

SUSCEPTIBILITY PATTERNS, RESISTANCE GENES,
RISK FACTORS AND OUTCOMES OF MULTIDRUG-
RESISTANT *Pseudomonas aeruginosa* IN
NORTHEASTERN STATE OF MALAYSIA: A
MULTICENTER STUDY

DR SITI NORFAIRUZ BINTI MD SALIM

Dissertation Submitted in Partial Fulfilment of the Requirements
for the Degree of Master of Pathology (Microbiology)



UNIVERSITI SAINS MALAYSIA

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SUPERVISOR:

PROF. DR ZAKUAN ZAINY DERIS

ACKNOWLEDGEMENT

Bismilahirrahmanirrahim

I praised Allah as The Most Gracious and The Most Merciful for His blessings that enables me to complete this dissertation. Indeed, without His help and will, nothing is accomplished.

First and foremost, I would like to express my respectful gratitude and appreciation to Professor Dr. Zakuan Zainy Deris as my principal supervisor and Head, Department of Medical Microbiology & Parasitology, University Sains Malaysia for the continuous support and patience in guiding me throughout the journey of completing this dissertation and my studies.

My token of appreciation also goes to Dr Nurul Izzah Md Din @ Mohamad, Dr Rosnita Rashid, Dr Sharifah Aisyah Sayed Hitam and staffs at the Medical Microbiology & Parasitology Laboratory of Universiti Sains Malaysia for the direct and indirect contribution in assistance, sample collection and technical support.

Ultimately, I would like to present special gratefulness to my husband, Dr Mohamad Hamzi bin Mohamad Noor, my beloved parent Md Salim bin Sulaiman and Sharifah Noorazillah binti Syed Abdullah, mother-in-law Hafsah binti Mat for their endless support and dua', family, friends and finally, to my two children, Hana Irdeena and Hafiy Uzayr for giving me space, time and being so cooperative along the process to completion this dissertation.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

Symbols / Abbreviations	Meaning
-	Negative or subtraction
+	Positive or addition
±	Plus-minus
<	Less than
>	More than
≤	Less than or equal to
≥	More than or equal to
=	Equal to
×	Times or multiplication
/	Division or ‘or’
%	Percentage
°	Degree
μ	Micro
α	Alpha
β	Beta
AMS	Antimicrobial stewardship
AST	Antimicrobial susceptibility test
BHI	Brain heart infusion
<i>bla</i>	B-lactamase
BLAST	Basic local alignment search tool
BSI	Blood stream infection
bp	Base pair
C	Celsius
CAUTI	Catheter associated urinary tract infection
CF	Cystic fibrosis
CLABSI	Central line associated blood stream infection
CLSI	Clinical Laboratory Standards Institute
COPD	Chronic obstructive pulmonary disease
C-T	Ceftolozane-tazobactam
CDC	Center for Disease Prevention and Control
DNA	Deoxyribonuclease acid
dNTP	Deoxyribonucleotide triphosphate
DTR	Difficult-to-Treat Resistance
et al.	Et alia (and others)
Exo	Exotoxin
ESBL	Extended spectrum β-lactamase
ECDC	European Center for Disease Prevention and Control
EUCAST	European Committee on Antimicrobial Susceptibility Testing

F	Forward
g	Gram
GNB	Gram negative bacilli
HCAI	Health care associated infection
HRPZ	Hospital Raja Perempuan Zainab II
HSIP	Hospital Sultan Ismail Petra
HTM	Hospital Tanah Merah
HUSM	Hospital Universiti Sains Malaysia
I	Intermediate
ICU	Intensive care unit
ID	Identification
IDSA	Infectious Diseases Society of America
IMP	Imipenemase metallo- β -lactamase
i.e.	Id est (In other words)
IPC	Infection prevention and control
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
L	Liter
LPS	Lipopolysaccharide
LIS	Laboratory information system
m	Mili
mm	Milimeter
M	Molar
MALDI-TOF-MS	Matrix assisted laser desorption ionization time-of-flight mass spectrometry
MBL	Metallo- β -lactamase
MDR	Multidrug-resistant
MDRO	Multidrug-resistant organism
MIC	Minimum inhibitory concentration
n	Number of samples
NC	Negative control
NDM-1	New Delhi metallo- β -lactamase 1
NHSN	National Healthcare Safety Network
NMRR	National Medical Research Register
OMP	Outer membrane protein
OR	Odds ratio
OXA	Oxacillin- β -lactamase
PA	<i>Pseudomonas aeruginosa</i>
PC	Positive control
PCR	Polymerase chain reaction
PDC	Pseudomonas derived cephalosporinase
PDR	Pan drug resistance
QS	Quorum sensing
R	Resistant or reverse

RN	Registration number
RR	Relative risk
RND	Resistance nodulation division
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RT	Real time
S	Susceptible
SD	Standard deviation
SIM	Sulphur, indole, motility
SPSS	Statistical Package for Social Sciences
SSI	Surgical site infection
T3SS	Type 3 secretion system
TBE	Tris/Borate/EDTA
TSI	Triple sugar iron
U	Unit
USM	Universiti Sains Malaysia
UTI	Urinary tract infection
UV	Ultraviolet
VAP	Ventilator associated pneumonia
VIM	Verona Integron encoded metallo- β -lactamase
WBC	White blood cell
WHO	World Health Organization
XDR	Extensive drug resistant

ABSTRACT

Background: Multi-drug resistant *Pseudomonas aeruginosa* (MDR-PA) is one of the emerging nosocomial Gram-negative organisms responsible for high morbidity and mortality. Gradual increase in antibiotic resistance left clinician with limited therapeutic options. Minimal data exist regarding MDR-PA in Malaysia. Therefore, this study aimed to investigate the common resistance genes, activity of ceftolozane-tazobactam (C-T), risk factors and outcomes of MDR-PA infection.

