

Article

Epidemiology of Multidrug-Resistant Community-Associated *Pseudomonas aeruginosa* Isolated from Urban Diagnostic Laboratories in Southern Morocco

Hafsa Mguild ^{1,2,†}, Idrissa Diawara ^{1,3,4,*,†} , Ihssane Benzaarate ^{2,5} , Asma Er-Regragui ^{1,4}, Amine Aiddi ^{2,6} , Aoubakr Khazaz ^{2,5} , Siham El Machrafi ⁶, Malak Snoussi ^{1,7,8} , Abdelfattah Chakib ^{1,7,8}, Nouzha Dini ^{1,7,8} and Kaotar Nayme ²

- ¹ Research Laboratory of Microbiology, Infectious Diseases, Allergology and Pathogen Surveillance (LARMIAS), Mohammed VI Faculty of Medicine, Mohammed VI University of Sciences and Health (UM6SS), Casablanca 82403, Morocco; hmguild@um6ss.ma (H.M.)
 - ² Molecular Bacteriology Laboratory, Institut Pasteur du Maroc, Casablanca 20360, Morocco; kaotar.nayme@pasteur.ma (K.N.)
 - ³ Mohammed VI Higher Institute of Biosciences and Biotechnologies, Mohammed VI University of Sciences and Health (UM6SS), Casablanca 82403, Morocco
 - ⁴ Mohammed VI Center for Research and Innovation (CM6RI), Rabat 10112, Morocco
 - ⁵ Microbiology and Antimicrobial Agents Research Team, LB2VE, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida 24000, Morocco
 - ⁶ Microbiology Unit, Laboratory of Ecology and Environment, Department of Biology, Faculty of Sciences Ben M'sik, Hassan II University, Casablanca 20670, Morocco
 - ⁷ Cheikh Khalifa International University Hospital, Mohammed VI Faculty of Medicine, Mohammed VI University of Sciences and Health (UM6SS), Casablanca 82403, Morocco
 - ⁸ Mohammed VI International University Hospital, Mohammed VI Faculty of Medicine, Mohammed VI University of Sciences and Health (UM6SS), Bouskoura 27182, Morocco
- * Correspondence: idiawara@um6ss.ma
† These authors contributed equally to this work.

Abstract

Pseudomonas aeruginosa is an important opportunistic pathogen increasingly associated with multidrug resistance (MDR) and therapeutic failure. Data on MDR community-associated *P. aeruginosa* in southern Morocco remain limited. This study evaluated the contribution of urban diagnostic laboratories to the surveillance of MDR *P. aeruginosa* in this underrepresented region. A retrospective multicenter study was conducted between January and December 2024 using data collected from urban medical diagnostic laboratories in southern Morocco. Clinical isolates of *P. aeruginosa* recovered from community-associated patients were identified using standard microbiological methods, and antimicrobial susceptibility testing was performed according to EUCAST guidelines. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes according to the international criteria. Associations between MDR status and demographic variables were assessed using Chi-square and Fisher's exact tests, with statistical significance set at $p < 0.05$. Among the 49 *P. aeruginosa* isolates included, 29 (59.2%, 95% CI: 45.4–72.0%) were classified as MDR. High resistance rates were observed for several β -lactams, whereas most isolates remained susceptible to amikacin and selected β -lactam/ β -lactamase inhibitor combinations. No statistically significant association was found between MDR status and demographic variables. These findings highlight the circulation of MDR *P. aeruginosa* in southern Morocco and suggest that urban diagnostic laboratories may provide valuable complementary data for community-level antimicrobial resistance surveillance.



Academic Editor: Niels
Frimodt-Møller

Received: 12 May 2026

Revised: 18 June 2026

Accepted: 28 June 2026

Published: 14 July 2026

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Keywords: *Pseudomonas aeruginosa*; multidrug resistance; antimicrobial resistance surveillance; community-associated infections; southern Morocco

1. Introduction

Antimicrobial resistance (AMR) is recognized as one of the major global public health threats, compromising the effectiveness of antimicrobial therapies and contributing to increased morbidity, mortality, and healthcare costs [1,2]. Among the Gram-negative pathogens, *P. aeruginosa* is of particular concern because of its intrinsic resistance mechanisms, remarkable adaptive capacity, and ability to acquire additional resistance determinants, leading to multidrug-resistant (MDR) phenotypes [3,4]. MDR *P. aeruginosa* is increasingly associated with therapeutic failure and severe infections involving the urinary tract, respiratory tract, skin and soft tissues, and bloodstream [5].

