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Preliminary study of biofilm formation properties and antibiotic susceptibility pattern of MRSA and MSSA isolates obtained in Yogyakarta, Indonesia

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ABSTRACT

Aims: Nowadays, *Staphylococcus aureus*, especially methicillin resistant *Staphylococcus aureus* (MRSA), emerged as a major pathogenic agent of nosocomial infection and sepsis worldwide. Infections caused by these bacteria are often difficult to treat because of the development of antibiotic resistance. Biofilm formation is an important factor in the pathogenicity of staphylococcal infections and one of the reason of antibiotic treatment failure. In this study, the relationship between biofilm formation properties, the presence of *mecA*, *icaA/D* genes and antimicrobial resistance pattern were investigated in 10 methicillin sensitive *Staphylococcus aureus* (MSSA) and 10 MRSA clinical isolates.
Methodology and results: Staphylococcal strains were identified by conventional microbiological methods, while determination of methicillin susceptibility was distinguished by the presence of *mecA* gene. To investigate biofilm production, congo red agar and microtiter plate test were performed. PCR was done to detect the presence of *ica*A/D genes, which responsible for biofilm production. Antibiotic sensitivity was carried out by agar diffusion method. The majority of MRSA isolates (90%) were not able to form biofilm, only one isolate (10%) showed capability of weak biofilm producer. Meanwhile, fully established biofilms were formed by all of MSSA isolates (100%). In addition, all MRSA and almost MSSA isolates (90%) harboured both *ica*A/D genes in their chromosomes. Antibiotic resistance profile of MRSA was more dominant than MSSA isolates.
Conclusion, significance, and impact of study: Biofilm production of staphylococci showed difference regulation with regard to methicillin susceptibility. Antibiotic resistance profile was more dominant in MRSA, however biofilm production was found mostly in MSSA isolates.

Keywords: MRSA; biofilm; *ica*A/D genes; *mec*A gene; antibiotic susceptibility

INTRODUCTION

Staphylococcus aureus is well recognized as an important nosocomial pathogen that causes various skin infections, bacteremia, pneumonia, osteomyelitis, endocarditis, myocarditis, meningitis and abscesses at different sites (Chambers, 1997). Recently, methicillin resistant *S. aureus* (MRSA) has emerged as a major clinical and epidemiological problem in hospitals. Among surgery patients in three academic hospitals in Indonesia, MRSA carriage rate was 4.3% (Santosaningsih *et al*., 2014). A distinctive feature of MRSA strains is their resistance not only to all β-lactam antibiotics, but also to a wide range of other antimicrobials, which makes MRSA infections difficult to manage and costly to treat (Chambers, 1997; Gould, 2005).

Moreover, this type of Gram positive bacteria is frequently regarded as a cause of blood stream infection related to foreign bodies and indwelling medical devices. On these inert surfaces the bacteria are able to grow as biofilms, which are refractory to antimicrobial agents. The difficulty of treating MRSA may be exacerbated by its ability to form biofilm in the body. Biofilms are also responsible for the failure of antibiotic therapy, because of: a) inhibition of antibiotic penetration, b) slow growing state of bacteria, and c) the resistance genes expression (Lewis, 2001). These infections are costly to treat, often requiring multiple rounds of antibiotics. Removal of the infected device is often the only possible clinical solution, thus inflicting the trauma to the patient and the cost of treatment. Additionally, according to data announced by World Health Organization (WHO), more than 65% of microbial infections are caused by biofilm (CDC, 2004).

Biofilm formation by *S. aureus* can be governed in part by the production of polysaccharide intercellular adhesin (PIA), a major component of the exopolysaccharide matrix that embeds bacterial cells in the biofilm (O'Gara, 2007). PIA is produced by enzymes encoded by the *ica* operon which comprises four intercellular adhesion genes: *icaA, icaB, icaC* and *icaD*. Among these, the most extensively

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studied genes responsible for PIA synthesis are *icaA* and *icaD*. Meanwhile, MRSA has the ability to transform PBP2 protein into PBP2a, a penicillin binding protein that has a lower affinity for β-lactam antibiotics. Its synthesis is encoded by the presence of the *mec*A gene in the chromosomes, allow the bacteria to become resistant to β-lactam class of antibiotics and their derivatives (Chambers, 1997). To support the appropriate management of MRSA infections, it is necessary to obtain accurate data on the sensitivity pattern of *S. aureus*, both MSSA and MRSA isolates.

