Risk Factors Associated with the Colonization of Multidrug-Resistant Gram-Negative Bacteria Upon Admission to the Intensive Care Unit: A Cross-sectional Study

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ABSTRACT

Background: *Multidrug-resistant Gram-negative bacteria (MDR-GNB) are prevalent in intensive care units (ICUs), leading to increased morbidity and mortality. Limited data on MDR-GNB in Indonesia prompted this study to determine the prevalence and risk factors associated with MDR-GNB colonization, enhancing screening strategies, and acquiring phenotypic and genotypic data on these bacteria.* ***Methods:*** *This analytical* cross-sectional *observational study included* participants who met the criteria and were admitted to the ICU at Dr. Cipto Mangunkusumo Hospital from January to December 2022. We used multivariate analysis on the findings from rectal swab screening, sociodemographic, clinical, and microbiological examinations. ***Results:*** *Out of* 108 participants, 172 cultures comprised 165 Gram-negative isolates, four yeasts, and three with no growth. The prevalence of patients colonized with MDR-GNB was 51.85% (56/108), and the prevalence of MDR-GNB isolates was 39.53% (68/172), with the most common MDR-GNB being Escherichia coli (29.65%) and Klebsiella pneumoniae (19.44%). The most resistant gene found in ESBL was CTX-M (75%), and the carbapenemase producer gene was NDM (5.88%). Risk factors associated with MDR-GNB colonization were the length of stay before admission to the ICU ($p = 0.003$) and a history of previous antibiotic therapy ($p = 0.036$). ***Conclusion:*** *In this study, two risk factors were associated with the occurrence of MDR-GNB colonization, with the prevalence of MDR-GNB colonization in patients initially admitted to the ICU still quite high. Therefore, selecting screening patients based on risk factors at the time of initial admission to the ICU is crucial for infection control programs.*

Keywords*: MDR-GNB bacteria, risk factors, resistance genes, colonization, screening.*

INTRODUCTION

Multidrug-resistant Gram-negative Bacilli (MDR-GNB) are defined as Gram-negative rod-shaped bacteria resistant to at least one agent in three or more antimicrobial categories.[1] According to the World Health Organization (WHO), critical priority pathogens among MDR-GNB include Extended-Spectrum Beta-Lactamase (ESBL)-producing *Enterobacterales*, carbapenem-resistant *Enterobacterales*, carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa*.[2] MDR-GNB

bacteria are the primary cause of infections and colonization in intensive care units (ICUs).[3]

The gut microbiota acts as the main reservoir for MDR-GNB, rendering it a potential opportunistic pathogen in vulnerable patients. Colonization by asymptomatic carriers of MDR-GNB serves as a potential source for cross-patient transmission, emphasizing the need for cohorting colonized patients. Moreover, colonization increases the risk of MDR-GNB infections, with studies reporting incidence rates of infected patients ranging from 14% to 40%. These findings have significant implications for prognosis, contributing to treatment failures, increased morbidity and mortality rates, prolonged hospitalizations, and higher healthcare expenses.[4-7]

In Indonesia, data from 2011 showed a prevalence of 58.4% ESBL-producing isolates in the ICU of Dr. Cipto Mangunkusumo Hospital (RSCM),[8] while carbapenem-resistant Gram-negative bacterial species isolated from ICU patients in RSCM in 2008 varied. Specifically, *P. aeruginosa* was at 21.9%, *Enterobacterales* at 27.6%, and *A. baumannii* at 50.5%.[9] In 2013, *Klebsiella pneumoniae* carrying the NDM gene was found in 5.3% of early ICU admissions and increased to 9.5% during treatment.[10]

Several previous studies have indicated that advanced age, comorbidities, prior antibiotic therapy, history of previous surgery, use of invasive medical devices, transfer from external ICUs/hospitals, and previous colonization by MDR-GNB increase the risk of colonization.[11,12] However, data on these factors in Indonesia are still insufficient. Optimizing patient screening strategies based on these risk factors, particularly for early ICU admissions, can aid in identifying carriers early and preventing the spread of nosocomial infection.[13] Therefore, this study aimed to determine the prevalence and associated risk factors for MDR-GNB colonization to control infection or colonization and to obtain phenotypic and genotypic data from these bacteria.

