. aeruginosa* involves formation of biofilm in the lungs of infected patients where the biofilm serves as a diffusion barrier to limit antibiotic access to the bacterial cells (Pang et al. 2019).

Previously, it was reported that *P. aeruginosa*, among other bacteria, caused 16.3% of wound infections in Tanzania in 2014 at Muhimbili National Hospital and between 27% and 40% of wound infections in 2016 at the Bugando Medical Centre (BMC) (Moremi et al. 2017). *P. aeruginosa* was found to be a major contributor to wound infections in both hospitals (Moremi et al. 2017). Despite the fact that hospital environments are among the global responsibilities that need to be given a focus they often face challenges such as overcrowding, resource constraints, and the emergence of antibiotic-resistant bacteria. Therefore, this present study investigated the prevalence of *Pseudomonas aeruginosa* in the selected hospital environments and its resistance profile to multiple antibiotics and screening for the selected resistance genes conferring resistance to selected antibiotics.

Materials and Methods

Sampling Location

Water samples and swab samples were collected from selected tertiary hospitals in Dar es Salaam, Tanzania. The hospitals were Muhimbili National hospital, Amana Regional Referral Hospital, Temeke Regional Referral Hospital and Mwananyamala Regional Referral hospital.

Sample collection

The sample collection was conducted across multiple hospitals. During the period from June 20, 2023, to December 20, 2023, a total of 108 samples were collected. Each hospital contributed a set of 27 samples, consisting of 15 water samples and 12 swab samples. The water samples were stored in 15ml falcon tubes, while the swab samples were preserved in Stuart transport medium. All samples were carefully transported in a cooler box to the Microbiology Laboratory at the University of Dar es Salaam for processing.

Bacteria Isolation

Both swab sample and water samples were enriched on nutrient broth, then a loopful of the enriched nutrient broth was streaked onto Cetrimide Agar, and then incubated at 42℃ for 24 hours. The presence of pigmented blue-green, mucoid colonies, smooth in shape with a fruity odor, was indicative of *Pseudomonas aeruginosa* according to Brown and Lowbury (1985). Subsequently, one or two colonies were selected from the cetrimide agar plates and sub cultured onto nutrient agar plates to obtain pure cultures of *Pseudomonas aeruginosa* that appeared as yellowish green, smooth in shape with flat edges and elevated center. For identification, molecular identification and

biochemical tests including indole, motility, citrate utilization, Voges-Proskauer, Methyl Red, oxidase, and catalase tests were performed on the pure cultures of *Pseudomonas aeruginosa*.

Biochemical tests for Identification of *Pseudomonas aeruginosa* isolates

Gram Staining

This test was done according to Aryal (2023), where by a spotlessly clean slide was selected. Then, a loopful of the resistant pure *P. aeruginosa* was carefully spread onto the slide to create a suspension smear. The smear was left to air dry and then heat-fixed to ensure the sample adhered firmly to the slide. Following this, Crystal Violet was applied and allowed to sit for 30 to 60 seconds before being rinsed off with water. Next, the slide was soaked in iodine for a minute and then rinsed again with water. Subsequently, the slide was washed with 95% alcohol for 20 seconds and rinsed once more. Safranin was then added to the slide and left for about a minute before being rinsed off with water. Finally, the slide was allowed to air dry, gently patted dry, and then examined under a microscope. This thorough procedure ensures that the 30 resistant *Pseudomonas aeruginosa* samples were properly prepared and stained

Catalase test

A loop was used to transfer a small amount of pure colony of *Pseudomonas aeruginosa* growth onto the surface of a clean, dry glass slide. Then, a drop of 3% H_2O_2 was placed onto the glass slide, ensuring it covered the area where the colony growth had been transferred. Subsequently, the slide was observed closely for the evolution of oxygen bubbles. These bubbles indicated the presence of catalase enzyme, where it catalyzed the breakdown of hydrogen peroxide into water and oxygen (Aryal 2023b).

Oxidase test

This test was done according to Al-Joda and Jasim (2021) whereby, a strip of filter paper was soaked with a little freshly made 1% solution of the tetramethyl-p-phenylene-diamine dihydrochloride. A colony of *Pseudomonas aeruginosa* was rubbed on it with a platinum loop. A positive reaction was indicated by an intense deep-purple hue, appearing within 10 seconds.

Motility

This test was done according to Patricia Shields and Cathcart (2011), where by a straight needle was touched to a colony of a *Pseudomonas aeruginosa*. It was then stabbed once to a depth of only 1cm in the middle of the tube, ensuring the needle remained in the same line it entered as it was removed from the medium. The media used was SIM media, comprising peptone (30g), beef extract (3g), ferrous ammonium sulphate (0.2g), sodium thiosulphate (0.02g), and agar (3g). The inoculated tubes were incubated at 35°-37°C and examined daily for up to 7 days. Observations were made for a diffuse zone of growth flaring out from the line of inoculation, indicative of the presence of *Pseudomonas aeruginosa*.

