**Antibiotic Susceptibility and Molecular Characterization of *Pseudomonas fluorescens* from Hospital Biomedical Waste in Kalahandi, India**

**Abstract**

The prevalent application of antibiotics and the rise of antibiotic resistance in healthcare settings present challenges. However, antibiotics and antibiotic resistance as potential environmental threats and hazards have received less attention. The increasing prevalence of antibiotic resistance among various organisms is a significant concern for modern medicine. Variations in prevalence and resistance rates highlight the need for standardized monitoring and control strategies. Our findings, based on 16S rRNA phylogenetic analysis, provide valuable insights into bacterial classification and evolutionary relationships. Additionally, Pseudomonas fluorescens is emerging as a multidrug-resistant (MDR) pathogen, with resistance patterns influenced by geographical factors and antibiotic exposure. The complete resistance of the MDR-08 strain to five antibiotics is particularly concerning, underscoring the urgent need for stricter antibiotic stewardship and improved hospital waste management practices.

**Key Words:** Pseudomonas fluorescens**, Antibiotic Susceptibility, Biomedical Waste, Kalahandi, antibiotic resistance**

**1. INTRODUCTION**

Antibiotic usage and the emergence of antibiotic resistance in medical facilities are widely recognised challenges, but antibiotics and antibiotic resistance as potential threats and hazards have received less attention. As a result, the growing prevalence of antibiotic resistance among a wide range of organisms is an important issue for modern medicine (Moges et al., 2014)Biomedical waste can be hazardous to public health and ecological balance because it contains many types of contaminants such as radioactive, chemical, and pharmaceutical wastes, as well as infectious pathogens(Sharpe, 2003). The unregulated and excessive consumption of antibiotics by both humans and animals leads to an outbreak of resistance to antibiotics and the transmission of resistance genes in environmental samples such as Biomedical waste (Iversen et al., 2002). According to studies, hospital Biomedical is a very susceptible environment that contributes to the high rates of resistant bacteria released in the natural environment (Yanget al., 2009). The quantity and quantity of pharmaceuticals utilised in both hospitals and private residences, as well as those released into drainage and municipal sewage, exhibits a selective effect on microorganisms. Biomedical waste discharge has a high concentration of resistant bacteria and antibiotic residues that can hinder the growth of vulnerable microorganisms. As a consequence, Biomedical waste effluent may increase the number of antibiotic-resistant bacteria in receiving sewage through methods of transfer and selection for multi-resistant bacteria (Al-Ahmad et al., 1999). Antibiotics are often classified based on their chemical structure, mechanism of action, and activity spectra. Antibiotics are classified into chemical or molecular structures, such as beta-lactams, macrolides, tetracyclines, quinolones, aminoglycosides, sulphonamides, glycopeptides, and oxazolidinones. The European Centre for Disease Control (ECDC) and the Centre for Disease Control and Prevention (CDC) in Atlanta define multidrug resistance (MDR) as acquired non-susceptibility to at least one antimicrobial agent in three or more categories(Minhas et al., 2024). In India, there are five infectious agents to be aware of: *Escherichia coli* (152,700), *Klebsiella pneumoniae* (123,200), *Staphylococcus aureus* (111,400), *Acinetobacter baumannii* (103,500), and *Mycobacterium tuberculosis* (98,600). These infectious agents cause more deaths than neoplasms, respiratory infections and tuberculosis, enteric infections, diabetes and kidney diseases, and maternal and neonatal disorders. In the GBD area of South Asia, India has the third highest age-standardized death rate among five countries. In 2019, 297,000 deaths were attributed to AMR, with 1,042,500 deaths related with it. The purpose of this study was to find multi-drug resistant bacteria in the Biomedical waste and determine their antibiotic resistance trends.

**2. MATERIALS AND METHODS**

**2.1. Samples Collection and isolation**

The Biomedical waste samples were randomly collected from Kalahandi District Headquarter hospital, Bhawanipatna, Kalahandiduring December 2024.The Biomedical waste effluent was mixed with distilled water and diluted 10−1 to 10−5. Following dilution, 0.05 mL of serial diluted mixture was transferred from each dilution tube to several culture plates, including nutrient agar, *Pseudomonas* agar, King B agar, Mac Conkey agar, and EMB. The samples were aseptically spread onto growth plates by using a sterile glass spreader. Finally, each Petri dish was incubated at 37°C overnight. Following an overnight incubation, different bacterial colonies were selected and sub-cultured for pure isolation on selective media (Mahmud et al., 2023).