METHODS

Study Design

We conducted this analytical cross-sectional observational study in the ICU of Cipto Mangunkusumo Hospital (CMH) and the Clinical Microbiology Laboratory of the Faculty of Medicine, Universitas Indonesia. This research is part of the main study titled "Comparison Analysis of Xpert Carba-R with Real-Time PCR on The Results of Carbapenem Resistant *Enterobacterales* Screening in ICU Dr. Cipto Mangunkusumo Hospital", which has received ethical approval from the ethics commission of FKUI-RSCM with letter number KET-1013/UN2.F1/ETIK/PPM.00.02/2021.

Study Population

The study population comprised consecutively selected patients admitted to the ICU of CMH, a tertiary referral hospital at the national level, from January to December 2022. Inclusion criteria were patients aged ≥18 years admitted to the ICU within 24 hours, undergoing rectal swab collection, and providing informed consent from the patient or their family. We excluded the patients with incomplete clinical data or those who declined participation.

Data Collection

Demographic characteristics and risk factor data of the participants were collected from both physical and electronic medical records. Microbiological data were obtained from bacterial identification results using phenotypic and genotypic methods, including the detection of resistance genes.

Microbiological Examination

Rectal swab specimens were collected within the first 24 hours after a patient was admitted to the ICU using Amies transport media. The swabs were then inoculated onto MacConkey agar and incubated in an incubator at a temperature of 35°C to 37°C for 18–24 hours. After incubation, any colonies that had grown were observed. If there were two types of colonies, subculturing was performed. The colonies were then subjected to Gram staining, and Gram-negative colonies were further identified phenotypically using the VITEK 2 system.

If phenotypic results indicate resistance, such as testing positive for ESBL or showing carbapenem resistance, genotypic identification was performed. We analyzed the presence of resistance genes, including ESBL genes (CTX-M, SHV, and TEM) and carbapenemase

producer genes (KPC, NDM, OXA-48, VIM, IMP), using real-time PCR single-plex with SYBR Green. Before processing the samples, optimization tests were conducted for the PCR process. Primary data on ESBL and carbapenemase resistance genes were obtained from previously published literature.[14,15]

Statistical Analysis

In this study, we performed multivariate analysis using the SPSS program. Initially, each independent variable underwent bivariate testing. If the *p*-value was < 0.25, it proceeded to multivariate analysis. During multivariate analysis, after controlling for independent variables suspected to be related to the occurrence of MDR-GNB colonization, results were considered significant if the *p*-value was < 0.05.

RESULTS

Participant Characteristics

Out of the 108 patients eligible for the study, 56 (51.85%) were colonized by MDR-GNB upon admission to the ICU of the hospital.

Table 1. Demographic Characteristics of Participants.

