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Submission date: 10-Dec-2020 04:24PM (UTC+0800)

Submission ID: 1470783317

File name: it-Patients-from-Tertiary-Care-Hospital-in-Jakarta-Indonesia.pdf (146.26K)

Word count: 2812

Character count: 15451

Characterization of Screening *Staphylococcus aureus* Isolates Harboring *mecA* Genes among Intensive Care Unit Patients from Tertiary Care Hospital in Jakarta, Indonesia

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Abstract—The objective of this study is to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring *mecA* genes from screening isolates among intensive care unit (ICU) patients. All MRSA screening isolates from ICU's patients of Cipto Mangunkusumo Hospital during 2011 and 2014 were included in this study. Identification and susceptibility test was performed using Vitek2 system (Biomereux®). PCR was conducted to characterize the SCCmec of *S. aureus* harboring the *mecA* gene on each isolate. Patient's history of illness was traced through medical record. 24 isolates from 327 screening isolates were MRSA positive (7.3%). From PCR, we found 17 (70.8%) isolates carrying SCCmec type I, 3 (12.5%) isolates carrying SCCmec type III, and 2 (8.3%) isolates carrying SCCmec type IV. In conclusion, SCCmec type I is the most prevalent MRSA colonization among ICU patients in Cipto Mangunkusumo Hospital.

Keywords—MRSA, *mecA* genes, ICU, colonization.

I. INTRODUCTION

STAPHYLOCCUS aureus is a gram-positive coccal bacterial pathogen and is a common cause of infections, such as skin and soft-tissue infections, endovascular infections, pneumonia, septic arthritis, osteomyelitis, and sepsis [1]. The proportion of *S. aureus* infection due to MRSA strains has been increasing during recent decades [2], [3]. Previously, MRSA has been associated with healthcare-associated MRSA [HA-MRSA]. However, these organisms have recently emerged as an important cause of community-associated infection, called CA-MRSA [4].

MRSA carriage is an important predisposing factor for developing MRSA infection [2]. Hospital commonly screens patients, especially the ICU patients for nasal carriage of MRSA. Knowing the prevalence of MRSA colonization is very important to implement infection control measures in the hospital setting.

HA-MRSA has been associated with multidrug resistance. Regarding to the Center for Disease Control and Prevention

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(CDC) guideline, HA-MRSA is more resistant than CA-MRSA to many antibiotics such as beta lactams, erythromycin, clindamycin, fluoroquinolone and tetracycline [5]. HA-MRSA carries staphylococcal cassette chromosome *mec* (SCCmec) types I, II, and III which harbor the methicillin resistance (*mecA*). On the other hand, CA-MRSA carries the smaller SCCmec types (type IV and V) which are more easily transferred to other strain of *S. aureus*, and Panton-Valentine leukocidin (PVL) genes [4], [6], [7]. In this study, we were focusing on characterizing SCCmec type using multiplex polymerase chain reactions (PCR) of screening *S. aureus* isolates. Antibiotics susceptibility test (AST) results and possible risk factors for colonization among these patients were also analyzed.

II. METHODS

A. Setting

This study is conducted at Cipto Mangunkusumo Hospital, Jakarta. Located at the capital city, Cipto Mangunkusumo Hospital is a top teaching hospital affiliated to the Faculty of Medicine, Universitas Indonesia.

B. Bacterial Isolates

All specimens in this study are taken from ICU's patients who were hospitalized during 2011 and 2014, and were examined for MRSA screening. The screening was conducted at the time of admission in ICU.

All isolates (2011 and 2014 isolates) were subcultured on manitol salt agar (MSA) at 35 °C incubation for 18-24 hours and continued with coagulase test using staphaurex (Remel®). Identification test and AST was performed using Vitek2 System (Biomereux®).

C. DNA Extraction

The DNA extraction was performed using boiling technique. One to five bacterial colonies from fresh culture were suspended to 50 µL sterile distilled water and heated at 99 °C for 10 min. After centrifugation at 20,000 g for 1 min, 2.5 µL of the supernatant was used as a PCR template.

D. PCR Amplification

Multiplex PCR was conducted to detect type of SCCmec genes. The primers used in this study are summarized in Table I. Those were the same primers used in [7]-[9]. All PCR

assays were performed directly from bacterial suspensions obtained after DNA extraction. An aliquot of 2.5 µl of this suspension was added to 22.5 µl of PCR mixture. The mixture contains 1X *Taq* PCR Buffer, 4 mM MgCl₂, 100 µmol dNTPmix, 12.5 pmol each primers, 0.75 unit Hotstar *Taq* DNA polymerase. The thermocycling conditions set at 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min, and 72 °C for 4 min. The amplicon was analyzed by electrophoresis in a 2% agarose gel.