Methodology: In this case-control study, two hundred subjects were recruited from four major hospitals in Kelantan State of Malaysia between March 2021 and February 2022. One hundred MDR-PA isolates were subjected to antimicrobial susceptibility tests and resistance gene detections. Whereas risk factors and outcomes were analysed by comparing 1:1 case (MDR-PA) and control (susceptible-PA).

Result: The new antibiotic, ceftolozane-tazobactam activity showed the best performance among β -lactams (53.0% of MDR-PA were resistant). The most prevalent gene was *OprD* (91.0%) and followed by *bla*-NDM-1 (46.0%). None of the isolates carry *bla*-OXA-48 gene.

0.028), genitourinary disorder (OR: 6.89; $p = 0.001$) and central venous catheter (OR: 3.18; $p = 0.001$). This study also showed MDR-PA acquisitions were associated with microbiological failure (41.1% vs 25.0%; $p = 0.001$) and mortality (40% versus 6%; $p < 0.001$).

The emergence of *Pseudomonas aeruginosa* harbouring *bla*-NDM1 gene in Kelantan state population should alarm clinicians since it can spread from person to person, causing outbreaks. Early anticipation in patient at risk of acquiring MDR-PA is crucial to facilitate case detection and infection control measures.

ABSTRAK

Latar belakang: Perintang antibiotik berganda *Pseudomonas aeruginosa* (MDR-PA) merupakan salah satu daripada organisma gram-negatif nosokomial yang bertanggungjawab terhadap morbiditi dan kematian yang tinggi. Peningkatan secara beransur-ansur dalam rintangan antibiotik menyebabkan doktor mempunyai pilihan terapeutik yang terhad. Kewujudan data mengenai MDR-PA di Malaysia juga adalah terhad. Oleh itu, kajian ini bertujuan untuk mengkaji gen rintangan biasa, aktiviti ceftolozane-tazobactam (C-T), faktor risiko dan kesan jangkitan MDR-PA.

Tatacara kajian: Dalam kajian kawalan kes ini, seramai dua ratus subjek telah direkrut dari empat buah hospital utama di negeri Kelantan, Malaysia di antara Mac 2021 dan Februari 2022. Sebanyak seratus isolasi MDR-PA telah dijalankan ujian kerentanan antibiotik dan pengesanan gen rintangan. Manakala, faktor risiko dan kesan penyakit di analisa dengan membandingkan 1:1 kes (MDR-PA) dan kawalan (susceptible-PA).

Keputusan: Aktiviti antibiotik terbaru iaitu ceftolozane-tazobactam mempamerkan prestasi yang terbaik antara antibiotik β -lactam yang lain (53.0% MDR-PA adalah rintang). Gen *OprD* adalah yang paling berleluasa (91.0%) dan diikuti dengan gen *bla*-NDM-1 (46.0%). Tiada isolasi yang membawa gen *bla*-OXA-48. Faktor-faktor yang dikaitkan dengan perolehan jangkitan MDR-PA adalah berumur (OR:1.02; $p = 0.028$), gangguan genitourinari (berkaitan btah pinggang, saluran kencing, pundi kencing dan organ seksual) (OR:1.02; $p = 0.001$). Kajian ini juga menunjukkan perolehan jangkitan MDR-PA adalah berumur (OR:1.02; $p = 0.001$). Kajian ini juga menunjukkan perolehan jangkitan MDR-PA berkaitan dengan kegagalan mikrobiologi (41.1% berbanding 25.0%; dengan nilai $p = 0.001$) dan kematian (40% berbanding 6%; dengan nilai $p < 0.001$).

Kemunculan *Pseudomonas aeruginosa* yang mengandung gen *bla*-NDM1 dalam populasi di negeri Kelantan sepatutnya membimbangkan para doktor kerana ia boleh merebak dari seorang ke seorang dan menyebabkan wabak. Jangkaan awal pesakit yang berisiko mendapat MDR-PA adalah penting untuk memudahkan pengesanan kes dan langkah-langkah kawalan jangkitan.

CHAPTER 1 : INTRODUCTION

1.1 Multidrug-resistant (MDR) *Pseudomonas aeruginosa*

1.1.1 History and Taxonomy

The genus *Pseudomonas* belongs to the wider family of Pseudomonadaceae and class of Gammaproteobacteria. Migula in 1894 was the first to describe *Pseudomonas* based on its morphologic characteristics (Gomila *et al.*, 2015). It is one of the most complex bacterial genera with the largest number of species among Gram-negative bacteria. There are about 144 recognized species in the genus however only a few can cause disease in humans and are associated with opportunistic infections. These include *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. luteola* and *P. stutzeri* (Iglewski BH, 1996). Other *Pseudomonas* species are thought to be less virulent, although they can still infect people, particularly those who are immunocompromised.

Among all *Pseudomonas* species, the most frequent isolate from clinical specimens is *P. aeruginosa*. It rarely causes illness in healthy individuals, but it poses major threat to hospitalized patients that can be fatal and result in extremely debilitating diseases. The name *P. aeruginosa* was given based on the distinctive blue-green coloration pigment produced during culture. Only until 1882, the first successful culture of *P. aeruginosa* was reported as a blue-green coloration on bandages with a distinct odor from patient's wound by a physician Carle Gessard (Lister *et al.*, 2009).

1.1.2 Epidemiology

P. aeruginosa infections are predominantly hospital acquired and it has been recognized globally as one of the most common Gram-negative organisms responsible for nosocomial and healthcare-associated infections (HCAIs). *P. aeruginosa* contributed to 7.1% of hospital infections in 2011, according to the United States Centers for Disease Control and Prevention (CDC) (Y. Wang *et al.*, 2021), which is comparable to 9% that was stated in the 2011 to 2012 HCAIs report in European hospitals (Qin *et al.*, 2022). Data from Report on Antimicrobial Administration and Antimicrobial Resistance in China 2017 found that *P. aeruginosa* is the third most common nosocomial pathogens accounting for 12.7% of all gram-negative bacteria behind *Escherichia coli* and *Klebsiella pneumoniae*.

