

Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa*: An 11-Year Experience in a Tertiary Care Hospital in Makkah, Saudi Arabia

Aiman M Momenah ¹, Rafat Ahmed Bakri², Naif A Jalal ¹, Sami S Ashgar¹, Rakan Fahad Felemban³, Farkad Bantun ¹, Sumyya H Hariri¹, Abeer A Barhameen¹, Hani Faidah¹, Hamdi M AL-Said¹

¹Department of Microbiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia; ²University Medical Center, Umm Al-Qura University, Makkah, Saudi Arabia; ³Department of Laboratory and Blood Bank, Al-Noor Specialist Hospital, Makkah, Saudi Arabia

Correspondence: Aiman M Momenah, Department of Microbiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia, Tel +966503501449, Email ammomenah@uqu.edu.sa

Purpose: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common causative pathogen in healthcare settings and displays increasing levels of resistance to common antimicrobial drugs. Its capacity to resist has been reported in multiple locations across the world. This study evaluates current levels of antibiotic resistance and seeks to understand antibiotic resistance patterns in the context of the clinical isolates of *P. aeruginosa*.

Methods: All clinical isolates were cultured at 37 °C for 24 h in different media: blood sheep agar, McConkey agar, and cystine–lactose–electrolyte-deficient agar (CLED), bacterial identification and antibiotic susceptibility patterns were determined using the Vitek-2 (bioMérieux) automated system.

Results: In total, there were 61,029 patient specimens, of which 5534 were identified as non-duplicated *P. aeruginosa* clinical isolates, most being from males aged over 60 years. The research findings revealed that the maximum antibiotic resistance associated with *P. aeruginosa* isolates was found in colistin (97%), which was followed by piperacillin/tazobactam (75.8%). The maximum resistance rates in *P. aeruginosa* isolates were found in relation to cefepime (42.7%), which was followed by ciprofloxacin (34.3%).

Conclusion: The antibiotic resistance rate during the first six years of the research period was notably higher than in the last years, due to the application of infection control protocols and strict policies to control antibiotic prescriptions in all Saudi hospitals.

Keywords: *Pseudomonas aeruginosa*, antimicrobial drug, drug resistance, cefepime

Introduction

In 1882, Gessard became the first person to successfully isolate *P. aeruginosa* from green pus.¹ This bacterium is classified as Gram-negative and aerobic, and it takes the form of a rod without spore formation.² “It is capable of growing at temperatures ranging from 4°C to 42°C, but its optimal growth temperature is 37°C”.³

Soil, decomposing organic matter, vegetation, and water are all environments favorable to pseudomonas. However, moist settings in hospital environments (eg, food, cut flowers, sinks, toilets, floor mops, equipment for respiratory therapy and dialysis, disinfectant solutions) are propitious to this bacterium as well.⁴ Indeed, being of an opportunistic nature, pseudomonas is frequently encountered in hospitals, where it can easily be transferred from the surrounding environment to human beings. Although the bacterium does not usually affect healthy people, it can infect people with a weak immune system, especially those receiving treatment with antibiotics ineffective against pseudomonas.⁵

Individuals admitted to the hospital with conditions like burns, cancer, and cystic fibrosis are vulnerable to infection with *P. aeruginosa*, which can give rise to endocarditis, pneumonia, and infections affecting the urinary tract, central nervous system, eye wounds, ears, skin, and musculoskeletal system.⁶ The bacterium proliferates in excessively crowded or improperly disinfected hospital settings, which is why it is considered to have an opportunistic character.⁵ However, knowledge remains limited about how exactly the pathogenicity of *P. aeruginosa* manifests. The bacterium achieves

human tissue colonization and immune response evasion through its effective production of virulence factors. Another important property is strong adherence to a range of surfaces and hydrolysis of different biocompatible materials.⁷

Statistics published by the National Healthcare Safety Network (NHSN) for 2015–2017 revealed that, in the US, *P. aeruginosa* was among the top three pathogenic bacteria of highest prevalence associated with cases of catheter-associated urinary tract infection (CAUTI) and ventilator-associated pneumonia (VAP).⁸ Similarly, high prevalence has been found in other countries as well. For instance, Diekema et al reported that *P. aeruginosa* was the third greatest source of bloodstream infections, based on the data gathered from locations in 45 countries within the context of a SENTRY antimicrobial surveillance program.⁹ In Saudi Arabia, *P. aeruginosa* is also highly prevalent and becoming more and more resistant to antibiotics.^{10–12} Yezli et al found that 11% of all infections developed in the hospital were caused by *P. aeruginosa* and Gram-negative isolates accounted for 31% of all nosocomial isolates. More specifically, 13–25% of wound infections, 16% of respiratory infections, 6–16% of urinary tract infections (UTIs), and 7–11% of bacteremia were caused by *P. aeruginosa* isolates.⁷

