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Editorial Adress:
d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo, Gedung Diagnostik Center Lt. IV
Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
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RESEARCH

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION AND SCREENING METHOD EFFECTIVENESS FOR PATIENTS ADMITTED TO THE INTENSIVE CARE

(Kejadian dan Ketepatgunaan Penapisan Kolonisasi Methicillin-Resistant Staphylococcus aureus di Pasien Perawatan Intensif)

Andaru Dahesihdewi[1], Budi Mulyono[1], Iwan Dwiprahasto[2], Supra Wimbarti[2]

ABSTRAK

Methicillin-resistant S.aureus (MRSA) menyebabkan banyak infeksi nosokomial (inos) dan penyebarannya menunjukkan mutu clean care. Kejadian inos MRSA meningkatkan angka kesakitan, kematian, lama rawat inap, kebutuhan antibiotika dan meluasnya resistensi, readmisi serta biaya kesehatan. Penapisan kolonisasi MRSA di pasien yang akan dirawat intensif diperlukan untuk mencegah penyebaran dan mengendalikan peresepan antibiotika. Pemeriksaannya diharapkan tepat guna untuk mendukung Sistem Jaminan Kesehatan Nasional. Penelitian dilaksanakan di Ruang Rawat Intensif RSUP Dr Sardjito Yogyakarta di seluruh pasien pada tahun 2015 saat masuk sesuai patokan kesertaan dan non-kesertaan. Spesimen dari nares anterior dan kulit (aksila-inguinal), diambil dalam 1x24 jam, dinilai kepositifan MRSA menggunakan beberapa media identifikasi di Instalasi Laboratorium Klinik. Perbandingan analitik dan praktikabilitas metode identifikasi dianalisis efektivitasnya. Kejadian kolonisasi S.aureus dan MRSA di pasien saat masuk perawatan Ruang Intensif 23,4% dan 9,7%. Faktor kebahayaan dominan kolonisasi MRSA adalah riwayat perawatan RS. Nares anterior dominan sebagai tempat kolonisasi S.aureus (74,2%) dan MRSA (33,7%). Terdapat 7,7% kolonisasi lolos deteksi bila sampling hanya dilakukan di nares. Kesepakatan hasil pemeriksaan antar metode baik (indeks Kappa >0,8) dengan metode yang disarankan adalah penggunaan media selektif MRSA langsung atau urutan metode deteksi menggunakan Blood Agar, pengecatan Gram, uji aglutinasi, media khromogenik selektif MRSA. Metode penapisan dengan tingkat deteksi dan praktikabilitas yang baik bermanfaat mendukung pengendalian infeksi di ruang berkebahayaan tinggi.

Kata kunci: *Kolonisasi MRSA, penapisan tepat guna, metode deteksi, pengendalian infeksi*

ABSTRACT

Methicillin-resistant *S.aureus* (MRSA) may cause hospital-acquired infection and indicate the quality of care at the hospital. The incidence of nosocomial MRSA may increase the morbidity, mortality, duration of hospitalization, antimicrobial usage and resistance, readmission and medical costs. MRSA screening in patients admitted to the intensive care is needed to prevent its spread and to control the prescription of antimicrobials. The laboratory methods should be effective in supporting health assurance system. This study was performed at the Intensive Care Unit of the Dr Sardjito Hospital in Yogyakarta for all patients in 2015 by inclusion and exclusion criteria. Specimens from nostrils and skin (axilla-inguinal), were taken during the first 24 hours of admission, determining MRSA using several identification media in Clinical Laboratory Department. Analytical and practicability comparisons between methods were analyzed. The prevalence of *S.aureus* and MRSA colonization was 23.4% and 9.7%. The previous hospitalization was the dominant risk factor for MRSA colonization. The nostrils were a principal site of *S.aureus* (74.2%) and MRSA (33.7%) colonization. Approximately 7.1% instances of colonization would not have been detected by a single investigation of the nostril. The agreement between methods was good (Kappa index >0.8) while the method suggestion was direct MRSA selective media or sequence method consisting of Blood Agar, Gram staining, agglutination test and selective MRSA chromogenic media. Screening method with a good detection rate and practicability was useful for infection control in high risk Units.