In this study, we investigated the contribution of methicillin susceptibility to the biofilm phenotype in *S. aureus*. To address this question, we characterized the biofilm phenotypes of a collection of 10 MRSA and 10 MSSA clinical isolates in Yogyakarta, Indonesia. The antibiotic resistance profile was determined, along with the ability to form biofilm on polystyrene surfaces. Identification of the genetic determinants of biofilm production and methicillin resistance, such as *icaA/D* and *mecA* genes were subsequently defined by PCR and results were compared to the other investigated characteristics.

MATERIALS AND METHODS

Bacterial isolates

A total of 20 clinical isolates *S. aureus,* isolated from pus and skin/mucose swab were collected from the clinical microbiology laboratory of Medicine Faculty, University Gadjah Mada, Yogyakarta, Indonesia, during the period January 2012 to January 2013. They were identified phenotypically by standard procedures on the basis of gram-staining properties, colony appearance on blood agar, catalase and coagulase test. Confirmation of MRSA and MSSA was done by PCR to detect the presence of *mecA* gene as described below. Clinical *S. aureus* isolates showed the presence of *mecA* gene was regarded as MRSA and considered as MSSA when *mecA* gene was absent.

Bacterial DNA extraction

DNA isolation was performed according to the manual procedure of Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific Inc, Lithuania). Overnight cultures of bacterial cells were pelleted by centrifugation, resuspended in 180 µL of lysis buffer, and subsequently treated with 200 µL of lysis solution containing 20 µL proteinase K (Sigma-Aldrich, Steinheim, Germany) (1 mg/mL) and incubated for 30 min at 56 °C. The DNA was purified and proceeded for genes detection assay.

Polymerase chain reaction (PCR) assay for *mecA* and *icaA/D* genes detection

Standard PCR was performed on an ICycler machine (PeqStar, PegLab, UK).Briefly, PCR reaction mixture was prepared containing 25 µL of DreamTaq Green PCR Master Mix (Thermo Scientific, USA), 10 pmol of each forward and reverse primer, template DNA and distilled water to a final volume of 50 µL. To amplify *mecA* gene, a 22 nucleotide forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3' and a 22 nucleotide reverse primer, 5'-AGTTCTGCAGTACCGGATTTGC-3', with the amplicon size of 532 bp were used (El-Mahallawy *et al*., 2009). Furthermore, a total of 40 cycles were run which consisted of DNA denaturation step at 94 °C for 30 sec, primers annealing at 55 °C for 30 sec, extension of the two strands occur at 72 °C for 60 sec and a final extension step of 4 min.

The strains were screened for the presence of *icaA/D* genes to determine the association between methicillin susceptibility and biofilm-forming capacities in *S. aureus* isolates. *S. epidermidis* ATCC 35984 was used as positive control and *S. epidermidis* ATCC 12228 as negative control for both genes. For the detection of *icaA*, 5'-TCTCTTGCAGGAGCAATCAA-3' was used as the forward primer (corresponding to nucleotides 4796–4815) and 5'-TCAGGCACTAACATCCAGCA-3' was used as the reverse primer corresponding to nucleotides 4964-4983. For *icaD*, 5'-ATGGTCAAGCCCAGACAGAG-3' was used as the forward primer (corresponding to nucleotides 5422–5441), and 5'-CGTGTTTTCAACATTTAATGCAA-3' was used as the reverse primer (corresponding to nucleotides 5616–5597). PCR condition for both genes were performed at the same condition, which consisted of 50 cycles, starting from incubation step at 94 °C for 5 min, followed by DNA denaturation step at 94 °C for 30 sec, annealing for the strands at 55.5 °C for 30 sec, extension at 72 °C for 30 sec and elongation at 72 °C for 1 min (Arciola *et al*., 2001). The amplified product were analyzed on a 2% agarose gel electrophoresis and the sizes estimated by comparison with 100 bp DNA ladder (Amersham Biosciences). Amplicons for *icaA* and *icaD* produced fragments of 189 and 195 bp, respectively.