The resistance displayed by *P. aeruginosa* to several antibiotics is a major obstacle in the treatment of infections caused by this bacterium. Multi-drug resistant (MDR) strains of *P. aeruginosa* have emerged recently owing to excessive antibiotic usage, especially in hospitals. This has had ramifications not only in terms of heightened morbidity and death rates, but also extended hospitalization and greater healthcare expenditure. Furthermore, MDR *P. aeruginosa* remains difficult to treat. Given these considerations, it is essential to monitor antibiotic efficacy closely to better understand antibiotic sensitivity patterns as well as to simplify the formulation of preliminary therapy, particularly in cases of critical illness.¹³

The purpose of the present work was to gain insight into the patterns and alterations in the antibiotics to which *P. aeruginosa* has become resistant in the last decade. To that end, data were gathered from cases of *P. aeruginosa* infections treated at a tertiary hospital with a capacity of 560 beds in western Saudi Arabia, namely, Alnoor General Hospital. Such a line of inquiry was motivated by the assumption that antibiotic treatment and prescription practices can be better understood and managed based on analysis of the modifications in the patterns of antibiotic resistance over an interval of a decade.

Materials and Methods

Ethics Statement

The Research Ethics and Advisory Committees of the College of Medicine at Umm Al-Qura University provided ethical approval for this study to take place (reference no HAPO-02-K-012-2022-02-946).

Study Design

This retrospective study was carried out in a tertiary hospital in Makkah, Saudi Arabia (with 560 beds) to examine the vulnerability patterns of all *P. aeruginosa* isolates. This study was carried out over an extended 11-year period from January 2011 to December 2021. Altogether, 86,631 clinical specimens were collected from various hospital departments, including urology, intensive care unit (ICU), and surgery units. Moreover, the samples collected included specimens of blood, pus, urine, sputum, swabs (ie, ear, catheter, urethral, nasal, throat, central venous puncture), bodily fluid (ie, semen, urethral discharge, pleural fluid) and these samples were sent to the microbiology laboratory for processing. A majority of the samples were cultured on sheep blood agar medium and incubated for 24–48 hours at 37°C in order to isolate *P. aeruginosa*. Additionally, Cystine Lactose Electrolyte Deficient agar (CLED) plates were used to inoculate the urine samples, after which they were incubated at 37°C for 24 hours. After the bacteria had grown on the solid medium, a Vitek-2 (bioMérieux) automated system was employed for identification purposes and to determine the antibiotic susceptibility testing of all *P. aeruginosa* isolates.

Subsequently, 0.5% sterile NaCl was added to the bacterial suspensions and the concentration was modified at 0.5–0.63 McFarland using the VITEK Densi CHEK (BioMérieux).

For purposes of identification, the GN-21341 cards were employed, after which the antibiotic susceptibility of the *P. aeruginosa* isolates could be examined using AST-N291 cards. Moreover, quality control was important, and thus the reference strain *P. aeruginosa* ATCC 27853 was employed. A number of antibiotics were tested in this examination,

including piperacillin/tazobactam, ceftazidime, cefepime, imipenem, gentamicin, ciprofloxacin, and colistin. The interpretation of the MIC results based on Clinical Laboratory Standard Institute (CLSI) guidelines.

Statistical Analysis

A database was created to store important data, such as the total number of patients, specimen type, and antibiograms. The antibiotic susceptibilities were recorded as a percentage of all *P. aeruginosa* isolates. Chi-square tests were carried out to compare the antibiotic susceptibility and sample types of different isolates from different years. Additionally, the analyses were all two-tailed. A p-value below 0.05 was determined to be statistically significant. Finally, Prism version 7 was employed to perform the statistical analysis.

Results

Patient Demographics

The current data collection study was conducted retrospectively over an 11-year period from January 1st, 2011, to December 30th, 2021. 61,029 clinical samples were gathered in total, of which 5534 (9.1%) revealed *P. aeruginosa* (Table 1). The proportion of samples in which *P. aeruginosa* was isolated significantly declined over the course of the study from 9.8% (2011) to 5.2% (2021) ($p < 0.05$).

Overall, male subjects exhibited a greater prevalence of infection with *P. aeruginosa* than their female counterparts. The proportions of men and women with a positive result for this bacterium in 2011 were 53.8% and 41.7%, respectively; equivalent data for 2021 were 63.8% and 36.2%, respectively.

