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Short Communication

Epidemiology and characterisation of carbapenem-non-susceptible *Pseudomonas aeruginosa* in a large intensive care unit in Jakarta, Indonesia

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ABSTRACT

The aim of this study was to describe the epidemiology and clinical impact of carbapenem-non-susceptible *Pseudomonas aeruginosa* (CNPA) in intensive care units (ICUs) of the national referral hospital of Indonesia. Adult patients admitted to ICUs were prospectively included. *Pseudomonas aeruginosa* were from clinical cultures and systematic screening. Environmental niches and healthcare workers (HCWs) were also screened. Susceptibility was determined phenotypically and the presence of carbapenemase genes was determined by PCR. Multiple loci variable-number tandem repeat analysis (MLVA) and multilocus sequence typing (MLST) were used for genotyping. Of the patients included in the study, 17/412 (4.1%) carried CNPA on admission and 34/395 (8.6%) became positive during their ICU stay. The acquisition rate was 18/1000 patient-days at risk. Of 16 environmental isolates, 12 (75.0%) were CNPA. HCWs screened negative. Acquisition of CNPA was associated with longer ICU stay (adjusted hazard ratio = 1.89, 99% confidence interval 1.12–3.13). Mortality was >40% among patients with CNPA versus <30% among those without CNPA ($P = 0.019$). Moreover, 83/119 (69.7%) CNPA carried either bla_{VIM} ($n = 36$), bla_{IMP} ($n = 23$) or bla_{GES-5} ($n = 24$). Four sequence types (STs) dominated (ST235, ST823, ST446 and ST357). Five major MLVA clusters were distinguished, two belonging to ST235 and the other three to ST823, ST446 and ST357. CNPA are introduced into these ICUs and some strains expand clonally among patients and the environment, creating endemic CNPA. VIM-, IMP- and GES-5 genes are prevalent. CNPA acquisition was associated with prolonged ICU stay and may affect ICU survival.

1. Introduction

The worldwide emergence of carbapenem-non-susceptible *Pseudomonas aeruginosa* (CNPA) compromises the treatment of pseudomonal infections [1]. Non-susceptibility to carbapenem antibiotics in *P. aeruginosa* is usually due to either a combination of mechanisms, including β-lactamase production, increased efflux pump activity and outer membrane modifications or to production of a carbapenemase as a single potent resistance mechanism; VIM, IMP and GES-5 carbapenemases are most commonly found around the world [2].

Little information exists on the epidemiology and impact of CNPA in Indonesia, the fourth most populous country in the world.

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In 2011, 21.9% of *P. aeruginosa* strains from the intensive care units (ICUs) of the national referral hospital in Jakarta were carbapenem-resistant, and four *P. aeruginosa* isolates contained the bla_{IMP} gene [3]. The aim of this study was to further delineate the clinical impact and molecular epidemiology of CNPA in ICUs of this hospital.

2. Materials and methods

2.1. Study design

A prospective observational study was performed from April–October 2013 and from April–August 2014 in the 12-bed adult ICU and 8-bed emergency room (ER)-ICU of Dr Cipto Mangunkusumo General Hospital (Jakarta, Indonesia), with an average of 1010 and 415 admissions per year, respectively. Both ICUs are open-plan wards [4].

Adult patients (age ≥18 years) admitted for >48 h were eligible. The first screening cultures were taken on the day of ICU admission. Informed consent was obtained from the patients or their relatives. Demographic and clinical characteristics were recorded on admission. Systemic inflammatory response syndrome (SIRS) and quick Sequential Organ Failure Assessment (qSOFA) score on admission were calculated.

The primary outcome measure was acquisition of a CNPA beyond 48 h of ICU admission. Acquisition of CNPA was defined as first detection of CNPA in a screening or clinical culture. Secondary outcome measures were ICU length of stay (LoS) and in-ICU mortality.

Screening cultures were obtained from the throat and rectum or stools on the day of admission, at the time of discharge from the ICU, and weekly if the patient's stay exceeded 7 days. Sterile cotton-tipped swabs were used for sampling, which were processed within 24 h. Additional samples were collected on clinical indication.