Although *P. aeruginosa* is traditionally considered a healthcare-associated pathogen, its emergence in community settings has been increasingly reported worldwide [6]. In low- and middle-income countries, the spread of MDR organisms is further promoted by inappropriate antibiotic use, limited microbiological diagnostic capacity, and insufficient antimicrobial stewardship programs [7]. In Morocco, national AMR surveillance is coordinated through the Direction de l'Épidémiologie et de Lutte contre les Maladies (DELM) and contributes to the World Health Organization Global Antimicrobial Resistance Surveillance System (GLASS) [8,9]. However, available epidemiological data remain largely hospital-centered and mainly originate from northern and central regions of the country, whereas southern Morocco remains poorly documented [10].

Furthermore, most Moroccan studies have focused on hospital-associated isolates, while data describing community-associated *P. aeruginosa* and its resistance patterns remain scarce. Consequently, the epidemiology of MDR *P. aeruginosa* circulating outside hospital settings in southern Morocco remains largely unknown.

Southern Morocco is characterized by vast geographic distances, a lower population density than northern regions, and disparities in access to specialized healthcare services [11,12]. In this context, diagnostic activities are concentrated in major urban centers such as Laayoune, Dakhla, and Guelmim, which function as referral hubs for surrounding populations [11,12]. These geographic and healthcare disparities may affect access to microbiological testing and contribute to gaps in regional AMR surveillance coverage.

Ambulatory diagnostic laboratories have increasingly been recognized as complementary sources of AMR surveillance data because they capture isolates from patients seeking care outside hospital settings. Although they cannot fully replace structured surveillance systems, they may provide valuable information regarding resistance trends circulating in the community [11]. These laboratories therefore represent a potentially important but underutilized source of AMR surveillance data, particularly for the early detection of emerging resistance patterns outside hospital settings.

In this context, the present study aimed to describe the epidemiology and antimicrobial resistance patterns of multidrug-resistant community-associated *P. aeruginosa* isolated through urban diagnostic laboratories in southern Morocco and to explore the potential value of these laboratories as complementary sources of AMR surveillance data.

2. Materials and Methods

2.1. Study Design

This retrospective multicenter cross-sectional analytical study aimed to determine the distribution of *P. aeruginosa* isolates recovered from routine clinical specimens and processed

in urban medical diagnostic laboratories across southern Morocco during 2024. The study encompassed the regions of Laayoune–Sakia El Hamra, Guelmim–Oued Noun, and Dakhla–Oued Ed-Dahab. According to the Moroccan High Commission for Planning [12], these regions account for approximately 1,023,443 inhabitants. Data were collected from eight major laboratories representing the main microbiological diagnostic facilities in the region (five in Laayoune, others in Dakhla, Tan-Tan, and Guelmim).

All consecutive eligible *P. aeruginosa* isolates recovered between January and December 2024 from participating laboratories were included in this study. Only the first non-duplicate *P. aeruginosa* isolate recovered from each patient during the study period was included. Duplicate isolates and polymicrobial cultures were excluded.

No formal sample size calculation was performed because this retrospective study included all eligible *P. aeruginosa* isolates recovered during the study period from the participating laboratories.

The participating diagnostic laboratories predominantly process specimens from ambulatory patients. Therefore, the *P. aeruginosa* isolates included in this study were considered to reflect community-associated infections. Demographic and clinical data, including age, sex, patient type, and antimicrobial susceptibility testing results, were systematically collected for analysis. However, due to the absence of detailed hospitalization history, the classification of isolates as community-associated was based on the ambulatory nature of the laboratory population and should be interpreted with caution.

2.2. Microbiological Analysis

All isolates were identified as *P. aeruginosa* using the VITEK® 2 Compact automated identification system (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing was performed using the disk diffusion (Kirby–Bauer) method on Mueller–Hinton agar. Plates were incubated at 35 ± 2 °C for 16–18 h. Susceptibility categories (S, I, and R) were interpreted according to the EUCAST [13] breakpoints, and results were interpreted according to the EUCAST guidelines. Quality control was ensured using standard reference strains (*Pseudomonas aeruginosa* ATCC 27853) [13]. To ensure the comparability of results, all isolates collected from participating laboratories were subjected to standardized antimicrobial susceptibility testing using the disk diffusion method according to the EUCAST recommendations. Quality control was performed using *Pseudomonas aeruginosa* ATCC 27853.

