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The Antibiotic Resistance Profile of *Pseudomonas Aeruginosa* in a Tertiary Medical Center from Malaysia

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Abstract

Background: *Pseudomonas aeruginosa is a notorious gram-negative bacterium that has become a global public health concern owing to the emergence of multi- and pandrug-resistant strains. This study sought to determine the antibiotic susceptibility profile of P. aeruginosa in a tertiary medical center from Malaysia.*

Materials and Methods: *Each isolate's identity was confirmed using the VITEK 2 GN kit, and subjected to antibiotic susceptibility testing using the VITEK 2 AST-N374 card (for testing against piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin and ciprofloxacin) and Etest strips (for testing against doripenem and polymyxin B). Isolates which were not susceptible to >1 carbapenem were tested for carbapenemase production using the modified carbapenem inactivation method (mCIM).*

Results: *Out of 102 isolates studied, 64 (62.7%) were fully susceptible to all the antibiotics tested. Twenty-six (25.5%) were resistant to >1 antibiotic from >2 antibiotic classes, and 21 (20.6%) were resistant to >1 antibiotic from >3 classes. Susceptibility was highest with polymyxin B (100%) and lowest with piperacillin-tazobactam (64.7%). Carbapenem susceptibility was between 78.4% to 81.4%. Out of 22 isolates which were not susceptible to >1 carbapenem, 18 (81.8%) were not susceptible to all three carbapenems.*

Conclusion: *More than half of our P. aeruginosa isolates were fully susceptible to all the anti-pseudomonal antibiotics tested. Multidrug-resistant strains accounted for between 20% to 25% of all our P. aeruginosa isolates. Through mCIM testing, carbapenemase production did not appear to be the dominant resistance mechanism.*

Keywords: *Carbapenem, multidrug resistance, piperacillin-tazobactam, polymyxin B, Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is an ancient bacterium first described more than a century ago by Migula in 1894.[1] It is a ubiquitous non-fermenting and motile gram-negative bacterium easily recognized from its characteristic blue-green pigment. This bacterium is not usually considered part of normal human flora, but owing to its inherent capability to survive on minimal nutritional requirements and produce biofilms, it is able to withstand harsh environmental conditions which has facilitated its persistence in hospital settings.[2,3] The gamut of diseases attributable to *P. aerugionosa* is extensive – it is implicated as a causative agent of serious infections such as bacteremia (often in association with neutropenia or vascular catheterization),

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pneumonia (particularly in mechanically ventilated patients), urinary tract infections (chiefly in patients with indwelling urinary catheters or stents), as well as skin and soft tissue infections (especially in the setting of skin burns or trauma). Moreover, *P. aeruginosa* infections have a propensity to become chronic and hard-to-eradicate.[3]

In 2008, a group of organisms was dubbed "the ESKAPE bugs" due to their legendary ability to escape the action of antimicrobial agents, with the "P" essentially referring to *P. aeruginosa.*[4] *P. aeruginosa* is regarded as an "ESKAPE bug" due to the presence of various antibiotic resistance mechanisms in this versatile bacterium. The bacterium may be endowed with low outer-membrane permeability to antimicrobial agents, various drug efflux pumps, or antibiotic-inactivating enzymes. These mechanisms may even be present concurrently to confer multidrug resistance.[5] Antimicrobial susceptibility testing is therefore essential in the management of *P. aeruginosa* infections.[5] Local institutional data on the antimicrobial susceptibility profile (antibiogram) of this bacterium will assist clinicians in choosing the most efficacious antibiotic for empirical treatment which may not necessarily be the most broad-spectrum or most financially costly drug. Therefore, the aim of this study was to construct a *P. aeruginosa* antibiogram to assist the formulation of institutional treatment guidelines that address infections likely to be caused by *P. aeruginosa.*

Materials and Methods

Study design

This cross-sectional study over a period of 18 months was conducted from January 2020 until June 2021. Non-duplicate *P. aeruginosa* isolates were collected from sterile specimens (e.g., blood, tissue, bone and body fluids) of patients who presented to Hospital Canselor Tuanku Muhriz (HCTM) in the capital city of Malaysia for various medical and surgical conditions.

