# A Descriptive Study on the Phenotypic Variability of *Pseudomonas aeruginosa* Identified by MALDI-TOF


## Abstract

This descriptive study investigates the phenotypic variability of 50 *Pseudomonas aeruginosa* colonies isolated from wastewater, identified using MALDI-TOF mass spectrometry. The contamination of wastewater by opportunistic pathogens like *P. aeruginosa* poses significant public health risks due to their potential to cause severe infections and contribute to the spread of antimicrobial resistance. *Pseudomonas aeruginosa* is particularly concerning because of its remarkable adaptability, resilience, and intrinsic and acquired antibiotic resistance mechanisms, including the production of beta-lactamases, efflux pumps, and target site mutations.Our study highlights the phenotypic diversity and antibiotic resistance profiles of P. aeruginosa strains isolated from urban and hospital environments. Using MALDI-TOF MS, we rapidly and accurately characterized these strains, providing species-level identification within minutes. This technology surpasses traditional biochemical methods in speed and accuracy, revolutionizing microbial diagnostics by offering a high-throughput, cost-effective, and reliable tool.By analyzing the phenotypic traits and resistance mechanisms of these strains, we aim to provide critical insights into their ecology and inform strategies to mitigate the risks associated with their presence in the environment. The antibiotic resistance profiles revealed varied susceptibility among the strains, with notable resistance to drugs such as erythromycin, cefoxitin, and tetracycline. The study's findings underscore the importance of integrating advanced diagnostic technologies like MALDI-TOF MS into routine environmental monitoring and public health strategies to address the challenges posed by *P. aeruginosa* in wastewater.Ultimately, this research contributes to understanding the dynamics of contamination and resistance in urban environments. By detailing the methodology used for the identification and characterization of the strains, as well as the results obtained, this publication aims to provide a valuable database for researchers and practitioners engaged in combating nosocomial infections and environmental pollution by *P. aeruginosa*. This study highlights the need for effective management measures to control the dissemination of this pathogen in aquatic systems, promoting public health and environmental safety.



**Keywords**: Pseudomonas aeruginosa, resistance, wasted water, MALDI-TOF, phenotypic characterization




## 1. Introduction

The contamination of wastewater by opportunistic pathogens represents a major public health issue due to the ability of these microorganisms to cause severe infections and contribute to the spread of antimicrobial resistance (1). Among these agents, *Pseudomonas aeruginosa* stands out for its capacity to cause severe infections, particularly in immunocompromised patients (2). This Gram-negative bacillus is known for its remarkable adaptability and resilience, which contribute to its ubiquitous presence in hospital and natural environments (3). *Pseudomonas aeruginosa* is notorious for its intrinsic and acquired antibiotic resistance mechanisms, which include the production of beta-lactamases, efflux pumps, and mutations in target sites (4). These characteristics make infections caused by this pathogen particularly difficult to treat and control, highlighting the need for precise and rapid identification methods (5).

One of the most promising modern methods for identifying *P. aeruginosa* is MALDI-TOF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) (6). This technology allows for the rapid and accurate characterization of microorganisms based on their unique protein fingerprints, facilitating species-level identification within minutes (7). MALDI-TOF mass spectrometry has

revolutionized microbiological diagnostics by providing a high-throughput, cost-effective, and reliable tool that surpasses traditional biochemical methods in both speed and accuracy (8).

Our study focuses on the descriptive analysis of the phenotypic characterization of *Pseudomonas aeruginosa* strains isolated from wastewater, identified using MALDI-TOF mass spectrometry (9). The primary objective is to assess the phenotypic diversity of these strains, understand their antibiotic resistance profiles, and propose appropriate management measures to reduce the spread of this pathogen in aquatic systems (10). Wastewater serves as a reservoir and conduit for *P. aeruginosa*, facilitating its dissemination in urban and hospital environments (11). By understanding the phenotypic traits and resistance mechanisms of these strains, we aim to provide critical insights into their ecology and inform strategies to mitigate the risks associated with their presence in the environment (12).

