Original Article

Risk Factors and Outcomes of Multidrug-resistant *Pseudomonas aeruginosa* in Kelantan, Malaysia: A Multicenter Case–control Study

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Abstract

Background: Increasing trend and spread of multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) in clinical settings is a great challenge in managing patients with infections caused by this pathogen.

Objective: To determine the risk factors and outcomes of MDR-PA acquisition in the northeastern state of Malaysia. In addition, this study also reported on the susceptibility pattern and common resistant genes among MDR-PA.

Materials and Methods: MDR-PA isolates obtained between March 2021 and February 2022 from all four major hospitals in the state of Kelantan, Malaysia, were submitted for susceptibility and resistant genes identification. The clinical data of the patients with MDR-PA were retrospectively reviewed. The risk factors and outcomes of MDR-PA acquired patients were analyzed by comparing with patients who acquired susceptible-PA while admitted to the same hospital during the study time.

Results: A total of 100 MDR-PA and 100 susceptible-PA cases were included. Ceftolozane–tazobactam was susceptible in 41.3% of MDR-PA compared to only 4%–8% with other β-lactams. About half (46%) of the MDR-PA isolates harbored the $bla_{\text{-NDM-1}}$ gene, but none had the $bla_{\text{-OXA-48}}$ gene. Factors independently associated with MDR-PA acquisitions were age (OR: 1.02; $P = 0.028$), genitourinary disorder (OR: 6.89; $P = 0.001$), and central venous catheter (OR: 3.18; $P = 0.001$). In addition, MDR-PA acquisitions were found to be associated with antimicrobial treatment failure (41.1% vs. 25.0%; $P = 0.001$) and mortality (40.0% versus 6.0%; $P < 0.001$).

Conclusion: Most of the MDR-PA strains in Kelantan tertiary hospitals harbored the $bla_{\text{-NDM-1}}$ gene, which is easily transmissible and can lead to an outbreak. Nonetheless, a significant number of the MDR-PA isolates were still susceptible to ceftolozane–tazobactam.

Keywords: $bla_{\text{-NDM-1}}$, ceftolozane-tazobactam, Malaysia, outcomes, *Pseudomonas aeruginosa*, risk factors

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INTRODUCTION

Pseudomonas aeruginosa strains are among the globally recognized causes of nosocomial infections such as pneumonia, bloodstream infections, urinary tract infections, and surgical site infections that are attributed to high morbidity and mortality. Globally, multidrug-resistant (MDR) and extensive drug-resistant *P. aeruginosa* (XDR-PA) cases are on the rise in the past decade, with prevalence rates varying from 5% to 30%.[1-4] The utmost common resistance mechanisms conferred by *P. aeruginosa* include low outer membrane permeability, expression of efflux pumps, and production of antimicrobial inactivating enzymes. Resistance genes found in MDR *P. aeruginosa* (MDR-PA) in Southeast Asia countries are associated with either a loss or downregulation of the *opr*D gene and metallo-β-lactamase -production (MBL) such as $bla_{\text{-IMP}}$ and $bla_{\text{-VIM}}$.[5,6]

Resistance to most antipseudomonal agents is constantly increasing, leaving clinicians with few effective treatment options. Ceftolozane-tazobactam (C-T), a novel drug, has a promising effect in treating infections caused by MDR/XDR-PA; however the potential emergence of resistance is worrisome.[4,7] The resistance is mainly mediated by MDR/XDR-PA harboring various extended-spectrum β-lactamases (OXA-14, OXA-19, OXA-35, GES-9, and PER-1), carbapenemases (GES-5, 1 IMP-8, and VIM-2), or both; or mutational overexpression of ampC mainly *amp*R gene that is responsible for inducing pseudomonas-derived cephalosporinase.[8,9] Population at greater risk of acquiring MDR-PA include those with comorbidities (diabetes mellitus, renal failure, and chronic lung disease), ICU stay, previous hospitalizations, immunocompromised status, and use of invasive devices.[1,10-12] As there are only a few antimicrobial options available, MDR-PA infection is attributed to worse clinical outcomes and treatment failure.[13,14]

There is limited data on the risk factors and outcomes of patients with MDR-PA infection in Malaysia. Therefore, this study aimed to analyze these MDR-PA features with a focus on Malaysia's northeastern region. Besides, this study also reported on the proportion of susceptibility among MDR-PA and common resistant genes found in MDR-PA isolates from this region.