Isolate identification

Any bacterium that produced a diffusible green, blue or blue-green pigment on routine bacteriological media was subjected to an oxidase test. The oxidase test was performed using a filter paper disk moistened with a freshly prepared 1% solution of tetramethyl-p-phenylenediamine dihydrochloride. The ability of the tested organism (grown on either blood or Mueller Hinton agar) to cause the oxidase reagent to turn from colourless to dark purple within 10 seconds of being smeared was regarded as oxidase-positive.

To confirm the identity of *P. aeruginosa*, a commercial biochemical identification kit for gram-negative bacteria, VITEK 2 GN (bioMerieux, Inc., USA), was utilized. The biochemical identification was performed as per the manufacturer's protocol. A VITEK 2 GN identification percentage of at least 85% was taken as evidence of correct identification. Biochemical identification was sought because other pseudomonads (e.g., *Pseudomonas fluorescens*) may also produce pigments which are visually similar to that released by *P. aeruginosa.*

Antibiotic susceptibility testing

Once pure growths of young (18-24 hours old) *P. aeruginosa* colonies were obtained, the isolates were subjected to antibiotic susceptibility testing using the VITEK 2 AST-N374 card for gram-negative bacteria (bioMerieux, Inc., USA), as per the manufacturer's instructions. The card provided the antibiotic minimal inhibitory concentrations (MICs) for, among others, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin and ciprofloxacin. All MIC values were interpreted according to the 2019 edition of the Clinical & Laboratory Standards Institute (CLSI) document M100[6].

Whenever the VITEK 2 AST-N374 card reported a carbapenem-resistant MIC, the isolate's MIC to doripenem and polymyxin B (which were not included in the card's panel) were determined using relevant Etest strips (bioMerieux SA, France), in accordance to the manufacturer's instructions. Before reading the MIC values, the Mueller Hinton (MH) agar plates containing the Etest strips were visually inspected to ensure no contamination had occurred during incubation and that a confluent bacterial lawn had been achieved. The MIC for each antibiotic was read by recording the point at which the inhibition ellipse intersected the side of the Etest strip.

Detection of carbapenemase production

A modified carbapenem inactivation method (mCIM) was perfomed as described by the CLSI whenever a *P. aeruginosa* isolate had either an intermediate or resistant MIC (i.e., non-susceptible) to even a single carbapenem agent. Essentially, for each *P. aeruginosa* isolate, a 10 ml inoculum obtained from a pure overnight colony was suspended in 2 ml of trypticase soy broth (TSB). A 10 g meropenem disk was added to the TSB suspension, followed by an incubation at 35°C in ambient air for 4 hours. Just before the completion of the TSB-meropenem disk incubation, a 0.5 McFarland suspension of a carbapenem-susceptible indicator organism (*Escherichia coli* ATCC^{Ò} 25922) was prepared in saline and seeded onto an MH plate within 15 minutes. After allowing the inoculated MH agar plate to dry for 5-10 minutes, the meropenem disk from the TSB suspension was removed and placed on the MH agar plate inoculated with *E. coli*. The

meropenem disk-containing MH agar plate was then incubated at 35°C in ambient air for 18-24 hours. The production of carbapenamase by a *P. aeruginosa* isolate was inferred either by a meropenem disk inhibition zone of £15 mm in diameter or the presence of colonies within the inhibition zone, while the absence of carbapenemase was indicated by meropenem inhibition zones of ³19 mm. Isolates which formed inhibition zones of between 16 and18 mm were interpreted as indeterminate for carbapenemase production.

Results

Demographic data of study subjects

Table 1 shows the demographic data of study subjects. A total of 102 non-repetitive *P. aeruginosa* isolates from 102 different patients were included in the study. There were slightly more male patients (52.9%) compared to female patients (47.1%). The proportion of fully susceptible *P. aeruginosa* isolates was higher in the female gender (69% vs. 57%). Most of the patients (30.4%) were aged between 60-69 years, with the median age being 63 years. For most age groups, the proportion of fully susceptible *P. aeruginosa* isolates predominated. However, for the 50-59 years age group, half of the *P. aeruginosa* isolates were resistant to >1 antibiotic. Regarding ethnicity, most patients were Malays (60.8%), followed by Chinese (34.3%) and Indians (2.9%). Irrespective the ethnicity, the percentage of fully susceptible *P. aeruginosa* isolates predominated.