Indole test

Sterilized test tubes containing 4 ml of tryptophan broth were prepared. The tubes were inoculated aseptically by taking the growth from 24 hrs. culture of *Pseudomonas aeruginosa*. The inoculated tubes were then incubated at 37°C for 24-28 hours to allow for growth and metabolic activity. Following the incubation period, 0.5 ml of Kovac's reagent was added to each broth culture. Observations were made for the presence or absence of a ring formation, indicative of indole production (Al-Joda and Jasim 2021b).

Voges Proskauer

Prior to inoculation, the medium was allowed to equilibrate to room temperature. Organisms taken from pure culture of *Pseudomonas aeruginosa* were lightly inoculated onto the medium. The inoculated medium was then incubated aerobically at 37℃ for 24 hours. Following the 24-hour incubation period, 2 ml of the broth were aliquoted to a clean test tube. The remaining broth was re-incubated for an additional 24 hours. After the additional incubation period, 6 drops of 5% alpha-naphthol were added to the aliquoted broth, and the mixture was well aerated by mixing. Subsequently, 2 drops of 40% potassium hydroxide were added and mixed well to aerate the solution. Observations were made for a pink-red color at the surface within 30 minutes. During this 30-minute period, the tube was shaken vigorously (Aryal 2023b).

Citrate test

The citrate utilization test uses a Simmon's Citrate which composes of Sodium Chloride 5.0g Sodium Citrate 2.0g Ammonium Dihydrogen Phosphate 1.0g Dipotassium Phosphate 1.0g Magnesium Sulfate 0.2g Bromothymol Blue 0.08 g and agar 15.0g. The slant of the agar was streaked back and forth with a light inoculum picked from the center of a well-isolated colony of *Pseudomonas aeruginosa*. The inoculated slants were then incubated at 35 to 37°C for up to 4days. Observations were made for a color change from green to blue along the slant, indicating citrate utilization by *Pseudomonas aeruginosa* (MacWilliams 2009).

Methyl Red Test

This test was done also according to (Aryal 2023b). The MR-VP broth was brought to room temperature then, tubes containing MR-VP broth were inoculated with a pure culture of *Pseudomonas aeruginosa*. After inoculation, the broth medium was incubated at 35°C for at least 48 hours. Following incubation, five drops of methyl red indicator solution were added to the first tube, and observations were made for any change in color in the tubes.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute guidelines (Syal et al. 2017). Sterile Petri – dishes of Mueller Hinton agar were prepared and 0.1ml of each morphologically identified bacterial isolates prepared directly from an overnight agar plate, equilibrated to match 0.5 McFarland standard were inoculated onto each of the Petri dishes containing Mueller-Hinton agar and allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs each containing the following antibiotics: Ceftazidime 30µg, Aztreonam 30µg, Ciprofloxacin 5µg, Norfloxacin 10µg, Gentamicin10µg and Amikacin 30µg were aseptically placed onto the surfaces of the inoculated agar plates with sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37℃ for 16-24hrs and zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (mm) using a meter ruler according to Hudzicki (2016).

Genomic DNA Extraction

Genomic DNA (gDNA) of extraction resistant *Pseudomonas* strains were done by CTAB protocol as previously reported by Singh Kumar et al. (2014) with minor modifications. The protocol was carried out as follows; a 2 ml of overnight bacterial culture (grown in nutrient broth) was transferred into 2 ml Eppendorf tubes and spun at 13000 rpm for 2 minutes to pellet bacterial cells and supernatant was

discarded. To pelleted cells, 600 µl 2% CTAB extraction buffer was added, mixed well and incubated at 50 – 60 ˚C for 1 hour (the mixture was well shaken at every 10 minutes interval). Tubes were cooled to room temperature, then 800 µl Chloroform: Octanol or Chloroform: Isoamyl (at 24:1 mixture ratio) was added, mixed well, and spun at 13000 rpm for 5 minutes. Upper layer (about 500 µl) was transferred into a new Eppendorf tube and to this layer, 600 µl or equal volume of chloroform was added, mixed well, and spun at 13000 rpm for 5 minutes. A 400 µl of upper layer was transferred to a new tube and 200 µl 3M sodium acetate and 800 µl cold isopropanol were added, mixed well by inverting tubes at least 20 times or for 1 min. Tubes were incubated at – 20 ˚ C for 1 hour. To pellet DNA, tubes were spun at 13000 rpm for 10 minutes and the supernatant was discarded. To wash the pellet, 500 µl 70% ethanol were added and spun at 13000 rpm for 5 minutes. Ethanol was discarded and the pellets were air-dried for 30 minutes and the DNA was eluted with 30 µl TE buffer. The concentration and purity of DNA was determined using NanoDropTM OneC (Thermo Fisher Scientific, USA) through 260/280 nm absorbance ratio and DNA visualization was done by using 1% agarose gel electrophoresis.