MATERIALS AND METHODS

Study settings and designs

This was a 1:1 case–control study where the patients and the isolates were recruited and collected, respectively, between March 01, 2021, and February 28, 2022, from all four hospitals with microbiology laboratory support in Kelantan state, i.e. Hospital Universiti Sains Malaysia (HUSM), Hospital Raja Perempuan Zainab II (HPRZ2), Hospital Tanah Merah (HTM), and Hospital Sultan Ismail Petra (HSIP). These hospitals cater for almost 1.5 million populations in the Northeastern part of Peninsular Malaysia, consisting of the entire Kelantan State and the Besut District of Terengganu State.

This study was on the MDR-PA acquisition during the study period. Any clinical samples with MDR-PA during the study period were included. The exclusion criteria were repeated isolates and polymicrobial co-infection. All cases that fulfilled the inclusion criteria were included in the study, without discrimination in either community or hospital-acquired MDR-PA. Controls for individual MDR-PA cases were patients who acquired susceptible *P. aeruginosa* (susceptible-PA) from the same hospital and were admitted during the study period. The definitions of MDR-PA and susceptible-PA are stated in the "Operational Definitions" section below. The isolates from HPRZ2, HTM and HSIP were submitted to the Microbiology Laboratory, HUSM, for confirmation of species and susceptibility profiles as well as for determination of resistant genes.

For the risk factor analysis, the sample size was calculated using a two proportion formula, as described by Nasir *et al*., 2015.[1] By adding a 10% dropout, the minimal subject was determined as 79. The sample size for outcomes was calculated as described by Matos *et al*.,[14] where the minimal sample size after adding 10% dropout was 100. Thus, calculated sample size for the study was 100 subjects from each arm.

Microbiology procedures

The isolates from different hospitals were transported to the Microbiology Laboratory, HUSM, in brain heart infusion broth with 20% glycerol (OXOID Ltd., UK) on ice slurry. The maximum transportation time from the hospitals to the HUSM was 2 hours. Upon arrival, the isolates were kept at –80°C before further experiments.

In the subsequent experiments, after retrieving from storage, *P. aeruginosa* was re-identified in the microbiology laboratory by phenotypic characteristics on standard culture media, including biochemical testing; i.e., oxidase, triple sugar iron, SIM (sulfur, indole, motility) media, citrate, and urease test and those with discrepancies were confirmed by Gram negative card ID Vitek® 2 (Biomerieux, France). Antibiotic susceptibility tests were performed using the

disc diffusion Kirby–Bauer method, according to the Clinical and Laboratory Standards Institute Guideline 32nd Edition.[15] The tested antibiotics were piperacillin–tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, and C-T. Conventional multiplex PCR was used for the detection of common resistance genes, i.e. $bla_{\text{-NDM-1}}$ and $bla_{\text{-OXA-48}}$. Primers were selected from previous works, *acs*A (internal control) and $bla_{\text{-OXA-48}}$ from Beig *et al*.[16] and $bla_{\text{-NDM-1}}$ from Shenoy *et al*.[17]

Clinical data collection

To avoid any interference with patient management, the clinical data were retrospectively collected from patients' records and transferred to a proforma checklist. The data retrieved for each patient included age, sex, underlying comorbidities, recent hospitalization, prolonged hospital stay, intensive care unit (ICU) stay, immunosuppressive therapy, and use of invasive devices such as mechanical ventilation, central venous catheter, Foley catheter and surgical drainage. The recorded outcomes were microbiological and clinical.

