Multidrug-Resistant Bacteria Colonization in Patients Admitted to Dr. Cipto Mangunkusumo Hospital Jakarta, Indonesia

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ABSTRACT

Background: *Antibiotic resistance is the main problem in infectious disease management. Multidrug-resistant (MDR) bacteria could be carried by admitted patients and become a source of spread in the hospital, causing infections in other patients or the patients themselves. However, the screening of MDR bacteria has not been a standard in developing countries. This study aimed to get the prevalence of MDR bacteria colonization in patients on admission to Dr. Cipto Mangunkusumo Hospital.* ***Methods:*** *Selective liquid media with added antibiotics were used for culturing the MDR bacteria. While admitted to the hospital, subjects were sampled and interviewed to fill out a questionnaire. The screening specimens used for this study were throat, navel, rectal, nasal, and armpit swabs. During hospitalization, hospital-acquired infections (HAIs) were recorded.* ***Results:*** *Of 100 patients included in the study, the prevalence of MDR bacteria colonization on admission was 63% (n=63) with the prevalence of CR-GNB, ESBL-PE, and MRSA were 11%, 54%, and 11%, respectively. Two-thirds of the patients with HAIs (n=8/12) were colonized with MDR bacteria. Factors associated with MDR bacteria colonization were the recent use of invasive medical devices and comorbidity, while a factor associated with CR-GNB colonization was the recent use of antibiotics.* ***Conclusion****: The prevalence of MDR bacteria colonization in patients on admission to Dr. Cipto Mangunkusumo Hospital in 2022 was 63% (n=63), of which 12.68% (n=8) experienced HAIs during hospitalization. MDR bacteria colonization was associated with the recent use of invasive medical devices and comorbidity. History of antibiotic use was associated with CR-GNB colonization.*

Keywords*: multi-drug resistance bacteria, colonization, healthcare-associated infections.*

INTRODUCTION

Antibiotic resistance is a major problem in the management of bacterial infections since it can lead to therapeutic failure, prolonged hospitalization, and increased treatment costs leading to an increased socioeconomic burden.[1] Resistant bacteria can be carried by newly admitted patients and become a source of the spread in the hospital, causing infections in other patients or for themselves.[2,3] Monitoring antibiotic-resistant bacteria is one of the first steps in reducing mortality and morbidity due

to these pathogenic infections.[4]

As the Indonesian national referral hospital, Dr. Cipto Mangunkusumo Hospital (CMH) has a high prevalence of resistant bacteria, especially in those with long-stay and intensive care.[5,6] The prevalence of carbapenem-resistant Gram-negative bacilli (CR-GNB) among patients admitted to the ICU of CMH reached 34.35%.[5] Among the Enterobacterales found in CMH, more than half (58.4%) of them produced extended spectrum beta-lactamase (ESBL) which can cause bacteria to become resistant to various antibiotics.[7-10] Based on blood culture results, the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) in RSCM in 2020 was 9.2%, the highest among multidrug-resistant (MDR) Gram-positive bacteria.[11]

It was difficult to determine whether the high prevalence of MDR bacteria was acquired during hospitalization or already present at the time of admission. Several studies have been conducted and described the benefits of monitoring MDR bacteria in hospitalized patients.[12-15] However, detection by culture using standard media has low sensitivity. The addition of antibiotics in the culture media is known to improve culture sensitivity for the detection of MRSA and ESBL-producing Enterobacterales (ESBL-PE).[16-18] Therefore, we used antibiotics as selective agents to the culture media to optimize the detection.

In this study, we conducted screening to determine the prevalence of MDR bacterial colonization in patients admitted to CMH, associated factors, and outcome at discharge. The MDR bacteria tested were critical priority pathogens defined by WHO, such as carbapenem-resistant *Acinetobacter baumannii,* carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant Enterobacterales, and ESBL-PE. In addition, MRSA, which is a bacterium that is often reported as a major cause of healthcare-associated infections (HAIs) with high morbidity and mortality, was tested as well.[19,20]

METHODS

We conducted a cohort study to determine the prevalence of MDR bacterial colonization in hospitalized patients at admission, its risk factors and incidence of HAIs. Screening specimens were collected and validated questionnaires were filled by interview in the first 24 hours from the admission. Bacterial culture for identification and antibiotic susceptibility testing was conducted at the Clinical Microbiology Laboratory of the Faculty of Medicine, Universitas Indonesia. Patient recruitment was held from March 1, 2022, to May 31, 2022.