**2.2. Characteristics of Isolated Bacteria**

**2.2.1 Gram’s staining**

Gram staining was performed according to Krishnamoorthy & Arjun (2012) with some modification where all isolated bacteria were stained with crystal violet as a primary stain, followed by iodine as a mordant the decolorized, and at final counterstained with safranin to differentiate between the gram positive and negative bacteria.

**2.3.2 Colony morphology**

The 24 hours incubated bacteria cultured were set to study the colonies' morphology which includes size, shape, colour, elevation, and texture (Krishnamoorthy & Arjun, 2012).

**2.3.3 Biochemical tests**

Biochemical tests were done according to Microbiology: A Laboratory Manual, such as the catalase test, oxidase test, citrate utilisation test, Methyl red test, Indole test, triple sugar iron test, and carbohydrate fermentation test, were carried out using isolated bacterial strains that displayed distinctive colony morphology (Kianpour et al., 2017).

**2.3.4 Molecular identification of the isolates**

DNA was isolated with in a MCT tube from the provided culture using Quick-DNA" Fungal/Bacterial Miniprep Kit Catalog No. D6005 from Zymo Research. Quality was evaluated on 1.8% Agarose Gel; asingle band of high-molecular weight DNA has been observed. Isolated DNA was amplified with 16s rRNA Specific Primer (27Fand 1492R) using Veriti®96 well Thermal Cycler. A single discrete PCR amplicon band of ~1500 bp was observed. The PCR amplicon was bead purified and further subjected to Sanger Sequencing. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with 27FGAGTTIGATCATGGCTCAG & 1492RTACGGTTACCTIGTTACGACTT primers using BDT v3.1 Cycle sequencing kit on ABI 3500Dx Genetic Analyzer(Mahmud et al., 2023).The MEGA6 program measured a phylogenetic tree by applying the neighbour joining method of 1,000 replicates used for bootstrapping (Tamura et al., 2013).

**2.3.5 Evolutionary relationship**

Further, to assess the genetic variation and evolutionary relationship between *P. fluorescens* strain MDR-08 and those in other publicly accessible databases. The phylogenetic analyses was conducted using 16S rRNA sequence with the 10 most closely related strains based on BLAST search similarity (Figure 2).

**2.3.6 Antibiotic susceptibility tests**

According to CLSI, the antibiotic sensitivity patterns of isolates were determined through agar disk diffusion techniques on Muller-Hinton agar plates. A total of 5 commercially available antibiotics, including Azithromycin (15μg), Chloramphenicol (30 μg), Erythromycin (15μg), Gentamicin (10 μg) and Vancomycin (30 μg) were used to analyse the Multi drug resistance capacity. All antibiotic disks were purchased from HiMedia Leading Bio Sciences Company. The antimicrobial sensitivity test was conducted according to the procedure Kirby-Bauer disk diffusion susceptibility test protocol.

 After morphological and biochemical identification, the broth culture of pure isolates was spread on Muller-Hinton agar and sterile forceps were used to place selected antibiotic discs. Subsequently, the Muller-Hinton agar plates were incubated at 37°C ± 2 for 24 hours, and the diameter zone of inhibition was determined using a millimetre scale (Wayne, 2015).

**3. RESULTS**

We got samples of Biomedical wastewater. The total viable counts in dilution 10⁻⁵ for sample 1 were found to be 4.5×10⁹ cfu/mL, and for sample 2, they were 4.8×10⁹ cfu/mL. We collected eight multidrug-resistant (MDR) bacterial strains from two distinct sources. Thestrains were mostly *Pseudomonas* species *(50%), Escherichia coli (25%), and Staphylococcus* species *(25%).* All of these can use TSI, and TSI confirmed the identification of the bacteria (Tables 2 and 3). It was confirmed that the bacteria were what they said they were by using biochemical tests like catalase, MR-VP, indole, and citrate (Tables 2 and 3).

*Pseudomonas fluorescens* was the predominant isolate in our study, accounting for 50% in sample number 1. In this study, most of the isolates were related to *P. fluorescens*, and representative isolates were identified by molecular method. To determine the genetic diversity and evolutionary relationship between *P. fluorescens* strain MDR-08 and with other strains available in NCBI sites; using 16S rRNA with the 10 most close strains based on BLAST search similarity (Table 4).

The most significant isolate in our research was *P.* *fluorescens*, which accounted for 50% of total sample collected. The majority of the isolates in this study were associated with *P. fluorescens*, and representative isolates were identified using molecular methods.