Operational definitions

MDR-PA was defined as a strain that was not susceptible to at least 1 drug in at least 3 of the following 5 antipseudomonal antimicrobial classes: extended-spectrum cephalosporin (cefepime, ceftazidime), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (amikacin, gentamicin, tobramycin), carbapenems (imipenem, meropenem, doripenem), and β-lactams-β-lactamase inhibitor (piperacillin–tazobactam).[12,13] Susceptible-PA was defined as a strain that was susceptible to all antipseudomonal antimicrobial agents, excluding those that do not fulfil the MDR-PA definition.[2] True infection was defined as the presence of clinical signs and symptoms, laboratory, or imaging findings compatible with clinical infection, microbiological detection in a relevant clinical specimen and the patient received treatment for the disease. This includes bloodstream infection, respiratory tract infection, urinary tract infection, and skin and soft tissue infection, as defined by the Centers for Disease Control and Prevention.[18,19] Colonization was defined as the isolation of *P. aeruginosa* from any clinical specimens in the absence of signs or symptoms of infections.[20] Microbiological responses were defined as follows: (a) successful (negative culture on repeat culture after 72 hours on therapy); (b) failure (persistent isolation at the site of infection); (c) new infection (found new organism at the site of infection); and (d) indeterminate (absence of subsequent culture to assess microbiological response).[18] Clinical outcomes were classified as (a) survived and (b) death at discharge that might be directly attributable to infection or death unrelated to infection.[20]

Statistical analysis

The descriptive statistics for the patients' demographics, antibiotic activities, and common resistance genes were presented. Data were expressed as numbers and percentages. The risk factors were examined using simple logistic regression analysis, and those with a P value <0.25 were selected for multiple logistic regression analysis. For microbiological and clinical outcomes, Pearson Chi-square or Fisher's exact test was applied to determine association in the categorical distribution. All tests were two-sided and P value <0.05 was considered significant. Effect estimates were expressed as odds ratio, with a 95% confidence interval. Statistical analyses were performed using SPSS version 26.

RESULTS

Subject recruitment

A total of 100 cases of MDR-PA acquisitions and 100 controls from susceptible-PA group (1:1) were collected in the study.

Antimicrobial susceptibility pattern

Table 1 shows the susceptibility patterns of β-lactam antipseudomonal agents among MDR-PA isolates. The novel antibiotic C-T was the most susceptible agent among β-lactams. Of 100 isolates that underwent C-T susceptibility test, 39.0% of MDR-PA were susceptible, 8.0% were intermediate, and 53.0% were resistant. The susceptibility to non-β-lactam antibiotics was low: 46.0% for amikacin and 41.0% for ciprofloxacin. Among the 89 carbapenem-resistant isolates, 41 (46.1%) were positive for $bla_{\text{-NDM-1}}$ gene and 31 (34.8%) were susceptible to C-T. Further analysis of the carbapenem-resistant $bla_{\text{-NDM-1}}$ gene positive subgroup demonstrated that nine (22.0%) MDR-PA isolates were susceptible to C-T.

Common resistance genes in multidrug-resistant *Pseudomonas aeruginosa*

The *acs*A gene was detected in all 100 isolates, which confirmed the species as *P. aeruginosa*. We detected 46 isolates (46.0%) harboring the $bla_{\text{-NDM-1}}$ gene. None of the isolates harbored the $bla_{\text{-OXA-48}}$ gene. Interestingly, in three

Table 1: Antibiotic susceptibility among multidrug-resistant *P. aeruginosa* isolates against β-lactam antipseudomonal agents (*N*=100)

Antibiotics	Susceptible, *n* (%)	Intermediate, *n* (%)	Resistance, *n* (%)
Ceftazidime	8 (8.0)	4 (4.0)	88 (88.0)
Cefepime	8 (8.0)	17 (17.0)	75 (75.0)
Imipenem	4 (4.0)	7 (7.0)	89 (89.0)
Meropenem	6 (6.0)	5 (5.0)	89 (89.0)
Piperacillin-tazobactam	8 (8.0)	27 (27.0)	65 (65.0)
Ceftolozane-tazobactam	39 (39.0)	8 (8.0)	53 (53.0)

MDR-PA isolates, neither the $bla_{\text{-NDM-1}}$ nor the $bla_{\text{-OXA-48}}$ genes were detected.