The inclusion criteria were being over 18 years old, having been admitted to RSCM for at least 24 hours, and agreeing to participate in the study. Subjects were excluded if a complete set of specimens was not possible to collect or if they were not competent enough to answer the questions in the questionnaire and were not accompanied by a competent guardian. Comorbidities were assessed by the Charlson comorbidity index (CCI) and high comorbidity was defined as a CCI of 3 or more.[21,22]

The study subjects were interviewed by the research team using a questionnaire instrument to determine demographic characteristics and factors associated with the occurrence of MDR bacterial colonization in patients. There were five types of screening specimens used in the study, namely throat swab, navel swab, rectal swab, nasal swab, and axillary swab. Specimens were collected using sterile cotton swabs inserted into Tryptic Soy Broth (TSB) medium as soon as the specimens were obtained. Swab specimens were transported at 2-7°C to the laboratory for further processing.

In the laboratory, the TSB medium is then supplemented with antibiotics based on the target MDR bacteria (**Figure 1**). Then, incubation was carried out at 35°C for 24 hours before subculturing to a selective agar plate medium. The growing bacterial colonies were identified using VITEK2 (bioMerieux). If *A. baumannii*, *P. aeruginosa*, or Enterobacterales were identified, a carbapenem susceptibility test was performed using the Kirby-Bauer method with Mueller Hinton agar plates. The growth of Enterobacterales on MacConkey agar plates was also followed by a double disc synergy test (DDST) using ceftazidime 30 mcg, cefepime 30 mcg, and cefotaxime 30 mcgwith amoxicillin-clavulanate (clavulanate 10 mcg) on Mueller Hinton agar plates according to

EUCAST guidelines to determine the ESBL production.[23] When *S. aureus* identified, a cefoxitin susceptibility test was performed to determine the methicillin-resistant strains.

Data processing and analysis were performed using the SPSS version 20 program for the