Throughout the duration of the study, in-patients' samples positive for *P. aeruginosa* (4644) outnumbered those from out-patients (890, $p < 0.05$) (Table 1). The frequency of the former over the 11-year study duration was between 74.6% and 88.8%. The number of in-patients detected in the initial two years of the study, ie 87.4% (2011) and 87.8% (2012), was greater than in the final two years, ie 74.7% and 74.6%.

When evaluated in terms of patients' age, the majority of instances of *P. aeruginosa* were isolated in those over 60 years (Figure 1). In all age-ranges, more in-patient than out-patient cases were documented ($p < 0.01$). The least frequency of *P. aeruginosa* was noted in patients aged 10 years or less.

Sample Demographics

During the entire study period, positive isolations of *P. aeruginosa* were most frequently identified in the wound (39.6%) and sputum (31.2%) specimens, followed by urine (8.7%) and blood (5.9%) (Figure 2). Irrespective of the sample origin, the mean *P. aeruginosa* isolate percentages were elevated in in-patient specimens as opposed to those from out-patients, eg 98% of sputum samples were obtained from in-patients; the remaining 2% were from out-patients.

Table 1 Demographic Data for *P. aeruginosa* Detection Throughout the 11-Year Study Duration

Year	Total Isolated Organisms	Total <i>P. aeruginosa</i> Isolates	In-Patient	Out-Patient	Male	Female
2011	8082	796 (9.8%)	696 (87.4%)	100 (12.56%)	464 (58.3%)	332 (41.7%)
2012	7241	789 (10.9%)	693 (87.8%)	96 (12.17%)	462 (58.6%)	327 (41.4%)
2013	6294	666 (10.6%)	554 (83.2%)	112 (16.82%)	449 (67.4%)	218 (32.7%)
2014	5217	608 (11.7%)	507 (83.4%)	101 (16.61%)	388 (63.8%)	220 (36.2%)
2015	5163	656 (12.7%)	555 (84.6%)	101 (15.40%)	404 (61.6%)	252 (38.4%)
2016	4722	464 (9.8%)	412 (88.8%)	52 (11.21%)	295 (63.6%)	196 (42.2%)
2017	4376	337 (7.7%)	284 (84.3%)	53 (15.73%)	231 (68.5%)	106 (31.5%)
2018	4980	350 (7%)	269 (76.9%)	81 (23.14%)	197 (56.3%)	153 (43.7%)
2019	4835	379 (7.8%)	309 (81.5%)	70 (18.47%)	214 (56.5%)	165 (43.5%)
2020	4029	174 (4.3%)	130 (74.7%)	44 (25.29%)	101 (58.0%)	73 (42.0%)
2021	6090	315 (5.2%)	235 (74.6%)	80 (25.40%)	201 (63.8%)	114 (36.2%)

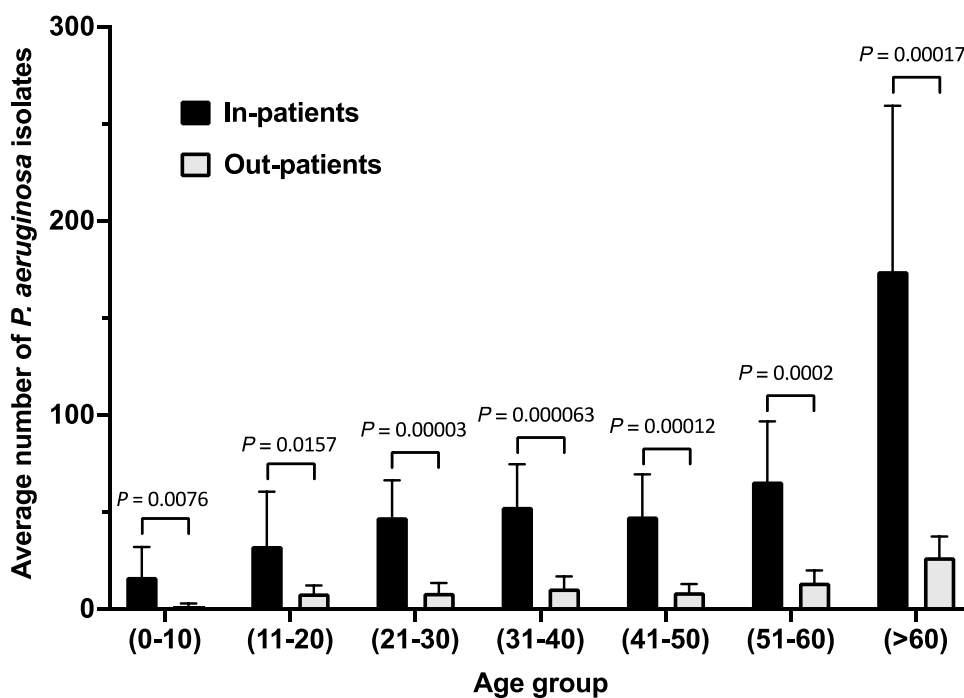


Figure 1 Proportions of the various sample types from which *P. aeruginosa* was isolated over the 11-year study duration.

