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ORIGINAL ARTICLE

Genomic Characterisation of Virulence and Antimicrobial Resistance Genes in *Pseudomonas aeruginosa* Isolates from Northern Tanzania

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a common cause of hospital-acquired infections and is classified as a World Health Organization (WHO) critical-priority pathogen because of its increasing resistance to key antibiotics, including carbapenems. In Tanzania, several studies that documented *P. aeruginosa* resistance profiles have relied on phenotypic or targeted Polymerase Chain Reaction (PCR) methods, limiting information on comprehensive antimicrobial resistance and virulence profiles. This study aimed to characterize genomic features of *P. aeruginosa* from a tertiary care hospital in northern Tanzania, focusing on antimicrobial resistance genes, virulence genes, sequence type and genetic relatedness.

Materials and Methods: A total of 16 isolates initially identified as *Pseudomonas* species were selected for whole genome sequencing analysis, of which 12 isolates were sequenced using Illumina NextSeq 550 and 4 with existing genomic data. Genomic species identification was confirmed using KmerFinder v3.2. Antimicrobial resistance and virulence genes were identified using ABRicate v1.0.1. Mobile genetic elements were identified using MobileElementFinder software v1.0.3 and database v1.0.2 from the Center for Genomic Epidemiology. Analysis of Inter-strain whole genome Single-Nucleotide Polymorphisms (SNPs) was performed using CSI Phylogeny v1.4.

Results: Of the 16 isolates, 13 were confirmed as *Pseudomonas* species by genomic analysis. Antimicrobial susceptibility testing showed the highest susceptibility to meropenem 11(84.6%) followed by gentamicin and ceftazidime 10(76.9%). Genome assemblies ranged from 6.4 to 6.9 Mb: MLST analysis revealed substantial genetic heterogeneity, including the presence of the high-risk ST235. All isolates carried intrinsic resistance genes, including blaPAO, blaOXA variants, *catB7, fosA, aph(3')-IIb*. Acquired resistance genes included *sul1/2, aadA2/6, ant(2")-Ia, blaCARB-2, tet(G)* and the carbapenemase gene blaDIM-1. The tmexC3/D3-TOprJ3 efflux system was detected. Mobile genetic elements (insertion sequences and transposons) were detected and co-localized with resistance genes. The highest proportion of resistance genes were observed among urine isolates 19(34.5%). Virulence genes associated with adhesion, secretion, biofilm, and toxin production were widely distributed. Phylogenetic analysis revealed genomic diversity ranging from 199 to 54,448 SNPs.

Conclusion: This study showed that multidrug-resistant *P. aeruginosa* from a tertiary hospital in Tanzania carried a wide range of resistance determinants, virulence factors, and high-risk clones. These preliminary findings support the need for strengthened antimicrobial stewardship and genomic surveillance to support clinical management and infection control.

BACKGROUND

Pseudomonas aeruginosa is a highly adaptable opportunistic pathogen and a major contributor to healthcare-associated infections worldwide.[1,2] It causes a wide range of severe infections, including blood infection, otitis, urinary tract infections, endophthalmitis, folliculitis, keratitis, osteomyelitis, enterocolitis, wound infections, pneumonia, chronic respiratory infections, and meningitis.[3,4] Its resilience to varied habitats, combined with intrinsic resistance and the ability to acquire additional resistance determinants, poses a critical threat globally.[5,6] The increasing emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains severely limits treatment options and contributes to higher morbidity and mortality worldwide.[7,8]

In Tanzania, *P. aeruginosa* is frequently reported among the leading causes of hospital-acquired infections, particularly among surgical and burn patients. [9,10] Previous studies have shown high levels of resistance to cephalosporins and carbapenems, raising concern for treatment challenges.[10,11] For example, *P. aeruginosa* isolates from tertiary hospitals showed approximately 89.5% resistance to third-generation cephalosporins

and 26.3% resistance to carbapenems.[12] Other reports from burn and wound infections describe multidrug resistance in up to 79.2% of *P. aeruginosa* isolates, with high resistance to meropenem and piperacillin (81.8%).[10] Furthermore, a recent whole genome sequencing (WGS) study on *P. aeruginosa* isolates from chronic leg ulcer patients identified clinically significant carbapenemase genes, including *blaVIM-2*, *blaDIM-1*, and *blaOXA*, and reported global high-risk sequence types such as ST233, ST244, and ST357.[4] Beyond antimicrobial resistance (AMR), *P. aeruginosa* possesses a broad repertoire of virulence factors, including biofilm-associated genes, siderophore systems, motility determinants, type III secretion system effectors, and quorum-sensing systems, that enhance its ability to colonize, persist, and cause severe disease.[13]

Whole genome sequencing (WGS) enables comprehensive detection of antimicrobial resistance genes, virulence determinants, and sequence types in a single assay, providing high-resolution data for AMR surveillance and outbreak investigation.[14] Despite this potential, genomic data in Tanzania remains limited. A recent systematic review from the East African community[15] reports that most studies in East Africa, including Tanzania relied on phenotypic susceptibility testing or targeted Polymerase Chain Reaction (PCR) assays, despite their limited ability to detect resistance mechanisms. The review also noted that antibiotic pressure within clinical settings drives the selection of increasingly resistant *P. aeruginosa* strains, yet the lack of comprehensive WGS limits understanding of the molecular basis of these phenotypes. The authors emphasized the urgent need for expanded genomic work to inform infection prevention and antimicrobial stewardship strategies within East Africa.

