RESEARCH NOTE

PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM NOSE AND THROAT OF PATIENTS ON ADMISSION TO MEDICAL WARDS OF DR SOETOMO HOSPITAL, SURABAYA, INDONESIA

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Abstract. Epidemiological data of methicillin resistant *Staphylococcus aureus* (MRSA) carriage in Indonesian hospitals are still scarce. These data are required for health management of infectious diseases in order to control hospital MRSA. The carriage rate of MRSA in nose and throat of patients on admission to Dr Soetomo Hospital Surabaya, Indonesia was 8.1% of 643 patients, 5.4% from throat, 3.9% from nose and 1.2% from both sites. Prevalence of MRSA among patients admitted to surgical and non-surgical ward was not different (8.2% and 8.0%, respectively). Although MRSA prevalence in Indonesian hospitals is low compared to many other countries worldwide, appropriate health strategies will be needed to be implemented if this infection is to be controlled.

Keywords: MRSA, prevalence, medical ward, surgical ward, nose, throat, Indonesia

INTRODUCTION

The problem of methicillin resistant *Staphylococcus aureus* (MRSA) is increasing worldwide, mainly in Asia (Chen and Huang, 2014). The spread of MRSA is through direct and indirect contacts among patients who have been colonized or infected with MRSA. For instance, in Germany the percent MRSA in *S. aureus* isolates derived from blood cultures has increased from 9 in 1999 to 20 in 2002 (Yang *et al*, 2010). Nosocomial infection caused by MRSA in Taiwan was also increased from 26.3% in 1986 to 77% in 2001 (Hsueh *et al*, 2004). Survey of MRSA in India by INSAR group, also indicated the higher rate of infection caused by MRSA (INSAR, 2013). MRSA has also been iden-

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tified in animals (Juhász-Kaszanyitzky *et al*, 2007), or associated with animal products, such as pig (van Cleef *et al*, 2010) and bovine (Tavakol *et al*, 2012).

To date, there has been limited data on MRSA in Indonesia. An early study conducted in 2001 identified 1 (0.3%) MRSA isolates among 329 *S. aureus* nares flora from 3,995 patients (Severin *et al*, 2008). By 2011, in three teaching hospitals (Denpasar, Semarang and Malang) in Indonesia, screening of 1,502 surgery patients at time of discharge by culturing nares, throat and skin lesion, revealed a MRSA carriage rate of 4% (Santosaningsih *et al*, 2014).

Carriers may acquire MRSA from the community, but acquisition and spread in hospitals have been found in health care settings worldwide (Bartoloni *et al*, 2013; Yamamoto *et al*, 2013; Santosaningsih *et al*, 2014). Accordingly, this study addresses the epidemiology and distribution of MRSA in surgical and other medical wards of Dr Soetomo Hospital, Surabaya, Indonesia. Such information is crucial to develop preventive strategies for combating emerging MRSA infection in the Indonesian health care system.

MATERIALS AND METHODS

Samples collection

Screening was conducted on patients at the time of admission to the wards of the Department of Surgery and Department of Internal Medicine of Dr Soetomo Hospital, Surabaya, Indonesia from June to September, 2014. Anterior nares and throat samples were obtained using sterile dry cotton swabs, one swab for both nostrils, from every patient enrolled in the study. Specimens were transported to the Microbiology Laboratory and inoculated into 5 ml of phenyl mannitol salt broth (Difco, Detroit, MI), incubated overnight at 37°C, then sub-cultured onto prepoured culture plates MRSA Chromagar medium (Brilliance™ MRSA Agar; Oxoid, Basingstoke, UK) and incubated for 24-48 hours at 37°C before being inspected for typical MRSA colonies of denim blue color. These colonies were picked and re-cultured on nonselective agar (Trypticase Soy Agar, Oxoid). The suspected bacteria were then confirmed by catalase test (3% H_2O_2), mannitol fermentation in Mannitol Salt Agar (MSA, Oxoid) plate and using Staphaurex (Remel Europe, Lenexa, KS).

The study protocol was approved by the Medical Ethics Committee of Dr Soetomo Hospital Surabaya (approval no. 181/Panke.KKE/III/2014).