Key words: MRSA colonization, screening effectiveness, detection method, infection control

[1] Department of Clinical Pathology, Faculty of Medicine Gadjah Mada University/Dr. Sardjito General Hospital Yogyakarta, Indonesia
E-mail: adahesihdewi@yahoo.com
[2] Clinical Epidemiology & Biostatistic Unit, Faculty of Medicine Gadjah Mada University Yogyakarta Indonesia

INTRODUCTION

Infection Control and Prevention (ICP) in hospitals stand as a mandatory effort to achieve safety for patients, health-care providers and hospital environment, an integral part of quality services. Hospital Acquired Infections (HAI's) is one of the most common adverse events around the world, including Indonesia. HAI's by multi-drug resistant organisms, the most common MRSA (Methicillin-resistant *S.aureus*), is declared as an indicator of clean care.[1-6]

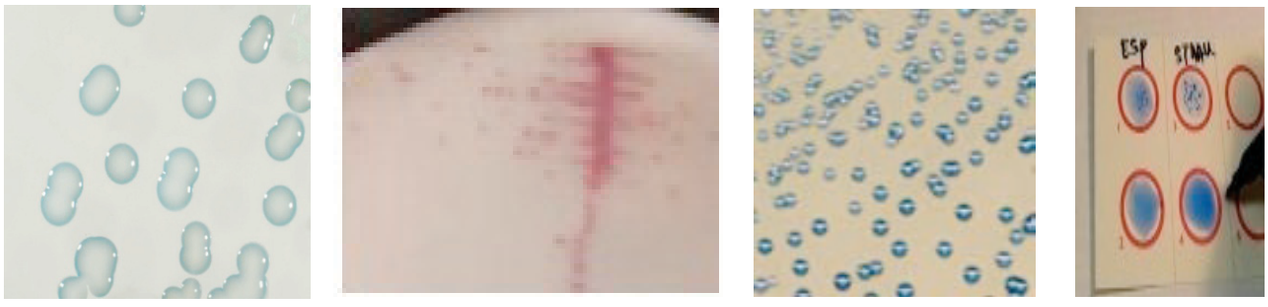
Methicillin-resistant *S.aureus* is a type of *S.aureus* bacteria resistant to beta-lactam group antibiotics and potentially resistant to many other antibiotics. The prevalence of MRSA in a lot of countries tends to increase[5-7] In Indonesia, related to its limited hospital resources, MRSA is reported to have raised from 2.5% in 1986, which soon after became 9.4% in 1993, to 23.5% in 2006.[8] The World Health Organization (WHO) has declared that MRSA is considered as one of 10 largest causes of pandemic infection due to their vast spread and significant burdens they have affected.[4,9]

Healthcare-associated MRSA (HA-MRSA), according to the Centers for Disease Control and Prevention (CDC), is a MRSA infection among individuals who have been hospitalized or have had surgery in the previous year, have a permanent medical device implanted, live in a long–term care facility, or a patient who underwent hemodialysis.[4,10] HA-MRSA infection is a serious HAI's due to its morbidity, mortality and medical cost. Recommendation for its transmission control strategy is searching and destroying method.[11,12] The ICP program also recommends cohorting principle in patient placement.[2,3]

MRSA detection can be done by the phenotype identification using selective chromogenic media or detection of Penicillin Binding Protein-2a (PBP2a) or detection of MecA gene. Chromogenic media contains selective chromogenic enzyme substrates for rapid growth of *S.aureus* and will give a specific color for MRSA. Detection of protein PBP2a which is coded by MecÃ gene could be done using a latex human fibrinogen and specific S.*aureus* monoclonal antibody sensitized agglutination method. MecA gene detection as the gold standard, could be done using nucleic acid amplification technic with Staphylococcal Cassette Chromosome (SCC) related DNA sequence as a specific target.[13-15] (Fig.1)

In Indonesia, a study by Endang Sri Lestari[16] reported that the rate of MRSA incidence among hospital discharged patients was 6.6%, among patients in the same room with MRSA positive patients was 16.5%, among health care workers was 1.7% and a 1.5% rate in the environment with the total prevalence rate of 7.3%.[16] In the Dr. Sardjito Hospital Yogyakarta (2011), a retrospective observational study reported a rate of 24.8% presumptive MRSA among *S.aureus* positive clinical specimens. The dominant characteristics of them were immunocompromized patients, longer length of stay and invasive device usage.[17] In the Dr. Muwardi Hospital Surakarta, a prospective observational study reported the prevalence rate of MRSA colonization at about 50–66.7% among randomized patients who were more than 72 hours in wards, at about 22.4%-64.5% among healthcare workers and at 68.4% among environmental specimens and instruments.[18]