Given the high clinical burden of *P. aeruginosa* and increasing resistance rates in Tanzania, hospital-based evidence suggests that *P. aeruginosa* contributes to infection burden and treatment challenges. For example, Masoud et al., found *P. aeruginosa* accounted for 179/276 (64.9%) of isolates in wounds of hospitalized patients, with carbapenem resistance detected in 33.5% of *P. aeruginosa*. Another study reported that among pus swab isolates from hospitalized patients, *P. aeruginosa* represented 25% of clinically significant isolates, alongside 89.5% resistance to 3rd-generation cephalosporins.[16]

These findings highlight the need for comprehensive characterization of the genetic determinants underlying antimicrobial resistance, virulence and the detection of high-risk lineages. Therefore, this study aimed to characterize the genomic features of *P. aeruginosa* isolates from a tertiary hospital in Northern Tanzania, with a focus on antimicrobial resistance, virulence genes, sequence type, and genetic relatedness. This baseline data will provide a foundation of genomic insights to inform infection prevention and antimicrobial stewardship strategies and guide large scale genomic surveillance in the region.

MATERIALS AND METHODS

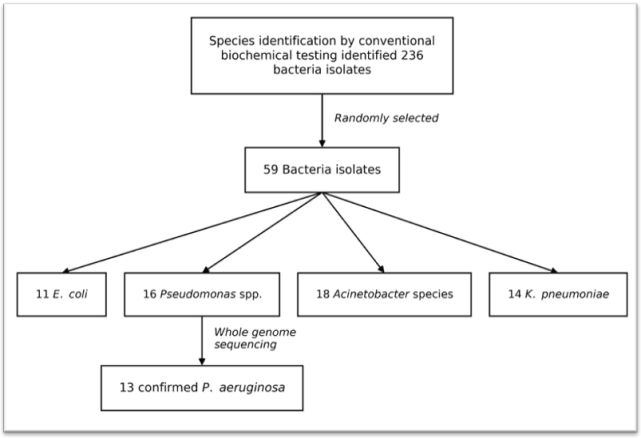
Study Design and Setting

The study was a mixed retrospective and prospective, laboratory-based genomic characterization involving clinical bacterial isolates obtained from inpatients admitted at surgical and medical wards at Kilimanjaro Christian Medical Center (KCMC) hospital, a tertiary referral hospital in Northern Tanzania that serves a catchment population of approximately eleven million. Clinical samples were prospectively collected in 2023, whereas bacterial isolates were retrieved from the laboratory archive. The genomic laboratory analyses were performed at the Kilimanjaro Clinical Research Institute (KCRI), a research institute equipped with microbiology, molecular biology and genomic laboratory facilities. The study utilized clinical bacterial isolates collected in 2018, 2020, and 2023.

Inclusion Criteria and Selection Process

A total of 236 bacterial isolates stored at −80°C were assigned unique identification numbers and entered into the sampling frame. Using simple random sampling, 59 isolates were selected from the 236 bacterial isolates. Random selection was based on the unique identification numbers, ensuring that each eligible archived isolate had an equal probability of selection. Of the 59 randomly selected isolates, 16 were identified as *Pseudomonas* species and included in the present study (Figure 1). The 16 *Pseudomonas* isolates were from clinical specimens, including wound/pus swabs (n=9), blood (n=2), urine (n=3), cerebrospinal fluid (n=1), and sputum (n=1), collected from patients admitted to surgical and medical wards. Where multiple isolates originated from the same patient, only one non-duplicate isolate was selected for analysis to avoid repeated representation of the same patient. All 16 isolates initially identified as *Pseudomonas* species were subjected to whole-genome sequencing. Following sequencing and genomic analysis, 13 isolates were confirmed as *P. aeruginosa* and met the criteria for inclusion in the final genomic analysis. Three isolates were excluded from the final *P. aeruginosa* analysis because genomic analysis identified them as other bacteria rather than *P. aeruginosa*.

FIGURE 1: Flow Diagram Showing the Selection of the Bacterial Isolates and Confirmation of *P. aeruginosa* by Whole Genome Sequencing



Bacterial Culture and Identification

Clinical samples collected in 2023 were cultured, and bacterial isolates were identified. Briefly, urine was cultured on CLED agar (Oxoid, Hampshire, United Kingdom) and blood agar (Himedia, Mumbai, India); wound or pus swab samples were cultured on MacConkey agar and blood agar (Himedia, Mumbai, India) and incubated at 37 °C for 24 h. Blood samples were incubated in the BACTEC system for a maximum of five days. Positive blood cultures were then cultured on MacConkey agar (Himedia, Mumbai, India) and blood agar (Oxoid, Hampshire, United Kingdom), and incubated for 24 h at 37 °C with 5% CO_2.

Archived bacterial isolates from the KCRI biorepository were cultured on blood agar (Oxoid, Hampshire, United Kingdom) for purity and viability. The original clinical samples for these isolates were not recultured. The 2023 bacterial isolates were identified while the identities of the archived bacterial isolates were reconfirmed using conventional biochemical tests, including triple sugar iron, sulfur indole motility, citrate, and urease. The identified isolates were stored at -80 °C.

Antimicrobial Susceptibility Testing (AST)

The disc diffusion method was used to perform antimicrobial susceptibility testing (AST) on Mueller–Hinton agar plates (Oxoid, Hampshire, United Kingdom) in accordance with the Clinical and Laboratory Standards Institute recommendations (CLSI 2021).[17] AST was carried out on all 16 isolates that were initially identified as *Pseudomonas* species. Five antimicrobial drugs were tested: piperacillin/tazobactam (100µg), gentamicin (10µg), ceftazidime (30µg), ciprofloxacin (5µg), and meropenem (10µg). The results were interpreted as Sensitive, Intermediate, or Resistant as per CLSI 2021.