Multidrug-resistant organisms (MDROs), particularly Gram-negative bacteria are continuously emerging at an alarming rate. Members of the Enterobacterales family, *P. aeruginosa* and *Acinetobacter baumannii* are classified as critical pathogens by World Health Organization (WHO) for which new drugs are urgently required due to their vast rates of emergence, acquisition and spread by different resistance mechanisms (Liao *et al.*, 2022). Recent years have witnessed an increasing prevalence of multidrug-resistant or extensive drug-resistant (MDR/XDR) *P. aeruginosa* strains, with rates varying from 5% to 30% depending on the region. According to the United States CDC, the incidence is 7.7% in 2020, whereas it can reach up to 31.8% in several European countries (European CDC, 2019). MDR *P. aeruginosa* (MDR-PA) isolates were estimated to be 8.2% and 6.9% in South America and Malaysia, respectively (Mirzaei *et al.*, 2020). *P. aeruginosa* causes substantial burden to healthcare system in terms of morbidity and

mortality. From a systematic review and meta-analysis, Nathwani et al. found that infection by MDR-PA can lead to fatality ranged between 25% to 60% (Druge *et al.*, 2019; Horcajada *et al.*, 2019; Nathwani *et al.*, 2014).

The consensus definition of MDR-PA that is currently being used is the one that Magiorakos *et al.* published in 2011. MDR-PA was defined as non-susceptibility (intermediate or resistant [I/R]) to at least one agent in at least three antibiotic classes, XDR as non-susceptibility to at least one agent in all but one or two antibiotic classes and pan drug-resistance (PDR) as non-susceptibility to all agents in all classes (Magiorakos, A.P. *et al.*, 2011).

1.1.3 Normal Habitat and Transmission

P. aeruginosa is a ubiquitous environmental pathogen and frequently isolated from water, plants, animals, and humans. Its ability to tolerate physical condition, minimal nutritional requirement and predilection to water allow *P. aeruginosa* to stay in both community and hospital settings. *P. aeruginosa* can be acquired either exogenously or endogenously. Variety of sources can act as reservoirs and lead to exogenous transmission such as medical equipment especially respiratory ventilators, dialysis machines, sinks, tubs or pools, solutions, and soil (Moore *et al.*, 2011). *P. aeruginosa* is recovered infrequently from the endogenous microbial flora in humans. Despite, risk of being colonized by *P. aeruginosa* exceeded more than 50% during hospitalization, particularly among patients with breach in cutaneous or mucosal barriers by mechanical ventilation, catheters, burns and greater in immunosuppressed person with exposure to broad spectrum antibiotics (Lister *et al.*, 2009; Nadeem *et al.*, 2006).

P. aeruginosa can be transmitted by several routes, including patient-to-patient and environmental contamination. Its ability to adapt nature and high surviving rate explain why it can survive on environmental surfaces in hospital environment (Spagnolo *et al.*, 2021). Furthermore, contamination of the operator's hands following contact with an infected or colonized patient can result in infections. Individual risk factors, such as patient characteristics and in-hospital events, as well as extrinsic variables, such as suboptimal infection control measures used by healthcare professionals and irrational antibiotic use, might contribute to the development of MDR-PA.

1.1.4 Virulence Factors

P. aeruginosa thought to be highly virulent organisms as they exhibit abundance virulence factors. Virulence factors of *P. aeruginosa* itself contribute to the pathogenesis of infection. It can be either present on the bacterial cell surfaces or secreted as extracellular products.

Lipopolysaccharide (LPS), an important classical structural component of the outer membrane in most gram-negative bacteria elicit robust innate inflammatory response and eventually causes dysregulated inflammation responses that contribute to morbidity and mortality (Rocha *et al.*, 2019). Presence of surface appendages like Type-IV pili and flagella aid in adherent to respiratory epithelium, motility and enhances the development of biofilm. Type 3 secretion system (T3SS) is one of the most powerful virulent factors of *P. aeruginosa* as it initiates bacterial invasion and exert cytotoxicity effect by directly inject exotoxins (ExoS, ExoT, ExoU and ExoY) into the cell cytoplasm (Liao *et al.*, 2022). This is reflected by the clinical manifestations and severity of infections. Poor outcomes, increased bacterial burden, infection persistence, and increased mortality have

been linked to the expression of T3SS in *P. aeruginosa* (Newman *et al.*, 2017; Reynolds and Kollef, 2021).

Alginate is an exopolysachharide composed of D-mannuronic acid and L-guluronic acid being secreted by *P. aeruginosa* which results in mucoid phenotype as seen in cystic fibrosis (CF). It impedes bacterial clearance by host due to its antiphagocytic activity and important for the formation of biofilms. A blue-green pigment called pyocyanin is a secreted metabolite that impairs cilia and damages tissue while pyoverdine a fluorescent pigment binds with iron to facilitate bacterial growth. Other toxins responsible in the pathogenesis of *P. aeruginosa* include ExolysinA, ExotoxinA, LipaseA, PhospholipaseC, LipoxygenaseA and Leukocidin.

Biofilm formation is the most significant hallmark of *P. aeruginosa* infection. There are four Quorum Sensing (QS) pathways exist in *P. aeruginosa*, where bacterial cells communicate with each other uses signalling molecules called autoinducer allowing the microbes to coordinate activity (Qin *et al.*, 2022). It is crucial for the formation of biofilms and aid in the production of multiple virulence factors.

