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ORIGINAL ARTICLE

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Prevalence and susceptibility profile of carbapenem-resistant pseudomonas aeruginosa (CRPA) at Dr. Soetomo Public Hospital, Surabaya, from January to December 2021



Andritta Febriana¹, Agung Dwi Widodo^{2*}, Muhammad Vitanata Arfijanto³

ABSTRACT

Introduction: Pseudomonas aeruginosa is a pathogen that frequently causes healthcare-associated infections (HAI), which has also been associated with high mortality and morbidity rates. Carbapenems have been widely utilized as empiric therapy for *P. aeruqinosa* infections since these organisms have intrinsic resistance to various antibiotics. Therefore, the high rate of CRPA infection became the reason for conducting this study to determine the prevalence and susceptibility pattern of CRPA in Dr. Soetomo Public Hospital, Surabaya, from January to December 2021.

Method: The researcher employed descriptive observational study from secondary data with the first isolate sample of P. aeruginosa per specimen per patient that has been identified by the BD Phoenix[™] automated identification and susceptibility testing system from specimens of urine, blood, sterile fluid, pus, tissue, and sputum that are phenotypically resistant to meropenem or imipenem antibiotics, examined at the Clinical Microbiology Unit of Dr. Soetomo Public Hospital, Surabaya, from January to December 2021.

Result: Of the total P. aeruginosa isolates, 149 CRPA isolates were obtained. The researcher conveyed that the majority of the samples were male (59.1%); the most comorbid cases were diabetes mellitus with complications (22.4%) found in the intensive care unit (40.3%); the majority of specimens were from the respiratory tract (43%); the highest antibiotic susceptibility was amikacin (62.4%); the prevalence of CRPA in Dr. Soetomo Public Hospital, Surabaya, from January to December 2021 reached 21.25%.

Conclusion: In this study, CRPA isolates showed the highest sensitivity to amikacin, and the highest distribution of CRPA events was found in the intensive care unit.

Keywords: CRPA, incidence rate, susceptibility profile.

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> immunocompromised patients, patients treated in the ICU, or patients with severe comorbidities.^{5,6} Thus, *P. aeruginosa* is commonly linked to healthcare-associated infections.7,8

> P. aeruginosa has an innate resistance to several antibiotics, such as ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone. ertapenem, tetracycline, and tigecycline.^{9,10} Carbapenem is a broad-spectrum β -lactam antibiotic with bacterial cell wall synthesis inhibitory activity. Carbapenem has extremely high potency against Gram-negative and Grampositive bacteria among other β-lactam drugs. In addition, carbapenem is a lastline drug for treating cases of patients with MDR pathogenic infections, including P.

aeruginosa.7,11,12 The cause of carbapenemresistant P. aeruginosa (CRPA) is generally acquired due to a chromosomal mutation that causes loss or inactivation of the OprD (Outer membrane protein D) porin and/or overexpression of the efflux pump. Resistance to carbapenems can also be obtained through the acquisition of horizontal gene transfer (HGT) of the gene encoding carbapenemase. This B-lactamase is capable of hydrolyzing carbapenems and provides resistance to all β-lactam antibiotics.^{8,13,14}

The increase in CRPA rates can be caused by the emergence of resistance in these organisms after exposure to carbapenems and can occur due to the spread of CRPA from patient to patient.¹⁵ Resistance to these carbapenems,

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INTRODUCTION

The worldwide increase in infection with multidrug-resistant gram-negative (MDR) pathogens is a crucial global health problem. Southeast Asia has a high prevalence of MDR gram-negative bacteria, including multidrug-resistant non-fermenters such as P. aeruginosa.^{1,2} P. aeruginosa is an opportunistic pathogen that requires minimal nutrition for its survival, enabling it to easily adapt to and survive in an environment with extreme conditions, such as a hospital environment.^{3,4} These pathogens are commonly found on abiotic surfaces such as medical equipment, rooms, and showers and even can contaminate distilled water, and are widely isolated, especially in

especially when mediated by transferable carbapenemase-coding genes, can spread rapidly and cause outbreaks.¹¹ The increase in CRPA cases has become significant health and economic issue that deserves more attention given the limited options for an effective and safe therapy.^{1,16} In addition, carbapenemaseproducing CRPA strains cannot be treated with classical β -lactamase inhibitors, thus, it can lead to poor clinical conditions, such as an increased risk of mortality, morbidity, length of hospitalization, and increased treatment costs.5