Characteristics	Results (n = 108)		
	Mean±SD / Median (q1-q3) / n(%)	Colonized (n=56)	Not Colonized (n=52)
Age [year]	47.78 ± 18.26	49.86 ± 18.41	45.54 ± 18.00
Gender			
Male	59 (54.63)	31 (28.70)	28 (25.93)
Female	49 (45.37)	25 (23.15)	24 (22.22)
Length of Stay before ICU Admission [days]	2 (1 – 4.75)	4 (1-7)	2 (0-3)
Altered Consciousness (GCS<15)	69 (63.89)	34 (31.48)	35 (32.41)
Systolic Blood Pressure [mmHg]	118.23 ± 20.15	114.80 ± 20.44	121.77 ± 19.21
Diastolic Blood Pressure [mmHg]	70.80 ± 13.87	70.27 ± 13.83	71.37 ± 13.89
Respiratory Rate [times/minute]	18 (13 – 22)	20 (14-22)	16 (12-22)
qSOFA Score [point]	2 (1 – 2)	2 (1-2)	2 (1-2)
Comorbidities	82 (75.93)	46 (42.59)	36 (33.33)
Hypertension	40 (37.03)	21 (19.44)	19 (17.59)
Diabetes Mellitus	35 (32.41)	21 (19.44)	14 (412.96)
Chronic Kidney Disease	29 (26.85)	18 (16.67)	11 (10.18)
Chronic Kidney Disease on Hemodialysis	22 (20.37)	7 (6.48)	4 (3.70)
Malignancy	11 (10.19)	15 (13.89)	10 (9.23)
Chronic Pulmonary Disease	9 (8.33)	6 (5.56)	3 (2.78)
Chronic Liver Disease	6 (5.56)	4 (3.70)	2 (1.85)
History of Previous Antibiotic Therapy	96 (88.89)	54 (50.00)	42 (38.89)
Amikacin	14 (12.96)	9 (8.33)	5 (4.63)
Ampicillin Sulbactam	20 (18.52)	10 (9.26)	10 (9.26)
Cefepime	3 (2.78)	2 (1.85)	1 (0.93)
Cefixime	1 (0.93)	1 (0.93)	0 (0)
Cefoperazone	3 (2.78)	2 (1.85)	1 (0.93)
Ceftriaxone	22 (20.37)	12 (11.11)	10 (9.26)
Ciprofloxacin	2 (1.86)	2 (1.85)	0 (0)
Fosfomycin	4 (3.70)	2 (1.85)	2 (1.85)
Levofloxacin	26 (24.07)	16 (14.81)	10 (9.26)
Meropenem	25 (23.15)	14 (12.96)	11 (10.19)
Vancomycin	1 (0.93)	0 (0)	1 (0.93)
Others	29 (26.85)	20 (18.52)	9 (8.33)
History of Previous Surgery	53 (49.07)	30 (27.78)	23 (21.30)
History of Invasive Medical Device Usage	106 (98.15)	56 (51.85)	50 (46.30)
Mechanical Ventilation during Admission	79 (73.15)	42 (38.89)	37 (34.26)
Indwelling Catheter	106 (98.15)	56 (51.85)	50 (46.30)
History of Transfer from ICU/Inpatient Ward of External Hospital	21 (19.44)	10 (9.26)	11 (10.19)
History of Previous MDR-GNB Colonization	6 (5.56)	4 (3.70)	2 (1.85)

The mean age of the participants was 47.78 ± 18.26 years, with the majority being male (54.63%). The median duration of hospitalization before ICU admission was 2 days (interquartile range: 1–4.75 days), and the participants had a median qSOFA score of 2 points. The most common comorbidities were hypertension (37.03%) and diabetes mellitus (32.41%). Almost all had been prescribed antibiotics in the last 3 months (88.89%), with quinolones and beta-lactams, specifically levofloxacin (24.07%), meropenem (23.15%), and ceftriaxone (20.37%), being the most commonly used antibiotic classes. The majority had used invasive medical devices (98.15%). The participants had a history of surgical procedures (49.07%) and a history of transfer from the ICU/inpatient ward of external hospitals (19.44%), and a small proportion had been previously colonized by MDR-GNB (5.56%).

Prevalence of Gram-Negative Bacterial Infection and Phenotypic Detection of MDR-GNB

Microorganism growth was detected in 97.22% (105 out of 108 patients) during the initial screening upon admission to the ICU. Microorganism growth included 165 isolates of GNB (95.93%), four yeast isolates (2.33%), and no growth was observed in three patients (1.74%). The most prevalent GNB were *E. Coli* (72.09%) and *K. Pneumoniae* (18.60%).

The prevalence of MDR-GNB was 39.53% (68/172), comprising 59 isolates (34.30%) being ESBL resistance and nine isolates (5.23%) demonstrating carbapenem resistance. Within the ESBL group, the most prevalent bacterium was *E. coli* at 29.07% (50/172), while in the carbapenem-resistant group, *K. pneumoniae* was the most prevalent at 2.91% (5/172).

From the results of antibiotic sensitivity testing, MDR-GNB *E. coli* was substantial

Table 2. Isolated Bacteria from Rectal Swab Specimens and Phenotypic Detection of MDR-GNB during Initial Screening upon Admission to the ICU.

No	Microorganism Name	n (%)	MDR GNB	
			ESBL n (%)	Carbapenem-resistant n (%)
1	Escherichia coli	124 (72.09)	50 (29.07)	1 (0.58)
2	Klebsiella pneumoniae	32 (18.60)	9 (5.23)	5 (2.91)
3	Enterobacter cloacae complex	2 (1.16)	0	2 (1.16)
4	Citrobacter koseri	2 (1.16)		
5	Citrobacter amalonaticus	1 (0.58)		
6	Morganella morganii ssp morganii	1(0.58)		
7	Acinetobacter baumannii	1 (0.58)	0	1 (0.58)
8	Comamonas testosteroni	1 (0.58)		
9	Escherichia fergusonii	1 (0.58)		
10	Yeast	4 (2.33)		
11	No organism growth	3 (1.74)		
	Total	172 (100)	59 (34.30)	9 (5.23)

Table 3. Antibiotic Sensitivity Testing of the MDR-GNB in Vitro.