Deoxyribonucleic Acid (DNA) Extraction and Sequencing

Whole genome sequencing was performed to characterize clinical isolates of *P. aeruginosa*. A total of 16 isolates initially identified as *Pseudomonas* species were included in the genomic analysis, of which 12 isolates were sequenced in this study, while 4 additional isolates with previously available genome sequence data were included for comparative analysis. Genomic DNA was extracted from pure bacterial cultures using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quantity was assessed before library preparation using a Qubit 2.0 Fluorometer (Invitrogen Life Technologies, Singapore).

Sequencing libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit following standard procedures, including DNA fragmentation, adapter ligation, size selection, barcoding, and pooling of libraries. The normalized libraries were combined with a PhiX control and sequenced on the Illumina NextSeq 550 platform.

Bioinformatics Analysis

Raw sequencing reads were obtained in FASTQ format. Adapter sequences and low-quality reads were trimmed using Trimmomatic v0.39 and quality was assessed using FastQC v0.12.1. De novo assembly was performed with SPAdes v4.0.0 to generate draft genome assemblies in FASTA format containing multiple contigs. Following de novo assembly, genome quality and contamination were assessed using the National Center for Biotechnology Information (NCBI) Foreign Contamination Screen (FCS). The assembled genomes were screened for foreign DNA and contamination, and the quality-controlled assemblies were used for downstream genomic analysis.

Species identification was confirmed using KmerFinder v3.2[18] against the Center for Genomic Epidemiology (CGE) database. Antimicrobial resistance genes, plasmid replicons, and virulence genes were identified using ABRicate v1.0.1 against ResFinder, PlasmidFinder, and Virulence Factors Database, respectively.[19] ABRicate was run using default minimum filtering threshold of ≥80% nucleotide identity and ≥80% query coverage for all gene matches. All bioinformatics tools were locally installed and run via the command line on a Linux-based system. Mobile genetic elements (MGEs) were identified in the assembled genome using the mobile genetic element finder, a tool available at the Center for Genomic Epidemiology (CGE).[20]

Phylogenetic Tree Analysis

For the analysis of inter-strain whole genome Single Nucleotide Polymorphisms (SNPs), the CSI Phylogeny version 1.4 pipeline hosted on the CGE server was used.[18] Read mapping and SNP calling were performed using *P. aeruginosa* PAO reference genome (RefSeq accession GCF_000006765.1). The analysis was performed using the default parameters, including minimum depth of 10x at SNP positions, minimum relative depth of 10%, minimum distance of 10bp between SNPs for pruning, minimum SNP quality of 30, and minimum read mapping quality of 25.

The analysis included 13 *P. aeruginosa* isolates sequenced in this study, together with seven publicly available *P. aeruginosa* genomes obtained from the NCBI database. The seven comparator genomes were from Tanzania and were included to provide additional genomic context, and expand the phylogenetic tree. CSI Phylogeny generated a high-quality concatenated SNP alignment, SNP matrix, and Newick format phylogenetic tree and enabled the comparison of the study isolates with previously available *P. aeruginosa* genomes from Tanzania. The resulting Newick tree was annotated and visualized using MEGA X v10.2.6.[21]

Statistical Analysis

Data were entered into Microsoft Excel and exported to Stata version 15 (STATA Corp LLC, College Station, TX, USA) for analysis. Descriptive statistics were used to summarize phenotypic antimicrobial resistance profiles and the distribution of antimicrobial resistance genes identified among *P. aeruginosa* isolates. Resistance genes were counted based on unique gene types identified, and repeated detection of the same gene across multiple isolates was considered as a single gene type. The proportion of resistance genes according to specimen source was calculated as the number of unique gene types detected in isolates from each specimen divided by the total number of unique resistance gene types identified across all isolates. The percentage agreement between phenotypic AST and genotypic WGS predictions was calculated by dividing the

total number of concordant isolate drug combinations by the total number of combinations evaluated. Results were presented as frequencies and percentages. The pie chart was used to visualize the distribution of antimicrobial resistance genes among isolates according to specimen type. No inferential statistics were performed.

Ethics Approval and Consent to Participate

The project received ethical approval from the KCMC University Research Ethics Review Committee (dated: 05/01/2023, approval number:2601). For prospective study in 2023, written informed consent was obtained from all participants before their inclusion in the study. Participants were informed about the purpose of the study, and confidentiality of personal information was maintained throughout the research process. For the archived isolates, permission to access and use the stored bacterial isolates was obtained from the respective parent project team. These archived isolates were used for the approved research purposes, and no additional participant recruitment or consent was undertaken for their genomic analysis. Genomic sequencing and analysis were conducted as part of the approved research protocol.

RESULTS

Species Confirmation

A total of 16 isolates were subjected to genomic analysis, of which 13 isolates were confirmed as *P. aeruginosa*. Three were identified as *Klebsiella pneumoniae* and *Acinetobacter baumannii*. These isolates were obtained from wound/pus swabs (n=6), blood (n=2), urine (n=3), cerebrospinal fluid (n=1), and sputum (n=1).

Antimicrobial susceptibility profile of *Pseudomonas aeruginosa*

Antimicrobial susceptibility testing of *P. aeruginosa* showed that susceptibility was highest to meropenem, with 11(84.6%), followed by gentamicin and ceftazidime with 10(76.9%). The highest resistance was observed for piperacillin/tazobactam at 7(53.8%) (Table 1).

TABLE 1: Antimicrobial Susceptibility Profile of *Pseudomonas aeruginosa* (N=13)

Antibiotics	Susceptible (n (%))	Resistance (n (%))
Ciprofloxacin	9(69.2%)	4(30.8%)
Gentamicin	10(76.9%)	3(23.1%)
Meropenem	11(84.6%)	2(15.4%)
Ceftazidime	10(76.9%)	3(23.1%)
Piperacillin-tazobactam	7(53.8%)	6(46.2%)

Genome Assembly Characteristics

The assembled genomes of *P. aeruginosa* ranged from 6.4 to 6.9 Mb. The overall genomic GC content was highly uniform across all isolates, averaging approximately 66%. The total number of assembled contigs per genome ranged between 151 and 873 (Table 2).