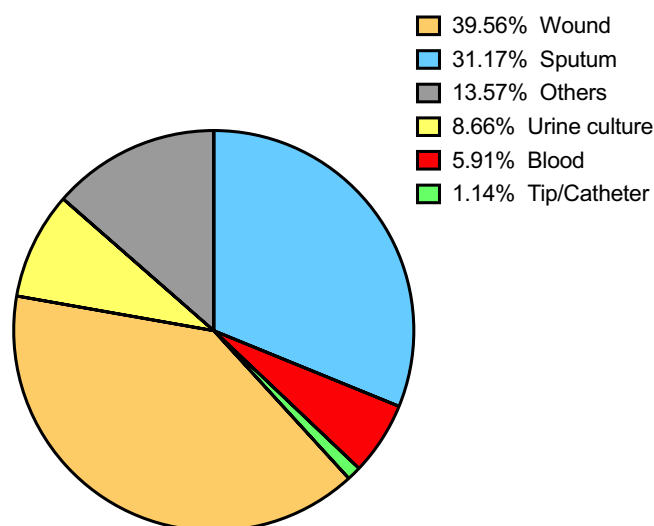


Figure 2 Distribution of *P. aeruginosa* isolates according to sample origin over the 11-year study duration.

The marked decrease in *P. aeruginosa* isolates identified in specimens from the last two years of the study period, 2020 and 2021, compared to the initial two years, 2011 and 2012, is shown in [Table 2](#).

Anti-Microbial Resistance Profiles of *P. aeruginosa*

In total, over the course of the study, 5534 positive cultures for *P. aeruginosa* isolates were identified.

- (i) Mean rates of resistance to sample drugs from different classes of anti-microbial agents, listed highest percentage first, were: cefepime, 42.7%; ciprofloxacin, 34.3%; imipenem, 30.5%; ceftazidime, 29.2%; gentamicin, 26%; piperacillin/tazobactam, 24.2%; and colistin, 3.1% ([Figure 3](#)).

Table 2 Distribution of *P. aeruginosa* Isolates According to Sample Origin with Respect to Year

Specimen Quantitative Values of Isolates (%)		Years										
		2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Sputum	<i>P. aeruginosa</i> isolates	293	298	233	219	237	160	102	101	99	29	70
	Total isolates	1868	1701	1417	1173	1086	912	778	842	785	803	897
	% of <i>P. aeruginosa</i>	15.7%	17.5%	16.4%	18.7%	21.8%	17.5%	13.1%	12.0%	12.6%	3.6%	7.8%
Blood	<i>P. aeruginosa</i> isolates	35	31	43	25	30	25	22	18	46	23	29
	Total isolates	1107	1094	1075	975	1015	972	1086	1083	1208	1075	2038
	% of <i>P. aeruginosa</i>	3.2%	2.8%	4.0%	2.6%	3.0%	2.6%	2.0%	1.7%	3.8%	2.1%	1.4%
Tip and catheter	<i>P. aeruginosa</i> isolates	10	13	5	5	5	5	6	3	5	1	5
	Total isolates	149	179	117	78	58	76	67	54	45	40	65
	% of <i>P. aeruginosa</i>	6.7%	7.3%	4.3%	6.4%	8.6%	6.6%	9.0%	5.6%	11.1%	2.5%	7.7%
Wound	<i>P. aeruginosa</i> isolates	239	286	225	252	279	194	145	159	166	97	147
	Total isolates	1827	1725	1615	1858	1667	1311	1096	1200	1400	899	1372
	% of <i>P. aeruginosa</i>	13.1%	16.6%	13.9%	13.6%	16.7%	14.8%	13.2%	13.3%	11.9%	10.8%	10.7%
Urine	<i>P. aeruginosa</i> isolates	96	77	68	25	45	40	26	33	29	12	28
	Total isolates	1629	1288	1050	523	674	774	417	918	759	565	948
	% of <i>P. aeruginosa</i>	5.9%	6.0%	6.5%	4.8%	6.7%	5.2%	6.2%	3.6%	3.8%	2.1%	3.0%
Miscellaneous	<i>P. aeruginosa</i> isolates	123	84	92	82	60	40	36	36	34	12	36
	Total isolates	1502	1254	1020	610	663	677	932	883	638	647	770
	% of <i>P. aeruginosa</i>	8.2%	6.7%	9.0%	13.4%	9.0%	5.9%	3.9%	4.1%	5.3%	1.9%	4.7%