PCR Amplification for Detection of 16S rRNA Resistance Genes

The extracted bacterial genomic DNA was subjected to PCR to screen for the presence of genes associated with resistance to selected drugs and for 16S rRNA for identification Specific sets of primers, both forward and reverse, were utilized for each targeted gene, as outlined in Table 1. The antibiotic resistance genes investigated included those for quinolones (*qnrA*), aminoglycosides (*AAC(3)-IV*), and beta-lactams (*blaVIM*, *blaCTX-M*, and *blaTEM*).

Carbapenems are hindered by the metallo-β-lactamase enzymes produced by the *blaVIM* gene (Botelho et al. 2018). The *qnrA* gene generates quinolone resistance factors that are mediated by plasmids and can withstand quinolone antibiotics (Cayci et al. 2014). The extended-spectrum β-lactamase enzymes *blaTEM* and *blaCTX-M*, which expand resistance to β-lactam antibiotics, are likewise encoded by the genes found in *P. aeruginosa* (Shams et al. 2019, Haghighifar et al. 2021). Moreover, the *aac(3)-IV* gene produces the enzyme 3-N-acetyltransferase, which helps promote resistance to aminoglycoside(Magalhaes and Blanchard 2005).

Table 1: Nucleotide Sequences of Primers for the Detection of Resistance Genes, and *16S rRNA*

Gene Target	Primer Sequence (5'-3')	Expected Amplicon (bp)	Annealing Temp. (℃)	Reference
Aac (3)-IV	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	55	Momtaz et al. 2012
qnrA	(F): GGATGCCAGTTTCGAGG (R): TGCCAGGCACAGATCTTG	670	50	Safain 2020
blaVIM	F: AGTGGTGAGTATCCGACAG R: ATGAAAGTGCGTGGAGAC	390	58	Safain 2020
BlaTEM	(F) ACATGGGGGATCATGTAACT (R) GACAGTTACAATGCTTACT	421	52	Safain 2020
BlaCTX-M	(F3) GACGATGTCACTGGCTGAGC (R2) AGCCGCCGACGCTAATACA	499	55	Safain 2020
16S rRNA	(27F) AGAGTTTGATCCTGGCTCAG (1495R) CTACGGCTACCTTGTTACGA	1500	55	Abd Alfadil et al. 2018

All the PCR reactions were performed in a final volume of 25 µL containing 3 µL (100-200 ng/µL) of extracted DNA as template, 12.5 µL of Taq 2X Master Mix (New England Biolabs), 1 µL (10 pmol) of each primer set and 8.5 µL of nuclease free water (Water for Molecular biology, BioConcept). Each PCR amplification cycle consisted of an initial denaturation step at 95 ℃ for 10 minutes, followed by 35 cycles of denaturation at 95 ℃ for the 30s, annealing for 30s at a temperature appropriate for each primer used, extension for 1 minute at 72 ℃ and a final extension at 72 ℃ for 10 minutes. The amplified PCR products were verified by electrophoresis in 1.5% agarose gels stained with safe view™ classic dye visualized in the Gel.LUMINAX Gel Documentation System - BioZEN Labs (Nagar, India). The 1kb plus DNA ladder (New England Biolabs, UK) was used as a molecular weight marker.

Results

Bacterial Isolation

Out of the 108 collected samples, only 68 (63%) indicated the presence of *Pseudomonas aeruginosa* and these 68 samples yielded 129 strains of *Pseudomonas aeruginosa*. Colonies in these strains displaying a distinct yellowish green, smooth in shape with flat edges and elevated center in Nutrient agar as shown in Figure 1

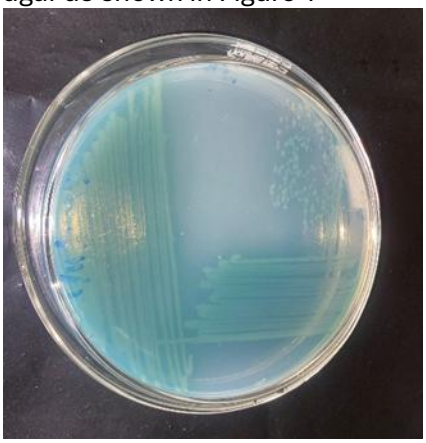

Figure 1: Nutrient agar plate showing the growth of *Pseudomonas aeruginosa*

Biochemical Tests Results

Following isolation of *Pseudomonas aeruginosa*, biochemical tests such as Indole test, Motility test, Citrate test, Voges Proskauer (VP), Methyl Red (MR), Oxidase test and Catalase test were used to identify *Pseudomonas aeruginosa* thereby indicating to be *Pseudomonas aeruginosa* species (Table:2)

Table 2: Results of Biochemical Tests on *Pseudomonas aeruginosa*

Biochemical Test	Inference
Citrate utilization	Positive
Motility	Positive
Gram staining	Negative
Catalase	Positive
Oxidase	Positive
Methyl Red (MR)	Negative
Voges Proskauer (VP)	Negative

