Methicillin-resistant *Staphylococcus aureus* in Indonesia

Dewi Santosaningsih

Methicillin-resistant *Staphylococcus aureus* in Indonesia

The printing of this thesis was financially supported by Erasmus University Rotterdam, the Netherlands

Printed by: UB Media

ISBN 9786026743480

Methicillin-resistant *Staphylococcus aureus* in Indonesia

Methicilline-resistente *Staphylococcus aureus* in Indonesië

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
Rector Magnificus
Prof.dr. H.A.P. Pols
and in accordance with the decision of the Doctorate Board

The public defense shall be held on
Thursday, 30 November 2017 at 13.30 hrs
by

Dewi Santosaningsih
born in Malang, Indonesia

Erasmus University Rotterdam

DOCTORAL COMMITTEE

Promoters:	Prof.dr. H.A. Verbrugh
	Prof.dr. S. Santoso
Other members:	Prof.dr. H.P. Endtz
	Prof.dr. H.F.L. Wertheim
	Prof.dr. K. Kuntaman
Copromoter:	Dr. J.A. Severin

Untuk yang tersayang

Suamiku Agung dan anakku Yusuf

Contents

Chapter 1

Introduction and outline of the thesis

INTRODUCTION

Staphylococcus aureus

Staphylococcus aureus (S. aureus) is a member of Staphylococcaceae (1). The staphylococci are gram-positive spherical cells, usually arranged in grapelike clusters (2). In 1880, Sir Alexander Ogston, a Scottish surgeon, named the clustered cells "staphylococci", from the Greek "staphyle", meaning bunch of grapes. Staphylococci grow well in most bacteriologic media under aerobic or anaerobic condition at 37°C. On solid media, colonies are round, smooth, raised, and shining. *S. aureus* usually forms grey to golden-yellow pigmented colonies. In 1884, Friedrich Julius Rosenbach, a German surgeon, named the *Staphylococcus aureus* from the Latin "aurum" for gold (3). Besides the golden pigment formation, *S. aureus* can be distinguished from other staphylococci species on the basis of positive results of coagulase, mannitol-fermentation, and deoxyribonuclease tests (4).

S. aureus is a common human commensal that may especially colonize the anterior nares, but also other body sites including skin, perineum, pharynx, vagina, gastrointestinal tract, and axillae (5). In general, approximately 20-30% of the population can be classified as persistent nasal carrier, 30-40% as intermittent carrier, and 30-50% as non-carrier. In addition, *S. aureus* is recognized as an important pathogen of a variety of infections such as skin and soft tissue infections, osteomyelitis, pneumonia, surgical site infections, and bloodstream infections (4,6). Its success as a pathogen can be explained by the production of numerous toxins, which may include enterotoxins, toxic shock syndrome toxin, exfoliative toxins, and leukocidin and a variety of enzymes (e.g. coagulase, protease, lipase, hyaluronidase) (4).

Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA)

Initially, penicillin, which was introduced in the early 1940s, was a highly effective treatment for staphylococcal infections. However, as early as 1942, the first penicillin-resistant *S. aureus* appeared in hospitals. Unfortunately, the resistance of *S. aureus* to penicillin due to penicillinase increased from 50% to 80% within a decade (7,8). In 1959, the penicillinase-resistant agent methicillin was introduced for staphylococcal infections therapy. Nevertheless, Dr. Patricia Jevons reported the first methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from wounds and anterior nares of patients and a nurse in one hospital in the United Kingdom (UK) two years later (8,9). Resistance in MRSA was soon found to be mediated by the *mecA* gene which encodes a penicillin-binding protein with a low affinity for methicillin and other beta-lactam antibiotics. The gene is located on a mobile element known as staphylococcal cassette chromosome *mecA* (SCC*mec*). Although the origin of the *mecA* gene is unclear, it was found among non-*aureus* staphylococcal species that are frequently isolated from animal and food products, including *Staphylococcus sciuri*, *Staphylococcus vitulinus*, and *Staphylococcus fleurettii* (8,10). The inter-species *mecA* gene transfer to methicillin-sensitive *S. aureus* (MSSA) in distinct lineages may have initiated the emergence of MRSA. Interestingly, a recent study suggested that this must have been occurred years before methicillin had been introduced into clinical practice (11). In 2014, another methicillin-resistant gene was described, the *mecC* gene (12).

MRSA prevalence in hospital settings throughout the world

Since the first report in 1961, MRSA has become pandemic with significant geographic differences in prevalence (Figure 1). In the United States, the percentage of MRSA among *S. aureus* isolates increased from

22% in 1995 to 57% in 2001, whereas the prevalence of MRSA in Latin America increased from 34% in 1996 to 40% in 2006 (13,14,15). Recent studies reported a high MRSA prevalence in Colombia (45%) and Peru (62%)(16). Geographic variation of MRSA prevalence was revealed among European countries, ranging from less than 5% in Iceland, Denmark, Estonia, Finland, the Netherlands, Norway, and Sweden to above 50% in Malta and Portugal. In the most recent report from the European Center for Disease Prevention and Control (ECDC), eight out of thirty European countries reported a prevalence above 25%, among which especially the Mediterranean countries (17). In the Middle-East, MRSA seems to be endemic with prevalence rates of 20% or higher. Studies reported the prevalence of MRSA between 30-50% in Saudi Arabia, Lebanon, Palestine and Turkey. However, higher MRSA prevalence rates were reported in Jordan (68%) and Iran (up to 90%)(18-23). A few studies on MRSA prevalence from hospitals in Africa were reported, including in the Democratic Republic of the Congo (25%) and South Africa (46%) (24,25).

In the Asia-Pacific region, the proportion of MRSA among clinical *S. aureus* isolates has also been variable. Countries located in the north Asia-Pacific region, including Japan, mainland China, Taiwan, and South Korea, reported high prevalences of MRSA from 50% to 70%, and even above 70% in South Korea. In the southern Asian countries, including Sri Lanka, India, Philippines, Thailand, Malaysia, and Vietnam, the prevalence was lower, between 20-40% (26-32). Little is known regarding the MRSA prevalence among clinical *S. aureus* isolates in Indonesia, the fourth largest populous country in the world, indicated with “white color” in figure 1.

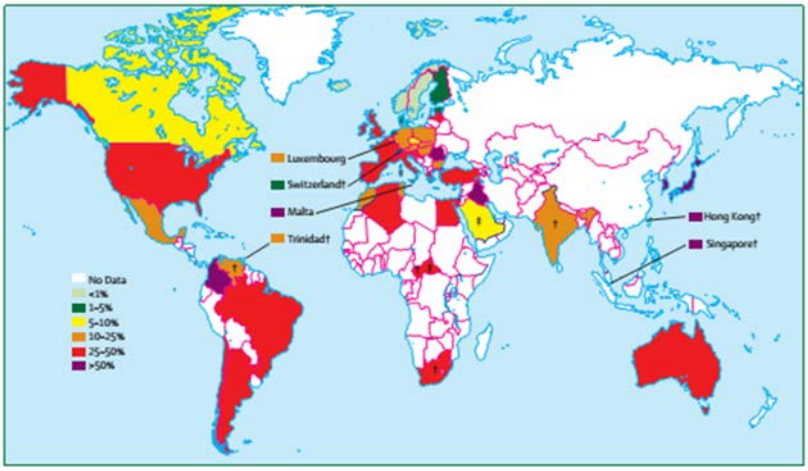

Figure 1. Prevalence of MRSA among clinical *S. aureus* isolates worldwide (33).

Community-associated MRSA

Community-associated MRSA (CA-MRSA) infections have emerged as a major public health problem in several countries threatening healthy individuals. According to the Centers for Disease Control and Prevention (CDC), CA-MRSA infection is defined as MRSA infection diagnosed in an outpatient or within 48 hours of hospitalization lacking the following traditional risk factors for MRSA infection: receipt of hemodialysis, surgery, residence in a long-term care facility, or hospitalization during the previous year; the presence of an indwelling catheter or a percutaneous device at the time culture samples were obtained; or previous isolation of MRSA. Various clinical manifestations of CA-MRSA have been reported ranging from minor skin and soft tissue infections to fatal necrotizing pneumonia, severe sepsis, and necrotizing fasciitis (13, 34-37). CA-MRSA isolates are associated with the presence of Panton-Valentine leukocidin (*pvl*) genes and a non-multidrug resistant phenotype. However, penetration of either hospital-associated MRSA (HA-

MRSA) to the community setting or invasion of CA-MRSA into the hospital has led to a somewhat blurred distinction between these MRSA categories.

CA-MRSA have spread in both developed and developing countries in the past two decades. In the United States and Canada, the prevalence of CA-MRSA infections was 17% and 5-7%, respectively, whereas 58% of community-associated *S. aureus* isolated from 16 European countries was MRSA (38,39,40). An increase of CA-MRSA prevalence was reported in Australia from 10% in 2000 to 16% in 2006 (41). Geographical variations of CA-MRSA infection prevalence have been observed in the African continent, with data from Madagascar (7%), Egypt (19%), and Nigeria (41%), as well as in the Asia-Pacific region with data from Taiwan, The Philippines, Vietnam, and Sri Lanka (30-39%), Korea and Japan (15-20%), and Thailand, India, and Hong Kong (3-9%) (27,34,42,43). Similar to the HA-MRSA, there are only limited data on CA-MRSA in Indonesia.

Impact of MRSA infection

Multiple studies have proven MRSA colonization as a risk factor for subsequent infection, by showing genetic relatedness between colonizing and infecting isolates (5,44,45). The reported rate of MRSA subsequent infection was 30% after detection of MRSA colonization. Infections caused by MRSA lead to significant impact on healthcare cost, morbidity, and mortality. For patients with an infection caused by MRSA total healthcare costs are two times higher than for those with an infection caused by MSSA. These costs include inpatient care, antimicrobial agents, other medicines, laboratory tests, imaging, surgical procedures, and physical medicine and rehabilitation (13,46). Patients with MRSA suffer significantly more often from complications than those with MSSA, and this may contribute to the increased costs (46). A meta-analysis of 31 studies reported a significant increase of mortality associated with MRSA bacteremia compared with MSSA bacteremia (odds ratio 1.93) (47).

Ineffectiveness of antibiotics causing suboptimal therapeutic response may contribute to the higher mortality, but virulence factors of MRSA strains or underlying diseases of affected patients may also play a role (13).

Although many studies regarding the epidemiology and clinical impact of HA-MRSA and CA-MRSA have been performed in both developed and developing countries, only a few publications report on MRSA in Indonesia, the fourth largest populous country in the world. Therefore, the overall aim of this thesis was to ascertain the emergence, the prevalence, and the genetic background of MRSA in Indonesia both in the healthcare and community setting. This can be specified in the following research questions:

1. What is the prevalence of MRSA among carriage and clinical isolates in the hospital and community settings in Indonesia?
2. What is the clonal relatedness of MRSA isolates and PVL-positive MSSA detected in the Indonesian population inside and outside hospitals?
3. What are the genetic characteristics of MRSA isolates and PVL-positive MSSA detected in the Indonesian population inside and outside hospitals?
4. What are the determinants of MRSA carriage among patients in the Indonesian hospitals?

OUTLINE OF THE THESIS

Chapter 2 describes the epidemiology of *S. aureus* with focus on the presence of *mecA* and *pvl* genes among surgery patients at discharge in tertiary care hospitals in Malang, Denpasar, and Semarang. Transmission of MRSA to contact patients, healthcare workers, and the innate hospital environment is also investigated. In addition, several risk factors for MRSA carriage and PVL-positive MSSA carriage among surgery patients are shown. In **chapter 3**, the first multicenter survey of clinical

S. aureus isolates from Indonesian hospitals is presented. The prevalence, antimicrobial susceptibility profiles, and clonal distribution of either MRSA or PVL-positive *S. aureus* obtained from clinical cultures in four tertiary care hospitals in Malang, Denpasar, Semarang, and Padang are determined.

In **chapter 4**, the first study on CA-MRSA among patients with skin and soft tissue infections in Indonesia is presented. The prevalence of virulence genes, including *pvl* genes and exfoliative toxin genes, encountered in *S. aureus* from patients with skin and soft tissue infection in community settings in Malang, Surabaya, and Denpasar is reported. In addition, risk factors for *S. aureus* skin and soft tissue infections and associations between virulence genes and clinical feature are shown.

Chapters 5, 6 and 7 are focused on efforts to control the dissemination of MRSA in a resource-limited hospital in Indonesia. In **chapter 5**, the effect on the MRSA acquisition rate after the implementation of a bundle of infection control measures, including hand hygiene, adapted isolation procedures, cleaning and disinfecting of hospital environment, disinfecting of instruments, screening, and decolonization therapy is presented. Hand hygiene is an important component of infection control strategies. **Chapter 6** is devoted to this topic, describing the effect of educational programs to improve the hand hygiene compliance and knowledge among healthcare workers. In **chapter 7**, risk factors for MRSA carriage among patients at admission in the surgery ward are explored. The possibility of using a selective screening approach as an infection control measure for MRSA in a low-resource hospital is discussed.

In **chapter 8,** the main findings of the studies in this thesis are discussed and suggestions for further investigations regarding MRSA and its control in Indonesia are given.

REFERENCES

1. Schleifer KH and Bell JA: Family VIII. Staphylococcaceae fam. nov. In: P. De Vos, GM Garrity, D. Jones, NR Krieg, W. Ludwig, FA Rainey, KH Schleifer, and WB Whitman (editors): Bergey's Manual of Systematic Bacteriology, second edition, vol 3 (The Firmicutes), Springer Dordrecht, Heidelberg, London, New York;2009:392.
2. Brooks GF, Carroll KC, Butel JS, Morse SA. Jawetz, Melnick, & Adelberg Medical Microbiology 24th ed. The McGraw Hill, Inc. USA;2007:224-32.
3. Licitra G. Etymologia: Staphylococcus. Emerging Infectious Diseases 2013;19:1553.
4. Lowy FD. *Staphylococcus aureus* Infections. NEJM 1998;339:520-32.
5. Wertheim HFL, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 2005;5:751-62.
6. van Belkum A, Verbrugh HA. 40 years of methicillin-resistant *Staphylococcus aureus*. BMJ 2001;323:644.
7. Chambers HF. The Changing Epidemiology of *Staphylococcus aureus*? EID 2001;7:178-82.
8. Moellering Jr RC. MRSA: the first half century. J Antimicrob Chemother 2012;67:4-11.
9. Jevons MP. Celbenin-resistant staphylococci. BMJ 1961;1:124-5.
10. Tsubakishita S, Kuwahara-Arai K, Sasaki T, and Hiramatsu K. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. Antimicrob Agents Chemother 2010;54:4352-9.
11. Harkins CP, Pichon B, Doumith M, et al. Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. Genome Biology 2017;18:130
12. Deplano A, Vandendriessche S, Nonhoff C, and Denis O. Genetic diversity among methicillin-resistant *Staphylococcus aureus* isolates carrying the *mecC* gene in Belgium. Journal of Antimicrobial Chemotherapy 2014;69:1457-60.

13. Boucher HW and Corey GR. Epidemiology of Methicillin-Resistant *Staphylococcus aureus*. Clin Infect Dis 2008;46:S344-9.
14. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
15. Mejía C, Zurita J, Gusmán-Blanco M. Epidemiology and surveillance of methicillin-resistant *Staphylococcus aureus* in Latin America. Braz J Infect Dis 2010;14:S79-S86.
16. Reyes J, Rincón S, Diaz L, et al. Dissemination of Methicillin-Resistant *Staphylococcus aureus* (MRSA), USA300 Sequence Type 8 Lineage in Latin-America. Clin Infect Dis 2009;49:1861-67.
17. European Center for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC;2017.
18. Eed EM, Ghonaim MM, Hussein YM, Saber TM, Khalifa AS. Phenotypic and molecular characterization of HA-MRSA in Taif hospitals, Saudi Arabia. J Infect Dev Ctries 2015;9:298-303.
19. Harastani HH, Araj GF, Tokajian ST. Molecular characteristics of *Staphylococcus aureus* from a major hospital in Lebanon. IJID 2014;19:33-8.
20. Laham NA, Mediavilla JR, Chen L, Abdelateef N, Elamreen FA, Ginocchio CC et al. MRSA Clonal Complex 22 Strains Harboring Toxic Shock Syndrome Toxin (TSST-1) Are Endemic in the Primary Hospital in Gaza, Palestine. PLoS ONE 2015;10:e120008.
21. Oksuz L, Dupieux C, Tristan A, Bes M, Etienne J, Gurlel N. The High Diversity of MRSA Clones Detected in a University Hospital in Istanbul. Int. J Med Scie 2013;10:1740-5.
22. Bazzoun DA, Harastani HH, Shehabi AA, Tokajian ST. Molecular typing of *Staphylococcus aureus* collected from a Major Hospital in Amman, Jordan. J Infect Dev Ctries 2014;8:441-7.

23. Moghadam SO, Pourmand MR, Mahmoudi M, and Sadighian H. Molecular characterization of methicillin-resistant *Staphylococcus aureus*: characterization of major clones and emergence of epidemic clones of sequence type (ST) 36 and ST 121 in Tehran, Iran. FEMS Microbiology Letters 2015;362:1-5.
24. Vandendriessche S, De Boeck H, Deplano A, et al. Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo. Eur J Clin Microbiol Infect Dis 2017;36:1163-71.
25. Perovic O, Iyaloo S, Kularatne R, et al. Prevalence and Trends of Staphylococcus aureus Bacteraemia in Hospitalized Patients in South Africa, 2010-2012: Laboratory-Based Surveillance Mapping of Antimicrobial Resistance and Molecular Epidemiology. PLoS One 2015;10:e0145429.
26. Kang C-I, and Song J-H. Antimicrobial Resistance in Asia: Current Epidemiology and Clinical Implications. Infect Chemother 2013;45:22-31.
27. Song J-H, Hsueh P-R, Chung DR, Ko KS, Kang C-I, Peck KR et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 2011;66:1061-9.
28. Nickerson EK, West TE, Day NP, Peacock SJ. *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and east Asia. Lancet Infec Dis 2009;9:130-5.
29. Boyce JM, Cookson B, Christiansen K, Hori S, Vuopio-Varkila J, Kogacöz S et al. Meticillin-resistant *Staphylococcus aureus*. Lancet Infect Dis 2005;5:653-63.
30. Kim HB, Park WB, Lee KD, Choi YJ, Park SW, Oh MD et al. Nationwide surveillance for *Staphylococcus aureus* with reduced susceptibility to vancomycin in Korea. J Clin Microbiol 2003;41:2279-81.

31. Chen F-J, Lauderdale T-L, Huang I-W, Lo H-J, Lai J-F, Wang H-Y et al. Methicillin-resistant *Staphylococcus aureus* in Taiwan. EID 2005;11:1761-3.
32. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, Khoon LY, Aziz MN, Hamat RA et al. Predominance and Emergence of Clones of Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* in Malaysia. J Clin Microbiol 2010;48:867-72.
33. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public health threat. Lancet 2006;368:874-85.
34. Chuang Y-Y, Huang Y-C. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Asia. Lancet Infect Dis 2013;13:698-708.
35. Lo W-T, Wang C-C. Panton-Valentine Leukocidin in the Pathogenesis of Community-associated Methicillin-resistant *Staphylococcus aureus* Infection. Pediatr Neonatol 2011;52:59-65.
36. Kale P, Dhawan B. The changing face of community-acquired methicillin-resistant *Staphylococcus aureus.* Indian J Med Microbiol 2016;34:275-85.
37. Witte W. Community-acquired methicillin-resistant *Staphylococcus aureus*: what do we need to know? Clin Microbiol Inf 2009;15 (Suppl. 7):17-25.
38. Rolo J, Miragaia M, Turlej-Rogacka A, et al. High Genetic Diversity among Community-Associated *Staphylococcus aureus* in Europe: Results from a Multicenter Study. PLoS One 2012;7:e35768.
39. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA et al. Methicillin-Resistant *Staphylococcus aureus* Disease in Three Communities. NEJM 2005; 352:1436-44.
40. Aureden K, Arias K, Burns LA, Creen C, Hickok J, Moody J et al. Guide to the Elimination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Transmission in Hospital Settings, 2nd Edition. APIC 2010.

41. Coombs GW, Nimmo GR, Pearson JC, Christiansen KJ, Bell JM, Collignon PJ et al. Prevalence of MRSA strains among *Staphylococcus aureus* isolated from outpatients, 2006. CDI 2009;33:10-20.
42. Enany S, Yaoita E, Yoshida Y, Enany M, Yamamoto T. Molecular characterization of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* isolates in Egypt. Microbiol Res 2010;165:152-62.
43. Abdulgader SM, Shittu AO, Nicol MP, and Kaba M. Molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. Front Microbiol 2015;6:348.
44. Stenehjem E, Rimland D. MRSA nasal colonization burden and risk of MRSA infection. Am J Infect Control 2013;41:405-10.
45. Davis KA, Stewart JJ, Crouch HK, Florez CE, and Hospenthal DR. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Nares Colonization at Hospital Admission and Its Effect on Subsequent MRSA Infection. CID 2004;39:776-82.
46. Filice GA, Nyman JA, Lexau C, Lees CH, Bockstedt LA, Como-Sabetti K, Lesher LJ, Lynfield R. Excess Cost and Utilization Associated with Methicillin Resistance for Patients with *Staphylococcus aureus* Infection. Infect Control Hosp Epidemiol 2010;31:365-73.
47. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, and Carmeli Y. Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Bacteremia: A Meta-analysis. Clinical Infectious Diseases 2003;36:53-9.

Chapter 2

Epidemiology of *Staphylococcus aureus* Harboring the *mecA* or Panton-Valentine Leukocidin Genes in Hospitals in Java and Bali, Indonesia

Dewi Santosaningsih[1], Sanarto Santoso[1], Nyoman S. Budayanti[2], Kuntaman Kuntaman[3], Endang S. Lestari[4], Helmia Farida[4], Rebriarina Hapsari[4], Purnomo Hadi[4], Winarto Winarto[4], Catarina Milheiriço[5], Kees Maquelin[6], Diana Willemse-Erix[6], Alex van Belkum[7], Juliëtte A. Severin[7], and Henri A. Verbrugh[7]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [2]Department of Microbiology, Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, Bali, Indonesia; [3]Department of Microbiology, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya, Indonesia; [4]Department of Microbiology, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia; [5]Laboratory of Molecular Genetics, Instituto de Tecnologia Quimica e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal; [6]Center for Optical Diagnostics and Therapy, Department of Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands; [7]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

The American Journal of Tropical Medicine and Hygiene 2014; 90(4):728–734. doi:10.4269/ajtmh.13-0734

ABSTRACT

Data of *Staphylococcus aureus* carriage in Indonesian hospitals are scarce. Therefore, the epidemiology of *S. aureus* among surgery patients in three academic hospitals in Indonesia was studied. In total, 366/1,502 (24.4%) patients carried *S. aureus*. The methicillin-resistant *S. aureus* (MRSA) carriage rate was 4.3%, whereas 1.5% of the patients carried Panton-Valentine leukocidin (PVL)-positive methicillin-sensitive *S. aureus* (MSSA). Semarang and Malang city (OR 9.4 and OR 9.0), being male (OR 2.4), hospitalization for more than five days (OR 11.708), and antibiotic therapy during hospitalization (OR 2.6) were independent determinants for MRSA carriage, while prior hospitalization (OR 2.5) was the only one risk factor for PVL-positive MSSA carriage. Typing of MRSA strains by Raman spectroscopy showed three large clusters assigned type 21, 24, and 38, all corresponding to ST239-MRSA-SCC*mec* type III. In conclusion, MRSA and PVL-positive MSSA are present among patients in surgical wards in Indonesian academic hospitals.

INTRODUCTION

Carriage of *Staphylococcus aureus* is a risk factor for subsequent infection in various settings (1). Antibiotic treatment of staphylococcal infections has become more challenging over the past decades with the emergence of methicillin-resistant *S. aureus* (MRSA). Nowadays, MRSA is a persistent problem in many healthcare settings around the world (2-8). Undetected MRSA-positive patients serve as reservoirs, and roommates of such patients as well as health-care personnel are at significant risk of becoming colonized (9-13). In addition, MRSA-positive persons contaminate the hospital's environment turning it into a reservoir for other patients (14-17). After discharge from hospital, MRSA-positive patients may transmit their strain to their household members (12).

Knowledge of the prevalence of MRSA colonization and the frequency of transmission is vital for the implementation of MRSA infection control measures in hospitals (18). However, little is known about the current epidemiology of MRSA in health care settings in Indonesia. Severin *et al.* reported that among 98 *S. aureus* isolates from 999 patients screened at discharge in 2001-2002 in two cities on the island of Java (Semarang and Surabaya), only two strains were identified as MRSA (carriage rate 0.2%). Among 263 isolates from healthy persons, patients in the community, and patients at the time of admission to the hospital, not a single MRSA was found. However, an unexpectedly high prevalence of Panton-Valentine leukocidin (PVL) genes among methicillin-sensitive *S. aureus* was documented among both patients and healthy individuals (19,20). This was of concern, since PVL–positive strains are associated with skin infections and severe necrotizing pneumonia (21). In the present study, we aimed to gain more insight in the more recent epidemiology of MRSA and *S. aureus* harboring the *pvl* genes in the Indonesian hospital setting in order to develop targeted preventive measures. Similar to the study described by Lestari *et al.*, we performed a multicenter study focused on patients at discharge (19). Molecular characterization of *S. aureus*

isolates was carried out and possible risk factors for colonization among these patients were analyzed.

MATERIALS AND METHODS

Setting

Three referral teaching hospitals participated in the study: Sanglah hospital in Denpasar (Bali; 704 beds), Dr. Kariadi hospital in Semarang (Central Java; 779 beds), and Dr. Saiful Anwar hospital in Malang (East Java; 810 beds).

Design

Surgery patients were screened for MRSA carriage at the time of discharge from hospital. All surgery patients were eligible for inclusion. However, surgery patients discharged within 48 hours after admission were excluded. In case a patient was found MRSA-positive (i.e. the "index case"), additional screening to detect secondary cases was carried out as follows: all patients that had been sharing the room with such an MRSA-positive patient were screened within one week as well as all attending health-care workers in that ward. In addition, screening of the innate hospital environment where index cases had been admitted was conducted. In case more than one index case was found within a week, the screening for secondary cases in hospital was conducted once that referred to those index cases. Four to six weeks after discharge of index cases, the household members and household environment of the cases were screened. Index cases living in the rural area or other cities than Denpasar and Semarang were excluded for household members and household environment screening. The additional screening of roommates, health-care workers, environment, and household members was not conducted in Malang. The study was performed from July 2007 to December 2008 in Denpasar, from February 2008 to October 2009 in Semarang and from January to March 2011 in Malang. The study was

approved by the medical ethics committee of the three academic hospitals.

Screening for *S. aureus* carriage

Samples were obtained using sterile dry cotton swabs (Deltalab, Rubí, Spain) after patients had given informed consent. Cultures of anterior nares, throat, and open skin lesion (if present) were taken from discharge patients and contact patients. Cultures of anterior nares and throat were taken from health-care workers and household members. The hospital environment was screened by taking ten samples minimal, from instruments (stethoscope, blood pressure cuff, and thermometer) and surfaces (bedrails, door handles, telephone handles, dust, sink faucet, and floor). Door handles, kitchen sink and appliances, bed, chairs, table tops, floor, and dust were taken from the household environment of index cases.

Bacterial isolates

Swabs were directly inoculated into 5 ml phenol red mannitol broth (BBL™, Le Pont de Claix, France) for overnight incubation at 37°C and then sub-cultured onto *Staphylococcus aureus* and MRSA Chromagar medium (ITK Diagnostics, Uithoorn, The Netherlands) for 24--48 hours incubation at 37°C. Typical colonies of *S. aureus* and MRSA were stored into trypticase soy agar. Confirmation of *S. aureus* was performed by Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France) and the Vitek®2 system (bioMérieux).

DNA extraction and detection of *mecA* and *pvl* genes

Bacterial DNA was extracted using a MagNa Pure LC™ DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) (22). The DNA concentration was measured spectrophotometrically and samples were stored at -20°C. Detection of *mecA* and *pvl* genes were performed by PCR as previously described (21,23).

SCC*mec* typing

The Staphylococcal Cassette Chromosome *mec* (SCC*mec*) of *S. aureus* isolates containing the *mecA* gene was characterized using a multiplex PCR that enables the identification of SCC*mec* types I to VI (24). Positive and negative control strains were included in each PCR run.

Raman spectroscopy

We performed Raman spectroscopy (SpectraCell*RA*® Bacterial Strain Analyzer, RiverD international BV, Rotterdam, The Netherlands) to assess clonal relationship among MRSA and PVL-positive MSSA isolates, as described previously (25,26). ATCC strains were included on each measurement day as a control for reproducibility. The analysis of spectra was performed using SpectraCell*RA* software version 1.9.0.13444:24 (RiverD international). In the SpectraCellRA software, the similarity of two spectra is calculated from the squared Pearson correlation coefficient (R^2) of the sample spectra and the known R^2 - distributions of identical and unrelated isolates (27). For comparing multiple isolates, a similarity or 2D plot is created where the similarity between each combination of isolates is represented as a color-coded square. In this plot, the similarity threshold was set at a 1% false positive rate, which means that for 1% of all indistinguishable isolates a misidentification as unrelated is allowed. Two isolates with a similarity below this value were considered unrelated and designated different Raman types. The cut-off value was set at a 3% false negative rate, so a misidentification as indistinguishable is allowed for 3% of all unrelated strains. Two isolates with a similarity above this value were considered indistinguishable and assigned the same RT. In case of a similarity value between both borders the isolates were considered potentially related.

MLST

A random selection of 10 *S. aureus* isolates from the largest clusters generated by Raman spectroscopy were further analyzed by MLST (28).

The MLST sequence type was assigned through the MLST website (http://www.mlst.net).

Risk factor analysis

Socio-demographic data, date of admission, date of discharge, ward that discharged the patient, prior hospitalization, ICU admission, surgery procedure, and antibiotic therapy during admission were included in the risk factor analysis. These data were collected from patient records and by interviewing the patients at the moment of discharge using a structured questionnaire. Data obtained from the questionnaires were recorded in a case record form (crf) program. Data were analyzed using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, USA). A *p* value less than 0.05 was considered as significant.

Definitions

Index cases were defined as patients from whom MRSA was found from any site by screening on discharge; contact patients were patients having shared the room with an index cases; secondary cases were patients, healthcare-workers, hospital environment, household members, and household environment from whom or which MRSA was found with a link in time and location to an index case, and the identical MRSA isolates were determined by Raman spectroscopy.

RESULTS

Carriage rate of MRSA and PVL-positive MSSA among discharge patients

We screened 488, 914, and 100 discharge patients in Sanglah hospital in Denpasar, Dr. Kariadi hospital in Semarang, and Dr. Saiful Anwar hospital in Malang, respectively (Table 1). The carriage rate of *S. aureus* among these was 9.4% (46/488) in Denpasar, 32.4% (296/914) in Semarang, and 24.0% (24/100) in Malang. Overall, the carriage rate of

MRSA among discharge patients was 4.3% (64/1,502). *S. aureus* was less frequently found in patients from Denpasar than in patients from Semarang and Malang, as well as the carriage rate of MRSA ($p<0.001$). MRSA isolates were found in cultures of nares (n=50 isolates; 54.4%), throat (n=30; 32.6%), and open skin lesion (n=12; 13.0%). All MRSA isolates were PVL-negative. PVL-positive MSSA was found among discharge patients in Semarang and Malang, but not in Denpasar (Table 1).

Table 1. Number of subjects and isolates analyzed for carriage rate of *S. aureus*, MRSA and PVL-positive MSSA

City	Group	No. of subjects screened	No. of cultures (environment)	No. of *S. aureus* isolates	No. of MRSA isolates (%)*	No. of PVL-positive MSSA isolates (%)*	*S. aureus* carriage rate (%)	MRSA carriage rate (%)	PVL-positive MSSA carriage rate (%)
Denpasar	Patients at discharge	488		56	4 (7.1)	0	46/488 (9.4)	2/488 (0.4)	0
	Contact patients	21		2	0	0	2/21 (9.5)	0	0
	Health care workers	58		3	0	0	3/58 (5.2)	0	0
	Hospital environment		50	0	0	0	0	0	0
	Household members	12		0	0	0	0	0	0
	Household environment		20	0	0	0	0	0	0
Semarang	Patients at discharge	914		423	76 (18.0)	24 (5.7)	296/914 (32.4)	54/914 (5.9)	21/914 (2.3)
	Contact patients	200		90	33 (26.7)	4 (4.4)	73/200 (36.5)	24/200 (12.0)	4/200 (2.0)
	Health care workers	58		60	0	10 (16.7)	39/58 (67.0)	0	6/58 (10.3)
	Hospital environment		132	13	1 (7.7)	0	13/132 (9.8)	1/132 (0.7)	0

Table 1. Number of subjects and isolates analyzed for carriage rate of *S. aureus*, MRSA and PVL-positive MSSA

Malang	Household members	10	4	2 (50.0)	0	2/10 (20.0)	1/10 (10.0)	0
	Household environment	15	0	0	0	0	0	0
	Patients at discharge	100	30	12 (40.0)	1 (3.3)	24/100 (24.0)	8/100 (8.0)	1/100 (1.0)
Total	Patients at discharge	1,502	509	92 (18.1)	25 (4.9)	366/1,502 (24.4)	64/1,502 (4.3)	22/1,502 (1.5)
	Contact patients	221	92	33 (35.9)	4 (4.3)	75/221 (33.9)	24/221 (10.9)	4/221 (1.8)
	Health care workers	116	63	0	10 (15.9)	42/116 (36.2)	0	6/116 (5.2)
	Hospital environment	182	13	1 (7.7)	0	13/182 (7.1)	1/182 (0.5)	0
	Household members	22	4	2 (50.0)	0	2/22 (9.1)	1/22 (4.5)	0
	Household environment	35	0	0	0	0	0	0

Note. MRSA, methicillin-resistant *S. aureus;* PVL, Panton-Valentine leukocidin; MSSA, methicillin-sensitive *S. aureus*. *The denominator is number of *S. aureus* isolates.

Carriage rate of MRSA and PVL-positive MSSA among contact patients, health-care workers, hospital environment, household members, and household environment

Secondary cases were only found in Semarang: contact patients, 24/200 (12.0%) from 54 index cases; hospital environment, 1/132 (0.8%) from 3 index cases; and household member, 1/10 (10.0%) from 1 index case. MRSA was not found among health-care workers and household environment. PVL-positive MSSA was only detected in Semarang which was 2.0% of contact patients and 10.3% among health-care workers.

Risk factor analysis for carriage of MRSA and PVL-positive MSSA among discharge patients

Multivariate analysis showed that more than five days hospitalization was independently associated with carriage of MRSA (odds ratio [OR] 11.708; 95% confidence interval [CI] 1.587-86.376) (Table 2). Other identified risk factors were being in the hospital in Semarang (OR 9.404; 95% CI 2.239-39.501) or Malang city (OR 9.003; 95% CI 1.752-46.271), being male (OR 2.374; 95% CI 1.127-5.000), and antibiotic therapy during hospitalization (OR 2.621; 95% CI 1.014-6.773). The only factor that was associated with carriage of PVL-positive MSSA was prior hospitalization (OR 2.466; 95% CI 1.028-5.916).