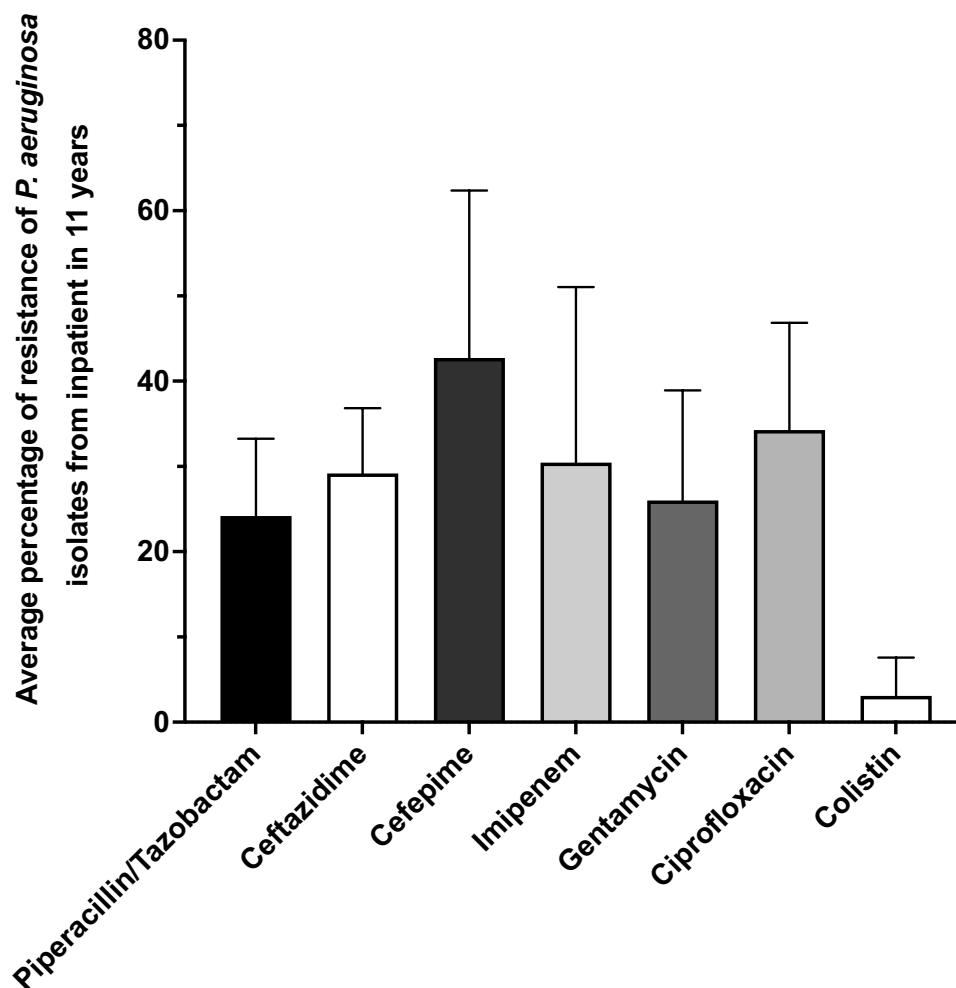


Figure 3 Average rates of resistance for the antibiotics tested amongst isolates of *P. aeruginosa*.

- (ii) The rates of resistance were equivalent regardless of whether the samples were obtained from in-patients or out-patients (Figure 4).
- (iii) The temporal data demonstrated a rise in the resistance trend for all the anti-microbials under investigation early in the study period and subsequently, a steep decline (Figure 5).
- (iv) The rate of resistance during the initial two years of the research period, 2011 and 2012, was notably higher than in the last two years, 2020 and 2021 ($p < 0.01$). This can be illustrated by cefepime, which had resistance rates in these time periods of 46.5% and 12.5%, respectively, exhibiting a fall of 34%, and gentamicin, for which the equivalent resistance rates were 39% and 6.5%, respectively, which indicated a reduction of 32.5% (Figure 6).