E. *MecA* Gene Confirmation

We performed latex agglutination test of Penicillin-Binding Protein (PBP)-2 (Oxoid®) to confirm the *MecA* gene detection. This test has similar sensitivity and specificity to PCR as a gold standard [10].

F. Ethical Clearance

This study has passed evaluation by the Ethics Committee of Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo Hospital.

TABLE I
PRIMERS USED IN THE STUDY

Primers	Sequences (5'-3')	Size (bp)	Type of SCCmec				
			I	II	III	IV	V
β	ATTGCCTTGATAATAGCCYTCT	ccrA2-B (937)		X		X	
α3	TAAAGGCATCAATGCACAAACACT	ccrC (518)			X		X
ccrCF	CGTCTATTACAAGATGTTAAGGATAAT	IS1272 (415)	X			X	
ccrCR	CCTTTATAGACTGGATTATTCAAAATAT	mecA-IS431 (359)					X
1272F1	GCCACTCATAACATATGGAA						
1272R1	CATCCGAGTGAAACCCAAA						
5RmecA	TATACCAAACCCGACAACTAC						
5R431	CGGCTACAGTGATAACATCC						

TABLE II
ANTIBIOTICS RESISTANCE PATTERN BASED ON SCCmec TYPE

Antibiotics	n (%) Resistant		
	SCCmec Type I	SCCmec Type III	SCCmec Type IV
Benzylopicillin	15 (88.2)	3 (100)	2 (100)
Gentamicin	3 (17.6)	3 (100)	1 (50)
Ciprofloxacin	1 (5.9)	3 (100)	0 (0)
Levofloxacin	1 (5.9)	3 (100)	0 (0)
Moxifloxacin	1 (5.9)	3 (100)	0 (0)
Erythromycin	2 (11.8)	1 (33.3)	0 (0)
Clindamycin	3 (17.6)	1 (33.3)	2 (100)
Quinipristin	1 (5.9)	0 (0)	0 (0)
Linezolid	1 (5.9)	0 (0)	0 (0)
Vancomycin	1 (5.9)	0 (0)	1 (50)
Tetracycline	11 (64.7)	3 (100)	1 (50)
Tigecyclin	17 (100)	3 (100)	2 (100)
Nitrofurantoin	17 (100)	3 (100)	2 (100)
Rifampin	1 (5.9)	3 (100)	0 (0)
Cotrimoxazol	1 (5.9)	1 (33.3)	0 (0)

III. RESULTS

Total isolates included in this study were 327 isolates. Once the isolate was confirmed as *S. aureus*, we identified whether that isolate is MRSA positive or negative. AST was performed on MRSA positive isolates, and the results are summarized on Table II.

Based on the results of identification test, we identified that 7.3% (24 isolates) of all isolates were MRSA positive. We examined those 24 MRSA positive isolates using PCR to detect the type of SCCmec, and we found SCCmec type I were 17 isolates, SCCmec type III were 3 isolates, and SCCmec type IV were 2 isolates. None of the isolates has either SCCmec type II or type V. Two MRSA positive isolates did not have SCCmec gene based on primers tested on this study.

We searched the risk factors of these patients which are written on patient's medical record and analyzed it using

statistical software SPSS version 16.0 to determine the relationship between various risk factors with the incidence of MRSA colonization. The results of risk factors analysis are summarized on Table III.