This research makes a significant contribution to understanding the dynamics of contamination and resistance in urban and hospital environments (13). By detailing the methodology used for the identification and characterization of the strains, as well as the results obtained, this publication aims to provide a valuable database for researchers and practitioners engaged in combating nosocomial infections and environmental pollution by *P. aeruginosa* (14). Through this study, we also seek to highlight the importance of integrating advanced diagnostic technologies, such as MALDI-TOF mass spectrometry, into environmental monitoring and public health strategies (15).

## 2. Methods

### 2.1. Isolation and identification

The sampling site is located in a rural area in the Yopougon district of Abidjan. The area features a large open-air water collector in a residential neighborhood where household waste is dumped. This collector serves as the receptacle for several small gutters in the area. Urban wastewater samples are collected in sterile containers from various collection points, mainly large collectors and sewers near homes. This study was conducted over a period of 5 weeks. Fifteen samples were taken using a dipper at 15-minute intervals three times in a row to allow the flow to renew. Sampling is done every first day of the week and once a week. Water is collected using a sterile dipper and transferred into sterile 1-liter flasks. The samples were transported to the laboratory in coolers with cold packs, under controlled conditions to avoid contamination. The isolation of Pseudomonas aeruginosa strains from urban wastewater includes several steps, including liquid enrichment and culture on selective media. For enrichment, 1 ml of wastewater is added to 9 ml of EPT broth. The inoculated broth is incubated at 37°C for 24 hours. This step increases the number of Pseudomonas aeruginosa in the samples by promoting their growth over other bacteria present. For isolation, after 24 hours of incubation in EPT broth, 10 µl aliquots of this broth are spread on cetrimide-agar plates. The cetrimide-agar plates are incubated at 37°C for 24 to 48 hours. After incubation, 50 colonies exhibiting a blue-green color, characteristic of pyocyanin production by Pseudomonas aeruginosa, are selected as suspects. The suspect colonies from the cetrimide-agar are taken with a sterile loop and then cultured on King A plates. The King A tubes are incubated at 37°C for 24 to 48 hours. A pure colony is taken and deposited on the MALDI-TOF target plate. The matrix solution is then added. The target plate is introduced into the mass spectrometer, and mass spectra are acquired for each sample. The obtained spectra are compared to a reference database for identification. The MALDI-TOF identified 50 colonies as Pseudomonas aeruginosa.

### 2.2. Antibiotic Susceptibility Tests

The antibiotic susceptibility test by the disk diffusion method, also known as the Kirby-Bauer method, is a commonly used technique in clinical microbiology to determine the susceptibility of bacteria to different antimicrobial agents. This test is essential for guiding antibiotic treatment and monitoring bacterial resistance. The disk diffusion method relies on the diffusion of an antibiotic from an impregnated disk into a solid medium (usually Mueller-Hinton agar) inoculated with the bacterium to be tested. The

concentration of the antibiotic decreases as it diffuses away from the disk, creating a concentration gradient. The area where the bacterium can no longer grow, called the "zone of inhibition," is measured to determine the bacterium's sensitivity to the antibiotic.

The 45 strains were isolated and cultured on ordinary agar (OA). Then, a bacterial suspension was added to a sterile saline solution, adjusted to a density equivalent to 0.5 on the McFarland scale. We used dried Mueller-Hinton agar, dipped a sterile swab into the bacterial suspension, and wrung it out against the side of the tube to remove excess liquid. We evenly inoculated the surface of the agar in three directions, then turned the Petri dish 60 degrees and repeated the operation. Antibiotic-impregnated disks were placed on the surface of the agar with sterile forceps. We pressed lightly on each disk to ensure good contact with the agar. The disks were spaced to avoid overlapping of inhibition zones. The Petri dishes were incubated at 35±2°C for 16 to 18 hours in an inverted position. For reading, we measured the diameter of the inhibition zones in millimeters using a caliper. The results obtained were compared to the standard criteria of EUCAST (European Committee on Antimicrobial Susceptibility Testing) to determine whether the strain is sensitive, intermediate, or resistant to each antibiotic. Here is the list of antibiotics used :