HAI-related CRPA cases have increased globally in recent years.5,17 In 2017, WHO classified CRPA as a priority pathogen of the 'critical pathogen' category for research and development of the latest antibiotics.18 In Indonesia, the incidence of CRPA from the ICU of Dr Cipto Mangunkusumo Hospital reached 21.9% in 2011.19 Based on the surveillance of antibiotic resistance in class A and B hospitals in Indonesia in 2020, the prevalence of CRPA amounted to 25% of all specimens and rooms from all hospitals.²⁰ Therefore, the high rate of CRPA infection became the reason for conducting this study to determine the prevalence and susceptibility pattern of CRPA in Dr. Soetomo Public Hospital, Surabaya, from January to December 2021.

MATERIAL AND METHODS

The research sample included all data on *Pseudomonas aeruginosa* isolates identified by the BD Phoenix[™] automated identification and susceptibility testing system from urine, blood, sterile fluid, sputum, pus, and tissue specimens that are phenotypically resistant to carbapenem antibiotics examined at the Clinical Microbiology Unit of Dr. Soetomo Public Hospital, Surabaya. The isolates taken were the first isolates from each type of specimen in the same patient from January 1 to December 31, 2021. This research was approved by the Health Research Ethics Committee of Dr. Soetomo Public Hospital, Surabaya, based on Ethical Clearance No. 1411/122/4/IV/2022.

The data analyzed in this study included CRPA distribution by age and sex, CRPA distribution by comorbidities, CRPA distribution by the patient clinical outcome, CRPA distribution by care unit, CRPA distribution by the specimen, and CRPA antibiotic susceptibility pattern.

The data on the results of the antibiotic susceptibility test of carbapenem resistant P. aeruginosa (CRPA) isolates were stated in the Susceptible, Intermediate, and Resistant categories. The methods employed were disk diffusion for testing the antibiotic, such as fosfomycin, and the semi-automatic BD Phoenix[™] automated identification and susceptibility testing system for testing antibiotics, such as gentamicin, amikacin, imipenem, meropenem, ceftazidime. cefepime, ciprofloxacin, levofloxacin, aztreonam, piperacillin-tazobactam, according to the group P. aeruginosa test at the Clinical and Laboratory Standards Institute (CLSI). The data obtained were presented in the form of tables containing frequency and percentage data for analysis.

RESULTS

This research collected 701 data from culture and susceptibility test of *P. aeruginosa*, which was divided into 149 (21.25%) carbapenem-resistant *P. aeruginosa* (CRPA) and 552 carbapenem susceptible *P. aeruginosa* (CSPA), which derived from all specimens of blood, sterile fluid, urine, sputum, pus, and tissue recorded at the Clinical Microbiology unit of Dr. Soetomo Public Hospital, Surabaya. Based on the distribution data of the study sample, CRPA was most commonly found

in the male sex, reaching 88 (59.06%) people, while in the female sex, it reached 61 (40.93%) people. Meanwhile, based on the distribution of samples by age, the most CPRA was found in patients aged > 45 years, as many as 78/149 (52.34%), which were divided into middle age adults (54 patients or 36.2%) and seniors adult (24 patients or 16.1%), as presented in table 1.

The distribution of CRPA patients with comorbid factors based on the Charlson comorbidity index indicated that 107/149 (71.81%) patients had comorbidities and one patient could have more than one comorbid. The highest frequency of comorbidity was diabetes, which reached 42 (28.18%) cases, which was divided into 18 (12.1%) cases of diabetes without complications and 24 (16.1%) cases of diabetes with complications. The second most common comorbidities were Cerebrovascular disease and chronic lung disease, each with 12 (8.1%) cases (Table 2).

This study revealed that more than 70% of patients were estimated to have comorbidities, but no specific comorbidities were associated with an increased risk of CRPA incidence. The most comorbid in this study was diabetes, which reached 28.18% of cases, divided into diabetes without complications (12.1%) and diabetes with complications (16.1%). The clinical outcomes of CRPA patients in this study demonstrated that 88 (59.06%) patients were alive and 61 (40.93%) patients were deceased (Table 3). From all care units, the highest number of CRPA was found in intensive care units, including ICU, NICU, ROI, and HCU rooms, which reached 60 (40.3%) cases, followed by surgical care unit, amounting to 32 (21.5%) cases (Table 4).