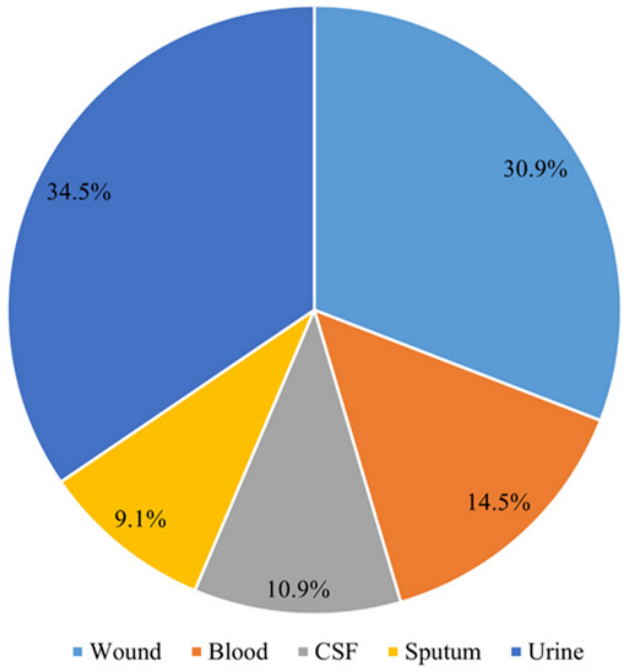
Multilocus Sequence Typing and Sequence Type

Multilocus sequence typing (MLST) confirmed genetic heterogeneity among the *P. aeruginosa* strains. The global high-risk clone ST235 was detected in isolates PM050 and WGS_45, while other isolates carried unique sequence types including ST3079, ST1076, ST612, ST1239, ST646, ST267, ST1405, and ST3714.

Distribution of Resistance Genes

All isolates carried intrinsic resistance genes including *blaPAO*, *aph(3')-IIb*, *catB7*, *fosA*, and *blaOXA* variants (*blaOXA-50*, *blaOXA-396*, *blaOXA-494*, *blaOXA-488*), while other isolates harbored additional acquired resistance determinants (*sul1/sul2*, *aadA2*, *aadA6*, *ant(2")-Ia*, *blaCARB-2*, *tet(G)*), and one isolate C099 carried a broader resistome, including *blaDIM-1*, *blaOXA-10*, *qnrD1*, *tmexC3D3-TOprJ3*, and *cmlA1* (Table 3). The distribution of the 55 unique antibiotics resistance genes detected across all samples is shown in Figure 2. The highest proportion of resistance genes were observed among urine isolates 19(34.5%), followed by wound 17(30.9%), blood 8(14.5%), cerebrospinal fluid 6(10.9%), and sputum 5(9.1%).

FIGURE 2: Distribution of Antimicrobial Resistance Genes Detected in *Pseudomonas aeruginosa* Clinical Isolates from Different Sample Sources



Mobile Genetic Elements (MGEs) and Plasmids among the Isolates

Several isolates were found to have MGEs, including transposons (Tn) and insertion sequences (ISs) as summarized in Table 3. In some of these, MGEs were on the same contig that contained resistance genes.

TABLE 2: Genome Assembly Statistics for *Pseudomonas aeruginosa*

ID	Genome size (Mb)	GC content (%)	N50 (bp)	Number of Contigs
T40	6.3	66.5	78.7kb	225
WGS_45	6.9	66	981.9kb	151
WGS_310	6.3	66.5	170.7kb	199
WGS_348	6.5	66	13.8kb	873
WGS_363	6.7	66.5	144kb	399
PM020	6.5	66.5	15.1kb	837
PM047	6.5	66.5	53.4kb	249
PM050	6.9	66	59.5kb	319
PM051	6.5	66.5	67.5kb	287
C018	6.5	66	73.4kb	218
C041	6.5	66	76.4kb	323
C060	6.4	66.5	56.1kb	254
C099	6.7	66	89.5kb	250

Tn6061 and ISPa7 were observed on the same contig as the resistance gene *blaCARB-2* in the isolate WGS_45. In isolate C099, IS6100 co-localized with the *aadA1* resistance gene, while ISKpn4 was detected in the same contig as *blaDIM-1* and *ant(2")-Ia*. ISVsa3 was observed in the same contig as *sul2* resistance gene in the isolate PM020. In PM051, ISPa6 and ISPa1 were found in the same contig as the resistance gene *fosA*. Furthermore, in C018, ISPa5, ISPa2, and cn_1130_ISPa5 were found in the same contig as the resistance gene *fosA*. Other ISs did not co-localize with resistance genes. In addition, plasmid-mediated resistance was limited. Only isolate C099 carried the quinolone resistance gene *qnrD1* on the Col3M plasmid (Table 3).

Integration Between Resistance Phenotypes and the Resistance Gene

Out of 65 total isolate drug combinations evaluated across 13 isolates, an overall genotype-phenotype percentage agreement (concordance) of 47(72.3%) was observed, revealing an overall discordance rate of 18(27.7%). Drug specific concordance was highest for meropenem at 12(92.3%) and lowest for ciprofloxacin at 3(23.1%) (Table 4).

The comparison at the isolate level further confirmed the occurrence of genotype-phenotype discordance, including detection of resistance-associated genes in phenotypically susceptible isolates, and phenotypic resistance in the absence of corresponding known resistance genes. These discordances were observed across several antimicrobial agents including ceftazidime, meropenem, piperacillin/tazobactam and gentamicin.