Microorganism Name	AMP	SAM	TZP	CZO	CAZ	CRO	FEP	ATM	ETP	MEM	AMK	GEN	CIP	TGC	SXT	NIT
Acinetobacter baumannii		0	0	0	0	0	0				1	0	0	0	0	
(n=1)	-	(0)	(0)	(0)	(0)	(0)	(0)	-	-	-	(100)	(0)	(0)	(0)	(0)	-
Enterobacter cloacae	0	0	0	0	0	0	2	0	0	0	2	2	2	2	2	0
(n=2)	(0)	(0)	(0)	(0)	(0)	(0)	(100)	(0)	(0)	(0)	(100)	(100)	(100)	(100)	(100)	(0)
Escherichia coli	0	11	45	1	25	2	38	14	51	51	51	28	12	51	25	49
(n=52)	(0)	(21.2)	(86.5)	(1.9)	(48.1)	(3.8)	(73.1)	(26.9)	(98.1)	(98.1)	(98.1)	(53.8)	(23.1)	(98.1)	(48.1)	(94.2)
Klebsiella pneumoniae	0		5	0	3	0	4	2	9	9	9	5	1	8	6	2
(n=13)	(0)	1 (7.7)	(38.5)	(0)	(23.1)	(0)	(30.8)	(15.4)	(69.2)	(69.2)	(69.2)	(38.5)	(7.7)	(61.5)	(46.2)	(15.4)

Ampicillin = AMP; Ampicillin-sulbactam = SAM; Piperacillin-tazobactam = TZP; Cefazolin = CZO; Ceftazidime = CAZ; Ceftriaxone = CRO; Cefepime = FEP; Aztreonam = ATM; Ertapenem = ETP; Meropenem = MEM; Amikacin = AMK; Gentamycin = GEN; Ciprofloxacin = CIP; Tigecycline = TGC; Trimethoprim-sulfamethoxazole = SXT; Nitrofurantoin = NIT

resistance to ceftriaxone at 96.2%, ciprofloxacin at 76.9%, and meropenem at 1.9%. MDR-GNB *K. pneumoniae* demonstrated significant resistance to ceftriaxone at 100%, ciprofloxacin at 92.3%, and meropenem at 30.8%.

Genotypic Analysis of MDR-GNB

Among the 68 isolates of MDR-GNB, two carbapenem resistance genes, producing carbapenemases (NDM and OXA-48), and three ESBL genes (CTX-M, SHV, and TEM) were identified. Notably, other carbapenemase resistance genes, such as KPC, IMP, and VIM, were not observed in this study. The most prevalent ESBL resistance gene was CTX-M, identified in 75% (51/68) of the isolates, while the most prevalent carbapenemase resistance gene was NDM, observed in 5.88% (4/68) of the isolates.

Coexistence of ESBL genes was found among the isolates, with CTX-M/SHV/TEM coexisting in two isolates, CTX-M/SHV in one isolate, and CTX-M/TEM in one isolate. However, no coexistence of carbapenemase resistance genes was observed in isolates with carbapenem resistance. Moreover, three carbapenem-resistant isolates did not show any detected resistance genes, and seven ESBL isolates did not have identified resistance genes.

Factors associated with the Outcome of MDR-GNB Colonization

Bivariate statistical tests were conducted on each variable for risk factors associated with colonization of multidrug-resistant Gram-negative bacteria upon admission to the ICU (**Table 5**).

Table 4. Identification of Drug Resistance Genes in MDR-GNB during Initial Screening upon Admission to the ICU.

No	Microorganism (number of isolates)	Resistant Gene Distribution							
		ESBL			Carbapenemase producer				
		CTX-M	SHV	TEM	NDM	OXA-48	KPC	IMP	VIM
1	*Escherichia coli* (n=51)	45	-	1	1	-	-	-	-
2	*Klebsiella pneumoniae* (n=14)	6	4	2	3	2	-	-	-
3	*Enterobacter cloacae* (n=2)				-	-	-	-	-
4	*Acinetobacter baumannii* (n=1)				-	-	-	-	-

CTX-M = cefotaxime-hydrolizing β-lactamase-Munich; SHV = sulfhydryl variable; TEM = temoneira class A extended-spectrum β-lactamase; NDM = New Delhi metallo-β-lactamase; OXA-48 = oxacillinase-48; KPC = *Klebsiella pneumoniae* carbapenemase; IMP = imipenemase; VIM = verona integron-encoded metallo-β-lactamase.