The environment was sampled twice in both ICUs (Supplementary Table S1). Screening of healthcare workers (HCWs) was performed once by throat and rectal swabs. HCWs were defined as all personnel (doctors, nurses, cleaning staff, administration staff, porters, nutritionist) working in the ICUs.

2.2. Microbiological methods

2.2.1. Isolation and identification

Swabs for screening were placed in 5 mL of trypticase soy broth (TSB) with 2 mg/L cefotaxime and 50 mg/L vancomycin, were incubated overnight and were subsequently subcultured on MacConkey agar, followed by identification of suspected colonies using VITEK®2 (bioMérieux, Marcy-l'Étoile, France). Blood cultures were collected in BACTEC® (BD, Franklin Lakes, NJ, USA) bottles. Other clinical specimens were inoculated onto blood and MacConkey agar plates and were processed as above. *P. aeruginosa* strains were stored at −80 °C in TSB with 10% v/v glycerol. Their identity was confirmed at Erasmus MC (Rotterdam, the Netherlands) by mass spectrometry (MALDI Biotyper®; Bruker, Coventry, UK).

2.2.2. Antimicrobial susceptibility testing

Susceptibility of screening isolates to carbapenems was determined by the Kirby–Bauer disk diffusion method using Mueller–Hinton plates (BD). Susceptibility of the clinical isolates was determined by VITEK®2. Carbapenem zone sizes and minimum inhibitory concentrations (MICs) were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2013 breakpoints (meropenem, <24 mm and MIC > 2 mg/L; imipenem, <20 mm and MIC > 4 mg/L). For detection of metallo-β-lactamases (MBLs), the imipenem/doripenem combination disk test with ethylene diamine tetra-acetic acid (EDTA) was performed [5].

2.2.3. DNA extraction and carbapenemase gene detection

DNA was extracted using InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA). PCR for Ambler class B MBLs (bla_{NDM}, bla_{VIM} and bla_{IMP}) was carried out using a T3000 Thermocycler (Biometra-Whatman, Germany) [6].

2.2.4. Clonal relatedness

Multiple loci variable-number tandem repeat analysis (MLVA) and in silico multilocus sequence typing (MLST) were used for typing (Supplementary Table S2) [7]. Briefly, 2 µL of 100 × diluted PCR product was analysed on an ABI3130xl Genetic Analyzer (Thermo Fisher Scientific, USA). Electropherograms were analysed using the MLVA plugin in BioNumerics® v.7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Typing data were analysed categorically.

MLST sequence types (STs) as well as carbapenemase gene subtypes were inferred from whole-genome sequencing data using the MLST plugin and sequence extraction tool from BioNumerics® v.7.6. The classical 7-digit in silico MLST profiles were obtained through BLAST using the PubMLST database hosted at https://pubmlst.org. Sequencing was performed using a HiSeq 2500 instrument (Illumina Inc., San Diego, CA, USA).

2.3. Statistical analysis

IBM SPSS Statistics v.24.0 (IBM Corp., Armonk, NY) was used for statistical analysis. Patients admitted to the adult ICU were compared with those admitted to the ER-ICU using χ^2 test or Fisher's exact test and Mann–Whitney *U*-test as appropriate. One-way analysis of variance (ANOVA) was used to compare patient characteristics according to their *P. aeruginosa* status. Univariate and multivariate analyses were performed to establish risk factors associated with mortality using a multivariate logistic regression model with backward selection and inclusion of variables with a *P*-value of <0.1 in the univariate analysis. Cox proportional regression was used to analyse risk factors for LoS. The Kaplan–Meier method was performed to construct survival curves. A *P*-value of <0.01 was considered statistically significant [8].

3. Results

3.1. Patient characteristics and outcomes

A total of 1211 patients were hospitalised in the ICUs (adult ICU, $n = 863$; ER-ICU, $n = 348$), of which 412 were included in this study (adult ICU, $n = 188$; ER-ICU, $n = 224$). Most of the non-eligible patients were excluded due to short LoS. There were no significant differences between patients in both ICUs, except that in the adult ICU most patients had been referred from another ward and more patients had malignancies (Supplementary Table S3). Therefore, data from the ICUs were analysed both separately and pooled.