The *P. fluorescens* strain MDR-08 strain from Bhawanipatna, Kalahandi, aligned to sequences previously added to the NCBI GenBank from around the world. MDR-08strain shared a high degree of similarity (98.64%) with *P. fluorescens* strain WCS365, accession number KP253039, followed by (98.57%) *Pseudomonas* species K94.37, and so on, with other strain sequences from all over the world that had previously been deposited in the NCBI GenBank. For the study of prokaryotic taxonomy, the tree derived from 16s rRNA has been used (Figure 2).

The result revealed that MDR-08strain shows multi-drug resistance to all five antibiotics as a prominent multi-drug-resistant bacterium. Rest seven bacteria show no major variations, only MDR-01 resists Chloramphenicol, followed by MDR-02 resists Erythromycin and MDR-05 resists Vancomycin (Table 5).

**Table 1 Percentage of bacterial populations in Biochemical waste**

|  |  |  |  |
| --- | --- | --- | --- |
| BacterialIsolates | Biomedical Waste (S1) | Biomedical Waste (S2) | Percentage (%) |
| *Pseudomonas fluorescens* | 2 | 2 | 4(50%) |
| *E. coli* | 1 | 1 | 2(25%) |
| *Staphylococcus* species | 1 | 1 | 2(25%) |
| Total isolates | 4 | 4 | 8 (100%) |

**Figure 1 Prevalence of bacterial population from Biochemical Waste**

**Table 2 Morphological analysis of isolated bacterial population**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **SL NO.** | **SHAPE** | **SIZE** | **SURFACE** | **COLOUR** | **OPACITY** | **ELEVATION** | **MARGIN** |
| **MDR-01** | CIRCULAR | SMALL | SMOOTH | YELLOW | OPAQUE | CONVEX | EVEN |
| **MDR-02** | CIRCULAR | SMALL | SMOOTH | ORANGE | OPAQUE | FLAT | EVEN |
| **MDR-03** | CIRCULAR | MEDIUM | SMOOTH | CREAM WHITE | OPAQUE | CONVEX | EVEN |
| **MDR-04** | CIRCULAR | MEDIUM | SMOOTH | ORANGE | OPAQUE |  | EVEN |
| **MDR-05** | IRREGULAR | MEDIUM | ROUGH |  | OPAQUE | RAISED |  |
| **MDR-06** | IRREGULAR | LARGE  | SMOOTH | WHITE | OPAQUE |  | WAVY |
| **MDR-07** | CIRCULAR | LARGE | GLISTINING | ORANGE | OPAQUE | CONVEX | EVEN |
| **MDR-08** | CIRCULAR | MEDIUM | GLISTINING | WHITE | OPAQUE | CONVEX | EVEN |

**Table 3Morphological and biochemical analysis of isolated bacterial population**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No.** | **CODE** | **INDOLE** | **MR** | **VP** | **CITRATE** | **TSI** | **CATALASE** | **OXIDASE** | **BIOFILM** |
| **1** | **MDR-01** | \_ | \_ |  | \_ | GLUCOSE | + | \_ | \_ |
| **2** | **MDR-02** | \_ | \_ |  | + | GLUCOSELACTOSESUCROSE | +++ | + | + |
| **3** | **MDR-03** | \_ | + |  | \_ | \_ |  | + | \_ |
| **4** | **MDR-04** | \_ | + |  | \_ | GLUCOSELACTOSESUCROSE | +++ | \_ | \_ |
| **5** | **MDR-05** | \_ | + |  | + | GLUCOSE | ++ | \_ | + |
| **6** | **MDR-06** | \_ | + |  | \_ | GLUCOSE | + | \_ | \_ |
| **7** | **MDR-07** | \_ | + |  | \_ | GLUCOSELACTOSESUCROSE | ++ | \_ | \_ |
| **8** | **MDR-08** | \_ | + |  | \_ | GAS PRODUCTION WITH GLUCOSELACTOSESUCROSE | + | \_ | \_ |

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**Figure 2** Phylogentic tree of*Pseudomonas fluorescens* isolated from Biomedical waste marked in yellow.