Antibiotics tested included: ticarcillin (75 µg), ticarcillin-clavulanic acid (75/10 µg), piperacillin (30 µg), piperacillin-tazobactam (30/6 µg), cefepime (30 µg), ceftazidime (10 µg), ceftazidime-avibactam (10/4 µg), ceftolozane-tazobactam (30/10 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg), tobramycin (10 µg), and gentamicin (10 µg). An isolate was classified as multidrug-resistant (MDR) if it displayed non-susceptibility to at least one agent in three or more antimicrobial categories according to the international criteria [14].

2.3. Statistical Analysis

Associations between MDR status and patient features (sex, age group, city) were evaluated. Age was analyzed in two groups (<50 years vs. ≥50 years) based on the sample distribution and to ensure sufficient observations within each subgroup. Associations between categorical variables were assessed using the Chi-square test or Fisher's exact test when appropriate. A *p*-value < 0.05 was established as statistically significant. All analyses were undertaken with Jamovi (version 2.5.4.0).

No missing laboratory data were identified for the variables included in the analysis. However, hospitalization history, prior antibiotic exposure, comorbidities, and other clinical

risk factors were not available in the laboratory records and were therefore not included in the analysis.

2.4. Ethical Considerations

The study was approved by the Ethics Committee of Mohammed VI University of Sciences and Health (UM6SS) (Approval No. CE/UM6SS/59/24). The study was conducted in accordance with the principles of the Declaration of Helsinki. Clinical and laboratory data were collected retrospectively and analyzed anonymously to ensure patient confidentiality. Given the retrospective nature of the study and the use of anonymized data, the requirement for individual informed consent was waived by the Ethics Committee.

3. Results

During the study period, a total of 13,230 clinical specimens were processed across participating laboratories, yielding 3357 positive cultures (25.4%). Among these, 49 non-duplicate *P. aeruginosa* isolates were identified and included in the study. These isolates originated from urban diagnostic laboratories located across southern Morocco and were analyzed to determine their antimicrobial resistance profiles and the occurrence of multidrug resistance.

3.1. Distribution by Laboratory and Sample Type

A total of 49 *P. aeruginosa* isolates were identified across the participating laboratories. The majority originated from Laayoune ($n = 36$; 73.5%), followed Dakhla ($n = 7$; 14.3%), Guelmim ($n = 5$; 10.2%) and Tan-Tan ($n = 1$; 2%).

Among the 49 *P. aeruginosa* isolates, 32 (65%) were recovered from urine samples, followed by 8 (16%) from pus and 2 (4%) from wound swabs. The remaining isolates were obtained from other specimen types in lower proportions.

Among the MDR isolates, urine samples represented the largest proportion (17/29). Pus samples accounted for a notably high proportion of MDR isolates (6/8). Because several specimen categories included only one or two isolates, percentage-based interpretations for these categories should be considered with caution. All cultures driven from auricular, blood, kidney, and biliary specimens were considered as MDR, whereas no MDR isolates were detected from pleural fluid and protected lung samples (Table 1).

Table 1. Distribution of *P. aeruginosa* isolates and multidrug-resistant (MDR) strains by specimen type in southern Morocco.

Sample Type	<i>P. aeruginosa</i> Isolates n (% of Total)	MDR <i>P. aeruginosa</i> Isolates n (%)
Urine	32 (65%)	17 (53.1%)
Pus	8 (16%)	6 (75%)
Wound swab	2 (4%)	1 (50%)
Otic swab	2 (4%)	2 (100%)
Pleural fluid	1 (2%)	0 (0%)
Biliary fluid	1 (2%)	1 (100%)
Blood	1 (2%)	1 (100%)
Renal tissue	1 (2%)	1 (100%)
Protected lung	1 (2%)	0 (0%)
Total	49 (100%)	29 (59.2%)

Note: Categories containing fewer than five isolates should be interpreted with caution because percentage estimates may be unstable.