This study aimed to determine the prevalence of MRSA colonization patients admitted to the Dr. Sardjito Hospital Intensive Care and evaluate the analytical performance of its various detection methods. It is a necessity to have an effective routine MRSA screening with efficient detection method for safer clinical management and better clinical outcome, particularly



(a) (b) (c) (d)

Figure 1. MRSA detection: Selective chromogenic media for MRSA culture (a-c); latexagglutination for PBP2a detection (d)

in the Intensive Care, in the era of National Health Insurance (JKN/Jaminan Kesehatan Nasional).

METHODS

The study was an analytical observational cross-sectional design. MRSA colonization screening was done in the Dr. Sardjito Hospital Intensive Care, consisting of Intensive Care Unit (ICU), Pediatric Intensive Care Unit (PICU), Burn Unit and Intensive Cardiac Care Unit, in January-December 2015. All patients who were introduced to the target room and agreed to participate in this study were consecutively included. Patients proven to be infected by MRSA when signed were excluded from screening and directly recorded as a potential source of MRSA infections. Characteristics of study subjects including age, gender, main diagnosis, history of hospitalization and history of antibiotics usage were collected through medical records.

Nostril, axilla and groin swabs were taken using a sterile cotton swab moistened with physiological saline. Nostril swab was obtained by carefully 5x rotating a swab in both nostrils reaching to the nasal bone. Axilla and groin swabs were taken by wiping the skin area repeatedly referred to 5 times. All swabs were performed by trained nurses within 24 hours of admitting patients to the target room, further inoculated in Trypticase Soy Broth (TSB) media and immediately sent to the Microbiology-Parasitology-Immunology (MPI) Clinical Laboratory Sub Lab Dr. Sardjito Hospital.

Cultivation and identification of MRSA were performed in the Microbiology-Parasitology-Immunology Sub Lab (MPI) Clinical Laboratory Dr. Sardjito Hospital using a general media and a selective chromogenic media. Blood Agar (BA) was compared with the Mannitol Salt Agar (MSA), continued with *S.aureus* identification through Gram staining, catalase test, DNase and agglutination test, subsequently, MRSA identification was performed using cefoxitin and oxacillin disc diffusion method on Mueller Hinton (MH) agar compared with using MRSA selective chromogenic media. Method reliability was performed based on quality control standard guidelines, consisting of sterility test, media quality test, antimicrobial disc quality test which was checked by *S.aureus* ATCC 25923 as control bacteria.[19-21]

All recruitment and research data collection obtained the ethical approval by the Faculty of Medicine UGM/Dr. Sardjito Hospital Ethical Board.

RESULT AND DISCUSSION

The total samples of MRSA colonization screening between January–December 2015 in target rooms was 3,774, consisting of nostril and skin swab (axilla and groin), from 1,877 patients (95.8% of total patients admitted to target rooms). The remaining patients were not sampled because they were not ready to provide consent for various reasons. The ward distribution of sample source was presented in Table 1.

Distribution of *S.aureus* and MRSA colonization based on patient's ward was presented in Table 2.

Table 1. The ward distribution of clinical specimens

Target room	n	%
ICU	541 x2	28.7
PICU	363 x2	19.2
Burn Unit	44 x2	2.3
ICCU	939 x2	49.8
Total	1887 x2	100.0

Table 2. Patient distribution with MRSA and *S.aureus* colonization

Target room	*S.aureus*		MRSA	
	n	%	n	%
ICU	108	19.96	51	9.4
PICU	171	47.1	43	11.8
Burn Unit	9	20.5	4	9.1
ICCU	154	16.4	85	9.1
Total	442	23.4	183	9.7