Overall, 145/412 (35.2%) patients had at least one positive culture with *P. aeruginosa*, whereas the remaining 267 patients were free from *P. aeruginosa* on admission and during their ICU stay (Table 1). A total of 83 patients (20.1%) already carried *P. aeruginosa* on the day of admission, of whom 66 carried a carbapenem-susceptible *P. aeruginosa* (CSPA) and 17 carried a CNPA (Supplementary Fig. S1). Moreover, 34 patients acquired a CSPA and 34 patients acquired a CNPA. For the 51 patients with CNPA, 35 CNPA were obtained from screening, 6 from clinical cultures and 10 from both screening and clinical samples; there were no CNPA-positive blood cultures.

The dynamics of acquisition of *P. aeruginosa* in the ICUs is shown in Fig. 1A. Patients who acquired a CSPA had their first

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Table 1
Patient characteristics and outcomes according to *Pseudomonas aeruginosa* status[a].

Characteristic	Group 1 ($n=267$)	Group 2 ($n=60$)	Group 3 ($n=17$)	Group 4 ($n=34$)	Group 5 ($n=34$)	*P*-value[b]
Age (years) [median (IQR)]	46 (31–58)	44 (32–56)	43 (27–58)	49 (33–60)	47 (37–55)	0.895
Sex [*n* (%)]						0.530
Male	137 (51.3)	27 (45.0)	11 (64.7)	20 (58.8)	19 (55.9)	
Female	130 (48.7)	33 (55.0)	6 (35.3)	14 (41.2)	15 (44.1)	
Underlying diseases [*n* (%)]						
Cardiovascular						0.851
Yes	16 (6.0)	4 (6.7)	1 (5.9)	3 (8.8)	1 (2.9)	
No	251 (94.0)	56 (93.3)	16 (94.1)	31 (91.2)	33 (97.1)	
Cerebrovascular						0.006
Yes	12 (4.5)	6 (10.0)	0 (0)	4 (11.8)	7 (20.6)	
No	255 (95.5)	54 (90.0)	17 (100)	30 (88.2)	27 (79.4)	
Chronic kidney disease						0.130
Yes	13 (4.9)	6 (10.0)	3 (17.6)	2 (5.9)	1 (2.9)	
No	254 (95.1)	54 (90.0)	14 (82.4)	32 (94.1)	33 (97.1)	
Diabetes mellitus						0.262
Yes	24 (9.0)	1 (1.7)	1 (5.9)	3 (8.8)	4 (11.8)	
No	243 (91.0)	59 (98.3)	16 (94.1)	31 (91.2)	30 (88.2)	
Malignancy						0.379
Yes	74 (27.7)	17 (28.3)	6 (35.3)	14 (41.2)	7 (20.6)	
No	193 (72.3)	43 (71.7)	11 (64.7)	20 (58.8)	27 (79.4)	
Indication for ICU admission [*n* (%)]						0.005
Medical	78 (29.2)	22 (36.7)	9 (52.9)	11 (32.4)	20 (58.8)	
Surgical	189 (70.8)	38 (63.3)	8 (47.1)	23 (67.6)	14 (41.2)	
Referral from [*n* (%)]						0.120
Other ward in this hospital	141 (52.8)	26 (43.3)	10 (58.8)	25 (73.5)	20 (58.8)	
Other hospital	46 (17.2)	15 (25.0)	3 (17.6)	4 (11.8)	9 (26.5)	
Directly from ER	80 (30.0)	19 (31.7)	4 (23.5)	5 (14.7)	5 (14.3)	
Antibiotic exposure pre-ICU admission [*n* (%)]						
Any antibiotic	189 (70.8)	52 (86.7)	12 (70.6)	28 (82.4)	30 (88.2)	0.020
Carbapenem	41 (15.4)	9 (15.0)	8 (47.1)	4 (11.8)	17 (50.0)	0.000**
SIRS score [*n* (%)]						0.530
≥2	240 (89.9)	56 (93.3)	17 (100)	31 (91.2)	33 (97.1)	
<2	27 (10.1)	4 (6.7)	0 (0)	3 (8.8)	1 (2.9)	
qSOFA score [*n* (%)]						0.014
≥2	205 (76.8)	51 (85.0)	17 (100)	29 (85.3)	32 (94.1)	
<2	62 (23.2)	9 (15.0)	0 (0)	5 (14.7)	2 (5.9)	
Procedures during ICU admission						
Mechanical ventilation (%)	233 (87.3)	55 (91.7)	17 (100)	33 (97.1)	33 (97.1)	0.126
Mechanical ventilation duration						0.000**
≥5 days	99 (37.1)	28 (46.7)	13 (76.5)	17 (50.0)	25 (73.5)	
<5 days	168 (62.9)	32 (53.3)	4 (23.5)	17 (50.0)	9 (26.5)	
Central venous catheter (%)	230 (86.1)	52 (86.7)	17 (100)	31 (91.2)	33 (97.1)	0.196
Central venous catheter duration						
≥5 days	125 (46.8)	33 (55.0)	13 (76.5)	23 (67.6)	29 (85.3)	
<5 days	142 (53.2)	27 (45.0)	4 (23.5)	11 (32.4)	5 (14.7)	
Urinary catheter [*n* (%)]	267 (100)	60 (100)	17 (100)	34 (100)	34 (100)	N/A
Urinary catheter duration						0.001
≥5 days	148 (55.4)	35 (58.3)	13 (76.5)	25 (73.5)	30 (88.2)	
<5 days	119 (44.6)	25 (41.7)	4 (23.5)	9 (26.5)	4 (11.8)	
Antibiotic therapy during ICU admission [*n* (%)]						
Any antibiotic	263 (98.5)	59 (98.3)	16 (94.1)	34 (100)	34 (100)	0.460
Carbapenem	114 (42.7)	31 (51.7)	14 (82.4)	12 (35.3)	28 (82.4)	0.000**
Outcomes						
ICU LoS (days) [median (IQR)]	4 (3–7)	5 (3–10)	6 (4–12)	7 (3–12)	16 (6–27)	0.000**
In-ICU mortality [*n* (%)]	74 (27.7)	18 (30.0)	8 (47.1)	5 (14.7)	14 (41.2)	0.064