Biofilm phenotypic characterization

The strains were cultured on Congo Red Agar (CRA) plates, prepared by adding 0.8 g of Congo Red (Sigma-Aldrich, Steinheim, Germany), 12 g bacto agar (Becton, Dickinson and Co, Sparks, MD, USA) and 36 g of saccharose (Merck, Darmstadt, Germany) to 1 L of BHI broth (Oxoid, Basingstoke, UK). The plates were subsequently incubated for 24 h at 37 °C and additionally overnight at room temperature. Black colonies were considered indicative of normal slime producing strains, while those coloured almost black were indicative of a weak slime production activity, very red to bordeaux red colonies were classified as non-slime producers (Christensen *et al*., 1982). The CRA plate assay was done in duplicate.

Biofilm formation assay

A modified quantitative biofilm assay was performed (Chri-

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-stensen *et al*., 1982). Briefly, 1:100 dilutions of overnight cultures in Triptic Soy Broth (TSB) (Oxoid, Basingstoke, UK) were used to inoculate wells in a microtiter polystyrene plate (Falcon, Becton Dickinson Labware, NJ, USA). After incubation for 24 h at 37 °C, the plates were gently washed two times with Phosphate Buffered Saline (PBS) (Oxoid, Basingstoke, UK), and stained with 1% (w/v) crystal violet solution; the excess stain was washed off with demineralised water. Subsequently the adherent cells were resuspended in acid-isopropanol (5% v/v 1M HCl in isopropanol), and finally, the absorbance was measured at 575 nm wave length (λ575) in an ELISA reader (Multiskan Ex, Thermo Scientific, Finlandia). *Staphylococcus epidermidis* RP62A (ATCC 35954) was used as biofilm positive control strain and *S. epidermidis* ATCC 12228 was included as negative biofilm control strain. All isolates were tested in triplicate and the mean optical density (OD) was calculated.

Strongly positive biofilm was indicated by the OD value of ≥1; weakly positive biofilm when 0.1< OD value < 1 (low grade positive biofilm); and negative biofilm was interpreted as OD value < 0.1(van Merode, 2006).

Antibiotic susceptibility test

The antibiotic susceptibility pattern was determined by the disk diffusion method. The antibiotics used in this study included the following group: penicillinase-labile penicillins (penicillin 10 U), penicillinase-stabile penicillins (cefoxitin 30 µg, amoxillin/clavulanic acid 20/10 µg), cephalosporins (cefuroxime 30 µg, ceftriaxone 30 µg, cefepime 30 µg, cefixime 5 µg), carbapenem (meropenem 10 µg), aminoglycosides (amikacin 30 µg, gentamicin 10 µg), macrolides (erythromycin 15 µg), fluoroquinolones (ciprofloxacin 5 µg, levofloxacin 5 µg), sulfa group (trimethoprim-sulfamethoxazole 1.25/23.75 µg), chloramphenicol 30 µg and tetracycline 30 µg (Oxoid, Basingstoke, U.K). This was carried out on Mueller-Hinton agar medium (Oxoid, Basingstoke, U.K) and growth inhibition zones were measured and interpreted according to the CLSI guidelines (CLSI, 2014). *S. aureus* ATCC 29213 was used as control strain.

RESULTS AND DISCUSSION

PCR detection of *icaA* and *icaD* genes

Staphylococcus aureus isolates used in this study has been characterized as MRSA and MSSA based on the presence of *mec*A gene in their chromosome. PCR revealed the presence of *icaA/D* in all of MRSA isolates (Figures 1 and 2) and almost all of MSSA isolates (90%). In addition, *icaA/D* were absent in one of MSSA isolate which was still able to synthesize fully established biofilm (Table 1).

Biofilm-producing phenotype and the capability to form biofilms

The biofilm-producing phenotype of 10 MSSA and 10 MR-SA clinical *S. aureus* isolates was determined on CRA. Duplicate runs showed consistent results. As shown in Table 2, it was demonstrated that 10 out of 10 (100%) MSSA isolates were able to form biofilm, as indicated by the appearance of black, dry and rough colonies on the CRA medium (Figure 3). However, only 1 out of 10 (10%) MRSA isolates have the capability to form biofilm, which demonstrated as bordeoux colonies on CRA.

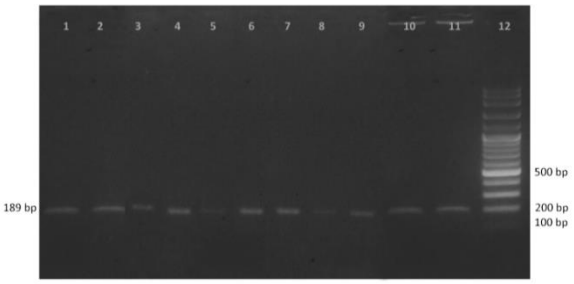

Figure 1: PCR detection of *icaA* from MRSA isolates is shown by amplicon of 189 bp; lane 1: *icaA* from control isolate (*S. epidermidis* 46), lane 2-11: MRSA isolates, lane 12 was applied for 100 bp marker (Vivantis, USA).