Table 2. Risk factors for carriage of MRSA and PVL positive MSSA among discharge patients in three Indonesian hospitals: univariate and multivariate analysis

Risk factors	Univariate		Multivariate (Backward LR)			Univariate		Multivariate (Backward LR)		
	No. of subjects (%)					No. of subjects (%)				
	MRSA (+) (n=55)	MRSA (-) (n=1351)	OR	95% CI	*p*	PVL (+) (n=21)	PVL (-) (n=1385)	OR	95% CI	*p*
City										NS
Denpasar	2 (3.6)	485 (35.9)	1			0	487 (35.2)			
Semarang	47 (85.5)	786 (58.2)	9.404	2.239-39.501	0.002	20 (95.2)	813 (58.7)			
Malang	6 (10.9)	80 (5.9)	9.003	1.752-46.271	0.009	1 (4.7)	86 (6.2)			
Gender#										
Male	46 (83.6)	875 (64.8)	2.374	1.127-5.000	0.023	12 (57.1)	909 (65.6)			
Female	9 (16.4)	476 (35.2)	1			9 (42.9)	476 (34.4)			
Age*#										
≤ 18 yo	11 (20)	279 (20.7)				3 (14.3)	287 (20.7)			
19 – 59 yo	36 (65.5)	900 (66.6)				16 (76.2)	920 (66.4)			
≥ 60 yo	8 (14.5)	172 (12.7)				2 (9.5)	178 (12.9)			

Table 2. Risk factors for carriage of MRSA and PVL positive MSSA among discharge patients in three Indonesian hospitals: univariate and multivariate analysis (cont'd)

Risk factors	Univariate		Multivariate (Backward LR)			Univariate		Multivariate (Backward LR)		
	No. of subjects (%)					No. of subjects (%)				
	MRSA (+) (n=55)	MRSA (-) (n=1351)	OR	95% CI	*p*	PVL (+) (n=21)	PVL (-) (n=1385)	OR	95% CI	*p*
Length of stay										NS
≤ 5 days	1 (1.8)	394 (29.2)	1			2 (9.5)	393 (28.4)			
> 5 days	54 (98.2)	957 (70.8)	11.708	1.587-86.376	0.016	19 (90.5)	992 (71.6)			
ICU admission*#										
Yes	3 (5.5)	113 (8.4)				1 (4.8)	115 (8.3)			
No	52 (94.5)	1238 (91.6)				20 (95.2)	1270 (91.7)			
Surgery *										NS
Yes	43 (78.2)	961 (71.1)				9 (42.9)	995 (71.8)			
No	12 (21.8)	390 (28.9)				12 (57.1)	390 (28.2)			

Table 2. Risk factors for carriage of MRSA and PVL positive MSSA among discharge patients in three Indonesian hospitals: univariate and multivariate analysis (cont'd)

Risk factors	Univariate		Multivariate			Univariate		Multivariate		
	No. of subjects (%)		(Backward LR)			No. of subjects (%)		(Backward LR)		
	MRSA (+) (n=55)	MRSA (-) (n=1351)	OR	95% CI	*p*	PVL (+) (n=21)	PVL (-) (n=1385)	OR	95% CI	*p*
Prior Hospitalization*										
Yes	13 (23.6)	336 (24.9)				10 (47.6)	339 (24.5)	2.466	1.028 – 5.916	0.043
No	42 (76.4)	1015 (75.1)				11 (52.4)	1046 (75.5)	1		
Antibiotic therapy during hospitalization										NS
Yes	50 (90.9)	1116 (82.6)	2.621	1.014-6.773	0.047	13 (61.9)	1153 (83.2)			
No	5 (9.1)	235 (17.4)	1			8 (38.1)	232 (16.8)			

Patients without complete data were not analyzed statistically

*Univariate analysis of MRSA carriage was not significant ($p>0.2$); #Univariate analysis of PVL-positive MSSA was not significant ($p>0.2$)

SCC*mec* typing

We performed SCC*mec* typing of 127 MRSA isolates obtained from discharge patients, contact patients, hospital environment, and household members. One other MRSA isolate from discharge patient was missing. Type III SCC*mec* was the main type (94.4%). However, we found few isolates containing type I, type I-*dcs*, type II-*kdp*, and type III variant (type III-*mecI*) in Semarang and Denpasar city. Type III-*mecI* is an isolate that was characterized by SCC*mec* multiplex PCR by the presence of a pattern very similar to the SCC*mec* type III but lacking the band corresponding to the amplification of the *mecI* gene. Interestingly, one SCC*mec* type V was detected from a discharge patient in Malang (Table 3).

Table 3. Distribution of SCC*mec* type among MRSA isolates from three Indonesian hospitals (Denpasar, Semarang, and Malang)*

SCC*mec* type	Number of isolates (%)			
	Denpasar (n=4)	Semarang (n=111)	Malang (n=12)	Total (n=127)
I	0	2 (1.8)	0	2 (1.6)
I - *dcs*	2 (50.0)	0	0	2 (1.6)
II	0	0	0	0
II – *kdp*	0	1 (0.9)	0	1 (0.8)
III	2 (50.0)	107 (96.4)	11 (91.7)	120 (94.4)
III - *mecI*	0	1 (0.9)	0	1 (0.8)
IV	0	0	0	0
V	0	0	1 (8.3)	1 (0.8)
VI	0	0	0	0

*SCC*mec*=staphylococcal cassette chromosome *mec*; MRSA= methicillin-resistant *Staphylococcus aureus.*

Raman spectroscopy

We carried out Raman spectroscopy for 162 *S. aureus* isolates consisting of 127 MRSA and 35 PVL-positive MSSA. The *S. aureus* isolates were obtained from discharge patients (112 isolates), contact patients (37 isolates), health-care workers (10 isolates), hospital environment (1 isolate), and household members (2 isolates). One other MRSA isolate and 4 other PVL-positive MSSA isolates from discharge patients were missing.

The Raman spectroscopic analysis showed 61 Raman types (RT) (Figure 1). The most frequently found type was RT 24 containing 75 *S. aureus* including 61 MRSA and 14 PVL-positive MSSA. The MRSA isolates were obtained from discharge patients (40 isolates), contact patients (18 isolates), hospital environment (1 isolate), and household members (2 isolates) which were mostly (54 isolates) from Dr. Kariadi hospital in Semarang. The RT 24 PVL-positive MSSA isolates were obtained from discharge patients (7 isolates), contact patients (1 isolates), and health-care workers (6 isolates) from Semarang. RT 38 and RT 21 clusters were the second and third most common Raman types including 26 and 7 *S. aureus* isolates, respectively.

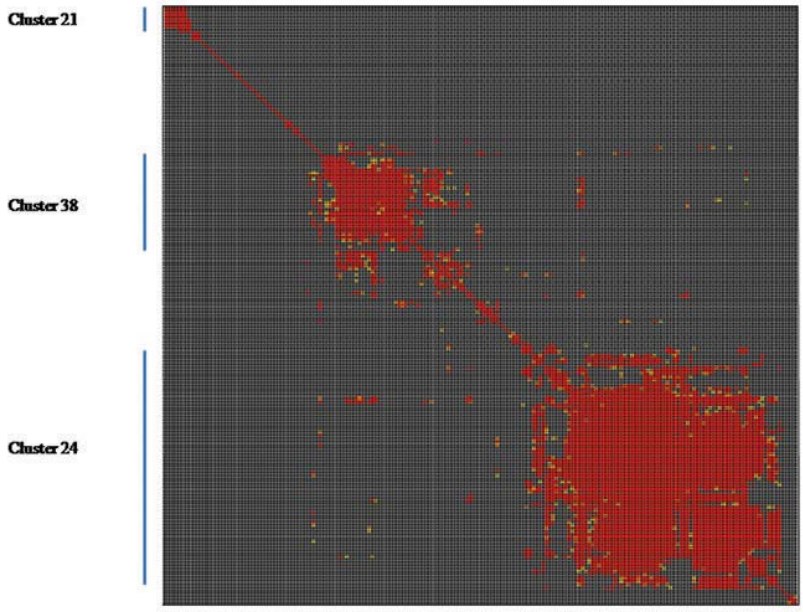

Figure 1. Clustering of MRSA and PVL positive MSSA isolates from discharge patients in three hospitals in Indonesia as determined by Raman spectroscopy (RT: Raman type).

Note: Figure displays a correlation matrix used to analyze Raman spectral relatedness between isolates. Red clusters indicate isolates that are indistinguishable based on the cut-off value. The grey areas indicate isolates that are non-related based on the similarity threshold. The potentially related isolates are shown by yellow areas to orange areas gradually. Cluster 21 includes MRSA isolates from Semarang: 5 isolates from discharge patients and 2 isolates from contact patients. Cluster 24 contains MRSA isolates from Semarang and Malang: 40 isolates from discharge patients, 18 isolates from contact patients, 1 isolate from hospital environment, 2 isolates from household members. In addition this cluster contains PVL-positive MSSA Semarang isolates: 7 isolates from discharge patients, 1 isolate from contact patient, and 6 isolates from health-care workers. Cluster 38 is consisted of MRSA isolates from Semarang and Malang: 21 isolates from discharge patients and 4 isolates from contact patients. One PVL-positive MSSA isolate belongs to cluster 38.

Figure 2 shows the endemicity profile of RT 24 MRSA in the surgery ward in Dr. Kariadi hospital. Interestingly, 7 MRSA isolates from Malang were included in the RT 24 together with MRSA isolates from Semarang. No MRSA isolate from Denpasar clustered in one of the three large clusters RT 24, RT 38, or RT 21. Instead, the four MRSA isolates from Denpasar could be designated RT 15 (2 isolates), RT 48 (1 isolate), and RT 51 (1 isolate).

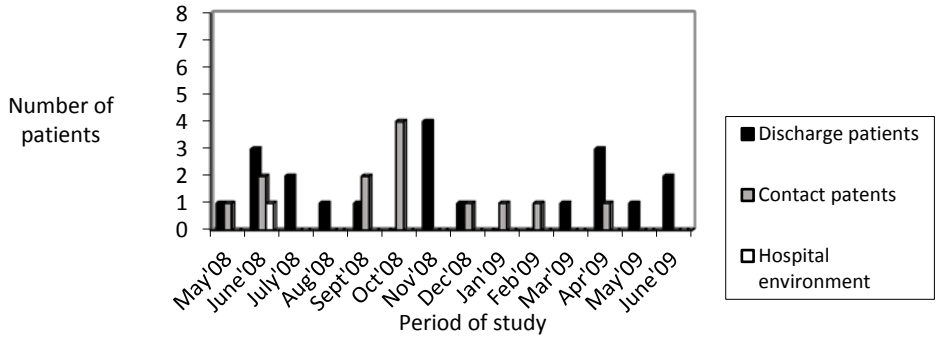

Figure 2. Endemicity profile of Raman type 24 MRSA among discharge patients, contact patients, and hospital environment in a surgery ward in Dr.Kariadi hospital, Semarang, Indonesia.

MLST analysis

We randomly selected 10 *S. aureus* isolates representing RT 21 (1 isolate), RT 24 (3 isolates), RT 35 (1 isolate), RT 38 (3 isolates), RT 40 (1 isolate), and RT 43 (1 isolate) for MLST. All seven MRSA isolates that were distributed among four different RT clusters belonged to ST239 (Table 4). The PVL-positive MSSA isolates were assigned to ST121 and ST188. One isolate was untypeable by MLST since no PCR product could be generated repeatedly for the *gmk* gene. All *S. aureus* isolates that were analyzed by MLST were from Semarang.

Table 4. Bacterial typing of selected MRSA isolates from discharge patients in Indonesia*

Isolates number	Raman type	SCC*mec* type	ST
7192	21	III	239
7237	24	III	239
7300	24	III	239
7337	24	III	239
7047	35	#	ND
7148	38	III	239
7254	38	III	239
7233	38	#	121
7244	40	#	188
7491	43	III	239

*SCCmec=staphylococcal cassette chromosome *mec*; MRSA= methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*; ST=sequence type; # = PVL-positive MSSA.

DISCUSSION

In the present study, we showed that MRSA were present in the hospital setting in Indonesia, although significant geographical variations exist. The carriage rate among surgery patients screened at discharge was highest in the two hospitals on the island of Java, i.e. 8.0% in Malang and 5.9% in Semarang, versus 0.4% in Denpasar on the island of Bali. Importantly, in our previous 2001-2002 study, no MRSA was found among patients at discharge in Semarang (19,29). We now uncovered endemicity of ST239-MRSA-SCC*mec* type III in the same hospital in Semarang several years later. It has been shown by mathematical modeling that the ST239-MRSA-SCC*mec* type III lineage exhibits an enhanced transmissibility compared to other lineages which could explain its successful spread (30). This clone is notable for causing prolonged epidemics that are difficult to control in hospitals worldwide,

and it is the dominant sequence type in Asia. Raman spectroscopy, a method that attains 95.2% concordance with pulsed-field gel electrophoresis (25), sub-divided isolates belonging to this clone into several sub-clones or RTs, of which RT 24 was the most common. This RT 24 was not only carried by discharge patients but also by contact patients, and contaminated and persisted in a surgical ward of the Semarang hospital over a 14-month-period. In such an endemic situation, it is not possible to determine a single (point) source of the MRSA. RT 24 was also the most frequently found type in Malang hospital, suggesting that this type has also become endemic in that setting. RT 38, another ST239 sub-clone, was prevalent in another surgical ward in the hospital in Semarang, and was also found in Malang hospital (n=4 isolates).

MRSA isolates from Sanglah hospital, Denpasar, on the Bali Island clustered in RT 15, RT 48, and RT 51, indicating that the epidemiology of MRSA in Bali differs from that in the two Javanese hospitals.

Interestingly, health care workers were not identified as carriers of MRSA. Although health care workers are generally considered reservoirs for MRSA, this may not be the case in these Indonesian settings. We hypothesize that health care workers may be colonized with other *Staphylococcus* species, such as *S. sciuri* (31), or PVL-positive MSSA, which may protect their mucosa from colonization by MRSA. This interference hypothesis, however, needs to be explored further.

In this study, we presented the first risk factors analysis related to MRSA carriage in Indonesian hospitals. According to the multivariate analysis, being male, length of hospitalization, and antibiotic therapy during admission were associated with MRSA carriage among discharge patients, in addition to the determinant city as described above ($p<0.05$). Indeed, these risk factors are in agreement with multiple studies on the nasal carriage of MRSA (1,32-35), however no information about these in Indonesian hospital setting has been presented before.

PVL-positive MSSA were detected in patients at discharge in Semarang and Malang hospitals (PVL-positive MSSA carriage rate ranged between 1.0 to 2.3%) and these data are in agreement with previous reports from Indonesia (20,36). However, the prevalence of PVL-positive MSSA among health-care workers in Semarang in this study was remarkably high (10.3%). The consequence of this finding is not yet clear. As hypothesized above, it could provide protection against colonization by other strains of *S. aureus*, including MRSA.

Two isolates of PVL-positive MSSA from patients hospitalized in Semarang were confirmed as ST121 and ST188 by MLST. Both sequence types were also found among PVL-positive MSSA in Surabaya, another city in East Java, Indonesia in 2001-2002 (20). A more recent study reported that ST188 PVL-positive MSSA was predominant among discharge patients in Malaysia (37).

In concordance with other studies we did not find PVL-positive MRSA in Indonesia (20, 36). However, the emergence of such strains is not unlikely because of possible horizontal transfer of the *mecA* gene to PVL-positive MSSA (38). Of note, some PVL-positive MSSA clustered in RT 24, together with ST239-MRSA-SCC*mec* type III isolates.

The present study has some limitations. Firstly, we did not ascertain whether the MRSA was acquired in the hospital or before admission, because we did not screen the patients at the time of their admission. Although Raman spectroscopy, MLST, and SCC*mec* typing indicated predominance of typical hospital-acquired MRSA strains, acquisition of such MRSA in the community setting may occur. For example, the single MRSA isolate with a type V SCC*mec* from a patient in Malang could have been acquired in the community. Secondly, screening of secondary cases in Dr. Saiful Anwar hospital, Malang was not conducted. Consequently, we could not analyze the possible MRSA transmission to the secondary cases at that study site. Thirdly, genetic confirmation with MLST was not performed for all *S. aureus* isolates.

In summary, the prevalence of MRSA among patients in surgery wards in Indonesian hospitals was high in comparison with our earlier analysis, although geographical variations exist. We showed that an endemic situation occurred in Semarang. Therefore, targeted intervention measures including hand hygiene, isolation procedures, cleaning of hospital environment, screening and decolonization of patients are required to reduce the MRSA acquisition rate in Indonesian hospitals. Recent studies reported that selective MRSA screening to high risk colonization patients (e.g. patients at ICU admission) and patients detected as MRSA carrier previously will reduce the prevalence of MRSA more efficiently than universal screening at hospital admission (6,39). Such a strategy should also be developed for other settings. In addition, it is necessary to study clinical isolates and the burden of disease caused by MRSA in Indonesian hospitals.

Acknowledgments

We thank the deans of the Faculty of Medicine, Brawijaya University, Malang, Indonesia, the Faculty of Medicine, Diponegoro University, Semarang, Indonesia, Faculty of Medicine, Airlangga University, Surabaya, Indonesia, and the Faculty of Medicine, Udayana University, Denpasar, Indonesia, the directors of the Dr. Saiful Anwar hospital, Malang, Indonesia, the Dr. Kariadi hospital, Semarang, Indonesia, the Dr. Soetomo hospital, Surabaya, Indonesia, and the Sanglah hospital, Denpasar, Indonesia who facilitated our work in these teaching hospitals. We also thank all staff members who have been involved in the isolation of bacteria. The crf program for recording the patients' database involved in this study was created by Arjen van Vliet. We thank Ketut Suata for valuable suggestions. The excellent technical assistance of Mitchell Laurens is gratefully acknowledged.

This study was supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands, and by RUTI grant number RUTI : SK Menristek No.

97/M/Kp/XI/2007, No. 194/M/Kp/X/2008, and No.110/M/Kp/X/2009 from the Ministry of Research and Technology, Republic of Indonesia.

REFERENCES

1. Kluytmans JA, van Belkum A, Verbrugh HA. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505-520.
2. Alvarez JA, Ramirez AJ, Mojica-Larrea M, et al. Methicillin-resistant *Staphylococcus aureus* at a general hospital: epidemiological overview between 2000-2007. Rev Invest Clin 2009;61:98-103.
3. Burlage RS and Mahdi N. A novel molecular pattern for methicillin-resistant *Staphylococcus aureus* in Milwaukee, WI clinical isolates. Diagn Microbiol Infect Dis. 2009;63:296-301.
4. Choi CS, Yin CS, Bakar AA, et al. Nasal carriage of *Staphylococcus aureus* among healthy adults. J Microbiol Immunol Infect 2006;39:458-464.
5. Grundmann HA-d-SM, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public health threat. Lancet 2006;368:874-875.
6. Johnston BL and Bryce E. Hospital infection control strategies for vancomycin-resistant Enterococcus, methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile*. CMAJ 2009;180:627-631.
7. Kwon JC, Kim SH, Park SH, et al. Molecular Epidemiologic Analysis of Methicillin-Resistant *Staphylococcus aureus* Isolates from Bacteremia and Nasal Colonization at 10 Intensive Care Units: Multicenter Prospective Study in Korea. J Korean Med Sci 2011;26:604-611.
8. Zinn CS, Westh H, Rosdahl VT, the Sarisa Study Group. An International Multicenter Study of Antimicrobial Resistance and Typing of Hospital *Staphylococcus aureus* Isolates from 21 Laboratories in 19 Countries or States. Microbial Drug Resistance 2004;10:160-168.

9. Ben-David D ML, Parenteau S. Methicillin-resistant *Staphylococcus aureus* transmission: the possible importance of unrecognized health care worker carriage. Am J Infect Control 2008;36:93-97.
10. Davis KA, Stewart JJ, Crouch HK, Florez CE, Hospenthal DR. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Nares Colonization at Hospital Admission and Its Effect on Subsequent MRSA Infection. CID 2004;39:776-782.
11. Fishbain JT, Lee JC, Nguyen HD, et al. Nosocomial Transmission of Methicillin-Resistant *Staphylococcus aureus*: A Blinded Study to Establish Baseline Acquisition Rates. Infection Control and Hospital Epidemiology 2003;24:415-421.
12. Lucet JC, Paoletti X, Demontpion C, et al. Carriage of methicillin-resistant *Staphylococcus aureus* in home care settings: prevalence, duration, and transmission to household members. Arch Intern Med 2009;169:1372-1378.
13. Moore C, Dhaliwal J, Tong A, et al. Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition in roommate contacts of patients colonized or infected with MRSA in an acute-care hospital. Infection Control and Hospital Epidemiology 2008;29:600-606.
14. Boyce JM. Environmental contamination makes an important contribution to hospital infection. J Hosp Infect 2007;65:50-54.
15. Hardy KJ, Oppenheim BA, Gossain S, Gao F, Hawkey PM. A Study of the Relationship Between Environmental Contamination with Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Patients' Acquisition of MRSA. Infection Control and Hospital Epidemiology 2006;27:127-132.
16. Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H. Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. J Hosp Infect 2006;62:187-194.

17. Shigeharu O, Suenaga S, Sawa A, Kamiya A. Association between Isolation Sites of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Patients with MRSA-Positive Body Sites and MRSA Contamination in Their Surrounding Environmental Surfaces. Jpn. J Infect Dis 2007;60:367-369.
18. Aizen E, Ljubuncic Z, Ljubuncic P, Aizen I, Potasman I. Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in a geriatric rehabilitation hospital. J Gerontol A Biol Sci Med Sci 2007;62:1152-1156.
19. Lestari ES, Severin JA, Fillus PMG et al. Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. Eur J Clin Microbiol Infect Dis 2008;27:45-51.
20. 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 Microbiol 2008;46:1989-1995.
21. Lina G, Plemont Y, Godall-Gamot F, et al. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. CID 1999;29:1128-1132.
22. Melles DC, Gorkink RFJ, Boelens HAM, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. The Journal of Clinical Investigation 2004;114:1732-1740.
23. 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;29:2240-2244.
24. Milheirico C, Oliveira DC, de Lencastre H. Update to the Multiplex PCR Strategy for Assignment of *mec* Element Types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2007;51:3374-3377.

25. Te Witt R, Vaessen N, Melles DC, et al. Good performance of SpectraCellRA system for typing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Clin Microbiol 2013;51:1434-1438.
26. Willemse-Erix DF, Scholtes-Timmerman MJ, Jachtenberg JW, et al. Optical fingerprinting in bacterial epidemiology: Raman spectroscopy as a real-time typing method. J Clin Microbiol 2009;47:652-659.
27. Willemse-Erix D, Bakker-Schut T, Slagboom-Bax F, et al. Rapid Typing of Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates by Use of SpectraCell RA. J Clin Microbiol 2012;50:1370-1375.
28. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008-1015.
29. Lestari ES, Duerink DO, Hadi U, et al. Determinants of carriage of resistant *Staphylococcus aureus* among *S. aureus* carriers in the Indonesian population inside and outside hospitals. Tropical Medicine and International Health 2010;15:1235-1243.
30. Cooper BS, Batra R, Wyncoll D, Tosas O, Edgeworth JD. Quantifying Type-Specific Reproduction Numbers for Nosocomial Pathogens: Evidence for Heightened Transmission of an Asian Sequence Type 239 MRSA Clone. PLOS Computational Biology 2012;8:1-13.
31. Severin JA, Lestari ES, Kuntaman K, et al. Nasal carriage of methicillin-resistant and methicillin sensitive strains of *Staphylococcus sciuri* in the Indonesian population: epidemiology and risk factors. Antimicrobial Agents and Chemotherapy 2010;54:5413-5417.
32. Jariyasethpong T, Tribuddharat C, Dejsirilert S, et al. MRSA carriage in a tertiary governmental hospital in Thailand: emphasis on prevalence and molecular epidemiology. Eur J Clin Microbiol Infect Dis 2010;29:977-985.

33. Mathanraj S, Sujatha S, Sivasangeetha K, Parija SC. Screening for methicillin-resistant *Staphylococcus aureus* carriers among patients and health care workers of a tertiary care hospital in south India. Indian Journal of Medical Microbiology 2009;27:62-64.
34. Sivaraman K, Venkataraman N, Cole AM. *Staphylococcus aureus* Nasal Carriage and its Contributing Factors. Future Microbiol 2009;4:999-1008.
35. Tacconelli E, De Angelis G, Cataldo MA, et al. Antibotic usage and risk of colonization and infection with antibiotic-resistant bacteria a hospital population-based study. Antimicrobial Agents and Chemotherapy 2009;53:4264-4269.
36. Deurenberg RH, Beisser PS, Visschers MJ, Driessen C, Stobberingh EE. Molecular typing of methicillin-susceptible *Staphylococcus aureus* isolates collected in the Yogyakarta area in Indonesia, 2006. Clinical Microbiology and Infection 2010;16:92-94.
37. Neela VK, Ehsanollah GR, Zamberi S, van Belkum A, Mariana NS. Prevalence of Panton-Valentine leukocidin genes among carriage and invasive *Staphylococcus aureus* isolates in Malaysia. International Journal of Infectious Diseases 2009;13:e131-e132.
38. Boyle-Vavra S and Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Laboratory Investigation 2007;87:3-9.
39. Gurieva T, Bootsma MCJ, Bonten MJM. Cost and Effects of Different Admission Screening Strategies to Control the Spread of Methicillin-resistant *Staphylococcus aureus*. PLOS Computational Biology 2013;9:1-11.

Chapter 3

Characterization of clinical *Staphylococcus aureus* isolates harboring *mecA* or Panton-Valentine leukocidin genes from four tertiary care hospitals in Indonesia

Dewi Santosaningsih[1], Sanarto Santoso[1], Nyoman S. Budayanti[2], Ketut Suata[2], Endang S. Lestari[3], Hendro Wahjono[3], Aziz Djamal[4], Kuntaman Kuntaman[5], Alex van Belkum[6,7], Mitchell Laurens[6,8], Susan V. Snijders[6], Diana Willemse-Erix[6,9], Wil H. Goessens[6], Henri A. Verbrugh[6] and Juliëtte A. Severin[6]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr.Saiful Anwar Hospital, Malang, Indonesia; [2]Department of Microbiology, Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, Bali, Indonesia; [3]Department of Microbiology, Faculty of Medicine, Diponegoro University/Dr.Kariadi Hospital, Semarang, Indonesia; [4]Department of Microbiology, Faculty of Medicine, Andalas University/Dr.M.Djamil Hospital, Padang, Indonesia; [5]Department of Microbiology, Faculty of Medicine, Airlangga University/Dr.Soetomo Hospital, Surabaya, Indonesia; [6]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Centre, Rotterdam, the Netherlands; [7]Microbiology Unit, Biomérieux, Inc., La Balme, France; [8]BaseClear BV, Leiden, the Netherlands; [9]Molecular Diagnostics, Jeroen Bosch Hospital, Tilburg, the Netherlands.

Tropical Medicine and International Health 2016; 21(5):610–618;
doi:10.1111/tmi.12692

Abstract

OBJECTIVES. To determine the prevalence, antimicrobial susceptibility profiles, clonal distribution, of either methicillin-resistant *Staphylococcus aureus* (MRSA) or Panton-Valentine leukocidin (PVL)-positive *S. aureus* obtained from clinical cultures in Indonesian hospitals.

METHODS. *S. aureus* isolates from clinical cultures of patients in four tertiary care hospitals in Denpasar, Malang, Padang, and Semarang were included. We assessed the antimicrobial susceptibility profiles using the Vitek2® system, determined the presence of the *mecA* gene and genes encoding PVL using PCR, and analyzed the clonal relatedness with Raman spectroscopy. SCC*mec* typing was performed for all MRSA isolates. Multilocus sequence typing (MLST) was performed for a subset of isolates.

RESULTS. In total, 259 *S. aureus* strains were collected. Of these, 17/259 (6.6%) and 48/259 (18.5%) were MRSA and PVL-positive methicillin-susceptible *S. aureus* (MSSA), respectively. The prevalence of MRSA and PVL-positive MSSA ranged between 2.5% - 8.9% and 9.5% - 29.1%, respectively and depended on geographic origin. PVL-positive MRSA were not detected. Raman spectroscopy of the strains revealed multiple Raman types with two predominant clusters. We also showed possible transmission of a ST239-MRSA-SCC*mec* type III strain and a ST121 PVL-positive MSSA in one of the hospitals.

CONCLUSIONS. We showed that MRSA and PVL-positive MSSA are of clinical importance in Indonesian hospitals. A national surveillance system should be set-up to further monitor this. To reduce the prevalence of MRSA in Indonesian hospitals, a bundle of intervention measures is highly recommended.

INTRODUCTION

Staphylococcus aureus is recognized as an important pathogen, both in the hospital and community settings (1). The emergence and spread of methicillin-resistant *S. aureus* (MRSA), covering both hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA), has been a major problem worldwide (2-6). Traditionally, HA-MRSA has been associated with multi-drug resistance and staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, and III, while CA-MRSA has been associated with SCC*mec* types IV and V and the presence of Panton-Valentine leukocidin (PVL) genes. However, this distinction has blurred in many countries as the CA-MRSA clones have been introduced into the hospital and the HA-MRSA in the community (7).

S. aureus infections, including MRSA infections, have long been underappreciated in resource-limited countries in south and east Asia, where the healthcare agenda prioritises other health care issues (8). With the rapid emergence of antimicrobial resistance worldwide, this topic has, however, increasingly gained interest. Still, only limited data are available on the epidemiology of *S. aureus* infections in Indonesia. We have recently shown that MRSA was carried among patients screened at discharge from hospital in Indonesia, but with significant geographical variation (9). Among the MRSA strains, the HA-MRSA clone sequence type (ST) 239-SCC*mec* III prevailed. In addition, a high prevalence of PVL was found among carriage and community-onset infectious strains of methicillin-susceptible *S. aureus* (MSSA) from Indonesia (9-13). However, data on clinical *S. aureus* from Indonesian hospitals are lacking. In this study, we determined the prevalence, antimicrobial susceptibility profiles and clonal distribution of either MRSA or PVL-positive *S. aureus* obtained from clinical cultures in four Indonesian hospitals.

MATERIALS AND METHODS

Setting

Four tertiary care hospitals located on Sumatra island, Java island, and Bali island (Figure 1) participated in this study: Sanglah hospital in Denpasar (Bali; 704 beds), Dr. Saiful Anwar hospital in Malang (East Java; 810 beds), Dr. M. Djamil hospital in Padang (West Sumatra; 688 beds), and Dr. Kariadi hospital in Semarang (Central Java; 779 beds). The study was performed from January 2008 to January 2009 in Padang and Denpasar, from October 2009 to January 2010 in Malang, and from April 2008 to October 2009 in Semarang. This study was approved by the medical ethics committee of Dr. Saiful Anwar hospital (Malang), Sanglah hospital (Denpasar), and Dr. Kariadi hospital (Semarang) related to the "MRSA study" in Indonesia. Isolates were obtained as part of routine diagnostic testing and analyzed anonymously for this study.

Figure 1. Map of Indonesia depicting the four cities involved in this study (squares). The capital city Jakarta is also indicated (circle).

Bacterial isolates

S. aureus strains were isolated and identified by clinically indicated culture in each hospital involved in this study. *S. aureus* from all departments were included. Isolates were stored in trypticase soy agar until further characterization could be performed. Confirmation of identification and antibiotic susceptibility testing of the *S. aureus* strains were carried out by the Vitek2® system (bioMérieux, Marcy l'Etoile, France). Antibiotics tested included macrolides (clindamycin, erythromycin), aminoglycosides (gentamicin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), glycopeptides (teicoplanin, vancomycin), trimethoprim-sulfamethoxazole, fosfomycin, fusidic acid, linezolid, mupirocin, nitrofurantoin, rifampicin, and tetracycline. Only one clinical culture with *S. aureus* per patient was included in this study.

DNA isolation and detection of *mecA* and PVL genes

Bacterial DNA was isolated using the MagNa Pure LC DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) (14)). The DNA concentration was measured spectrophotometrically and the samples were stored at -20°C. PCRs to detect *mecA* and PVL (*lukF-PV and lukS-PV*) genes were performed as previously described (15, 16).

SCC*mec* typing

Multiplex PCR to characterize the SCC*mec* of *S. aureus* harbouring the *mecA* gene was conducted as previously described (17).

Raman spectroscopy

The clonal relationship among MRSA and PVL-positive MSSA isolates was analyzed using Raman spectroscopy (SpectraCell*RA* Bacterial Strain Analyzer, RiverD international BV, Rotterdam, The Netherlands), as previously described (9,18-20).

The Raman spectral analysis was performed using SpectraCell*RA* software version 1.9.0.13444:24 (RiverD international). The squared Pearson correlation coefficient (R^2) determined the similarity of the sample spectra and the known R^2 distribution of the identical and unrelated strains. In five different measurements, we included the ATCC (American Type Collection Culture) 43300 strain as a reproducibility control. A two-dimensional plot was created to compare the similarity of multiple isolates; the similarity of two isolates was presented by a color scale. The clonal relatedness was determined by setting the similarity threshold and cut off value as previously described (9).

Multilocus sequence typing (MLST)

Fourteen *S. aureus* isolates were further analyzed by MLST to allow international comparison (21). From each Raman cluster with at least two isolates, one to three isolates (depending on the cluster size) were selected for MLST. For larger clusters, we selected isolates from both the center and the fringe of the Raman cluster. The MLST sequence type was assigned through the MLST website (http://www.mlst.net).

Statistical analysis

Chi-square (x^2) test was applied to compare the antibiotic resistance rates between MRSA and MSSA isolates as well as PVL-positive MSSA and PVL-negative MSSA isolates using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, IL). This analysis was only performed in case resistance was present in 10 or more isolates per group. A *P* value less than 0.05 was considered significant.

RESULTS

Prevalence of MRSA and PVL-positive MSSA

A total of 259 *S. aureus* were collected consecutively by clinical culture: Denpasar (Sanglah hospital), 40 strains ; Malang (Dr. Saiful Anwar

hospital), 77 strains; Padang (Dr. M. Djamil hospital), 79 strains; Semarang (Dr. Kariadi hospital), 63 strains. Table 1 shows the origin of *S. aureus* isolates i.e. ward or outpatient clinic and type of specimen. The majority of the *S. aureus* strains were frequently found from pus (144/259 [55.6%]) , followed by other sites of infection such as sterile sites (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, tissue, urine, and vaginal discharge (61/259 [23.6%]) , sputum (37/259 [14.3%]) , and blood (17/259 [6.6%]) .The majority of *S. aureus* from pus were obtained from mixed wards (29.2%) and surgery wards (26.4%). Overall, the prevalence of MRSA among clinical *S. aureus* isolates was 6.6%, whereas the prevalence of PVL-positive MSSA was 18.5%. The highest prevalence of both MRSA (8.9%) and PVL-positive MSSA (29.1%) was found in Padang. None of the MRSA isolates was PVL-positive (Table 2). Fifteen out of 17 (88.2%) of the MRSA strains and 38/48 (79.2%) of the PVL-positive MSSA strains were isolated from pus, and of these, five MRSA (5/15, 33.3%) and 13 PVL-positive MSSA (13/38, 34.2%) were from surgery wards. We did not find any MRSA isolates among the blood culture isolates.

Table 1. Origin (ward / outpatient clinic and specimen) of *S. aureus* isolates collected in the study

Ward/outpatient clinic	No. of isolates (%)			
	Blood (n=17)	Pus (n=144)	Sputum (n=37)	Other[4] (n=61)
Surgery	0	38 (26.4)[a,i]	1 (2.7)	0
Internal medicine	0	21 (14.6)[b,j]	13 (35.1)[e]	2 (3.3)[q]
Pediatric	0	5 (3.5)[k]	0	5 (8.2)[r]
Emergency	1 (5.9)	6 (4.2)[l]	0	0
ICU	5 (29.4)[g]	2 (1.4)[m]	5 (13.5)	0
Mixed ward[1]	11 (64.7)[h]	42 (29.2)[c,n]	3 (8.1)	11 (18.0)[f]
Outpatient clinic[2]	0	30 (20.8)[d,o]	15 (40.5)[p]	28 (45.9)[s]

Unidentified ward[3]	0	0	0	15 (24.6)
MRSA (n=17)	0	15/144 (10.4)	1/37 (2.7)	1/61 (1.6)
PVL-positive MSSA (n=48)	4/17 (23.5)	38/144 (26.4)	2/37 (5.4)	4/61 (6.6)

Note. MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*; ICU, intensive care unit.

[1]occupied by adults only (no children) separated between male and female for mixed medical cases (i.e. internal medicine, neurology, surgery); [2]consisted of outpatient clinics of : surgery, ear nose and throat, dentistry, internal medicine, pulmonology, and dermatology ; [3]missing ward identity; [4]sterile sites (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, urine, tissue, and vaginal discharge; MRSA (n=17): [a]5 (29.4%), [b]3 (17.6%), [c]3 (17.6%), [d]3 (17.6%), [e]1 (5.9%), [f]2 (11.8%). PVL-positive MSSA (n=48): [g]1 (2.1%), [h]3 (6.3%), [i]13 (34.2%), [j]4 (8.3%), [k]2 (4.2%), [l]1 (2.1%), [m]1 (2.1%), [n]8 (16.7%), [o]8 (16.7%), [p]2 (4.2%), [q]1 (2.1%), [r]1 (2.1%), [s]3 (6.3%).