The highest incidence of CRPA derived from respiratory tract specimens which

 Table 1.
 CRPA distribution by age and sex.

| Age (years) | Toddler (0-5) | Child (6-11) | Teenager (12-25) | Adult (26-45) | Middle Age Adult (46-65) | Senior Adult (> 65) | Total |
|-------------|------------------|-----------------|---------------------|------------------|-----------------------------|------------------------|----------|
| Male | 15 | 5 | 10 | 14 | 35 | 9 | 88 |
| | (17%) | (5.7%) | (11.4%) | (15.9%) | (39.8%) | (10.2%) | (59.06%) |
| Female | 5 | 0 | 7 | 15 | 19 | 15 | 61 |
| | (8.2%) | (0.0%) | (11.5%) | (24.6%) | (31.1%) | (24.6%) | (40.93%) |
| Total | 20 | 5 | 17 | 29 | 54 | 24 | 149 |
| | (13.4%) | (3.4%) | (11.4%) | (19.5%) | (36.2%) | (16.1%) | (100%) |

reached 64 (43%) specimens, which consisted of 23 spontaneous sputum specimens, 40 endotracheal tube aspirates, and 1 bronchial lavage. CRPA isolates from pus specimens were 41 (27.5%) specimens, from urine specimens were 30 (20.8%) specimens, from blood specimens were 8 (5.4%) specimens, and from sterile fluid specimens were 5 (3.4%) specimens (Table 5). The results of the antibiotic susceptibility test of all CRPA isolates demonstrated the highest susceptibility to *amikacin* as much as 93/149 (62.4%), followed by *piperacilintazobactam* at 57/149 (38.3%), *aztreonam* at 40/149 (26.8%), *gentamicin* at 38/148 (25.7%), *ceftazidime* at 31/148 (20.9%), *cefepime* at 28/148 (18.9%), *ciprofloxacine* (17.4%), *levofloxacine* (16.2%), *meropenem* (13.5%), *fosfomycin* (10.7%),

Table 2. CRPA distribution by comorbidity.

| Comorbidity | Total (n= 107) |
|-----------------------------------|-------------------|
| Diabetes with complications | 24 (22.4%) |
| Diabetes without complications | 18 (16.8%) |
| Cerebrovascular disease | 12 (11.2%) |
| Chronic lung disease | 12 (11.2%) |
| Mild liver disease | 11 (10.3%) |
| Malignant solid tumor | 11 (10.3%) |
| Kidney illness | 10 (9.3%) |
| Peripheral vascular disease | 8 (7.5%) |
| Tumors with metastases | 5 (4.7%) |
| Congestive heart failure | 3 (2.8%) |
| Acute myocardial infarction | 2 (1.9%) |
| HIV/AIDS | 2 (1.9%) |
| Hemiplegia/paraplegia | 2 (1.9%) |
| Dementia | 1 (0.9%) |
| Leukemia | 1 (0.9%) |
| Lymphoma | 1 (0.9%) |
| Rheumatism | 0 (0%) |
| Moderate to serious liver disease | 0 (0 %) |
| Peptic ulcer | 0 (0%) |

Table 3. CRPA distribution by patient clinical outcome.

| Living Patient | Deceased patient | Total |
|----------------|------------------|------------|
| n (%) | n (%) | n (%) |
| 88 (59.06%) | 61 (40.93%) | 149 (100%) |

Table 4. CRPA distribution by care unit.

| Care Units | CRPA n (%) | |
|----------------------------|---------------|--|
| ICU, NICU, ROI, HCU | 60 (40.3) | |
| Surgical Care Unit | 32 (21.5) | |
| Medical Care Unit | 27 (18.1) | |
| Pediatric Care Unit | 15 (10.1) | |
| Special Isolation Room | 5 (3.4) | |
| Obstetrics and Gynaecology | 4 (2.7) | |
| Emergency Room | 6 (4) | |
| Total | 149 (100) | |

Table 5. CRPA distribution by specimen.

| Blood | Sterile fluid | Sputum | Urine | Pus and tissue | Total |
|---------|---------------|---------|-----------|----------------|-----------|
| n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
| 8 (5.4) | 5 (3.4) | 64 (43) | 31 (20.8) | 41 (27.5) | 149 (100) |

and *imipenem* (2.7%). The highest number of CRPA isolates resistant to *imipenem* antibiotics was 140/149 (94%) isolates. *Fosfomycin* revealed resistance of 122/140 (87.1%), *cefepime* and *levofloxacine* each of 120/148 (80.5%), *ciprofloxacine* of 119/149 (79.9%), *meropenem* of 117/148 (79.1%), and *gentamicin* of 106/148 (71.6%) (Figure 1).