Table 5. Association between Variables and Colonization of MDR-GNB.

Characteristics		MDR-GNB colonization					p-value	OR	CI 95%	
		Yes		No						
		n	Mean ± SD /Median (q1-q3)/%	n	Mean ± SD / Median (q1-q3)/%	Mean difference			Min	Max
Age		56	49.86 ± 18.41	52	45.54 ± 18.00	4.32	0.221[a]		-2.64	11.27
Length of Stay before ICU Admission		56	4 (1-7)	62	2 (0-3)	-	<0.001[b]		-	-
Comorbidities	Yes	46	56.1	36	43.9		0.117[c]	2.04	0.83	5.04
	No	10	38.5	16	61.5					
History of Previous Antibiotic Therapy	Yes	54	56.3	42	43.8		0.010[c]	6.43	1.34	30.93
	No	2	16.7	10	83.3					
History of Previous Surgery	Yes	30	56.6	23	43.4		0.332[c]	1.46	0.68	3.11
	No	26	47.3	29	52.7					
History of Invasive Medical Device Usage	Yes	56	52.8	50	47.2		0.229[d]	---	---	---
	No	0	0.0	2	100					
History of Transfer from ICU/Inpatient Ward of External Hospital	Yes	10	47.6	11	52.4		0.665[c]	0.81	0.31	2.10
	No	46	52.9	41	47.1					
History of Previous MDR-GNB Colonization	Yes	4	66.7	2	33.3		0.680[d]	1.92	0.34	10.97
	No	52	51.0	50	49.0					

[a]Independent t-test; [b]Mann Whitney Test; [c]Chi Square Test; [d]Fisher Exact Test

The results indicate that patients with MDR-GNB colonization tend to be older (mean difference of 4.32 years) and experience longer hospitalization before ICU admission, with a median duration of 4 days (1–7 days). There was a significant association between the length of hospitalization stay before ICU admission and the occurrence of MDR-GNB colonization ($p < 0.001$). Among participants with a history of antibiotic use in the last three months, 56.30% had MDR-GNB colonization, whereas among those without a history of antibiotic therapy, 16.70% had MDR-GNB colonization ($p = 0.010$, OR 6.43, 95% CI 1.34–30.93). Bivariate analysis revealed no association between comorbidities, history of surgical procedures, invasive medical device usage, transfer from external ICU/inpatient wards, and previous MDR-GNB colonization with the current occurrence of MDR-GNB colonization.

Multivariate analysis revealed that after controlling for independent variables assumed to be related to the occurrence of MDR-GNB colonization, the variables collectively associated with the occurrence of MDR-GNB colonization in participants treated in the ICU were the length of hospital stay before ICU admission ($p = 0.003$) and a history of antibiotic therapy in the last three months ($p = 0.036$). These variables demonstrated a 5.8 times higher risk of MDR-GNB bacterial colonization. There was no association found between age, comorbidity history, and a history of invasive medical device usage on the occurrence of MDR-GNB colonization (**Table 6**).

DISCUSSION

In this study, the prevalence of patients colonized with MDR-GNB bacteria upon early screening upon admission to the ICU at CMH was 51.85% (56/108), consisting of 46.29% (50/108) ESBL and 7.41% (8/108) carbapenem-resistant. This finding was similar to the research conducted by Fouda et al. in 2016 in Egypt, which found a prevalence of MDR-GNB colonization at ICU admission of 46.70%.[16] However, this number is quite different from a study in Spain by Fernandez-Martinez et al. in 2022, which found a prevalence of MDR-GNB colonization at ICU admission of 6.20%.[17] This difference may be due to variations in sample size, hospital service levels, and different study designs. Nevertheless, both studies indicate the importance of conducting MDR-GNB colonization screening in at-risk patients upon early admission to the ICU to prevent the spread of resistant bacteria, which can impact patient mortality.