Table 2. Prevalence and sources of MRSA and PVL-positive MSSA among clinical specimens in four Indonesian hospitals

City	Clinical specimen	Number of strains (%)		
		S. aureus	MRSA	PVL-pos MSSA
Denpasar	Total	40	1 (2.5)	7 (17.5)
	Blood	5 (12.5)	0	1 (14.3)
	Pus	14 (35.0)	0	5 (71.4)
	Sputum	2 (5.0)	0	0
	Other	19 (47.5)	1[a] (100)	1[a] (14.3)
Malang	Total	77	4 (5.2)	12 (15.6)
	Blood	0	0	0
	Pus	33 (42.3)	3 (75.0)	9 (75.0)
	Sputum	24 (30.8)	1 (25.0)	1 (8.3)
	Other	20 (25.9)	0	2[b] (16.7)
Padang	Total	79	7 (8.9)	23 (29.1)
	Blood	1 (1.3)	0	1 (4.3)
	Pus	69 (87.3)	7 (100.0)	21 (91.4)
	Sputum	5 (6.3)	0	0
	Other	4 (5.1)	0	1[c] (4.3)
Semarang	Total	63	5 (7.9)	6 (9.5)
	Blood	11 (17.5)	0	2 (33.3)
	Pus	28 (44.4)	5 (100.0)	3 (50.0)
	Sputum	6 (9.5)	0	1 (16.7)
	Other	18 (28.6)	0	0
All cities	Total	259	17 (6.6)	48 (18.5)
	Blood	17 (6.6)	0	4 (8.3)
	Pus	144 (55.6)	15 (88.2)	38 (79.2)
	Sputum	37 (14.3)	1 (5.9)	2 (4.2)

Other	61 (23.6)	1 (5.9)	4 (8.3)

Note. MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-sensitive *Staphylococcus aureus.*; PVL, Panton-Valentine leukocidin.

Others=body fluid (amnion fluid, joint fluid, pleural fluid), eye secretion, ear secretion, nose, throat, urine, tissue, vaginal discharge).

[a]urine; [b]amnion fluid (1 isolate), pleural fluid (1 isolate); [c]pleural fluid.

Antibiotic susceptibility testing

Table 3 shows the resistance rates of the *S. aureus* isolates. The majority of *S. aureus* isolates were resistant to penicillin (219/259, 84.6%). The MRSA isolates were significantly more resistant to the aminoglycosides (gentamicin and tobramycin), the
fluoroquinolones (ciprofloxacin and levofloxacin), and tetracycline. For the other tested antibiotics, this comparison could not be performed because of statistical limitations. The overall resistance rate of MSSA isolates to tetracycline, either PVL-positive or PVL-negative MSSA, was also high (104/242, 43.0%). Of the PVL-negative MSSA, 9.5% were susceptible to all tested antibiotics, 31.4% were resistant to penicillin only, 29.8% were resistant to penicillin and tetracycline, and 14.5% were resistant to more than two classes of antibiotics.

Table 3. Antibiotic resistances of *S. aureus* isolated from clinical specimens in four Indonesian tertiary care hospitals

Antibiotics*	Resistance rate (%)		p^1	Resistance rate (%)		p^2
	MRSA (n=17)	MSSA (n=242)		PVL (+) MSSA (n=48)	PVL (-) MSSA (n=194)	
PEN	17 (100)	202 (83.5)	0.083	38 (79.2)	164 (84.5)	0.388
CLI	5 (29.4)	9 (3.7)	ND	2 (4.2)	7 (3.6)	ND
ERY	6 (35.3)	13 (5.4)	ND	3 (6.2)	10 (5.2)	ND
GEN	17 (100)	14 (5.8)	<0.001	0	14 (7.2)	ND
TOB	17 (100)	12 (5.0)	<0.001	0	12 (6.2)	ND
CIP	17 (100)	28 (11.6)	<0.001	3 (6.2)	25 (12.9)	ND
LVX	17 (100)	11 (4.5)	<0.001	1 (2.1)	10 (5.2)	ND
MXF	10 (58.8)	5 (2.1)	ND	0	5 (2.6)	ND
SXT	5 (29.4)	2 (0.8)	ND	0	2 (1.0)	ND
FOF	1 (5.9)	6 (2.5)	ND	0	6 (3.1)	ND
FUA	0	23 (9.5)	ND	7 (14.6)	16 (8.2)	ND
LZD	0	0	-	0	0	-
MUP	0	0	-	0	0	-
RIF	7 (41.2)	15 (6.2)	ND	2 (4.2)	13 (6.7)	ND
TET	17 (100)	104 (43)	<0.001	20 (41.7)	84 (43.3)	0.872
TEC	0	0	-	0	0	-
VAN	0	0	-	0	0	-

Note. MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PVL, Panton Valentine leucocidin; ND, not determined because of low number of resistance case (less than 10); *Abbreviations of antibiotics tested: PEN, penicillin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; SXT, trimethoprim-sulfamethoxazole; FOF, fosfomycin; FUA, fucidic acid; LZD, linezolid; MUP, mupirocin; RIF, rifampicin; TET, tetracycline; TEC, teicoplanin; VAN, vancomycin

All PVL positive strains were *mecA* negative; p^1 = significance value between MRSA and MSSA; p^2 = significance value between PVL (+) MSSA and PVL (-) MSSA.

SCC*mec* typing

The SCC*mec* typing was performed for all MRSA isolates (n=17). SCC*mec* type III was predominant in this study, as it was found in isolates from Denpasar (n=1), Malang (n=4), Padang (n=7), and Semarang (n=4). We found only one isolate, cultured in Semarang, with SCC*mec* type V. The SCC*mec* type V isolate was resistant to aminoglycosides (gentamicin and tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), trimethoprim-sulfamethoxazole, and tetracycline.

Raman spectroscopy

We performed Raman spectroscopy of the 17 MRSA and the 48 PVL-positive MSSA isolates. Raman spectroscopic analysis generated 48 Raman types (RTs) (Figure 2). The results of the quality control as performed with the ATCC strain in five different measurements are shown in Figure 2 as well. RT10 was the most frequently found and included 6 *S. aureus* isolates (Malang: MRSA-SCC*mec* type III, 1 isolate; Padang: MRSA-SCC*mec* type III, 1 isolate and PVL-positive MSSA, 1 isolate; Semarang: MRSA-SCC*mec* type III, 1 isolate and MRSA-SCC*mec* type V, 1 isolate; Denpasar: PVL-positive MSSA, 1 isolate). The second most common type was RT18, which consisted of 5 PVL-positive MSSA isolates from Padang.

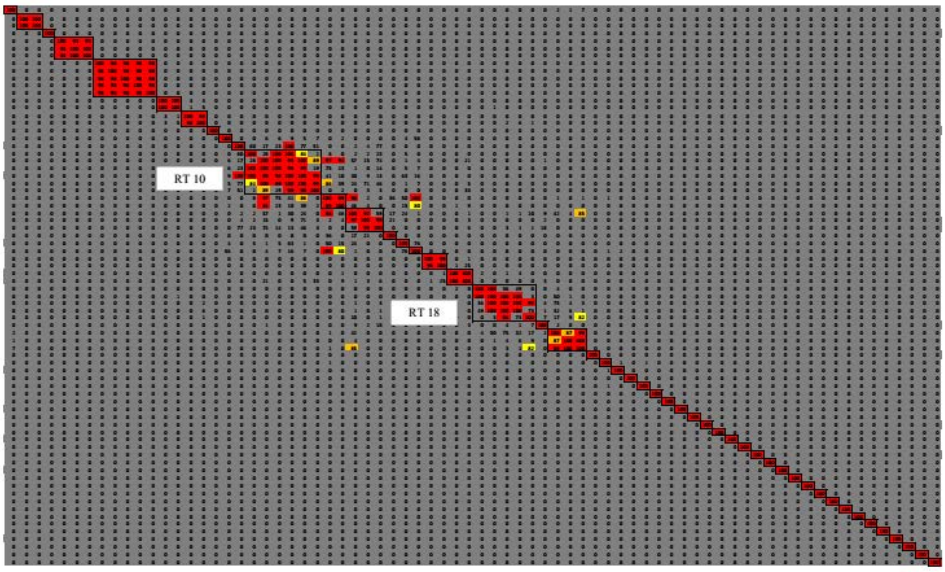

Figure 2. Clustering of MRSA and PVL-positive MSSA isolates from clinical isolates in four hospitals in Indonesia as determined by Raman spectroscopy (RT: Raman type). Note: Figure displays a correlation matrix used to analyze Raman spectral relatedness between isolates. Red clusters indicate isolates that are indistinguishable based on the cut-off value. The grey areas indicate isolates that are non-related based on the similarity threshold. The potentially related isolates are shown by yellow areas to orange areas gradually.

MLST

Similar to our previous study (9), we randomly selected 14 strains of *S. aureus* based on Raman spectroscopy analysis for MLST. MLST was performed for 3 MRSA isolates representing RT10 (1 isolate) and RT16 (2 isolates) as well as 11 PVL-positive MSSA isolates representing RT2 (1 isolate), RT4 (2 isolates), RT5 (2 isolate), RT6 (1 isolate), RT18 (3 isolates), and RT20 (2 isolates) (Table 4). The RT10 isolate (MRSA-SCC*mec* type V from Semarang) belonged to ST672, whereas the RT16 MRSA isolates (with SCC*mec* type III) were assigned to ST239. In addition, ST1 was presented by one RT2 and one RT4 PVL-positive MSSA. RT5, RT6, and

RT18 PVL-positive MSSA were assigned to ST121. We found different sequence types within the same Raman type of PVL-positive MSSA: (i) RT4 isolates belonged to both ST1 and ST188, (ii) RT18 isolates were assigned to both ST121 (allele profile 6-5-6-2-7-14-5) and a new sequence type, (iii) RT20 isolates belonged to ST2696 (allele profile 6-5-6-6-7-14-5) and a new sequence type. Interestingly, the allele profile of both new sequence types detected in RT18 and RT20 were the same, 158-5-6-2-7-199-5.

Table 4. Clonality of MRSA and PVL-positive MSSA clinical isolates in four hospitals in Indonesia

Isolate number	City	Raman type	*SCCmec* type	ST	Specimen	Ward
P47	Padang	2	*	1	Pus	Surgery
P66	Padang	4	*	1	Pus	Surgery
P24	Padang	4	*	188	Pleural fluid	Outpatient clinic
P109 a	Padang	5	*	121	Pus	Surgery
P116 a	Padang	5	*	121	Pus	Surgery
5196	Denpasar	6	*	121	Pus	Unidentified
16	Semarang	10	V	672	Tissue	Mixed
P63 b	Padang	16	III	239	Pus	Internal medicine
P37 b	Padang	16	III	239	Pus	Internal medicine
P101 c	Padang	18	*	121	Pus	Surgery
P119 c	Padang	18	*	121	Pus	Surgery
P111	Padang	18	*	New ST	Blood	Mixed
P107	Padang	20	*	2696	Pus	Ear, nose,

						throat
P114	Padang	20	*	New ST	Pus	Internal medicine

Note. MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; ST, sequence type.

a=clone "a" showing the concordance of RT5, PVL-positive MSSA, and ST121 on P109 and P116; b=clone "b" showing the concordance of RT16, SCC*mec* type III, and ST239 on P63 and 37; c=clone "c" showing the concordance of RT18, PVL-positive MSSA, and ST121 on P101 and P119; * = PVL-positive MSSA

DISCUSSION

This study presents the first multicenter survey of clinical *S. aureus* isolates from Indonesian hospitals. Similar to our previous study (9), we found geographic variation in the prevalence of MRSA among tertiary hospitals in Indonesia. Nevertheless, comparability of the prevalences is limited because of the different study period among hospitals involved. Overall, the prevalence of MRSA detected in this study was 6.6% which is considered lower than most other countries, but higher than countries in the north of Europe (4,22-26). Among *S. aureus* from blood cultures we did not find any MRSA, however the number of isolates from blood was low (n=17). In the European Antimicrobial Resistance Surveillance Network (EARS-Net), the country would be given the color "green". Among *S. aureus* from pus, the prevalence of MRSA was 10.4% (15/144 isolates). The prevalence of PVL-positive MSSA in Indonesian hospitals was high (18.5%) which is consistent with previous studies from Indonesia, but the phenomenon has also been reported recently from other countries (9,11,13,27,28). In such situations, the emergence of PVL-positive MRSA is a possibility in case the *mecA* gene is transferred to the PVL-positive MSSA strains (9). Both MRSA and PVL-positive MSSA were most frequently isolated from pus, especially from patients in the surgery

ward, suggesting the occurrence of surgical site infections caused by MRSA and PVL-positive MSSA.

Compared to the MSSA strains, the MRSA isolates were resistant to significantly more classes of antibiotics. More than 95% of MRSA strains were resistant to aminoglycosides (gentamicin, tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin), and tetracycline indicating that these antibiotics were often used as antibiotic therapy. Unfortunately, no clinical data were available to confirm that the patients had been treated with any of these antibiotics before the culture was obtained. Although the PVL-negative MSSA were mostly resistant to penicillin only, they have broadened their resistance profile, now including penicillin and tetracycline as shown before (29). The addition of tetracycline resistance may be due to the acquisition of the *tet*K and *tet*M genes via plasmid transfer (30).

SCC*mec* typing showed that SCC*mec* type III was predominant among clinical isolates of MRSA, suggesting the presence of HA-MRSA in Indonesian hospitals. In addition, similar with our previous study (9), we found one PVL-negative, SCC*mec* type V MRSA, representing a CA-MRSA, among the isolates from Dr. Kariadi hospital, Semarang, which was resistant to penicillin, fluoroquinolones, tetracycline, and trimethoprim-sulfamethoxazole. However, the strain was also resistant to gentamicin indicating that the CA-MRSA strains may have penetrated into the hospital setting, and even caused hospital-onset or healthcare-associated infections in Indonesia.

All MRSA and PVL-positive MSSA were analyzed using Raman spectroscopy, and a subset of isolates using MLST. Two MRSA isolates from Padang (P63 and P37) were identified as ST239-MRSA-SCC*mec* type III and belonged to RT16. Both MRSA isolates were obtained from wound cultures of patients admitted to the internal medicine ward which is suggestive for cross-transmission. The ST239-MRSA-SCC*mec* type III clone is a single-locus variant of USA300 (21) and is common in Asian countries (31). In our previous study, we also identified the carriage of

ST239-MRSA-SCC*mec* type III among discharged patients, particularly in Dr. Kariadi hospital, Semarang (9). Interestingly, the MRSA isolate from Semarang that clustered in RT10 was identified as ST672-SCC*mec* type V that was also reported as emerging in India (32). Two PVL-positive MSSA from Padang (P101 and P119) were assigned to the second largest Raman cluster, RT18, which corresponded to ST121. Both identical PVL-positive MSSA were isolated from wound cultures of surgery patients, hence nosocomial transmission may have occurred. The other two PVL-positive MSSA from Padang (P109 and P116) were assigned to the smaller Raman cluster, RT5, which also corresponded to ST121. Both identical PVL-positive MSSA were isolated from wound cultures of surgery patients indicating nosocomial transmission may also have occurred. Thus, the ST121 PVL-positive MSSA obtained from wound culture of surgery patients in Padang expressed two different phenotypic Raman cluster. New sequence types were identified in both RT18 and RT20 with an allele profile that is similar to ST121 featuring *arcC* and *tpi* variants.

The same Raman type could be assigned to different sequence types that might belong to a single clonal complex. This situation was also encountered in RT4 isolates that were assigned to both ST1 and its double locus variant ST188, both members of clonal complex 1 according to BURST analysis (33). These discrepancies are not unexpected given the different technical background of the two typing methods. In previous reports we (20) and others (34) found Raman typing to produce results that were >95% concordant with PFGE, the gold standard for assigning staphylococci to genetic clones. Discrepancies remained, however, albeit at a low rate. In this study we found two strains with the same RT (4) to have two different ST's; however, the two strains were genetically closely related since one (ST188) is a double locus variant of the other (ST1). Apparently, such limited variation in the allelic profile of a few housekeeping genes does not necessarily translates into differences in the strain's Raman spectra. However, for a valid comparison of the two methods, a much larger collection has to be analyzed.

Our study has certain limitations. First, it was based on a convenience sample of strains isolated from routine clinical cultures and additional clinical information including some potential risk factors for acquisition of MRSA and the time of culture related to the admission date was not collected. Therefore, a true distinction between community-acquired and healthcare-associated infection was not possible. Second, as four hospitals from three islands participated, our data should not be considered representative for the whole country, but the data may be used as a point reference (35). However, a national surveillance system should be set up in which MRSA and PVL prevalence, epidemic clonal shifts, clone emergence, and transmission between community and healthcare settings can be monitored. This would be challenging given the extent of the country and the still limited availability of clinical microbiology services in many areas. Finally, Raman spectroscopy and MLST were not performed for all *S. aureus* strains; consequently, we could not provide the clonal relatedness of PVL-negative MSSA strains.

In summary, infection with HA-MRSA occurred in Indonesian hospitals, but with geographic variation. The possible penetration of CA-MRSA in Dr. Kariadi hospital, Semarang is of concern. In this situation, a bundle of intervention measures to reduce the prevalence of MRSA in Indonesian hospitals is highly recommended.

Acknowledgments

We thank the deans of the Faculty of Medicine, Brawijaya University, Malang, Indonesia; the Faculty of Medicine, Diponegoro University, Semarang, Indonesia; Faculty of Medicine, Andalas University, Padang, Indonesia, and the Faculty of Medicine, Udayana University, Denpasar, Indonesia, the directors of the Dr. Saiful Anwar hospital, Malang, Indonesia; the Dr. Kariadi hospital, Semarang, Indonesia; the Dr. M. Djamil hospital, Padang, Indonesia; and the Sanglah hospital, Denpasar, Indonesia who facilitated our work in these teaching hospitals. We also thank all staff members who have been involved in the isolation of bacteria. The excellent technical assistance of Michelle de Regt is gratefully acknowledged.

This study was financially supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands.

REFERENCES

1. Lowy F. Mapping the Distribution of Invasive *Staphylococcus aureus* across Europe. PLoS Med 2010;7:e1000205.
2. Shittu AO & Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in Kwa Zulu-Natal province, South Africa. BMC Infectious Diseases 2006;6:1-13.
3. Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z, et al. Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. International Journal of Medical Microbiology 2011;301: 347-53.
4. Kock R, Becker K, Cookson B, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010;15:1-9.
5. Muttaiyah S, Coombs G, Pandey S, et al. Incidence, Risk Factors, and Outcomes of Panton-Valentine leukocidin-Positive Methicillin-Susceptible *Staphylococcus aureus* Infections in Auckland, New Zealand. J Clin Microbiol 2010;48:3470-74.
6. Song, K-H, Kim ES, Sin H-y, et al. Characteristics of invasive *Staphylococcus aureus* infections in three regions of Korea, 2009-2011: a multi- center cohort study. BMC Infectious Diseases 2013;13:1-8.
7. Huang H, Flynn NM, King JH, et al. Comparison of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Hospital-Associated MRSA Infections in Sacramento, California. J Clin Microbiol 2006;44;2423-27.
8. Nickerson EK, West TE, Day NP, et al. Staphylococcus disease and drug resistance in resource-limited countries in south and east Asia. Lancet Infect Disease 2009;9:130-35.
9. 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.

10. Deurenberg RH, Beisser PS, Visschers MJ, et al. Molecular typing of methicillin-susceptible *Staphylococcus aureus* isolates collected in the Yogyakarta area in Indonesia, 2006. Clin Microbiol Infect 2010;16:92-94.
11. Buntaran L, Hatta M, Sultan AR, et al. SCCmec type II gene is common among clinical isolates of methicillin-resistant *Staphylococcus aureus* in Jakarta, Indonesia. BMC Research Notes 2013;6:1-7.
12. Salasia SIO, Tato S, Sugiyono N, et al. Genotypic characterization of *Staphylococcus aureus* isolated from bovines, humans, and food in Indonesia. J Vet Sci 2011;12:353-61.
13. 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 Microbiol 2008;46: 1989-95.
14. Melles DC, Gorkink RFJ, Boelens HAM, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. The Journal of Clinical Investigation 2004;114:1732-40.
15. Lina G, Plemont Y, Godall-Gamot F, et al. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. Clinical Infectious Diseases 1999;29:1128-32.
16. Murakami K, Minamide W, Wada K, et al. Identification of Methicillin-Resistant Strains of Staphylococci by Polymerase Chain Reaction. J Clin Microbiol 1991;29:2240-44.
17. Milheiriço C, Oliveira DC, and de Lencastre H. Update to the Multiplex PCR Strategy for Assignment of *mec* Element Types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2007;51:3374-77.

18. Willemse-Erix DFM, Scholtes-Timmerman MJ, Jachtenberg J-W, et al. Optical fingerprinting in bacterial epidemiology: Raman spectroscopy as a real-time typing method. J Clin Microbiol 2009;47:652-59.
19. Willemse-Erix D, Bakker-Schut T, Slagboom-Bax F, et al. Rapid Typing of Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates by Use of SpectraCell RA. J Clin Microbiol 2012;50:1370-75.
20. Te Witt R, Vaessen N, Melles DC et al. Good performance of SpectraCellRA system for typing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Clin Microbiol 2013;51:1434-38.
21. Enright MC, Day NPJ, Davies CE, et al. Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of *Staphylococcus aureus.* J Clin Microbiol 2000;38:1008-15.
22. Ghaznavi-Rad E, Shamsudin MN, Sakawi Z, et al. Predominance and Emergence of Clones of Hospital Acquired Methicillin-Resistant *Staphylococcus aureus* in Malaysia. J Clin Microbiol 2010;48:867-72.
23. Kang C-I & Song J-H. Antimicrobial Resistance in Asia: Current Epidemiology and Clinical Implications. J Infect Chemother 2013;45:22-31.
24. Robert J, Tristan A, Cavalie L, et al. Panton-Valentine Leukocidin-Positive and Toxic Shock Syndrome Toxin 1-Positive Methicillin-Resistant *Staphylococcus aureus*: a French Multicenter Prospective Study in 2008. Antimicrobial Agents and Chemotherapy 2011;55:1734-39.
25. Lozano C, Porres-Osante N, Crettaz J et al. Changes in genetic lineages, resistance, and virulence in clinical methicillin-resistant *Staphylococcus aureus* in a Spanish hospital. J Infect Chemother 2013;19:233-42.

26. Naidoo R, Nuttall J, Whitelaw A et al. Epidemiology of *Staphylococcus aureus* Bacteraemia at a Tertiary Children's Hospital in Cape Town, South Africa. PLoS ONE 2013;8:e78396.
27. Harastani HH, Araj GF, and Tokajian ST. Molecular characteristics of *Staphylococcus aureus* isolated from a major hospital in Lebanon. International Journal of Infectious Diseases 2014;19:33-38.
28. van der Meeren BT, Millard PS, Scacchetti M et al. Emergence of methicillin resistance and Panton-Valentine leukocidin positivity in hospital- and community-acquired *Staphylococcus aureus* infections in Beira, Mozambique. Tropical Medicine and International Health 2014;19:169-76.
29. Lestari ES, Duerink DO, Hadi U, et al. Determinants of carriage of resistant *Staphylococcus aureus* among *S. aureus* carriers in the Indonesian population inside and outside hospitals. Tropical Medicine and International Health 2010;15:1235-43.
30. Tenover FC and Goering RV. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J Antimicrobial and Chemotherapy 2009;64:441-46.
31. Ko KS, Lee J-Y, Suh JY, et al. Distribution of Major Genotypes among Methicillin-Resistant *Staphylococcus aureus* Clones in Asian Countries. J Clin Microbiol 2005;43:421-426.
32. Khedkar S, Prabhakara S, Malarini R, et al. Draft Genome Sequence of *Staphylococcus aureus* ST672, an Emerging Disease Clone from India. J Bacteriol 2012;194:6946.
33. Feil EJ, Cooper JE, Grundmann H, et al. How clonal is *Staphylococcus aureus?* J Bacteriol 2003;185:3307-16.
34. Wulf MW, Willemse-Erix D, Verduin CM, et al. The use of Raman spectroscopy in the epidemiology of methicillin-resistant *Staphylococcus aureus* of human- and animal-related clonal lineages. Clin Microbiol Infect 2012;18:147-52.
35. Zinn CS, Westh H, Rosdahl VT, et al. An International Multicenter Study of Antimicrobial Resistance and Typing of Hospital

Staphylococcus aureus Isolates from 21 Laboratories in 19 Countries or States. Microbial Drug Resistance 2004;10:160-68.

Chapter 4

Prevalence and characterization of *Staphylococcus aureus* causing skin and soft tissue infections in the community setting in Java and Bali, Indonesia

Dewi Santosaningsih[1,9], Sanarto Santoso[1], Nanik Setijowati[2], Harun A Rasyid[2], Nyoman S Budayanti[3], Ketut Suata[3], Dicky B Widhyatmoko[4], Priyo B Purwono[4], Kuntaman Kuntaman[4], Damayanti Damayanti[5], Cita RS Prakoeswa[5], Mitchell Laurens[6,9], Josephine WI van Nierop[7,9], Geraldine L Nanninga[9], Neline Oudenes[8,9], Michelle de Regt[9], Susan V Snijders[9], Henri A Verbrugh[9], Juliëtte A Severin[9]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [2]Department of Public Health, Faculty of Medicine, Brawijaya University, Malang, Indonesia; [3]Department of Microbiology, Faculty of Medicine, Udayana University/Sanglah Hospital, Denpasar, Indonesia; [4]Department of Microbiology, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya, Indonesia; [5]Department of Dermatology and Venereology, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya, Indonesia; [6]BaseClear BV, Leiden, the Netherlands; [7]Department of Otorhinolaryngology, Head and Neck Surgery, Radboud University Medical Center, P.O. The Netherlands; [8]Department of Virology, University Medical Center, Utrecht, the Netherlands; [9]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands.

ABSTRACT

OBJECTIVES To define the role of *Staphylococcus aureus* in community settings among patients with skin and soft tissue infections (SSTI) in Indonesia.

METHODS *S. aureus* were cultured from anterior nares, throat, and wound of 567 ambulatory patients presenting with SSTI. The *mecA* gene and genes encoding Panton-Valentine leukocidin (PVL; *lukF-PV* and *lukS-PV*) and exfoliative toxin (ET; *eta* and *etb*) were determined by PCR. Clonal relatedness among methicillin-resistant *S. aureus* (MRSA) and PVL-positive *S. aureus* was analyzed using Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) typing, and multilocus sequence typing (MLST) for a subset of isolates. Staphylococcal cassette chromosome *mec* (SCC*mec*) was determined for all MRSA isolates. Moreover, determinants for *S. aureus* SSTI, and PVL/ET-positive versus PVL/ET-negative *S. aureus* were assessed.

RESULTS *S. aureus* were isolated from SSTI wounds of 257 (45.3%) patients, eight (3.1%) of these were MRSA. Genes encoding PVL and ETs were detected in 21.8% and 17.5% of methicillin-susceptible *S. aureus* (MSSA), respectively. PVL-positive MRSA was not detected. Nasopharyngeal *S. aureus* carriage was an independent determinant for *S. aureus* SSTI (odds ratio (OR) 1.8). Primary skin infection (OR 5.4) and previous antibiotic therapy (OR 3.5) were associated with PVL-positive MSSA. Primary skin infection (OR 2.2) was the only factor associated with ET-positive MSSA. MLVA typing revealed two predominant MSSA clusters. One ST1-MRSA-SCC*mec* type IV isolate and a cluster of ST239-MRSA-SCC*mec* type III were found.

CONCLUSIONS Community-acquired SSTI in Indonesia was frequently caused by PVL-positive MSSA, and the hospital-associated ST239-MRSA may have spread from the hospital into the community.

INTRODUCTION

Staphylococcus aureus is known as an important pathogen of skin and soft tissue infections (SSTI) in the community setting (1). The emergence and transmission of community-associated methicillin-resistant *S. aureus* (CA-MRSA) has become a major problem in several countries (2-7). In general, CA-MRSA are more susceptible to non-beta-lactam antibiotics compared to hospital-associated MRSA (HA-MRSA). However, CA-MRSA tend to be more virulent than HA-MRSA and may cause highly invasive, rapidly progressive, and life threatening diseases (1). This phenomenon has been associated with the presence of Panton-Valentine leukocidin (PVL). CA-MRSA are also associated with staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V which differentiates them further from HA-MRSA that carry SCC*mec* type I, II, or III. Nevertheless, the distinction between CA-MRSA and HA-MRSA has blurred in many countries as CA-MRSA clones have been introduced into the hospital and, vice versa, HA-MRSA have spread from health care settings into the community (8).

Only few data are available on the epidemiology of CA-MRSA in Indonesia, the fourth most populous nation in the world. Only a few case reports of the finding of a SCC*mec* type V carrying MRSA strain, one regarding a patient discharged from Dr. Saiful Anwar hospital in Malang and one describing a patient admitted to the Dr. Kariadi hospital in Semarang have been published previously (9, 10). Interestingly, a high prevalence of PVL-positive methicillin-susceptible *S. aureus* (MSSA) was observed among carriage and community-onset infection strains in Indonesia (11, 12). In the present study, *S. aureus* isolates from multiple community settings in Indonesia targeting patients suffering from community-acquired SSTI were collected. The presence of *mecA*, genes for PVL and exfoliative toxins were assessed. Further, their clonal relatedness and sequence type (ST) were determined and we

subsequently correlated these data with patients' disease characteristics and outcomes.

MATERIALS AND METHODS

Setting

Eight primary healthcare centers and two dermatology outpatient clinics of teaching hospitals located on Java and Bali islands participated in this study: Java (Malang city; five primary healthcare centers, Surabaya city; one primary healthcare center and one dermatology outpatient clinic in Dr. Soetomo academic hospital), Bali (Denpasar city; two primary healthcare centers and one dermatology outpatient clinic in Sanglah academic hospital). Enrollment of patients into the study was from July 2009 to February 2010.

Study population

Patients with SSTI including superficial skin infections (impetigo, ecthyma, erysipelas and furuncles), more deep-seated infections (carbuncles, cellulitis, subcutaneous abscesses and paronychia), and, as a separate category, secondary infections complicating pre-existing skin diseases or traumatic lesions presenting to the participating centers were eligible for enrollment. Patients with a history of prior hospitalization within the past 12 months or other established risk factors, such as surgery, residence in a long-term care facility, dialysis or indwelling percutaneous medical devices and urinary catheters were excluded. The study was approved by the medical ethics commitee of Faculty of Medicine, Brawijaya University, Malang, Indonesia (the ethical clearance was not assigned by a number).

Bacterial isolates

Patients with SSTI who were eligible for the study and who had given written informed consent were cultured by swabbing their skin lesions.

Screening for MSSA and MRSA carriage among patients was performed by culturing their anterior nares and throat. Samples were transported using Amies transport medium (Becton Dickinson). Swabs were directly inoculated into 5 mL phenol red mannitol broth (BBL, Le Pont de Claix, France) for overnight incubation at 37°C and subsequently sub-cultured onto *S. aureus* and MRSA Chromagar medium (ITK Diagnostics, Uithoorn, The Netherlands) and incubated for 24–48 hours at 37°C. Typical colonies of *S. aureus* and MRSA were stored into trypticase soy agar until further characterization could be carried out. Confirmation of *S. aureus* was performed by Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France) and the Vitek2 system (bioMérieux). A subset of *S. aureus* isolates from Denpasar was additionally confirmed by matrix-assisted laser desorption/ionisation (Maldi Biotyper, Bruker Microflex LT, Bruker, London, UK). Antibiotic susceptibility tests on the MRSA isolates was conducted by Vitek2 system (bioMérieux) and included macrolides (clindamycin and erythromycin), aminoglycosides (gentamicin and tobramycin), fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), glycopeptides (teicoplanin and vancomycin), trimethoprim-sulfamethoxazole, fosfomycin, fusidic acid, linezolid, mupirocin, nitrofurantoin, rifampicin, and tetracycline.

DNA extraction and detection of *mecA*, SCC*mec*, and genes for PVL and exfoliative toxins A and B

Bacterial DNA was extracted using the MagNa Pure LC™ DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) (13). For a subset of isolates, bacterial DNA was extracted using DNA Mini Kits (QiaAmp DNA Mini Kits; Qiagen Inc, Germany) according to the manufacturer's instruction. The DNA concentration was measured spectrophotometrically and samples were stored at -20°C. Detection of *mecA*, PVL genes *lukF-PV* and *lukS-PV,* and exfoliative toxin A (*eta*) and B (*etb*) genes were performed by PCR as previously described (14-16). The SCC*mec* of *S. aureus* isolates containing the *mecA* gene was

characterized using a multiplex PCR that enables the identification of SCC*mec* types I to VI (17). Positive and negative control strains were included in each PCR run.

Multilocus Variable-Number Tandem-Repeat Analysis (MLVA)

MLVA typing was carried out based on the combination of six loci (SIRU01, SIRU05, SIRU07, SIRU13, SIRU15, and SIRU21) as described previously (18). Amplification of SIRUs (Staphylococcal Interspersed Repeat Units) and the assignment of MLVA type were derived from a previous study by Ikawati et al. (19).

Multilocus sequence typing (MLST)

A selection of 12 randomly chosen *S. aureus* isolates was further analyzed by MLST (20) as described before with a minor modification, i.e. the amplicon was purified using a supplement, ExoSAP-IT (Affymetrix product no. 78200; Isogen), before sequence reactions were carried out. The MLST sequence type (ST) was assigned through the MLST website (www.mlst.net).

Risk factor analysis

Socio-demographic data, atopic history, *S. aureus* carriage status, family with similar disease, and number of family members were included in the risk factor analysis of *S aureus* SSTI in the community. Since the *S. aureus* carriage status is important in this analysis, only patients that were actually screened for nasopharyngeal carriage were included in this analysis. Duration and type of skin lesion, same complaint before, antibiotic therapy in the previous month, and diabetes mellitus were included in the analysis to determine association between the presence of genes encoding PVL and exfoliative toxins and the clinical features and background of the staphylococcal SSTI. These data were collected from patient records and by interviewing the patients using a structured questionnaire. Potential risk factors for *S. aureus* SSTI were tested

univariately using Pearson Chi-Square or Fisher analysis. All variables with a *P* value ≤ 0.2 were included in a multivariate logistic regression model. Backward selection based on the likelihood-ratio test was used to identify significant variables. Likewise, determinants for PVL-positive versus PVL-negative *S. aureus* and exfoliative toxin-positive versus exfoliative toxin-negative *S. aureus* were analyzed. Data were analyzed using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, USA). A *p* value less than 0.05 was considered significant.

RESULTS

Prevalence of MRSA, PVL- and exfoliative toxin-positive MSSA

A total of 567 patients with SSTI were enrolled in this study, 200 in Denpasar (Bali island), 196 in Malang (Java island), and 171 in Surabaya (Java island). The prevalence of *S. aureus* obtained from wound culture among patients with SSTI was 257/567 (45.3%) with some variation between the three cities (Table 1). In total, we found that eight out of 257 (3.1%) *S. aureus* infected patients had an MRSA infection. In Surabaya, MRSA was significantly more common among the SSTI causing *S. aureus* compared to other cities (7/74 in Surabaya versus 1/183 in other cities; $p \leq 0.001$). In Malang, none of the *S. aureus* were MRSA, which was also statistically significant (Table 1). Remarkably, all MRSA isolates were PVL-negative. In contrast, PVL-positive strains were frequently found among the methicillin-susceptible isolates of *S. aureus* (MSSA) 56/249 (22.5%) with significant variation between the three cities. Likewise, exfoliative toxin-coding genes were only detected in MSSA isolates, overall 45/249 (18.1%), only *eta* (29/249, 11.6%), only *etb* (5/249, 2.0%). Exfoliative toxin-positive strains were found in all three cities at approximately similar frequencies (16.0% - 19.0%) (Table 1). All patients enrolled in this study were screened for nasopharyngeal MRSA carriage, except in Surabaya where 69 out of 171 patients enrolled were screened. Table 1 shows the significant variation of *S. aureus* carriage between the

three cities either among patients with *S. aureus* SSTI (p<0.001) or patients with non-*S. aureus* SSTI (p<0.001). We did not find any MRSA among the 136 *S. aureus* carriage patients.

Table 1. Prevalence of methicillin-resistant, PVL-, and exfoliative toxin-positive *S. aureus* among patients with staphylococcal skin and soft tissue infections in the community setting in Indonesia.