Detection of *mecA*

Bacterial DNA was extracted by the TE boil extraction method, a modification of the bacterial DNA extraction method as described previously (Li *et al*, 2003). Briefly, a tip of bacterial colony was suspended in 100 µl TE buffer [10 mM Tris-HCl, 1 mM Na_2EDTA, (pH 8.0)], and the briefly mixed on a vortex mixer. The suspension was placed on the block heater at 95°C for 10 minutes and then centrifuged at 12,000 rpm for 1 minute. Primers for amplification of MRSA *mecA* were 5′ AAAATCGATGGTAAAGGTTGGC 3′ and 5′ AGTTCTGCAGTACCGGATTTGC 3′ (Mukarami *et al*, 1991). DNA amplification was carried out in 20-µl reaction solution consisting of 10 µl of 2X Master Mix (Intron Biotechnology; Gyeonggi-do, Korea), 1 µl (10 pmol) of each primer, 5 µl of DNA template, and 3 µl of distilled water. Thermocycling (conducted in Bioer GeneTouch Thermal Cycler; Alpha Laboratories; Hampshire, UK) was performed as follow: 94°C for 4 minutes; followed by 30 cycles of 94°C for 45 seconds, 55°C

Table 1
Distribution of MRSA carriages among wards in Dr Soetomo Hospital, Surabaya, Indonesia, June to September, 2014.

Ward	Number of patients screened	Number of MRSA carriage (%)
Surgical ward A[1]	38	7 (18)
Surgical ward B[2]	51	5 (10)
Surgical ward C[3]	17	0 (0)
Surgical ward D[4]	27	1 (4)
Surgical ward E[5]	67	6 (9)
Surgical ward G[6]	74	5 (7)
Surgical ward H[7]	42	2 (5)
Female Internal Medicine ward[8]	128	9 (7)
Male Internal Medicine ward[1] ward[9]	47	3 (6)
Male Internal Medicine ward[2,10]	56	6 (11)
Female Tropical Disease ward[11]	61	6 (10)
Male Tropical Disease ward[12]	35	2 (6)
Total	643	52 (8)

[1,2]General elective surgery, [3]elective surgery for mild classification, [4]urology surgery, [5]orthopedic surgery, [6,7]post-operative from Emergency Department, [8]mainly patients with diabetes mellitus, [9,10]chronic diseases, such as hepatic disease/cirrhosis, [11,12]also ward for hematology/oncology patients.

for 45 seconds, and 72°C for 45 seconds; then one cycle of 72°C for 10 minutes. Amplicon (533 bp) was detected by electrophoresis in 1.5% agarose gel (Sigma, St Louis, MO), staining with RedSafe™ DNA Staining Solution (Intron) and visualization under UV illumination (Sage Creation, Beijing, China).

RESULTS

Among the 643 (279 males and 364 females) patients enrolled in the study (316 and 327 from the surgical and other medical ward, respectively), based on culture and presence of *mecA* (data not shown) a total of 60 MRSA isolates (from 52 patients) were detected, 35 from nose (17 and 18 from surgical and medical ward, respectively); 25 from throat (11 and 14 from surgical and medical ward, respectively), and 16 from both nose and throat (4 and 12 from surgical and medical ward, respectively). There is no significant difference in MRSA colonization rate of patients on admission between surgical and medical wards (Table 1).

DISCUSSION

The MRSA carriage (8%) among patients admitted to surgical and other medical wards of Dr Soetomo Hospital, Surabaya is as high as that previously reported among discharged patients from a teaching hospital in Malang (Santosaningsih *et al*, 2014), a 16-fold increase since the first study in 2001 (Severin *et al*, 2008). These results highlight the continuing high prevalence of MRSA among patients in hospitals in Indonesia. The fact that the MRSA carriages were detected prior to hospital admission would indicate that the infection was community acquired.

Among the patients on admission to Dr Soetomo Hospital, Surabaya with positive colonization of MRSA, 15% of the patients had colonization of either nose or throat, and so it is important that these two sites are swabbed simultaneously.

Huang *et al* (2007), in an analysis of community (CA, 26 isolates) and hospital (HA, 382 isolates) acquired MRSA stored between 1999 and 2005 at the National University of Taiwan, showed that PFGE-pulsotype C was identified in SSCmec V type of 10 CA and 4 HA MRSA. A study of the Emergency Intensive Care Unit (EICU) at a tertiary teaching hospital of Chonnam National University, Republic of Korea, showed that 129/282 (46%) patients are colonized with MRSA, 106 (82%) in throat and 48 (47%) in nares, and that infection rate of MRSA during stay in EICU rises to 19% compared with 3% upon admission (Jang *et al*, 2014). All the above facts show that patients without MRSA colonization on admission to hospital are at risk of acquiring MRSA infection during their hospital stay. Krishnamurthy *et al* (2014) showed that 9.2% of nursing students working in a hospital in the town of Tumkur in southern India between September 2010 and February 2012, harbor MRSA in either nose or throat, whereas those not daily contact with patients have a prevalence of 4%.