**Table 4 Ten most close strains based on BLAST search**

|  |  |  |  |
| --- | --- | --- | --- |
| **SL NO.**  | **DESCRIPTION** | **Accession** | **PER IDENTITY** |
| 1 | [*Pseudomonas* fluorescens strain WCS365 16S ribosomal RNA gene, partial sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_786481768) | [KP253039.1](https://www.ncbi.nlm.nih.gov/nucleotide/KP253039.1?report=genbank&log$=nucltop&blast_rank=2&RID=XSFBRX8M016) | 98.64% |
| 2 | [*Pseudomonas* sp. K94.37 16S ribosomal RNA gene, complete sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_91694082) | [DQ453820.1](https://www.ncbi.nlm.nih.gov/nucleotide/DQ453820.1?report=genbank&log$=nucltop&blast_rank=3&RID=XSFBRX8M016) | 98.57% |
| 3 | [*Pseudomonas* brassicacearum strain Delaware 16S ribosomal RNA gene, partial sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_926574377) | [KT695846.1](https://www.ncbi.nlm.nih.gov/nucleotide/KT695846.1?report=genbank&log$=nucltop&blast_rank=4&RID=XSFBRX8M016) | 98.50% |
| 4 | [*Pseudomonas* sp. B21-010 chromosome, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2289914695) | [CP087198.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP087198.1?report=genbank&log$=nucltop&blast_rank=5&RID=XSFBRX8M016) | 98.50% |
| 5 | [*Pseudomonas* brassicacearum strain L13-6-12 chromosome, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1070525623) | [CP014693.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP014693.1?report=genbank&log$=nucltop&blast_rank=6&RID=XSFBRX8M016) | 98.50% |
| 6 | [*Pseudomonas* brassicacearum strain 37.15 16S ribosomal RNA gene, partial sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2743013808) | [PP907906.1](https://www.ncbi.nlm.nih.gov/nucleotide/PP907906.1?report=genbank&log$=nucltop&blast_rank=7&RID=XSG1MAE7013) | 98.50% |
| 7 | [*Pseudomonas* sp. P9\_32 chromosome, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2624831633) | [CP125374.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP125374.1?report=genbank&log$=nucltop&blast_rank=8&RID=XSG1MAE7013) | 98.50% |
| 8 | [*Pseudomonas* brassicacearum strain LBUM300 chromosome, complete genome](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_955697512) | [CP012680.1](https://www.ncbi.nlm.nih.gov/nucleotide/CP012680.1?report=genbank&log$=nucltop&blast_rank=9&RID=XSG88K6K013) | 98.50% |
| 9 | [*Pseudomonas* sp. K93.2 16S ribosomal RNA gene, complete sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_91694080) | [DQ453818.1](https://www.ncbi.nlm.nih.gov/nucleotide/DQ453818.1?report=genbank&log$=nucltop&blast_rank=10&RID=XSG88K6K013) | 98.50% |
| 10 | [*Pseudomonas* sp. strain RS2040 16S ribosomal RNA gene, partial sequence](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2425865757) | [OQ236299.1](https://www.ncbi.nlm.nih.gov/nucleotide/OQ236299.1?report=genbank&log$=nucltop&blast_rank=11&RID=XSG88K6K013) | 98.50% |

**Table 5 Result of antibiotics susptibilty test of isolated strain**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bacterial strains** | **AZM (IN CM)** | **GEN (IN CM)** | **E(IN CM)** | **VA (IN CM)** | **C (IN CM)** |
| **MDR-01** | **S (1.5)** | **S (2.3)** | **S (1.0)** | **S (1.7)** | **R** |
| **MDR-02** | **S (0.7)** | **S (2.0)** | **R** | **S (1.8)** | **S (0.7)** |
| **MDR-03** | **S (1.7)** | **S (2.1)** | **S (1.4)** | **S (1.6)** | **S (1.6)** |
| **MDR-04** | **S (1.8)** | **S (2.4)** | **S (1)**  | **S (1.6)** | **S (1.9)** |
| **MDR-05** | **S (0.5)** | **S (2.0)** | **S (0.5)** | **R** | **S (1.0)** |
| **MDR-06** | **S (1.4)** | **S (2.2)**  | **S (2.4)** | **S (1.5)**  | **S (1.4)** |
| **MDR-07** | **S (1.4)** | **S (2.2)** | **S (1.3)** | **S (2.2)** | **S (2)** |
| **MDR-08** | **R** | **R** | **R** | **R** | **R** |

**AZM- Azithromycin (15μg),C- Chloramphenicol (30 μg), E- Erythromycin (15μg), GEN- Gentamicin (10 μg) and VA- Vancomycin (30 μg)**

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**Figure 3 Result of antibiotics susceptibility test**

