IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS COLLECTED AT A REFERRAL HOSPITAL, JAKARTA, INDONESIA IN 2013

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Abstract. The study identified and determined antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) strains collected from patients at a referral hospital in Jakarta, Indonesia from August to October 2013. *S. aureus* strains were identified using a matrix-assisted laser desorption ionization-time of flight mass spectrometry and confirmed by PCR-based detection of staphylococcal *nuc*, and MRSA by presence of *mecA*. Antibiotic susceptibility was determined using a disk diffusion method. Thirty-seven out of 133 *S. aureus* strains were identified as MRSA strains, 84% of which were collected from hospital wards, followed by emergency (8%), and outpatient (5%) units. Clinical specimens harboring MRSA were swabs (27%), sputum (27%), blood (16%), pus (11%), and urine (8%). MRSA strains were susceptible to sulphamethoxazole-trimethoprim (81%) followed by chloramphenicol (59 %), tetracycline (41%), erythromycin (41%), and gentamicin (30%), but in general, were still less sensitive than methicillin-sensitive strains. These data show the necessity of consistent surveillance and monitoring of prevalence, distribution and antimicrobial resistance of MRSA both in hospitals and community in Indonesia.

Keywords: methicillin-resistant *Staphylococcus aureus,* antimicrobial susceptibility, clinical specimen, Indonesia

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global health problem responsible for most global *S. aureus* bacteremia cases compared to methicillin-sensitive *S. aureus* (MSSA) and is associated with poorer clinical outcome (Hassoun *et al,* 2017). Resistance in MRSA is usually conferred by acquisition of *mecA* encoding a penicillin-binding protein with a significantly lower affinity for β-lactams carried on mobile genetic element (Peacock and Paterson, 2015). Prevalence of nosocomial and community-acquired MRSA are reported to be highest in Asia (Chen and Huang, 2014).

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Most hospitals in Asia are endemics for MRSA, with an estimated proportion ranging from 28% to >70% among all clinical *S. aureus* isolates in early 2010 (Chen and Huang, 2014). In Indonesia during this period (2008 to 2010) of 259 clinical *S. aureus* isolates from patients in four tertiary care hospitals (in Denpasar, Malang, Padang and Semarang) 17 (7%) isolates are MRSA strains, with a prevalence of 2-9% depending on the hospital (Santosaningsih *et al,* 2016). Colonization of MRSA in nose and throat of patients on admission to Dr Soetomo Hospital, Surabaya in 2014 is 52 (8.1%) of 643 patients (Kuntaman *et al,* 2016). More recently, Santosaningsih *et al* (2018) reported 8 MRSA from 257 *S. aureus* isolates from skin and soft tissue infections, and 60/384 (16%) elective surgery patients at a referral hospital in Jakarta carried *S. aureus* with 3 isolates being MRSA (Nelwan *et al,* 2018).

In Indonesia, risk factors of MRSA carriage among patients at admission to a surgery ward in a resource-limited hospital are referral from another hospital, transfer from surgery acute care unit, having a surgical procedure within three months prior to admission, and immune-compromised status (Santosaningsih *et al,* 2017). This study investigated MRSA prevalence among patients in a referral hospital in Jakarta during 2013 to provide the epidemiology of MRSA infection that can be applied in the development of strategies to treat and control MRSA infection in the country.

MATERIALS AND METHODS

S. aureus isolates

One hundred thirty-six clinical presumptive *S. aureus* isolates were collected as part of a routine test at the Department of Clinical Pathology, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia from August to October 2013. This 900-bed Hospital is a referral hospital at the national level and has a turnover of approximately 36,000 in-patients per year (Nelwan *et al,* 2018). The presumptive *S. aureus* isolates were from sputum (n=38), swabs (n=30), blood (n=13), pus/abscess (n=22), urine (n=13), and other sources (n=9). These isolates were transferred to the Molecular Bacteriology Unit, Eijkman Institute for Molecular Biology, Jakarta and stored in skim milk-tryptone-glucose-glycerol medium (prepared in-house) at -80C until used.

The study was approved by the ethic's committee of the Faculty of Medicine, University of Indonesia, Jakarta (ref. no. 824/H2.F1/ETIK/VIII/2013).