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	Indole	Negative

Distribution of *Pseudomonas aeruginosa* Growth in Hospital Settings

Table 3 presents the distribution of collected samples based on recorded demographic information. Samples obtained at Muhimbili National Hospital had a higher growth of *Pseudomonas aeruginosa* than other sampling sites (p = 0.3916). It's worth noting that only 8 out of 48 swab samples (17%) from the treatment units of Muhimbili National Hospital, specifically from beds, trolleys, ventilators and suctions from the ICU, showed the presence of *Pseudomonas aeruginosa*. Conversely, no *Pseudomonas aeruginosa* growth was found in swab samples from other hospitals. However, every hospital's sewage water drainage samples exhibited more *Pseudomonas aeruginosa* (p = 0.406) than swab samples from hospital units. This highlights the prevalence of this bacterium in sewage water drainages originating from the bathroom and toilet facilities within hospital units.

Table 3: Distribution of Samples collected among Different demographic categories

Characteristics	Category	Growth	No growth	Total
All	Samples	68(63%)	40(37%)	108(100%)
Muhimbili	ICU	3(75%)	1(25%)	4(100%)
	Theatre	1(25%)	3(75%)	4(100%)
	Wards	4(100%)	0(0%)	4(100%)
	Sewage Water Drainage	15(100%)	0(0%)	15(100%)
Mwananyamala	ICU	0(0%)	4(100%)	4(100%)
	Theatre	0(0%)	4(100%)	4(100%)
	Wards	0(0%)	4(100%)	4(100%)
	Sewage Water Drainage	15(100%)	0(0%)	15(100%)
Temeke	ICU	0(0%)	4(100%)	4(100%)
	Theatre	0(0%)	4(100%)	4(100%)
	Wards	0(0%)	4(100%)	4(100%)
	Sewage Water Drainage	15(100%)	0	15(100%)
Amana	ICU	0(0%)	4(100%)	4(100%)
	Theatre	0(0%)	4(100%)	4(100%)
	Wards	0(0%)	4(100%)	4(100%)
	Sewage Water Drainage	15(100%)	0(0%)	15(100%)
Samples obtained from	Beds	2(17%)	10(83%)	12(100%)
	Trolleys	1(8%)	11(92%)	12(100%)
	Ventilators	3(25%)	9(75%)	12(100%)
	Suctions sewage Water Drainage	2(17%) 60(100%)	10(83%) 0(0%)	12(100%) 60(100%)
Type of Sample	Swabs	8(17%)	40(83%)	48(100%)

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	sewage Drainage	Water	60(100%)	0(0%)	60(100%)

Antimicrobial Resistance Profile among *Pseudomonas aeruginosa* Isolates

A total of 108 samples were initially collected, out of which 68 samples showed growth of *Pseudomonas aeruginosa*. From these 68 samples, 129 isolates of *Pseudomonas aeruginosa* were obtained. The disc diffusion method was then employed to conduct antimicrobial susceptibility testing on these 129 isolates using six antibiotics: Ciprofloxacin (CIP), Amikacin (AK), Ceftazidime (CAZ), Gentamicin (GEN), Norfloxacin (NOR), and Aztreonam (ATM), as depicted in Figure 2.Generally this test yielded a total of 30 resistant isolates of *Pseudomonas aeruginosa*.