**Figure 4 Antibiotic resistant pattern of MDR**

**4. Discussion**

A variety of bacterial species have been prevalent. A variety of bacterial species are frequently found across diverse environments, such as soil, water, and the human body. The bacteria that are most commonly identified include: *Pseudomonas* species represent opportunistic pathogens present in Biomedical waste, with a particular emphasis on *Pseudomonas* aeruginosa (Park et al., 2009; Mwaikono et al., 2015; Mhamunkar et al., 2025). Staphylococcus species, particularly Staphylococcus aureus, are recognised for their antibiotic resistance and significant involvement in hospital-acquired infections (Park et al., 2009; Nascimento et al., 2015). Escherichia coli is a prevalent contaminant found in hospital liquid waste (Budıartı et al., 2018). Klebsiella species are linked to hospital-acquired infections and can be found in Biomedical waste (Maina et al., 2018; Selim et al., 2023). Hospital waste frequently includes Bacillus species (Maina et al., 2018; Egbenyah et al., 2021). In our investigation, we identified three bacterial species: *Pseudomonas* species, Staphylococcus species, and Escherichia coli. These species were identified through meticulous sampling and thorough analysis of the hospital waste.

our study found that Pseudomonas species accounted for **50%** of the total isolated MDR strains. **Similar Findings** reported Pseudomonas species as the most frequently isolated pathogen (45%) from Biomedical waste in hospital environments. Another study by Mahmud et al., (2023) identified Pseudomonas species in **41.67%** of waste samples, showing its strong ability to persist in contaminated environments. However, there are some **Contrasting** studies have reported lower prevalence rates. For instance, Nikita, (2024) found Pseudomonas species in only **24.66%** of hospital waste samples, with E. coli and Staphylococcus species being more dominant. Further the study of Geta, & Kibret, (2022) also reported that the *Pseudomonas* bacterial species percentage was 17.17% with E. coli and Staphylococcus species being more dominant.

Our study demonstrates that the P. fluorescens strain MDR-08 from Bhawanipatna, Kalahandi, aligns closely with previously deposited sequences in NCBI GenBank, sharing a high degree of similarity with Pseudomonas fluorescens strain WCS365 (98.64%) and Pseudomonas sp. K94.37 (98.57%). In this study, most of the isolates were related to *P. fluorescens*, and representative isolates were identified by molecular method by sequencing 16s rRNA. But the data availablibity is scare. and the isolation of *P. fluorescens* from Biomedical waste is not common , however it is common in soil. *P. fluorescens* encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium. As its name implies, it secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability (Ganeshan & Manoj Kumar, 2005). Certain members of the *P. fluorescens* have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases (Hoffland et al., [1996](https://www.tandfonline.com/doi/full/10.1080/17429140600907043); Wei et al., [1996](https://www.tandfonline.com/doi/full/10.1080/17429140600907043)). To determine the genetic diversity and evolutionary relationship between *P. fluorescens* strain MDR-08 and with other strains available in NCBI sites; using 16S rRNA with the 10 most close strains based on BLAST search similarity Variations in prevalence could be due to differences in sampling locations, waste handling methods, or environmental conditions. Our isolated strain MDR-8 P. fluorescens constituted **50%** of the bacterial isolates from Biomedical waste, while other Pseudomonas species, such as P. aeruginosa, were more prevalent. **And** reported a lower prevalence of P. fluorescens and suggested that environmental conditions, hospital waste disposal practices, and selective pressures from antibiotic use could influence its distribution.

Till date there is no evidence about pathogenicity of P. fluorescens species, but other species of *Pseudomonas* like P. aeruginosa (Roulová et al., 2022), is MDR, suggesting geographical or environmental factors may influence resistance patterns. The high prevalence of Pseudomonas species in Biomedical waste raises concerns due to its role in hospital-acquired infections. Its ability to survive in diverse conditions and resist antibiotics makes it a major threat in waste management and public health. Pseudomonas species are often highly drug resistant, P. aeruginosa is a member of the ESKAPE group (Johansson et al., 2023) and has been classified as a Priority One Pathogen by the World Health Organization. Disinfectants are critical to reducing the microbial burden of surfaces and preventing transmission from contaminated surfaces to patients (Lineback et al., 2018). As well as being able to resist antimicrobial drugs, Pseudomonas species are often able to tolerate high levels of disinfectants (Bakht et al., 2022). Whilst P. aeruginosa is acknowledged as an important nosocomial pathogen, less is known about other Pseudomonas species which occupy the same niches (de Abreu et al., 2026), such as hospital sinks. Non-aeruginosa Pseudomonas species have been increasingly documented as causing a range of clinical infections typically involving vulnerable patients such as neonates (Aumeran et al., 2007; Bouallegue et al., 2004). A substantial proportion of these infections have been linked to healthcare settings and equipment, for example contaminated IV fluids led to 19 cases of P. fulva bacteriaemia in a Chinese hospital (Liu et al., 2014).