Subjects' demographics, specimen types, and clinical diagnosis

The demographics, type of samples, and clinical diagnosis are shown in Table 2. The mean ages were 48.55 ± 22.03 years and 40.83 ± 23.63 years for MDR-PA and susceptible-PA groups, respectively. The subjects were predominantly male in both groups. In the MDR-PA group, most isolates were from respiratory samples (47.0%), followed by urine (25.0%) and pus/bone/tissue (19.0%), whereas in the susceptible-PA group, the majority of samples came from respiratory sample (42.0%), followed by pus/bone/tissue (35.0%) and urine (8.0%). Among MDR-PA acquisitions, 28.0% were regarded as colonization and 72.0% as true infections, whereas only 10% of the susceptible-PA were regarded as colonization and 90.0% as true infections [Table 2]. This difference was statistically significant (P <0.01).

Risk factors of multidrug-resistant *Pseudomonas aeruginosa* acquisition

Based on simple logistic regression, factors found to be associated with MDR-PA acquisitions were age (P = 0.019), genitourinary disorder (i.e., disease of kidneys, ureters, bladder, urethra and genital organs) (P = 0.015), malignancy (P = 0.044), ICU stay (P = 0.011), prolong hospitalization (P = 0.114), on chemotherapy (P = 0.005), central venous catheter (P = 0.024), urinary catheter (P <0.001), and mechanical ventilator (P = 0.007) [Table 3]. Diabetes and prolonged hospitalization were also included in the multiple logistic regressions because of P <0.25.

In the final multiple logistic regression analysis, factors independently associated with acquisition of MDR-PA were age [adjusted Odds Ratio (aOR)= 1.02, 95% CI 1.00-1.03; P = 0.028], genitourinary disorder [aOR = 6.89, 95% CI 2.23-21.22; P = 0.001] and central venous catheter [aOR = 3.18, 95% CI 1.66-6.12; P = 0.001]. Patients on chemotherapy were significantly more in the susceptible-PA group [aOR = 0.09, 95% CI 0.02-0.44; P = 0.003] [Table 4].

Microbiological and clinical outcomes in multidrug-resistant *Pseudomonas aeruginosa* infection

In this study, only 56 subjects had repeated samples after 72 hours of initiation of antibiotics. Those with the absence of subsequent cultures were excluded from microbiological treatment outcome analysis. A significant association was found between antimicrobial treatment failure and MDR-PA acquisitions (P = 0.001). MDR-PA acquisitions were also associated with more mortality (P <0.001) [Table 5].

DISCUSSION

P. aeruginosa is an opportunistic pathogen in patients with comorbidities or pre-existing illnesses. In hospital settings with prolonged stays, colonization can occur in critically ill and immunocompromised individuals. In the current study, about a quarter of MDR-PA acquisitions were colonization. Thus, the acquisition of MDR-PA needs to be correlated with the patient's clinical condition and to be carefully interpreted, either causing an infection or a colonizer. The proportion of colonization among MDR-PA acquisitions in this study is comparable with another study, where 26% of isolates were considered as colonization.[18] In the present study, the MDR-PA respiratory isolates (47%) were most

Table 2: Demographics, type of samples, and clinical diagnosis among multidrug-resistant *P. aeruginosa* and susceptible *P. aeruginosa* cases

Variables	Total (*N*=200), *n* (%)	MDR-PA (*n*=100), *n* (%)	Susceptible-PA (*n*=100), *n* (%)
Age (years), mean±SD	44.69±23.12	48.55±22.03	40.83±23.63
Gender			
Male	138 (69.0)	67 (67.0)	71 (71.0)
Female	62 (31.0)	33 (33.0)	29 (29.0)
Type of samples			
Blood	22 (11.0)	9 (9.0)	13 (13.0)
Body fluid	2 (1.0)	0	2 (2.0)
Pus/tissue/bone	54 (27.5)	19 (19.0)	35 (35.0)
Respiratory sample	89 (44.5)	47 (47.0)	42 (42.0)
Urine	33 (16.5)	25 (25.0)	8 (8.0)
Clinical diagnosis			
Infection	162 (81.0)	72 (72.0)	90 (90.0)
Blood stream infection	18 (9.0)	7 (7.0)	11 (11.0)
Pneumonia	82 (41.0)	42 (42.0)	40 (40.0)
Urinary tract infection	15 (7.5)	10 (10.0)	5 (5.0)
Soft tissue infection	42 (21.0)	10 (10.0)	32 (32.0)
Abdominal infection	5 (2.5)	3 (3.0)	2 (2.0)
Colonization	38 (19.0)	28 (28.0)	10 (10.0)