1.1.5 Antibiotic Resistance Mechanism

In recent years, the prevalence of MDR-PA is on a rise internationally. The ability of *P. aeruginosa* to resist antibiotics by employing their high levels of intrinsic and acquired resistance mechanisms has rendered treatment difficult. *P. aeruginosa* has one of the largest bacterial genomes, giving it a great genetic capacity and is among the largest in the bacterial world allowing for great genetic capacity as well as a high level of adaptation to environmental changes (Pang *et al.*, 2019). *P. aeruginosa* has a wide range of intrinsic

and acquired mechanisms for resistance, thus demonstrating high rates of resistance to different antibiotic classes (Spagnolo *et al.*, 2021). Four most common mechanisms of resistance in *P. aeruginosa* has been proposed. Firstly, through plasmid conjugation this species can obtain potent enzymes called β -lactamases such as metallo- β -lactamases (MBLs) and oxacillin hydrolyzing β -lactamase (OXA). Secondly, inactivation or downregulation of the carbapenem-specific porin *OprD*, a trans-membrane protein which is used by the carbapenems to cross the bacterial outer membrane. Thirdly, the bacteria can activate a pump-based efflux mechanism termed MexAB-OprM, which drives antibiotics out of the bacterial cell. Fourthly, *P. aeruginosa* has a chromosomal gene coding for AmpC cephalosporinase responsible for resistance to antipseudomonal penicillins and cephalosporins.

The production of MBLs (Ambler class B) can degrade all the broad-spectrum antibiotics like carbapenems and beta-lactamase inhibitors like clavulanate, sulbactam and tazobactam. In a large-scale multicenter study in Spain (2017), 16.7% produced either Extended-spectrum β -lactamase (ESBL) or carbapenamases with MBLs were the most frequent carbapenamases detected, particularly of Verona integron-encoded metallo β -lactamse (VIM) type (Del Barrio-Tofin  *et al.*, 2019). Variants of the Imipenem (IMP) and VIM genes have been found in Malaysia, Thailand, Singapore, Vietnam and other Southeast Asian countries healthcare facilities (Suwantararat and Carroll, 2016; Tran *et al.*, 2021). The extremely rare New Delhi metallo β -lactamase-1 (NDM-1) is one of the most clinically significant MBLs. This NDM-1 strain is incredibly hazardous since it has the capacity to spread by plasmid transfer and encodes multiple resistance determinants to different antibiotics leads to MDR/XDR-PA (Farajzadeh Sheikh *et al.*, 2020). OXA-48 (Ambler Class D) is a unique carbapenemase since it weakly hydrolyzes both

carbapenems and broad spectrum cephalosporins though occasionally has ESBL co production. This gene has progressively disseminated throughout Europe, Middle East, and North African countries. However, the detection of OXA-48 is always obscured and difficult to detect by standard laboratory methods due to the weak hydrolytic profile, thus increase the likelihood that treatment failure would occur (Bakthavatchalam *et al.*, 2016; Stewart *et al.*, 2018).

The most well-known porin on *P. aeruginosa* outer membrane is called *OprD*. Hydrophilic antibiotics like carbapenems use this porin for penetration through the lipid membrane. Mutations in the *OprD* gene lead to downregulation or loss of the *OprD* protein (Shen *et al.*, 2015). Almost 90% of carbapenem-resistant *P. aeruginosa* are related with decrease in the *OprD* production (Turk, 2011). It imparts resistance to imipenem, and to meropenem and doripenem to a lesser extent. However in cases with both overproduction of AmpC and *OprD* inactivation these will confer resistance to all anti-pseudomonal beta-lactams (Al-Orphaly *et al.*, 2021).

MexAB-OprM was the first multidrug efflux pump belongs to resistance nodulation division (RND) family discovered by Poole *et al.*, that play important role in expelling drugs from several different classes out of the cell (Lister *et al.*, 2009). It confers resistance to a wide range of drugs, including β -lactams, fluoroquinolones, tetracyclines, macrolides, chloramphenicol, trimethoprim, and sulfamethoxazole. This pump is intrinsically produced and overexpression of MexAB-OprM contributing to the emergence of MDR-PA. Multiple lines of evidence point that the ability of *P. aeruginosa* to resist antibiotics which is mediated by the efflux pump as the primary means for the organism's success in an infection. The presence of MexAB-OprM genes among MDR-

PA isolates have been reported by previous studies ranging from 58.8% to 100% (Abbas *et al.*, 2018; Bhandari *et al.*, 2022; Kishk *et al.*, 2020).

P. aeruginosa, like other several members of Enterobacterales harbors a chromosomal drug inducible gene, AmpC encoding for Class C β -lactamase. It confers natural resistance towards aminopenicillins, first- and second generation cephalosporins. When induced, AmpC β -lactamase hyperproduction can degrade anti-pseudomonal drugs such as piperacillin, aztreonam, ceftazidime and cefepime except for carbapenems. This differs from AmpC inducible Enterobacterales which remain susceptible to cefepime. AmpC cephalosporinase prevalent in *P. aeruginosa* has been found in over 20% of clinical isolates (Horcajada *et al.*, 2019).

1.1.6 Risk Factors

Pseudomonas infections commonly occur in immunocompromised (diabetes, severe kidney failure, hematological malignancies), structural lung disease (bronchiectasis), chronic obstructive pulmonary disease (COPD) patients with frequent steroid and/or antibiotic use, recent and prolonged hospital stay, Intensive care unit (ICU) admission, severe burns, presence of indwelling device, mechanical ventilation and previous antimicrobial exposure (Mandell *et al.*, 2007; Alhussain *et al.*, 2021; Litwin *et al.*, 2021;).

Published studies show that majority of MDR-PA strains was isolated from surgical ward and ICU (Pachori *et al.*, 2019; Alhussain *et al.*, 2021). Intensive care unit especially create an environment for infection to occur because the debilitating effects of severely ill patient, bed ridden, prolonged hospitalization and the application of multiple invasive equipment's compromise protective barrier such as mechanical ventilation, Foley

catheter, central venous catheter (Farooq *et al.*, 2019). ICU patient care, which demands close contact with healthcare workers is prone to several opportunities. The care of ICU patients which requires intensive contact with health-care provider are prone to multiple opportunities for failure of infection control measure serving as a vector for the transmission of these MDROs.