Evidences demonstrating higher prevalences of MRSA carriers in other countries indicate that MRSA infection may increase in Indonesia in the near future. The 'Search and Destroy" strategy recently applied in Denmark may provide a good strategy for controlling MRSA infection in Indonesia as well (Bocher *et al*, 2010). Of course, the health policy and health management systems in Indonesia should anticipate this problem. Up to date information regarding the spread of MRSA among patients in hospitals is a requirement towards implantation of such a policy in Indonesia.

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REFERENCES

Bartoloni A, Pallecchi L, Fernandez C, *et al*. Low prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in urban and rural community settings in Bolivia and Peru. *Int J Infect Dis* 2013;17: e339-42.

Bocher S, Knudsen MA, Guardabassi L, *et al*, The search and destroy strategy prevents spread and long-term carriage of methicillin-resistant *Staphylococcus aureus*: result from the follow-up screening of a large ST22 (E-MRSA 15) outbreak in Denmark. *Clin Microbiol Infect* 2010; 16: 1427-34.

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

Huang YH, Tseng SP, Hu JM, Tsai JC, Hsueh PR, Teng LJ. Clonal Spread of SSCmec type IV methicillin resistant *Staphylococcus aureus* between community and hospital. *Clin Microbiol Infect* 2007; 13: 717-24.

Hsueh PR, Teng LJ, Chen WH, *et al.* Increasing prevalence of methicillin-resistant *Staphylococcus aureus* cuasing nosocomial infections at a university hospital in Taiwan from 1986 to 2001. *Antimicrob Agents*

Chemother 2004; 48: 1361-4.

INSAR-India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. *Indian J Med Res* 2013; 137: 363-9.

Jang HC, Choi OJ, Kim GS, *et al*. Active aurveillance of the trachea or throat for MRSA is more sensitive than nasal surveillance and a better predictor of MRSA infections among patients in intensive care. *PLOS One* 2014; 9: 1-7.

Juhász-Kaszanyitzky E, Jánosi S, Somogyi P, *et al*. MRSA transmission between cows and humans. *Emerg Infect Dis* 2007; 13: 630-2.

Krishnamurthy V, Saha A, Renushri BV, Nagaraj ER. Methicillin resistant *Staphylococcus aureus* carriage, antibiotic resistance and molecular pathogenicity among healthy individuals exposed and not exposed to hospital environment. *J Clin Diagn Res* 2014; 8: 4-8.

Li M, Gong J, Cottrill M, *et al*. Evaluation of QIAamp DNA Stool Mini Kit for ecological studies of gut microbiota. *J Microbiol Methods* 2003; 54: 13-20.

Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 1991; 29: 2240-4.

Santosaningsih D, Santoso S, Budayanti NS, *et al*. Epidemiology of *Staphylococcus aureus* harboring the *mecA* or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. *Am J Trop Med Hyg* 2014; 90: 728-34.

Severin JA, Lestari ES, Kuntaman K, *et al*. Unusually high prevalence of Panton-Valentine leukocidin genes among methicillin-sensitive *Staphylococcus aureus* strains carried in the Indonesian population. *J Clin Microb* 2008; 46: 1989-95.

Tavakol M, Riekerink RGMO, Sampimon OC, *et al*. Bovine-associated MRSA ST398 in The Netherlands. *Acta Vet Scand* 2012 May 1; 54: 28.

van Cleef BA, Verkade EJM, Wulf MW, *et al*. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. *PLOS One* 2010; 5: e9385.

Yamamoto T, Hung WC, Takano T, Nishiyama A. Genetic nature and virulence of community- associated methicillin-resistant *Staphylococcus aureus*. *Biomedicine* 2013; 3: 2-18.

Yang ES, Tan J, Eells S, Rieg G, Tagudar G, Miller LG. Body site colonization in patients with community-associated methicillin-resistant *Staphylococcus aureus* and other types of *S. aureus* skin infections. *Clin Microbiol Infect* 2010; 16: 425-31.