**Factors Contributing to High** P. fluorescens **Prevalence in our Study**, P. fluorescens is highly adaptable and can survive in diverse conditions, including Biomedical waste. Resistance to disinfectants and antibiotics enables its persistence in hospital environments. The difference in prevalence rates across studies may be due to: **Geographical variation** in hospital waste management practices. **Sampling techniques** (e.g., solid waste vs. liquid waste). **Selective pressure** from antibiotic works in different regions. **Antibiotic Resistance and Public Health Concern,** Several studies have identified P. fluorescens as an emerging multidrug-resistant (MDR) pathogen in hospital environments. Research by Silverio **et al. (2022)** found that P. fluorescens isolates from Biomedical waste exhibited MDR properties. Its increasing prevalence raises concerns about its role in opportunistic infections, particularly in immune compromised patients. Recently Silverio et al. (2025) offered valuable insight into the evolution of antimicrobial resistance in *P. fluorescens*, particularly in extreme environments, such as Antarctica. *P. fluorescens* is significantly less virulent than *P. aeruginosa* and is a rare cause of invasive hospital-acquired infections, with most common site of infection being the bloodstream (Arega et al., 2017; Shah et al., 2007). It has been isolated in respiratory samples from patients with lung transplants (Dickson et al., 2014), ventilator-associated pneumonia (Bahrani-Mougeot et al., 2007), cystic fibrosis (CF) (Heirali et al., 2016) and rice-field drowning-associated pneumonia (Yamawaki et al., 2016).

Our study revealed that P. fluorescens strain MDR-08 exhibited **multidrug resistance (MDR) to all five tested antibiotics**, making it the most resistant strain among the isolates. The remaining seven bacterial strains showed resistance to only specific antibiotics, with MDR-01 resisting **Chloramphenicol**, MDR-02 resisting **Erythromycin**, and MDR-05 resisting **Vancomycin**. Our finding that P. fluorescens MDR-08 is resistant to multiple antibiotics. The ability of P. fluorescens to form biofilms enhances its survival against antibiotics. The spread of resistance genes through plasmids and integrons may explain why your MDR-08 strain is highly resistant.

**5. Conclusion**

Pseudomonas fluorescens is emerging as a multidrug-resistant (MDR) pathogen, with resistance patterns influenced by geographical factors and antibiotic exposure. The complete resistance of the MDR-08 strain to five antibiotics is particularly concerning, underscoring the urgent need for stricter antibiotic stewardship and improved hospital waste management practices.