The extent of prior antibiotic usage in the emergence of MDR-PA is well discussed in the literature (Fernández-Barat *et al.*, 2017; Druge *et al.*, 2019;). Result from a meta-analysis and systematic review by Raman *et al.*, confirmed that prior use of broad spectrum cephalosporin, quinolones, aminoglycosides and carbapenems predicted an increased risk of MDR/XDR-PA infection (Nasir *et al.*, 2015; Palavutitotai *et al.*, 2018; Raman *et al.*, 2018). The initial inappropriate use of those antibiotics will lead to selective pressure allowing MDR-PA strains to proliferate over susceptible *P. aeruginosa* (S-PA) strain.

1.1.7 Types of Infection

Ventilator-associated Pneumonia (VAP)

VAP was diagnosed based on new or progressive chest radiographs, fever $> 38^{\circ}\text{C}$, leukocytosis $\geq 12,000 \text{ WBC/mm}^3$, tachypnoea, crepitation, worsening gas exchange with positive microbiological results from tracheal aspirate occurring > 48 hours after endotracheal intubation (NHSN, 2022). *P. aeruginosa* is the most frequent cause of ventilator-associated pneumonia of all gram-negative bacteria. MDROs such as *A. baumannii* and *P. aeruginosa* are associated with late onset VAP with high fatality rate. Even with appropriate antibiotic treatment, death from VAP caused by *P. aeruginosa* can reach up to 13.5% with prevalence of 4% to 38%. The intubation process causes break of

the airway mucosa and lead to tissue invasion by *P. aeruginosa* colonization. Risk factors attributable to acquisition of *P. aeruginosa* VAP are prior antibiotic exposure, prolonged mechanical ventilation and translocation of endogenous colonization in chronic lung diseases patient following intubation (Pachori *et al.*, 2019).

Bloodstream infection (BSI)

After urinary tract infection (UTI) and surgical site infection (SSI), BSI is one of the most prevalent nosocomial infections with serious life-threatening conditions. Patients who had either fever ($> 38^{\circ}\text{C}$), chills, or hypotension and one positive blood culture for *P. aeruginosa* after 48 hours of admission were considered as nosocomial BSI based on the criteria of The European Centre for Disease Prevention and Control (ECDC, 2016). *P. aeruginosa* BSI were common, accounting for 51% of cases, with 4% of those being central line associated blood stream infection (CLABSI). Nosocomial BSI are implicated with the highest fatality rate ranging from 39% to 60% with MDR-PA strains poses two-threefold higher compared with non-MDR strains (Bennett, J.E., Dolin, R. and Blaser, 2020).

Catheter-associated Urinary Tract Infection (CAUTI)

P. aeruginosa was the third most frequent pathogen implicated in nosocomial UTI, particularly CAUTI, accounting for 10% of all nosocomial infections with up to 16% occurrence in ICU patients (Reynolds and Kollef, 2021). This organism is a known uropathogen particularly secondary to instrumentation of urinary system such as catheter, nephrostomy, and stent. The insertion of a catheter, which breaches the mucosal epithelial layers acts as an entry point and further facilitates bacterial colonization. Antimicrobial resistance rates are very high among *P. aeruginosa* strains causing CAUTI, depending on

local antimicrobial resistance patterns. Surveillance study of the Asia-Pacific region reported that resistance rates of > 40% for antibiotics like imipenem, ceftazidime, cefepime and ciprofloxacin (Bennett, J.E., Dolin, R. and Blaser, 2020). Bacteremia is one of the possible complications from nosocomial UTI by *P. aeruginosa* led to high morbidity and death.

Other Nosocomial Infections

P. aeruginosa play major role in SSI with prevalence 27.4%. The most frequent types of surgery where *P. aeruginosa* was identified to be a secondary cause of SSI were breast and cardiac operations (Reynolds and Kollef, 2021). Apart from that, patients with extreme burn injuries are vulnerable to MDR-PA infection due to loss of skin barrier in primary host. Burn wound infections were one of the sequelae, and their fatality rate can reach 75% (Al-Dahmoshi *et al.*, 2021).

1.1.8 Outcomes

P. aeruginosa infections are often severe and life threatening thus significantly increases duration of hospital stay, cost, morbidity and mortality rates, thus resulting in devastating outcomes (Phoon *et al.*, 2018). In other circumstances, these MDR-PA strains may spread from one patient to another, resulting in outbreaks that place a substantial burden on the healthcare system. According to numerous studies, mortality rate in MDR-PA was 15% to 80% (Cao *et al.*, 2004; Tam *et al.*, 2010; Alhussain *et al.*, 2021). Relative to non-MBL-producing strains, MBL-producing strains contribute to a greater fatality rate (23 out of 93 [25%] vs 10 out of 80 [13%] deaths; relative risk [RR]: 1.98; 95% CI: 1.00-3.90; $p = 0.05$) (Laupland *et al.*, 2019).

1.1.9 Laboratory Identification

Microscopy and Culture

P. aeruginosa appears as thin, gram-negative rods in laboratory settings after Gram staining. It is aerobic-facultative anaerobe organism and culturable on many types of solid media such as blood agar at 37°C incubation temperature. After 24 hours of incubation, the colonies of *P. aeruginosa* appear as large irregular grey and some smooth round exhibit beta-hemolysis on blood agar and grow well on MacConkey agar producing non lactose fermenter colonies with green pigmentation. A fruity grape-like odor is one of the typical characteristics in *P. aeruginosa*.