Distribution of Virulence Genes

P. aeruginosa isolates in this study harbored a wide repertoire of virulence associated genes. Several important virulence genes were observed across isolates. These include exotoxin A gene (*toxA*), alginate biosynthesis-related genes (*algU*, *algD,mucA*), and motility genes such as *fliA*, *fleN*, *fleQ*, *motC*, *motD*. Additionally, genes associated with the type III secretion system (T3SS), such as *exoS*, *exoT*, *exoY*, *exoU*, and *pcrV*, quorum sensing and biofilm formation (*lasA*, *lasB*, *rhlA*, *rhlB*, *rhlI*), siderophore production (*pchA*, *pvdA*, *fptA*, *fpvA*), and phenazine biosynthesis (*phzA*, *phzM*, *phzS*) were widely distributed among isolates (Table 5).

Phylogenetic Relationship

Core genome SNP-based phylogenetic analysis revealed clear genetic clustering among the study isolates. A total of 13 *P. aeruginosa* isolates and reference genomes were compared using whole genome alignment against *P. aeruginosa* PAO (RefSeq accession GCF_000006765.1). Pairwise SNP analysis revealed genomic diversity ranging from 199 to 54,448 SNPs. The closest relationship was observed between isolates PM050 and WGS_45, with 199 SNP differences. Isolate T40 showed the highest divergence from 47,849 to 54,000 SNPs from other isolates (Figure 3).

TABLE 3: Distribution of Resistance Genes, Mobile Genetic Elements, and Plasmids among *Pseudomonas aeruginosa (n=13)*

Isolate ID	Sequence Type (ST)	Core resistance genes	Acquired resistance genes	Mobile genetic elements	Plasmids
T40	Unknown	blaPAO_3, blaOXA-494_1, aph(3′)-IIb_2, catB7_1, fosA_4	ND	ND	ND
WGS_45	235/534	blaPAO, blaOXA-488, aph(3′)-IIb, catB7, fosA	sul1, ant(2'')-Ia, aadA2, blaCARB-2, dfrB2, tet(G)	Tn6061, ISPa7	ND
WGS_310	Unknown	blaOXA-396, aph(3′)-IIb, catB7, fosA	ND	ISPa2, ISPa32	ND
WGS_348	Unknown	blaOXA-50, aph(3′)-IIb, catB7	crpP	ISPa1	ND
WGS_363	Unknown	blaPAO, blaOXA-50, aph(3′)-IIb, catB7, fosA	crpP	ISPa6, ISPa32	ND
PM020	Unknown	blaPAO_2, blaOXA-486_1, aph(3′)-IIb_1, catB7_1, fosA_4	crpP_1, sul2_2	ISVsa3, ISUnCu4, ISPa6, ISPa2	ND
PM047	267	blaPAO_1, blaOXA-488_1, aph(3′)-IIb_2, catB7_1, fosA_4	ND	ISPa32, ISPa6, ISPa5, ISPa1, ISPst3, ISPa37, cn_1132_ISPa5	ND
PM050	235	blaPAO_2, blaOXA-488_1, aph(3')-IIb_1, catB7_1, fosA_4	crpP_1, sul1_5, aadA6_1	ISPa86, ISPa6, ISPre3, ISPa7	ND
PM051	1405	blaPAO_3, blaOXA-396_1, aph(3′)-IIb_2, catB7_1, fosA_4	crpP_1	ISPa6, ISPa1, ISPa22	Col3M
C018	3076	blaPAO_2, blaOXA-50_1, aph(3′)-IIb_2, catB7_1, fosA_4	crpP_1	ISPa5, ISPa2, cn_1130_ISPa5, ISPa22, ISPa11	ND
C060	612	blaPAO_2, blaOXA-50_1, aph(3′)-IIb_2, catB7_1, fosA_4	crpP_1	ISPa32, ISPa6, ISPa1	Col(BS512), Col(MG828)
C041	1076	blaPAO_4, blaOXA-395_1, aph(3′)-IIb_2, catB7_1, fosA_4	ND	ISPa6, ISPa5	ND
C099	3714	blaPAO_1, blaOXA-396_1, blaOXA-494, aph(3′)-IIb_2, catB7_1, fosA_4	sul1_5, ant(2″)-Ia_1, ant(3″)-Ia, blaOXA-10_1, blaDIM-1_1, ARR-3_4, cmlA1_1, qnrD1, tmexC3, tmexD3, TOprJ3	IS6100, ISKpn4, ISPen2	Col3M

ND =Not detected

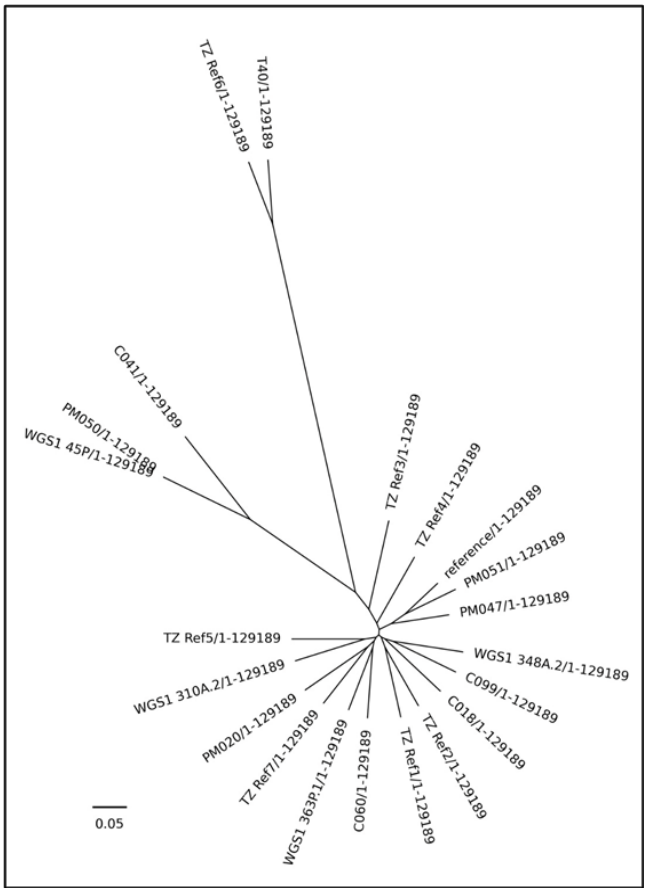
TABLE 5: Distribution of Key Virulence Genes among *Pseudomonas aeruginosa*