	Patients enrolled in				*P*
	Denpasar (n=200)	Malang (n=196)	Surabaya (n=171)	Total (n=567)	
S. aureus clinical[a]	80/200 (40.0)	103/196 (52.6)	74/171 (43.3)	257/567 (45.3)	0.035
S. aureus carriage[b]	7/80 (8.8)[c]	65/103 (63.1)[c]	1/18 (5.6)[c]	73/201 (36.3)[c]	<0.001
	12/120 (10.0)[d]	33/93 (35.5)[d]	18/51 (35.3)[d]	63/264 (23.9)[d]	<0.001
MRSA[a]	1/80 (1.2)	0/103 (0)	7/74 (9.5)	8/257 (3.1)	[e]0.441
					[f]<0.001
					[g]0.023
PVL (+)[a,h]	28/80 (35.0)	10/103 (9.7)	18/74 (24.3)	56/257 (21.8)	[e]0.001
					[f]0.617
					[g]<0.001
eta (+)[a]	11/80 (13.8)	10/103 (9.7)	8/74 (10.8)	29/257 (11.3)	[i]0.819
etb (+)[a]	2/80 (2.5)	2/103 (1.9)	1/74 (1.4)	5/257 (1.9)	
eta and *etb* (+)[a]	1/80 (1.2)	5/103 (4.9)	5/74 (6.8)	11/257 (4.3)	

Data are absolute numbers of patients (%); MSSA=methicillin-susceptible *S. aureus;* MRSA=methicillin-resistant *S. aureus;* PVL=Panton-Valentine Leukocidin; *eta*=exfoliative toxin A gene; *etb*=exfoliative toxin B gene; [a]*S. aureus* were obtained from wound culture; [b]*S. aureus* were obtained from either nose only, throat only, or both nose and throat; [c]*S. aureus* carriage among patients with *S. aureus* SSTI; [d]*S. aureus* carriage among patients with non-*S. aureus* SSTI; [e]Denpasar vs rest (Fisher); [f]Surabaya vs rest (Fisher); [g]Malang vs rest (Fisher); [h]all PVL-positive strains were MSSA; [i]either in conjunction or independent *eta* and *etb* genes.

Antibiotic susceptibility testing

Antibiotic susceptibility tests showed that the seven MRSA isolates from Surabaya were all resistant to aminoglycosides, macrolides, ciprofloxacin, levofloxacin, tetracycline, and trimethoprim-sulfamethoxazole, whereas four of the seven MRSA isolates were resistant to moxifloxacin as well. All of seven MRSA isolates were sensitive to the remaining antibiotics tested. In contrast, the single MRSA isolate from Denpasar showed susceptibility to all non-betalactam antibiotics in the test panel.

SCC*mec* and MLVA typing

SCC*mec* typing was performed for the eight MRSA isolates from wound culture of patients with SSTI. Interestingly, the seven MRSA isolates from Surabaya harboured SCC*mec* type III and the single MRSA isolate from Denpasar harboured SCC*mec* type IV. MLVA typing was carried out for a subset of 56 *S. aureus* isolates consisting of the eight MRSA isolates from wound cultures and 48 PVL-positive MSSA from different sources (wound: 34 isolates; nose screening: six isolates; throat screening: eight isolates). Eight PVL-positive MSSA isolates could not be retrieved from storage for MLVA typing. The MLVA revealed 30 MLVA types (MT) (Table 2) and one isolate was not determinable. The most frequently found type was MT11 consisting of seven PVL-positive MSSA obtained from wound cultures in three cities involved in this study, except one isolate from Malang that was obtained from a nose culture. The second largest cluster was MT19 containing five MRSA isolates from wound cultures, all isolated in Surabaya.

Table 2. MLVA typing results of *S. aureus* obtained from three cities in the community setting in Indonesia

Isolate number	City	Source	SIRU profile						MLVA type
			SIRU01	SIRU05	SIRU07	SIRU13	SIRU15	SIRU21	
2241	Malang	T	1	999	2	1	1	10	1
W492	Denpasar	W	1	999	2	2	1	6	2
1121	Malang	N	1	999	3	999	999	999	3
2091	Malang	T	1	999	3	999	4	5	4
W138	Denpasar	W	1	999	3	2	1	5	5
3195	Malang	W	1	999	3	2	1	6	6
10115	Surabaya	W	1	999	3	2	1	6	6
3107	Malang	W	1	999	3	2	2	6	6
3122	Malang	W	1	999	3	3	4	5	7
3222	Malang	W	1	999	3	3	4	5	7
W387	Denpasar	W	1	999	3	3	4	5	7
W546	Denpasar	W	1	999	3	3	4	5	7
TS448	Denpasar	T	1	1	2	1	3	7	8
2089	Malang	T	1	1	3	1	3	7	9
W372	Denpasar	W	1	1	3	1	3	7	9
10124	Surabaya	W	1	1	3	1	3	7	9
W305	Denpasar	W	1	1	3	2	3	1	10
3087	Malang	W	1	1	3	2	3	8	11
1086	Malang	N	1	1	3	2	3	8	11
W501	Denpasar	W	1	1	3	2	3	8	11
W378	Denpasar	W	1	1	3	2	3	8	11

Table 2. MLVA typing results of *S. aureus* obtained from three cities in the community setting in Indonesia (cont'd)

Isolate number	City	Source	SIRU profile						MLVA type
			SIRU01	SIRU05	SIRU07	SIRU13	SIRU15	SIRU21	
10093	Surabaya	W	1	1	3	2	3	8	11
10022	Surabaya	W	1	1	3	2	3	8	11
3178	Malang	W	1	1	3	2	1	8	11
W396	Denpasar	W	1	1	5	2	3	7	12
1223	Malang	N	2	999	1	2	2	7	13
3011	Malang	W	2	0	3	2	3	9	14
2010	Malang	T	2	0	3	2	3	9	14
3106	Malang	W	2	0	3	2	3	9	14
W357	Denpasar	W	2	1	3	1	3	8	15
10069	Surabaya	W	2	8	1	5	1	8	16
W474	Denpasar	W	2	10	1	4	1	9	17
W31	Denpasar	W	3	999	3	2	4	9	18
12*	Surabaya	W	3	1	2	1	1	6	19
13*	Surabaya	W	3	1	2	1	1	6	19
14*	Surabaya	W	3	1	2	1	1	6	19
18*	Surabaya	W	3	1	2	1	1	6	19
19*	Surabaya	W	3	1	2	1	1	6	19
15	Surabaya	W	3	1	3	1	1	6	20
10066	Surabaya	W	3	1	3	1	1	6	20
W486	Denpasar	W	3	1	3	1	3	8	21

Table 2. MLVA typing results of *S. aureus* obtained from three cities in the community setting in Indonesia (cont'd)

Isolate number	City	Source	SIRU profile						MLVA type
			SIRU01	SIRU05	SIRU07	SIRU13	SIRU15	SIRU21	
W375	Denpasar	W	3	1	3	1	3	8	21
1128	Malang	N	3	1	3	2	2	8	22
3130	Malang	W	3	1	3	2	2	8	22
10005	Surabaya	W	3	2	3	2	2	8	23
2214	Malang	T	3	4	2	5	4	10	24
2216	Malang	T	3	4	3	3	4	3	25
P24	Surabaya	W	3	4	3	3	4	9	26
3007	Malang	W	3	4	3	3	4	9	26
W320	Denpasar	W	3	4	3	3	4	10	27
W429	Denpasar	W	3	4	3	3	4	10	27
W402	Denpasar	W	4	999	1	3	5	7	28
W335	Denpasar	W	4	999	1	3	4	8	29
1193	Malang	N	4	9	2	3	5	4	30
2194	Malang	T	4	9	2	3	5	4	30
1240	Malang	N	999	999	999	999	999	999	ND

SIRU= Staphylococcal Interspersed Repeat Units; MLVA= Multilocus Variable-Number Tandem-Repeat Analysis; ND=not determinable; W=wound swab; N=nose swab; T=throat swab; *MRSA isolates; 999=no amplification of SIRU.

MLST analysis

Twelve *S. aureus* strains were selected for MLST. MLST was performed for two MRSA isolates representing MT2 (one isolate from Denpasar) and MT19 (one isolate from Surabaya); they belonged to ST1-MRSA-SCC*mec* type IV and ST239-MRSA-SCC*mec* type III, respectively. In addition, MLST was conducted for 10 PVL-positive MSSA representing MT5 (one isolate), MT6 (one isolate), MT7 (one isolate), MT10 (one isolate), MT11 (two isolates), MT18 (one isolate), MT22 (two isolates), and MT23 (one isolate). The MLST typing identified ST1301 as a predominant sequence type among PVL-positive MSSA isolates corresponding to MT10, MT11, MT22, and MT23, MLVA types with largely concordant SIRU profiles (Table 3).

Table 3. Bacterial typing of 12 selected *S. aureus* isolates from community setting in Indonesia

Isolate number	City	SCC*mec* type	SIRU profile	MLVA type (MT)	ST
W492	Denpasar	IV	1.999.2.2.1.6	2	1
W138	Denpasar	#	1.999.3.2.1.5	5	97
3107	Malang	#	1.999.3.2.2.6	6	1
W546	Denpasar	#	1.999.3.3.4.5	7	188
W305	Denpasar	#	1.1.3.2.3.1	10	1301
W501 [a]	Denpasar	#	1.1.3.2.3.8	11	1301
10022 [a]	Surabaya	#	1.1.3.2.3.8	11	1301
W31	Denpasar	#	3.999.3.2.4.9	18	3035
12	Surabaya	III	3.1.2.1.1.6	19	239
CA1128 [b]	Malang	#	3.1.3.2.2.8	22	1301
CA3130 [b]	Malang	#	3.1.3.2.2.8	22	1301
10005	Surabaya	#	3.2.3.2.2.8	23	1301

SCC*mec*=Staphylococcal Cassette Chromosome *mec*; ST=sequence type; SIRU=Staphylococcal Interspersed Repeat Units; MLVA=Multilocus Variable_Number

Tandem_Repeat Analysis; #=methicillin-susceptible *Staphylococcus aureus*; a, clone 'a' showing the concordance of PVL-positive MSSA, MLVA type 11, and ST1301; b, clone 'b' showing the concordance of PVL-positive MSSA, MLVA type 22, and ST1301; 999=no amplification of SIRU.

Risk factor analysis

For the risk factor analysis all patients from Malang and Denpasar were included, however from Surabaya only 69 patients were included, since screening for nose and throat carriage was not performed among the remaining 102 patients. When SSTI due to *S. aureus* was compared univariately to SSTI due to other species, it appeared that *S. aureus* infected patients were younger than patients suffering from SSTIs by other pathogens (P=0.015). *S. aureus* infected patients were more likely to carry *S. aureus* in their nasopharynx (P=0.004).

By multivariate analysis, nasopharyngeal carriage of *S. aureus* was independently associated with *S. aureus* infection (odds ratio (OR) 1.8; 95%CI 1.172-2.649), whereas age was not. Gender, the number of household members, the ethnicity and the presence of atopy were not associated with *S. aureus* SSTI as compared to SSTI by other pathogens (Table 4).

Table 4. Risk factors of *Staphylococcus aureus* (*S. aureus*) skin and soft tissue infection

Factors		Univariate analysis			Mutivariate analysis		
		No. of subjects (%)		*p*			
		S. aureus infection (n= 201)	Not *S. aureus* infection (n=264)		OR	95%CI	*p*
Age				0.015			NS
	≤ 18 yrs	148 (73.6)	158 (59.8)				
	19 – 59 yrs	43 (21.4)	86 (32.6)				
	≥ 60 yrs	9 (4.5)	15 (5.7)				
	Unknown	1 (0.5)	5 (1.9)				
Gender				0.354			
	Male	123 (61.2)	147 (55.7)				
	Female	78 (38.8)	116 (43.9)				
	Unknown	0	1 (0.4)				
Ethnic group				0.615			
	Javanese	135 (67.2)	167 (63.3)				
	Balinese	54 (26.9)	85 (32.2)				
	Maduranese	7 (3.5)	7 (2.7)				
	Other*	5 (2.4)	5 (1.8)				
Atopic				0.929			
	Yes	64 (31.8)	82 (31.1)				
	No	136 (67.7)	180 (68.2)				
	Unknow	1 (0.5)	2 (0.7)				

Table 4. Risk factors of *Staphylococcus aureus* (*S. aureus*) skin and soft tissue infection (cont'd)

Factors	Univariate analysis			Mutivariate analysis		
	No. of subjects (%)		*p*			
	S. aureus infection (n= 201)	Not *S. aureus* infection (n=264)		OR	95%CI	*p*
Nasopharyngeal carriage	73 (36.3)	63 (23.9)	0.004	1.762	1.172-2.649	0.006
Family with similar disease	48 (23.9)	49 (18.6)	0.361			
Number of family member			0.890			
1-4	104 (51.7)	145 (54.9)				
5-8	85 (42.3)	106 (40.2)				
≥9	7 (3.5)	7 (2.7)				
Unknown	5 (2.5)	6 (2.3)				

*Bataknese, Chinese, Lomboknese, foreigner.

Furthermore, the associations of PVL-positive or exfoliative toxin-positive strains with premorbid history and clinical features of the SSTI were investigated (Table 5). The results showed that PVL-positive MSSA was associated with primary skin infection (OR 5.4; 95%CI 2.1-13.6), $p<0.001$) and a history of antibiotic therapy in the previous month (OR 3.5; 95%CI 1.7-7.1, $p=0.001$), whereas exfoliative toxin-positive MSSA was only associated with primary skin infection (OR 2.2; 95%CI 1.0-4.9, $p=0.047$).

According to the questionnaires obtained from the eight patients with MRSA SSTI, six patients were between 2-13 years old and the remaining two were between 20-24 years old. None of these eight patients had been hospitalized within the previous year. However, two of these patients had had a previous episode of SSTI and had received antibiotic therapy in the month preceding enrollment in this study.

Table 5. Association of genes encoding PVL and exfoliative toxins to the clinical feature and background of the staphylococcal skin and soft tissue infections.

Factors	Univariate analysis					
	No. of subjects (%)			No of subjects (%)		
	PVL (+) MSSA (n=56)	PVL (-) MSSA (n=193)	*p*	ETs (+) MSSA (n=45)	ETs (-) MSSA (n=204)	*p*
Duration of skin lesion			0.327			0.659
1 – 3 days	15 (26.8)	35 (18.1)		6 (13.3)	44 (21.6)	
4 – 7 days	23 (41.1)	85 (44.0)		24 (53.3)	84 (41.2)	
8 – 14 days	13 (23.2)	36 (18.7)		8 (17.8)	41 (20.1)	
15 – 30 days	3 (5.4)	23 (11.9)		4 (8.9)	22 (10.8)	
> 30 days	1 (1.8)	12 (6.2)		2 (4.4)	11 (5.4)	
Unknown	1 (1.8)	2 (1.0)		1 (2.2)	2 (1.0)	
Type of skin infection			<0.001			0.087
Primary infection[1]	49 (87.5)	110 (57.0)		34 (75.6)	125 (61.3)	
Secondary infection[2]	7 (12.5)	83 (43.0)		11 (24.4)	79 (38.7)	
Same complaint before	21 (37.5)	58 (30.1)	0.396	12 (26.7)	67 (32.8)	0.072
Antibiotic therapy previous month	22 (39.3)	27 (14.0)	<0.001	6 (13.3)	43 (21.1)	0.026
Diabetes mellitus	1 (1.8)	6 (3.1)	0.555	2 (4.4)	5 (2.5)	0.066
Atopic history	25 (44.6)	66 (34.2)	0.257	14 (31.1)	77 (37.7)	0.072
Family with similar disease (yes/no question)	17 (30.4)	37 (19.2)	0.142	7 (15.6)	47 (23.0)	0.055

Table 5. Association of genes encoding PVL and exfoliative toxins to the clinical feature and background of the staphylococcal skin and soft tissue infections (cont'd).

Factors	Multivariate analysis		
	OR	95% CI	*P*
PVL-positive MSSA			
Type of skin infection			
Primary infection[1]	5.417	2.137-13.734	<0.001
Secondary infection[2]	1		
Antibiotic therapy previous month	3.463	1.693-7.086	0.001
Family with similar disease			NS
ETs positive MSSA			
Type of skin infection			
Primary infection[1]	2.233	1.010-4.936	0.047
Secondary infection[2]	1		
Same complaint before			NS
Antibiotic therapy previous month			NS
Diabetes mellitus			NS
Atopic history			NS
Family with similar disease			NS

MSSA=methicillin susceptible *S. aureus;* PVL=Panton-Valentine Leukocidin; ETs=exfoliative toxins (either in conjunction or independent exfoliative toxin A gene and exfoliative toxin B gene); [1]impetigo, ecthyma, erysipelas, furuncles, carbuncles, cellulitis, subcutaneous abscesses and paronychia; [2]secondary infections complicating pre-existing skin diseases or traumatic lesions.

DISCUSSION

This is the first multicenter survey of *S. aureus* SSTI in the community setting in Indonesia. Similar to our previous studies (9,10), we found significant geographic variation in the prevalence of MRSA in Indonesia. Overall, the prevalence of MRSA detected in this study was 3.1%, which is within the reported range of 2.5% - 39% of MRSA among CA-*S. aureus* in Asian countries (2). Of importance, two previous studies from Indonesia, including one from Surabaya, did not find MRSA in the community setting (11,12). In the present study, we uncovered a clone of ST239-MRSA-SCC*mec* type III consisting of six MRSA isolates in Surabaya several years later, indicating infiltration of an HA-MRSA clone into this urban community. Furthermore, one isolate from Denpasar was ST1-MRSA-SCC*mec* type IV which represents a typical CA-MRSA, although, in this case, PVL genes were absent in the isolate. The antibiotic susceptibility patterns among MRSA isolates were in accordance with the HA-MRSA and CA-MRSA susceptibility characteristics reported before (8,21). Similar to previous findings in Indonesia, all MRSA isolates were PVL-negative, whereas the prevalence of PVL among MSSA strains causing SSTIs was high, since more than 20% of the isolates carried these cytotoxin-coding genes (9,10,12). Bacterial typing showed that two PVL-positive MSSA isolates from different patients in Malang (CA1128 and CA3130) were assigned to the same clone (ST1301/MT22) which is suggestive of cross-transmission in the community setting. However, we did not investigate a possible epidemiological link between them. Another two PVL-positive MSSA isolates (W501, Denpasar and 10022, Surabaya) were also assigned to the same clone (ST1301/MT11), a finding that is compatible with spreading of this clone between two islands, Bali and Java. Furthermore, this ST1301 clone that was frequently found among PVL-positive MSSA isolates in this study, was also detected among clinical *S. aureus* in several hospitals in China (22,23). Clonal dissemination of this strain

might, thus, have been influenced by travel of persons between Indonesia and China.

Concordant with a previous study (19), multiple MLVA types within one ST were present; ST1301 included MT10, MT11, MT22, and MT23. We also found single locus variants: MT10 and MT11 (SIRU21), MT11 and MT22 (SIRU01), MT22 and MT23 (SIRU05), and two locus variants: MT10 and MT22 (SIRU01 and SIRU21) and MT10 and MT23 (SIRU01 and SIRU05), that indicates that these strains are possibly clonally related (24).

In the community setting, transmission between animals and humans of certain *S. aureus* clones may occur and may be difficult to prevent. In Denpasar we isolated a *S. aureus* strain belonging to ST97, a clone that has been described in pigs and bovine animals in Europe and Japan (25-27). However, the MLST profile of *S. aureus* carried by livestock in Indonesia has yet to be determined. In order to ascertain the level of the transmission of *S. aureus* from animal to human, further investigations on genetic background of livestock-associated *S. aureus* in Indonesia are needed.

This study yielded a MRSA clone represented by ST239-SCC*mec* type III that belonged to MT19, suggesting penetration of a typical HA-MRSA clone into the community setting. However, all patients infected with this typical hospital clone reported not to have had hospital admission in the preceding 12 months. Therefore, this HA-MRSA might have gained a community foothold some time before this survey, and can now be acquired through social contact in the community setting such as daycare center or at home (28). However, PVL and SCC*mec* PCR differentiating between CA- and HA-MRSA are not routinely performed.

According to the multivariate analysis, nasopharyngeal *S. aureus* carriage was associated with *S. aureus* SSTI, a finding similar to that in multiple previous studies on *S. aureus* carriage (29,30). Decolonization may therefore be beneficial in such cases. Nevertheless, decolonization of *S. aureus* nasal carriage to prevent recurrent SSTI using mupirocin nasal

ointment remains controversial because of the cross sectional design of the studies performed so far, and because of the high prevalence of *S. aureus* carriage among patients without skin infection. Therefore, a causal relationship between carriage status and SSTI remains to be proven by prospective randomized trials of preventive eradication of *S. aureus* carriage (31).

Notably, an association between primary SSTI and either PVL- or exfoliative toxin-positive MSSA was found in this study. The association with PVL-positive MSSA was stronger (OR 5.4) than the association with exfoliative toxin-positive MSSA (OR 2.2), suggesting a more significant role for PVL in the pathogenesis of primary SSTI (Table 5). Other virulence factors of *S. aureus* might be associated with secondary SSTI but further investigation is needed. Antibiotic therapy in the previous month was also associated with PVL-positive MSSA, however, we did not find any study reporting the effect of prior antibiotic therapy on the occurrence of PVL-encoding genes in *S. aureus*. Nevertheless, the effect of antibiotics on PVL released by PVL-producing *S. aureus* has been reported (32,33).

The present study has certain limitations. First, data from three cities in Java and Bali cannot considered to be representative for the whole country but it may serve as point of reference. Therefore, a national surveillance system should be developed to obtain more representative and longitudinal data. Second, we did not ascertain the association of the many virulence factors of *S. aureus* other than PVL and exfoliative toxins to SSTI, for which further investigation is recommended. Third, due to limited resources MLVA and MLST were not conducted for all *S. aureus* isolates, hence the clonal relatedness of all strains causing SSTI could not be provided.

In summary, SSTI caused by either CA-MRSA or HA-MRSA were discovered in the community setting in Indonesia. The penetration of a typical HA-MRSA clone in the community setting probably mediated by undetected carriage deserves increased awareness of public health

authorities. Promoting household hygiene in general and proper hand hygiene in particular may be a simple and cost-effective method for containing the spread of MRSA in the community setting. Simultaneously reducing the use of antibiotics in ambulatory care would synergize with such efforts.

Acknowledgements:

We thank the deans of the Faculty of Medicine, Brawijaya University, Malang, Indonesia, the Faculty of Medicine, Airlangga University, Surabaya, Indonesia, and the Faculty of Medicine, Udayana University, Denpasar, Indonesia, the directors of the Dr. Soetomo Hospital, Surabaya, Indonesia, the Sanglah Hospital, Denpasar, Indonesia, and the heads of primary healthcare centers in Lawang, Singosari, Pakisaji, Sumberpucung, and Gondanglegi, Malang, Indonesia who facilitated our work. We also thank Suyati Pujiani and Slamet Riyanto for their technical assistance.

This study was financially supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands.

REFERENCES

1. Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Laboratory Investigation 2007;87:3-9.
2. Chuang Y-Y, Huang Y-C. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. Lancet 2013;13:698-708.
3. Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z *et al*. Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. International Journal of Medical Microbiology 2011;301:347-53.
4. Kawaguchiya M, Urushibara N, Kuwahara O *et al*. Molecular Characteristics of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Hokkaido, Northern Main Island of Japan: Identification of Sequence Types 6 and 59 Panton-Valentine Leukocidin-Positive Community-Acquired Methicillin-Resistant *Staphylococcus aureus*. Microbial Drug Resistance 2011;17:241-50.
5. Coombs GW, Monecke S, Pearson JC, et al. Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region. BMC Microbiology 2011;11:215.
6. Labreche MJ, Lee GC, Attridge RT, et al. Treatment Failure and Cost in Patients with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Skin and Soft Tissue Infections: A South Texas Ambulatory Research Network (STARNet) Study. JABFM 2013;26:508-517.
7. Kock R, Becker K, Cookson B, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010: 15:1-9.
8. Huang H, Flynn NM, King JH, et al. Comparison of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Hospital-Associated MRSA Infections in Sacramento, California. J Clin Microbiol 2006;44:2423-27.

9. 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.
10. Santosaningsih D, Santoso S, Budayanti NS, et al. Characterization of clinical *Staphylococcus aureus* harbouring *mecA* or Panton-Valentine leukocidin genes from four tertiary care hospitals in Indonesia. Trop Med Int Health 2016;21:610-18
11. Deurenberg RH, Beisser PS, Visschers MJ, et al. Molecular typing of methicillin-susceptible *Staphylococcus aureus* isolates collected in the Yogyakarta area in Indonesia, 2006. Clin Microbiol Infect 2010;16:92-94.
12. 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 Microbiol 2008;46:1989-95.
13. Melles DC, Gorkink RFJ, Boelens HAM, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus.* The Journal of Clinical Investigation 2004;114:1732-40.
14. Murakami K, Minamide W, Wada K, et al. Identification of Methicillin-Resistant Strains of Staphylococci by Polymerase Chain Reaction. J Clin Microbiol 1991:29:2240-44.
15. Lina G, Plemont Y, Godall-Gamot F, et al. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. CID 1999;29:1128-32.
16. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. J Clin Microbiol 2000;38:1032-35.
17. Milheirico C, Oliveira DC, de Lencastre H. Update to the Multiplex PCR Strategy for Assignment of *mec* Element Types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2007;51:3374-77.

18. Hardy KJ, Oppenheim BA, Gossain S, et al. Use of variations in staphylococcal interspersed repeat units for molecular typing of methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol 2006;44:271-73.
19. Ikawati R, Willems RJL, Box ATA, et al. A novel multiple locus variable number tandem repeat (VNTR) analysis for rapid molecular typing of human *Staphylococcus aureus*. J Clin Microbiol 2008;46:3147-51.
20. Enright MC, Day NPJ, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008–15.
21. Vysakh PR, Jeya M. A Comparative Analysis of Community Acquired and Hospital Acquired Methicillin Resistant *Staphylococcus aureus*. J Clin Microbiol 2013;7: 1339-42.
22. Liu C, Chen Z, Sun Z, et al. Molecular characteristics and virulence factors in methicillin susceptible, resistant, and heterogenous vancomycin-intermediate *Staphylococcus aureus* from central-southern China. Journal of Microbiology, Immunology, and Infection 2015;48:490-96.
23. Zhao H, Hu F, Jin S, et al. Typing of Panton-Valentine Leukocidin-Encoding Phages and lukSF-PV Gene Sequence Variation in *Staphylococcus aureus* from China. Front. Microbiol 2016;7:1200.
24. Bloemendaal ALA, Fluit AC, Jansen WTM, et al. Colonization with Multiple *Staphylococcus aureus* Strains among Patients in European Intensive Care Units. Infect Control Hosp Epidemiol 2009;30:918-20.
25. Hata E, Katsuda K, Kobayashi H, et al. Genetic variation among Staphylococcus aureus Strains from Bovine Milk and Their Relevance to Methicillin-Resistant Isolates from Humans. J Clin Microbiol 2010;48:2130-39.
26. Espinosa-Gongora C, Moodley A, Lipinska U, et al. Phenotypes and Genotypes of Old and Contemporary Porcine Strains Indicate a

Temporal Change in the S. aureus Population Structure in Pigs. PLoS ONE 2014;9:e101988.

27. Ikawaty R, Brouwer EC, Jansen MD, et al. Characterization of Dutch *Staphylococcus aureus* from bovine mastitis using a Multiple Locus Variable Number Tandem Repeat Analysis. Vet. Microbiol 2009;136:277-84.
28. Davoodabadi F, Mobasherizadeh S, Mostafavizadeh K, et al. Nasal colonization in children with community acquired methicillin-resistant *Staphylococcus aureus*. Adv Biomed Res 2016;5:86.
29. Nouwen JL, Ott A, Kluytmans-Vandenbergh MFQ, et al. Predicting the *Staphylococcus aureus* Nasal Carriage State : Derivation and Validation of a "Culture Rule". CID 2004;39:806-11.
30. Pires FV, da Cunha MdLRdS, Abraão LM, et al. Nasal Carriage of *Staphylococcus aureus* in Botucatu, Brazil: A PopulationBased Survey. PLoS ONE 2014;9:e92537.
31. Davido B, Dinh A, Salomon J, et al. Recurrent furunculosis: Efficacy of the CMC regimen--skin disinfection (chlorhexidine), local nasal antibiotic (mupirocin), and systemic antibiotic (clindamycin). Scand J Infect Dis 2013;45:837-41.
32. Dumitrescu O, Boisset S, Badiou C, et al. Effect of antibiotics on *Staphylococcus aureus* Producing Panton-Valentine Leukocidin. Antimicrob Agents Chemother 2007;51:1515-19.
33. Dumitrescu O, Badiou C, Bes M, et al. Effect of antibiotics, alone and in combination, on Panton-Valentine leukocidin production by a *Staphylococcus aureus* reference strain. Clin Microbiol Infect 2008;14:384-88

Chapter 5

Reducing transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in a surgical ward of a resource-limited hospital in Indonesia: an intervention study

Dewi Santosaningsih[1,5], Dewi Erikawati[1], Iffa A. Hakim[1], Sanarto Santoso[1], M. Hidayat[2], Ayu H. Suwendha[2], Vicky Puspitasari[3], Irhamni Irhamni[3], Kuntaman Kuntaman[4], Andreas L. E. van Arkel[5], Luke G. Terlouw[5], Neline Oudenes[5,6], Diana Willemse-Erix[5,7], Susan V. Snijders[5], Nicole S. Erler[8], Henri A. Verbrugh[5], Juliëtte A. Severin[5]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [2]Department of Orthopedic Surgery, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [3]Department of Pharmacy, Dr. Saiful Anwar Hospital, Malang, Indonesia; [4]Department of Microbiology, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital, Surabaya, Indonesia; [5]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands; [6]Department of Virology, University Medical Center, Utrecht, the Netherlands; [7]Molecular Diagnostics, Jeroen Bosch Hospital, Tilburg, the Netherlands; [8]Department of Biostatistics, Erasmus University Medical Center, Rotterdam, the Netherlands

ABSTRACT

Objective. To evaluate the effect of the introduction of a bundle of preventive measures on the transmission and acquisition of MRSA.

Design. Before-after trial: pre-intervention (seven months), intervention (two months), and post-intervention phase (five months).

Setting. A surgery ward in a tertiary care hospital in Indonesia.

Participants. In total, 1,937 patients were eligible for MRSA carriage screening consecutively on admission and at discharge during the study; patients discharged within 48 hours, missing for discharge screening, and discharge upon personal request were excluded. Patients withdrawn due to adverse effects were not detected, finally 1,120 patients completed the study. Additionally, 214 healthcare workers were screened for MRSA carriage.

Interventions. (i) Hand-hygiene educational program; (ii) Cohorting all MRSA-positive patients behind the screen; (iii) Decolonization therapy of all MRSA-positive patients and healthcare workers consisting of mupirocin dermatological cream 2% to both nares twice daily for five days plus chlorhexidine medicated soap 4% for seven days, and suggesting trimethoprim/sulfametoxazole oral therapy 960 mg twice daily for seven days; (iv) Cleaning and disinfection the environment ward twice per phase using sodium hypochlorite 0.05% (surfaces) and alcohol 70% (instruments).

Results. Hand-hygiene compliance rate improved from 15% in pre- to 64% in post-intervention phase (p<0.001). The MRSA acquisition decreased from 9/1,000 patient-days at risk in the pre- to 3/1,000 patient-days at risk in the post-intervention phase (p=0.08).

Conclusion. The introduction of a bundle of preventive measures may reduce MRSA transmission and acquisition among surgery patients in a resource-limited hospital in Indonesia.

Registered to ISRCTN registry with ID ISRCTN22906231 (http://www.isrctn.com/ISRCTN22906231)

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a prevalent antimicrobial-resistant microorganism causing hospital-acquired infections. Although the prevalence varies considerably between countries or regions, MRSA has been detected in most countries worldwide (1-8). Infections caused by MRSA are associated with excess morbidity and mortality (9). Guidelines with measures to prevent the spread of MRSA within healthcare facilities has not been developed in developing countries so far, because of limited resources and other healthcare issues such as HIV-AIDS, malaria, and tuberculosis that are more prioritized on the public healthcare agendas (8,10-13). In a previous study, we have shown an MRSA carriage rate of 4.3% among surgery patients at discharge from Indonesian hospitals (14). In Dr. Saiful Anwar hospital in Malang, Indonesia, the carriage rate was the highest, 8.0%. In the present study, we aimed to design feasible actions to prevent further transmission of MRSA in the surgery ward of this resource-limited hospital, and to measure the effect of introducing these preventive measures on the transmission and acquisition of MRSA. We performed bacterial typing in order to analyze the clonal relatedness of the MRSA isolates circulating in the ward.

METHODS

Setting

The study was carried out in two rooms of the surgical ward in the Dr. Saiful Anwar Hospital in Malang, Indonesia, which is an 810-bed tertiary care academic hospital. The baseline situation was as follows: (a) Room A: male general surgery room, shared by 50 adult patients, with a nurse-to-patient ratio of 1:5-10; two sinks were available (b) Room B: female general surgery room, shared by 22 adult patients, with a nurse-to-patient ratio of 1:3-6; one sink were available. In each room, two bottles

of 500 mL alcohol based liquid in wall dispensers were available located in the middle of the room, no handrub container at the bedsides, and no separate isolation facilities for patients with contact precautions.

Study design and participants

A before-and-after intervention study was conducted during a period of 14 months as described in Table 1. Culture-based screening of MRSA among patients, healthcare workers (HCW), and the environment was performed during each phase. Patients were screened on admission and at discharge; however, routine screening on day 5 of hospitalization was also carried out to anticipate the missing of discharge screening. All patients admitted to the study rooms were eligible for inclusion; patients leaving the room within 48 hours of admission were excluded from the analysis. Only patients with complete culture sets, i.e. nose and throat swab taken on admission and either at day-5 hospitalization or at discharge (or both), were included into the statistical analysis. When a patient was screened on day 5 of hospitalization and/or at discharge in a different phase of this study than in which the admission screening was performed, the patient was analyzed within the phase at the moment of admission. Screening of HCW (nurse, nurse assistant, cleaning staff, pharmacist, and dietician) and environment was conducted in the first week and the last week of the pre- and post-intervention phase, but in the middle of the intervention phase. Doctors on duty in both rooms were included in the screening, but not in the intervention phase.

The hand-hygiene compliance among HCW was observed directly by three trained observers several times a week during various time slots except on the weekend during the study using the hand-hygiene compliance observation sheet based on the WHO tools[15]. The study was approved by the medical ethics committee of Dr. Saiful Anwar hospital, Malang, Indonesia (No.129/EC/KEPK-JK/05/2012).

Interventions

During the intervention phase, a bundle of preventive measures was introduced in the ward as described in Table 1 (8,16-18).

Microbiological procedures

For screening of MRSA carriage among patients and HCW, the anterior nares, throat, and skin lesions, if present, were sampled. The hospital environment was screened by taking samples from bedrails, bedside cabinets, thermometers, stethoscopes, blood pressure cuffs, nurses' tables, door handles, telephone handles, sink handles, intravenous line stands, and trolley handles. All cultures were performed using cotton-tipped swabs and transported in Amies agar medium without charcoal (Copan Italia, Brescia, Italia). Swabs were directly inoculated into 5 ml phenol red mannitol broth (BBL™, Le Pont de Claix, France) for overnight incubation at 37°C and then sub-cultured onto *Staphylococcus aureus* and MRSA Chromagar medium (ITK Diagnostics, Uithoorn, the Netherlands) for 24-48 hours incubation at 37°C. Typical colonies of *S. aureus* and MRSA confirmed with Staphaurex®Plus (Remel, PT. Dipa Puspa Labsains, Indonesia) were stored into trypticase soy agar. After a subsequent identification test performed by mass spectrometry (MALDI-TOF, Bruker, the Netherlands), the colonies were stored into trypticase soy broth containing 15% glycerol at -80°C until further analysis. Clinically indicated cultures were performed on the patients involved in this study. However, the *S. aureus* isolates obtained from clinical cultures were not included in the further microbiological analysis.

DNA isolation and detection of *mecA* and PVL genes

Bacterial DNA was extracted using a MagNa Pure LC DNA system (DNA isolation kit III; Roche Molecular Biochemicals, Mannheim, Germany) (19). *mecA* and PVL (*lukF-PV* and *lukS-PV*) genes were detected by PCR (20,21).