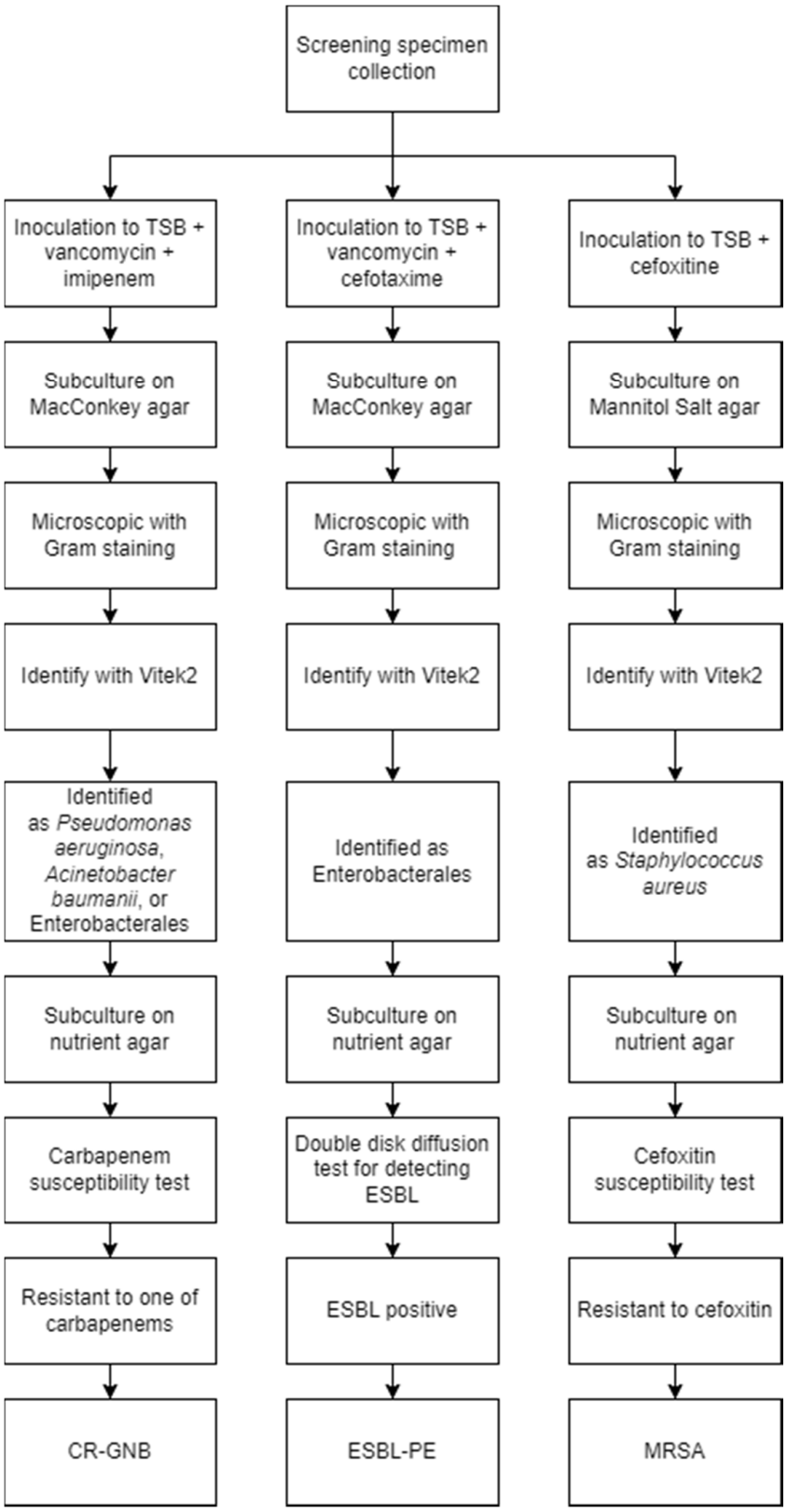

Figure 1. Laboratory test for detecting multidrug-resistant bacteria

Windows system. From the data obtained, analysis was carried out to determine the factors associated with MDR bacterial colonization in patients. The analysis began with bivariate analysis, then continued with multivariate for factors with a p-value of less than 0.25.[24] Bivariate analysis was also performed to determine the association of MDR bacterial colonization with the occurrence of HAIs in patients. Factors were considered associated if the p-value was less than 0.05. The research proposal has received ethical approval from the Ethics Committee of FKUI-RSCM with number KET-1235/UN2.F1/ETIK/PPM.00.02/2021 dated December 27, 2021.

RESULTS

A total of 100 patients who met the inclusion and exclusion criteria were selected (**Figure 1**). The median length of stay was 5 (3-9.5) days with 49% of the patients staying for 1-5 days. The demographic characteristics of the subjects can be seen in **Table 1**. There were 15 patients with the infection on admission, however, the infections did not occur in the area of specimen collection for screening purposes . Therefore, any

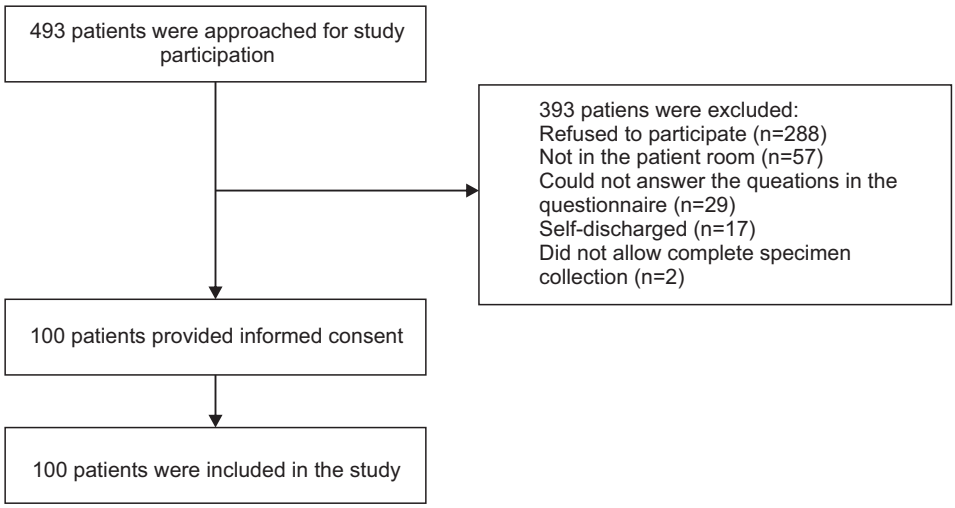

Figure 1. Patients recruitment.

Table 1. Demographic characteristics of subjects.