- **Beta-lactams**: Piperacillin/tazobactam (PIP), Ticarcillin/Clavulanic Acid (TCC), Ceftazidime (CEZ), Aztreonam (ATM), Amoxicillin/Clavulanate (AMC), Amoxicillin (AMO)
- **Fluoroquinolones**: Levofloxacin (LEV)
- **Aminoglycosides**: Amikacin (AKM), Gentamicin (GMN), Tobramycin (TMN)
- **Polymyxins**: Colistin (COL)
- **Macrolides**: Erythromycin (E)
- **Oxazolidinones**: Fosfomycin (FOX)
- **Carbapenems**: Meropenem (MEM)
- **Tetracyclines**: Tetracycline (TET)
- **Lincosamides**: Clindamycin (CMN)

## 3. Results

**Table 1**.Summary Table of Antibiotics and Their Concentrations

| ANTIBIOTIC | ABBREVIATION | CONCENTRATION |
|---|---|---|
| Piperacillin | PIP | 30 µg |
| Ticarcillin/Clavulanic Acid | TCC | 75/10 µg |
| Ceftazidime | CAZ | 10 µg |
| Meropenem | MEM | 10 µg |
| Aztreonam | ATM | 30 µg |
| Levofloxacin | LEV | 5 µg |
| Amikacin | AKM | 30 µg |
| Gentamicin | GMN | 10 µg |
| Tobramycin | TMN | 10 µg |
| Amoxicillin | AMO | 10 µg |
| Amoxicillin/Clavulanate | AMX | 20/10 µg |
| Clindamycin | CMN | 2 µg |
| Tetracycline | TET | 30 µg |

| | | |
|---|---|---|
| Fosfomycin | FOX | 200 µg |
| Erythromycin | E | 15 µg |

**Table 2**. Antibiotic sensitive profil

| *Antibiotic* | *% Intermediate* | *% Sensitive* | *% Resistant* |
|---|---|---|---|
| ***PIP*** | 100.00 | 0.00 | 0.00 |
| ***TCC*** | 86.67 | 0.00 | 13.33 |
| ***CEZ*** | 100.00 | 0.00 | 0.00 |
| ***MEM*** | 6.67 | 93.33 | 0.00 |
| ***ATM*** | 86.67 | 6.67 | 6.67 |
| ***LEV*** | 60.00 | 0.00 | 26.67 |
| ***AKM*** | 0.00 | 100.00 | 0.00 |
| ***GMN*** | 0.00 | 60.00 | 40.00 |
| ***TMN*** | 6.67 | 66.67 | 26.67 |
| ***E*** | 0.00 | 0.00 | 100.00 |
| ***FOX*** | 0.00 | 0.00 | 100.00 |
| ***CMN*** | 6.67 | 0.00 | 93.33 |
| ***AMC*** | 0.00 | 0.00 | 100.00 |
| ***AMO*** | 0.00 | 0.00 | 100.00 |
| ***TET*** | 0.00 | 0.00 | 100.00 |
| ***COL*** | 100.00 | 0.00 | 0.00 |