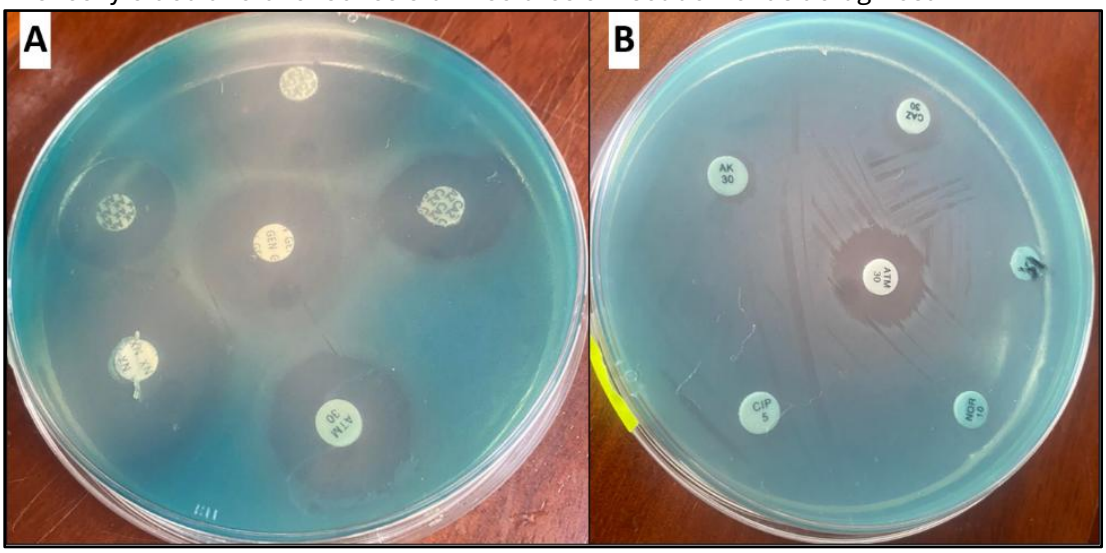

Figure 2: Phenotypic Screening of Antibiotics against *Pseudomonas aeruginosa* Isolates.
Where A is a susceptible Isolate, and B is the resistant Isolate. The Antibiotics Shown on the Plate Includes Ciprofloxacin (CIP), Amikacin (AK), Ceftazidime (CAZ), Gentamicin (GEN), Norfloxacin (NOR), Aztreonam (ATM)

Table 4: Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* Isolates

Antibiotic	Susceptibility Status (n (%))			
	Resistant (R)	Intermediate (I)	Susceptible (S)	Total
Aztreonam	15(11.6%)	74(57.4%)	40(31%)	129
Ceftazidime	10(7.8%)	29(22.5%)	90(69.8%)	129
Norfloxacin	3(2.3%)	0(0%)	126(97.7%)	129
Ciprofloxacin	4(3.1%)	0(0%)	125(96.9%)	129
Gentamicin	11(8.5%)	21(16.3%)	97(78.9%)	129
Amikacin	4(3.1%)	2(1.6%)	123(95.3%)	129

Multi-drug Resistance (MDR) Profile of *Pseudomonas aeruginosa* Isolates

The six (6) tested antibiotics [fluoroquinolones (norfloxacin and ciprofloxacin), aminoglycosides (gentamicin and amikacin), monobactam (aztreonam) and third-generation cephalosporins (ceftazidime)] covered four (4) antibiotic classes based on their modes of actions. Among 129 *Pseudomonas aeruginosa* isolates, a total of thirty (30) isolates exhibited resistance to at least one antibiotic, and three (3) isolates from Muhimbili National Hospital only resisted against at least one antibiotic in more than three (3) antibiotic classes hence were observed to be multidrug-resistant (Table 5).

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The isolates that displayed MDR profiles were for those against Norfloxacin, Ciprofloxacin, Gentamicin, Ceftazidime, Amikacin and Aztreonam

Table 5: Categorical Abundance of MDR *Pseudomonas aeruginosa* Isolates

Sampling Hospital	Antibiotic Category						MDR
	R1	R2	R3	R4	R5	R6	(n)
Muhimbili	7	1	0	1	2	0	3
Mwananyamala	4	1	0	0	0	0	0
Temeke	1	0	0	0	0	0	0
Amana	9	4	0	0	0	0	0
Total	**21**	**6**	**0**	**1**	**2**	**0**	**3**

Whereby, R1= resistant to one antibiotic; R2= resistant to two antibiotics; R3= resistant to three antibiotics; R4= resistant to four antibiotics; R5= resistant to five antibiotics; R6= resistant to six antibiotics

Frequency of *16S rRNA* and Antibiotic Resistance Genes

PCR amplification of 16s rRNA gene of their genomic DNA using specific primer as described in table 1 above. As expected, the amplicon size was around 1500 bp

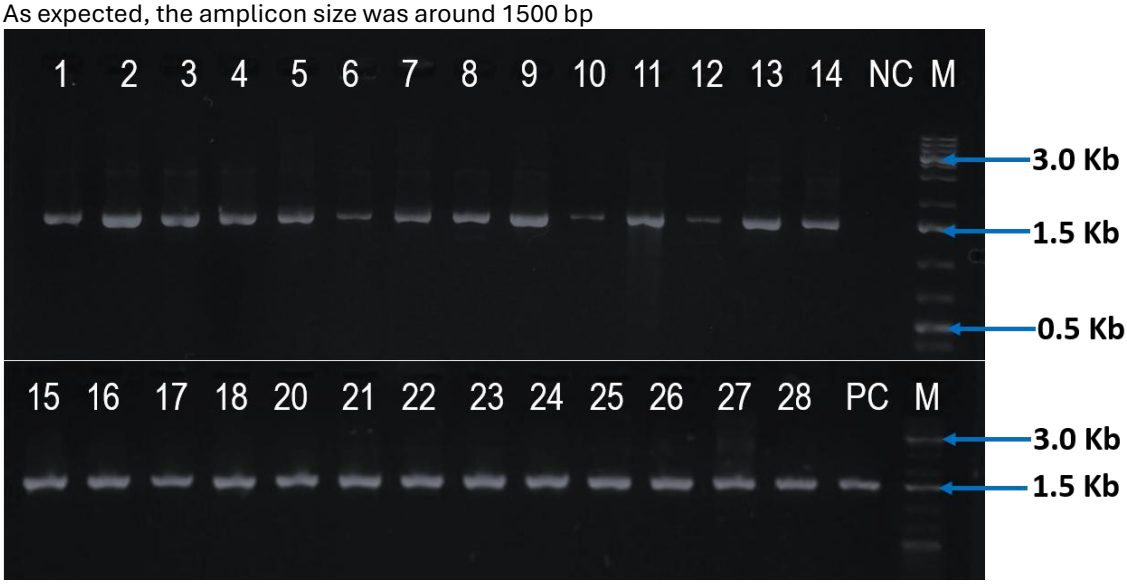

Figure 3: Gel Electrophoresis of 16S rRNA Genes in Lane 1-28 (1.5 Kb);
Where by **M** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

The resistant genes were screened in respective to antibiotic-resistant isolates as shown (Figures 3,4, 5, 6 and 7, respectively).