Table 1. Description of the bundle of intervention measures

Preventive measures against MRSA transmission	Period		
	Pre-intervention phase (July 2012-January 2013)	Intervention phase (February 2013-March 2013)	Post-intervention phase (April 2013-August 2013)
Hand hygiene promotion	Posters of hand-hygiene procedure according to the WHO guideline created by infection control team were placed on the wall near the sinks. No systematic and sustainable educational program.	The existing posters were maintained. In addition, we placed 2 bigger posters on the wall of each study ward. Reminders of "five hand-hygiene opportunities" were placed on the cover of each medical record. Each health-care workers working in the study ward must read the information sheet regarding hand-hygiene procedure. Weekly presentation was delivered in the study ward attended by nurses, nurse assistants, pharmacists, and dieticians.	
Hand-rub solution access	Two bottles of 500 ml alcohol based liquid were placed through wall-fixed dispensers and located in the middle of the study ward.	A bottle of 500 ml chlorhexidine-containing hand glycerin alcohol 0.5% was placed at each bedside.	
Hand hygiene compliance observation	The compliance was observed and measured 7 times.	The compliance was observed and measured 7 times.	The compliance was observed and measured 15 times

Table 1. Description of the bundle of intervention measures (cont'd)

Preventive measures against MRSA transmission	Period		
	Pre-intervention phase (July 2012-January 2013)	Intervention phase (February 2013-March 2013)	Post-intervention phase (April 2013-August 2013)
Screening of MRSA	Screening of patients[1], HCW[2], and hospital environment[2]	Screening of patients[1], HCW[3], and hospital environment[3]	Screening of patients[1], HCW[2], and hospital environment[2]
Cohorting	Not yet implemented.	Patients with MRSA positive detected at admission were grouped separately from MRSA negative patients behind a screen in a designated area.	
Decolonization therapy	None.	Patients with MRSA positive detected at admission and MRSA-positive HCW received decolonization therapy consisting of mupirocin dermatological cream 2% (Bactoderm cream, PT. Ikapharmindo Putramas, Indonesia) to both nares twice daily for five days plus washing their bodies with chlorhexidine medicated soap 4% (Hibiscrub, Astra Zeneca) for seven days. Patients and HCW who carried MRSA in their throat were additionally offered trimethoprim/sulfamethoxazole oral therapy 960 mg twice daily for seven days	
Cleaning and disinfecting of hospital environment	Cleaning and disinfecting of surfaces was conducted once per week using sodium hypochlorite 0.05%.		
Disinfecting of instruments	Not regularly.	Disinfecting of instruments was conducted once per week using alcohol 70%	

Note. WHO, World Health Organization; MRSA, methicillin-resistant *Staphylococcus aureus*; HCW, healthcare workers. [1]At admission and either at day-5 or at discharge; [2]In the first week and at the end of the phase; [3]In the middle of the phase.

Raman spectroscopy

We assessed the clonal relatedness among MRSA isolates using Raman spectroscopy (SpectraCellRA Bacterial Strain Analyzer, River D international BV, Rotterdam, the Netherlands) (22,23). Raman spectral analysis was performed using SpectraCellRA software version 1.9.0.13444:24 (RiverD international) as previously described (14). The squared Pearson correlation coefficient (R2) determined the similarity of the sample spectra and the known R2 distribution of the identical and unrelated strains. Sixteen isolates were measured in duplicate as a reproducibility control. A two dimensional plot was created to compare the similarity of multiple isolates; the similarity of two isolates was presented by a color scale. The clonal relatedness was determined by setting the similarity threshold and cut-off value as previously described.

Multilocus sequence typing (MLST)

Ten MRSA isolates from the largest clusters of Raman spectra were selected randomly and analyzed by multilocus sequence typing (MLST) for international comparison purposes (24). We selected isolates from both the center and the edge of the Raman cluster. The MLST sequence type was assigned through the MLST website (www.mlst.net).

Definitions

- Prevalence of MRSA at admission was proportion of patients screened positive for MRSA within 48 hours of admission.
- Prevalence of MRSA carriage was proportion of either HCW or environment items screened positive for MRSA.
- An acquisition of MRSA was an MRSA-positive screening test either at day-5 hospitalization or at discharge that followed an initial negative test on admission (25).
- Acquisition rate of MRSA was the number of acquisition events of MRSA divided by the number of patient-days at risk times 1,000.

- Prevalence of environmental contamination by MRSA was the percentage of environmental samples showing MRSA positive culture.
- Hand-hygiene compliance rate was the percentage of correct hand-hygiene actions undertaken on moments when hand-hygiene was considered necessary according to the WHO "five moments" (15).
- Definition of handwashing and handrubbing was as described in the WHO guideline (15).

Statistical analysis

Data of MRSA prevalence among patients at admission, HCW, and environment were analyzed using statistical software packages SPSS version 16.0 (SPSS Inc., Chicago, USA). Differences in prevalence between study phases were determined using chi-square tests or fisher's exact test (when numbers were small). The effect of intervention measures was analyzed with an Exact Wilcoxon-Mann Whitney test of the acquisition rate measurements during pre- and post-intervention but not the intervention phase using R version 3.3.2 (2016-10-31) statistical software. A *p* value less than 0.05 was considered as significant for all analysis.

RESULTS

Prevalence of MRSA carriage

In total, 1,937 patients were screened during the study, however 817 patients were excluded from data analysis because the patients moved to another room or ward within 48 hours in the pre- and post-intervention phase (3/426, 0.7%; 1/305, 0.3%), discharged upon personal request in the pre-intervention phase (1/426, 0.2%), missed to be screened at admission (27/426, 6.3%; 9/86, 10.5%; 25/305, 8.2%) and at discharge (395/426, 92.7%; 77/86, 89.5%; 277/305, 90.8%) in all phases.

Therefore 1,120 patients were included in the statistical analysis (Table 2).

The MRSA prevalence among patients, HCW and the environment is presented in Table 2. The prevalence of MRSA among patients at admission was not significantly affected by the intervention. The MRSA carriage rate among HCW increased in the intervention phase (p=0.340), however we did not find MRSA-positive HCW in the post-intervention phase (compared to pre-intervention phase, p=0.420). Although the MRSA carriage rate was low among HCW, many HCW carried methicillin-susceptible *S. aureus* (MSSA); we identified 71% (48/68), 46% (26/56), and 60% (54/90) of HCW that were positive for MSSA in the pre-, intervention, and post-intervention phase, respectively. Among doctors, we found 78% (21/27) positive for MSSA in the pre- and 83% (25/30) in the post-intervention phase. Furthermore, we found the hospital environment to be contaminated with MRSA in the pre- (2 items: intravenous line stand and bedrail) and post-intervention phase (one item: patient table). We did not find MRSA contamination of the environment during the intervention phase, but the number of samples taken during this phase was lower (Table 2). The PVL-positive rate was 17% among 90 MRSA isolates obtained in this study.

Prevalence of MRSA among clinical cultures

We found 10 MRSA isolates from clinical cultures among patients enrolled in this study (pre-intervention: 7; intervention: 1; post-intervention: 2). All MRSA isolates were isolated from wound cultures except one isolate obtained from a sputum culture. Overall, the nosocomial MRSA clinical culture rate was 1.5 per 1000 patient-days at risk. The nosocomial MRSA clinical culture rate decreased from 2.2 to 0.6 per 1,000 patient-days at risk in the pre- to post-intervention phase, respectively (p=0.055).

MRSA acquisition

After implementing the bundle of preventive measures, the acquisition rate of MRSA was lower but this decrease did not reach statistical significance (9 to 3 per 1,000 patient-days at risk; p=0.08). The highest acquisition rate of MRSA was 41 per 1,000 patient–days at risk and occurred in the fourth month of pre-intervention phase (Figure 1).

Table 2. The admission prevalence, acquisition rate, and carriage rate of methicillin-resistant *Staphylococcus aureus*

Group	Phase	Number of subjects screened	Number of patients without complete screening	Number of patients analyzed	Number of cultures (environment)	Prevalence of MRSA at admission (%;CI_{95})	MRSA acquisition event	MRSA acquisition rate[2] (median; range)	Prevalence of MRSA carriage (%;CI_{95})
Health-care workers	PI	68							1/68 (1.5; 0.1-7.9)[3]
	I	56							3/60 (5.0; 1.7-13.7)
	PoI	90							0/94[3]
Patients	PI	998	426	572		18/572 (3.1; 2.0-4.9)[1]	30	5.3; 0.0-41.0	
	I	174	86	88		1/88 (1.1; 0.1-6.2)	2	2.8; 0.0-5.6	
	PoI	765	305	460		11/460 (2.4; 1.3-4.2)[1]	8	1.7; 0.0-6.7	
Environment	PI				201				2/201 (1.0; 0.3-3.6)[4]
	I				100				0/100
	PoI				200				1/200 (0.5; 0.0-2.8)[4]

Note. MRSA, methicillin-resistant *Staphylococcus aureus*; PI, pre-intervention; I, intervention; PoI, post-intervention.

[1]p=0.625; [2]Number of acquisition events divided by number of patient days at risk (per 1000 patient days); [3]p=0.420; [4]Contamination rate (p=1.000).

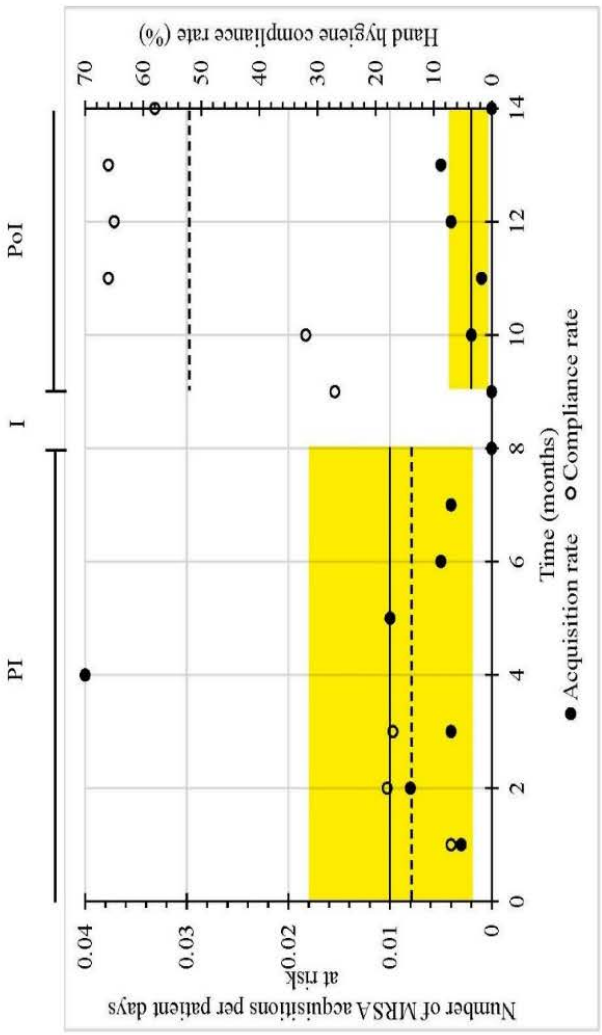

Figure 1. Methicillin-resistant *Staphylococcus aureus* acquisitions among patients versus hand hygiene compliance rate among healthcare workers. The solid horizontal line represents the average of acquisition rate, the yellow area represents the 95% confidence interval around that mean. The dashed horizontal line represents the average of hand hygiene compliance rate. PI=pre-intervention phase; I=intervention phase; PoI=post-intervention phase.

Adherence to hand-hygiene procedure

In general, the overall hand-hygiene compliance rate (by either washing with soap and water or with handrubbing) increased significantly from 15% (CI_{95}, 12-19%) in the pre- to 30% (CI_{95}, 28 - 33%) in the intervention, and to 64% (CI_{95}, 62-66%) in the post-intervention phase. In the pre- and intervention phase, the compliance rate of handrubbing was low, 11% (CI_{95}, 4-18%) and 25% (CI_{95}, 20-30%), respectively. However, in the post-intervention phase the proportion of HCW performing hand-hygiene by handrubbing was much higher compared to handwashing (p<0.001) (Figure 2). When comparing the five moments of hand-hygiene recommended by WHO guideline, the compliance rate was significantly improved (p<0.001) for all five moments of hand-hygiene, but most spectacularly for the moment before the HCW touched patients and performed a clean or aseptic procedure (Table 3). The increase of hand-hygiene compliance rate was accompanied by a decrease of MRSA acquisition rate (Figure 1).

Table 3. The improvement of hand-hygiene compliance rate after intervention

Hand hygiene moment	Total		
	Compliance rate (%)		OR (95% CI)
	PI and I	PoI	
1	9.5	85	52.8 (31.2 – 89.2)[a]
2	3.6	40	17.8 (8.6 – 36.7)[a]
3	37	70	4.1 (2.1 – 8.0)[a]
4	40	65	2.8 (2.1 – 3.8)[b]
5	35	62	3.0 (2.4 – 3.7)[a]

Note. OR, odds ratio; PI, pre-intervention phase; I, intervention phase; PoI, post-intervention phase; moment 1, before touching a patient; moment 2, before clean/aseptic procedure; moment 3, after body fluid exposure risk; moment 4, after touching a patient; moment 5, after touching patient surroundings.

[a]p<0.001 (Fisher's exact test); [b]p<0.001 (χ^2).

Raman spectroscopy

We performed Raman spectroscopy for the 90 MRSA isolates obtained in the pre- (patients: 31 isolates; healthcare workers: 1 isolate), intervention (patients: 4 isolates; healthcare workers: 1 isolate), and post-intervention phase (patients: 52 isolates; environment: 1 isolate). The Raman spectroscopic analysis showed 22 Raman types (RT) (Figure 3). However, a single clone, RT9, was the most frequently found RT including 39 MRSA isolates from patients (pre-intervention: 15 isolates and post-intervention: 24 isolates) and one MRSA isolate from the environment in the post-intervention phase. The second most common clone was RT11 including 15 MRSA isolates from patients (pre-intervention: 2 isolates, intervention: 3 isolates, post-intervention: 10 isolates) followed by RT8 containing 10 PVL-positive MRSA isolates from patients in the post-intervention phase. The two MRSA isolates from HCW were unique.

The endemicity profile of MRSA isolates from patients is shown in Figure 4. The dominant Raman type 9 strain and its closely related strain RT11 were found either at admission or during admission in both the pre- and post-intervention phase of this study, indicating that this clonal complex was endemic in the hospital. The RT9 strain was isolated from both rooms, whereas the RT11 strain was only found in the male room (data not shown). Although, the RT11 clone was not cultured from patients at the time of admission in the pre-intervention phase, it was found among admissions both in the intervention and post-intervention phase. Interestingly, the third most common type RT8 was found only in the post-intervention phase of the study, suggesting a recent introduction and spread of a new MRSA clone in this setting. Other unique RTs were detected only briefly during the study period.

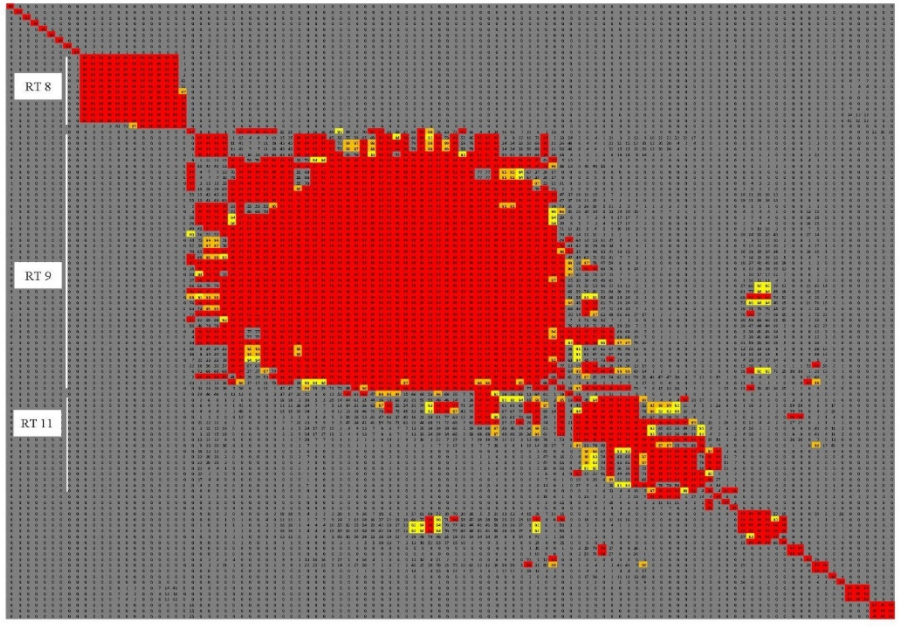

Figure 3. Raman spectra of methicillin-resistant *Staphylococcus aureus* isolates. Note: The correlation matrix displayed is used to analyze the relatedness between isolates. Red clusters show isolates that are indistinguishable based on the cut-off value. The grey areas indicate isolates that are not related based on the similarity threshold. Yellow areas to orange areas gradually show the potentially related isolates.

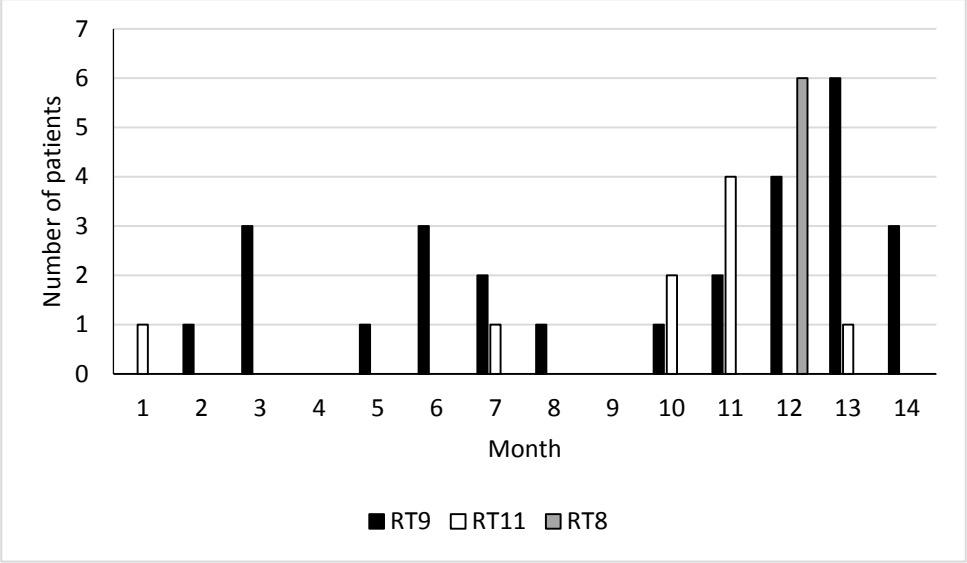

Figure 4. Endemicity profile of large clusters of methicillin-resistant *Staphylococcus aureus* (MRSA) assigned to Raman type (RT) 9, RT11, and RT8. Month 1 to 7=pre intervention phase; month 8 to 9=intervention phase; month 10 to 14=post-intervention phase.

MLST analysis

Ten MRSA isolates randomly selected for MLST were presented in Table 4. All seven MRSA isolates that belonged to RT9 and RT11 were assigned to ST239 indicating that these two clones were indeed genetically closely related. One PVL-positive MRSA isolate belonging to RT8 was assigned to ST772.

Table 4. Clonality of selected methicillin-resistant *Staphylococcus aureus* isolates

Isolates number	Raman type	Sequence type
PPoM 10458[a]	8	772
PPiM 10293[b]	9	239
PPiM 10756[b]	9	239
PPoM 20544[a]	9	239
PPoM 20673[a]	9	239
HI 1097[c]	10	8
PPoH 10221[d]	11	239
PPoM 10212[a]	11	239
PIK 10127[e]	11	239
PPiM 10495[b]	15	789

Note. [a]isolated from patients at admission in the post-intervention phase; [b]isolated from patients at admission in the pre-intervention phase; [c]isolated from health-care worker in the intervention phase; [d]isolated from patients at day-5 admission in the post-intervention phase; [e]isolated from patients at discharge in the intervention phase.

DISCUSSION

This study is the first intervention study aimed at reducing MRSA transmission in a resource-limited hospital in Indonesia. The intervention significantly improved hand-hygiene compliance among HCW and we observed a three-fold reduction in the rate of MRSA acquisitions after the intervention. Although this decline in MRSA acquisition rate did not reach statistical significance, possibly due to the limited duration of the follow-up phase, the observed decline in the MRSA acquisition rate indicates that the bundle of preventive measures might be effective in controlling MRSA transmissions in this setting. Contact precautions and dedicated medical equipment for MRSA positive patients were not implemented well in our study because isolation room, personal protective equipment, and medical equipment in our hospital were limited. These limitations may well have restricted the impact of

introducing the bundle of preventive measures aimed at reducing the MRSA acquisition rate in this study. Other studies regarding the impact of MRSA control programs have likewise showed varied levels of effectiveness for reducing the incidence rate of hospital acquired MRSA (11,25-28).

We also found that the admission prevalence of MRSA was lower, albeit not statistically significant so, in the post-intervention phase compared to the baseline phase of this study. Therefore, selective MRSA screening at admission and other preventive measures should be developed to control the spread of MRSA in the hospital setting. In concordance with a previous study (25), the MRSA prevalence at admission was prognostic of either MRSA acquisition rate or prevalence of nosocomial MRSA among clinical cultures and vice versa. We found the decrease of MRSA prevalence at admission after intervention to coincide with a decreasing MRSA acquisition rate and prevalence of nosocomial MRSA among clinical culture. After intervention, the MRSA acquisition rate was three times lower than in the baseline phase.

Two strains belonging to multilocus sequence type ST239 MRSA but distinguished by Raman spectroscopy and assigned to RT9 and RT11 were particularly endemic in the male ward throughout the study. Furthermore, the number of patients already colonized on admission with either one of these two dominant MRSA clones even increased in the-post intervention phase. It is known that ST239-MRSA is the predominant MRSA sequence type in most Asian countries, highly transmissible and difficult to control in hospital setting (1,14,29,30).

Other potential MRSA reservoirs found in this study were HCW and the hospital environment, reservoirs that we did not find in a previous study (14). However, after intervention measures, MRSA carriage of HCW was successfully eliminated whereas one item of hospital environment remained contaminated with MRSA.

Contrary to the earlier studies (14,31,32), we now observed the emergence of a PVL-positive MRSA clone in the post-intervention phase of

this study. The PVL-positive MRSA representing RT8 was introduced to the male surgery room by five patients who were cultured positive at the time of admission. Three patients were successfully eradicated by the intervention measures, however two patients carried the strains at discharge. This PVL-positive MRSA belonged to ST772-MRSA which has been reported before as a community-associated (CA) MRSA infiltrating in hospital settings in India (33,34). It is suggested that both hospital-associated (HA) MRSA and CA-MRSA pose a threat to patients in the hospital setting in Indonesia. Thus, intervention actions applied in the hospital setting are necessary to control MRSA transmission in both hospital and community setting.

Adherence of healthcare workers to hand-hygiene procedure is known as an important factor to control MRSA transmission (35,36). In this study, the hand-hygiene compliance improved significantly in the post-intervention phase. Furthermore, we reported the increased use of handrubbing with alcohol, clearly overtaking handwashing for hand-hygiene practices. Accessibility to handrub containers supported with systematic and sustainable hand-hygiene promotion and education were likely instrumental in the improvement of hand-hygiene compliance. However, high workload, understaffing, skin irritation and a sensation of stickiness of the handrub solution might hamper the adherence to the hand-hygiene procedure(37).

This study has some limitations. First, we performed the study in a single tertiary care center in Malang and only in the surgery ward. This intervention should be implemented in some other healthcare settings or wards to assess its effectiveness. However, this was not feasible because of limited resources. To overcome this limitation, mathematical and computational modelling can be applied to evaluate hospital infection control in different settings (38). Second, HCW may have improved their compliance with hand-hygiene guidelines because they were aware of being observed (Hawthorn effect) (39,40). Third, we did not compare the hand-hygiene compliance rate among doctors, nurses, and students.

Consequently, we could not identify the group who contributed most to the level of adherence with hand-hygiene procedures. However, previous publications reported lower hand-hygiene compliance rates of doctors than nurses (35,37). Fourth, the clonal background of MRSA strains obtained from clinical cultures was not determined. The concordance of MRSA clones between screening and clinical cultures isolates would have confirmed or refuted the notion that MRSA acquisition will contribute to nosocomial MRSA infections. Last, we did not observe compliance with contact precautions by HCW caring for MRSA positive patients. The limited number of nurses and medical equipment including personal protective equipment and medical instruments in our hospital may well have had impact on the quality of care for MRSA-positive patients. In summary, a bundle of intervention measures including hand-hygiene, isolation procedures, cleaning and disinfecting of hospital environment, disinfecting of instruments, and screening, and decolonization therapy may help to control the MRSA transmission in surgery wards in resource-limited hospitals in Indonesia.

Acknowledgements

We thank the dean of the Faculty of Medicine, Brawijaya University, Malang, Indonesia and the director of the Dr. Saiful Anwar hospital, Malang, Indonesia, who facilitated our work in this teaching hospital. We also thank all staff members who were involved in the implementation of the preventive measures in the study. The laboratory technical support of Suyati Pujiani, Yuanita Mulyastuti, Siwipeni I. Rahayu, and Bethania S. Tadjong is gratefully acknowledged.

This study was supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

REFERENCES

1. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, et al. Predominance and Emergence of Clones of Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* in Malaysia. J Clin Microbiol 2010;48:867-872.
2. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. Lancet Infect Dis 2013;13:698-708.
3. Naidoo R, Nuttall J, Whitelaw A, Eley B. Epidemiology of *Staphylococcus aureus* Bacteraemia at a Tertiary Children's Hospital in Cape Town, South Africa. PLoS ONE 2013;8:e78396.
4. Espadinha D, Faria NA, Miragaia M, et al. Extensive Dissemination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) between the Hospital and the Community in a Country with a High Prevalence of Nosocomial MRSA. PLoS ONE 2013;8:e59960.
5. Labreche MJ, Lee GC, Attridge RT, et al. Treatment Failure and Costs in Patients with Methicillin-Resistant Staphylococcus aureus (MRSA) Skin and Soft Tissue Infections: A South Texas Ambulatory Research Network (STARNet) Study. J Am Board Fam Med 2013;26:508-517.
6. Santos HB, Machado DP, Camey SA, Kuchenbecker RS, Barth AL, Wagner MB. Prevalence and acquisition of MRSA among patients admitted to a tertiary-care hospital in Brazil. BMC Infectious Diseases 2010;10:328.
7. Costello ME, Huygens F. Diversity of community acquired MRSA carrying the PVL gene in Queensland and New South Wales, Australia. Eur J Clin Microbiol Infect Dis 2011;30:1163-1167.
8. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public health threat. Lancet 2006; 368:874-85.

9. Kang CI and Song JH. Antimicrobial Resistance in Asia: Current Epidemiology and Clinical Implications. Infect Chemother 2013;45:22-31.
10. van Rijen and Kluytmans. Adjusment of the MRSA Search and Destroy policy for outpatients in the Netherlands: a prospective cohort study with repeated prevalence measurement. Antimicrobial Resistance and Infection Control 2014;3:3.
11. Lee AS, Cooper BS, Malhotra-Kumar S, et al. Comparison of strategies to reduce meticillin-resistant *Staphylococcus aureus* rates in surgical patients: a controlled multicenter intervention trial. BMJ Open 2013;3:e003126.
12. Raboud J, Saskin R, Simor A, et al. Modelling Transmission of Methicillin-Resistant *Staphylococcus aureus* among patients admitted to a hospital. Infect Control Hosp Epidemiol 2005;26:607-615.
13. Hotez PJ, Remme JHF, Buss P, Alleyne G, Morel C, Breman JG. Combating Tropical Infectious Diseases: Report of the Disease Control Priorities in Developing Countries Project. Clin Inf Dis 2004;38:871-8.
14. 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.
15. The First Global Patient Safety Challenge team and the Guidelines' editor. WHO guidelines on hand hygiene in health care. WHO press. Geneva. 2009.
16. Calfee DP, Salgado CD, Classen D, et al. Strategies to prevent transmission of MRSA in acute care hospitals. Infect Control and Hospital Epidemiology 2008;29:S62-S80.
17. Rutala WA. APIC guideline for selection and use of disinfectants. Am J Infect Control 1996;24:313-42.
18. Gamage B. A Guide to Selection and Use of Disinfectants. BC Centre for Disease Control. 2003.

19. Melles DC, Gorkink RF, Boelens HA, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J Clin Invest 2004;114:1732-1740.
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;29:2240-2244.
21. Lina G, Plemont Y, Godall-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. CID 1999;29:1128-1132.
22. Te Witt R, Vaessen N, Melles DC et al. Good performance of SpectraCellRA system for typing of methicillin-resistant Staphylococcus aureus (MRSA). J Clin Microbiol 2013;51:1434-1438.
23. Willemse-Erix DF, Scholtes-Timmerman MJ, Jachtenberg JW, et al. Optical fingerprinting in bacterial epidemiology: Raman spectroscopy as a real-time typing method. J Clin Microbiol 2009;47:652-659.
24. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;28:1008-1015
25. Jones M, Ying J, Huttner B, et al. Relationships Between the Importation, Transmission, and Nosocomial Infections of Methicillin-Resistant *Staphylococcus aureus*: An Observational Study of 112 Veterans Affairs Medical Centers. Clin Infect Dis 2014;58:32-9.
26. McLaws ML, Pantle AC, Fitzpatrick KR, Hughes CF. More than hand hygiene is needed to affect methicillin-resistant *Staphylococcus aureus* clinical indicator rates: clean hands save live, Part IV. MJA 2009;191:526-531.
27. Nicolle LE, Dyck B, Thompson G, et al. Regional Dissemination and Control of Epidemic Methicillin-Resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol 1999;20:202-205.

28. Geraci DM, Giuffrè M, Bonura C, et al. Methicillin-Resistant *Staphylococcus aureus* Colonization: A Three Year Prospective Study in a Neonatal Intensive Care Unit in Italy. PLoS ONE 2014;9:e87760.
29. Ko, KS, Lee JY, Suh JY, et al. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. J. Clin. Microbiol 2005;43:421–426.
30. Cooper BS, Batra R, Wyncoll D, Tosas O. Edgeworth JD. Quantifying type-specific reproduction numbers for nosocomial pathogens: evidence for heightened transmission of an Asian sequence Type 239 MRSA clone. PLOS Computational Biology 2012;8:1-13.
31. Santosaningsih D, Santoso S, Budayanti NS, et al. Characterization of clinical *Staphylococcus aureus* isolates harbouring *mecA* or Panton-Valentine leukocidin genes from four tertiary care hospitals in Indonesia. Trop Med Int Health 2016;21:610-18.
32. 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 Microbiol 2008;46:1989-95.
33. Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B. Emergence of hospital-and community-associated panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. J Clin Microbiol 2012;50:841-7.
34. Pittet D, Hugonnet S, Harbarth S, et al. Effectivenss of a hospital wide programme to improve compliance with hand hygiene. The Lancet 2000;356:1307-1312.
35. Johnston BL, Boyce E. Hospital infection control strategies for vancomycin resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile*. CMAJ 2009;180:627-631.

36. Cheng VC-C, Tai JW-M, Chau P-H, et al. Minimal Intervention for Controlling Nosocomial Transmission of Methicillin-Resistant *Staphylococcus aureus* in Resource Limited Setting with High Endemicity. PLoS ONE 2014;9:e100493.
37. Santosaningsih D, Erikawati D, Santoso S, et al. Intervening with healthcare workers' hand hygiene compliance, knowledge, and perception in a limited-resource hospital in Indonesia: a randomized controlled trial study. Antimicrob Resist Infect Control 2016;6:1-10.
38. Sadsad R, Sintchenko V, McDonnell GD, Gilbert GL. Effectiveness of Hospital-Wide Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection Control Policies Differs by Ward Specialty. PLoS ONE 2013;8:e83099.
39. Srigley JA, Furness CD, Baker GR, Gardom M. Quantification of the Hawthorn effect in hand hygiene compliance monitoring using an electronic monitoring system: a retrospective cohort study. BMJ Qual Saf 2014;23:974–80.
40. Rosenthal VD, McCormick RD, Guzman S, Villamayor C, Orellano PW. Effect of education and performance feedback on handwashing; The benefit of administrative support in Argentian hospitals. Am J Infect Control. 2003;31:85–92.

Chapter 6

Intervening with healthcare workers' hand hygiene compliance, knowledge, and perception in a limited-resource hospital in Indonesia: a randomized controlled trial study

Dewi Santosaningsih[1,2,3], Dewi Erikawati[1], Sanarto Santoso[1], Noorhamdani Noorhamdani[1,2], Irene Ratridewi[2], Didi Candradikusuma[2], Iin N. Chozin[2], Thomas E. C. J. Huwae[2], Gwen van der Donk[3], Eva van Boven[3], Anne F. Voor in 't holt[3], Henri A. Verbrugh[3], Juliëtte A. Severin[3]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [2]Infection Prevention and Control Committee, Dr. Saiful Anwar Hospital, Malang, Indonesia; [3]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands

Antimicrobial Resistance and Infection Control 2017; 6(23):1-10
doi: 10.1186/s13756-017-0179-y

ABSTRACT

Background: Hand hygiene is recognized as an important measure to prevent healthcare-associated infections. Hand hygiene adherence among healthcare workers is associated with their knowledge and perception. This study aimed to evaluate the effect of three different educational programs on improving hand hygiene compliance, knowledge, and perception among healthcare workers in a tertiary care hospital in Indonesia.

Methods: The study was performed from May to October 2014 and divided into a pre-intervention, intervention, and post-intervention phase. This cluster randomized controlled trial allocated the implementation of three interventions to the departments, including role model training-pediatrics, active presentation-surgery, a combination of role model training and active presentation-internal medicine, and a control group-obstetrics-gynecology. Both direct observation and knowledge-perception survey of hand hygiene were performed using WHO tools.

Results: Hand hygiene compliance was observed during 2,766 hand hygiene opportunities, and knowledge-perception was assessed among 196 participants in the pre-intervention and 88 in the post-intervention period. After intervention, the hand hygiene compliance rate improved significantly in pediatrics (24.1% to 43.7%; $p<0.001$), internal medicine (5.2% to 18.5%; $p<0.001$), and obstetrics-gynecology (10.1% to 20.5%; $p<0.001$). The nurses' incorrect use of hand rub while wearing gloves increased as well ($p<0.001$). The average knowledge score improved from 5.6 (SD=2.1) to 6.2 (SD=1.9) ($p<0.05$). In the perception survey, "strong smell of hand alcohol" as a reason for non-compliance increased significantly in the departments with intervention (10.1% to 22.9%; $p=0.021$).

Conclusion: The educational programs improved the hand hygiene compliance and knowledge among healthcare workers in two out of three intervention departments in a limited-resources hospital in Indonesia.

Role model training had the most impact in this setting. However, adjustments to the strategy are necessary to further improve hand hygiene.

BACKGROUND

Healthcare-associated infections (HAIs) are known to be a threat in healthcare facilities, affecting morbidity, mortality, and length of stay of patients, and increase costs worldwide (1-5). One of the most important measures to control the transmission of pathogens that may cause HAIs is hand hygiene (6-7). In 2005, the World Health Organization (WHO) launched the Clean Care is Safer Care campaign to encourage Member States to advocate hand hygiene. To support local improvement, a range of tools was published that were based on a multi-modal strategy with the following five components: system change, training and education, evaluation and feedback, reminders in the workplace, and institutional safety climate (1,8). Although the WHO guidelines and tools were designed in a way that would be of use in any setting regardless of the resources available and the cultural background, it was recognized that adaptation according to local needs, resources, and settings would be necessary (6). Especially in developing countries, hand hygiene improvement requires a different approach than in developed countries (9). In Indonesia, a low-middle income country, many efforts have been made over the past decade to improve the overall quality of healthcare including a national hospital accreditation program that incorporates infection control components. However, hospitals are still facing problems that typically occur in a developing country, such as overcrowding of wards and shortage of certain supplies (10,11). It is unknown which of the elements of the WHO multi-modal approach would have the greatest impact on the improvement of hand hygiene in such a setting (8). Additionally, there is also only limited data on hand hygiene barriers (12). This kind of information is necessary to redesign the approach into a suitable and feasible program for Indonesia and similar countries. This study aimed to assess the healthcare workers' (HCWs') hand hygiene compliance, knowledge and perception in a limited-resource hospital in

Indonesia before and after the implementation of three different educational programs.

METHODS

Setting

The study was performed in Dr. Saiful Anwar hospital, a 902-bed tertiary care hospital, in Malang, Indonesia. In this hospital, there are four classes of care, including VIP (very important person), class I, II and III related to the room and care facilities. In this study, four departments including pediatrics, surgery, internal medicine, and obstetrics-gynecology (obst-gyn) were involved with characteristics as presented in Table 1. The alcohol-based hand rub that is used in the hospital is produced by the hospital pharmacist according to the WHO formulation II (6). A hand rub container was attached to the footboard of each bed and next to each entrance door.