3.2. Distribution by Sex and Age

Among the 49 isolates, both genders were represented, with a minor preponderance of female patients.

A slight predominance of female patients was observed (51%). Patients aged ≥ 50 years represented the majority of cases (63%).

3.3. Resistance Profile

The antimicrobial resistance patterns of *P. aeruginosa* isolates showed considerable variability across antibiotic classes. Within the β -lactam group, high resistance rates were observed for ticarcillin (69.4%), ceftazidime (63.3%), and meropenem (79.6%), while moderate resistance was noted for ticarcillin/clavulanic acid (52%), piperacillin (46%), piperacillin/tazobactam (46.9%), cefepime (46.9%), and ceftazidime/avibactam (30.6%). These findings highlight a concerning level of resistance to commonly used β -lactams in the region. In contrast, ceftolozane/tazobactam (10.2%) and aztreonam (6.1%) exhibited the lowest resistance rates.

Carbapenem resistance showed variability, with a relatively low resistance rate to imipenem (12.2%) compared to a markedly higher resistance to meropenem. Among the fluoroquinolones, resistance levels were moderate, ranging from 22.4% for ciprofloxacin to 26.5% for levofloxacin. Aminoglycosides demonstrated the most preserved activity, particularly amikacin (10.2% resistance), while higher resistance was observed for gentamicin (51%) and moderate resistance for tobramycin (28.69%) (Table 2).

Table 2. Resistance rates of the 49 *P. aeruginosa* isolates to commonly used antibiotics.

Antibiotic	Resistance <i>n</i> (%)
Beta-lactam family	
- Ticarcillin (TIC)	34 (69.4%)
- Ticarcillin/clavulanic acid (TTC)	25 (51%)
- Piperacillin (PRL)	23 (46.9%)
- Piperacillin/tazobactam (TZP)	23 (46.9%)
- Cefepime (FEP)	23 (46.9%)
- Ceftazidime (CAZ)	31 (63.3%)
- Ceftazidime/avibactam (CZA)	15 (30.6%)
- Ceftolozane/tazobactam (CLT)	5 (10.2%)
- Aztreonam (ATM)	3 (6.1%)
- Imipenem (IMP)	6 (12.2%)
- Meropenem (MEM)	39 (79.6%)
Quinolone family	
- Ciprofloxacin (CIP)	11 (22.4%)
- Levofloxacin (LEV)	13 (26.5%)
Aminoglycoside family	
- Amikacin (AK)	5 (10.2%)
- Tobramycin (TOB)	14 (28.6%)
- Gentamicin (CN)	25 (51%)

3.4. Association Between Demographics and MDR Status

Among the 49 *P. aeruginosa* isolates, 29 (59.2%; 95% CI: 45.4–72.0%) were classified as MDR. MDR prevalence was similar between males (14/24; 58.3%) and females (15/25; 60.0%), with no statistically significant association ($p = 1.0$). No statistically significant association was observed between age group and MDR status ($p = 0.07$). Although a higher proportion of MDR isolates was observed among patients aged < 50 years (14/18; 77.8%) compared with those aged ≥ 50 years (15/31; 48.4%), this difference did not reach statistical significance (Table 3).

Table 3. Association between demographic characteristics and MDR status among *P. aeruginosa* isolates.

		Total	MDR	Non-MDR	p-Value
		n = 49	n = 29	n = 20	
Sex	Male	24 (49%)	14 (48.3%)	10 (50.0%)	1.0
	Female	25 (51%)	15 (51.7%)	10 (50.0%)	
Age	<50	18 (37%)	14 (48.3%)	4 (20%)	0.07
	≥50	31 (63%)	15 (51.7%)	16 (80%)	

p-value obtained using Fisher’s exact test to determine the association between MDR and non-MDR *P. aeruginosa* and patient-related variables. MDR: multidrug resistant.

4. Discussion

The present study provides regional data on community-associated *P. aeruginosa* isolates and their antimicrobial resistance patterns in southern Morocco, a region where such information remains limited. Despite the relatively small number of isolates included in the study, *P. aeruginosa* remains clinically important because of its intrinsic resistance mechanisms and its ability to acquire multidrug resistance determinants [15]. All non-duplicate isolates identified during the study period were included, providing an overview of regional antimicrobial resistance patterns in 2024.