PA – *Pseudomonas aeruginosa;* MDR-PA – Multidrug-resistant-PA; SD – Standard deviation

Table 3: Analysis of risk factors of multidrug-resistant *P. aeruginosa* acquisitions by simple logistic regression

Factors	MDR-PA (*n*=100), *n* (%)	Susceptible-PA (*n*=100), *n* (%)	Crude OR (95% CI)	*P*
Age (years), mean±SD	48.55±22.03	40.83±23.63	1.02 (1.00–1.03)	0.019
Gender				
Male	67 (33.5)	71 (35.5)	1	
Female	33 (16.5)	29 (14.5)	1.21 (0.66–2.20)	0.541
Co-morbidity				
Diabetes mellitus				
No	55 (27.6)	63 (31.7)	1	0.216
Yes	45 (22.6)	36 (18.1)	1.43 (0.81–2.53)	
Dialysis				
No	97 (48.7)	93 (46.7)	1	
Yes	3 (1.5)	6 (3.0)	0.48 (0.12–1.97)	0.308
Pulmonary disease				
No	89 (44.5)	91 (45.5)	1	
Yes	11 (5.5)	9 (4.5)	1.25 (0.49–3.16)	0.638
Gut disorder				
No	81 (40.5)	93 (46.5)	1	
Yes	19 (9.5)	7 (3.5)	3.12 (1.25–7.79)	0.015
Malignancy				
No	88 (44.0)	77 (38.5)	1	
Yes	12 (6.0)	23 (11.5)	0.46 (0.21–0.98)	0.044
ICU stays				
No	42 (21.0)	60 (30.0)	1	
Yes	58 (29.0)	40 (20.0)	2.07 (1.18–3.64)	0.011
Prolong hospitalization (>2 weeks)				
No	35 (17.5)	46 (23.0)	1	
Yes	65 (32.5)	54 (27.0)	1.58 (0.90–2.79)	0.114
Recent hospitalization				
No	69 (34.5)	66 (33.0)	1	
Yes	31 (15.5)	34 (17.0)	0.87 (0.48–1.58)	0.651
Chemotherapy				
No	98 (49.0)	85 (42.5)	1	
Yes	2 (1.0)	15 (7.5)	0.12 (0.03–0.52)	0.005
Prolong steroid use (>2 weeks)				
No	100 (0.0)	98 (49.0)	1	
Yes	0	2 (1.0)	0.00 (0.00–0.00)	>0.950
Devices present during admission				
Central venous catheter				
No	43 (21.5)	59 (29.5)	1	
Yes	57 (28.5)	41 (20.5)	1.83 (1.05–3.21)	0.024
Urinary catheter				
No	17 (8.5)	45 (22.5)	1	
Yes	83 (41.5)	55 (27.5)	4.00 (2.08–7.68)	<0.001
Mechanical ventilator				
No	45 (22.5)	64 (32.0)	1	
Yes	55 (27.5)	36 (18.0)	2.17 (1.23–3.83)	0.007
Surgical drainage				
No	78 (39.0)	77 (38.5)	1	
Yes	22 (11.0)	23 (11.5)	0.94 (0.49–1.83)	0.866

PA – *Pseudomonas aeruginosa;* MDR-PA – Multidrug-resistant-PA; OR – Odds ratio; CI – Confidence interval; ICU – Intensive care unit; SD – Standard deviation

prevalent, followed by urine (25%) and pus (19%). These findings were in line with those of a previous study.[21]