Isolate ID	Biofilm & Adherence	Quorum Sensing & Secreted Enzymes	Motility (Flagella/Pili)	Type III Secretion System (T3SS)	Iron Acquisition (Siderophores)	Phenazine Synthesis & Toxins	Other Mechanisms (T6SS / Effector)
T40	algD, algU, mucA	lasB, lasA, rhlA-B,	pilT, motD, motC, fliC, fleO, fleN, fimT	exoS, exoT, pcrV	fpvA, pvdA	toxA, phzM-S, phzA	hcp1, hsi, vipB, tse3, Tse1
WGS_45	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
WGS_363	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
WGS_310	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
WGS_348	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
PM020	algD, algU, mucA	lasA-B rhlA	pilT, motD, motC, fleN	exoS, exoT, exoY, pcrV	fpvA, pvdA, pchA, pchR	toxA, phzM-S, phzA-B	hcp1, tse3
PM047	algD, algU, mucA	lasA-B rhlA-B, rhlI	pilT, motD, motC, fleN, fleQ, fliA	exoS, exoY	fpvA, pvdA, pvdS, pchA	toxA, phzM-S phzA-B	hcp1, tse3
PM050	algD, algU, mucA	lasB, lasI, rhlA, aprA	pilT, motD, motC, fleQ, fliA	exoU, exoY, exoT, pcrV	pvdA, pvdS, pchA	toxA, phzM-S	hcp1, tse3, vgrG1a
PM051	algD, algU, mucA	lasA-B lasI, rhlA-B	pilT, motD, motC, fleQ, fliA, fleN	exoT, pcrV	fpvA, pvdA, pchA	toxA, phzM-S, phzA-B	hcp1, tse3
C018	algD, algU, mucA	lasA-B lasI, aprA	pilT, motD, motC, fleN, fleQ, fliA, fliQ	exoS, exoT, pcrV	fptA, pvdS, pchA	toxA, phzM-S, phzA-B,	hcp1, tse3, vgrG1a
C041	algD, algU, mucA	lasA-B, rhlA-B	pilT, motD, motC, fleN, fliA, fleQ	exoU, exoY, pcrV	fptA, pvdS, pchA	toxA	hcp1, tse3
C060	algD, algU, mucA	lasA, rhlA-B	pilT, motD, motC, fleN, fleQ	exoS, exoT, exoY	fptA, pvdA, pvdS, pchA	toxA, phzM-S, phzA-B	hcp1, tse3, pchA
C099	algD, algU, mucA	lasA-B, lasI, rhlA-B	pilT, motD, motC, fleN, fleQ, fliA	exoS, exoT, exoY	pvdA, pvdM, pchA	toxA, phzM-S	hcp1, tse3, vgrG1a, aprA

TABLE 4: Genotype-phenotypes Concordance and Discordance Rate Across *Pseudomonas aeruginosa* (N=13)

Antibiotic	Total Tested	Concordant n(%)	Discordant n(%)
Meropenem	13	12(92.3%)	1(7.7%)
Ceftazidime	13	11(84.6%)	2(15.4%)
Gentamicin	13	11(84.6%)	2(15.4%)
Piperacillin/Tazobactam	13	10(76.9%)	3(23.1%)
Ciprofloxacin	13	3(23.1%)	10(76.9%)
Total	65	47(72.3%)	18(27.7%)

FIGURE 3: Phylogenetic Relationship of *P. aeruginosa* Clinical Isolates Based on SNP Analysis.



The tree comprises 13 *P. aeruginosa* clinical isolates and 7 publicly available *P. aeruginosa* genomes obtained from the NCBI database. Branch lengths represent genetic distance. Closely clustered isolates indicate greater genomic relatedness and fewer SNP differences. The scale bar represents 0.05 substitution per nucleotide site.

DISCUSSION

This study aimed to characterize the genomic sequences of clinical isolates of *P. aeruginosa* obtained from different samples. The findings provide important insights into genetic diversity, antimicrobial resistance genes, virulence determinants, and phylogenetic relationships of *P. aeruginosa*.