The Dr. Saiful Anwar hospital has an infection prevention control team that consists of eight infection prevention control nurses (IPCN), each representing a specific ward (internal medicine, surgery, obst-gyn, pediatrics, intensive care unit, VIP unit, emergency unit, and operation room unit). These nurses work part time as an infection control practitioner and part time as a nurse providing patient care in the wards. The IPCN coordinate a larger team of 48 infection prevention control-linked nurses (IPCLN) who are selected among senior nurses from the different wards. IPCN and IPCLN were recruited based on Pedoman Pencegahan dan Pengendalian Infeksi di Rumah Sakit dan Fasilitas Pelayanan Kesehatan Lainnya (Guideline of Infection Prevention and Control for Hospitals and other Healthcare Services) published by Ministry of Health of the Republic of Indonesia, 2008. Before the start of the study and partly during the study period, the hospital was preparing for a national hospital accreditation. Therefore, the hand hygiene procedure, according to the existing guideline, had been introduced to

HCW by the IPCN and IPCLN in collaboration with the hospital accreditation team. In addition, posters presenting the hand hygiene procedures had been posted in the workplaces. Nevertheless, observations of the hand hygiene compliance had not been conducted by the infection prevention control team until the present study started.

Design

The design of the study was a pilot cluster randomized controlled trial, with a total duration of 24 weeks. The study was divided into three phases: pre-intervention (May to June 2014; 8 weeks), intervention (July to August 2014; 8 weeks), and post-intervention (September to October 2014; 8 weeks). The interventions consisted of three different educational programs: (1) active presentations; (2) role model training; (3) a combination of active presentations and role model training. By drawing lots, the four departments were randomly assigned to either one of the three educational interventions or to no intervention (Table 1). Active presentations to the HCW were held on at least three different occasions per ward to ensure that all HCW could participate and focused on the threat of HAIs and hand hygiene procedures (6). In the intervention with role model training, IPCLN, as role models, received training about the hand hygiene educational program focusing on hand hygiene training techniques, including active presentations, discussions, practicing the hand hygiene procedure and the observation method. The theoretical part of HAIs and their prevention through high hand hygiene compliance was also presented to IPCLN. Therefore, they were able to motivate other HCW to better adhere to the hand hygiene procedures in the ward.

Table 1. Characteristics of participating wards.

Dept.	Type of ward involved	Facilities	Number of patients per room	Ratio nurse:patients	Type of intervention
IM	General ward: 4 rooms	Class I[a] (1 room)	1	1:5	Active presentations and
		Class II[b] (1 room)	7-8	1:8	role model training
		Class III[c] (2 rooms)	30	1:8	
SUR	Acute surgery unit: 1 room	Class III[c]	30	1:4	Active presentations
	General ward: 1 room	Class II[b]	7-8	1:6	
OBG	General ward: 2 rooms	Class III[c]	30	1:5	No intervention (control group)
PED	High care unit: 1 room	Class II[b]	14	1:2	Role model training
	Neonatology ward: 6 rooms	Class II[b]	7	1:5	

Note. IM, internal medicine; SUR, surgery; OBG, obstetrics-gynecology; PED, pediatrics.

[a]patients have to share the bathroom with another patient; [b]patients share a bathroom together; [c]only one bathroom per room.

The combination of active presentations and role model training was executed separately from the other interventions.

The main outcome and secondary outcome of the study was the hand hygiene compliance among HCW, including doctors, nurses, and students (either nursing students or medical students), and knowledge-perception regarding HAIs and hand hygiene among HCW obtained by a survey in the pre-intervention phase compared to the post-intervention phase, respectively. The direct observation method was applied to establish hand hygiene compliance rates, because this is considered the gold standard (13). The observations were carried out several times a week during differing time slots, but not during the weekend. Every moment of observation lasted about 30 to 60 minutes. The outcomes of these observations were presented as percentages of compliance representing the fraction of the number of times when hand hygiene should have taken place correctly, and the number of times it had actually taken place correctly. The hand hygiene compliance observation sheet as well as knowledge and perception questionnaires were based on the WHO tools (6). The knowledge survey consisted of three single item and three multiple item (i.e. more than one answer) questions on the following topics: transmission of microorganisms, source of HAIs, and hand hygiene indications. A correct answer was awarded with one point, with a maximum score of 12 points for 12 correct answers. A wrong answer led to one score deduction for multiple item questions, and a score of zero for a wrong answer to a single item question. The perception survey consisted of 12 yes/no questions, 13 of a 4-Likert-items scale questions (the last two points of the scale were considered as positive perception), and 3 open-ended questions, and included: intention to adhere to hand hygiene, risk of cross-transmission and HAIs related to non-compliance, social norms concerning hand hygiene, and hand hygiene methods, indications, importance, promotion, and compliance barriers. In addition, the three open-ended questions concerned the perception of HCW on the risk of patients acquiring HAIs, the hand

hygiene compliance rate that should be achieved, and self-reporting of the hand hygiene compliance level. When the HCW were providing patient care in the wards and filling out the surveys, two observers concurrently recorded their clothing regarding jewellery on the arms or fingers, long sleeves, and nail polish.

The study was approved by the medical ethics committee (No 129/EC/KEPK-JK/05/2012). Informed consent was not obtained, since it involved little risk of harm for the participants and the study was regarded as a hospital infection control program. Anonymity of HCW was guaranteed in the knowledge and perception survey.

Statistical analysis

The overall compliance to hand hygiene with confidence intervals (95%CI) was calculated using the standard normal distribution. To assess differences in compliance and each of the WHO five moments of hand hygiene at the different departments between pre- and post-intervention, the Pearson Chi-Square statistic or the Fisher's exact test was used when applicable. If the compliance in pre- and post-intervention at the departments with intervention (i.e. pediatrics, surgery and internal medicine) was significantly different from the department without intervention (i.e. obst-gyn), the Pearson Chi-Square statistic was used followed by the Mantel-Haenszel statistic. In addition, knowledge and perception improvement were analyzed using the independent T-test and the Chi-Square test, respectively. Backward multiple logistic regression analysis was performed to determine factors associated with hand hygiene compliance and included department, class, room type, nurse-to-patient ratio, moment of hand hygiene, and HCWs' professions before and after intervention. A p value of <0.05 was considered statistically significant and all analyses were performed using IBM SPSS version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Compliance to hand hygiene

During the study period, 2,766 potential hand hygiene opportunities were observed at the 4 participating departments. The overall compliance to hand hygiene was 19.5% (95%CI: 18.0 to 20.9). After intervention, the hand hygiene compliance rate increased both in the departments with intervention (i.e. pediatrics, internal medicine, and surgery) and in the department without intervention (i.e. obst-gyn) from 16.1% to 27.1% and from 10.1% to 20.5%, respectively. Departments pediatrics, internal medicine and obst-gyn improved significantly when comparing pre-intervention to post-intervention (p<0.001). However, the intervention did not significantly improve hand hygiene compliance in the surgery department (p=0.05). When considering the different types of HCW, hand hygiene compliance of doctors and nurses improved significantly post-intervention (p<0.001), but not among students (p=0.840). For the 538 opportunities with good hand hygiene compliance, it was observed that HCW used hand rub at 379 (70.6%) opportunities, whereas at 159 (29.6%) opportunities they washed their hands. We did not find a significant increase in the use of hand rub in the departments with intervention (Table 2). For the 2,228 opportunities with non-compliance, we found that HCW used hand rub while wearing gloves (GA) at 74 (3.3%) opportunities, wore gloves when it was not necessary at 157 (7.0%) opportunities, and did not perform hand hygiene at all at 1,997 (89.6%) opportunities. When comparing pre-intervention and post-intervention phases, the nurses who did not perform hand hygiene at all and wore gloves when it was not necessary decreased significantly (p=0.024 and p=0.046, respectively), however their use of hand rub while wearing gloves increased (p<0.001). Similarly, the students who wore gloves when it was not necessary decreased but the use of hand rub while wearing gloves increased significantly (p<0.001) (Table 3). With regard to clothing among nurses in the pre-intervention phase, 17% of the nurses wore

jewelry, 31% of the nurses had long sleeves and 33% wore both jewelry and had long sleeves. Thus, a total of 81% did not wear appropriate clothing.

Based on the five moments of hand hygiene recommended by the WHO, the highest compliance was to moment 4 (27.4%) (i.e. after touching a patient). The lowest compliance was to moment 5 (12.2%) (i.e. after touching patient surroundings). Table 4 shows compliance considering the five moments pre-intervention and post-intervention at the four participating departments.

Hand hygiene compliance pre-intervention compared to post-intervention

Independent of phase (i.e. pre-intervention and post-intervention), we observed a statistically significant difference between compliance at departments obst-gyn and surgery (p<0.001), and between obst-gyn and pediatrics (p<0.001). When adding phase as a confounding factor, the relationships remained significant (p=0.001 and p<0.001, respectively). Independent of phase (i.e. pre-intervention and post-intervention), we did not observe a statistically significant difference between compliance at departments obst-gyn and internal medicine (p=0.207). When adding phase as confounding factor, the relationship remained non-significant (p=0.069).

Factors associated with hand hygiene compliance

Multivariate analysis showed that when comparing the pre- and post-intervention phase, the pediatric and surgery department was significantly associated with hand hygiene compliance improvement among HCW (odds ratio (OR) 4.078 and 1.963; 95%CI 1.513-10.994 and 1.178-3.270, respectively). Other factors associated with the hand hygiene compliance at the different departments were general adult room (OR 1.710; 95%CI 1.002-2.918), class III room facilities (OR 1.993; 95%CI 1.168-3.400), WHO moment of before touching a patient and after

touching a patient (OR 1.442; 95%CI 1.057-1.968 and OR 2.333; 95%CI 1.850-2.943, respectively). Professional categories being either a doctor or a nurse was also associated with hand hygiene compliance improvement in the post-intervention phase (OR 1.366; 95%CI 1.012-1.843 or OR 1.279; 95%CI 1.019-1.604) (Table 5).

Knowledge and perception

A total of 284 HCW participated in the knowledge and perception survey regarding hand hygiene and HAIs in the pre- (total n = 196; internal medicine, 56; surgery, 33; obst-gyn, 47, and pediatrics, 60) and post-intervention phase (total n=88; internal medicine, 33; surgery, 18; obst-gyn, 15; pediatrics, 22). Overall, the average score was 5.8 (SD = 2.1), the median score was 6 and the mode score was 7/12, whereas the minimum and maximum score were 1/12 and 11/12, respectively. After interventions, the average of knowledge score improved from 5.6 (SD = 2.1) to 6.2 (SD = 1.9) (p<0.05). We classified the knowledge score to be low level (0-5) and high level (6-12) and noted a significant increase in the proportion of high level scores in the pediatrics department (p<0.05) after the intervention. There was not a significant change identified in other departments (Table 2). Also, we did not find a significant increase in the proportion of high level scores among doctors, nurses, and students in the four departments. The results of the perception survey on intention to adhere to hand hygiene, risk of cross-transmission and HAIs related to non-compliance, social norms concerning hand hygiene, hand hygiene indications, methods and promotion are presented in Table 6. In the departments with intervention, positive perception was demonstrated by 69.1% to 98.7% of HCW in the pre-intervention phase and 67.1% to 98.6% of HCW in the post-intervention phase to all perception items. The survey in the department without an intervention showed that 63.8% to 100% of HCW in the pre-intervention phase and 80% to 100% of HCW in the post-intervention phase answered with positive response. There was no significant improvement of the hand hygiene perception before and

after the interventions. However, “strong smell of hand-alcohol” as a reason not to perform hand hygiene increased significantly in the departments with intervention. The perception of HCW in the departments with intervention regarding the average percentage of hospitalized patients who will develop a HAI increased significantly from 49.7% to 58.6% ($P < 0.05$) in the post-intervention phase. In addition, the self-reporting of hand hygiene compliance rate decreased from 85.5% to 75.1% ($p<0.001$). We did not find any significant difference in the perception survey in the department without intervention between pre- and post-intervention (Table 6).

Table 2. Compliance and knowledge to hand hygiene in pre- and post-intervention.

Department[a]		Compliance Rate[b]			Proportion of HCW based on Knowledge Score Group			
HCW	Overall % (95%CI)	Pre-intervention (%)	Post-intervention (%)	*p* value	Score	Pre-intervention (%)	Post-intervention (%)	*p* value
PED	32.4 (28.6-36.2)	80/332 (24.1)	107/245 (43.7)	< 0.001	0 – 5	30/60[d] (50.0)	5/22 (22.7)	0.043
HR		53/80 (66.2)	70/107 (65.4)	1.000	6 – 12	30/60[d] (50.0)	17/22 (77.3)	
HW		27/80 (33.8)	37/107 (34.6)					
Doctor		21/84 (25.0)	55/117 (47.0)	0.002	0 – 5	8/17 (47.1)	0/2 (0)	0.322
					6 – 12	9/17 (52.9)	2/2 (100)	
Nurse		23/105 (21.9)	45/87 (51.7)	< 0.001	0 – 5	13/23 (56.5)	5/18 (27.8)	0.063
					6 – 12	10/23 (43.5)	13/18 (72.2)	
Student		36/143 (25.2)	7/41 (17.1)	0.280[c]	0 – 5	8/17 (47.1)	0/2 (0)	0.322
					6 – 12	9/17 (52.9)	2/2 (100)	
IM	12.3 (10.0-14.6)	19/364 (5.2)	74/399 (18.5)	< 0.001	0 – 5	28/56 (50.0)	11/33 (33.3)	0.184
HR		11/19 (57.9)	42/74 (56.8)	1.000	6 – 12	28/56 (50.0)	22/33 (66.7)	
HW		8/19 (42.1)	32/74 (43.2)					
Doctor		3/40 (7.5)	10/37 (27.0)	0.032	0 – 5	5/13 (38.5)	NA	NA
					6 – 12	8/13 (61.5)	NA	
Nurse		6/180 (3.3)	55/295 (18.6)	< 0.001	0 – 5	11/26 (42.3)	8/24 (33.3)	0.570
					6 – 12	15/26 (57.7)	16/24 (66.7)	

Table 2. Compliance and knowledge to hand hygiene in pre- and post-intervention (cont'd).

Department[a]		Compliance Rate[b]		*p* value	Proportion of HCW based on Knowledge Score Group			*p* value
HCW	Overall % (95%CI)	Pre-intervention (%)	Post-intervention (%)		Score	Pre-intervention (%)	Post-intervention (%)	
Student		11/144 (7.6)	9/67 (13.4)	0.181	0 – 5	9/12 (75.0)	2/5 (40.0)	0.280
					6 – 12	3/12 (25.0)	3/5 (60.0)	
SUR	21.3 (18.3-24.3)	83/440 (18.9)	73/293 (24.9)	0.05	0 – 5	16/33[e] (48.5)	10/18 (55.6)	0.771
HR		69/83 (83.1)	64/73 (87.7)	0.501	6 – 12	17/33[e] (51.5)	8/18 (44.4)	
HW		14/83 (16.9)	9/73 (12.3)					
Doctor		7/57 (12.3)	8/30 (26.7)	0.091	0 – 5	4/8 (50.0)	NA	NA
					6 – 12	4/8 (50.0)	NA	
Nurse		31/238 (13.0)	46/118 (39.0)	< 0.001	0 – 5	7/12 (58.3)	6/7 (85.7)	0.333
					6 – 12	5/12 (41.7)	1/7 (14.3)	
Student		45/145 (31.0)	19/145 (13.1)	<0.001[c]	0 – 5	2/8 (25.0)	4/11 (36.4)	1.000
					6 – 12	6/8 (75.0)	7/11 (63.6)	
OBG	14.6 (12.0-17.2)	40/395 (10.1)	61/298 (20.5)	< 0.001	0 – 5	20/47[f] (42.6)	3/15[g] (20.0)	0.138
HR		32/40 (80.0)	38/61 (62.3)	0.078	6 – 12	27/47[f] (57.4)	12/15[g] (80.0)	
HW		8/40 (20.0)	23/61 (37.7)					
Doctor		0/18 (0)	3/12 (25.0)	0.054	0 – 5	5/10 (50.0)	NA	NA
					6 – 12	5/10 (50.0)	NA	

Table 2. Compliance and knowledge to hand hygiene in pre- and post-intervention (cont'd).

Department[a]		Compliance Rate[b]		*p* value	Proportion of HCW based on Knowledge Score Group			*p* value
HCW	Overall % (95%CI)	Pre-intervention (%)	Post-intervention (%)		Score	Pre-intervention (%)	Post-intervention (%)	
Nurse		24/173 (13.9)	30/157 (19.1)	0.199	0 – 5	2/12 (16.7)	1/7 (14.3)	1.000
					6 – 12	10/12 (83.3)	6/7 (85.7)	
Student		16/204 (7.8)	28/129 (21.7)	<0.001	0 – 5	10/21 (47.6)	2/7 (28.6)	0.662
					6 – 12	11/21 (52.4)	5/7 (71.4)	

Note. IM, internal medicine; SUR, surgery; OBG, obstetrics-gynecology; PED, pediatrics; HCW, healthcare workers; HR, handrubbing; HW, handwashing; *NA* not available.

[a]Departments of Pediatrics, Surgery and Internal Medicine with intervention, Department of Obstetrics without intervention; [b]the percentage of correct hand hygiene actions undertaken on moments when hand hygiene was considered necessary according to the WHO "five moments" ; [c]Significantly worse instead of significantly better; [d]3 HCWs did not mention the profession in the questionnaire; [e]5 HCWs did not mention the profession in the questionnaire; [f]4 HCWs did not mention the profession in the questionnaire; [g]1 HCW did not mention the profession in the questionnaire; score range 0-5=0-42% correct; score range 6-12=50%-100% correct.

Table 3. Behavior of HCW at moments of non-compliance (n= 2,228 out of n=2,766 observed moments).

Behavior	Phase	Total	HCW					
			Doctors (%)	*P* value	Nurses (%)	*p* value	Students (%)	*p* value
GA	Pre	4/1308 (0.3)	0/168 (0)	NA	1/612 (0.2)	< 0.001	3/528 (0.6)	< 0.001
	Post	70/920 (7.6)	0/120 (0)		41/481 (8.5)		29/319 (9.1)	
Gloves*	Pre	114/1308 (8.7)	1/168 (0.6)	1.000	67/612 (10.9)	0.046	46/528 (8.7)	< 0.001
	Post	43/920 (4.7)	0/120 (0)		35/481 (7.3)		8/319 (2.5)	
No HH	Pre	1190/1308 (90.9)	167/168 (99.4)	1.000	544/612 (88.9)	0.024	479/528 (90.7)	0.292
	Post	807/920 (87.7)	120/120 (100.0)		405/481 (84.2)		282/319 (88.4)	

Note. HCW, healthcare workers; HH, hand hygiene; GA, gloves and alcohol (using an alcohol based hand rub while wearing gloves); Pre, pre-intervention; Post, post-intervention; *wearing gloves when it was not necessary.

Table 4. Compliance to the five different WHO moments of hand hygiene pre-intervention and post intervention.

WHO moment	Total (%)	Compliance (%)		*p* value
		Pre-intervention	Post-intervention	
1: before	86/438 (19.6)	57/267 (21.3)	29/171 (17.0)	0.259
Pediatrics		30/89 (33.7)	9/39 (23.1)	0.298
Internal medicine		6/64 (9.4)	7/87 (8.0)	0.774
Surgery		7/67 (10.4)	11/22 (50.0)	< 0.001
Obstetrics		14/47 (29.8)	2/23 (8.7)	0.069
2: before	16/123 (13.0)	7/87 (8.0)	9/36 (25.0)	0.017
Pediatrics		4/20 (20.0)	4/8 (50.0)	0.172
Internal medicine		0/31 (0)	2/14 (14.3)	0.092
Surgery		2/20 (10.0)	2/4 (50.0)	0.115
Obstetrics		1/16 (6.3)	1/10 (10.0)	1.000
3: after	3/12 (25.0)	2/9 (22.2)	1/3 (33.3)	1.000
Pediatrics		0/4 (0)	1/1 (100)	0.200
Internal medicine		0/1 (0)	0/0 (0)	NA
Surgery		1/3 (33.3)	0/0 (0)	NA
Obstetrics		1/1 (100)	0/2 (0)	0.333
4: after	299/1093 (27.4)	112/544 (20.6)	187/549 (34.1)	< 0.001
Pediatrics		40/133 (30.1)	79/119 (66.4)	< 0.001
Internal medicine		12/96 (12.5)	38/139 (27.3)	0.006
Surgery		42/178 (23.6)	42/180 (23.3)	0.953
Obstetrics		18/137 (13.1)	28/78 (35.9)	< 0.001
5: after	134/1100 (12.2)	45/624 (7.2)	89/476 (18.7)	< 0.001
Pediatrics		6/86 (7.0)	14/45 (31.1)	< 0.001
Internal medicine		2/172 (1.2)	27/159 (17.0)	< 0.001
Surgery		31/172 (18.0)	18/87 (20.7)	0.605
Obstetrics		6/194 (3.1)	30/185 (16.2)	< 0.001

Note. WHO, World Health Organization.

1=before touching a patient; 2=before a procedure; 3=after a procedure or body fluid exposure risk; 4=after touching a patient; 5=after touching a patient's surroundings.

Table 5. Multivariate analysis of the factors associated with hand hygiene compliance in the pre-and post-intervention phase.

Factors	Univariate		*p* value	Multivariate		*p* value
	Number of HH compliance (%)			OR	95% CI	
	PI (n=223)	PoI (n=315)				
Department:			< 0.001			
Obst-gyn	40 (17.9)	61 (19.4)		1		
Internal medicine	20 (9.0)	74 (23.5)		-	-	NS
Pediatric	80 (35.9)	107 (34.0)		4.078	1.513-10.994	0.005
Surgery	83 (37.2)	73 (23.2)		1.963	1.178-3.270	0.010
Class of room facilities:			< 0.001			
Class I	1 (0.4)	20 (6.3)		1		
Class II	108 (48.4)	175 (55.6)		-	-	NS
Class III	114 (51.1)	120 (38.1)		1.993	1.168-3.400	0.011
Room type:			< 0.001			
Neonatology	50 (22.4)	72 (22.9)		-	-	NS
General ward	92 (41.3)	192 (61.0)		1.710	1.002-2.918	0.049
High/acute care unit	81 (36.3)	51 (16.2)		1		
Ratio nurse : patients:						NS
1:2	30 (13.5)	35 (11.1)	0.015			
1:4-6	174 (78.0)	226 (71.7)				
1:8	19 (8.5)	54 (17.1)				
Moment of HH:			< 0.001			
Moment 1	57 (25.6)	29 (9.2)		1.442	1.057-1.968	0.021
Moment 2	7 (3.1)	9 (2.9)			-	NS

Table 5. Multivariate analysis of the factors associated with hand hygiene compliance in the pre-and post-intervention phase (cont'd).

Factors	Univariate		*p* value	Multivariate		*p* value
	Number of HH compliance (%)			OR	95% CI	
	PI (n=223)	PoI (n=315)				
Moment 3	2 (0.9)	1 (0.3)			-	NS
Moment 4	112 (50.2)	187 (59.4)		2.333	1.850-2.943	< 0.001
Moment 5	45 (20.2)	89 (28.3)		1		
HCW categories:			< 0.001	-		
Doctor	31 (13.9)	76 (24.1)		1.366	1.012-1.843	0.042
Nurse	84 (37.7)	176 (55.9)		1.279	1.019-1.604	0.034
Student	108 (48.4)	63 (20.0)		1		

Note. HH, hand hygiene; PI, pre-intervention; PoI, post-intervention; HCW, healthcare workers.

Moment 1: before touching a patient; Moment 2: before a procedure; Moment 3: after a procedure or body fluid exposure risk; Moment 4: after touching a patient; Moment 5: after touching a patient's surroundings.

Table 6. Perception associated with HAIs and hand hygiene adherence among HCW between departments.

Perception	No. of HCW (%)					
	Departments with intervention		*p* value	Department without intervention		*p* value
	Pre (n=149)	Post (n=73)		Pre (n=47)	Post (n=15)	
Formal training on HH within 3 years[a]	107 (71.8)	51 (70.8)	0.875	30 (63.8)	13 (86.7)	0.118
Intention to adhere to HH[a]	137 (91.9)	70 (95.9)	0.396	45 (95.7)	15 (100.0)	1.000
The impact of a HAIs on a patient's clinical outcome[b]	115 (77.2)	64 (87.7)	0.119	41 (87.2)	14 (93.3)	0.759
Effectiveness of HH in preventing HAIs[b]	140 (94.0)	65 (89.0)	0.220	41 (87.2)	12 (80.0)	0.674
Importance of HH in the ward among all patient safety issues[b]	136 (91.3)	63 (86.3)	0.480	40 (85.1)	14 (93.3)	0.676
Performing HH as WHO recommended method[b]	126 (84.6)	62 (84.9)	0.944	45 (95.7)	13 (86.7)	0.244
Importance that the head of department attach to the HH behavior[b]	121 (81.2)	61 (83.6)	0.292	41 (87.2)	13 (86.7)	1.000
Importance that the colleagues attach to the HH behavior[b]	103 (69.1)	56 (76.7)	0.147	37 (78.7)	13 (86.7)	0.713
Importance that the patients attach to the HH behavior[b]	109 (73.2)	49 (67.1)	0.557	37 (78.7)	12 (80.0)	1.000
Effort to perform HH as WHO recommended method[b]	135 (90.6)	68 (93.2)	0.768	42 (89.4)	14 (93.3)	0.824

Table 6. Perception associated with HAIs and hand hygiene adherence among HCW between departments (cont'd).

Perception		No. of HCW (%)					
		Departments with intervention		*p* value	Department without intervention		*p* value
		Pre (n=149)	Post (n=73)		Pre (n=47)	Post (n=15)	
e)	Strong smell of hand-alcohol	14 (10.1)	16 (22.9)	0.021	7 (15.2)	1 (7.1)	0.667
f)	The hand-alcohol substance is not convenient (sticky)	25 (17.9)	13 (19.4)	0.848	7 (15.9)	5 (33.3)	0.263
g)	The hand becomes sweaty	27 (19.6)	9 (13.4)	0.331	7 (15.9)	0	0.178
h)	Feeling dirty hand after using hand-alcohol	11 (8.1)	4 (6.0)	0.777	6 (14.0)	1 (7.1)	0.669
Hand hygiene procedure as WHO guideline[a]:							
a)	Information about five moments for HH is known well	129 (86.6)	67 (91.8)	0.369	45 (95.7)	15 (100.0)	1.000
b)	Information about six steps of HH is known well	146 (98.0)	68 (93.2)	0.134	46 (97.9)	15 (100.0)	1.000
c)	Know when to apply HH	143 (96.0)	69 (94.5)	0.838	46 (97.0)	15 (100.0)	1.000
d)	Know how to apply HH	147 (98.7)	72 (98.6)	0.221	47 (100.0)	15 (100.0)	-
	Enough reminders in the ward	121 (81.2)	59 (80.8)	0.998	43 (91.5)	13 (86.7)	0.626

Table 6. Perception associated with HAIs and hand hygiene adherence among HCW between departments (cont'd).

Perception	% (95% CI)					
	Departments with intervention		*p* value	Department without intervention		*p* value
	Pre (n=149)	Post (n=73)		Pre (n=47)	Post (n=15)	
Average percentage of hospitalized patients who will develop a HAIs	49.7 (44.9-54.5)	58.6 (52.8-64.4)	0.026	57.7 (51.6-63.8)	64.0 (51.3-76.7)	0.320
Average percentage of situations HCW perform HH when required	69.3 (65.2-73.3)	68.1 (63.4-72.8)	0.736	75.3 (70.0-80.6)	76.3 (62.8-89.9)	0.860
Percentage of situations requiring HH do the HCW actually perform HH, either by handrubbing or handwashing (self-reporting)	85.5 (82.6-88.4)	75.1 (70.5-79.7)	< 0.001	81.8 (76.8-86.7)	85.3 (78.8-91.8)	0.425

Note. HAIs, healthcare associated infections; HH, hand hygiene; HCW, healthcare workers.

[a]"yes" response; [b]high/very high response.

DISCUSSION

We report the first cluster randomized controlled trial evaluating the effect of three different educational programs on HCWs' hand hygiene compliance and knowledge-perception in a limited-resource hospital in Indonesia. Particularly in our hospital, educational programs on hand hygiene were not applied regularly. Therefore, the educational programs used in this study were introduced in our hospital for the first time. In the departments with an intervention of role model training (i.e. pediatrics and internal medicine), the hand hygiene compliance improved, but only pediatrics department with the sole intervention of role model training was significantly better than the control group. The hand hygiene compliance improvement co-occurred with a statistically significant improvement of the knowledge score. Therefore, we conclude that role model training has the most impact on improving hand hygiene compliance in this setting. Erasmus *et al.* and other studies have also pointed out the importance of role models (14-16). However, it is possible that the factor of positive role models is even more important in societies where job seniority plays a great role, such as in Indonesia.

The improvement in the pediatrics department might also be associated with fewer activities related to hand hygiene opportunities in patient care (n = 577) compared to internal medicine (n = 763), surgery (n = 733), and obst-gyn (n = 693). Pittet *et al.* reported the inverse relationship of activity level in the ward with hand hygiene compliance rate (17,18). The low activity level might also be associated with the improvement of hand hygiene adherence in general in wards and in rooms with class III type facilities. Overall, however, the hand hygiene compliance rate was low. Compared to Pakistan, also a low-middle income country (1), overall hand hygiene compliance rate in our study was lower. On the other hand, the HCW assured that they performed hand hygiene very well based on the perception survey (85.5% and 75.1% in the pre- and post-intervention phase, respectively). Therefore, the HCW may not change

behavior (12). Additionally, only good knowledge about the hand hygiene procedure did not lead to the high hand hygiene compliance among HCW. Other factors including awareness, action control, facilitation, social influence, attitude, self-efficacy, and intention might also be associated with the adherence to hand hygiene procedure. However, further investigation is needed (19).

Although hand hygiene compliance improved after intervention, we noted higher compliance rates after a procedure or body fluid exposure risk (although for only a low number of observed opportunities) and after touching a patient than before performing patient care. The lowest adherence was at the moment after touching a patients' surroundings. Therefore, the reason to perform hand hygiene was more to protect the HCW themselves than patients (1,17,20,21). In addition, effectiveness of hand hygiene to prevent HAIs was hampered by inappropriate clothing such as hand-accessories and long sleeves by most HCW, so transmission of pathogens was unavoidable.

Based on healthcare profession, hand hygiene adherence improved among doctors and nurses in general, although it was not significant in the surgery department. The hand hygiene performance among students did not improve significantly, and even decreased in the surgery and pediatrics departments. This might be associated with the weekly rotation of students' traineeships in our hospital leading to missing education programs, the attitudes of mentors and role models, curriculum enforcement, beliefs, and the use of gloves (21). In such situations, students may transmit the pathogens causing HAIs from patient to patient (22).

Our data showed that wearing gloves regardless of the recommendation for gloves during patient care (i.e. wearing gloves when writing in the patient medical record) hampered HCWs' hand hygiene adherence. WHO observed such misuse of gloves not only in limited-resource hospitals, but also in hospitals where gloves are widely available (6). After intervention, wearing gloves without indication decreased but shifted to

handrubbing while using gloves during patient care. Then, HCW did not change gloves between patients or between contacts of different sites on the same patient. Nurses declared that glove decontamination resulted from a limited examination gloves supply in our hospital (750 pairs per room in Class III). However, WHO does not recommend glove decontamination (6) because of material damage, which can endanger the protective function of gloves. Similar problems were encountered by the WHO in Ebola-affected countries, where gloves were frequently disinfected with chlorine solutions (23).

This study has some limitations. Firstly, the preparation of national hospital accreditation was held in the same period as this study, which may have influenced the knowledge and perception on hand hygiene among HCW. In addition, the HCW were busy preparing the accreditation, so participation in the knowledge and perception survey after intervention was limited. Secondly, the HCW may have changed behavior during hand hygiene observation because of their awareness of the observer (Hawthorn effect) (24,25). This could also be an additional explanation for the significant improvement in hand hygiene compliance in the control department. Thirdly, the study was performed in a tertiary academic hospital that included medical students and nursing students, in the delivery of patient care. Modification of the hand hygiene educational program is suggested when it is applied in either secondary or non-academic hospitals according to the hospital resources.

CONCLUSIONS

In summary, role model training as part of a multi-model strategy has the most impact on knowledge and perception regarding hand hygiene and HAIs among HCW, and improves the hand hygiene compliance in a limited-resource hospital in Indonesia. However, we showed that the hand hygiene compliance rate remained rather low, therefore, the multi-modal hand hygiene strategy should be re-customized considering local resources, administrative support, and education/training focused on the barriers of non-established practice (1,6,27,28).

Acknowledgments

We thank the dean of the Faculty of Medicine, Brawijaya University, Malang, Indonesia and the director of the Dr. Saiful Anwar hospital, Malang, Indonesia who facilitated our work in this teaching hospital. We also thank Irwan Subekti, Tjutjuk Hardiyanto, Anis Chabibah as infection prevention control nurses as well as Muhammad Yasin and Arina Dita as infection prevention control-linked nurses who were involved in the distribution and collection of questionnaires among HCW, and supported the hand hygiene education program in this study.

REFERENCES

1. Allegranzi B, Gayet-Ageron A, Damani N, Bengaly L, McLaws M-L, Moro M-L, Memish Z, Urroz O, Richet H, Storr J, Donaldson L, Pittet D. Global Implementation of WHO's multimodal strategy for Improvement of hand-hygiene: a quasi-experimental study. Lancet Infect Dis. 2013;13:843-51.
2. Huis A, van Achterberg T, de Bruin M, Grol R, Schoonhoven L, Hulscher M. A systematic review of hand hygiene improvement strategies: a behavioral approach. Implementation Science. 2012;7:1-14.
3. Gurley ES, Zaman RU, Sultana R, Bell M, Fry AM, Srinivasan A, Rahman M, Rahman MW, Hossain MJ, and Luby SP. Rates of Hospital Acquired Respiratory Ilness in Bangladesh Tertiarty Care Hospitals: Results from a Low-Cost Pilot Surveillance Strategy. Clin Infect Dis. 2010;50:1084-90.
4. Duerink O, Roeshadi D, Wahjono H, Lestari ES, Hadi U, Wille JC, De Jong RM, Nagelgerke NJ, Van den Broek PJ, Study Group 'antimicrobial Resistance In Indonesia Prevalence and Prevention' Amrin. Surveillance of Health-care Associated Infections in Indonesian hospitals. J Hosp Infect. 2006;62:219-29.
5. Murni IM, Duke T, Kinney S, Daley AJ, Soenarto Y. Reducing hospital-acquired infections and improving the rational use of antibiotics in a developing country: an effectiveness study. Arch Dis Child. 2014;0:1-6.
6. World Health Organization. WHO Guidelines on Hand Hygiene in Health Care. France: WHO press;2009.
7. Asadollahi M, Bostanabad MA, Jebraili M, Mahallei M, Rasooli AS, Abdolalipour M. Nurses' Knowledge Regarding Hand Hygiene and Its Individual and Organizational Predictors. J Caring Sci. 2015;4:45-53.

8. World Health Organization. A Guide to the Implementation of the WHO Multimodal Hand Hygiene Improvement Strategy. WHO press;2009.
9. Jumaa PA. Hand hygiene: simplex and complex. Int J Infect Dis. 2005;9:3-14.
10. Peabody JW, Taguiwalo MM, Robalino DA, Frenk J. Improving the Quality of Care in Developing Countries. In: Jamison DT, Breman JG, Measham AR editors. Disease Control Priorities in Developing Countries. 2nd ed. New York: Oxford University Press; 2006. p. 1293-1307.
11. Nejad SB, Allegranzi B, Syed SB, Ellis B, Pittet D. Health-care-associated infection in Africa: a systematic review. Bull World Health Organ. 2011;89:757-65.
12. Duerink DO, Hadi U, Lestari ES, Roeshadi D, Wahyono H, Nagelkerke NJD, Van der Meulen RG, Van den Broek PJ. A tool to Assess Knowledge, Attitude and Behavior of Indonesian Health Care Workers Regarding Infection Control. Acta Med Indones. 2013;45:206-15.
13. Boyce JM. Update on hand hygiene. Am J Infect Control. 2013;41:S94-6.
14. Erasmus V, Brouwer W, van Beeck EF, Oenema A, Daha TJ, Richardus JH, Vos MC, Brug J. A qualitative exploration of reasons for poor hand hygiene among hospital workers: lack of positive role models and of convincing evidence that hand hygiene prevents cross transmission. Infect Control Hosp Epidemiol. 2009;30:415-9.
15. Lee SS, Park SJ, Chung MJ, Lee JH, Kang HJ, Lee JA, Kim YK. Improved Hand Hygiene Compliance is Associated with the Change of Perception toward Hand Hygiene among Medical Personnel. J Infect Chemother. 2014;46:165-71.
16. Buffet-Bataillon S, Leray E, Poisson M, Michelet C, Bonnaure-Mallet M, Cormier M. Influence of job seniority, hand hygiene education,

and patient to nurse ratio on hand disinfection compliance. J Hosp Infect. 2010;76:32-5.