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) mass spectrometry assay

MALDI-TOF MS was employed to confirm the identity of the presumptive *S. aureus* isolates (Josten *et al,* 2013; Manukumar and Umesha, 2017). In brief, a direct colony smear (DCS) on a ground plate was prepared by spreading a single colony from an overnight culture grown at 37°C, air dried at room temperature and then was added with 1 µl aliquot of matrix solution (α-cyano-4-hydroxycinnamic acid) and air dried at room temperature. Analysis was conducted using a Microflex LT MALDI-TOF MS spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a flex control software (Bruker Daltonics). Measurements were taken in the linear positive-ion mode with an accelerating voltage of 20 kV over a mass range of 2–20 kDa (Loucif *et al,* 2014), and results are reported as numeric score (0-3.00) based on collective comparison of experimental protein spectra compared to MALDI Bruker's Biotyper-specific

database, with scores <1.69 reported as non-reliable genus ID, 1.70-1.99 as probable genus ID, 2.00-2.29 as secure genus ID, and 2.30-3.00 as highly probable species ID (Josten *et al*, 2013; Manukumar and Umesha, 2017).

PCR detection of MRSA *nuc* and *mecA*

PCR amplification of staphylococcal *nuc* (encoding nuclease) and *mecA* (encoding methicillin-resistance factor) was performed as described previously (Nelwan *et al*, 2018). In short, DNA was prepared by incubating a bacterial cell suspension in TE buffer (10 mM Tris-HCl pH 8.0 containing 1 mM Na_2EDTA,) at 100°C for 10 minutes, immediately cooled at -20°C for 5 minutes and centrifuged at 1,000*g* for 10 minutes. Primers for amplification of *nuc* were 5′-TCAGCAAATGCATCACAAACAG-3′ and 5′-CGTAAATGCACTTGCTTCAGG-3′, and those of *mecA* were 5′-GGGATCATAGCGTCATTATTC-3′ and 5′-AACGATTGTGACACGATAGCC-3′. The reaction mixture (25 µl) contained GoTaq Green Master Mix (Promega, Madison, WI), 10 µM primers, and 1 µl of DNA template. Thermocycling was conducted in Perkin Elmer PE 9700 Thermal Cycler (Thermo Fisher Scientific, Norwalk, CT) as follows: 5 minutes at 94°C; followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute; with a final step at 72°C for 10 minutes. Amplicons (255 and 527 bp of *nuc* and *mecA*, respectively) were detected by 1.5% agarose gel-electrophoresis and SYBR safe DNA gel staining (Invitrogen, Carlsbad, CA) (Nelwan *et al*, 2018).

Antibiogram determination

Antibiograms were determined using a disk diffusion method on Muller-Hinton 5% sheep blood agar (prepared in-house) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). Seven different antibiotic disks (Oxoid, Hampshire, UK) used in the assay were chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.5 g) (Safari *et al*, 2015). Clinical and Laboratory Standards Institute (2013) guidelines were used for interpreting zones of inhibition. *S. aureus* ATCC 25923 was used as control (Nelwan *et al*, 2018).

RESULTS

Identification of *S. aureus* by MALDI-TOF MS

Of the 136 presumptive *S. aureus* strains evaluated by MALDI-TOF MS, 83 (61%) isolates had score values of 2.00-2.99 indicative of secure genus ID and 30 (22%) with score values of 2.30-3.00, probable genus ID (Table 1). Three isolates were identified as *S. epidermidis (n=1)* and *S. saprophyticus* (*n*=2) with scores >1.70 by MALDI-TOF MS.

PCR-based detection of MRSA strains

All 136 presumptive *S. aureus* strains were subjected to PCR amplification of staphylococcal *nuc* and *mecA*, resulting in 98% positivity for *nuc* and 28% positivity for *nuc+mecA* (MRSA) (Table 1). The majority of MRSA strains were from swabs (27%) and sputum (27%), with 84% collected from patients in the wards (Table 2).

Antibiogram profiles

Using the disc diffusion method, the majority of MRSA strains were susceptible to sulphamethoxazole/trimethoprim (81%) and chloramphenicol (59%) (Table 3). In general, the MRSA strains were less susceptible to the seven test antibiotics than MSSA strains.

Table 1
Identification of *Staphylococcus aureus* strains collected from clinical specimens, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, August-October 2013.