CNPA, carbapenem-non-susceptible *P. aeruginosa*; CSPA, carbapenem-susceptible *P. aeruginosa*; ER, emergency room; ICU, intensive care unit; IQR, interquartile range; LoS, length of stay; N/A, not applicable; qSOFA, quick Sepsis-related Organ Failure Assessment; SIRS, Systemic Inflammatory Response Syndrome.

[a] Group 1, no *P. aeruginosa* on admission and negative for *P. aeruginosa* during ICU admission; group 2, CSPA on admission, no CNPA acquisition during ICU admission; group 3, CNPA on admission, considered as positive during ICU admission (regardless of results of follow-up cultures); group 4, no *P. aeruginosa* on admission, acquisition of CSPA during ICU admission; and group 5, either no *P. aeruginosa* or CSPA on admission, acquisition of CNPA during ICU admission.

[b] Significance was calculated using one-way analysis of variance (ANOVA), Pearson's χ^2 test and Fisher's exact test. A *P*-value of <0.01 was considered statistically significant.

** $P < 0.001$.

positive culture 2 days earlier than patients that acquired a CNPA ($P=0.065$). The acquisition rate of CSPA was 19/1000 patient-days at risk (adult ICU, 22; ER-ICU, 14) compared with 18/1000 patient-days at risk for CNPA (adult ICU, 15; ER-ICU, 21).

Patients who acquired CNPA had a significantly longer LoS (median [interquartile range (IQR)] LoS, 15 [6–26] days; adjusted hazard ratio (aHR) = 1.89, 99% confidence interval (CI) 1.12–3.13; $P=0.002$) (Supplementary Table S4; Fig. 1B) compared with the other groups of patients, of whom ≥80% were discharged from the ICU within 7–12 days. These latter groups included patients who were always free from *P. aeruginosa*, patients who already carried *P. aeruginosa* (either CSPA or CNPA) at the time of ICU admission, and patients who became positive for CSPA during their ICU stay.