Presumptive Identification via Phenotypical Characteristic

On nutrient agar, *P. aeruginosa* produces a characteristic green-blue coloration due to the production of pyocyanin (blue) and pyoverdine (green). Other pigments produced by some strains include pyorubin (dark red) and pyomelanin (black). *P. aeruginosa* is differentiated from other *Pseudomonas* species including *P. fluorescence* and *P. putida* by growth at 42°C. Identification of *P. aeruginosa* therefore is based on colony morphology, pigment production, oxidase positive, and growth at 42°C (Phillips, 1969). Other biochemical reactions include alkaline/neutral in Triple Sugar Iron (TSI) test and positive citrate utilization. Automated identification systems like The Vitek[®] 2 and Phoenix can be used to correctly identify *P. aeruginosa* with 78.8% and 90.98% accuracy, respectively (Tang *et al.*, 2017).

Identification via Genotypic Methods

Currently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has provided an accurate and robust tool that enables rapid and reliable microbial identification in clinical diagnostic microbiology setting. The results from MALDI-TOF MS for detection of *P. aeruginosa* were consistent and it has been utilized in *P. aeruginosa* drug resistance evaluation such as carbapenamase. Numerous molecular assays have been developed and often used for detecting pathogens. The polymerase chain reaction (PCR) technique has been widely employed in rapidly detecting *P. aeruginosa* that specifically targets the 16S ribosomal ribonucleic acid (rRNA) or other specific genes such as *acsA*, *ecfX*, *oprL*, *algD*, *fliC* and *gyrB* (AL-Shimmary *et al.*, 2019; Beig *et al.*, 2020; C. Wang *et al.*, 2022).

Antimicrobial Susceptibility Testing (AST)

Ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, cefotaxime, ceftriaxone, ertapenem, trimethoprim, tigecycline, and chloramphenicol are all known to be ineffective against *P. aeruginosa*. Laboratories all over the world adopt established guidelines when interpreting AST result, regardless using Kirby Bauer disk diffusion method, broth microdilution, or commercial AST products which are the Clinical Laboratory Standard Institute (CLSI) guidelines and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. However using EUCAST breakpoints for *P. aeruginosa* interpretation results in lower susceptible percentages and higher resistant percentages notably in amikacin and piperacillin-tazobactam due to 1-2 doubling dilutions below CLSI interpretative breakpoints (Badal, R. *et al.*, 2009; Wolfensberger *et al.*, 2013). The latest editions of the CLSI and EUCAST guidelines now

include new antimicrobial drugs namely ceftolozane-tazobactam, ceftazidime-tazobactam, imipenem-relebactam, meropenem-vaborbactam and cefiderocol which can be used in the treatment of MDR-PA.

1.1.10 Treatment

The use of traditional anti-pseudomonal agents to treat *P. aeruginosa* infections has become increasingly ineffective due to the emergence of multidrug-resistant strains. Current therapeutic options for MDR/XDR-PA infections are the use of different antibiotic combinations and development of novel antibiotics. Novel antibiotics are more superior, effective and decreased likelihood of developing resistance lower frequency of resistance development given their unique modes of action and resist bacterial enzyme modification (Pang *et al.*, 2019).

Ceftolozane-tazobactam (C-T) is a new drug consist of a novel anti-pseudomonal cephalosporins with β -lactamase inhibitor. Ceftolozane is stable against AmpC enzymes and not affected by neither efflux pump nor porin channel. Tazobactam does not have any activity against *P. aeruginosa* just that it protects ceftolozane from destruction by ESBL. A multicenter trial carried out in the United States found that C-T is successful in treating MDR-PA with overall success rate around 73.7% (Gallagher *et al.*, 2018). A systematic study found that 76.2% of patients with Difficult-to-Treat Resistance (DTR) *P. aeruginosa* infections from a wide range of disease were successfully treated (Maraolo *et al.*, 2020). Monotherapy C-T has been approved for the treatment of complicated urinary tract infection, complicated intraabdominal infection, and nosocomial pneumonia. Additionally, it is recommended for use as an empiric treatment along with aminoglycoside in the treatment of nosocomial pneumonia in ICU patients who are

susceptible to *P. aeruginosa* infection (Reynolds and Kollef, 2021). However, C-T resistance in MDR-PA has been reported but varies regionally mainly due to alterations in Pseudomonas-derived cephalosporinase (PDC), the chromosomally encoded class C beta-lactamase (Bitar *et al.*, 2022; Tamma *et al.*, 2022).

Other novel combination antimicrobials with antipseudomonal activity available for use in MDR-PA are ceftazidime-avibactam (combination of cephalosporin with non- β -lactam β -lactamase inhibitor), imipenem-cilastatin-relebactam (combination of carbapenem- β -lactamase inhibitor that inhibits class A carbapenemases and class C cephalosporinases) and meropenem-vaborbactam (combination carbapenem- β -lactamase inhibitor). Several multicenter randomized control trials demonstrated the non-inferiority of these drugs (ceftazidime-avibactam and imipenem-cilastatin-relebactam) compared to conventional drugs (Torres *et al.*, 2018; Titov *et al.*, 2021).

A newer antibiotic, cefiderocol, is a siderophore cephalosporin which binds to iron and facilitates bacterial cell entry, further binds to penicillin binding protein 3 causing inhibition of bacterial cell wall synthesis. Infectious Disease Society of America (IDSA) 2022 on Guidance on the treatment of DTR-PA suggested cefiderocol as alternative treatment option in MDR/XDR-PA infections outside the urine when the newer β -lactam- β -lactamase inhibitors are unavailable, inactive or intolerance (Tamma *et al.*, 2022).

Plazomicin is a semisynthetic aminoglycoside which able to resist broad spectrum of aminoglycoside modifying enzymes. When combined with cefepime, imipenem or piperacillin-tazobactam, this drug has in vitro synergistic activity propose that plazomicin can be use as combination therapies in MDR-PA treatment (Pang *et al.*, 2019). Despite

plazomicin is unlikely to provide any benefit if DTR-PA is shown to resistant to all aminoglycosides.