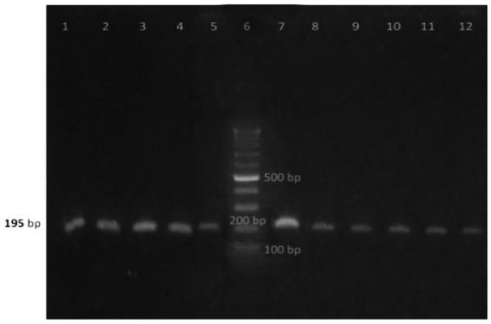

Figure 2: PCR detection of *icaD* from MRSA isolates is shown by amplicon of 195 bp; lane 1: *icaD* from control isolate (*S. epidermidis* 46), lane 2-5 and 7-12: MRSA isolates, lane 6 was applied for 100 bp marker (Vivantis, USA). Images of PCR products of *icaA/D* gene from MSSA isolates are not shown.

The remaining MRSA isolates (90%) revealed as red and smooth colonies on CRA indicating non biofilm-producing strains. Furthermore, microtiter plate assay was used to confirm the biofilm formation properties of both isolates. As reported by Hassan *et al.* (2011), microtiter plate assay is more reliable and considered as the standard test for the detection of biofilm formation. It was shown that 100% of MSSA isolates were able to form biofilm on polysterene surfaces (Figure 4) and considered as strong biofilm-producing strain as indicated by higher absorbance values (more than 1). On the contrary, ninety percent of MRSA isolates tested in this study had a very low absorbance values (less than 0.1) and considered as non-biofilm producer. Furthermore only one isolate (10%) of MRSA was regarded as a weak biofilm-producing strain (Table 2).

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Table 1: Association between biofilm formation characteristics, presence of *icaA* and *icaD* genes and antibiotic resistance profile in MSSA and MRSA isolates.

Isolate	Presence of related genes		Biofilm production	Antibiotic resistance profile
	icaA	*icaD*		
MSSA				
947	+	+	Strong	P,E, CRO,CFM,FEP
957	+	+	Strong	AMC,CFM
758	+	+	Strong	E,C,CFM
913	+	+	Strong	P,CFM
746	+	+	Strong	P,CFM,T
120	-	-	Strong	P,CFM,T
944	+	+	Strong	CFM
1642	+	+	Strong	CFM
38	+	+	Strong	P,CFM
28	+	+	Strong	P,C,CRO,CFM
MRSA				
1227	+	+	Negative	P,FOX,AMC,A,G,C,Cip,Lev,CXM,CRO,CFM,FEP, MEM,SXT
1646	+	+	Weak	P,FOX,AMC,Cip,CXM,CRO,CFM,FEP,MEM,T
1629	+	+	Negative	P,FOX,AMC,C,Cip,CXM,CRO,CFM,FEP,MEM,T
70	+	+	Negative	P,FOX,AMC,E,C,Cip,CXM,CRO,CFM,FEP,T
124	+	+	Negative	P,FOX,AMC,A,G,E,C,Cip,Lev,CXM,CRO,CFM,FE P,MEM,SXT,T
4477	+	+	Negative	P,FOX,AMC,A,G,E,Cip,Lev,CXM,CRO,CFM,FEP, MEM,SXT,T
112	+	+	Negative	P,FOX,AMC,A,G,C,Cip,Lev,CXM,CRO,CFM,FEP, MEM,T
50	+	+	Negative	P,FOX,AMC,G,C,Cip,Lev,CXM,CRO,CFM,FEP,M EM,T
5068	+	+	Negative	P,FOX,A,G,E,Cip,Lev,CXM,CRO,CFM,FEP,MEM
5736	+	+	Negative	P,FOX,AMC,A,G,E,Cip,Lev,CXM,CRO,CFM,FEP, MEM,SXT,T