Parameter	N =100	Bacterial Colonization of MDR[a] Positive N = 63	Negative N = 37
Sex, n (%)			
Male	63	42 (66.7)	21 (56.8)
Female	37	21 (33.3)	16 (43.2)
Age (year), median (IQR)	51.0 (18-81)	52.0 (18-81)	50.0 (19-77)
Diagnosis on admission, n (%)			
Tumors and malignancies	39	24 (38.1)	15 (40.5)
Trauma	13	8 (12.7)	5 (13.5)
Kidney disease	12	11 (17.5)	1 (2.7)
Cardiovascular disease	4	1 (1.6)	3 (8.1)
Autoimmune diseases	3	1 (1.6)	2 (5.4)
Other diagnosis[b]	23	18 (28.6)	11 (29.7)
Infection on admission[c], n (%)			
Yes	15	7 (11.1)	8 (21.6)
No	85	56 (88.9)	29 (78.4)
BMI[e], mean (SD)	21.6 (4.8)	21.9 (5.1)	21.1 (4.1)

[a]Colonization of multidrug-resistance bacteria, including carbapenem-resistant Gram-negative bacilli, extended-spectrum beta-lactamase-producing Enterobacterales, and methicillin-resistant *Staphylococcus aureus*
[b]Other diagnosis: Liver abscess, stroke, acute cholangitis, gastric ulcers, anemia, diabetes melitus, digital abscess, Internal hemorroid, arthiritis, hydropneumothorax, condyloma acuminata, systemic sclerosis, choledocolithiasis, achalasia, palatum fistula, hernia, multiple gangrene, urethral stenosis, hirschsprung, anal stenosis
[c]infection at admission did not occur around specimen collection for screening purpose [d]BMI = body mass index

positive results from those screening specimens were marked as colonization. The infections were acute cholangitis (n=3), musculoskeletal infection (n=3), community-acquired pneumonia (n=2), urinary tract infection (n=2), and others (n=5).

MDR Bacterial Colonization

The prevalence of MDR bacterial colonization in patients admitted to RSCM in 2022 was 63%. Of the 100 patients examined, 54 patients were ESBL-PE colonized, 11 patients were CR-GNB colonized, and 11 patients were MRSA colonized (**Table 2**).

Risk Factors for MDR Bacterial Colonization

The most commonly 3-month prior antibiotics used were cephalosporins (18 of 37 subjects), such as cefixime (10 subjects), ceftriaxone (4 subjects), and cefoperazone (4 subjects). The number of subjects who had high comorbidity was 50 subjects, of which 72% were colonized with MDR bacteria. The median total score of living behavior was 24 (IQR 14-28), therefore the subjects were characterized as having healthy living behavior if the score were ≥24.

Based on the multivariate analysis, history of invasive medical device use (OR 3.48, 95%CI 1.23-9.79) and high comorbidity (OR 2.41, 95%CI 1.02-5.72) were associated with MDR

Table 2. List of CR-GNB and ESBL-PE found on the basis of specimen type.

Swab of throat - TSB-VI (N=2/100)	Navel swab - TSB-VI (N=2/100)	Rectal swab– TSB-VI (N=8/100)	Rectal swab – TSB-VC (N=54/100)	Nasal swab – TSB-Cx (N=10/100)	Axillary swab – TSB-Cx (N=10/100)
K. pneumoniae CR (N=1) *P. aeruginosa* CR (N=1)	*A. baumannii* CR (N=1) *P. aeruginosa* CR (N=1)	*K. pneumoniae* CR (N=3)* *E. coli* CR (N=3)* *P. aeruginosa* CR (N=2) *A. baumannii* CR (N=2)	*E. coli* ESBL (N=48) *K. pneumoniae* ESBL (N=5) *Salmonella typhi* ESBL (N=1)	MRSA (N=10)	MRSA (N=10)

*2 the patient has co-colonization *K. pneumoniae* CR dan *E. coli* CR on a rectal swab
TSB-VI = Trypticase soy broth with vancomycin and imipenem
TSB-VC = Trypticase soy broth with vancomycin and cefotaxime
TSB-Cx = Trypticase soy broth with cefoxitine
CR-GNB = carbapenem-resistant Gram negative bacilli
ESBL-PE = extended-spectrum beta lactamase-producing Enterobacterales
MRSA= methicillin resistant *Staphylococcus aureus*

Table 3. Bivariate analysis of risk factors for MDR bacterial colonization.