Discussion

As described by Ahmed et al, *Pseudomonas aeruginosa* is the most commonly isolated non-fermenting bacterium from clinical samples, representing a substantial problem in treating both community-acquired and nosocomial infection.¹⁴ To achieve the best clinical outcome, analyzing and selecting the first-line of antibiotic defense is critical. The potential of antibiotic resistance is of great importance, and the World Health Organization (WHO) report (2015) recommends that continuous surveillance of antimicrobial resistance (AMR) is conducted to both identify the infection caused by resistant organisms and to monitor the emergence of resistant strains.¹⁵ As noted by Ahmed and Farah et al, 2019 in the Kingdom of Saudi Arabia, even against the backdrop of a need for more study of resistance trends, only a few such studies

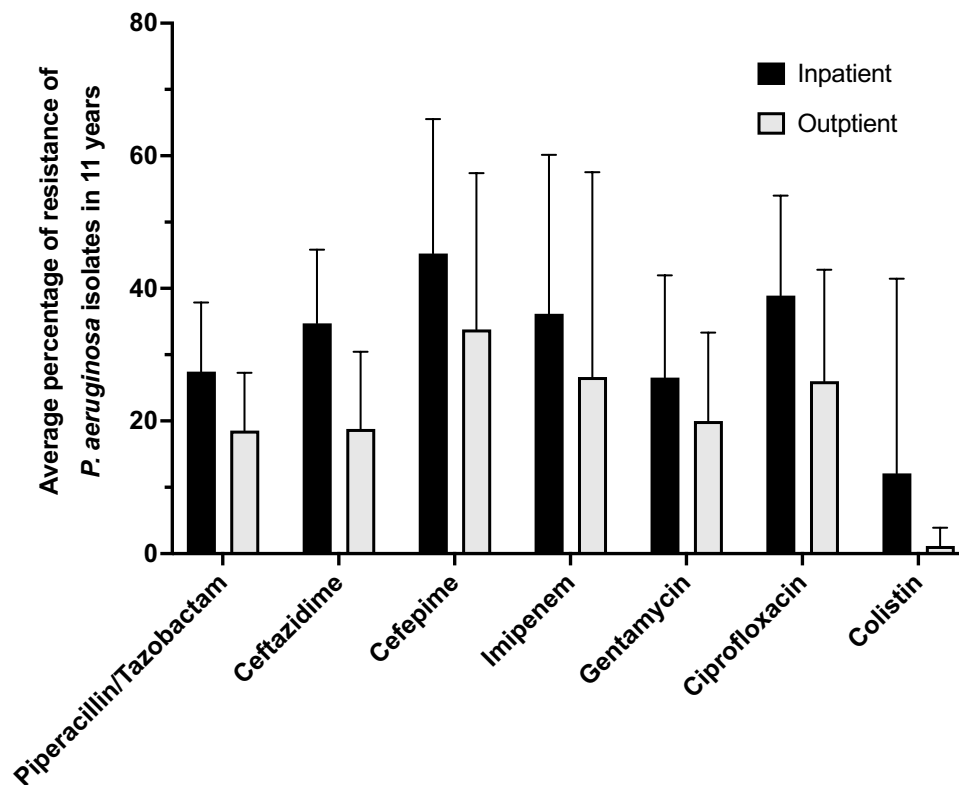


Figure 4 Average rates of resistance for the antibiotics tested with respect to in-patient or out-patient sample source.

of *P. aeruginosa* have been conducted.^{16,17} Herein, we provide data and analysis that fills the void in the current understanding of AMR patterns. To meet this aim, we retrospectively completed an analysis of AMR in *P. aeruginosa* samples obtained over an eleven-year period (2011 to 2021) from a tertiary hospital in Saudi Arabia. Our longer-term study enables the analysis of variation in the patterns of resistance over a long period. In contrast, other studies have been cross-sectional in design, consequently providing resistance data at a single time point.

The prevalence rate of 9.1% of *P. aeruginosa* (5543 isolates from 61,029 total clinical isolates) identified in our study is comparable to that of Ahmed, who reported a rate of 8.7% in a survey conducted in Makkah, Saudi Arabia.¹⁶ However, both of these values are substantially lower than the prevalence rates described^{17,18}. They reported rates of 24% and 23.8%, respectively, in their Saudi-based studies. The rates identified in two Iraqi sites by Al-Derzi (2012) were substantially lower – compared to the current study – at 5.2% (Mosul study) and 2.1% (Duhok study). These apparent location-related variances may be attributed to a number of possible causes; clinical specimen type, the population from which the samples were collected, the treating hospital, and the geographic location.⁵

In our analysis, a comparison of the rate of detection of *P. aeruginosa* isolates from the initiation date (2011/2012) and the endpoint of our study (2020/2021) identified a significant decrease in isolates, with a drop from 10% to 5%, respectively. There are a number of policy areas which may have led to this decrease. During this time, the Ministry of Health in Saudi Arabia established the specialized body, the “Health Facilities Infection Control General Department”, with a remit to address the issue of hospital-acquired infection and AMR. Also, in 2020 the Ministry of Health introduced a policy preventing the sale of antibiotics without a prescription.¹⁹ In addition, the decrease in the number of isolates identified in 2020/2021 may also be attributed to the global Covid-19 pandemic and the reduced number of individuals traveling during this time.²⁰