P, Penicillin; FOX, Cefoxitin; AMC, Amoxillin Clavulanic acid; A, Amikacin; G, Gentamicin; E, Erythromycin; C, Chloramphenicol; Cip, Ciprofloxacin; Lev, Levofloxacin; CXM, Cefuroxime; CRO, Ceftriaxone; CFM, Cefixime; FEP, Cefepime; MEM, Meropenem; SXT, Trimethoprim/sulfamethoxazole; T, Tetracyclin

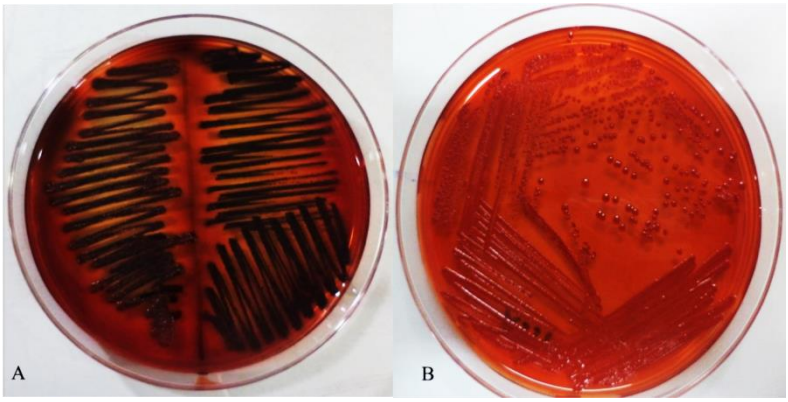

Figure 3: Colonies appearance of biofilm producer of MSSA isolates (A) and non-biofilm producer of MRSA isolates (B) on CRA. Biofilm producer of MSSA isolates showed black, rough and dry colonies, while non-biofilm-producing strain demonstrated smooth and red colonies.

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Table 2: Biofilm formation capability of MSSA and MRSA isolates performed using CRA and microtiter plate assay.

Isolate	CRA isolate number (%)		Microtiter assay isolate number (%)	
	Biofilm +	Biofilm -	Biofilm +	Biofilm -
MSSA	10 (100)	0 (0)	10 (100)	0 (0)
MRSA	1 (10)	9 (90)	1 (10)	9 (90)

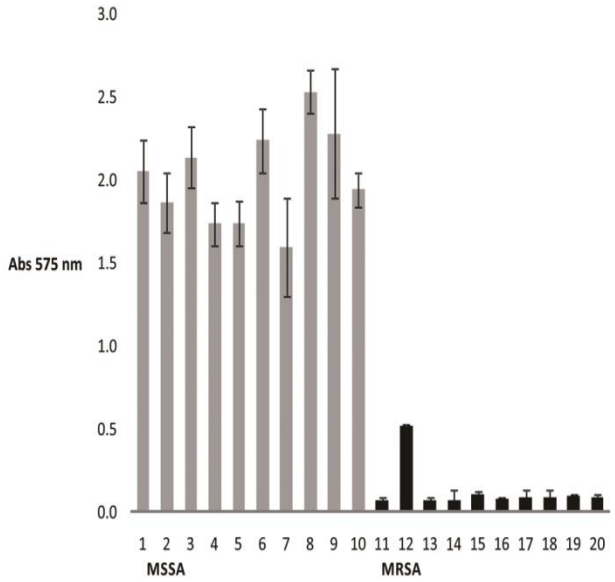

Figure 4: Microtiter plate assay results of MSSA and MRSA isolates with 1% crystal violet staining. This data is presented as an average of three times replication for each well. The x-axis represents the test isolates: grey bars (no.1-10) demonstrated the result of MSSA isolates, black bars (no.11-20) depicted the MRSA isolates. Y-axis represents the absorbance value at 575 nm wavelength.

Antibiotic susceptibility pattern of MRSA and MSSA isolates

MRSA and MSSA isolates used in this study were assayed for antibiotic resistance using disk diffusion technique (CLSI, 2014). MRSA isolates tested in this study showed different resistance pattern compared with MSSA isolates, which the more dominant resistance is MRSA isolates (Table 1). MRSA resistance against antibiotics acting on the cell wall such as penicillin, ampicillin, cephalosporins groups and carbapenem (meropenem) are very high (80-100%). Additionally, the sensitivity of MRSA isolates to aminoglycoside (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin) and tetracycline are very low as more than 50% isolates showed resistance to those antibiotics. Moreover, trimethoprim/sulfamethoxazole and chloramphenicol demonstrated as a potent drug choice for about 60% of MRSA isolates tested in this study. Antibiotic sensitivity patterns of MSSA isolates are still quite good, especially to aminoglycoside, fluoroquinolones, third and fourth generation of cephalosporine, carbapenem (meropenem) and trimethoprim/sulfamethoxazole as shown by the data of 0% resistance rate. Antibiotic resistance rate of MSSA isolates did not achieve yet 100% to all of antibiotics tested (Table 3).