A longer LoS was independently associated with mechanical ventilation ≥5 days (median [IQR] LoS, 10 [7–15] days; aHR = 3.09, 99% CI 1.98–4.83; $P < 0.001$) (Supplementary Table S4) and use of a urinary catheter ≥5 days (median [IQR] LoS, 8 [5–12] days; aHR = 3.03, 99% CI 1.73–5.30; $P < 0.001$) (Supplementary Table S4).

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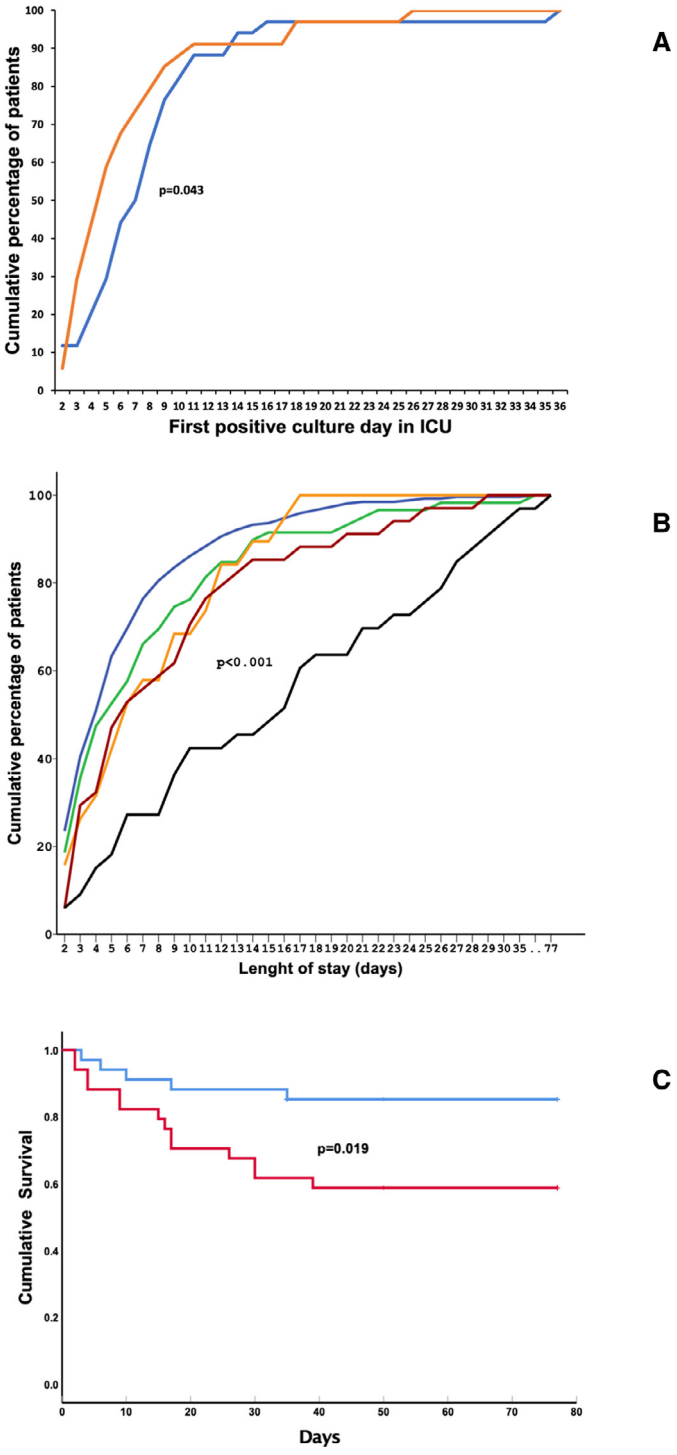