For MDR-PA, this study demonstrates that all β-lactam antibiotics such as carbapenems, cephalosporins, and piperacillin-tazobactam exhibit low susceptibility, ranging from 4%–8%. This supports the theory that the widespread use of antibiotics causes the emergence of drug resistance. The susceptibility rate of C-T was found to be as high as 94.1% in some regions, as described in a European study.[22] However, lower susceptibility rates have been reported among MDR/XDR-PA strains worldwide.[23] In our study, C-T had a susceptibility of 39.0% against MDR-PA and was the most susceptible compared with other β-lactam anti-pseudomonal drugs. Our result is comparable to a study done in Thailand, with the C-T susceptibility rate of 44.5% among β-lactams non-susceptible-PA.[24] To a certain extent, this result provides an evidence that C-T with optimal dosage and duration is valid as a therapeutic option to address MDR- and XDR-PA infections.[24-26] In this study, C-T was found to be active against approximately one-third of the carbapenem-resistant-PA and one-fifth

Table 4: Analysis of risk factors of multidrug-resistant *P. aeruginosa* acquisitions by multiple logistic regression model

Factors	MDR-PA (*n*=100), *n* (%)	Susceptible-PA (*n*=10), *n* (%)	Adjusted OR (95% CI)	*P*
Age, years (mean±SD)	48.55±22.03	40.83±23.63	1.02 (1.00–1.03)	0.028
Genitourinary disorder				
No	81 (40.5)	93 (46.5)	1	
Yes	19 (9.5)	7 (3.5)	6.89 (2.23–21.22)	0.001
Chemotherapy				
No	98 (49.0)	85 (42.5)	1	
Yes	2 (1.0)	15 (7.5)	0.09 (0.02–0.44)	0.003
Central venous catheter				
No	44 (22.0)	59 (29.5)	1	
Yes	56 (28.0)	41 (20.5)	3.18 (1.66–6.12)	0.001

Multiple logistic regression was applied. Most of the assumptions were met (classification table=68.1%, Hosmer Lemeshow *P*=0.994, area under the curve ROC=0.74 [0.67–0.81]). PA – *Pseudomonas aeruginosa;* MDR-PA – Multidrug-resistant-PA; OR – Odds ratio; CI – Confidence interval; SD – Standard deviation; ROC – Receiver operating characteristic

Table 5: Microbiological and clinical outcomes of multidrug-resistant *Pseudomonas aeruginosa* acquisitions compared to susceptible *P. aeruginosa* acquisitions

Outcomes	MDR-PA, *n* (%)	Susceptible-PA, *n* (%)	*P*
Microbiological (*n*=56)			
Success	2 (8.0)	17 (54.8)	0.001[a]
Failure	23 (92.0)	14 (45.2)	
Mortality (*n*=200)			
Survived	59 (59.0)	94 (94.0)	<0.001[b]
Died	41 (41.0)	6 (6.0)	

[a]Fisher's exact test; [b]Pearson Chi-square test was applied.
PA – *Pseudomonas aeruginosa;* MDR-PA – Multidrug-resistant-PA

of the carbapenem-resistant-PA that carry NDM metallo-β-lactamase.

The current study found that $bla_{\text{-NDM-1}}$ was detected in 46.0% of the MDR-PA isolates, suggesting that the presence of $bla_{\text{-NDM-1}}$ gene among MDR-PA isolates is high in the region. In 2018, Liew *et al*. reported the first case of $bla_{\text{-NDM-1}}$ among 53 MDR-PA isolates in Malaysia.[6] Subsequently, Lee *et al*. demonstrated in 2022 that 61 (24.9%) isolates of carbapenem-resistant *P. aeruginosa* in the Asia-Pacific Region harbored the $bla_{\text{-NDM}}$ gene.[27] The actual burden of NDM producer among MDR-PA is still underreported. The present study also revealed that none of the MDR-PA harbored $bla_{\text{-OXA-48}}$, in line with previous reports that indicated class D β-lactamases have rarely been identified in *P. aeruginosa*.[28]