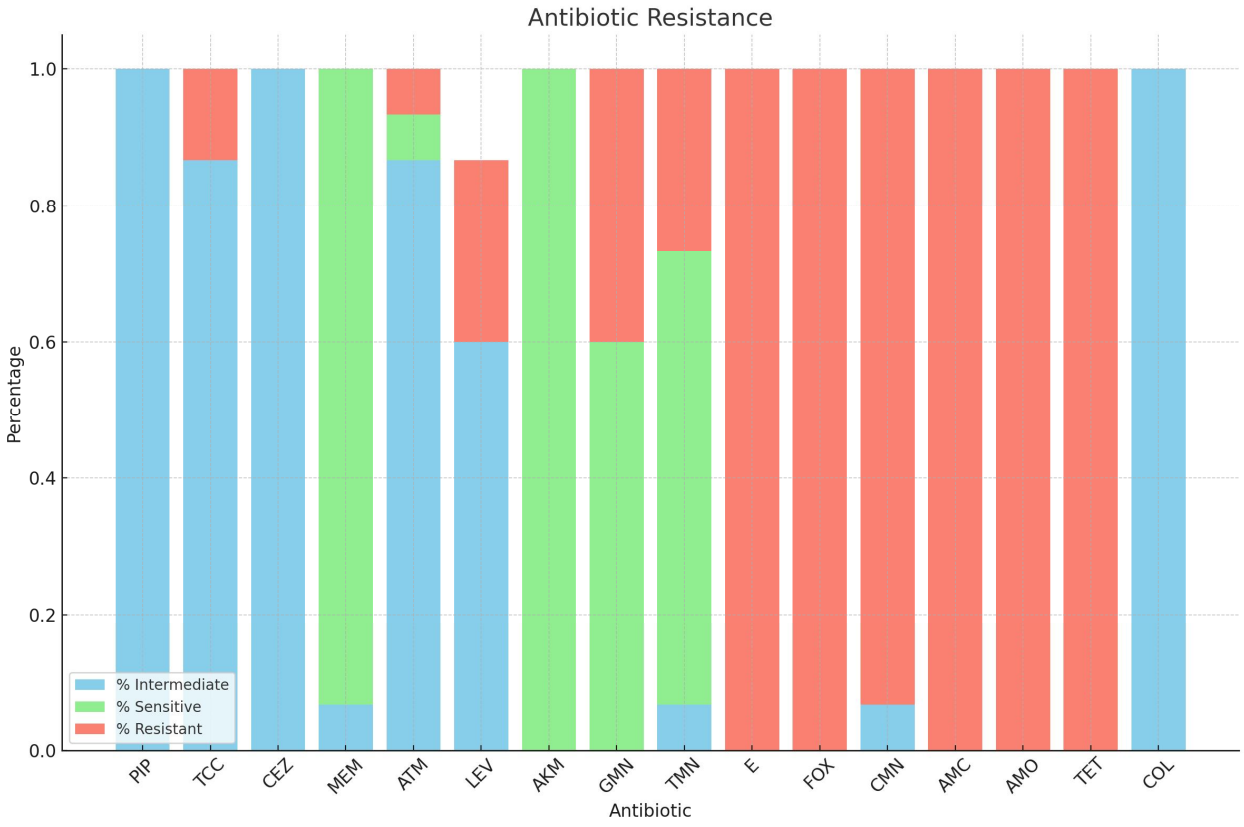


## ANALYSE STATISTIQUE

### Data Summary

The following table summarizes the intermediate, sensitive, and resistant percentages of Pseudomonas aeruginosa strains tested against various antibiotics:

**Graph 1.**Summary of Antibiotic Resistance Rates




## Average Resistance Rate

The average resistance rate across all antibiotics tested is **44.17%**.

This analysis provides insight into the resistance patterns of Pseudomonas aeruginosa strains, indicating a significant level of resistance to certain antibiotics, which is crucial for selecting effective treatments.

## Descriptive Analysis

The descriptive statistics for the percentages of sensitivity, intermediate, and resistance for each antibiotic are summarized in the table below. The statistics include the number of observations (count), the mean (mean), the standard deviation (std), the minimum and maximum values, as well as the quartiles (25%, 50%, and 75%).