In this study, 13 of 16 sequenced isolates were confirmed as *P. aeruginosa* by genomic analysis. This indicates that conventional microbiological methods failed to correctly identify three isolates. This discrepancy highlights the limitations of phenotypic and biochemical methods, which often fail with unusual strains due to variable expression or silencing of traits. Previous studies have also reported misidentification of bacterial species using phenotypic methods.[21,22]

The antimicrobial susceptibility testing showed that *P. aeruginosa* tested were generally more susceptible to meropenem, ceftazidime and gentamicin, suggesting that these antibiotics remained effective against most of the isolates studied. In contrast, the high resistance to piperacillin/tazobactam, suggests that this antibiotic may be commonly used as empiric antipseudomonal therapy. Comparable susceptibility to meropenem and ceftazidime has been reported in another study. For example, Mtenga et al., found high susceptibility to meropenem and ceftazidime, similar to our finding, and reported fully susceptible *P. aeruginosa* to piperacillin/tazobactam, in contrast to high resistance observed in our study[23]. In contrast, other surveillance studies have documented variable patterns; some report better activity of piperacillin/tazobactam than meropenem, while others describe high non-susceptibility to both piperacillin/tazobactam and meropenem, particularly in ICU and multidrug-resistant populations.[24–26] Similarly, other studies have also reported high resistance to piperacillin–tazobactam, consistent with our results.[27,28] These discrepancies likely reflect differences in geographic region, specimen source, sample size, and temporal trends in antibiotic use. Therefore, AMR response actions in Tanzania should include periodic review of empiric antipseudomonal treatment guidance using local susceptibility trends, together with strengthened antimicrobial stewardship to reduce unnecessary exposure to broad-spectrum agents.

MLST analysis showed genetic diversity among the *P. aeruginosa* isolates with detection of multiple sequence types, including the globally recognized high-risk clone ST235, alongside several unique STs (ST3079,

ST1076, ST612, ST1239, ST646, ST267, ST1405, and ST3714). This finding indicates the presence of diverse *P. aeruginosa* lineages among the isolates analyzed in the present study. Previous studies have demonstrated ST235 to be a globally high-risk clone and have reported its association with multidrug resistance and hospital outbreaks. [29,30] The presence of this high-risk clone in our hospital environment is concerning, suggesting the circulation of a strain with enhanced resistance potential. This finding is consistent with previous studies on MLST analysis reporting a dominant high-risk clone and several uncommon lineages. [31,32] This diversity indicates that the hospital environment harbors both globally disseminated high-risk clones and uncommon lineages, highlighting the complexity of *P. aeruginosa* infection.

Genomic analysis also revealed widespread genetic diversity in resistance mechanisms among *P. aeruginosa* isolates within the hospital environment. The presence of core resistance genes, such as *blaPAO*, *blaOXA* variants, *catB7*, *fosA*, and *aph(3')-IIb*, indicates the intrinsic resistome of *P. aeruginosa*. Previous studies have reported that these chromosomally encoded genes are commonly present in *P. aeruginosa* and contribute to its intrinsic reduced susceptibility to multiple antibiotics. [33,34] These indicate that *P. aeruginosa* possesses intrinsic resistance mechanisms enabling continuous adaptation to antibiotics. In addition to the core resistome, several isolates harbored acquired resistance genes including *sul1/sul2*, *aadA6*, *tmexC3D3-TOprJ3*, and *blaDIM-1*, highlighting the contribution of horizontal gene transfer to the evolving resistome of *P. aeruginosa*. Notably, *blaDIM-1*, a carbapenem hydrolyzing gene, is of particular clinical and public health concern because carbapenem resistant *P. aeruginosa* is recognized among WHO priority pathogens.[35] The findings align with the Tanzania national action plan on AMR (NAP-AMR), which prioritizes strengthening AMR surveillance, optimizing antimicrobial use, improving infection prevention and control.[36]

Genomic analysis further showed that some of the resistance genes were detected on the same contig as MGEs. The observed association of some resistance genes with mobile genetic elements suggests that horizontal gene transfer may contribute to the evolving resistance landscape. Previous studies reported that several acquired resistance genes are located in mobile genetic elements, including transposons and ISs. [37,38] The detection of *blaDIM-1*, which hydrolyzes carbapenems like meropenem and imipenem, is alarming as it confers resistance to last-resort antibiotics, contributing to MDR, and is linked to difficult-to-treat infections globally. [39] Other studies have reported an increasing prevalence of carbapenemases in *P. aeruginosa*. For instance, in Iraq, 56.25% of *P. aeruginosa* isolates had *blaVIM* genes, while 25% of isolates carried *blaNDM* genes. [40] Similarly, in Iran, 90%, 40%, and 5% of carbapenem-resistant *P. aeruginosa* carried the bla_{IMP}, bla_{VIM}, and bla_{NDM} genes, respectively. [41] In another study done in Sudan, the most detected carbapenemase genes were bla_{VIM} (9%), bla_{NDM} (31%), *blaOXA-48* (22%), and *blaKPC* (49%). [42] These findings indicate the increasing global spread of the carbapenem resistance genes in *P. aeruginosa*, raising major concerns. Tanzania NAP-AMR should emphasize measures that reduce the risk of transmission and dissemination, including adherence to contact precautions, and improved cleaning and disinfection practices.

This study detected limited plasmid-mediated resistance. Only col-type plasmids were detected in three isolates. Col3M was observed to co-localize with the quinolone resistance gene *qnrD1*. Other col-type plasmids did not show co-localization with resistance genes. This result indicates that chromosomal and MGE-associated mechanisms are major contributors to resistance in these isolates. Other studies have also reported similar findings, where several resistance genes in *P. aeruginosa* were located on chromosomal mobile genetic elements rather than plasmids.[37,43]

The genotype-phenotype comparison showed an overall agreement of 72.3%, indicating that most of the resistance phenotypes were reflected by resistance determinants identified by whole genome sequencing. However, the 27.7% discordance between genotype and phenotype suggest that genomic prediction does not consistently correspond to the phenotypic susceptibility profile. Discordance varied across antibiotics, with high concordance for meropenem (92.3%) and lower for ciprofloxacin at 23.1%. The differences suggest that the ability of WGS based susceptibility prediction may depend on the specific resistance mechanisms underlying each antimicrobial phenotype, including cases where resistance may be mediated by determinants not captured by the genotype assessed, or where phenotypic expression is influenced by factors beyond the detected resistance genes. Previous studies have also reported discordance between phenotype and genotype, where isolates that harbored resistance determinants remained phenotypically susceptible or displayed phenotypic resistance without harboring resistance determinants. [44,45]