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**6. References**

1. Al-Ahmad, A., Daschner, F. D., & Kümmerer, K. (1999). Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. *Archives of environmental contamination and toxicology*, *37*(2), 158-163.
2. Arega, B., Wolde-Amanuel, Y., Adane, K., Belay, E., Abubeker, A., & Asrat, D. (2017). Rare bacterial isolates causing bloodstream infections in Ethiopian patients with cancer. *Infectious Agents and Cancer*, *12*, 1-6.
3. Aumeran, C., Paillard, C., Robin, F., Kanold, J., Baud, O., Bonnet, R., ... & Traore, O. (2007). *Pseudomonas* aeruginosa and *Pseudomonas* putida outbreak associated with contaminated water outlets in an oncohaematology paediatric unit. *Journal of Hospital Infection*, *65*(1), 47-53.
4. Bahrani-Mougeot, F. K., Paster, B. J., Coleman, S., Barbuto, S., Brennan, M. T., Noll, J., ... & Lockhart, P. B. (2007). Molecular analysis of oral and respiratory bacterial species associated with ventilator-associated pneumonia. *Journal of clinical microbiology*, *45*(5), 1588-1593.
5. Bakht, M., Alizadeh, S. A., Rahimi, S., Kazemzadeh Anari, R., Rostamani, M., Javadi, A., ... & Nikkhahi, F. (2022). Phenotype and genetic determination of resistance to common disinfectants among biofilm-producing and non-producing *Pseudomonas* aeruginosa strains from clinical specimens in Iran. *BMC microbiology*, *22*(1), 124.
6. Bouallegue, O., Mzoughi, R., Weill, F. X., Mahdhaoui, N., Salem, Y. B., Sboui, H., ... & Grimont, P. A. D. (2004). Outbreak of *Pseudomonas* putida bacteraemia in a neonatal intensive care unit. *Journal of Hospital Infection*, *57*(1), 88-91.
7. Budıartı, S., Lıngga, R., Rusmana, İ., &Wahyudı, A. T. (2018). Antibiotics resistant Escherichia coli from hospital liquid waste. *Journal of Applied Biological Sciences*, *12*(1), 36-40.
8. de Abreu, P. M., Farias, P. G., Paiva, G. S., Almeida, A. M., & Morais, P. V. (2014). Persistence of microbial communities including *Pseudomonas* aeruginosa in a hospital environment: a potential health hazard. *BMC microbiology*, *14*, 1-10.
9. Dickson, R. P., Erb-Downward, J. R., Prescott, H. C., Martinez, F. J., Curtis, J. L., Lama, V. N., & Huffnagle, G. B. (2014). Cell-associated bacteria in the human lung microbiome. *Microbiome*, *2*, 1-10.
10. Egbenyah, F., Udofia, E. A., Ayivor, J., Osei, M. M., Tetteh, J., Tetteh-Quarcoo, P. B., & Sampane-Donkor, E. (2021). Disposal habits and microbial load of solid medical waste in sub-district healthcare facilities and households in Yilo-Krobo municipality, Ghana. *Plos one*, *16*(12), e0261211.
11. Ganeshan, G., & Manoj Kumar, A. (2005). *Pseudomonas* fluorescens, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions*, *1*(3), 123-134.
12. Geta, K., & Kibret, M. (2022). Antibiotic resistance profiles of Bacteria isolated from hotspot environments in Bahir Dar City, northwestern Ethiopia. *Journal of Multidisciplinary Healthcare*, 1403-1414.
13. Heirali, A., McKeon, S., Purighalla, S., Storey, D. G., Rossi, L., Costilhes, G., ... & Parkins, M. D. (2016). Assessment of the microbial constituents of the home environment of individuals with cystic fibrosis (CF) and their association with lower airways infections. *PLoS One*, *11*(2), e0148534.
14. Hoffland, E., Hakulinen, J., & Van Pelt, J. A. (1996). Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. *Phytopathology*, *86*(7), 757-762.
15. Iversen, A., Kühn, I., Franklin, A., & Möllby, R. (2002). High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology*, *68*(6), 2838-2842.
16. Johansson, M. H., Aarestrup, F. M., & Petersen, T. N. (2023). Importance of mobile genetic elements for dissemination of antimicrobial resistance in metagenomic sewage samples across the world. *PLoS One*, *18*(10), e0293169.
17. Kianpour, S., Ebrahiminezhad, A., Mohkam, M., Tamaddon, A. M., Dehshahri, A., Heidari, R., & Ghasemi, Y. (2017). Physicochemical and biological characteristics of the nanostructured polysaccharide‐iron hydrogel produced by microorganism Klebsiella oxytoca. *Journal of basic microbiology*, *57*(2), 132-140.
18. Krishnamoorthy, M., & Arjun, P. (2012). Probiotic and antimicrobial activity of bacteria from fermented toddy of Cocus nucifera. *J Acad Indus Res*, *1*(3), 127-131.
19. Lineback, C. B., Nkemngong, C. A., Wu, S. T., Li, X., Teska, P. J., & Oliver, H. F. (2018). Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and *Pseudomonas* aeruginosa biofilms than quaternary ammonium compounds. *Antimicrobial Resistance & Infection Control*, *7*, 1-7.