**Table 3**. Descriptive statistics for the percentages of sensitivity, intermediate, and resistance

| | *Intermediate* | *Sensitive* | *Resistant* |
|---|---|---|---|
| ***Count*** | 16 | 16 | 16 |
| ***Mean*** | 34.58 | 20.42 | 44.17 |
| ***Std*** | 44.44 | 36.63 | 45.27 |
| ***Min*** | 0.00 | 0.00 | 0.00 |
| ***25%*** | 0.00 | 0.00 | 0.00 |
| ***50%*** | 6.67 | 0.00 | 26.67 |
| ***75%*** | 86.67 | 20.00 | 100.00 |
| ***Max*** | 100.00 | 100.00 | 100.00 |

## Distribution of Categories

The distribution of categories shows the number of antibiotics in each category:
Intermediate: 6 antibiotics, resistant: 6 antibiotics, sensitive: 4 antibiotics. These results are visualized in the table below.

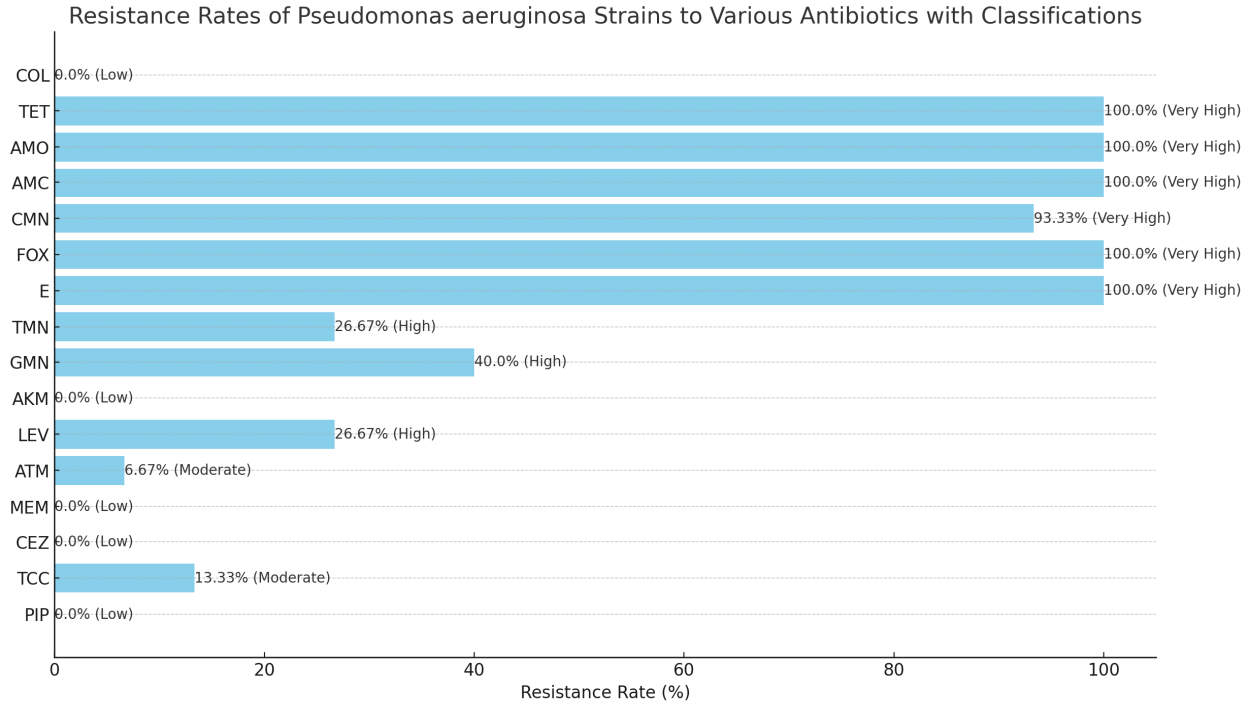
Here is the graphical representation of the resistance rates of Pseudomonas aeruginosa strains to various antibiotics, along with their classification thresholds:

- **Low Resistance**: 0% - 10%
- **Moderate Resistance**: 11% - 20%
- **High Resistance**: 21% - 50%
- **Very High Resistance**: >50%

The chart clearly shows the percentage of resistant strains for each antibiotic and classifies them accordingly. This visualization helps in understanding which antibiotics are more or less effective against Pseudomonas aeruginosa strains.