The development of alternative therapeutic strategies for the treatment of *P. aeruginosa* has gained attention in the past decade. These therapeutic strategies can act alone or in conjunction with conventional antimicrobial drugs to combat *P. aeruginosa* infection. Examples are quorum sensing and bacterial lectins inhibitors, use of iron chelators, phage therapy and nanoparticles (Liao *et al.*, 2022).

1.1.11 Prevention and control measures

The following WHO recommendations should be implemented to prevent the spread of MDR-PA in healthcare facilities: hand hygiene, contact precautions, patient isolation, environmental cleaning, and surveillance (Reynolds and Kollef, 2021). Overtreatment is a major concern in MDROs. The concept of Antimicrobial Stewardship (AMS) was promulgated by the IDSA in 2007. In addition to Infection prevention and control (IPC) and patient safety, AMS is one of three elements of an integrated strategy to strengthen the health system (Majumder *et al.*, 2020). Implementing the AMS Program aids the prevention of MDROs by optimising antibiotic usage. Initial antipseudomonal therapy should only be started in those patients at risk of *P. aeruginosa* infection followed by de-escalation to narrow spectrum antibiotic once culture sensitivities are available. ICU specifically need to strictly adhere to IPC measures which are the care “bundles” (additional to standard care) for instance bundles for prevention of VAP, bundles for prevention of CLABSI and bundles for prevention of CAUTI due to higher risk of infection with MDROs and transmission linked with ICU-specific working procedures (Teerawattanapong *et al.*, 2017; Spagnolo *et al.*, 2021).

1.2 Rationale of study

There are several major reasons why the emergence and dissemination of MDR and XDR *P. aeruginosa* strains have recently become issues of public health concern. First, *P. aeruginosa* causes severe infections, particularly in health care settings and in immunocompromised patients. Second, it has an outstanding capacity for being selected and for spreading antimicrobial resistance in vivo. Third, the successful worldwide spread of the so-called “high-risk” clones of *P. aeruginosa* poses a threat to global public health that needs to be studied and warrants proper infectious control measures to be taken. Therefore, continuous surveillance of MDR-PA is fundamental to monitor this resistant pathogen and to appropriately guide the clinician in choosing a new develop antimicrobial agent. To date, there are no study yet pertaining MDR-PA infection in Kelantan and limited multicenter studies in Malaysia due to limited manpower, logistic and financial support.

This study will give a better representative distribution regarding common mutation of MDR-PA in Kelantan. This study will also highlight the activity of C-T in MDR-PA strains that potentially to be used widely as first line therapy in near future. Apart from that, identifying patients who are at risk for acquisition of MDR-PA in the hospital setting will assist in precautionary measures and providing timely and appropriate treatment. Based on this study we want to see an association between MDR-PA and the outcome so that findings from this study will alert the managing team and prompt for prescribing antibiotic judiciously.

1.3 Research question

1. What are the common resistance genes in MDR *P. aeruginosa* isolates in Kelantan?
2. What is the activity of ceftolozane-tazobactam in MDR *P. aeruginosa* isolates in Kelantan?
3. What are the risk factors and outcomes of MDR *P. aeruginosa* infection in Kelantan?

1.4 Study objective

1.4.1 General objective

The aim of this research is to study on MDR *P. aeruginosa* infection from multicenter in Kelantan.

1.4.2 Specific objective

1. To describe common resistance genes, namely *bla*_{-NDM-1}, *bla*_{-OXA-48} and *OprD* in MDR *P. aeruginosa* isolates.
2. To describe activity of ceftolozane-tazobactam in MDR *P. aeruginosa* isolates.
3. To determine risk factors and outcomes associated with MDR *P. aeruginosa* infection.