Fig. 1. Acquisition of carbapenem-susceptible *Pseudomonas aeruginosa* (CSPA) and carbapenem-non-susceptible *P. aeruginosa* (CNPA) and its effect on intensive care unit (ICU) stay and survival. (A) Acquisition of CSPA (orange) and CNPA (blue) (*P*-value by independent Mann–Whitney *U*-test). (B) Length of ICU stay by *P. aeruginosa* status: patients who were always *P. aeruginosa* negative (blue); patients already positive for CSPA on admission (green); patients already positive for CNPA on admission (yellow); patients who acquired CSPA during their ICU stay (red); and patients who acquired CNPA during their ICU stay (black) (*P*-value by Cox regression). (C) Survival of patients acquiring a CSPA (blue) compared with a CNPA (red) during their ICU stay (*P*-value by binary regression).

Acquisition of *P. aeruginosa* was not associated with in-ICU mortality: 27.7% (74/267) of patients who remained free of *P. aeruginosa* died versus 14.7% (5/34) and 41.2% (14/34) of patients who acquired a CSPA or CNPA, respectively [Supplementary Table S5; adjusted odds ratio (aOR) = 0.41, 99% CI 0.10–1.71; $P = 0.109$; and aOR = 1.08, 99% CI 0.34–3.46; $P = 0.867$]. The group of patients who acquired CSPA had the lowest mortality rate, and the probability of ICU survival was higher for patients who acquired a CSPA compared with patients who acquired CNPA (aHR = 4.06, 99% CI 0.87–18.88; $P = 0.019$) (Fig. 1C). Likewise, the ICU mortality among all patients with CNPA was 22/51 (43.1%) versus 97/361 (26.9%) among patients without CNPA ($P = 0.016$). The admission SIRS and qSOFA scores of patients with or without *P. aeruginosa* acquisition did not differ, indicating that significant differences in the risk of dying were not present at the time of ICU admission but emerged later during their ICU stay (Supplementary Table S5). In multivariate comparison, patients who acquired a CNPA during their ICU stay were more likely to have had prior exposure to antibiotics, especially carbapenems (aOR = 2.67, 99% CI 0.94–7.62; $P = 0.015$).

3.2. Phenotypic and molecular characterisation of carbapenem-non-susceptible Pseudomonas aeruginosa

Overall, 107/281 (38.1%) isolates from 51/145 patients were found to be non-susceptible to carbapenems. Moreover, 12/16 (75.0%) *P. aeruginosa* isolates from the environment were CNPA (Supplementary Table S6). None of 25 isolates from HCWs were CNPA. Thus, 119 CNPA were subjected to further analyses. Phenotypic testing showed that 68/119 (57.1%) isolates produced a MBL. PCR demonstrated the presence of bla_{VIM} in 36 and bla_{IMP} in 23, including isolates from patients and the environment. None of the 119 isolates were positive for bla_{NDM}. The presence of non-MBL bla_{GES-5} was detected in 24 isolates.

3.3. Clonal relatedness

MLST revealed four major clusters (ST235, ST823, ST446 and ST357) as well as several new sequence types. By MLVA, five major clusters were distinguished, two belonging to ST235 and the others corresponding to ST823, ST446 and ST357 (Fig. 2A). These four major genetic clusters included 97/107 (90.7%) CNPA from patients (ICU-imported and ICU-acquired) as well as 11/12 (91.7%) environmental isolates (Fig. 2B). Most isolates belonged to ST235 (10 imported, 32 acquired patient isolates and 4 environmental isolates), of which 22 isolates harboured bla_{IMP}, 24 isolates harboured bla_{GES-5} but no isolates contained bla_{VIM}. All ST823 isolates harboured bla_{VIM} (Supplementary Tables S6 and S7).

4. Discussion

Reports describing the emergence of *P. aeruginosa* isolates harbouring carbapenemase genes come from different parts of the world, including Southeast Asia [9]. In the current study, it was found that 12.4% of patients who stayed in our ICUs for >2 days carried CNPA and 4.1% of patients were already colonised at ICU admission. Patients may become colonised elsewhere in the same hospital or in another hospital from which they are referred, or they may come with such a strain directly from the community, possibly having acquired the strain during previous healthcare contact or indirectly from exposure to relatives carrying such strains or unknown environmental niches.