Parameter	MDR bacterial colonization - n (%)		Value of p	OR (IK 95%)
	Yes	No		
History of antibiotic consumption			0.57	0.79
Yes	22 (34.9)	15 (40.5)		(0.34-1.82)
No	41 (65.1)	33 (59.5)		
Inpatient history			0.81	1.11
Yes	39 (61.9)	22 (59.5)		(0.48-2.54)
No	24 (3.1)	15 (40.5)		
History of invasive medical equipment use			0.02	3.18 (1.16-8.74)
Yes	24 (38.1)	6 (16.2)		
No	39 (61.9)	31 (83.4)		
CCI			0.06	2.19
High	36 (57.1)	14 (37.8)		(0.95-5.03)
Low	27 (42.9)	23 (62.2)		
Lifestyle			0.50	1.32
Unhealthy	35 (55.6)	18 (48.6)		(0.58-2.98)
Healthy	28 (44.4)	19 (51.4)		

CCI = Charlson comorbidity index
MDR = multi-drugs resistant

bacterial colonization. History of antibiotic use in the last 3 months increased the risk to 5.48 (95%CI 1.31-22.87) times for CR-GNB colonization. No risk factor was associated with ESBL-PE and MRSA colonization.

During hospitalization, 12 patients got HAIs, of which 4 were diagnosed with hospital acquired pneumonia (HAP), 3 with catheter-associated urinary tract infection (CAUTI), 2 with bloodstream infection (BSI), 2 with surgical site infection (SSI), and 1 with hospital acquired gastrointestinal infection. A total of 12.68% (N=8/63) patients with MDR bacterial colonization experienced HAIs during hospitalization with a relative risk of 1.18 (95%CI 0.38-3.63, p=0.52) compared to patients without MDR bacterial colonization.

DISCUSSION

The prevalence of MDR bacterial colonization in patients admitted to RSCM in 2022 was very high (64.3%) and dominated by ESBL-producing *E. coli*. The prevalence of CR-GNB colonization was found to be lower (11%) than previous studies on the prevalence of carbapenem-resistant *A. baumannii*.[25] ESBL-PE colonization occurred in more than half of the patients admitted to RSCM (54%).[10] The high prevalence can be a source of spread of MDR bacteria in the hospital environment and can cause HAIs if the implementation of IPC, especially contact precaution in hospitals is not carried out optimally, in vulnerable populations. MRSA colonization at the beginning of treatment was found to be 11%, higher than the previous study (0.8%).[26] This may be influenced by the use of antibiotics for culture medium supplementation that can increase MRSA culture positivity. Several reports show the benefits of using throat swabs in detecting MRSA.[27] In this study, MRSA screening was only performed on anterior nasal swabs and axillary swabs, while throat swabs were used for Gram-negative screening.

The prevalence of MDR bacterial colonization was higher in patients with a history of antibiotic use in the past 3 months although it was not statistically significant. In this study, the history of antibiotic use was only associated with CR-GNB colonization. Previous studies related to carbapenem resistance have also shown an association between antibiotic use and colonization of carbapenem-resistant bacteria.[28] History of hospitalization in the past year was not associated with MDR bacterial colonization, unlike previous reports.[29,30] This suggests that the resistant bacteria in these patients were acquired from the community. A history of using invasive medical equipment increases the risk of MDR bacterial colonization by almost 3.5 times, so MDR bacterial screening should be done in this group of patients. In this study, patients with high comorbidity had a 2.4 times higher risk of MDR bacterial colonization.

The prevalence of HAIs in patients in this study was high (12%), in line with the results of the study by Goh et al.[31] who reported the prevalence of HAIs in Southeast Asia ranged from 8.4-30.4%, with risk factors for HAIs being the most common. Given the high prevalence of HAIs that occur, it is important to implement IPC in hospitals. IPC problems that are often encountered in Indonesia include communication problems with management, limited antimicrobial sensitivity testing, and not enough IPC nurses available.[32]

The limitation of this study was that the data regarding the history of antibiotic consumption within three months was obtained from interview only relying on the subjects' memories and knowledge. Therefore, there is a possibility of recall bias in collecting this data. Although MDR bacterial colonization in this study was not statistically proven to be associated with the occurrence of HAIs, patients who are colonized at admission have the potential to experience HAIs during treatment. Efforts that must be made in preventing the spread of MDR bacteria from the body of colonized patients to the surrounding environment are the application of contact precautions and isolation or cohorting. The commitment of hospital leaders is needed by making policies for handling patients who enter the hospital, especially those with risk factors.

CONCLUSION

The prevalence of MDR bacterial colonization (consisting of CR-GNB, ESBL-PE, and MRSA

in patients during inpatient admissions at RSCM in 2022 was 63%, of which 12.68% experienced HAIs during hospitalization. Risk factors for MDR bacterial colonization in patients were history of invasive medical device use and high comorbidities. History of antibiotic use was associated to CR-GNB colonization. Screening of MDR bacterial colonization in patients with risk factor is needed to prevent its spread.

ACKNOWLEDGMENTS

This study was funded by Dr. Cipto Mangunkusumo Hospital.

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