Table I : *Demographic data of study subjects*

Characteristic	Total (n=102)	Susceptible to all antibiotic agents (n=64)	Resistant to at least one antibiotic agent (n=31)	Resistant to at least one agent from >2 antibiotic classes (n=26)	Resistant to at least one agent from >3 antibiotic classes (n=21)
Gender					
Male	54 (52.9%)	31 (48.4%)	18 (58.1%)	16 (61.5%)	13 (61.9%)
Female	48 (47.1%)	33 (51.6%)	13 (41.9%)	10 (38.5%)	8 (38.1%)
Age group (yr)					
<9	6 (5.9%)	6 (9.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
10-19	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
20-29	5 (4.9%)	4 (6.2%)	1 (3.2%)	1 (3.9%)	1 (4.8%)
30-39	4 (3.9%)	2 (3.1%)	1 (3.2%)	1 (3.9%)	1 (4.8%)
40-49	12 (11.8%)	9 (14.1%)	3 (9.7%)	3 (11.5%)	3 (14.3%)
50-59	12 (11.8%)	5 (7.8%)	5 (16.1%)	4 (15.4%)	4 (19.0%)
60-69	31 (30.4%)	15 (23.4%)	14 (45.2%)	11 (42.3%)	8 (38.1%)
70-79	25 (24.5%)	17 (26.6%)	7 (22.6%)	6 (23.1%)	4 (19.0%)
>80	7 (6.9%)	6 (9.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ethnicity					
Malay	62 (60.8%)	37 (57.8%)	20 (64.5%)	18 (69.2%)	15 (71.4%)
Chinese	35 (34.3%)	22 (34.4%)	11 (35.5%	8 (30.8%)	6 (28.6%)
Indian	3 (2.9%)	3 (4.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Foreigner	2 (2.0%)	2 (3.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Antibiotic susceptibility profile of isolates

Out of 102 isolates, 64 (62.7%) were fully susceptible to all the 10 antibiotics from a total of six antibiotic classes, viz., anti-pseudomonal penicillins, anti-pseudomonal cephalosporins, carbapenems, fluoroquinolones, aminoglycosides and polymyxins. Thirty-eight (37.3%) isolates were resistant to at least one antibiotic agent. Twenty-six (25.5%) isolates were resistant to at least one agent from >2 antibiotic classes, and 21 (20.6%) were resistant to at least one agent from >3 classes (Table 1). As shown in Table 2, non-susceptibility was highest with the anti-pseudomonal penicillin class (typified by piperacillin-tazobactam), followed by the cephalosporin (represented by ceftazidime and cefepime), the carbapenem (represented by meropenem, imipenem and doripenem), the fluoroquinolone (typified by ciprofloxacin), and the aminoglycoside (represented by gentamicin and amikacin) classes. None of the isolates were resistant to polymyxin B. For each antibiotic tested, 50% of the isolates could be inhibited by an MIC which was low enough to be interpreted as susceptible, although (with the exception of polymyxin B) only an MIC which was high enough to be interpreted as resistant could inhibit 90% of our isolates.

As presented in Table 3, out of 22 *P. aeruginosa* isolates which were not susceptible (i.e., testing either intermediate or resistant) to at least one carbapenem, 18 (81.8%) were non-susceptible to all three carbapenems tested and four were still susceptible to at least one carbapenem class member. The mCIM test was only found to be positive in one-third of the pan-carbapenem-non-susceptible isolates and negative in all the carbapenem-non-susceptible isolates which were still susceptible to at least one carbapenem agent. There was no statistically significant association between the extent of carbapenem non-susceptibility and mCIM positivity.