Hence, screening cultures are indispensable for early detection and infection control. This will also guide rational antibiotic use since it has been shown that colonisation with CNPA is a risk factor for infection [10].

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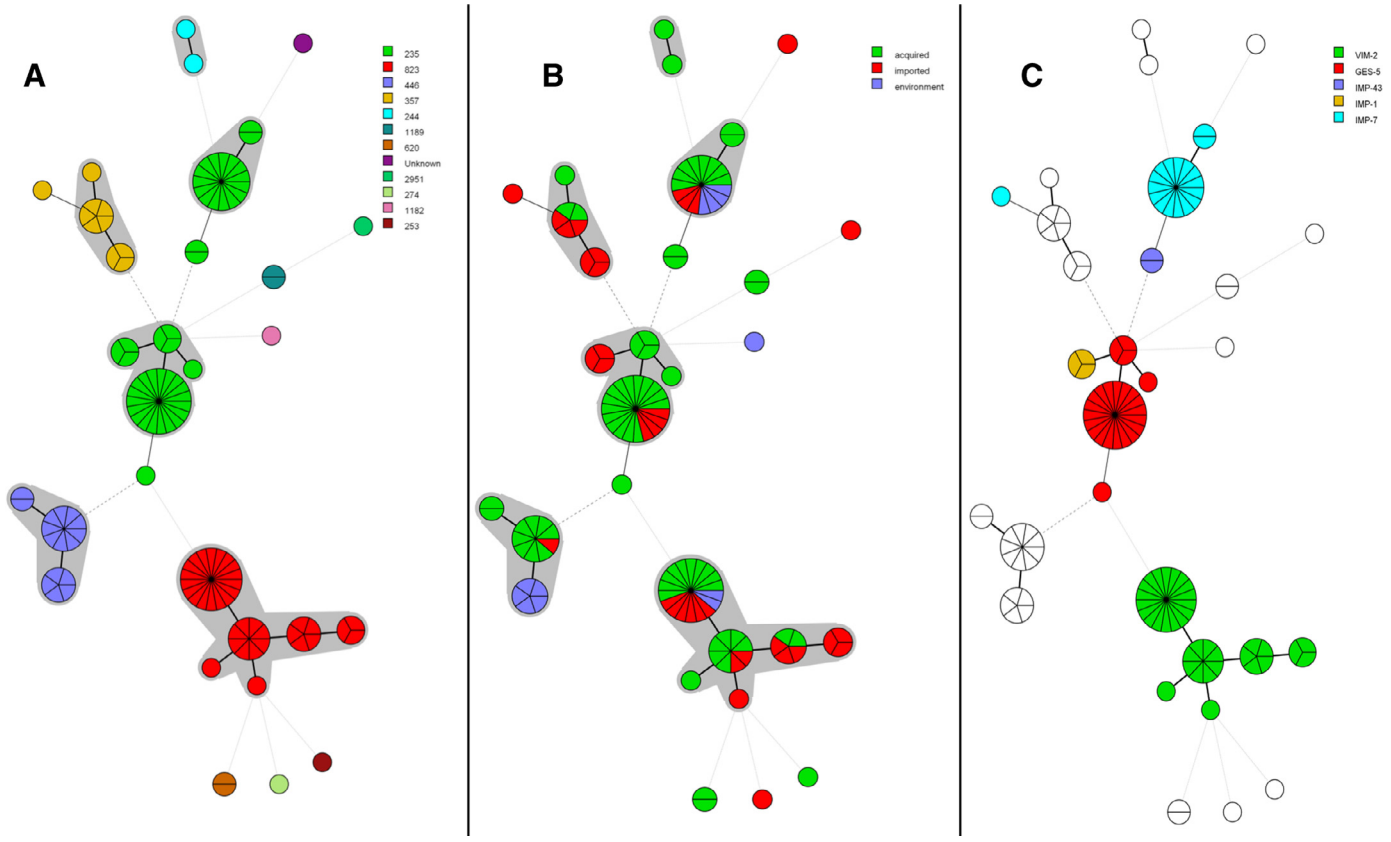