DISCUSSION

In this study, the prevalence of CRPA reached 21.25% which is in line with previous study which revealed the prevalence of CRPA from the ICU of Dr. Cipto Mangunkusumo Hospital in 2011 was 21.9%.²¹ However, the prevalence of CRPA in this study was not restricted to the ICU. The results of this study were higher than the study conducted by Tartof et al. (2018) which demonstrated a prevalence of CRPA of 20.2% in hospitalized patients in 14 medical centers from 2011 to 2015.²² Meanwhile, other study revealed the prevalence of HAIs caused by CRPA was 40%, however, this study did not distinguish between HAIs patients and non-HAI patients.19

Based on the aforementioned data, CRPA was mostly found in male patients (59.06%). These data are in accordance with a study conducted by Kang et al. (2003), which demonstrated that most patients with CRPA were male (66.2%).²¹ Similarly, in their study, Tartof et al. (2018) also revealed that most patients with CRPA were male (59.4%).²² In this study, patients over 45 were identified to have the highest prevalence of CRPA. Male patients from middle age adults to senior adults included in the category with higher rates of infection acquisition. This result is in line with other research findings that age has become a significant risk factor for P. aeruginosa infection and that nosocomial infection generally occurs in males.^{23,24} Additionally, elderly patients are more susceptible to infection with multiresistant microorganisms. This is brought on by a decline in immune system activity, which also affects the body's structure and function, leading to other conditions such as the necessity for a catheter, malnutrition, and prolonged treatment.5,26

The most comorbid in this study was diabetes, in a study conducted by

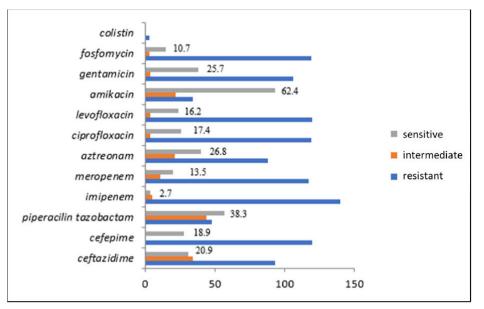


Figure 1. CRPA antibiotic susceptibility pattern.

Palavutitoi (2018), diabetes was the third most common comorbid after hypertension and chronic kidnev disease (CKD).7 Patients with CRPA infection have a higher risk of receiving appropriate antimicrobial therapy late, resulting in prolonged hospitalization, increased risk of subsequent antibioticresistant infections, as well as increased morbidity and mortality.^{22,27} The presence of virulence factors such as flagella, pili, and lipopolysaccharides as well as biologically active processes such as toxin secretion, biofilm formation, and quorum sensing has an impact on the outcome of P. aeruginosa infection.²⁵ In this study, the mortality rate reached 40.93%. Suarez et al., (2009) stated that CRPA was not associated with a higher mortality rate in hospitalized patients with bacteremia.²⁸

The global incidence of nosocomial infections caused by *P. aeruginosa* was estimated at 10-15% and around 10-20% was obtained from intensive care units.^{29,30} These research findings are in line with the research conducted by Tartof et al. (2018).²² The high rate of cross-transmission in intensive care units (Zavascki et al., 2006) and the use of a variety of broad-spectrum antibiotics in the ICU (Alhussain, 2021) contributed significantly to the incidence of CRPA infection.^{23,31} In addition, the use of invasive medical devices, such as the installation of ventilators and urinary catheters, are also at risk for infection

with CRPA. Patients in the intensive care unit are patients with severe diseases who frequently require invasive medical devices, which increases their risk of recurrent infections and complications.⁷ Several studies have discovered that using invasive medical devices is a significant determinant of CRPA.²² Patients on a ventilator have eleven times higher risk of infection with *P. aeruginosa* than those without a ventilator.²³

In this study, the most CRPA specimens were obtained from respiratory tract samples, i.e., sputum by 43%, where this result is in line with research conducted by Zavascki et al. (2006) with 50.3% specimens³¹; Karuniawati (2011)¹⁹ with 61/81 (75%) specimens; Tartof et al. (2018) with 61.45% specimens²²; and Alhussain (2021) with 60% specimens¹⁴. In this regard, Hospital Acquired Pneumonia Ventilator-Associated (HAP) and Pneumonia (VAP) were the most common nosocomial infections.³² P. aeruginosa was the most common cause of hospital pneumonia, the third most common cause of urinary tract infection, the fourth most common cause of surgical site infection, and the seventh most common pathogen isolated from blood.