20. Liu, Y., Liu, K., Yu, X., Li, B., & Cao, B. (2014). Identification and control of a *Pseudomonas* spp (P. fulva and P. putida) bloodstream infection outbreak in a teaching hospital in Beijing, China. *International Journal of Infectious Diseases*, *23*, 105-108.
21. Mahmud, M. S., Hosen, M. A., Hossion, M. I., SadikSabuj, M. S., Rumi, N. A., Hossain, M. K., ... &Bourhia, M. (2023). Isolation, identification, and characterization of resistant bacteria to antibiotics from pharmaceutical effluent and study of their antibiotic resistance. *Frontiers in Microbiology*, *14*, 1307291.
22. Maina, S. M., Nyerere, A. K., &Ngugi, C. W. (2018). Isolation of bacterial diversity present in medical waste and health care settings in hospitals in Kenya. *African Journal of Microbiology Research*, *12*(26), 606-615.
23. Mhamunkar, N. N., Kadam, J. H., &Patil, S. (2025). Emergence of Antibiotic-Resistant Isolates Due to Hospital Waste. In *Hospital Waste Management and Toxicity Evaluation* (pp. 135-162). IGI Global Scientific Publishing.
24. Minhas B, Bali P and Minhas N (2024): Isolation and characterization of multi-drug resistant (MDR) and extensively drug resistant (XDR) bacteria from diverse environmental niches. Int J Pharm Sci & Res; 15(2): 398-08. doi: 10.13040/IJPSR.0975-8232.15(2).398-08.
25. Moges, F., Endris, M., Belyhun, Y., & Worku, W. (2014). Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. *BMC research notes*, *7*, 1-6.
26. Mwaikono, K. S., Maina, S., Sebastian, A., Kapur, V., &Gwakisa, P. (2015). 16S rRNA amplicons survey revealed unprecedented bacterial community in solid Biomedical wastes.
27. Nascimento, T. C., da Silva, V. L., Ferreira-Machado, A. B., &Diniz, C. G. (2015). Potential spread of multidrug-resistant coagulase-negative staphylococci through healthcare waste. *The Journal of Infection in Developing Countries*, *9*(01), 029-034.
28. Nikita, Sain ML, Goklaney D, Vaishali CD. The study of bacterial flora and their antibiogram pattern isolated from Biomedical waste. International Journal of Veterinary Sciences and Animal Husbandry. 2024; SP-9(3): 97-101.
29. Park, H., Lee, K., Kim, M., Lee, J., Seong, S. Y., &Ko, G. (2009). Detection and hazard assessment of pathogenic microorganisms in medical wastes. *Journal of Environmental Science and Health Part A*, *44*(10), 995-1003.
30. PPCP, C. (2003). High on pollution: drugs as environmental contaminants. *J. Environ. Monit*, *5*.
31. Roulová, N., Mot’ková, P., Brožková, I., & Pejchalová, M. (2022). Antibiotic resistance of *Pseudomonas* aeruginosa isolated from hospital wastewater in the Czech Republic. *Journal of Water and Health*, *20*(4), 692-701.
32. Selim, S. B., Tabassum, N., &Ananna, J. N. (2023). *Isolation and Characterization of Klebsiellapneumoniae from medical waste and adjoining community water* (Doctoral dissertation, Brac University).
33. Shah, S. S., Kagen, J., Lautenbach, E., Bilker, W. B., Matro, J., Dominguez, T. E., ... & Bell, L. M. (2007). Bloodstream infections after median sternotomy at a children’s hospital. *The Journal of thoracic and cardiovascular surgery*, *133*(2), 435-440.
34. Silverio, M. P., Kraychete, G. B., Rosado, A. S., & Bonelli, R. R. (2022). *Pseudomonas* fluorescens complex and its intrinsic, adaptive, and acquired antimicrobial resistance mechanisms in pristine and human-impacted sites. *Antibiotics*, *11*(8), 985.
35. Silverio, M. P., Schultz, J., Parise, M. T., Parise, D., Viana, M. V. C., Nogueira, W., ... & Rosado, A. S. (2025). Genomic and phenotypic insight into antimicrobial resistance of *Pseudomonas* fluorescens from King George Island, Antarctica. *Frontiers in Microbiology*, *16*, 1535420.
36. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. doi: 10.1093/molbev/mst197
37. Wayne, P. A. (2015). Clinical and laboratory standards institute (CLSI). *Performance standards for antimicrobial susceptibility testing*.
38. Wei, G., Kloepper, J. W., & Tuzun, S. (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions.
39. Yamawaki, S., Nakashima, K., Suzuki, F., Otsuki, A., Watanabe, J., Takai, M., ... & Aoshima, M. (2016). Rice-field drowning-associated pneumonia in which *Pseudomonas* species, Aspergillus fumigatus, and Cunninghamella sp. are isolated. *Internal medicine*, *55*(7), 825-829.
40. Yang, C. M., Lin, M. F., Liao, P. C., Yeh, H. W., Chang, B. V., Tang, T. K., ... & Liou, M. L. (2009). Comparison of antimicrobial resistance patterns between clinical and sewage isolates in a regional hospital in Taiwan. *Letters in applied microbiology*, *48*(5), 560-565.