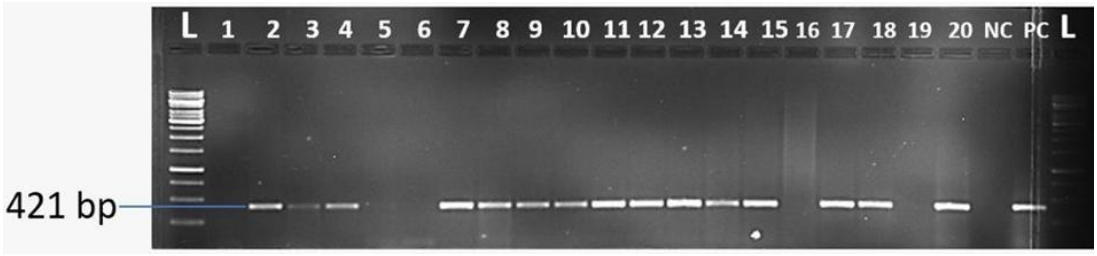

Figure 4: Gel Electrophoresis of *blaTEM* Resistance Genes;

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Whereby; **L** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

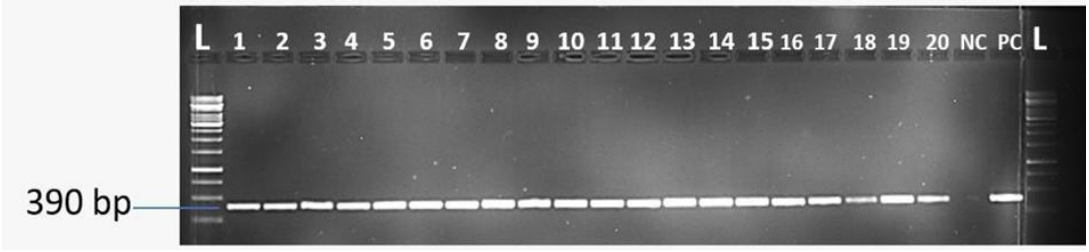

Figure 5: Gel Electrophoresis of *blaVIM* Resistance Genes;
Whereby; **L** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

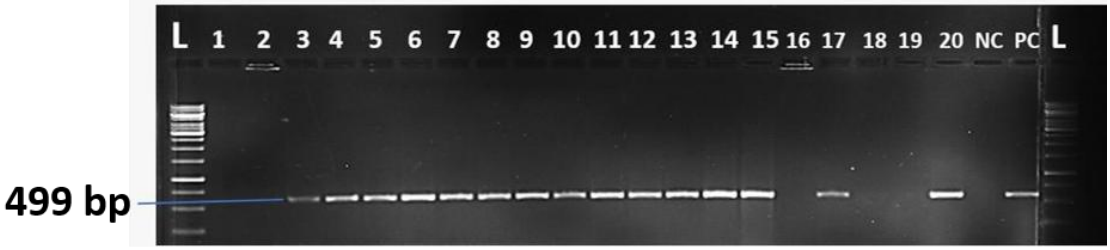

Figure 6: Gel Electrophoresis of *blaCTX-M* Resistance Genes;
Whereby; **L** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

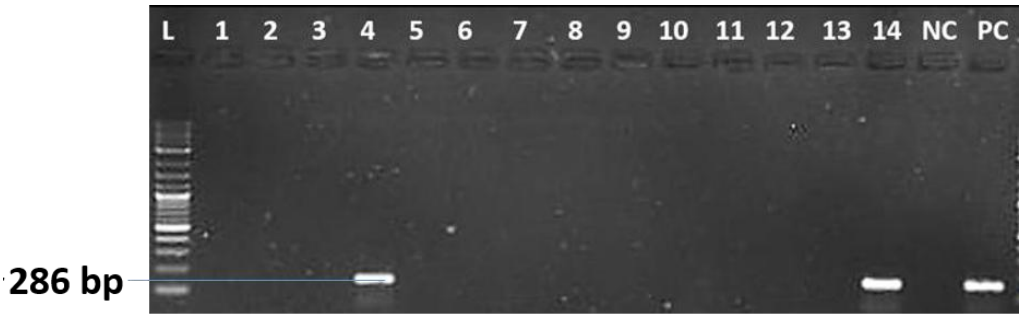

Figure 7: Gel Electrophoresis of *Aac (3)-IV* Resistance Genes;
Whereby; **L** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