Table 2 : *Antibiotic susceptibility profile of Pseudomonas aeruginosa*

Antibiotic	Susceptible, no. (%)	Intermediate, no. (%)	Resistant, no. (%)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Piperacillin-tazobactam	66 (64.7)	8 (7.8)	28 (27.5)	8	e”128*
Ceftazidime	74 (72.5)	2 (2.0)	26 (25.5)	2	e”64*
Cefepime	76 (74.5)	7 (6.9)	18 (17.6)	2	e”32*
Meropenem	80 (78.4)	5 (4.9)	17 (16.7)	0.5	e”16*
Imipenem	83 (81.4)	0 (0.0)	19 (18.6)	2	e”16*
Doripenem	83 (81.4)	4 (3.9)	15 (14.7)	0.38	e”32*
Ciprofloxacin	87 (85.3)	1 (1.0)	14 (13.7)	0.25	e”4*
Gentamicin	88 (86.3)	0 (0.0)	14 (13.7)	d”1	e”16*
Amikacin	90 (88.2)	1 (1.0)	11 (10.8)	4	e”64*
Polymyxin B	102 (100.0)	0 (0.0)	0 (0.0)	1.5	2

MIC: minimal inhibitory concentration, MIC_{50} and MIC_{90}: antibiotic MIC capable of inhibiting 50% and 90% of isolates, respectively, *indicates resistant MIC

Table 3 : *mCIM results for carbapenem-non-susceptible Pseudomonas aeruginosa*

Extent of carbapenem non-susceptibility	mCIM result		p-value*
	Positive	Negative	
All three carbapenems	6 (27.3%)	12 (54.5%)	0.541
Less than three carbapenems	0 (0.0%)	4 (18.2%)	

mCIM: modified carbapenem inactivation method, *derived from Fisher’s exact test

Discussion

Over the years, multidrug-resistant (MDR) *P. aeruginosa* has been increasingly isolated from hospitalized patients and is of particular concern in critically ill and immunocompromised patients. The global prevalence of MDR *P. aeruginosa* is now expected to be between 15% and 30% in multiple geographical areas.[7] Drug-resistant *P. aeruginosa* infections give rise to higher mortality, higher morbidity, additional resource utilization and increased financial costs. A meta-analysis found that patients infected with any resistant *P. aeruginosa* have an all-cause mortality of 34%, compared to 22% for those infected with susceptible *P. aeruginosa.*[8] The same meta-analysis also reported that there is at least a 2-fold increased risk of mortality with MDR *P. aeruginosa*.[8] Of noteworthy concern is resistance to carbapenems, which can catapult the mortality rate to as high as 71%.[9] MDR *P. aeruginosa* are also adept at spreading or transferring resistance determinants in vivo, particularly those encoding carbapenemases or extended-spectrum â-lactamases.[10] Thus, it is evident that knowing the prevalence of drug-resistant *P. aeruginosa* in any healthcare setting is no longer merely of academic interest.

Unfortunately, comparing prevalence or incidence data with other healthcare facilities is not so straightforward for the simple reason that the definition of "multidrug resistance" is inconsistent. Depending on the study or centre, "multidrug resistance" can either refer to resistance to at least one agent from >2 antibiotic classes,[11,12] or resistance to at least one agent from >3 antibiotic classes.[13-15] A study conducted in Hospital Kuala Lumpur (which is a healthcare facility located H"12 km away from HCTM) found that 19.6% of *P. aeruginosa* isolates were resistant to >2 antibiotic classes in 2007.[11] One and a half decades later, by employing the same MDR definition as Pathmanathan *et al.*, our rate of MDR *P. aeruginosa* appears to be higher at 25.5%. However, by defining MDR *P. aeruginosa* as resistance to >3 antibiotic classes, our centre's MDR *P. aeruginosa* frequency of 20.6% is still considerably lower compared to other major Asian nations, such as China (29.0%) and India (31.7%).[13,14] We are also fortunate to have no pandrug-resistant (defined as resistance to all anti-pseudomonal antibiotics, including polymyxins) *P. aeruginosa* isolates in our centre to date, although globally pandrug-resistant *P. aeruginosa* strains have already been reported in the literature for more than a decade.[16]