When investigating the prevalence of *P. aeruginosa* within the in- and outpatient settings, our findings concurred with those of Farah et al, who found that, in their Saudi-based study, *P. aeruginosa* was one of the most frequently identified

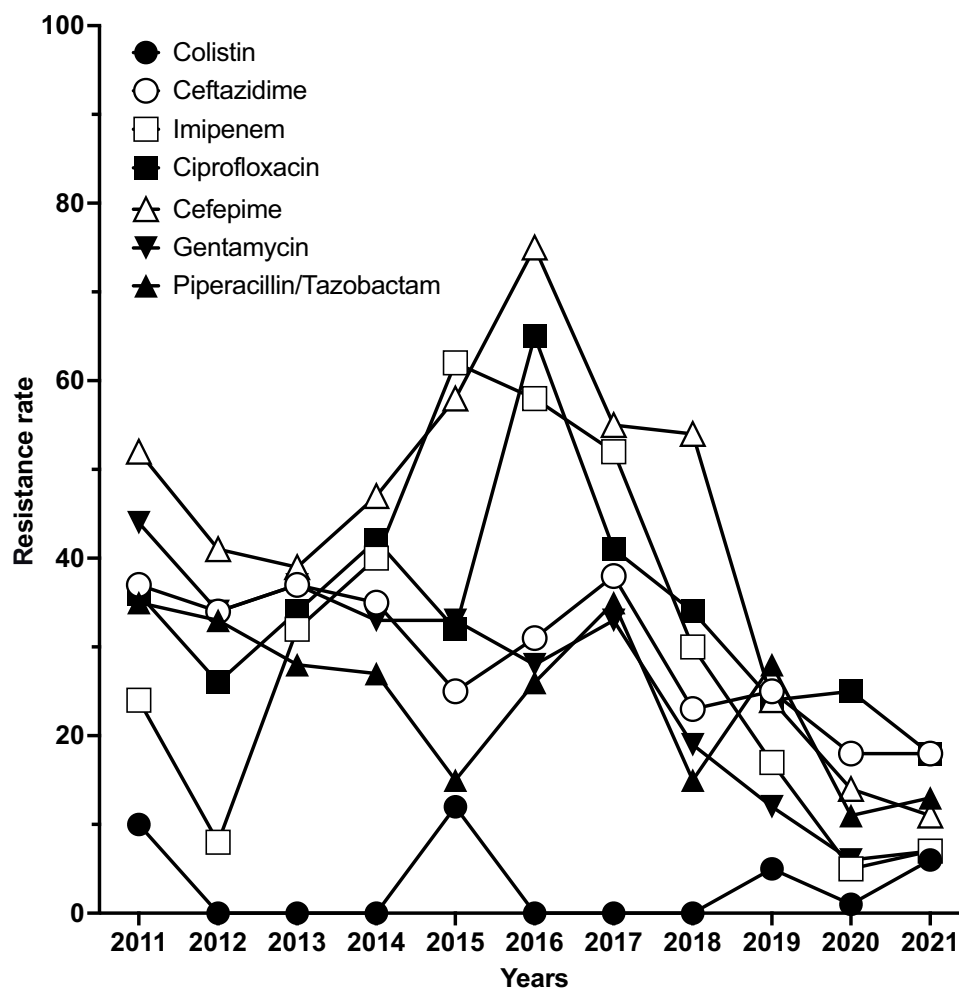


Figure 5 Schematic demonstrating the evolution of resistance to anti-microbial agents over the study period, 2011–2021.

isolates in inpatients. Specifically, in intensive care units, *P. aeruginosa* is noted as one of the leading causes of hospital-acquired infection.¹⁷

Sputum and wound swab samples account for 70% of the *P. aeruginosa* identified in our study. However, Javiya et al, in their Gujarat, India, study identified urine, pus, and sputum as the primary sources of isolates, with each accounting for 27%, and endotracheal secretion contributing 14% of the *P. aeruginosa* isolates.²¹ Once again there are a number of possible contributory factors which led to these differences, specifically the study period, patient population and size, and geographic location. To the best of our knowledge, no other recent studies in Saudi Arabia have determined and reported on the distribution of *P. aeruginosa* in clinical samples.