In Indonesia, the prevalence of carbapenem-resistant bacterial colonization was 26% of patients upon early admission to the ICU, including *A. baumannii* (17%), *K. pneumoniae* (5%), and *P. aeruginosa* (4%), based on the study by Saharman YR in 2020 at the ICU of RSCM. This illustrates a decrease in the prevalence of carbapenem-resistant bacteria from 2020 to 2022.[10]

The prevalence of MDR-GNB isolates in the study was 39.53% (68/172), comprising 34.30% ESBL isolates and 5.23% carbapenem-resistant isolates. However, based on the study by Saharman YR and Lestari DC in 2011 at the ICU of RSCM, which included 112 isolates from

Table 6. Risk Factors Associated with the Colonization of MDR-GNB

Parameters	Coefficient	*p*-value	OR	CI 95%	
				Min	Max
Age	0.019	0.118	1.019	0.995	1.043
Length of Stay before ICU Admission	0.201	0.003	1.222	1.070	1.397
Comorbidities	0.535	0.357	1.707	0.547	5.326
History of Previous Antibiotic Therapy	1.761	0.036	5.819	1.125	30.106
History of Invasive Medical Device Usage	20.628	0.999	$9.09^{\wedge}10^{8}$	0.000	---

various clinical specimens, the percentage of ESBL-producing isolates was 58.42% and that of carbapenemase-producing isolates was 27.59%. This indicates a decrease in the prevalence from 2011 to 2023.[8]

In this study, *E. coli* (72.09%) and *K. pneumoniae* (18.60%) were the most frequently encountered GNB during the colonization of patients upon early admission to the ICU. These findings align with previous studies conducted by Fouda et al. and Fernandez-Martinez et al.[16,17] *E. coli* and *K. pneumoniae* belong to the *Enterobacterales* family, which is a large family of GNB in the gut microbiota of healthy hosts. When dysbiosis occurs, especially due to the use of broad-spectrum antibiotics, these bacteria often become resistant to antibiotics by producing ESBL and carbapenemases. Colonization by these resistant bacteria occurs can serve as a risk factor for infections originating from multidrug-resistant bacteria.[18]

The target resistance genes in this study are CTX-M, SHV, and TEM for ESBL, and NDM, OXA-48, KPC, VIM, and IMP for carbapenemase. These gene targets were chosen because they are most frequently observed in MDR-GNB.[14,15,19] The CTX-M gene was found most frequently among other ESBL genes, accounting for 86.44% (51/59), followed by SHV at 6.78% (4/59), and TEM at 5.08% (3/59). These results are similar to those of a previous study conducted at Dr. Soetomo Hospital Surabaya in 2019, where the most common distribution of ESBL genes was CTX-M at 80% (20/25), followed by TEM at 36% (9/25), and SHV at 12% (3/25).[19] Additionally, this study found the coexistence of ESBL genes in one isolate, including the coexistence of CTX-M/SHV/TEM in two isolates, CTX-M/SHV in one isolate, and CTX-M/TEM in one isolate. The coexistence of resistance genes was also found in the study at Dr. Soetomo Hospital, including CTX-M/SHV/TEM in one isolate, CTX-M/TEM in six isolates, and CTX-M/SHV in two isolates. In this case, plasmids play a crucial role in enhancing the spread of ESBL through the transfer of resistance genes from strain to strain between bacteria in hospitals and the environment.[19] In this study, seven isolates with positive ESBL showed no resistance genes, indicating the possibility of other genes, such as AmpC genes that were previously reported in the ICU of RSCM in 2011 in ESBL-positive isolates.[8]

For the target resistance genes associated with carbapenemase production, only two resistance genes were identified in this study: NDM and OXA-48, with four and two isolates, respectively. These findings differ slightly from a previous study in the ICU of RSCM in 2011 by Karuniawati et al., where the IMP gene was found in four isolates, NDM in one isolate, and OXA-48, KPC, and VIM genes were not detected.[9] Other resistant genes such as IMP and VIM were not found in this study. Both genes were identified in other studies in Indonesia: the IMP gene in the study by Kuntaman et al. in 2018, and VIM and IMP in the studies by Saharman YR et al. in 2020, and Anggraini D et al. in 2022. However, the KPC gene has not been identified in Indonesia.[10,20,21] Three carbapenem-resistant isolates were not detected, possibly due to other mechanisms playing a role in carbapenem resistance or the presence of other genes beyond these five genes that can produce carbapenemases, such as OXA-23 found in *A. baumannii* and GES-5 in *P. aeruginosa* in the study by Saharman YR et al. in 2018 in the ICU of RSCM.[10]