The predominance of isolates from Laayoune is consistent with its role as the main urban and healthcare center in southern Morocco, characterized by higher population density and more developed diagnostic infrastructure. As a regional referral hub, Laayoune likely receives patients from neighboring rural and semi-urban areas, contributing to increased laboratory activity. In contrast, the lower number of isolates recovered from cities such as Tan-Tan and Guelmim may reflect disparities in healthcare accessibility and diagnostic capacities across southern regions [16,17]. Dakhla, despite its smaller population size, is undergoing important demographic and economic growth that may influence healthcare utilization and microbiological testing.

However, the present findings should be interpreted in light of the study design. Because surveillance was based exclusively on isolates recovered from urban diagnostic laboratories serving predominantly ambulatory patients, the study population may not fully represent all individuals affected by *P. aeruginosa* infections in southern Morocco. Patients living in remote areas, those with limited access to diagnostic services, and individuals managed outside participating laboratories may have been underrepresented, potentially introducing selection bias.

Urinary samples represented the main source of *P. aeruginosa* isolation, highlighting the importance of this pathogen in urinary tract infections in both community and healthcare-associated settings. Similar observations have been reported in other Moroccan regions, including Agadir, Marrakech, and Casablanca, where urinary specimens constitute a major source of *P. aeruginosa* isolates [18,19]. Isolates recovered from pus, wound, and respiratory specimens further illustrate the wide clinical distribution of this opportunistic pathogen.

A higher proportion of isolates was observed among patients aged ≥50 years, which may be explained by the increased susceptibility of older individuals due to comorbidities, repeated healthcare exposure, and antibiotic use [20]. However, no statistically significant association was identified between demographic characteristics and MDR status, suggesting that multidrug resistance may be influenced by multiple microbiological and environmental factors rather than demographic variables alone.

The antimicrobial resistance profile observed in this study is concerning. High resistance rates were observed for meropenem (79.6%), ticarcillin (69.4%) and ceftazidime

(63.3%), suggesting substantial selective pressure associated with β -lactam use in the region. Similar resistance trends have been described in previous Moroccan studies and are commonly associated with resistance mechanisms such as AmpC overproduction, efflux pump overexpression, and OprD porin alterations [21–23].

A notable discrepancy was observed between resistance rates to meropenem (79.6%) and imipenem (12.2%). Although similar findings have occasionally been reported, this difference remains uncommon and may be associated with differences in resistance mechanisms, including efflux pump overexpression, porin alterations, or local antimicrobial selection pressure. However, molecular investigations would be required to confirm the mechanisms underlying this observation.

Lower resistance rates to ceftolozane/tazobactam and ceftazidime/avibactam are encouraging, as these combinations remain among the most effective therapeutic options for MDR *P. aeruginosa* infections. Aminoglycosides, particularly amikacin, also retained good activity against most isolates, whereas higher resistance to gentamicin may reflect its more frequent use in routine clinical practice [18,19].

Future molecular investigations are needed to characterize the genetic determinants underlying antimicrobial resistance and to determine whether high-risk international clones are circulating in southern Morocco. Such studies would complement phenotypic surveillance data and provide a more comprehensive understanding of the epidemiology and dissemination of multidrug-resistant *P. aeruginosa* in the region.

Overall, these findings highlight the circulation of MDR *P. aeruginosa* in southern Morocco and underscore the need for continued antimicrobial resistance monitoring in this underrepresented region. Although the present study was not designed to formally evaluate surveillance performance, the findings suggest that urban diagnostic laboratories may provide useful complementary information on community-associated resistance patterns. Strengthening laboratory capacities and improving the integration of regional data into national surveillance programs could contribute to a better understanding of AMR dynamics in southern Morocco.

Limitations

Several limitations should be considered when interpreting the findings. First, the relatively limited number of *P. aeruginosa* isolates reduced the statistical power of subgroup analyses and precluded robust multivariable analyses. Second, hospitalization history was unavailable, and isolates were classified as community-associated based on outpatient attendance, which may have resulted in the misclassification of some cases. Third, important clinical variables, including prior antibiotic exposure, comorbidities, catheterization, and recent hospitalization, were not available. Fourth, the absence of molecular characterization prevented an investigation of the genetic determinants of antimicrobial resistance and the potential circulation of high-risk clones. The relatively small number of isolates contributed by several participating laboratories precluded robust laboratory-specific comparisons of antimicrobial resistance patterns. Because several participating laboratories contributed only a limited number of isolates, statistically robust inter-city and laboratory-specific comparisons could not be performed. Finally, because the study was restricted to isolates recovered from urban diagnostic laboratories serving predominantly ambulatory patients, the findings may not fully represent the antimicrobial resistance patterns across all populations in southern Morocco, particularly rural communities and hospital settings, introducing potential selection bias.