When analysing the association between gender and *P. aeruginosa* infection rates, our findings concurred with those of a number of other researchers. Specifically, we found that the number of cases in males is significantly higher than in females. Senthamarai et al study found that over three quarters (76.6%) of *P. aeruginosa* isolates were identified in male patients.²² Earlier,⁵ had proposed that occupational incidents are more likely to occur in males, a theory extended to include infection via outdoor activity, personal habits, work, and exposure to sources of contamination, such as soil, water, and other sources of infection by.²²

Our data did not fully agree with that of study.²³ We found that *P. aeruginosa* isolates exhibited high levels of resistance to a panel of antibiotics, whereas the work presented by Ahmad et al demonstrated generally lower levels of resistance. Specifically, we recorded 42% for cefepime, 34.3% for ciprofloxacin, 30.5% for imipenem, 29.2% for ceftazidime, 26% for gentamicin, 24% for piperacillin/tazobactam and 3.1% to colistin, while the other study showed

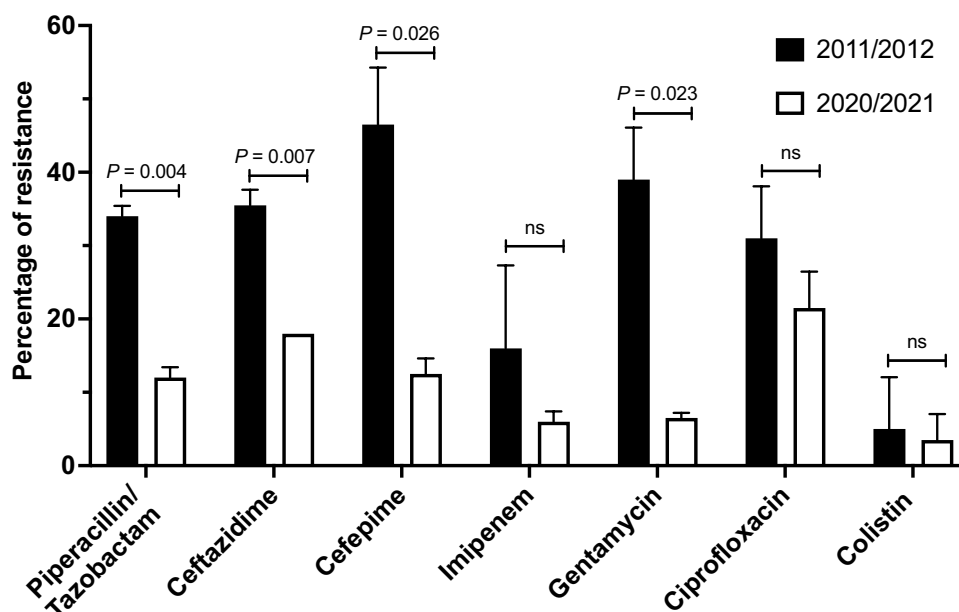


Figure 6 Antibiotic resistance rates of *P. aeruginosa* recognized during the initial (2011 and 2012) and final (2020 and 2021) two years of the study.

36.7% for imipenem, 16.7% for ceftazidime, 13.3% to ciprofloxacin, and 10% to cefepime.²³ The observed variance may be a consequence of the study period, sample size, and patient population, as well as geographic location.²¹

One prominent area of agreement between our study and others – conducted in Saudi Arabia and in other regions – is the high resistance level to ciprofloxacin. Our study, Ahmed’s study,¹⁶ and studies by²⁴ in Iran, and²⁵ in Turkey, produced rates of 34%, 24.1%, 58%, and 48.9%, respectively. The high level of resistance found in *P. aeruginosa* isolates to ciprofloxacin may be a consequence of the use of this antibiotic driving high levels of resistance in the bacterium. As a consequence, both clinical and economic outcomes are affected.¹⁶

Similarly, high resistance levels were observed in *P. aeruginosa* to cefepime; in our study, 42% resistance, and in Ibrahim’s Saudi Arabian study, 67% resistance.¹⁸ Therefore, we propose that earlier overuse of this antibiotic may have induced a rapid re-emergence of cefepime resistance in *P. aeruginosa*.²⁶

Our study demonstrates a relatively low (24%) level of resistance to piperacillin-tazobactam, while other Saudi-based studies have shown substantially higher resistance levels of 38% to this antibiotic.¹⁶ These variances may result from multiple factors, including total numbers of isolates assessed and screening duration, and may also represent setting-specific antibiotic usage and selective pressure.²⁷

Conclusion

Our study revealed that colistin was the most effective antimicrobial agent against *P. aeruginosa* isolates and the greatest resistance was seen with cefepime, and the results showed a significant decrease in the resistance to several antibiotics, including piperacillin/tazobactam, cefepime, ceftazidime, and gentamicin, especially in the period from 2017 to 2021, this due to application of infection control protocols and strict policies to control antibiotic prescriptions in all Saudi hospitals.

Disclosure

The authors report no conflicts of interest in this work.

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