The total number of *S.aureus* colonization was 442 (23.4%), as a source from only nostril swab was 151 (34.2%), from only skin swabs (both axilla and groin) was 114 (25.8%) and from both nostril and skin swab was 177 (40%). Patients who were admitted to PICU showed the highest prevalence of colonization, consistent with a previous surveillance result (semester-2 2014). This was assumed to be due to less personal hygiene among children, as stated in several other studies.[22,23] According to the result of several studies on prevalence and epidemiology of *S.aureus* colonization, the prevalence rate was reported at about 6.5%-38%.[24-28] The total nostril colonization in this study was 74.2% out of the positive *S.aureus* and was equal to the prevalence rate reported by a study in the Intensive Care Unit of the University of Brazil Hospital at the level of 80.4%, but was higher than the prevalence rate in Canada at a level of 67%.[22,29] There were 6.1% patients in this screening detected to have *S.aureus* colonization only on the skin, increasing rate compared with previously reported (4.9%), of which 7.1% of these colonies were MRSA, indicating

Table 3. Distribution of *S.aureus* and MRSA colonization sources among *S.aureus* positive patients

Target room	*S. aureus*						MRSA					
	nostrils		skin		nostrils & skin		nostrils		skin		nostrils & skin	
	n	%	n	%	n	%	n	%	n	%	n	%
ICU	45	41.6	23	21.3	40	37.0	27	25	9	8.3	15	13.9
PICU	31	18.1	35	20.5	105	61.4	32	18.7	8	4.7	3	1.8
Burn Unit	4	44.4	2	22.2	3	33.3	1	11.1	2	22.2	1	11.1
ICCU	32	20.8	54	35.1	68	44.2	51	33.1	15	9.7	19	12.3
Total	151	34.2	114	25.8	177	40.0	111	25.1	34	7.7	38	8.6

the consistent need of screening from both nostrils and skin swab (Table 3). Identification rate of skin colonization solely, was slightly higher in this study compared with a similar study reported in USA in 2007-2008 at the level of 5%.[30]

The total number of MRSA colonization was 183 (9.7% of the total patients or 41.4% of *S.aureus* colonization), source from the nostril swab was 111 (25.1% of *S.aureus* colonization), source from the skin swab was 34 (7.7% of *S.aureus* colonization) and both nostrils and skin swab was 38 (8.6% of *S.aureus* colonization) (Table 3), relatively similar to a previous surveillance. Several prevalences and epidemiology studies of MRSA colonization among patients in Intensive Care Unit reported the prevalence rate at about 6.74–15.4% out of all patients admitted.[22-27]

Overall amount of 41.4% of patients currently entering the Intensive Unit with *S.aureus* colonization was MRSA, increased compared to the previous rate (39.5%), higher than the result of a study in Brazil (2003) which amounted to 34.5%.[22] Most of MRSA colonization could be detected from nostril swabs (33.7%) while 7.7% was only detectable from skin swab. This detection rate is an important consideration as the basic cohorting of patient's placement in order to control its spread in Intensive Unit.[31]

Table 4 showed no significant differences in the characteristics of patients with *S.aureus* or MRSA

Table 4. Characteristics of patients with *S.aureus* and MRSA colonization

Characteristics	Colonization		p[a]
	S.aureus (%) n=442	MRSA (%) n=183	
Gender			
Male	235 (53.2)	105 (57.4)	0.103
Female	207 (46.8)	78 (42.6)	
Age			0.141
Up to 18 y/o	174 (39.4)	40 (21.9)	
>18 up to 45 y/o	72 (16.3)	34 (18.6)	
>45 up to 65 y/o	118 (26.7)	67 (36.6)	
>65 y/o	78 (17.6)	42 (22.9)	
Main diagnosis			
Infection	36 (8.1)	30 (16.4)	0.099
Malignancy	95 (20.8)	27 (14.8)	
Cardiac and vascular	154 (34.8)	47 (25.7)	
Methabolic degenerative	102 (23.1)	56 (30.6)	
Surgery/Trauma	55 (12.4)	23 (12.5)	
Hospitalization history			
Yes	261(59.1)	131 (71.6)	<0.001*
No	181 (40.9)	52 (28.4)	
Outpatient history			
Yes	244 (55.2)	102 (55.7)	0.412
No	198 (44.8)	81 (44.3)	
Medical intervention history			
Antibiotics (+)	172 (38.9)	89 (48.6)	0.137
(–)	270 (61.1)	94 (51.4)	
Surgery (+)	168 (38.1)	74 (40.4)	0.201
(–)	274 (61.9)	109 (59.6)	

[a] Proportional difference in MRSA colonization (chi square test)