1.5 Study flowchart

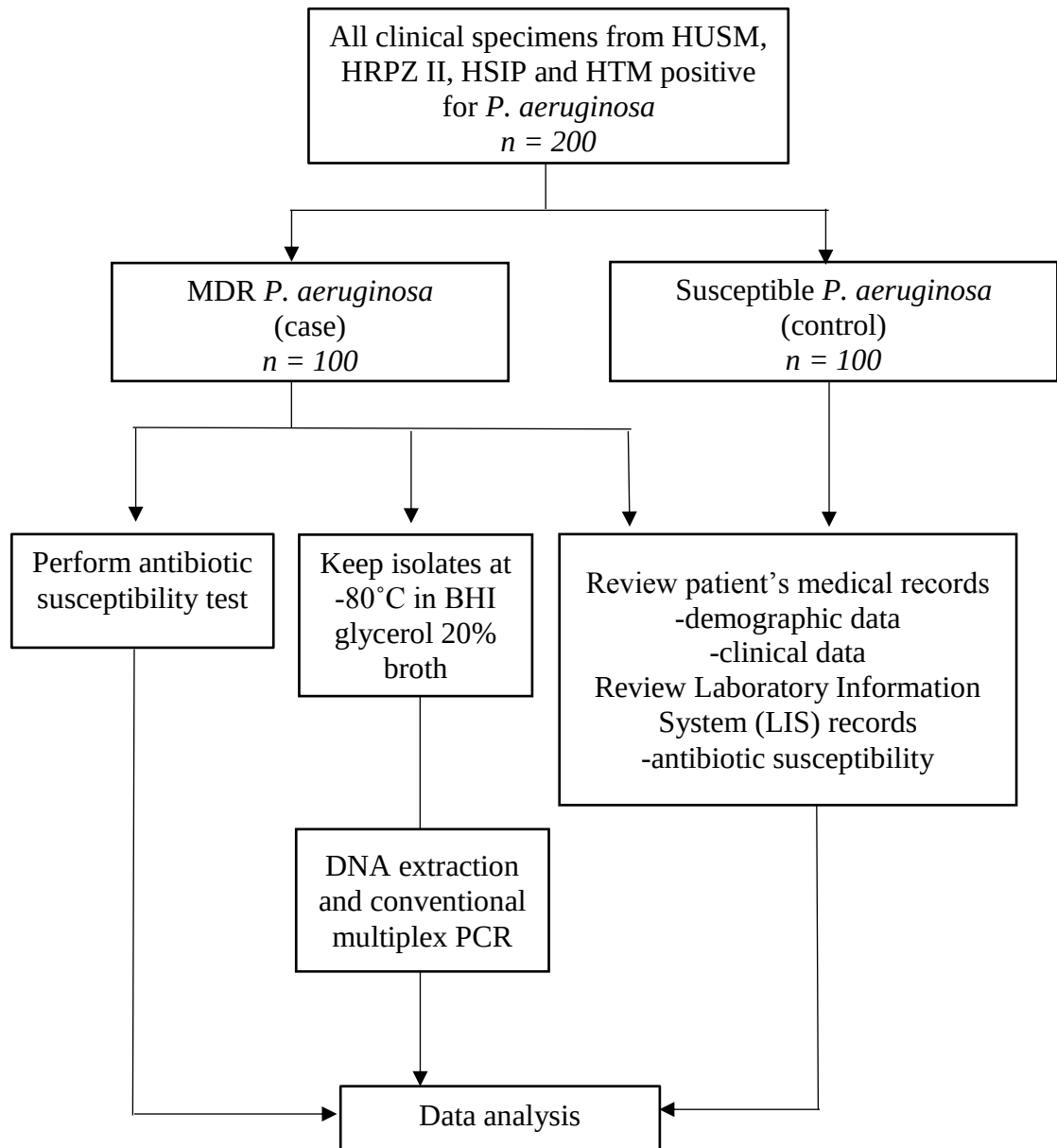


Figure 1-1 Flow chart of the study

1.6 Conceptual framework

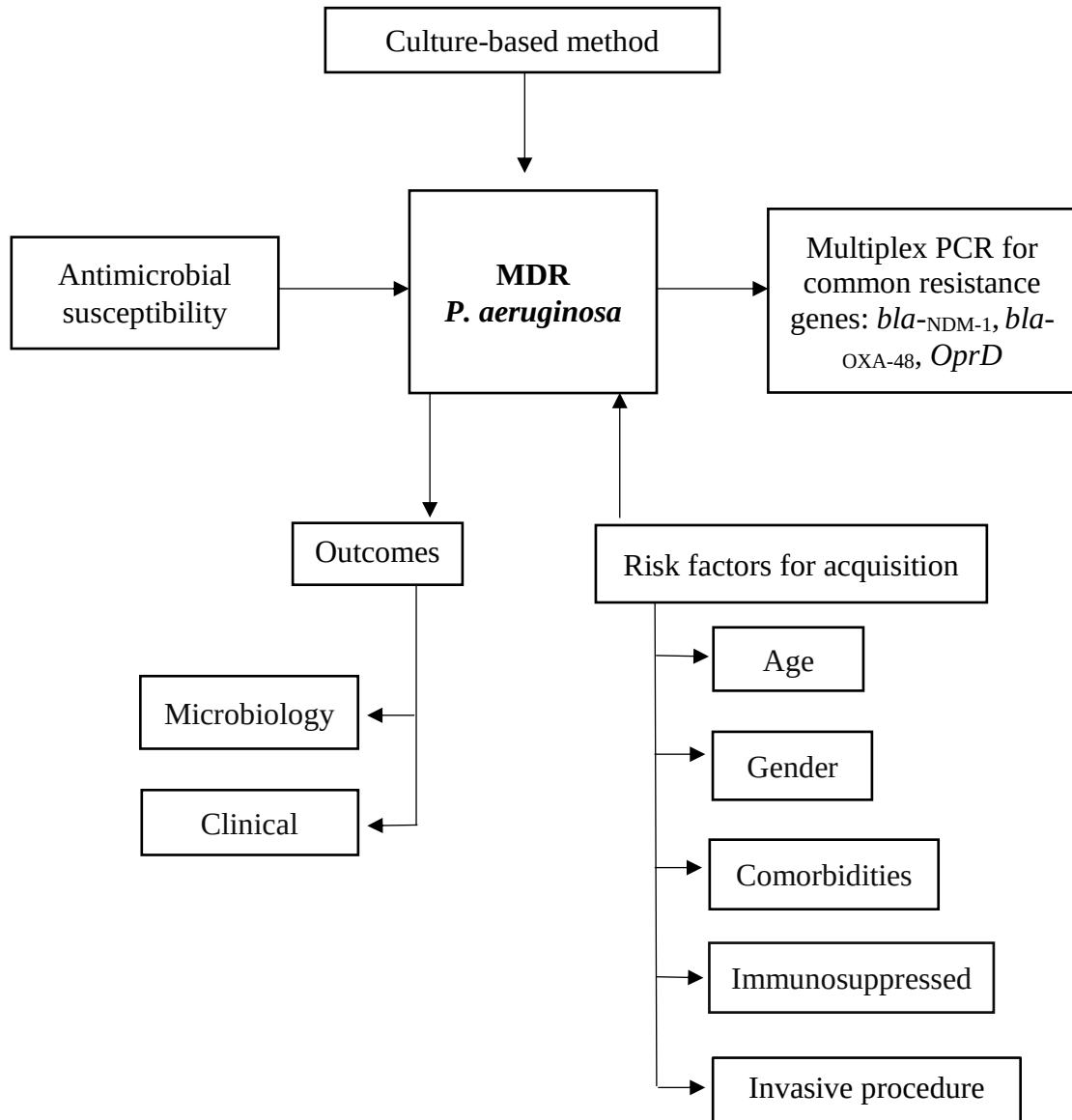


Figure 1-2 Conceptual framework of the study

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