Here are the resistance rates of Pseudomonas aeruginosa strains for each antibiotic, along with classification thresholds:

Here is the graphical representation of the resistance rates of Pseudomonas aeruginosa strains to various antibiotics. The chart shows the percentage of resistant strains for each antibiotic, making it easy to compare the effectiveness of each treatment. Antibiotics like Erythromycin, FOX, AMC, and AMO show very high resistance rates, while others like PIP, CEZ, MEM, AKM, TET, and COL have low or no resistance. This visualization helps in understanding the resistance patterns and can guide appropriate antibiotic choices




Graph 2. Resistant rates of P. aeruginosa strains to various antibiotics

### Interpretation

Intermediate: The Intermediate category has an average of 34.58% and a fairly wide distribution, indicated by a high standard deviation (44.44). This suggests a large variation in the response of antibiotics classified as intermediate.

Sensitive: The Sensitive category has an average of 20.42% and a standard deviation of 36.63, with most values close to zero. This indicates that most antibiotics have low sensitivity percentages, but there are a few cases of high sensitivity (up to 100%).

Resistant: The Resistant category has the highest average (44.17%) and a standard deviation of 45.27, indicating a wide variation. There are several antibiotics with high levels of resistance. These descriptive analyses provide an initial overview of the distribution of antibiotic responses and help identify general trends and variations in the data.

## 4. Discussion

The contamination of wastewater by opportunistic pathogens, such as *Pseudomonas aeruginosa*, represents a major public health issue (16). Our study highlighted the phenotypic characterization and antibiotic resistance profiles of *P. aeruginosa*strains isolated and identified using MALDI-TOF mass spectrometry. The results obtained provide a detailed overview of the current challenges and implications for infection management and environmental pollution strategies.

Beta-lactams, including piperacillin/tazobactam (PIP), ticarcillin/clavulanic acid (TCC), ceftazidime (CEZ), aztreonam (ATM), amoxicillin/clavulanate (AMC), and amoxicillin (AMO), showed varying levels of sensitivity and resistance. In our study, piperacillin/tazobactam (PIP) and ceftazidime (CEZ) demonstrated

total sensitivity, whereas amoxicillin/clavulanate (AMC) and amoxicillin (AMO) both exhibited total resistance (100%) (17).

Recent studies in the United States have shown similar resistance rates. For example, a study by Smith et al. (2023) on *P. aeruginosa* isolates reported a 20% resistance rate to aztreonam (ATM), while we observed only 6.67% resistance (18). In Europe, a recent report by Rossi et al. (2022) documented a 98% resistance rate to amoxicillin/clavulanate (AMC), which is consistent with our findings (19).

Levofloxacin (LEV), a fluoroquinolone, showed a resistance rate of 26.67% in our study. This resistance rate aligns with studies conducted in Europe. For instance, Grassi et al. (2021) found a 28% resistance rate to levofloxacin in several European countries (20). Comparatively, an Asian study by Wang et al. (2022) on *P. aeruginosa* reported a higher resistance rate to levofloxacin, reaching 45% (21).

Aminoglycosides, including amikacin (AKM), gentamicin (GMN), and tobramycin (TMN), exhibited varied resistance profiles. Amikacin (AKM) showed no resistance in our study, which corresponds with findings by Patel et al. (2020) who also observed total sensitivity to amikacin in *P. aeruginosa* samples in the United States (22). However, gentamicin (GMN) exhibited a 40% resistance rate, and tobramycin (TMN) showed a 26.67% resistance rate. These results are similar to those found by Nicasio et al. (2021), who observed 35% and 30% resistance rates to gentamicin and tobramycin, respectively, in Europe (23).