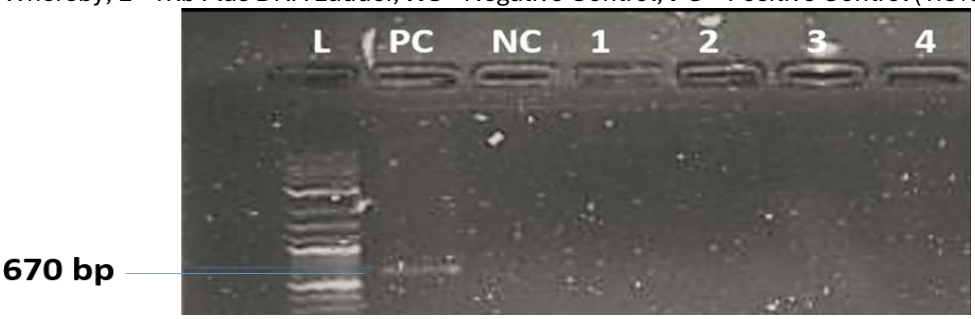

Figure 8: Gel Electrophoresis of *qnrA* Resistance Genes;
Whereby; **L** - 1Kb Plus DNA Ladder, **NC** - Negative Control; **PC** – Positive Control (1.5% Agarose Gel).

Table 6: Frequency of Occurrence of Antibiotic Resistance Genes

Antibiotics class	Antibiotics	Gene	Phenotypic Expression	Genotypic Expression	Percentage Occurrence
Quinolones	Ciprofloxacin	*qnrA*	2	0	0%
	Norfloxacin	*qnrA*	2	0	0%
Aminoglycosides	Gentamicin	*AAC (3)-IV*	13	2	15.3%
	Amikacin	*AAC (3)-IV*	1	0	0%
Beta lactams	Aztreonam	*blaVIM*	15	15	100%
	Aztreonam	*blaCTX-M*	15	13	86.7%
	Aztreonam	*blaTEM*	15	10	66.7%
	Ceftazidime	*blaVIM*	5	5	100%
	Ceftazidime	*blaCTX-M*	5	2	40%
	Ceftazidime	*blaTEM*	5	5	100%

Discussion

Occurrence of *Pseudomonas aeruginosa* Among Hospitals

Out of the 108 samples collected from the four hospitals, 68 (63%) showed the growth of *Pseudomonas aeruginosa*, while 40 (37%) did not show any growth. This indicates a significant presence of *Pseudomonas aeruginosa* in the hospital environments. The presence of *Pseudomonas aeruginosa* in hospital environments is concerning due to its ability to cause various infections, especially in immunocompromised patients. The high percentage (63%) of samples showing growth indicates that *Pseudomonas aeruginosa* is relatively prevalent within the hospital settings studied.

Notably, all 60 samples from sewage water drainage showed growth of *Pseudomonas aeruginosa,* similar to the study conducted by Ullah et al. (2012), suggesting that these areas may serve as reservoirs for the bacteria. Sewage water drainage systems often harbour bacteria due to organic matter and moisture, providing an ideal environment for bacterial growth (McLellan and Roguet 2017). Among the swab samples, only 8 out of 60 showed growth of *Pseudomonas aeruginosa*, and all of these positive samples were from Muhimbili Hospital, specifically from the ICU, theatre, and wards, similar to the study conducted by De Abreu et al. (2014).

The presence of *Pseudomonas aeruginosa* in specific areas of Muhimbili Hospital could be attributed to factors like patient populations, frequency of medical interventions, or cleanliness protocols (WHO 2009). Also, Muhimbili Hospital is a national referral hospital where patients nationwide are referred for specialized care and treatment. Consequently, the hospital may experience more patients with diverse medical conditions (Keniston et al. 2022), potentially contributing to increased microbial colonization and transmission within its facilities. The absence of growth in swab samples from Mwananyamala, Temeke and Amana regional hospitals suggests variability in bacterial distribution and highlights the importance of site-specific surveillance and control measures. Differences in hospital infrastructure, patient demographics, hygiene practices,

and environmental conditions may contribute to variations in the prevalence of *Pseudomonas aeruginosa* among the hospitals studied (Spagnolo et al., 2021). Understanding these differences can inform targeted interventions to reduce the transmission and impact of healthcare-associated infections across healthcare facilities.