17. Pittet D. Improving Adherence to Hand Hygiene Practice: A Multidisciplinary Approach. Emerg Infect Dis. 2001;7:234-40.
18. Pittet D. Compliance with hand disinfection and its impact on hospital acquired infections. J Hosp Infect. 2001;48 (Supplement A):S40-6.
19. Teker B, Ogutlu A, Gozdas HT, Ruayercan S, Hacialioglu G, Karabay O. Factors Affecting Hand Hygiene Adherence at a Private Hospital in Turkey. Eurasian J Med. 2015;47:208-12.
20. Randle J, Arthur A, Vaughan N. Twenty-four-hour observational study of hospital hand hygiene compliance. J Hosp Infect. 2010;76:252-5.
21. al Kadi A, Salati SA. Hand Hygiene Practices among Medical Students. Interdiscip Perspect Infect Dis. 2012;1-6.
22. Reem H, Kharraz R, Alshanqity A, AlFawaz D, Eshaq AM, Abu-Zaid A. Hand Hygiene: Knowledge and Attitudes of Fourth-Year Clerkship Medical Students at Alfaisal University, College of Medicine, Riyadh, Saudi Arabia. Cureus. 2015;7:e310.
23. Hopman J, Kubilay Z, Allen T, Edrees H, Pittet D, Allegranzi B. Efficacy of chlorine solution used for hand hygiene and gloves disinfection in Ebola settings: a systematic review. Antimicrob Resist Infect Control. 2015;4 (Suppl 1):O13.
24. Srigley JA, Furness CD, Baker GR, Gardom M. Quantification of the Hawthorn effect in hand hygiene compliance monitoring using an electronic monitoring system: a retrospective cohort study. BMJ Qual Saf. 2014;23:974-80.
25. Rosenthal VD, McCormick RD, Guzman S, Villamayor C, Orellano PW. Effect of education and performance feedback on handwashing; The benefit of administrative support in Argentian hospitals. Am J Infect Control. 2003;31:85-92.

26. Pittet D, Hugonnet S, Harbarth S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. The Lancet. 2000;356:1307-12.
27. Allegranzi B, and Pittet D. Role of hand hygiene in healthcare-associated infection prevention. J Hosp Infect. 2009;73:305-15.

Chapter 7

Risk factors for methicillin-resistant *Staphylococcus aureus* carriage among patients at admission to the surgical ward in a resource-limited hospital in Indonesia

Dewi Santosaningsih[1,2,3], Sanarto Santoso[1], Henri A. Verbrugh[3], Juliëtte A. Severin[3]

[1]Department of Microbiology, Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang, Indonesia; [2]Infection Prevention and Control Committe, Dr. Saiful Anwar Hospital, Malang, Indonesia; [3]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotteram, The Netherlands

American Journal of Tropical Medicine and Hygiene 2017
doi: 10.4269/ajtmh.16-0993

ABSTRACT

This study aimed to identify risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among patients at admission to the surgery ward in a resource-limited hospital in Indonesia. A case-control study was performed including 65 MRSA carriage patients and 132 non-MRSA carriage patients screened at admission to surgery wards in a hospital in Malang, East-Java. For MRSA screening, swabs were obtained from nares and throat, cultured in an enrichment broth followed by subculturing onto CHROMagar™ MRSA; suspected colonies were confirmed by PCR. Patients referred from other hospitals, patients transferred from the surgical acute care unit, patients that had a surgical procedure within 3 months prior to admission, and immunocompromised patients were more likely to be a MRSA carrier at admission to the surgery wards. Selective MRSA screening of patients according to such risk factors at admission would efficiently detect MRSA carriers and may help control MRSA dissemination in surgery wards in limited-resource settings.

Healthcare-associated infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), including bloodstream infection, pneumonia, and surgical site infection, lead to high-cost patient care, extended length of stay, high morbidity and mortality (1, 2). Nasal carriage plays a role in the transmission of MRSA in the hospital setting (3). Our previous study revealed a high prevalence of MRSA carriage at discharge from surgery wards (8.0%) (4). In such endemic settings, active screening of all patients for MRSA carriage at admission is recommended for infection prevention and control purposes. However, such universal screening is unlikely to be conducted in low-resource settings in developing countries including Indonesia because of the relatively high costs. Selective screening based on risk factor analysis is potentially much more efficient and, therefore, more feasible in such settings. This study aimed to analyze the risk factors for MRSA carriage among patients at admission to surgery wards of a tertiary care hospital in Indonesia.

We performed a case-control study including 65 MRSA carriage patients and 132 non-MRSA carriage patients screened at admission in the surgery ward of Dr. Saiful Anwar hospital (tertiary care hospital; 810 beds), Malang, Indonesia, between July 2012 and August 2013. The study was approved by the medical ethics committee (No 129/EC/KEPK-JK/05/2012). A case of MRSA carriage was defined as a patient from whom MRSA could be isolated from either anterior nares or throat. A non-MRSA carriage patient was defined as a patient from whom MRSA could not be isolated from either anterior nares or throat (this patient could harbor methicillin-susceptible *S. aureus* carriage (MSSA) or other species of bacteria at these sites). Patients were enrolled prospectively. All surgery patients were eligible for inclusion. However, surgery patients already admitted more than 48 hours before the moment of enrollment were excluded. The MRSA screening was carried out by culturing of anterior nares and throat using dry cotton swabs (Deltalab, Rubi, Spain) after patients had given informed consent within 48 hours of admission. One swab was used for both anterior nares by rubbing the swab in each

nostril (the “nose-picking area”). The throat was cultured with another swab by rubbing the tonsillar fossa. Swabs were directly inoculated into phenol red mannitol broth (BBL, Le Pont de Claix, France), incubated overnight at 37°C and then subcultured onto MRSA CHROMagar™ medium (ITK Diagnostics, Uithoorn, the Netherlands) for 24-48 hours at 37°C. Typical colonies of *S. aureus* were confirmed with Staphaurex®Plus (Remel, PT.Dipa Puspa Labsains, Indonesia) and subsequently identified by mass spectrometry (MALDI-TOF, Bruker, the Netherlands). Detection of *mecA* gene was performed by PCR (5). Data on age, gender, prior admission room, hospitalization within 12 months, surgical procedure within three months, immunocompromised status (patients with diabetes mellitus, chronic kidney disease, malignancy with either chemotherapy or radiotherapy, congestive heart disease, patients with HIV infection, and those with regular steroid usage), and open skin lesions were included in the risk factor analysis. Data were analyzed with multiple logistic regression analysis using statistical software packages SPSS version 16.0. A *P* value <0.05 was considered as significant.

Among the 65 MRSA carriers, we isolated MRSA from the nares only in 31 (47.7%) cases, from the throat only in 19 (29.2%) cases and from both nares and throat in 15 (23.1%) cases. In addition, we found that 42/132 (31.8%) patients that were free from MRSA did carry MSSA strains. Factors associated with MRSA carriage among patients at admission to surgical wards in the univariate analysis were prior admission room (P<0.001), hospitalization within 12 months (P=0.007), surgical procedure within three months (P=0.008), immunocompromised status (P=0.084), and open skin lesions (P=0.098). Multiple logistic regression analysis showed that patients referred from other hospitals (odds ratio [OR 7.7], 95% confidence interval [CI_{95}] 1.2–49.1), patients transferred from the surgical acute care unit (OR 5.6, CI_{95} 2.7–11.6), patients that had a surgical procedure within three months prior to admission (OR 6.7, CI_{95} 2.3-19.4), and immunocompromised patients (OR 7.3, CI_{95} 1.6–33.9) were

more likely to be MRSA carrier at admission to the surgery wards (Table 1).

This study is the first risk factors analysis that identified four groups of patients at admission to an Indonesian hospital with an increased risk of carrying MRSA. Similar to previously published studies, patients directly referred from another hospital, immunocompromised patients, and patients that had a surgical procedure within three months of admission were more likely to be MRSA carrying patients at admission in the surgery ward (6, 7). In contrast to other studies, we found that open skin lesions and previous hospitalization were not significantly associated with MRSA carriage at admission in this study (7, 8). Moreover, we found that patients transferred from the acute surgery unit (in the emergency suite) were more likely to carry MRSA at the time of transfer and admission to the general surgery wards. This finding suggests that patients experiencing an acute illness are at high risk of becoming colonized with MRSA during their stay in the acute surgery unit. However, further investigation is needed to explore the sources and routes of transmission of MRSA in this particular setting. The four high-risk groups detected in this study could be the target of selective screening of MRSA in Dr. Saiful Anwar hospital, but these findings cannot be generalized for other hospitals since different geographical areas and health care settings may yield different risk factors for patients to be MRSA positive at the time of their admission to surgery. However, when and wherever risk factor analysis for MRSA have yet to be conducted in most hospitals in Indonesia, the four high-risk population groups identified in this study could be used as surrogates for targeted screening of MRSA carriage among patients at admission. Indeed, similar studies are recommended to be conducted in other referral and district hospitals in Indonesia to detect additional risk factors that were not identified in this study. Patients referred from secondary care district hospitals had the highest chance to be MRSA carrier at admission in our referral hospital. However, the presence and spread of MRSA in the second tier hospitals in

Indonesia has not yet been investigated and published. Therefore, developing a network of infection control and antibiotic stewardship programs between secondary and tertiary care hospitals is needed to control MRSA in the Indonesian healthcare system. In addition, it is evidently necessary to gain more insight into the epidemiology of MRSA in the secondary care hospitals in order to design effective targeted intervention measures for controlling MRSA in Indonesia.

Statistical analysis revealed selective screening based on the four risk factors would have a sensitivity and specificity to detect MRSA carriage at admission to the surgery wards of 72.3% and 71.3%, respectively. Only 53/197 (26.9%) patients had one or more of the risk factors, thus eliminating most patients from those that need to be screened. Including additional risk factors such as antibiotic use within six months, the use of medical devices, and contact with a known MRSA carrier may further increase the sensitivity rate. Although further risk factors analysis is needed, we have identified the high-risk populations who should be screened for MRSA carriage at admission to surgery wards. By detecting MRSA carriers on admission, barrier nursing and decolonization therapy can be applied to prevent hospital-acquired MRSA infections in these patients and to prevent the cross contamination of pathogenic MRSA to other patients, to hospital personnel and to the innate environment of the hospital.

This study has some limitations. First, we did not include the use of medical devices, previous contact with a MRSA carrier, and previous antibiotic therapy in the risk factor analysis. Consequently, such potential risk factors on MRSA carriage could not be identified. Second, the high-risk population groups identified in this study was limited to the surgery ward with relatively small number of patients involved. Further investigation for other wards is required to discover risk factors of MRSA carriage among patients at admission to most or all wards of the entire hospital. Third, a cost-effective analysis of the proposed targeted screening in this limited-resource hospital was not carried out.

In summary, we found that patients referred from other hospitals, patients transferred from the surgical acute care unit, patients that had a surgical procedure within three months prior to admission, and immunocompromised patients were at increased risk of carrying MRSA at admission to the surgery ward in a tertiary care hospital in Indonesia. Selective screening for MRSA carriage at admission of these high-risk groups would be an efficient strategy to significantly reduce the prevalence of either MRSA colonization or infection in resource-limited hospitals. The early detection of MRSA carriage among patients at admission is important for infection prevention and control purposes.

Acknowledgements

We thank the director of the Dr. Saiful Anwar hospital, Malang, Indonesia, who facilitated our work in this hospital. The laboratory technical support of Dewi Erikawati, Suyati Pujiani, Yuanita Mulyastuti, Siwipeni I. Rahayu, and Bethania S. Tadjong is gratefully acknowledged.

This study was financially supported by the Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands.

Table 1. Analysis of risk factors for MRSA carriage among patients at admission in the surgery wards of a limited-resource hospital in Indonesia

Risk factors	No. of subjects (%)		Multivariate analysis (backward LR)		
	MRSA (+) (n=65)	MRSA (-) (n=132)	OR	95%CI	*p*
Age*					
< 18 yrs	6 (9.2)	18 (13.6)			
19-59 yrs	51 (78.5)	101 (76.5)			
> 60 yrs	8 (12.3)	13 (9.8)			
Gender*					
Male	50 (76.9)	105 (79.5)			
Female	15 (23.1)	27 (20.5)			
Prior admission room[#]					
Outpatient clinics	30 (46.2)	105 (79.5)	1		
ICU	0 (0)	1 (0.8)	NA		
Acute care unit	30 (46.2)	24 (18.2)	5.6	2.7 – 11.6	< 0.001
Burn unit	1 (1.5)	0 (0)	NA		
Referred from other hospitals	4 (6.2)	2 (1.5)	7.7	1.2 – 49.1	0.030
Hospitalization within 12 months	18 (27.7)	15 (11.4)			NS
Surgery procedure within 3 months	13 (20.0)	9 (6.8)	6.7	2.3 – 19.4	< 0.001
Immunocompromised[§]	6 (9.2)	4 (3.0)	7.3	1.6 – 33.9	0.011
Open skin lesions	0 (0)	7 (5.3)			NS

Note. No, number; MRSA, methicillin-resistant *Staphylococcus aureus*; LR, logistic regression; OR, odds ratio; CI, confidence interval; yrs, years; ICU, intensive care unit; NS, not significant.

*Univariate analysis was not significant (P>0.2); [§]patients with diabetes mellitus, chronic kidney disease, malignancy with either chemotherapy or radiotherapy, congestive heart disease; Note that patients with HIV infection or who use steroids on a regular basis were not among those enrolled in this study; [#] Prior admission room indicates what type of physical contact the patient had with hospitals prior to admission to the general surgery wards. Almost 80% of the MRSA-negative patients had visited outpatient clinics only, whereas the majority (54%) of MRSA-positive patients had had a prior admission to a hospital.

REFERENCES

1. Nickerson EK, West TE, Day NP, Peacock SJ. *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and east Asia. Lancet Infectious Diseases 2009;9:130-135.
2. Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, Mielke M, Peters G, Skov RL, Struelens MJ, Tacconelli E, Navarro Torné A, Witte W, Friedrich AW. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010;15:41.
3. Wertheim HFL, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 2005;5:751-762.
4. Santosaningsih D, Santoso S, Budayanti NS, Kuntaman K, Lestari ES, Farida H, Hapsari R, Hadi P, Winarto W, Milheiriço C, Maquelin K, Willemse-Erix D, van Belkum A, Severin JA, Verbrugh HA. 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;90:728–734.
5. 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;29:2240–2244.
6. Eveillard M, Lancien E, Barnaud G, Hidri N, Gaba S, Benlolo JA, Joly-Guillou M-L. Impact of screening for MRSA carriers at hospital admission on risk-adjusted indicators according to the imported MRSA colonization pressure. J Hosp Infect 2005;59:254-258.
7. McKinnel JA, Miller LG, Eells SJ, Cui E, Huang SS. A Systematic Literature Review and Meta-Analysis of Factors Associated with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Colonization at Time of Hospital or Intensive Care Unit Admission. Infect Control Hosp Epid 2013;34:1077-1086.

8. Fukuta Y, Cunningham CA, Harris PL, Wagener MM, Muder RR. Identifying the Risk Factors for Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection among Patient Colonized with MRSA on Admission. Infect Control Hosp Epid 2012;33:1219-1225.

Chapter 8

General Discussion

INTRODUCTION

In this thesis, we have studied the epidemiology of *Staphylococcus aureus* (*S. aureus*) carriage and infections both in hospitals and in community settings in Indonesia. The prevalence, clonal relatedness, and molecular characteristics of methicillin-resistant *S. aureus* (MRSA) have been investigated. In addition, we investigated the effect of infection control measures on MRSA transmission among patients, healthcare workers, and hospital environment in a resource-limited hospital. In this chapter, the main findings are summarized and discussed on the basis of four research questions. Furthermore, suggestions for further research on the different topics are presented.

RESEARCH QUESTION 1 – Prevalence of MRSA

What is the prevalence of MRSA among carriage and clinical isolates in the hospital and community settings in Indonesia?

In total, we screened 1,861 individuals for *S. aureus* carriage. These individuals consisted of surgery patients on the day of discharge after at least 48 hours of hospitalization (patients at discharge group, n=1,502), patients that had been sharing the room with a MRSA positive patient (contact patients group, n=221), all attending healthcare workers in the ward where a MRSA positive patient had been hospitalized (healthcare workers group, n=116), and household members of MRSA positive patients (household members group, n=22). In addition, a total of 217 cultures of the innate environment were taken. This included the hospital environment in which a MRSA positive patient had been admitted (n=182) and household environment of MRSA carriers (n=35).

Overall, the rate of *S. aureus* carriage among individuals was 25.9% which is much higher than in a previous study from Indonesia [1]. However, the finding is in agreement with a report from the neighboring

country Malaysia [2]. We found that 4.9% of individuals screened carried MRSA. Of importance, MRSA was not found among patients at discharge from the Dr. Kariadi academic hospital in Semarang in an earlier study performed in 2001-2002 [1]. In contrast, we discovered that in 2008-2009 5.9% of patients at discharge from the same hospital in Semarang carried MRSA. This is a clear indication that MRSA emerged in this hospital.

From the same period, a total of 259 clinical *S. aureus* isolates were collected from four tertiary care hospitals in Indonesia. We found that the prevalence of MRSA among clinical *S. aureus* isolates was 6.6%, which is considered lower than most other Asian countries e.g. Malaysia, Sri Lanka, and Korea [3,4], but higher than countries in northern Europe [5]. The clinical MRSA isolates were mainly detected from wound cultures. We did not find any MRSA among *S. aureus* from blood cultures. In the European Antimicrobial Resistance Surveillance Network (EARS-Net), Indonesia would be assigned the "green" color. However, direct comparison may not be appropriate due to large differences between Europe and Southeast Asia in the availability, quality and usage of diagnostic microbiological facilities.

The prevalence of *S. aureus* from wound cultures of 567 patients with skin and soft tissue infections in community settings in Indonesia was 45.3%. Furthermore, 3.1% of these *S. aureus* isolates were MRSA, which is within the reported range of 2.5% - 39% of MRSA among community-acquired (CA)-*S. aureus* in Asian countries [6]. Although the prevalence of MRSA in the community is considered low in this study, it should be noted that no MRSA was detected in the community setting in Surabaya several years before [1].

We have shown that there is an increasing prevalence of MRSA carriage in the hospital settings in Indonesia suggesting continuous nosocomial MRSA transmission. Therefore, a bundle of preventive measures including hand hygiene, isolation procedures, cleaning of hospital environment, screening and decolonization of patients is required to

reduce MRSA transmission in Indonesian hospitals. In addition, we presented evidence indicating an increasing prevalence of MRSA in the community settings compared to the previous study [1]. An explanation for this may be spread of hospital-associated MRSA to the community in combination with selective pressure by antimicrobial chemotherapy in the community settings. Hence, in addition to the introduction of bundles of preventive measures for hospitals, an antibiotic policy for ambulatory and primary care in Indonesia is required.

RESEARCH QUESTION 2 – Clonal relatedness

What is the clonal relatedness of MRSA isolates and PVL-positive MSSA detected in the Indonesian population inside and outside hospitals?

MRSA is a major problem worldwide. Typing of MRSA strains is important for the detection of clonal relatedness and understanding transmission routes, so that targeted preventive measures can be applied. We analyzed the clonal relatedness of *S. aureus* harboring the *mecA* gene using either Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) or Raman spectroscopy, a method that achieves 95% concordance with pulse-field gel electrophoresis [7]. Subsets of isolates were further analyzed by Multilocus-Sequence Typing (MLST) for international comparison. Such analyses were also carried out for *S. aureus* harboring Panton-Valentine leukocidin (*pvl*) genes because these strains may cause severe infections in the community and in hospitals.
According to Raman spectra analysis, a clonal relatedness between MRSA isolates obtained from patients at discharge in Dr. Kariadi hospital, Semarang and Dr. Saiful Anwar hospital, Malang was detected. In addition, we found that clinical MRSA isolates from Dr. M. Djamil hospital, Padang, Dr. Saiful Anwar hospital, Malang, and Dr. Kariadi hospital, Semarang belonged to the same clone. The MRSA isolates either from carriage or clinical cultures from Sanglah hospital, Denpasar were

unique. The clinical PVL-positive methicillin-susceptible *S. aureus* (MSSA) isolates from Dr. M. Djamil hospital, Padang and Sanglah hospital, Denpasar were clonally related.

In the community settings, we found a clone containing MRSA isolates obtained from wound cultures of patients with skin and soft tissue infections in Surabaya that was assigned MLVA type (MT) 19. However, the largest clone, MT 11, consisted of PVL-positive MSSA isolates from three cities in Indonesia (Denpasar, Malang, and Surabaya). Travel of humans between cities and islands in Indonesia may have contributed to the finding of clonally related *S. aureus* isolates in different cities.

According to MLST analysis, sequence type (ST) 239-MRSA isolates were found among both carriage and clinical isolates in Indonesia. The ST239-MRSA clone is a single-locus variant of the USA300 clone [8], and is common in Asian countries [9]. In Singapore [10], this clones' success has been attributed to resistance to non-beta-lactam and non-fluoroquinolone antibiotics, and antiseptics. The Arginine Catabolic Mobile Element could also play a role, since it is associated with enhanced skin colonization. An in-depth analysis of the Indonesian isolates could reveal these and additional characteristics to explain its success in Indonesian hospitals. Similar to the previous study [11] ST121 and ST188 PVL-positive MSSA were also identified in our study, not only among carriage but also clinical isolates.

Interestingly, our study identified ST97 PVL-positive MSSA from the wound culture of a patient with a skin and soft tissue infection in Denpasar. The ST97 has been described from pigs and bovine animals in Japan and Europe [12-14]. Transmission of ST97 from animal to human in the community settings possibly occurred, however, the genetic background of livestock-associated *S. aureus* in Indonesia, particularly in Denpasar is not known yet. A One Health approach involving the public health authorities, and medical and veterinary organizations should be developed to understand and control *S. aureus* and MRSA transmissions between animals and humans.

RESEARCH QUESTION 3 – Genetic characteristics

What are the genetic characteristics of MRSA isolates and PVL-positive MSSA detected in the Indonesian population inside and outside hospitals?

Methicillin-resistant *S. aureus* has been considered as an important nosocomial pathogen causing pandemic waves over the past decades [15]. *S. aureus* with the staphylococcal cassette chromosome *mec* (SCC*mec*) type I, II, and III are recognized as the prototype clones of hospital-associated MRSA (HA-MRSA). In Asian hospitals, SCC*mec* type II is prevalent in Japan and Korea whereas SCC*mec* type III is frequently found in China and Southeast Asian countries, including Indonesia [9]. In the 1990's, CA-MRSA emerged worldwide causing a wide range of diseases from minor skin infections to fatal necrotizing pneumonia. The typical CA-MRSA clones are genetically characterized by SCC*mec* type IV and V [16], and the presence of Panton-Valentine leukocidin (*pvl*) genes. PVL is a cytotoxin causing leukocyte destruction and tissue necrosis [17]. The importance of PVL as a potential virulence factor led us to investigate the prevalence of PVL-positive *S. aureus* both in hospitals and community settings in Indonesia.

Our study revealed a high prevalence of PVL-positive *S. aureus* isolates from wound cultures of patients with skin and soft tissue infections in the community settings (21.8%) as well as from clinical cultures in Indonesian hospitals (18.5%). In contrast, the prevalence of PVL-positive *S. aureus* among Indonesian carriage isolates in the hospital settings was low (2.8%). All PVL-positive isolates were methicillin-susceptible *S. aureus* (MSSA). However, five years later, we found PVL-positive MRSA (16.7%) among Indonesian carriage isolates in a resource-limited hospital. The SCC*mec* may have been transferred into existing successful Indonesian PVL-positive MSSA clones, or new clones may have been introduced.

Several studies have reported the same ST of MRSA isolates carrying a different SCC*mec* element, therefore, an MRSA clone was defined as a group of strains with an identical ST and SCC*mec* type [18]. In concordance with a previous study [9], ST239-MRSA-SCC*mec* type III isolates were found in Indonesian hospitals among both carriage and clinical isolates. Furthermore, we reported for the first time the existence of ST239-MRSA-SCC*mec* type III in the population outside hospitals, suggesting penetration of a typical HA-MRSA clone into the community setting in Indonesia. Vice versa, we discovered a possible infiltration of CA-MRSA represented by a clinical isolate of ST672-MRSA-SCC*mec* type V in Dr. Kariadi hospital, Semarang. Other SCC*mec* type V MRSA isolates were found among carriage isolates in Dr. Saiful Anwar hospital, Malang and Dr. Soetomo hospital, Surabaya [11]. In this situation, a bundle of intervention measures to reduce the prevalence of MRSA in Indonesian hospitals is highly recommended. The penetration of a typical HA-MRSA clone in the community setting probably mediated by undetected carriage is of concern for public health authorities.

RESEARCH QUESTION 4 – Determinants of MRSA carriage

What are the determinants of MRSA carriage among patients in the Indonesian hospitals?

We presented the first risk factor analysis of MRSA carriage of patients at the time of admission and discharge to Indonesian hospitals, more specifically to the surgery ward. Determinants of MRSA carriage were analyzed for patients at discharge (n=1,406) and, separately, for patients at admission (n=197). Multivariate analysis showed that being in the hospital in Semarang or Malang city, being male, and antibiotic therapy during hospitalization were associated with MRSA carriage among patients at discharge. However, the factor that had the strongest association with MRSA carriage at discharge was more than five days of

hospitalization (odds ratio 11.7). Thus, shortening the length of stay in the surgery ward to less than five days may reduce the MRSA carriage rate of patients at discharge.

Patients directly referred from another hospital, patients transferred from the surgical acute care unit, immunocompromised patients, and patients that had a surgical procedure in the three months before admission were more likely to be MRSA carriers at the time of admission to the surgery ward. Knowledge of such determinants is required for Indonesian hospitals to develop a screening strategy for preventing MRSA cross-transmission from newly admitted patients, allowing selective application of preventive measure including isolation precautions, and decolonization therapy for patients screened positive for MRSA at their admission.

RECOMMENDATION FOR FURTHER RESEARCH

Our studies were conducted in a limited number - three to five - tertiary care hospitals and regions in Indonesia. Since Indonesia is a large country and the fourth most populous in the world, the data presented in this thesis do not represent the entire country of Indonesia. In the future, a national surveillance system for MRSA should be developed to provide more representative national data of MRSA prevalence, epidemic clonal shifts, and clonal transmission between healthcare and community setting. To cover the whole country, however, the clinical microbiology services in many areas in Indonesia should be improved or even newly developed.

An endemic MRSA situation involving ST239-MRSA-SCC*mec* type III was uncovered in Dr. Kariadi hospital, Semarang and Dr. Saiful Anwar hospital, Malang. The MRSA clone was found among patients at admission and at discharge suggesting that the MRSA transmission occurred in these hospitals settings. Early detection of MRSA carriage among patients at admission is required for infection control purposes. Selective screening according to certain risk factors for MRSA carriage at

admission would be more applicable in developing countries such as Indonesia compared to universal screening. However, the determinants of MRSA carriage at admission have only been investigated in the surgery ward of a single hospital in Indonesia so far. Different determinants may be identified for other wards or other hospitals. This should be further explored in a large multi-center study.

We reported a decrease of the MRSA acquisition rate after implementation of preventive actions in a resource-limited hospital. In order to confirm or refute these findings that has more predictive power, a similar study should be carried out in multiple hospitals and with a longer follow-up phase. Importantly, we have shown that the intervention for reducing MRSA transmission conducted in our study is feasible for most other Indonesian hospitals. Therefore, the effectiveness of a bundle of intervention measures to reduce MRSA transmission in Indonesian hospitals can and should be further explored.

RECOMMENDATION FOR CONTROL MEASURES

We noted an increase of MRSA prevalence in Indonesian hospitals compared to a previous study conducted several years before [1]. Furthermore, the ST239-MRSA-SCCmec type III, a typical HA-MRSA, has become endemic in Indonesian hospitals. Because of the increased morbidity, mortality, and costs following MRSA infections, strategies to control nosocomial transmission of MRSA, including hand hygiene practice, cleaning and disinfection of hospital environment, isolation precautions, and decolonization therapy have been implemented in other endemic areas in Asia, Europe, and North America [19]. For hospitals in developing countries, such strategies are more challenging to implement since resources are limited. Particularly in Indonesia, the hospital buildings are of an outdated design with crowded multi-bed patient rooms, and very limited number of isolation facilities that are currently prioritized for patients with sputum-positive cases of tuberculosis.

Therefore, we have developed a feasible bundle of actions to prevent MRSA transmission in a surgery ward of a resource-limited hospital in Indonesia.

This bundle of preventive measures was implemented in an intervention study to reduce the MRSA acquisition rate in a resource-limited hospital in Indonesia and consisted of the following elements: (1) introducing hand hygiene practice according to WHO guideline; particularly for the hand hygiene intervention study that was conducted separately, local production of hand alcohol according to WHO formula was used. In addition an education program through either active presentation or role model training was performed to the healthcare workers; (2) contact precautions devoid of single room isolation facilities, therefore, we applied a simple form of cohorting MRSA-positive patients using mobile screens in open wards; (3) thoroughly cleaning and disinfection of surfaces and instruments using a chlorine-based solution (0.05%) and alcohol (70%), respectively; (4) decolonization therapy was performed for both patients and healthcare workers (HCWs) carrying MRSA, consisting of mupirocin dermatological (nasal formulation is not yet available in this country) cream 2% to both nares twice daily for five days plus washing their bodies with chlorhexidine-medicated soap 4% for 7 days. Patients and HCWs who carried MRSA in their throat were additionally offered trimethoprim/sulfamethoxazole oral therapy 960 mg twice daily. After implementation of the preventive actions for 8 weeks, the hand hygiene compliance rate increased significantly followed by a decrease of the MRSA acquisition rate, although this decline did not reach statistical significance.

Such a bundle approach is feasible for resource-limited hospitals in Indonesia, not only for tertiary care referral hospitals but also for secondary care hospitals. We noted that patients referred from another hospital had an increased risk of carrying MRSA at admission in the surgery ward of a tertiary care hospital. The prevalence of MRSA at admission influences the success of the MRSA control program during

hospitalization. Therefore, regional MRSA control networking of tertiary care and secondary care hospitals should be developed.

REFERENCES

1. Lestari ES, Severin JA, Filius JMG, et al. Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylcoccus aureus* in the Indonesian population inside and outside hospitals. Eur J Clin Microbiol Infect Dis 2008;27:45-51.
2. Choi CS, Yin CS, Bakar AA, et al. Nasal carriage of *Staphylococcus aureus* among healthy adults. J Microbiol Immunol Infect 2006;39:458-64.
3. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, et al. Predominance and Emergence of Clones of Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* in Malaysia. J Clin Microbiol 2010;48:867-72.
4. Kang C-I and Song J-H. Antimicrobial Resistance in Asia: Current Epidemiology and Clinical Implications. Infect Chemother 2013;45:22-31.
5. Köck R, Becker K, Cookson B, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010;15:pii=19688.
6. Chuang Y-Y and Huang Y-C. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. Lancet Infect Dis 2013;13:698-708.
7. te Witt R, Vaessen N, Melles DC, et al. Good Performance of the SpectraCell*RA* System for Typing of Methicillin-Resistant *Staphylococcus aureus* Isolates. J Clin Microbiol 2013;51:1434-38.
8. Enright MC, Day NPJ, Davies CE, Peacock SJ, and Spratt BG. Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008-15.

9. Ko KS, Lee J-Y, Suh JY, et al. Distribution of Major Genotypes among Methicillin-Resistant *Staphylococcus aureus* Clones in Asian Countries. J Clin Microbiol 2005;43:421-26.
10. Hsu L-Y, Harris SR, Chlebowicz MA, et al. Evolutionary dynamics of methicillin-resistant *Staphylococcus aureus* within a healthcare-system. Genome Biology 2015;16:81.
11. 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 Microbiol 2008;46:1989-95.
12. Hata E, Katsuda K, Kobayashi H *et al*. Genetic variation among *Staphylococcus aureus* Strains from Bovine Milk and Their Relevance to Methicillin-Resistant Isolates from Humans. J Clin Microbiol 2010;48:2130-2139.
13. Espinosa-Gongora C, Moodley A, Lipinska U *et al*. Phenotypes and Genotypes of Old and Contemporary Porcine Strains Indicate a Temporal Change in the *S. aureus* Population Structure in Pigs. PLoS ONE 2014;9:e101988.
14. Ikawaty R, Brouwer EC, Jansen MD, et al. Characterization of Dutch *Staphylococcus aureus* from bovine mastitis using a Multiple Locus Variable Number Tandem Repeat Analysis. Vet. Microbiol 2009;136:277-284.
15. Song J-H, Hsueh P-R, Chung DR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 2011;66:1061-69.
16. Milheiriço C, Oliveira DC, and de Lencastre H. Update to the Multiplex PCR Strategy for Assignment of *mec* Element Types in *Staphylococcus aureus*. Antimicrob Agents and Chemother 2007;51:3374-77.

17. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in Primary Skin Infections and Pneumonia. Clin Infect Dis 1999; 29:1128-32.
18. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infection, Genetics, and Evolution 2008;8:747-63.
19. Cheng CC-C, Tai JW-M, Chau P-H, et al. Minimal intervention for Controlling Nosocomial Transmission of Methicillin-Resistant *Staphylococcus aureus* in Resource Limited Setting with High Endemicity. PLoS ONE 2014;9:e100493.

Chapter 9

Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known as an important pathogen both in hospital and community settings worldwide. Infections caused by MRSA lead to significant impact on healthcare costs, morbidity, and mortality. The studies in this thesis aimed to ascertain the emergence, transmission, and genetic basis of MRSA in Indonesian hospitals and households. Risk factors for MRSA carriage among patients were determined. In addition, the preventive actions on transmission and acquisition of MRSA were introduced and evaluated, particularly the hand hygiene of healthcare workers. This information is necessary for the development of an evidence-based guideline to control MRSA transmission in Indonesian hospitals.

Chapter 2 presents the results of a prospective study on the epidemiology of *S. aureus* among discharge patients in hospitals in Java and Bali, Indonesia. It was found that 24.4% of patients carried *S. aureus*, the MRSA carriage rate was 4.3%, whereas 1.5% patients carried Panton–Valentine leukocidin (PVL)-positive methicillin-susceptible *S. aureus* (MSSA). PVL-positive MRSA were not found. Semarang and Malang city, being male, hospitalization for more than 5 days, and antibiotic therapy during hospitalization were identified as independent determinants for MRSA carriage, whereas prior hospitalization was the only one risk factor for PVL-positive MSSA carriage. Raman spectra analysis showed three large clusters assigned type 21, 24, and 38, all corresponding to ST239-MRSA-SCC*mec* type III.

Chapter 3 reports the prevalence, antimicrobial susceptibility profiles and clonal distribution of either MRSA or PVL-positive *S. aureus* obtained from clinical cultures in Indonesian hospitals. Among 259 *S. aureus* isolates from four tertiary hospitals, 6.6% were MRSA and 18.5% were PVL-positive MSSA. Similar to the study in chapter 2, we did not identify PVL-positive MRSA in this study. Raman spectroscopy revealed two predominant clusters. We discovered possible transmission of a ST239-

MRSA-SCC*mec* type III strain and a ST121 PVL-positive MSSA in one of the hospitals.

In **chapter 4**, the role of *S. aureus* in community settings among patients with skin and soft tissue infections (SSTI) in Indonesia was explored. We found that 3.1% of *S. aureus* isolated from wound cultures of patients with skin and soft tissue infections were MRSA. Genes encoding PVL and exfoliative toxins (ETs) were detected in 21.8% and 17.5% of MSSA, respectively. PVL-positive MRSA was also not detected in this study. Thus, community-acquired SSTI in Indonesia were frequently caused by PVL-positive MSSA. Nasopharyngeal *S. aureus* carriage was an independent determinant for *S. aureus* SSTI. Primary skin infections and previous antibiotic therapy were associated with PVL-positive MSSA, whereas primary skin infection was the only factor associated with ET-positive MSSA. Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) typing revealed two predominant MSSA clusters. A cluster of ST239-MRSA-SCC*mec* type III was found as well. We hypothesized that this hospital-associated ST239-MRSA may have spread from the hospital into community settings.