Fig. 2. Minimum spanning tree analysis of carbapenem-non-susceptible *Pseudomonas aeruginosa* (CNPA) isolates based on multiple loci variable-number tandem repeat analysis (MLVA). Grey shading in each panel indicates MLVA complexes. (A) Five most prevalent MLVA clusters corresponded to ST235 (green), ST823 (red), ST446 (purple), ST357 (yellow) and ST244 (light blue) as determined by multilocus sequence typing (MLST). Minor clones are indicated by other colours. (B) Red segments indicate *P. aeruginosa* isolates that were imported into the intensive care unit (ICU) by patients, green segments indicate those that were acquired in the ICU by patients, and purple segments indicate isolates from the ICU environment. (C) Coloured segments indicate the carbapenemase genes VIM-2 (green), GES-5 (red), IMP-7 (light blue), IMP-1 (yellow) and IMP-43 (purple). Colourless segments represent isolates without carbapenemase genes.

The current data show that patient acquisition of CNPA during ICU care is associated with prolonged ICU stay. Acquisition of CSPA or CNPA was statistically not associated with mortality compared with patients free of *P. aeruginosa*. However, the observed mortality rate was much higher among patients with CNPA versus those without. A study in Taiwan in 2016 also did not find carbapenem resistance to be associated with mortality [11], whereas another study revealed a relationship between CNPA carriage and mortality [12].

Among the 107 CNPA, bla_{VIM}, bla_{IMP} and bla_{GES-5} were the most prevalent carbapenemase genes. These genes are widely distributed in the world. The first MBL found in *P. aeruginosa* was IMP-1, identified in Japan in 1988. VIM was first identified in Italy in 1997, but reached Southeast Asia [2,9]. GES-5-producing *P. aeruginosa* was first detected in China in 2004 and has been isolated globally since [13,14].

In a recent review it was shown that use of carbapenems and medical devices are the leading risk factors for carriage of CNPA [15]. The authors also identified environmental sources of CNPA, with sinks being the most frequently reported reservoirs [16]. More recent reports of outbreaks of CNPA also demonstrate an association with environmental contamination [17–19]. This phenomenon was also observed in our setting. The four environmental bla_{IMP}-positive ST235 CNPA were all cultured from wet sources in the common cleaning room. In this room, located adjacent to the adult ICU, all reusable items are manually cleaned and stored. These types of 'wet' rooms may serve as a persistent source and route of transmission for resistant bacteria and should be targeted by infection control.

MLST revealed four major clusters (ST235, ST823, ST446 and ST357) and several new clones. ST235 is the most prevalent of the so-called 'international' clones that are associated with poor clinical outcomes [20]. The Indonesian offspring of this clone, identified in this study, always harboured bla_{IMP} or bla_{GES-5}, but not bla_{VIM}. All ST823 isolates consistently harboured only bla_{VIM}, and only bla_{IMP} was found in ST357, but no genes in ST446. Three dominant clusters included isolates from the ICU environment (ST235, ST823 and ST446). This epidemiological information should be used when designing interventions to reduce the acquisition of CNPA in ICUs in Indonesia and similar settings elsewhere.

This study has limitations. First, it was a single-centre study, therefore the data are not representative for the whole country. Second, we were unable to evaluate the effect of several other possible confounders of CNPA acquisition, including long-term kidney dialysis, use of inotropes, surgery and previous hospital admission.

5. Conclusion

This large prospective study describes the epidemiology and clinical impact of CNPA in ICUs in Indonesia. Acquisition of CNPA in ICUs was independently associated with prolonged LoS and possibly survival. ST235 was the dominant clone, as were IMP, VIM and GES carbapenemases. Controlling CNPA requires admission

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screening of patients and identifying and containing reservoirs within the ICU environment.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.08.003.

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Competing interests: ACP, AvB and CM are employees of bioMérieux, a company developing, marketing and selling tests in the infectious diseases domain; the opinions expressed in the manuscript are those of the authors and do not necessarily reflect company policies. All other authors declare no competing interests.

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