This observation highlights the complexity of antimicrobial resistance beyond simple gene presence and a limitation of WGS. Phenotypic susceptibility despite the presence of known resistance genes may suggest that the genes are transcriptionally silent, poorly expressed, or functionally impaired under the tested conditions. Such variability can be mediated by regulatory mechanisms, including those consistent with epigenetics, where DNA methylation and other factors influence gene expression without altering the underlying sequence.[46] In addition, regulatory mutations and inducible resistance mechanisms can alter the expression of resistance determinants, which means presence of the resistance determinant does not necessarily result in detectable resistance phenotype.[47] Conversely, the occurrence of phenotypic resistance in the absence of identifiable resistance genes may reflect alternative mechanisms such as efflux pump overexpression, porin mutations or reduced membrane permeability, and chromosomal mutations that affect antimicrobial targets or regulatory pathways that are not captured in resistance databases, or yet undescribed resistance determinants.[48,49] Thus, for a more comprehensive characterization of antimicrobial resistance, an integrative approach combining genomic, transcriptomic, and phenotypic data is desirable.

The virulence gene profile showed both conserved and strain specific virulence genes, highlighting the adaptability and pathogenic potential of the bacteria. Genes involved

in quorum sensing and biofilm formation, including *lasA*, *lasB*, *rhlA* and *rhlB*, were widely distributed, indicating a strong capacity for surface attachment, biofilm formation, and coordinated regulation of virulence factors. These biofilms not only shield bacteria from host defense and antibiotics but also contribute to chronic infection and treatment challenges.[50]

Motility related genes such as *fliA*, *fleN*, *fleQ*, *motC*, *motD* were also observed, indicating the ability of the bacteria to move, colonize, and establish the infection in different positions of the host. Motility is among the key virulence factors in *P. aeruginosa* contributing to host colonization, biofilm formation, and acute infection.[50] The presence of T3SS genes (*exoS*, *exoT*, *exoY*, *exoU*, *and pcrV*) in several isolates was observed, indicating high virulence potential. These genes encode major effector proteins involved in cytotoxicity and immune evasion, and are widely distributed among clinical strains, underscoring their role in promoting severe disease. [51,52] In particular, *exoU* in some isolates suggests certain lineages have enhanced pathogenicity that can give rise to more severe diseases. Several studies reported the association of strains harboring *exoU* with acute cytotoxicity, resulting in serious disease outcomes and higher antibiotic resistance. [53–55]

Furthermore, genes related to biosynthesis of alginate were found in the majority of the isolates, which underscores the ability of the *P. aeruginosa* to synthesise extracellular polysaccharides that are important for biofilm formation and evasion of host immunity. Previous studies reported that alginate production plays a key role in biofilm structure and protects bacteria from immune clearance.[56,57]

All strains carried siderophore biosynthesis (*pchA*, *pvdA*, *fptA*, *fpvA*), suggesting that these isolates possess efficient iron acquisition mechanisms under nutrient limited conditions, a key determinant of survival and virulence. A previous study demonstrated the essential roles of pyoverdine and pyochelin systems in microbial competition and host interaction.[58] Additionally, observation of the genes responsible for phenazines such as *phzA*, *phzM*, and *phzS* aligns with previous studies reporting that phenazines serve as antimicrobial metabolites contributing to competitive advantage against other microbes and enhancing *Pseudomonas* pathogenicity.[59–61]

Core genome SNP-based phylogenetic analysis revealed genetic diversity among the *P. aeruginosa* isolates, indicating the presence of multiple distinct lineages. Some isolates, such as WGS_45 and PM050, were closely related and differed by 199 SNPs, suggesting shared ancestry. While others, such as T40, showed high SNP distances, indicating that genetically distinct lineages. This pattern is consistent with previous reports showing that *P. aeruginosa* populations display a highly conserved core genome, but variation at the single-nucleotide level contributes to distinct phylogenetic clusters.[62] Furthermore, studies of genetically diverse strains from cystic fibrosis lungs have similarly shown that diverse lineages frequently coexist within the same host or environment.[63]

Therefore, the current findings provide an evidence base for implementing key NAP-AMR strategies and responding to the WHO bacterial priority pathogens. The findings underscore the need for integrating WGS into AMR surveillance to monitor high-risk clones and carbapenemases, updating empiric therapy guidelines for *Pseudomonas* based on local susceptibility and genomic data, strengthening infection prevention and control measures in high-risk units when ST235 or carbapenemase-producing isolates are detected.

Limitations of the Study

Despite this study providing valuable information on the antimicrobial resistance and virulence of *P. aeruginosa*, it is limited by the number of isolates included. In addition, all isolates were from inpatients; thus, the study largely captured the strains causing severe or complicated infections and cannot be generalized to the whole patient population, as strains causing mild infections are likely underrepresented. Furthermore, most isolates were collected between 2018 and 2023, while these findings are being reported in 2026. Given the rapid evolution of antimicrobial resistance in *P. aeruginosa*, our data may not fully capture the current landscape.

CONCLUSION

This study showed that multidrug-resistant *P. aeruginosa* from a tertiary hospital in Tanzania carried a wide range of resistance determinants, virulence factors, and high-risk clones. These preliminary findings provide genomic surveillance data on antibiotic resistance, virulence determinants and genetic diversity and emphasize the need for careful prescribing practices, particularly with carbapenems as last resort agents, and targeted stewardship to limit the emergence and spread of multidrug and pan-drug resistant strains. This genomic analysis provides a crucial baseline for enhancing surveillance programs and infection prevention in resource-limited settings.

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