Chapter 5 describes the effect of the introduction of a bundle of preventive measures on the transmission and acquisition of MRSA in the surgery ward of a resource-limited hospital in Indonesia. The study consisted of three phases: a pre-intervention, intervention, and post-intervention phase. After intervention, the hand hygiene compliance rate was significantly improved from 15% in the pre-intervention phase to 65% in the post-intervention phase ($p<0.001$). In addition, the MRSA acquisition decreased from 9/1,000 patient-days at risk in the pre-intervention phase to 3/1,000 patient-days at risk in the post-intervention phase ($p=0.08$). Contrary to the previous studies, we found 16.7% of MRSA to carry PVL genes in this study. Raman type 9 which belonged to ST239 was the dominant MRSA clone. Although the MRSA

acquisition rate was not significantly reduced, the implementation of such a bundle of preventive measures was shown to be feasible for a resource-limited hospital. The declining trend in our study may be an indication that the bundle will reduce MRSA transmission and acquisition among surgery patients in resource-limited hospitals in Indonesia.

Chapter 6 presents the effect of three different educational programs on hand hygiene compliance, knowledge, and perception among healthcare workers in a tertiary care hospital in Indonesia. After intervention, the hand hygiene compliance and knowledge had improved ($p<0.001$ and $p<0.05$, respectively). However, the nurses' incorrect use of hand rub while wearing gloves increased as well ($p < 0.001$). In the perception survey, the "strong smell of hand-alcohol" as a reason for non-compliance of hand hygiene practices increased significantly. The role model training had the most impact on the compliance in this setting compared to the control department with no educational intervention.

Chapter 7 describes a case-control study to identify risk factors for MRSA carriage among patients at admission to the surgery ward in a resource-limited hospital in Indonesia. We identified that patients referred from other hospitals, patients transferred from the surgical acute care unit, patients that had a surgical procedure within 3 months prior to admission, and immunocompromised patients were more likely to be a MRSA carrier at admission to the surgery wards. Selective MRSA screening of patients according to such risk factors at admission would efficiently detect MRSA carriers and may help control MRSA dissemination in surgery wards in resource-limited settings.

In **chapter 8 and 9**, the main results are discussed and summarized. Suggestions for measures to prevent MRSA transmission and acquisition among patients in hospital settings and for further research are provided.

Nederlandse samenvatting

Dutch summary

Meticilline-resistente *Staphylococcus aureus* (MRSA) staat wereldwijd bekend als een belangrijk pathogeen, zowel in ziekenhuizen als in de open bevolking. Infecties veroorzaakt door MRSA hebben een aanzienlijke impact op de kosten van de gezondheidszorg en gaan gepaard met een verhoogde ziektelast en sterfte ten opzichte van infecties met meticilline-gevoelige *S. aureus* (MSSA). De studies in dit proefschrift hadden als doel de opkomst, verspreiding en genetische kenmerken van MRSA in ziekenhuizen en de open bevolking in Indonesië in kaart te brengen. Tevens werden risicofactoren voor MRSA dragerschap bij patiënten onderzocht. Daarnaast werden infectiepreventiemaatregelen om de verspreiding van MRSA te stoppen onderzocht in een ziekenhuis met beperkte middelen. De handhygiëne van gezondheidsmedewerkers was hiervan een belangrijk onderdeel. Deze informatie is nodig om een op wetenschappelijk bewijs gebaseerde richtlijn te ontwikkelen om verspreiding van MRSA in Indonesische ziekenhuizen te voorkomen.

In **hoofdstuk 2** worden de resultaten gepresenteerd van een prospectieve studie over de epidemiologie van *S. aureus* bij patiënten die werden ontslagen uit een ziekenhuis in Java of Bali, Indonesië, na een opname van ten minste 48 uur. Op de dag van ontslag werd bij 24,4% van de patiënten *S. aureus*gevonden, bij 4,3% MRSA, en bij 1,5% van de patiënten een Panton-Valentine leukocidine (PVL) -positieve meticilline-gevoelige *S. aureus* (MSSA). PVL-positieve MRSA werden niet gevonden. De steden Semarang en Malang, mannelijk geslacht, ziekenhuisopname voor meer dan vijf dagen en behandeling met antibiotica tijdens ziekenhuisopname werden geïdentificeerd als onafhankelijke determinanten voor MRSA dragerschap, terwijl een eerdere

ziekenhuisopname de enige risicofactor voor PVL-positieve MSSA dragerschap was. Analyse van Raman spectra liet drie grote clusters zijn, namelijk type 21, 24 en 38, allemaal overeenkomend met ST239-MRSA-SCC*mec* type III.

Hoofdstuk 3 beschrijft het voorkomen, de antimicrobiële gevoeligheidsprofielen en klonale verdeling van MRSA en PVL-positieve *S. aureus* verkregen uit klinische kweken in Indonesische ziekenhuizen. Van 259 *S. aureus* isolaten uit vier tertiaire ziekenhuizen, was 6,6% MRSA en 18,5% PVL-positieve MSSA. Evenals de studie in hoofdstuk 2, identificeerden we geen PVL-positieve MRSA in deze studie. Met Raman spectroscopie werden twee overheersende clusters zichtbaar gemaakt. We ontdekten mogelijke overdracht van een ST239-MRSA-SCC*mec* type III stam en een ST121 PVL-positieve MSSA in een van de ziekenhuizen.

In **hoofdstuk 4** werd de rol van *S. aureus* bij patiënten met huid- en weke deleninfecties buiten het ziekenhuis in Indonesië onderzocht. We vonden dat 3,1% van *S. aureus* geïsoleerd uit wondkweken van patiënten met deze infecties MRSA was. Genen die coderen voor PVL en exfoliatieve toxines (ET's) werden gedetecteerd in respectievelijk 21,8% en 17,5% van de MSSA. PVL-positieve MRSA werd ook in deze studie niet gevonden. Huid- en weke deleninfecties in de open bevolking in Indonesië worden dus vaak veroorzaakt door PVL-positieve MSSA. Neus- en/of keeldragerschap van *S. aureus* was een onafhankelijke determinant voor een huid- en weke deleninfectie met *S. aureus*. Primaire huidinfecties en eerdere antibiotica therapie waren geassocieerd met PVL-positieve MSSA, terwijl primaire huidinfectie de enige factor was die geassocieerd was met ET-positieve MSSA. Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) liet twee overheersende MSSA clusters zien. Een cluster van ST239-MRSA-SCC*mec* type III werd ook gevonden, welke wordt beschouwd als een typische 'ziekenhuiskloon'. Het is onze hypothese dat

deze ST239-MRSA zich heeft verspreid vanuit het ziekenhuis naar de open bevolking.

Hoofdstuk 5 beschrijft het effect van de introductie van een bundel van infectiepreventiemaatregelen op de verspreiding van MRSA in de afdeling Heelkunde van een ziekenhuis in Indonesië. De studie bestond uit drie fasen: een pre-interventie, interventie en post-interventie fase. Na de interventie bleek de mate van naleving van handhygiëne significant verbeterd van 15% in de pre-interventiefase tot 65% in de post-interventiefase (p <0.001). Daarnaast daalde de MRSA-acquisitie van 9 per 1.000 patiëntendagen in de pre-interventiefase tot 3 / 1.000 patiëntdagen in de post-interventiefase (p = 0,08). In tegenstelling tot de studies in hoofdstuk 2 tot en met 4, vonden we dat 16,7% van de MRSA isolaten in deze studie PVL genen hadden. Raman type 9, die behoorde tot ST239, was de dominante MRSA kloon. Hoewel de acquisitie van MRSAniet significant verminderd was, is de bundel van preventieve maatregelen wel haalbaar gebleken voor een ziekenhuis met beperkte middelen, en zou de neerwaartse trend een aanwijzing kunnen zijn dat met de bundel de verspreiding van MRSA bij chirurgische patiënten in een ziekenhuis in Indonesië beperkt zou kunnen worden.

Hoofdstuk 6 presenteert het effect van drie verschillende scholingsprogramma's op mate van naleving van handhygiëne (compliance), kennis over en perceptie van handhygiëne bij gezondheidsmedewerkers in een tertiaire zorg ziekenhuis in Indonesië. Na interventie was de handhygiëne compliance en kennis verbeterd (p <0,001 en p <0,05, respectievelijk). Het onjuiste gebruik van handalcohol tijdens het dragen van handschoenen door verpleegkundigen was evenwel verhoogd (p <0,001). In de perceptie-vragenlijst was de "sterke geur van handalcohol" als reden voor het niet-naleven van handhygiënepraktijken aanzienlijk gestegen. Het opleiden van handhygiëne rolmodellen had de

meeste impact op de naleving in deze instelling in vergelijking met de controle afdeling zonder scholingsinterventie.

Hoofdstuk 7 beschrijft een case-control studie om risicofactoren voor MRSA dragerschap bij patiënten te identificeren bij opname op de afdeling Heelkunde in een ziekenhuis in Indonesië. We hebben gevonden dat patiënten die vanuit andere ziekenhuizen werden verwezen, patiënten die werden overgebracht van de acute chirurgie afdeling, patiënten die in de drie maanden voorafgaand aan de opname een chirurgische procedure hadden ondergaan en patiënten met een verminderde afweer een groter risico hadden om MRSA-drager te zijn op het moment van opname op de afdeling Heelkunde. Selectieve MRSA-screening van patiënten met dergelijke risicofactoren bij opname zou MRSA-dragers op efficiënte wijze detecteren en kunnen helpen om MRSA verspreiding te beheersen in chirurgische afdelingen in soortgelijke ziekenhuizen.

In **hoofdstukken 8 en 9** worden de belangrijkste resultaten besproken en samengevat. Voorstellen voor maatregelen ter voorkoming van MRSA verspreiding in ziekenhuizen worden gedaan, en suggesties voor verder onderzoek worden gegeven.

Ringkasan dalam bahasa Indonesia

Indonesian summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) dikenal sebagai patogen penting baik di rumah sakit maupun di komunitas di seluruh dunia. Infeksi yang disebabkan oleh MRSA menyebabkan dampak signifikan pada biaya kesehatan, morbiditas, dan mortalitas. Penelitian ini bertujuan untuk mengetahui keberadaan, transmisi, dan dasar genetik MRSA di rumah sakit dan komunitas di Indonesia. Faktor risiko karier MRSA pada pasien ditentukan. Selain itu, tindakan pencegahan transmisi dan akuisisi MRSA diperkenalkan dan dievaluasi, terutama kebersihan tangan petugas kesehatan. Hasil penelitian ini diperlukan untuk pengembangan panduan pengendalian transmisi MRSA di rumah sakit di Indonesia sesuai *evidence-based*.

Bab 2 memaparkan hasil studi prospektif tentang epidemiologi *S. aureus* pada pasien yang keluar dari rumah sakit di Jawa dan Bali, Indonesia. Hasil penelitian menunjukkan bahwa 24,4% pasien merupakan karier *S. aureus*, 4,3% pasien berstatus karier MRSA, sedangkan 1,5% pasien merupakan karier methicillin-susceptible *S. aureus* (MSSA) yang mengandung gen Panton-Valentine leukocidin (PVL). MRSA yang mengandung gen PVL tidak diketemukan pada penelitian ini. Kota Semarang and Malang, jenis kelamin laki-laki, perawatan di rumah sakit lebih dari 5 hari, dan terapi antibiotik selama perawatan di rumah sakit merupakan penentu karier MRSA, sedangkan perawatan di rumah sakit sebelumnya merupakan satu-satunya faktor risiko untuk karier PVL-positive MSSA. Analisis Raman spectra menunjukkan tiga kelompok besar MRSA yang ditandai dengan type 21, 24, and 38, yang semuanya merupakan ST239-MRSA-SCC*mec* type III.

Bab 3 melaporkan prevalensi, profil kepekaan antimikroba, dan distribusi *clone* baik MRSA atau *S. aureus* yang mengandung gen PVL yang diperoleh dari kultur spesimen klinik di rumah sakit di Indonesia. Di antara 259 isolat *S. aureus* isolate dari empat rumah sakit tersier, 6,6% merupakan MRSA dan 18,5% merupakan MSSA yang mengandung gen PVL. Sama seperti di dalam bab 2, kami tidak menemukan MRSA yang mengandung gen PVL pada penelitian ini. *Raman spectroscopy* menunjukkan dua kelompok besar *S. aureus*. Kami menemukan adanya kemungkinan transmisi strain ST239-MRSA-SCC*mec* type III dan ST121 PVL-positive MSSA di satu rumah sakit.

Di dalam **bab 4**, kami mengekplorasi peran *S. aureus* di komunitas khususnya pada pasien dengan infeksi kulit dan jaringan lunak. Kami menemukan bahwa 3,1% dari *S. aureus* yang diisolasi dari kultur luka pasien dengan infeksi kulit dan jaringan lunak merupakan MRSA. Gen yang mengkode PVL and exfoliative toxins (ETs) berturut-turut terdeteksi pada 21,8% and 17,5% dari MSSA. MRSA yang mengandung gen PVL juga tidak diketemukan pada penelitian ini. Jadi, infeki kulit dan jaringan lunak di komunitas di Indonesia sering disebabkan oleh MSSA yang mengandung gen PVL. Karier *S. aureus* di nasofaring merupakan faktor risiko terjadinya infeksi kulit dan jaringan lunak yang disebabkan oleh *S. aureus*. Infeksi kulit primer dan terapi antibiotik sebelumnya terkait dengan MSSA yang mengandung gen PVL, sedangkan infeksi kulit primer merupakan satu-satunya faktor yang terkait dengan MSSA yang mengandung gen ETs. Multilocus Variable-Number Tandem-Repeat Analysis (MLVA) typing menunjukkan dua kelompok besar MSSA. Kelompok strain ST239-MRSA-SCC*mec* type III diketemukan juga pada penelitian ini. Hipotesis kami adalah bahwa ST239 MRSA telah menyebar dari rumah sakit ke komunitas.

Bab 5 menggambarkan efek serangkaian tindakan pencegahan transmisi dan akuisisi MRSA di bangsal bedah dari suatu rumah sakit dengan

sumber daya terbatas di Indonesia. Penelitian ini terdiri dari tiga fase: fase pre-intervensi, intervensi, dan post-intervensi. Setelah intervensi, angka kepatuhan cuci tangan meningkat secara bermakna dari 15% pada fase pre-intervensi menjadi 65% pada fase post-intervensi (p<0,001). Selain itu, akuisisi MRSA menurun dari 9/1000 *patient-days at risk* pada fase pre-intervensi menjadi 3/1000 *patient-days at risk* pada fase post-intervensi (p=0,08). Berbeda dengan penelitian sebelumnya, pada penelitian ini kami menemukan 16,7% dari MRSA mengandung gen PVL. Raman type 9 yang terkait dengan ST239 merupakan satu kelompok dominan MRSA. Meskipun, angka akuisisi MRSA tidak menurun secara bermakna, serangkaian tindakan pencegahan dapat menurunkan transmisi dan akuisisi MRSA pada pasien bedah di suatu rumah sakit dengan sumber daya terbatas di Indonesia.

Bab 6 memaparkan efek tiga program pendidikan yang berbeda tentang kepatuhan, pengetahuan dan persepsi petugas kesehatan dalam melakukan prosedur cuci tangan di suatu rumah sakit tersier di Indonesia. Setelah intervensi, kepatuhan dan pengetahuan petugas dalam melakukan cuci tangan meningkat (berturut-turut p<0,001 dan p<0,05). Namun demikian, penggunaan handrub oleh perawat pada saat menggunakan sarung tangan juga (p < 0,001). Di dalam survey persepsi, terdapat peningkatan jumlah responden yang memilih "bau yang menyengat dari hand-alcohol" sebagai suatu alasan untuk tidak melakukan prosedur hand hygiene. Jika dibandingkan dengan kelompok kontrol, pelatihan role model menunjukkan dampak yang baik untuk meningkatkan kepatuhan melakukan prosedur hand hygiene.

Bab 7 menggambarkan suatu studi *case-control* untuk mengidentifikasi faktor risiko karier MRSA pada pasien saat masuk bangsal bedah di suatu rumah sakit dengan sumber daya terbatas di Indonesia. Kami mengidentifikasi bahwa pasien rujukan dari rumah sakit lain, pasien pindah dari unit perawatan bedah akut, pasien yang mengalami proses

pembedahan dalam 3 bulan sebelum masuk bangsal bedah, dan pasien dengan status *immunocompromised* merupakan faktor risiko karier MRSA pada saat masuk bangsal bedah. Skrining MRSA secara selektif sesuai dengan faktor risiko tersebut terhadap pasien yang masuk di bangsal bedah akan mendeteksi karier MRSA secara efisien dan dapat membantu mengendalikan penyebaran MRSA di bangsal bedah di rumah sakit dengan sumber daya terbatas di Indonesia.

Di dalam **bab 8 dan 9**, hasil-hasil penting penelitian didiskusikan dan dirangkum. Saran-saran untuk tindakan pencegahan transmisi dan akuisisi MRSA pada pasien di rumah sakit dan untuk penelitian selanjutnya dipaparkan.

Acknowledgements

Ucapan terima kasih

First of all, thank God that I was given the opportunity to do my PhD study in Erasmus University Rotterdam, the Netherlands.

Furthermore, I am grateful to the government of Republic of Indonesia for granting a doctoral scholarship provided by the Directorate of Higher Education Ministry of National Education (now Ministry of Research Technology and Higher Education), Republic of Indonesia.

I would like to thank the Rector of Brawijaya University, Prof. Dr. Ir. Yogi Sugito and his successor Prof. Dr. Ir. Mohammad Bisri, MS for their permission and support to conduct my PhD study. I would also like to thank the deans of the Faculty of Medicine Brawijaya University during the period of my PhD study: Dr. dr. Samsul Islam, SpMK, MKes, Dr. dr. Karyono Mintaroem, SpPA, and Dr. dr. Sri Andarini, MKes for granting permission and support for my PhD study. In addition, I would like to thank the heads of the Laboratory of Microbiology, Faculty of Medicine Brawijaya University/Dr. Saiful Anwar hospital during my PhD study period, Prof. Dr. dr. Sanarto Santoso, SpMK(K), Prof. Dr. dr. Noorhamdani AS, SpMK(K), and Dr. dr. Dwi Yuni Nur Hidayati, MKes for always supporting me to finish my PhD study.

The studies described in this thesis would not have been possible without the support of many people. I would like to thank all of you. I also want to express my deep gratitude to the patients and healthcare workers who were so kind to participate in the projects.

I would like to thank the deans of the Faculty of Medicine, Brawijaya University, Malang; the Faculty of Medicine, Diponegoro University, Semarang; the Faculty of Medicine, Andalas University, Padang, and the Faculty of Medicine, Udayana University, Denpasar, the directors of Dr. Saiful Anwar Hospital, Malang; Dr. Kariadi Hospital, Semarang; Dr. M.

Djamil Hospital, Padang; and Sanglah Hospital, Denpasar, and the heads of primary healthcare centers (Puskesmas) in Malang including Puskesmas Lawang, Puskesmas Singosari, Puskesmas Pakisaji, Puskesmas Sumberpucung, and Puskesmas Gondanglegi, who facilitated these studies.

I would like to address a special word of gratitude to the following persons:

Prof. dr. Henri A. Verbrugh, PhD, my promoter. I am very grateful for your ever-valuable encouragement, support, supervision, suggestions, and corrections. The underlined valuable suggestions are about optimism, good collaboration, and honesty in the research life. In addition, you helped me in the aspects other than my PhD study such as getting accommodation and public transportation for the first time I stayed in Rotterdam. Thank you very much for all your help. Many thanks also to your family for the hospitality whenever I stayed in Rotterdam.

Prof. Dr. dr. Sanarto Santoso, SpMK(K), my promoter. I am also very grateful for your support. Your knowledge and suggestions has been of great value for the study.

Dr. Juliëtte A. Severin, PhD, my co-promoter. I would like to express my deep gratitude for your encouragement, supervision, suggestions, and corrections. You supported me to pass the “bumpy road” of the studies from the beginning until the end. Thank you very much for all your help. Many thanks also to your family for the hospitality when I was staying in Rotterdam.

Prof. Alex van Belkum, PhD, PhD, dear Alex. You encouraged me to improve my laboratory skills at the beginning of my PhD study. Thank you for all your support. In addition, your valuable suggestions to the manuscripts were greatly appreciated.

Prof. Dr. dr. Kuntaman, SpMK(K), Prof. Dr. dr. Ketut Suata, SpMK(K), Prof. Dr. dr. Winarto, SpMK(K), Prof. Dr. dr. Hendro Wahjono, SpMK(K), Prof. Dr. dr. M. Hidayat, SpB, SpOT(K), saya ucapkan terima kasih yang

setinggi-tingginya atas dukungan dan supervisi profesor terhadap studi MRSA di Indonesia, sehingga studi multicenter ini dapat terlaksana dengan baik.

Dr. dr. Nyoman S. Budayanti, SpMK(K), Dr. dr. Agung D.W. Widodo, MKes, dr. Rebriarina Hapsari, MSc, SpMK, dr. Helmia Farida, MKes, SpA, PhD, dr. Purnomo Hadi, MSi.Biotek, SpMK, dr. Priyo B. Purwono, MSi, dr. Dicky B. Widhyatmoko, dr. Aziz Djamal, MSc, SpMK(K), dr. Damayanti, SpKK, Dr. dr. Cita RS Prakoeswa, SpKK(K), dr. Nanik Setijowati, MKes, dr. Harun A. Rasyid, MPH, dr. Dewi Erikawati, MSi, Iffa A. Hakim, dr. Ayu H. Suwendha, SpOT, Vicky Puspitasari, SFarm, Apt, dan Irhamni, SFarm, Apt, saya mengucapkan terima kasih yang tak terhingga atas kebersamaan dan kerjasama kita dalam tim studi MRSA yang membuat saya bersemangat untuk dapat menyelesaikan studi tersebut.

Dr. Endang S. Lestari, PhD, dear Mbak Endang. Penghargaan saya sampaikan kepada Mbak Endang yang telah bergabung bersama kami dalam studi multicenter MRSA di Indonesia. Data dan isolat dari Semarang yang dikerjakan oleh Mbak Endang sangat membantu keberhasilan studi MRSA ini. Saya juga mengucapkan terima kasih yang tak terhingga atas bimbingan Mbak Endang sebagai senior yang telah menjalani studi PhD di Erasmus University Rotterdam lebih dahulu daripada saya, sehingga memudahkan saya untuk beradaptasi terutama di awal masa studi.

Dr. Catarina Milheiriço, dear Catarina. You have performed confirmation of SCC*mec* typing of our isolates. Thank you for all your help. In addition, your valuable suggestions to the manuscript were greatly appreciated.

I feel grateful to Kees Maquelin and Diana Willemse-Erix for analyzing and consultation regarding the clonality of *Staphylococcus aureus* collected in the MRSA study in Indonesia with Raman spectroscopy.

Neline Oudenes and Michelle de Regt, thank you for technical support and help with PCR and Raman spectroscopy of *Staphylococcus aureus* isolates.

Nicole Lemmens-den Toom, Susan Snijders, and Mitchell Laurens, my special thanks for your technical support, consultation, and help. I have learned a lot how to organize the laboratory work from all of you. Honestly, I am jealous with the perfect DNA bands always found in your gels!

Arjen van Vliet, dear Arjen. You have created the case record form for the MRSA study in Indonesia. With this, the multicenter data could be recorded in a safe and similar way.

Anne F. Voor in 't holt, dear Anne. You have improved the statistical analysis of the "Hand Hygiene manuscript". Thank you for all your help. In addition, your valuable suggestions to the manuscript were greatly appreciated.

Nicole S. Erler, dear Nicole. You have improved the statistical analysis of the "Intervention Study manuscript". Thank you for all your help. In addition, your valuable suggestions to the manuscript were greatly appreciated.

I would like to thank medical students of Erasmus University Medical Center Rotterdam: Josephine W.I. van Nierop, Geraldine L Nanninga, Jessica Bakker, Nadine Rasenberg, Andreas L.E. van Arkel, Luke G. Terlouw, Eva van Boven, and Gwen van der Donk who helped me to collect the data of the MRSA study and the Hand Hygiene study in Indonesia. In addition, I appreciate your great spirit for doing laboratory work in Indonesia. Thank you very much for all your help guys!

Saya mengucapkan terima kasih sebesar-besarnya kepada seluruh keluarga besar Laboratorium Mikrobiologi, Fakultas Kedokteran Universitas Brawijaya/RSUD Dr. Saiful Anwar, Malang (yang belum saya sebutkan di atas) dan sejawat di rumah sakit jejaring yang telah memberikan dukungan terhadap terlaksananya studi MRSA Indonesia sejak dimulainya studi tersebut pada tahun 2007: dr. Sjoekoer M. Dzen, SpMK (alm), dr. Roekistiningsih, MS, SpMK(K) (almh), Prof. Dr. dr. Sumarno, SpMK(K), Dr. Dra. Sri Winarsih, Apt, MSi, Dr. drh. Sri Muwani, MP, dr. Yuanita Mulyastuti, MSi, dr. Siwipeni I. Rahayu,

MBiomed, dr. Bethania F. Tadjong, dr. Andrew W. Tulle, dr. Nurima D. Hastuti, dr. Etty Fitria Ruliatna, SpMK, dr. Rendra Brahmanti, SpMK, dr. Dewi Retno, SpMK, Slamet Riyanto, Soeyati Pujiani, Ali Sabet, Winarsih, Nina P. Ningsani, Novia A. Maulitta, Nuraini W. Riska, Diah S. Chumala, Suwarso, Musa HA. Bana, Anita Agustiani, Tino Oktavian, Fajar R. Rudiansyah, Samsul Arifin, Abdul Tholib, Sri Haryati, Hendri Setyanata, Novi Andrianto, Mega Andriawati, Safitri Nurdamayanti, dan Esti H. Putri.

Terima kasih yang tak terhingga dan mohon maaf yang sedalam-dalamnya kepada guru-guru saya, teman-teman, dan saudara yang tidak dapat saya sebutkan satu per satu di dalam ucapan terima kasih ini.

Terima kasih yang mendalam dan penghargaan yang tak ternilai untuk ayahanda Rochadi dan ibunda Supadmi (almh) yang dengan penuh kasih sayang telah mengasuh, membimbing, memberikan landasan pendidikan hidup yang kuat, dan mendorong saya untuk selalu belajar dan mencapai pendidikan tinggi. Terima kasih sebesar-besarnya juga saya haturkan kepada ayahanda dan ibunda (almh) yang telah dengan setulus hati membantu mengasuh Yusuf, anak saya, selama saya belajar di Belanda.

Matur nuwun sanget kagem ibunda mertua Marpu'ah yang telah membantu memberikan perhatian dan mengasuh Yusuf saat saya belajar di Belanda.

Kepada kakak, adik, kakak ipar, dan adik-adik ipar saya, perhatian kalian sangat saya hargai.

Untuk yang tercinta suamiku Agung Witjoro dan anakku M. Yusuf Airlangga, pengorbanan kalian tak ternilai. Terima kasih yang tak terhingga atas perhatian, pengertian, kasih sayang, kesabaran, dukungan, dan keikhlasan ayah dan Yusuf. Maafkan mama yang tidak dapat mendampingi secara fisik ketika Yusuf dirawat di rumah sakit karena saat itu mama sedang belajar di Belanda. Doa selalu mama panjatkan untuk kesehatan dan keselamatan ayah dan Yusuf, semoga Allah swt selalu melindungi keluarga kita. Aamiin YRA.

Curriculum Vitae

Dewi Santosaningsih

Dewi Santosaningsih was born on March 29, 1971 in Malang, East Java, Indonesia. She graduated from elementary school (Taman Muda I – Malang) in 1983, junior high school (SMP Negeri 5 – Malang) in 1986, and senior high school (SMA Negeri 3 – Malang) in 1989. After completing her education from Faculty of Medicine, Brawijaya University, Malang as medical doctor in 1996, she worked at Ngudi Waluyo hospital in Wlingi, Blitar, East Java, Indonesia for one year. Since 1998, she has held a position at the Department of Microbiology, Faculty of Medicine, Brawijaya University, Malang. Since 2000, she has also been a staff member in the Laboratory of Clinical Microbiology, Dr. Saiful Anwar hospital, Malang. She joined a master program on Medical Microbiology in Airlangga University, Surabaya, Indonesia from 2001 to 2003. She was involved in the MRSA study in Indonesia from 2007-2014, collaborating with Department of Microbiology, Faculty of Medicine, Diponegoro University/Dr. Kariadi hospital, Semarang, Department of Microbiology, Faculty of Medicine, Udayana University/Sanglah hospital, Denpasar, Department of Microbiology, Faculty of Medicine, Airlangga University/Dr. Soetomo hospital, Surabaya, Department of Microbiology, Faculty of Medicine, Andalas University/Dr. M. Djamil hospital, Padang, and Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands. In 2009, she started her PhD period in Erasmus University Medical Center, Rotterdam, the Netherlands. Since 2014 she has been the chair of the infection prevention and control committee and a member of the antibiotic resistance control committee in Dr. Saiful Anwar hospital, Malang, Indonesia. Dewi is married to Agung Witjoro and they have a son, Muhammad Yusuf Airlangga (2004).

List of Publications

Kuntaman K, Santoso S, Wahjono H, Mertaniasih NM, Lestari ES, Farida H, Hapsari R, Firmanti SC, Noorhamdani AS, **Santosaningsih D**, Purwono PB, Kusumaningrum D. The Sensitivity Pattern of Extended Spectrum Beta Lactamase-Producing Bacteria against Six Antibiotics that Routinely Used in Clinical Setting. J Indon Med Assoc 2011; 61:482-6.

Santosaningsih D, Santoso S, Budayanti NS, Kuntaman K, Lestari ES, Farida H, Hapsari R, Hadi P, Winarto W, Milheiriço C, Maquelin K, Willemse-Erix D, van Belkum A, Severin JA, Verbrugh HA. 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.

Santosaningsih D, Santoso S, Budayanti NS, Suata K, Lestari ES, Wahjono, Djamal A, Kuntaman K, van Belkum A, Laurens M, Snijders SV, Willemse-Erix D, Goessens WH, Verbrugh HA, Severin JA. 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.

Kuntaman K, Hadi U, Setiawan F, Koendori EB, Rusli M, **Santosaningsih D**, Severin J, Verbrugh HA. 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.

Santosaningsih D, Erikawati D, Santoso S, Noorhamdani N, Ratridewi I, Candradikusuma D, Chozin IN, Huwae TECJ, van der Donk G, van Boven E, Voor in 't holt AF, Verbrugh HA, Severin JA. Intervening with

healthcare workers' hand hygiene compliance, knowledge, and perception in a limited-resource hospital in Indonesia: a randomized controlled trial study. Antimicrobial Resistance and Infection Control 2017; 6(23):1-10.

Santosaningsih D, Santoso S, Verbrugh HA, Severin JA. Risk factors for methicillin-resistant *Staphylococcus aureus* carriage among patients at admission to the surgical ward in a resource-limited hospital in Indonesia. American Journal of Tropical Medicine and Hygiene 2017; doi: 10.4269/ajtmh.16-0993.

Santosaningsih D, Sanarto S, Setijowati N, Rasyid HA, Budayanti NS, Suata K, Widhyatmoko DB, Purwono PB, Kuntaman K, Damayanti D, Prakoeswa CRS, Laurens M, van Nierop JWI, Nanninga GL, Oudenes N, de Regt M, Snijders SV, Verbrugh HA, Severin JA. Prevalence and characterization of *Staphylococcus aureus* causing skin and soft tissue infections in the community setting in Java and Bali, Indonesia. Submitted.

Santosaningsih D, Erikawati D, Hakim IA, Sanarto S, Hidayat M, Suwendha AH, Puspitasari V, Irhamni I, Kuntaman K, van Arkel ALE, Terlouw LG, Oudenes N, Willemse-Erix D, Snijders SV, Verbrugh HA, Severin JA. Reducing transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in a surgical ward of a resource-limited hospital in Indonesia: an intervention study. Submitted.

PhD Portfolio

Name PhD student	: Dewi Santosaningsih
Erasmus MC department	: Medical Microbiology and Infectious Diseases
PhD period	: 2008 - 2017
Research school	: Postgraduate School Molecular Medicine
Promoters	: Prof. dr. H. A. Verbrugh
	Prof. dr. S. Santoso
Copromoter	: Dr. J. A. Severin

1. PhD Training	**Year**	**Workload**	
		Hour	**ECTS**
Courses			
– Antimicrobial Susceptibility Testing, Surabaya, Indonesia.	2015	40	1.4
– Basic Course on Infection Prevention and Control, Malang, Indonesia.	2015	16	0.6
– Courses on Clinical Microbiology, Denpasar, Indonesia	2016	40	1.4
Workshops			
– Antibiotic Stewardship, Bali, Indonesia.	2012	16	0.6
– Expertise in Antimicrobial Susceptibility Testing, Jakarta, Indonesia.	2013	8	0.3
– Prevalence and Screening Solutions of MRSA, ESBL, and VRE, Jakarta, Indonesia.	2013	8	0.3
– Hospital Antimicrobial Resistance Control Management, Semarang, Indonesia.	2013	8	0.3
– Infected Wound Care and Patient Safety, Jakarta, Indonesia.	2014	8	0.3
– Multidisciplinary Approach on Infection Prevention and Control, Lawang, Indonesia.	2014	8	0.3
– Improving Quality of Analytic Phase: From Colony Selection to AST, Jakarta, Indonesia.	2014	8	0.3

– Rationale Interpretation of Antibiotic and Susceptibility Testing, Medan, Indonesia.	2015	8	0.3
– Antibiotic Stewardship for Regional Trainer, Jakarta, Indonesia.	2016	40	1.4
– Carbapenemase Resistance Enterobacteriaceae: Phenotypic and Genotypic Identification, Solo, Indonesia.	2016	8	0.3

(Inter) national Conferences

– 13th International Symposium on Staphylococci and Staphylococcal Infections, Cairns, Australia (poster presentation)	2008	1
– Symposium on Controlling the Infectious Diseases to Improve the Quality of Life, Yogyakarta, Indonesia.	2009	1
– Annual Scientific Meeting of Indonesian Society for Clinical Microbiology, Bandung, Indonesia (oral presentation).	2010	1
– 6th National Symposium of Indonesia Antimicrobial Resistance Watch, Jakarta, Indonesia (poster presentation-runner up best poster).	2010	1
– 7th National Symposium of Indonesia Antimicrobial Resistance Watch, Jakarta, Indonesia (poster presentation-runner up best poster).	2011	1
– International Conference on Biomedical Science, Bandung, Indonesia (poster presentation)	2012	1
– Indonesia Infectious Disease Update Symposium, Malang, Indonesia	2012	1
– 8th National Congress of Indonesian Society for Clinical Microbiology, Bali, Indonesia (poster presentation).	2012	1
– Annual Scientific Meeting of Indonesian Society for Clinical Microbiology, Semarang, Indonesia (poster presentation-the best poster).	2013	1
– International Symposium on Multicenter Research		

	Study, Surabaya, Indonesia	2013	1
–	8th National Symposium of Indonesia Antimicrobial Resistance Watch, Jakarta, Indonesia (poster presentation).	2013	1
–	9th National Symposium & Workshop of Indonesia Antimicrobial Resistance Watch and Annual Scientific Meeting of Indonesian Society for Clinical Microbiology, Jakarta, Indonesia (poster presentation-runner up best poster).	2014	1
–	9th National Congress of Indonesian Society for Clinical Microbiology and 10th National Symposium of Indonesia Antimicrobial Resistance Watch (poster presentation-runner up best poster)	2015	1
–	Annual Scientific Meeting of Indonesian Society for Clinical Microbiology, Solo, Indonesia (oral presentation).	2016	1
–	Annual Scientific Meeting of Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.	2016	1
–	Symposium Jakarta Antimicrobial Update in Conjunction with Indonesian Sepsis Forum Meeting, Jakarta, Indonesia.	2016	1

2.	**Teaching**			
–	Teaching for medical students	2008-2009	60	2
–	Supervising 9 medical students from International Federation of Medical Students' Associations (IFMSA) for MRSA and Hand Hygiene research projects (@80 hours).	2010-2015	720	24
–	Supervising 4 medical students from Erasmus University Medical Center, Rotterdam, the Netherlands for MRSA and Hand Hygiene research projects (@480 hours).	2012-2014	480	17
–	Supervising 3 orthopedic residents of Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar	2011-2015	30	1

	hospital, Malang, Indonesia for researches and theses (@10 hours).			
–	Supervising medical students of Faculty of Medicine, Brawijaya University, Malang, Indonesia for researches and theses.	2009-2015	160	5
Total			1,666	72.8