Our *P. aeruginosa* isolates were least susceptible to piperacillin-tazobactam, with a susceptibility rate of 64.7%. This observation is in stark contrast to the Hospital Kuala Lumpur study which reported that piperacillin-tazobactam was the most active anti-pseudomonal antibiotic, with a susceptibility rate of 91.8%.[11] This difference in susceptibility could be due to variations in antibiotic prescribing practices between hospitals, which in turn result in different drug selection pressures being applied on the organism. For instance, if piperacillin-tazobactam is favored over cefepime for the empirical treatment of high-risk adult patients with neutropenic sepsis, one could expect a higher resistance rate to be recorded for the former antibiotic. With a susceptibility of 100%, polymyxin B appeared to the best antibiotic against *P. aeruginosa* in our study, although we are cognizant that the in vitro susceptibility testing of polymyxin B is fraught with difficulties.[17] The CLSI recommends only broth microdilution as the reference method to perform polymyxin B susceptibility testing, although we utilized an alternative method (i.e., Etest), akin to what Falagas *et al.* performed in their study.[16] Notwithstanding the CLSI's recommendation, the Etest (which is fundamentally a gradient diffusion method) has been shown to correlate well with the reference broth microdilution method.[18]

Susceptibility testing issues aside, polymyxin B has been found to be mediocre to other anti-pseudomonal antibiotics, as signified by the higher rate of in-hospital mortality associated with its usage.[19] Moreover, being the "antibiotic of last resort" in our antibiotic arsenal against *P. aeruginosa* (and other gram-negative pathogens), polymyxin B should be prescribed judiciously and sparingly. Some authorities advocate combination empirical therapy (sans the polymyxins) for severe *P. aeruginosa* infections, especially among critically ill patients. The rational for combination therapy is to enhance the prospect of selecting an effective antibiotic for empirical therapy, rather than to hamper resistance from developing during definitive therapy, or to obtain benefit from antibiotic synergism.[9] Looking at our own data, agents from the aminoglycoside and fluoroquinolone classes (which have documented susceptibilities in excess of 85%) are excellent pairing partners to the beta-lactam agents (including the carbapenems).[20]

The mechanisms conferring resistance to carbepenems can be expediently divided into carbapenemase-producing (CP) and non-CP mechanisms. The former is particularly pertinent for infection control and epidemiologic reasons, because the carbapenemase genes are borne on mobile genetic elements (e.g., plasmids and transposons) that can be transmitted horizontally to other gram-negative organisms.[21,22] Additionally, it is believed that CP bacteria are more virulent than their carbapenem-resistant but non-CP counterparts.[23] The mCIM and its predecessor (known

simply as CIM) were initially developed to detect carbapenemase production in carbapenem-resistant Enterobacteriaceae.[21] However, the mCIM has also been found to possess excellent sensitivity (98%) and specificity (95%) in identifying carbapenemase production in *P. aeruginosa*, and is now recommended by the CLSI for the same purpose.[6,22] Consistent with the published literature that the most frequent mechanism of carbapenem resistance in *P. aeruginosa* is related to oprD porin mutations, it is not surprising that most of our own isolates with pan-carbapenem non-susceptibility were actually mCIM-negative.[22] The fact that we found no statistically significant association between mCIM positivity and the extent of carbapenem resistance in our study essentially means that most of our *P. aeruginosa* with pan-carbapenem-resistant phenotypes are not CP bacteria, and thus do not pose infection control nightmares.

Conclusions

More than half of our center's *P. aeruginosa* isolates were still fully susceptible to all commonly used anti-pseudomonal antibiotics. MDR strains only accounted for, depending on the definition used, either 20% to 25% of all *P. aeruginosa* isolates. Antibiotic resistance was most evident with piperacillin-tazobactam and least pronounced with polymyxin B, with the latter documenting zero resistance. Although most of our isolates were fully carbapenem-susceptible, slightly less than one-fifth were pan-carbapenem-non-susceptible. Fortunately, mCIM testing showed that amongst our pan-carbapenem-non-susceptible *P. aeruginosa* isolates, carbapenemase production was not the dominant resistance mechanism.

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Conflicts of interest: None

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