5. Conclusions

This study provides valuable data on the antimicrobial resistance patterns of community-associated *P. aeruginosa* isolates recovered from urban diagnostic laboratories in southern Morocco, a region where such information remains limited. Although the number of isolates included was relatively small, the findings highlight regional differences that may be influenced by healthcare infrastructure, diagnostic capacity, and access to microbiological testing, particularly the central role of Laayoune as a regional referral hub and the growing contribution of Dakhla.

The high proportion of MDR isolates, together with elevated resistance rates to major β -lactams and carbapenems, underscores the need to strengthen antimicrobial stewardship and improve access to microbiological diagnostic tools across the region. In contrast, the preserved activity of amikacin and selected β -lactam/ β -lactamase inhibitor combinations remains clinically relevant and emphasizes the importance of continuous resistance monitoring.

Although urban diagnostic laboratories cannot fully represent all healthcare settings, they may provide useful complementary data for community-level antimicrobial resistance surveillance. Better integration of these laboratories into national surveillance frameworks could contribute to a more comprehensive understanding of regional resistance patterns.

Overall, these findings highlight the need for harmonized and regionally adapted AMR surveillance strategies in southern Morocco. Further large-scale studies are needed to better characterize resistance trends and support evidence-based infection control and public health policies.

Author Contributions: Conceptualization: H.M., I.D. and K.N. Data collection: H.M. Statistical analysis: H.M. and A.E.-R. Methodology: H.M., I.B., A.A., S.E.M., M.S., A.C., N.D., I.D. and A.K. Project administration: K.N. Visualization: H.M. Writing—original draft: H.M. Writing—review & editing: S.E.M., M.S., A.C., N.D., I.D. and K.N. All authors have read and agreed to the published version of the manuscript.

Funding: Hafsa Mguild is the recipient of a scholarship from Mohammed VI Foundation of Sciences and Health (FM6SS), Morocco, awarded in support of her doctoral research. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Mohammed VI Foundation of Sciences and Health (FM6SS). The use of trade names and commercial sources is for identification purposes only and does not imply endorsement.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of UM6SS (protocol code CE/UM6SS/59/24 on 29 November 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to sincerely thank the laboratories that collaborated with us for their technical support and provision of resources during the study, especially to: El Kamar Mohammed and Mguild Mohammed from El Kamar laboratory, Laayoune; Khitass Fahd from Medicina laboratory, Laayoune; Boufrioua El Ghali from Mekka laboratory, Laayoune; Boumesmar Sakina from Sakia Alhamra International laboratory, Laayoune; Halim Ibtissam from Laayoune laboratory, Laayoune; Gareh Fatma from Tantan laboratory, Tantan; and El Houari El Bachir from Dakhla laboratory, Dakhla. The authors would also like to sincerely thank the Research Technology Platforms of Mohammed VI University of Sciences and Health (PTR-UM6SS), Casablanca, Morocco, for their valuable methodological and experimental support.

Conflicts of Interest: The authors declare no competing interests.

Abbreviations

AK	Amikacin
AMR	Antimicrobial resistance
ATM	Aztreonam
CAZ	Ceftazidime
CIP	Ciprofloxacin
CLT	Ceftolozane-tazobactam
CN	Gentamicin
CZA	Ceftazidime-avibactam
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended-spectrum beta-lactamase
FEP	Cefepime
IMP	Imipenem
LEV	Levofloxacin
MDR	Multidrug-resistant
MEM	Meropenem
OprD	Outer membrane protein D
PA	<i>Pseudomonas aeruginosa</i>
PRL	Piperacillin
TIC	Ticarcillin
TOB	Tobramycin
TTC	Ticarcillin-clavulanic acid
TZP	Piperacillin-tazobactam
XDR	Extensively drug-resistant

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