Table 3: Antibiotic resistance rate of MSSA and MRSA isolates against tested panels of antibiotics.

Antibiotic	MSSA (% resistance)	MRSA (% resistance)
PEN	60	100
FOX	0	100
AMC	10	90
A	0	60
G	0	70
E	10	50
C	20	40
Cip	0	100
Lev	0	70
CXM	0	100
CRO	10	100
CFM	90	100
FEP	0	100
MEM	0	80
SXT	0	40
T	20	80

Discussion

Both MRSA and MSSA isolates showed consistent results of biofilm formation properties by the CRA inoculation test and microtiter plate assay. The agreement between these two assays is still debated, it is reported that CRA method has drawbacks because it only detects amyloid in the biofilm matrix polysaccharide and bind with congo red, whereas if the biofilm matrix is predominantly composed of proteins or extracellular DNA, the CRA will not be able to detect the formation of biofilm (Pantanella *et al*., 2013). Nevertheless, some researchers also reported that CRA is an early detection test of biofilm formation which is quite simple, inexpensive and easy to work (Hassan *et al*., 2011; Pantanella *et al*., 2013).

Staphylococcus aureus has a variety of virulence factors, including extracellular toxins and products of surface proteins that facilitate tissue colonization, evasion of the immune system, and tissue damage. The production of these virulence factors are controlled by the network of regulator genes, such as *agr* and *SarA*, which coordinates the expression of genes appropriate for different stages of infection (O'Gara, 2007). In addition to acute disease, *S. aureus* can cause chronic infections, particularly those mediated by the ability of these pathogens to attach to medical devices and form biofilm. Growth of *S. aureus* in a biofilm requires an adaptive response by the organism (O'Gara, 2007). Biofilm formation capacity is an important virulence factor in determining staphylococci infection related medical

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equipment. Production of polysaccharide adhesion, or often called polysaccharide intercellular adhesion (PIA) or polymeric N-acetyl-glucosamine (PNAG) in *Staphylococcus* is synthesized by enzymes encoded *ica* operon, now widely understood as basis mechanism of biofilm formation related infection (O'Gara, 2007).

In this study, the data revealed that *icaA/D* were present in any isolate of MRSA and MSSA, regardless of the biofilm production capability, except in one MSSA isolates. As shown in Table 2, 100% of MRSA isolates demonstrated the presence of *icaA/*D gene, however 90% of those isolates do not have the ability to form biofilm on the polysterene surface, and only 1 isolates (10%) was able to form biofilm in the category of weak biofilm formers. Meanwhile, one of the MSSA isolates did not have both *ica*A and *ica*D genes, but demonstrated capability of biofilm production. Importantly, MSSA isolates in this study which proven not harbouring *mec*A gene, mostly *ica*A/D gene were present, and they were able to form thick biofilms. Our finding reported that on the majority of MRSA isolates, the presence of *ica* genes do not support the biofilm-forming capacities. Moreover, the observation of majority MSSA isolates revealed that the presence of *ica* genes support the formation of biofilms, which was considered as strong biofilm producer. This data is in accordance with the previous study reported the important differences in the mechanism and regulation of biofilm formation in clinical isolates of MRSA and MSSA (O'Gara, 2007; O'Neil *et al*., 2007). Regulation of biofilm formation in MRSA isolates is mostly via an *ica*-independent mechanism, whereas the MSSA isolates use *ica*-dependent and PIA-mediated biofilms mechanism more often (O'Gara, 2007). Lasa and Penades (2006) first reported the regulation of biofilm formation by *S. aureus* that produced more dominant matrix proteins, which is governed through gene regulation by *bap* (*biofilm associated protein*). This protein component will play a role in the initiation of attachment and biofilm maturation process. The release of extracellular DNA (eDNA) and cell surface expression of a number of sortase anchored proteins such as the fibronectin binding proteins, and the major autolysin have been implicated in the colonization of surfaces and biofilm accumulation of MRSA (McCarthy *et al*., 2015). Acquisition of methicillin resistance appears to repress polysaccharide-type biofilm production and promote formation of proteinaceous-type biofilms (Pozzi *et al*., 2012)