Staphylococcus aureus identification method	Number (%)
Presumptive isolate	136
MALDI-TOF MS Biotyper identification (matched genus ID score)	
2.30-3.00[a]	30 (22)
2.00-2.99[b]	83 (61)
1.70-1.99[c]	20 (15)
< 1.69[d]	0
Others[e]	3 (2)
PCR-positive for *nuc*	133 (98)
PCR-positive for *nuc* and *mecA* (MRSA)	37 (28)

[a]Highly probable. [b]Secure. [c]Probable. [d]Non-reliable. [e]ID score > 1.7 as *S. epidermidis* (*n*=1) and *S. saprophyticus* (*n*=2).

Table 2
Specimen type and source of MRSA strains isolated from patients, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, August-October 2013.

Specimen type and source	Number of *Staphylococcus aureus* strains	Number of MRSA strains (%) (*n*=37)
Type		
Sputum	39	10 (27)
Swab[a]	30	10 (27)
Blood	19	6 (16)
Pus	22	4 (11)
Urine	13	3 (8)
Others	10	4 (11)
Source		
Ward	74	31 (84)
Emergency unit	17	3 (8)
Outpatient unit	25	2 (5)
Others	17	1 (3)

[a]Corneal (*n*=4), nasal (*n*=2), pustule (*n*=1), throat (*n*=1), ulcer (*n*=4), vaginal (*n*=2), wound (*n*=10), and others (*n*=6).

DISCUSSION

This study identified 28% of *S. aureus* strains isolated from patients at Cipto Mangunkusumo General Hospital, Jakarta from August to October 2013 as MRSA strains, with the majority (84%) from inpatients mainly from Gedung A ward, an integrated inpatient services including different departments such as obstetrics and gynecology, surgery, neurosurgery, internal medicine, ophthalmology, anes-

Table 3
Antibiogram profiles *Staphylococcus aureus* strains isolated from patients, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, August - October 2013.

Antimicrobial agent	Number (%) of susceptible strains		
	All (*N*=133)	MSSA (*n*=96)	MRSA (*n*=37)
Oxacillin	96 (72)	96 (100)	0
Erythromycin	97 (73)	82 (85)	15 (41)
Chloramphenicol	105 (79)	83 (86)	22 (59)
Gentamicin	96 (72)	85 (89)	11 (30)
Sulphamethoxazole/trimethoprim	123 (92)	93 (97)	30 (81)
Tetracycline	70 (53)	55 (57)	15 (41)

thesiology, dermatology, and geriatrics. Previously, Liana (2014) reported a MRSA prevalence of 32% in the same referral hospital in 2010, with majority collected from Gedung A ward. Prevalence of MRSA in Jakarta has steadily declined from 36% in 2008 to 28% in 2013 (current study) (Liana, 2014).

Matrix-assisted laser desorption/ionization time-of-flight (MALDITOF) mass spectrometry (MS) fingerprinting has become a powerful tool in microbiological diagnostics (Klein *et al*, 2012). Previously, MALDI-TOF MS was used for screening suspected clinical isolates of staphylococcus (Silva *et al*, 2015; Manukumar and Umesha, 2017; de Almeida *et al*, 2018) and it was report that the species identification power of MALDI-TOF MS allowed accurate identification of Staphylococcus (Chan *et al*, 2015). In this study, we found that all presumptive *S. aureus* strains that were identified as *S. aureus* by MALDI TOF MS with were also 100% positivity for *nuc* gene by PCR. da Motta *et al*, (2014) reported that the genotypic identification schedule based simultaneously on the detection of *coa*, *nuc* and *23S rDNA* genes and showed correspondence of 100% with the MALDI-TOF MS technique.

The lower susceptibility of MSSA compared with MSSA strains observed in the current study is in line with Santosaningsih *et al* (2016) who found in the four tertiary care hospitals in Denpasar, Malang, Padang, and Semarang MRSA compared to MSSA strains are significantly more resistant to aminoglycosides (gentamicin and tobramycin), quinolones (ciprofloxacin and levofloxacin) and tetracycline.

In conclusion, although the prevalence of MRSA strains in hospitals in Indonesia is slowly, but steadily declining, other characteristics, such as types of infected clinical specimens and susceptibility to antibiotics, remained relatively unchanged. Such information should be used in the development of strategies to treat and control MRSA infection in the country.