*significant difference (p<0,05)

colonization, such as gender, age, main diagnosis and antibiotics usage (p>0.05). Variables in this screening showed a significant difference in MRSA colonization patients was the history of hospitalization in the last 1 year (p<0.001). These results were consistent with a study report in Brazil (2003) as well as in the United States University Hospital.[22,32,33] The absence of difference in the use of antibiotics in both colonization populations was very likely due to the limited record of its history that could be referenced (recall bias). Similar studies in the United States and Australia reported that an important risk factor for MRSA colonization was the use of antibiotics.[23,24,34]

Among the 200 patient's swab *of S.aureus* and MRSA colonization screening randomly selected, evaluation of *S.aureus* selectivity recovery in the MSA compared to the BA, was presented in Table 5. The agreement of *S.aureus* recovery and identification between both the MSA dan BA was 0.81.[19,20] There were 13 isolates of MSA that did not indicate the specific growth with yellow pigment but gave a specific macroscopic and hemolytic type in the BA. On the other hand, there was 4 specific isolates outgrowth in the MSA that did not give a specific hemolytic type in the BA. In further identification tests, 17 isolates were Gram-positive cocci in cluster or chain form, positive catalase test, with negative agglutination test result. The disagreement then is concluded as no growth of *S.aureus* and did not distinguish the benefits of both media in its screening.

Table 5. Comparison of the result of presumptive *S.aureus* on MSA vs BA (n=200)

		BA		Total
		+	-	
MSA	+	124	4	128
	-	13	59	72
	Total	137	63	200

Furthermore, among 100 randomly selected samples from the consistently positive, comparative evaluation of agglutination test result provided MSA positivity of 65% in comparison to 75% in BA. Comparison of other identification tests (catalase, DNase) gave a 100% positive result agreed. *S.aureus* identification using the BA had a slightly higher probability of detection without confirming their specificity, consistent with the result of a previous evaluation.

Evaluation of the MRSA detection using antibiotic disc diffusion method (towards combination oxacillin-cefoxitin) compared with selective chromogenic media gave a good agreement with Kappa index of 0.83 (Table 6), relatively consistent with previous evaluation result (Kappa index of 0.89).[20] The selective chromogenic method provided more MRSA detection than the disc diffusion method without confirmation of specificity. There was no MRSA detected by the disc diffusion method which was not detected by the selective chromogenic method.

Table 6. Comparison of the result of presumptive MRSA on selective A chromogenic media vs antibiotic disc diffusion (n=75)

		Disc diffusion		Total
		+	-	
Selective chromo-genic media	+	23	6	29
	-	0	46	46
	Total	23	52	75

A high detection rate (sensitivity) without confirmation of specificity on the good level agreement is an important diagnostic performance for screening method. The advantage of direct inoculation to MRSA selective chromogenic media is time-saving, not needed in certain inoculum concentration, ready to use so it was more practical. However, readymade media have limitations such as shorter expiration date and are relatively more expensive. Use of the BA continued agglutination test yielded a higher detection rate of *S. aureus*. This step could be followed by using Muller Hinton (MH) or MRSA selective chromogenic media for MRSA identification. This method limitation is relatively complicated producing the BA than the MSA and the need for standardization on the inoculum preparation (0.5 McFarland) as well as on the production of the MH agar (4-5 mm thickness, pH) for the antibiotic sensitivity disc diffusion method.[21]

CONCLUSION AND SUGGESTION

The prevalence of *S.aureus* and MRSA colonization in patients on admission to the intensive care in the Dr. Sardjito Hospital period January-December 2015 was consistent with a previous surveillance period indicating that a considerable level became routine screening. The level of MRSA colonization of the nostril swab and exclusively of the skin swab indicated the screening needs of both swabs among patients within a 1 last year hospitalization as the priority.

Based on the MRSA screening method agreement and its practical aspect, it is suggested to select a method with a high probability detection including

direct clinical specimen inoculation on the selective chromogenic media. An alternative for cost saving, is using the BA as the first test followed by Gram staining, agglutination test and identification on MRSA selective chromogenic media. If it is done simultaneously, maximally within 2x24 hours, the results could be used to manage patient placement and infection control precaution practice in wards, also to plan the initial empiric antibiotic when the patients show signs of infection. This study can be a scientific evidence underlying recommendation to select MRSA detection method in the laboratory to support its screening program in the Intensive Care Unit, particularly when resources are limited.

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