Antibiotic susceptibility test

The antibiotic susceptibility testing results indicated a low resistance among the isolated *Pseudomonas aeruginosa* isolates. *Pseudomonas aeruginosa* isolates were highly resistant to aztreonam, with only 11.6%. This alarming antimicrobial resistance prevalence observed in this study has also been reported in previous studies (Gasink et al. 2007, Al-Daraghi and Al-Badrwi 2020). The high antimicrobial resistance is primarily caused by widespread use and misuse of these antibiotics, which creates selective pressure favouring the survival and the spread of the resistant strain (The United Republic of Tanzania 2022), which ultimately leads to the development of antimicrobial resistance (Davies and Davies 2010). These findings suggest that aztreonam may no longer be effective in treating infections caused by some strains of *Pseudomonas aeruginosa*. Given the emergence of antimicrobial resistance, exploring alternative treatment strategies is imperative. One such approach is the use of combination antibiotic therapy.

On the other hand, *Pseudomonas aeruginosa* strains were less resistant to Norfloxacin, Amikacin and Ciprofloxacin, with 2.3%, 3.1% and 3.1%, respectively. This low resistance observed against norfloxacin, amikacin and ciprofloxacin is consistent with the study conducted by Al-Daraghi and Al-Badrwi (2020) and Karruli et al. (2023). This explains that these antibiotics exert their antibacterial activity by targeting bacterial DNA gyrase and topoisomerase IV, which are enzymes involved in DNA replication and repair (Pang et al. 2019)However, it is important to note that even though these antibiotics are currently effective against a significant proportion of Pseudomonas aeruginosa, continuous monitoring of resistance patterns is essential to identify emerging trends and guide treatment decisions.

Multidrug Resistant *Pseudomonas aeruginosa*

The emergence and spread of multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains represent a critical challenge in healthcare settings globally (Jin 2024). In this study, three isolates were identified as MDR, showcasing resistance to multiple classes of antibiotics. They demonstrated resistance to fluoroquinolones, aminoglycosides, third-generation cephalosporin and Monobactam. The resistance mechanisms employed by *Pseudomonas aeruginosa* may involve various genetic mutations, efflux pumps, and enzymatic modifications, enabling them to evade the effects of antimicrobial agents (Pang et al. 2019). These findings underscore the complexity and urgency of addressing MDR *Pseudomonas aeruginosa* infections. The resistance profiles observed in these isolates significantly limit treatment options, increasing the risk of treatment failures, prolonged hospital stays, and adverse patient outcomes (Pachori et al. 2019). Furthermore, developing new antimicrobial agents and alternative treatment strategies is imperative to combat the growing threat of antimicrobial resistance in *Pseudomonas aeruginosa* and other pathogens.

Phenotypic and Genotypic Agreement among Resistant *Pseudomonas aeruginosa* Strains

The high occurrence trend (> 40%) of beta-lactam resistance genes, such as *blaVIM* (100%), (Table 6) , indicates the prevalence of these genetic determinants among the studied *Pseudomonas aeruginosa* isolates similar to a study conducted in Mwanza region (Moyo et al. 2015). Beta-lactam resistance genes encode enzymes like Metallo-beta-lactamases (e.g., VIM), which hydrolyze beta-lactam antibiotics, rendering them ineffective against the bacteria (Pournaras et al. 2002). The

absence of the quinolone resistance gene (qnrA) in any of the tested isolates suggests that mechanisms other than qnrA-mediated resistance may be responsible for the observed resistance to quinolone antibiotics (Kiula and Makene, 2023). *Pseudomonas aeruginosa* can develop resistance to quinolones through mutations in genes encoding DNA gyrase and topoisomerase IV, as well as through efflux pump mechanisms, which may not involve qnrA (Kiula and Makene 2023). The aminoglycoside resistance gene *AAC(3)-IV*, which confers resistance to amikacin, suggests that this genetic determinant plays a role in the observed resistance to amikacin among the tested isolates (Zhu et al. 2020). However, the lower occurrence of *AAC(3)-IV*-mediated resistance to gentamicin indicates that other mechanisms may contribute more significantly to gentamicin resistance in these isolates. This showed similar results as (Poole 2005).

The correlation between the phenotypic and the genotypic expression was 0.83; this indicates a strong positive relationship between phenotypic and genotypic expression. This suggests that genetic factors are strongly associated with observable traits, supporting the idea that genotype significantly influences phenotype. The correlation agreement between phenotypic resistance and resistance mechanisms underscores the role of specific resistance genes in mediating resistance to antibiotics. The absence of specific resistance genes and variations in their occurrence rates reflect the diversity of resistance mechanisms employed by *Pseudomonas aeruginosa*, highlighting the need for comprehensive surveillance and understanding of resistance mechanisms to guide antibiotic treatment strategies.

Conclusion

The research underlines the widespread prevalence of *Pseudomonas aeruginosa* in hospital environments and how concerning resistance is to several antibiotics, particularly aztreonam. The presence of the *blaVIM* gene emphasizes the danger of multidrug resistance and the need for more investigation into the mechanisms driving antibiotic resistance. Researching the prevalence and distribution of resistance genes like *blaVIM* can help develop strategies for treating diseases that are resistant to multiple medications

Declarations

Ethical Clearance was obtained from the University of Dar es Salaam Research Ethics Committee (UDSM-REC)

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Competing Interests

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