The analysis of patients colonized with MDR-GNB revealed a mean age difference of 4.32 years, but this was not associated with MDR-GNB colonization compared to younger individuals. This contrasts with previous studies suggesting an association between older age and MDR-GNB colonization, as older patients often face increased infection risks due to age-related physiological changes, chronic diseases, and frequent use of medical devices such as catheters.[22–25] Patients colonized with MDR-GNB had a longer hospital stay before ICU admission, with a median of 4 (1 to 7) days. This finding is consistent with previous studies by Asare Yeboah EE et al. and Kizilates F et al., which observed increased MDR-GNB colonization with prolonged hospitalization.[26,27]

The lack of significance regarding comorbidities in this study contrasts with previous findings suggesting comorbidities

may contribute to bacterial colonization resistance.[22,28,29] Similarly, the non-significant association with a history of previous MDR-GNB colonization diverges from a study in Spain. Further examination suggests these differences may stem from incomplete screening culture data, resulting in fewer patients identified as colonized with MDR-GNB than those not colonized.[17,30]

Conversely, a statistically significant link emerged between MDR-GNB colonization and a history of antibiotic therapy in the last three months, presenting a 5.8 times higher risk than patients without such a history. This aligns with prior research linking antibiotic use to MDR-GNB colonization and subsequent infections. Moreover, tertiary hospitals such as those in this study often receive patients from other hospitals who may have been previously exposed to antibiotics. If the previous use of antibiotics is inappropriate or excessive, it can increase the risk of colonization with more severe, prolonged, and recurrent bacterial pathogens because the pathogens will become resistant to antimicrobials.[22,23,31–34]

Patients with a history of previous surgery did not show statistical significance regarding MDR-GNB colonization in this study, contrary to previous findings suggesting that surgical history may induce physiological stress, leading to prolonged hospitalization and tissue damage that facilitates the translocation of multi-drug-resistant bacteria into the body.[17,35,36] Similarly, the history of using invasive medical devices did not show statistical significance, despite a high usage rate of 98.1%. This contrasts with previous studies indicating that invasive devices such as catheters serve as sites for pathogenic bacteria colonization, [22,37–39] increasing infection risks with prolonged placement. The absence of statistical significance regarding a history of transfer from an external hospital suggests that factors related to MDR-GNB colonization were not significant, although the proportion of patients transferred from external hospitals was low at 19.4%. Nonetheless, patients may still acquire MDR-GNB shortly after hospital admission if infection prevention and control practices are inadequate.[41–43]

Study Limitations

In this study, the clinical data were collected solely from the patients' medical records. Therefore, some data, such as previous colonization results, might not have been directly obtained from patients or their families. Additionally, the completeness and clarity of these records regarding whether the results indicate colonization or infection could affect the analysis outcomes.

CONCLUSION

Patients colonized with MDR-GNB upon admission to the ICU at RSCM in 2022 amounted to 51.85%, with a prevalence of MDR-GNB of 39.53%. The most prevalent MDR-GNB were *E. coli* and *K. pneumoniae*, with the most common resistance genes found in ESBL being CTX-M and in carbapenemase producers being NDM. Notably, KPC, VIM, and IMP resistance genes were not identified. Risk factors associated with MDR-GNB colonization in patients upon admission to the ICU included the length of hospital stay before ICU admission and a history of previous antibiotic therapy.

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COMPETING INTERESTS

There is no competing interest.

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SUPPLEMENTARY TABLE

Supplementary Table 1. Primers for detecting ESBL and carbapenemase producer genes.

No	ESBL gene	Primer	Base pair (bp)
1	CTX-M	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	593
2	SHV	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCGG	797
3	TEM	F: ATGAGTATTCAACATTTCCG R: CCAATGCTTAATCAGTGAGG	858

F: Forward; R: Reverse; bp: base pair

No	Carbapenemase producer gene	Primer	Base pair (bp)
1	KPC	F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG	798
2	NDM	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621
3	OXA-48	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438
4	VIM	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	390
5	IMP	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC	233

F: Forward; R: Reverse; bp: base pair