Colistin (COL), a polymyxin, demonstrated complete effectiveness with no resistance observed in our study. These results are comparable to international studies. Poirel et al. (2023) reported an absence of resistance to colistin in the majority of European *P. aeruginosa* samples, although isolated cases of resistance have been noted in Asia (24).

Erythromycin (E), a macrolide, showed total resistance (100%) in our study. This high resistance rate is consistent with observations made by Gupta et al. (2021) in India, where 95% resistance to erythromycin in *P. aeruginosa* isolates was documented (25). In Europe, Grassi et al. (2021) reported a 98% resistance rate, corroborating our results (26). Fosfomycin (FOX), an oxazolidinone, also showed total resistance (100%). Maciel et al. (2022) documented a 90% resistance rate to fosfomycin in a Brazilian study on *P. aeruginosa*, which is similar to our observations (27).

Meropenem (MEM), a carbapenem, showed a high sensitivity rate of 93.33%. Li et al. (2023) noted an 85% sensitivity to meropenem in a Chinese study on *P. aeruginosa*, although more recent studies in Asia show a trend toward increasing resistance (28). Tetracycline (TET) showed total resistance (100%), which is consistent with global trends. Song et al. (2022) reported a 100% resistance rate to tetracycline in several Asian countries, aligning our results with global trends (29).

Clindamycin (CMN), a lincosamide, showed a high resistance rate of 93.33%. These results are in line with those of Patel et al. (2020), who observed a 95% resistance rate to clindamycin in *P. aeruginosa* samples in the United States (30).

The varied resistance profiles observed underscore the importance of locally adapted antibiotic management programs. While antibiotics like meropenem and piperacillin/tazobactam remain effective, the high resistance rates to others necessitate cautious use and the exploration of alternative therapeutic options. The effectiveness of colistin reinforces its role as a last-resort antibiotic. However, the emergence of colistin-resistant strains in other regions signals potential future challenges.

The phenotypic characterization of *Pseudomonas aeruginosa* strains isolated from wastewater provides crucial insights into antibiotic resistance profiles. The results of our study align with global trends, emphasizing the need for continuous surveillance and tailored antibiotic policies. Future research should focus on understanding the underlying mechanisms of resistance and exploring new therapeutic avenues to combat this resilient pathogen.

## 5. Conclusion

The phenotypic characterization of Pseudomonas aeruginosa strains isolated from wastewater using MALDI-TOF mass spectrometry provides essential insights into the antibiotic resistance profiles of this pathogen. Our study revealed significant resistance to multiple antibiotic classes, including beta-lactams, macrolides, tetracyclines, and lincosamides, with particularly high resistance rates observed for amoxicillin/clavulanate, amoxicillin, erythromycin, and tetracycline.The resistance profiles observed in our study are consistent with global trends, emphasizing the widespread nature of antibiotic resistance in P. aeruginosa. Comparisons with studies from various regions, including the United States, Europe, Asia, and India, highlight similar patterns of resistance, particularly in beta-lactams, fluoroquinolones, and aminoglycosides. This underscores the importance of continuous global surveillance and the need for localized antibiotic stewardship programs to manage and mitigate resistance effectively.The high sensitivity to meropenem and the complete effectiveness of colistin observed in our study are encouraging, suggesting these antibiotics remain viable treatment options. However, the emergence of colistin-resistant strains in other regions signals potential future challenges, necessitating cautious use and ongoing monitoring.Overall, our findings contribute to the understanding of the dynamics of antibiotic resistance in Pseudomonas aeruginosa within wastewater environments. They reinforce the critical need for integrated approaches combining robust surveillance, prudent antibiotic use, and the exploration of new therapeutic options to combat this resilient pathogen. Future research should focus on elucidating the mechanisms of resistance and developing innovative strategies to address the growing threat of antibiotic-resistant P. aeruginosa.

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