P. aeruginosa has a strong tendency to become a pathogen that is resistant to various intrinsic and acquired antibiotics.⁸ *P. aeruginosa* susceptibility decreased significantly to carbapenem, ceftazidime, and ciprofloxacin (Alhussain, 2021).¹⁵ Additionally, *P. aeruginosa* is able to survive for a long time even under unfavorable conditions. These bacteria have various virulence factors, one of which produces biofilms that play a major role in the occurrence of nosocomial infections and resistance to antibiotics.^{33,34}

CRPA is often resistant to various drugs, causing difficulties in treating and controlling the infection.35 In this study, CRPA resistance ranged from 22.8 - 80.5% with the highest susceptibility to amikacin (62.4%), while susceptibility to other antipseudomonal ranged from 2.7 - 38.3%. This study is in line with the research conducted by Alhussain et al. (2021) which indicated the highest susceptibility to amikacin at 86.7%.23 Meanwhile, research conducted by Anggraini et al. (2018) showed that MDR-PA had the highest susceptibility to amikacin at 50.9%, lower than the susceptibility of amikacin in non-MDR-PA strains of 76.9%.³⁶ CRPA has more resistance than CSPA.^{22,35} Amikacin is a semisynthetic aminoglycoside that is extremely resistant to enzyme modification, so many bacteria are sensitive to this antibiotic. A fairly good susceptibility to amikacin indicates that the use of amikacin is not too frequent considering that amikacin may cause the side effects of ototoxicity and nephrotoxicity.36

Colistin is the last line of therapy for CRPA multidrug treatment. The analytical study conducted by Goli et al. (2016) and Sheikh et al., (2019) revealed that colistin still has a good susceptibility value to MDR CRPA infection. However, these antibiotics are rarely used since they are nephrotoxic and neurotoxic.37,38 Although the prevalence of resistance to colistin is still low, the incidence of resistance to colistin has been reported in various countries.^{37,38} In this study, only three CRPA isolates were tested against colistin with 100% of the isolates being resistant. Due to the limited number, this statement cannot generally be concluded.

The increased incidence of imipenem resistance in *P. aeruginosa* is often associated with resistance to other antipseudomonal drugs. This statement is in line with the research conducted by Palavutitotai et al. (2018), which

demonstrated that among the CRPA strains, 53.5% were XDR-PA, and 24.8% were MDR-PA. Patients with the XDR-PA strain tended to receive inappropriate empiric therapy and as many as 78.6% of them received carbapenem monotherapy.⁷

CRPA is generally acquired as a result of chromosomal mutations leading to loss or inactivation of the OprD porin and/or overexpression of the efflux pump. Resistance to carbapenems can also be obtained through the acquisition of horizontal gene transfer (HGT) of gene encoding carbapenemase. the This B-lactamase is able to hydrolyze carbapenems and provides resistance to all classes of β -lactam antibiotics (Botelho et al., 2019).⁵ Additionally, increased CRPA can be caused by selective pressure due to exposure to antibiotics and transmission through health workers or between patients. Thus, it requires efforts to control and prevent nosocomial infections as well as strict regulation and monitoring in the wise and rational use of antibiotics.¹⁵ This study had several limitations, including (1) The sample size of this study was limited to 149 inpatients at one hospital. (2) this research was conducted in Surabaya, so the situation may be different in other countries. (3) This study did not routinely test susceptibility to colistin. (4) Clinical data, laboratory and duration of use of antibiotics were not included in this study.

CONCLUSION

In this study, CRPA isolates showed the highest sensitivity to amikacin, and the highest distribution of CRPA events was found in the intensive care unit. The findings of this study are of paramount relevance for case identification, control measures, and optimal treatment strategies of *P. aeruginosa* infection in ICUs. The increasing prevalence of antimicrobial resistance among hospitalized patients continues to pose a challenge for practitioners.

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CONFLICT OF INTEREST

The authors declare that they have no financial or personal conflicts of interest in this work.

AUTHOR CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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