Several studies have reported that the prevalence of MRSA isolates to form biofilm vary greatly. Study in Scotlandia (Smith *et al*., 2008) reported 74% of MRSA isolates and 71% of MSSA isolates were able to form biofilm. Additionally, O'Neil (2007) stated that in standard laboratory using BHI medium, 98% of MSSA isolates and only 8% of MRSA were detected as biofilm-producing strains. The current study using MRSA and MSSA isolates from Yogyakarta reported only 10% of our MRSA isolates were capable of biofilm development, whereas 100% of MSSA isolates revealed as strongly biofilm producer. Although majority of staphylococci showed harbouring *ica* operon, expression of the *ica* operon and biofilm production is tightly regulated depending on the environmental conditions (Rachid *et al*., 2000; O'Gara, 2007). Various signals from the environment, such as CO_2 concentration, antibiotic exposure, anaerobic conditions, glucose levels, and osmotic stress, can alter the regulation of *ica* operon expression and biofilm formation (Rachid *et al*., 2000).

Antibiotic resistance of MRSA caused by the presence of the *mec*A gene on the bacterial chromosome which is part of the staphylococcal cassette chromosome mec (SCCmec), a mobile DNA element and its length approximately 21-60 kb (Chambers, 1997). In this study, the data demonstrated that MRSA isolates showed a higher level of resistance than MSSA isolates. Although MRSA isolates in this current study mostly are still sensitive to chloramphenicol and cotrimoxazole, treatment of this infection in clinical setting should consider the clinical syndromes associated with MRSA disease, including skin and soft tissue infections (SSTI), bacteremia and endocarditis, pneumonia, bone and joint infections and central nervous system (CNS) infections. Vancomycin has been the mainstay of parenteral therapy for MRSA infections, other agent empirically used are linezolid, tetracycline, trimethoprim/sulphamethoxazole, clindamycin and rifampin plus fluoroquinolones (Liu *et al.*, 2011). Furthermore, only 10 to 20% of MSSA tested in this study are resistant to penicillin and cefixime. Aminoglycosides, fluoroquinolones, third and fourth generation of cephalosporins, carbapenem and cotrimoxazole are still effective for MSSA treatment. However, published guideline for MSSA management was penicillinase-resistant semisynthetic penicillins, such as flucloxacillin, first-generation cephalosporins, such as cefazolin and the cyclic lipopeptide daptomycin (Corey, 2009). Kaddora (2010) reported that the observed MRSA has a high resistance to erythromycin, ciprofloxacin, levofloxacin and still sensitive to gentamicin, trimethoprim/sulfamethoxazole and tetracycline. As presented by Zabielinski *et al.* (2013), compared with MSSA, MRSA isolates are more sensitive to ciprofloxacin, while MSSA is more resistant to ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole. The high prevalence of MRSA resistance against many antibiotics occurred through the presence of a region sequence among SSCmec gene, which is the insertion sites of genes encoding antibiotic resistance, including transposons, plasmid and chromosomal cassette rekombinanase (ccr); facilitating easiness for MRSA isolates carrying resistance to various classes of antibiotic (Chambers, 1997).

The data of this study support the theory argued by previous researcher (Pozzi *et al*., 2012) that antibiotic resistance due to the presence of *mecA* gene, would weaken the virulence of MRSA in the attachment to the host cell or biomaterial surfaces, in this case related to biofilm formation. Our finding reported that compared with MSSA, MRSA isolates were notable to form biofilm and the presence of *ica* genes may not be expressed because of repression by PBP2a, the protein product of the *mecA* gene; either directly or indirectly through regulation by other genes such as *agr* gene (Pozzi *et al*., 2012). This

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situation will provide benefits for MRSA to adapt in clinical situations where antibiotic exposure occur excessively. Subsequently MRSA must take into account the energy for metabolism and choose the mechanism that should be prioritized to survive in a threatening situation (O'Neil *et al*., 2007; Pozzi *et al*., 2012).

CONCLUSION

In summary, we showed here that our clinical MSSA strains were more likely to produce an *icaAD*-dependent biofilm, while MRSA strains preferred to express the antibiotic resistance rather than biofilm formation phenotype that might be benefit to achieve successful adaptation to a specific niche within the hospital setting with abundant antibiotics exposure.

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