TABLE III
PATIENTS' CHARACTERISTICS AND RISK FACTORS

No	Variable	n (%) patient		OR 95% CI	p value
		MRSA	MSSA		
1.	Gender				
	Male	13(54.2)	8(36.4)		0.180
2.	Female	11(45.8)	14(63.6)		
	Age				
3.	Mean	47.8	35.3		
	Median (range)	53.5(23-88)	34(11-66)		
	< 65 years	22(91.7)	21(95.5)	0.524	0.533
	≥ 65 years	2(8.3)	1(4.5)	0.044-6.216	
4.	Antibiotics History				
	Yes	9(37.5)	10(45.5)	0.720	0.402
5.	No	15(62.5)	12(54.5)	(0.222-2.338)	
	Comorbidity				
6.	Yes	4(16.7)	2(9.1)	2	0.376
	No	20(83.3)	20(90.9)	(0.328-12.184)	
7.	Invasive Devices				
	Urine catheter	22(91.7)	16 (72.2)	0.242(0.43-1.361)	0.096
	Infus line	17(70.8)	17(77.3)	1.4(0.37-5.294)	0.437
	CVC	16(66.7)	10 (45.5)	0.417(0.126-1.374)	0.125
8.	Mechanical ventilation	17(70.8)	16(72.7)	1.098(0.303-3.975)	0.574
	Hospitalized History in Last 1 year				
9.	Yes	19(79.2)	18(81.8)	0.844	0.559
	No	5(20.8)	4(18.2)	(0.195-3.652)	

IV. DISCUSSION

The percentage of MRSA carriage (7.3%) found on this study is similar with other countries such as Taiwan, whereas the prevalence was around 10.7% [11]. While study conducted

by Santosaningsih et al. in 3 cities in Indonesia (Semarang, Malang, and Bali) found the percentage of MRSA carriage is 4.3% [3]. The low numbers of MRSA colonization found on this study may be caused by infection control programs that have been implemented better than previous years.

In this study, we found MRSA colonization in Cipto Mangunkusumo Hospital is dominated by SCCmec type I. Our finding in this study was different with [3]. They found MRSA colonization is dominated by SCCmec type III [3]. Thus, those 2 types of SCCmec are categorized as HA-MRSA. Although screening was conducted at the time of admission in ICU, almost all the patients have history of treatment in another ward or other hospitals. In contrast to other study in Korea which found that 80% (n=40) isolates of MRSA colonization was dominated by SCCmec type IV and categorized as CA-MRSA [12].

Two isolates in this study were phenotypically MRSA, but we could not identify the type of the SCCmec. We assumed that those isolates have other type of SCCmec that we did not tested on this study i.e. SCCmec type VI-XI. Methicillin resistance could be caused by other mechanisms, for example beta-lactamase hyperproduction, alteration of PBP target, inactivation of *fem* and *ilm* *Alt* genes, and activation of *chr* gene. Other studies also suggest that methicillin resistance could be influenced by novel *MecA* homologue gene called *MecC* [13], [14]. We did not consider those mechanisms because latex agglutination test of PBP-2 have shown positive result.

Based on the genotype characteristic, MRSA isolates in this study are categorized as HA-MRSA, but the antibiotics susceptibility test result was uncommon. HA-MRSA shows high resistance to many antibiotics such as beta lactams, erythromycin, clindamycin, fluoroquinolone and tetracycline [5]. However, this study found all isolates were resistant to tigecycline and nitrofurantoin, and mostly resistant to benzylpenicillin and tetracycline. We also found one isolate resistant to vancomycin and linezolid. This finding calls our imperative awareness that VRSA are already existed in the hospital.

Oh and Tan studied with 136 samples and found that the risk factors for MRSA colonization are prolonged hospitalization, history of MRSA infection previously, and diabetes mellitus as comorbid. No statistically significant relationship obtained for age and use of invasive devices [15]. Ridgway et al. found that 21.1% of patients colonized with MRSA have a history of hospitalization in recent years. [16] However, such a study confirmed no statistically significant relationship between age, use of invasive devices, history of prior treatment (within 1 year), history of antibiotics usage, and presence of comorbidities with the MRSA colonization.

MRSA colonization is the precursor for nosocomial infections. Knowing the prevalence of MRSA colonization is very important to implement infection control measure in the hospital setting. One effective way to prevent the transmission is by implementing the five moments of hand hygiene. In addition, it is necessary to perform routine surveillance to understand the changes of MRSA epidemiology.

This study has a few limitations to be considered. The small sample size and two groups compared (MRSA and MSSA) were unmatched, which may lead to statistical bias. Better analytical results could be obtained by designing case-control study, which is conducted by selecting the criteria for each case and control groups specifically to avoid bias.

V. CONCLUSION

Based on genotype characteristic, SCCmec type I is the most prevalent MRSA colonization, thus classified as HA-MRSA. Although age, use of invasive devices, history of prior treatment (within 1 year), history of antibiotics usage, and presence of comorbidities are statistically insignificant, they could not be ruled out as risk factors for MRSA colonization.

ACKNOWLEDGMENT

This work was fully supported by National Institutes of Health Research and Development, Ministry of Health, Indonesia (2014).

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