Previously, studies had identified a few risk factors, including comorbidities (such as COPD and diabetes mellitus), ICU stays, prior hospitalizations, lengthy hospital stays, use of devices (such as central venous catheters and mechanical ventilators), and prior antipseudomonal exposure (e.g., cephalosporins, carbapenems, quinolones), that are linked to the development of MDR-PA.[10,19] Based on our findings, the independent risk factors that contribute to MDR-PA include age, genitourinary disorder and central venous catheter. Our study showed an independent association between age and MDR-PA, in concordance with a case–control study conducted in France.[29] As with increasing age, there were 2% higher odds of experiencing the presence of MDR-PA. In contrast, many other studies found no association between age and MDR-PA acquisition.[18,30,31] Patients with genitourinary disease, particularly those who have structural or functional abnormalities of the urinary tract, served as a reservoir for MDR organisms. The presence of the central venous catheter demonstrates that the impact of this variable in MDR-PA and susceptible-PA increases the risk of MDR-PA by three times. Interestingly, we found that significantly more patients were on chemotherapy in the susceptible-PA group than in the MDR-PA group. This finding is corroborated by another research that discovered malignancy could protect against MDR-PA,[18] likely because rigorous infection control measures in place for such patients.

Infections caused by MDR organisms are associated with poor outcomes. We found that MDR-PA was associated with higher antimicrobial treatment failure ($P = 0.001$) and hospital all-cause mortality rate ($P < 0.001$), compared to controls. Antimicrobial treatment failure was observed in MDR-PA, which was similar to that observed in a previous study conducted in Bangkok, Thailand.[19] The low rates of microbiological clearance in this study could also be attributed to the small number of repeat cultures taken to determine successful eradication. In MDR, one of the main consequences is inadequate empirical antibiotic therapy resulting in the persistence of MDR-PA infection. Conversely, a recent multicenter retrospective analysis of C-T in MDR/XDR-PA infection reported a 42.1% microbiological eradication rate, indicating a promising treatment choice.[24] Our study showed that the all-cause mortality rate in patients with MDR-PA was 41%. This finding is in accordance with other reports,[13,20,32] thereby supporting the hypothesis that poorer prognosis is associated with MDR-PA. Patients with several comorbidities are at risk of having MDR/XDR-PA

colonization and infection. Substantial pre-existing illness may contribute to mortality in these patients. However, a multicenter study demonstrated that people with MDR-PA treated with C-T had improved mortality and clinical cure rates.[33]

Limitations

The major strength of this study is that it included four major hospitals to collectively reflect the circulating mechanisms of resistance in *P. aeruginosa* in Kelantan state of Malaysia. However, there are limitations to this study. The study has the inherent limitations of a retrospective study, including the inability to establish causation and data collection quality. Another limitation was that the recruitment of control (susceptible-PA) was not as the same type of infection as the MDR-PA. In addition, due to budget limitations, we were unable to analyze the outer membrane porin *oprD* level by qRT-PCR, which is necessary to demonstrate the downregulation of the *oprD* gene. This prevents drawing a complete picture of the common resistance gene in MDR-PA. Future studies are needed to investigate the mechanism of resistance among C-T subpopulation. Prospective studies with a larger sample size, longer duration, and a more in-depth analysis are needed to derive more conclusive findings.

CONCLUSION

This study highlights that the majority of the MDR-PA strains isolated in Kelantan tertiary hospitals harbored the $bla_{\text{-NDM-1}}$ gene, which is easily transmissible and can lead to an outbreak. However, a significant number of the MDR-PA isolates were still susceptible to ceftolozane–tazobactam. Given that MDR-PA infection results in poorer outcomes, there is need for judicious use of antibiotics, invention of new antipseudomonal drugs, and implementation of antimicrobial stewardship and assertive infection control measures in hospitals.

Ethical considerations

This study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (Ref no.: NMRR-20-2932-57800 [IIR]) and Human Research Ethics Committee, Universiti Sains Malaysia, Kubang Kerian, Kelantan (JEPeM Code: USM/JEPeM/21010074). Requirement for patient consent was waived owing to the study design. The study adhered to the principles of the Declaration of Helsinki, 2013.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: S.N.M.S. and Z.Z.D.; Methodology: S.N.M.S., N.I.M.D., R.R., and S.A.S.H.; Data analysis: S.N.M.S. and Z.Z.D.; Writing–original draft preparation: S.N.M.S.; Writing – review and editing: Z.Z.D.; Supervision: Z.Z.D.

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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