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REFERENCES

Chan WS, Chan TM, Lai TW, *et al*. Complementary use of MALDI-TOF MS and real-time PCR-melt curve analysis for rapid identification of methicillin-resistant staphylococci and VRE. *J Antimicrob Chemother* 2015; 70: 441-7.

Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect* 2014; 20: 605-23.

Clinical and Laboratory Standards Institute. (CLSI). Performance Standards For antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. Document M100-S23. Wayne: CLSI, 2013.

da Motta CC, Rojas ACCM, Dubenczuk FC, *et al*. Verification of molecular characterization of coagulase positive Staphylococcus from bovine mastitis with matrix-assisted laser desorption ionization, time off light mass spectrometry (MALDI-TOF MS) mass spectrometry. *Afr J Microbiol Res* 2014; 8: 3861-6.

de Almeida CC, Pizauro LJL, Soltes GA, *et al*. Some coagulase negative *Staphylococcus* spp isolated from buffalo can be misidentified as *Staphylococcus aureus* by phenotypic and Sa442 PCR methods. *BMC Res* 2018; Notes 11: 346.

Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. *Crit Care Lond Engl* 2017; 21: 211.

Josten M, Reif M, Szekat C, *et al*. Analysis of the matrix-assisted laser desorption ionization-time of flight mass spectrum of *Staphylococcus aureus* identifies mutations that allow differentiation of the main clonal lineages. *J Clin Microbiol* 2013; 51: 1809-17.

Klein S, Zimmermann S, Köhler C, *et al*. Integration of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in blood culture diagnostics: a fast and effective approach. *J Med Microbiol* 2012; 61: 323-31.

Kuntaman K, Hadi U, Setiawan F, *et al*. Prevalence of methicillin resistant *Staphylococcus aureus* from nose and throat of patients on admission to medical wards of Dr. Soetomo Hospital, Surabaya, Indonesia. *Southeast Asian J Trop Med Public Health* 2016; 47: 66-70.

Liana P. Description of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Microbiology Laboratory of the Clinical Pathology Department, Dr. Cipto Mangunkusumo Hospital (RSCM) period January-December 2010. *Maj Kedokt Sriwij* 2014; 46: 171-5.

Loucif L, Bendjama E, Gacemi-Kirane D, *et al*. Rapid identification of Streptomyces isolates by MALDI-TOF-MS. *Microbiol Res* 2014; 169: 940-7.

Manukumar HM, Umesha S. MALDI-TOF-MS based identification and molecular characterization of food associated methicillin-resistant *Staphylococcus aureus*. *Sci Rep* 2017; 7: 11414.

Nelwan EJ, Sinto R, Subekti D, *et al*. Screening of methicillin-resistant *Staphylococcus aureus* nasal colonization among elective surgery patients in referral hospital in Indonesia. *BMC Res Notes* 2018; 11: 56.

Peacock SJ, Paterson GK. Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annu Rev Biochem* 2015; 84: 577-601.

Safari D, Harimurti K, Khoeri MM, *et al*. *Staphylococcus aureus* and *Streptococcus pneumoniae* prevalence among elderly adults in Jakarta, Indonesia. *Southeast Asian J Trop Med Public Health* 2015; 46: 465-71.

Santosaningsih D, Santoso S, Budayanti NS, *et al*. Characterisation of clinical *Staphylococcus aureus* isolates harbouring *mecA* or Panton-Valentine leukocidin genes from four tertiary care hospitals in Indonesia. *Trop Med Int Health* 2016; 21: 610-8.

Santosaningsih D, Santoso S, Setijowati N, *et al*. Prevalence and characterisation of *Staphylococcus aureus* causing community acquired skin and soft tissue infections on Java and Bali, Indonesia. *Trop Med Int Health* 2018; 23: 34-44.

Santosaningsih D, Santoso S, Verbrugh HA, *et al*. Risk factors for methicillin-resistant *Staphylococcus aureus* carriage among patients at admission to the surgical ward in a resource-limited hospital in Indonesia. *Am J Trop Med Hyg* 2017; 97: 1310-12.

Silva MB, Ferreira FA, Garcia LNN, *et al*. An evaluation of matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the identification of *Staphylococcus pseudintermedius* isolates from canine infections. *J Vet Diagn Investig* 2015; 27: 231-5.