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Detection of *ica*AD Gene of Biofilm-Producing *Staphylococcus aureus* Carriage Isolates Obtained from Health Care Workers and Healthy Communities in Banyumas, Indonesia

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Abstract

Background: Asymptomatic biofilm-producing *Staphylococcus aureus* carriage play a pivotal role as a reservoir pathogen and increase the transmission rate in hospital as well as in healthy community. Biofilm- producing *S. aureus* which is regulated by the the *ica* AD gene reduce the antimicrobial ability in eliminating the pathogen.

Objective: The aim of this study was to detect the *ica*AD gene of biofilm-producing *Staphylococcus aureus* carriage isolates obtained from healthcare workers and healthy Community in Banyumas, Indonesia.

Methods: This descriptive observational study enrolled 60 healthcare workers and 60 healthy communities in Banyumas district. Antibiotic susceptibility test was using disc diffusion according to Clinical laboratory Standard Institute (CLSI) 2019. Biofilm-producing ability identified by using microtiter plate biofilm assay and the positivity of *ica*AD gene was performed by using PCR method.

Results: The results showed that one of 60 healthcare workers (0,017%) showed MRSA, four of 60 healthcare workers (0,07%) were MSSA and 2 samples from community (0,03%) were MSSA. Total of 7 samples underwent biofilm examination, one sample was moderate biofilm, two samples were weak biofilm, and four samples were no biofilm. It was known that three biofilm-producing *S.aureus* were positive *ica A/D* gene.

Conclusion: The *ica A/D* gene was found positive in both biofilm-producing MRSA and MSSA strain from both healthcare workers group and the healthy communities group. The presence of icaAD genes in both strains shows the potential for antibiotic resistance in these strains regulated by different mechanisms.

Keywords: Antibiotic resistance; Microtiter plate biofilm assay; MRSA; MSSA; Nasal swab

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INTRODUCTION

Methicillin-Resistant Staphylococcus aureus (MRSA) has become the main pathogen in developing countries and known as one of the Healthcare-associated infections (HAIs) agents as well as a carriage in general healthy community.[1] Asymptomatic MRSA carriage plays a pivotal role as a reservoir pathogen and increases the transmission rate in hospitals as well as in a healthy community.[2] Republic Indonesian ministry of health reported that the use of unprescribed antibiotics in the general community of Indonesia as high as 86,1%.[3] The study of Anjarwati et al. (2010) reported that MRSA carriage in healthcare workers of a government hospital were 25% and 14% in private hospital in Banyumas District, Central Java, Indonesia.[4]

The ability of *S. aureus* to produce biofilm allows this organism to survive from the host's immune response and is considered as the beginning of chronic or persistent infection, by producing this biofilm which protects bacteria from the opsonophagocytic process and antimicrobial agents.[5,6] *ica*A gene and *ica*D gene were reported to have a big role in producing biofilm and have enzymatic activity (N-acetyl glucosaminyl transferase).[7]

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There are few epidemiology data of biofilm-producing *S.aureus* and its correlation with antibiotic resistance mechanisms reported in Indonesia. The study of molecular characteristics and epidemiology of antibiotic resistance mechanisms is important in controlling multidrug resistant- antibiotics (MDR-AB) both in community and hospital. The high use of antibiotics that is not wise in hospitals and the community has the potential to increase the carriage of resistant strains and increase the source of transmission of these strains. Therefore the detection of *icaAD* regulatory genes of biofilm-producing *S.aureus* carriage in health workers and the community needs to be done, especially in areas that have not yet carried out massive control of antibiotic resistance such as in Banyumas district.

MATERIAL AND METHODS

This descriptive observational study was to detect the icaAD gene biofilm-producing *Staphylococcus aureus* carriage isolates obtained from 60 healthcare workers of TK.III Wijayakusuma Hospital, Purwokerto, and 60 healthy individuals in Banyumas, Central Java, Indonesia. The total samples were 120 respondents. The nasal sample was taken by an Amies collection swab after they had informed consent. Sample were taken from the Amies collection swab was then identified by using standard microbiology method. *Staphylococcus aureus* isolate was then tested for antibiotic susceptibility test using disc diffusion according to the Clinical Laboratory Standard Institute (CLSI) 2019.[8] *Staphylococcus aureus* which is identified as an MRSA strain is the bacteria that resistance to 30 µg cefoxitin as a standard antibiotic (the diameter of clear zona surrounding the disc is ≤21mm), while MSSA is the bacteria which has the diameter of clear zona surrounding the disc about ≥22mm.

Biofilm-producing MRSA and MSSA isolate measured by microtiter plate biofilm assay method. After microtiter plate biofilm assay, average Optical Density (OD) was counted. Average OD needs Optical Density cut-off value (ODc) which is obtained from negative control average's OD + 3x control negative's SD.[9] ODc interpretation result then divided into non-biofilm, weak biofilm, moderate biofilm, and strong biofilm.

The next examination was measuring the biofilm regulatory gene, *ica*AD gene, with the PCR method. Isolation of biofilm DNA using Quick DNA Fungal / Bacteria Miniprep Kit (Zymo Research Coorp.) The primer sequences of *ica*A gene Forward was 5'-CCT AAC TAA CGA AAG GA G and Reverse was 5'-AAG ATA TAG CGA TAA GTG C. Meanwhile Primer sequence of *ica*D gene Forward was 5'-AAA CGT AAG AGA GGT G and the Reverse was 5'-AGC AAT ATG ATC AAG ATA C.

The identification of biofilm-producing MRSA was carried out at the Medical Microbiology Laboratory of Jenderal Soedirman University. While the PCR examination of the *ica*AD gene was carried out at the Research Laboratory of Medical Faculty of Jenderal Soedirman University, Purwokerto, Central Java, Indonesia.

This study approved by the health research ethics committee of the medical faculty of Jenderal Soedirman University, Purwokerto. The ethical approval number was Ref: 1491/KEPK/III/2019.

RESULTS

S. aureus that were identified, then being tested for antibiotic susceptibility test using disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) 2019.[10] The results showed that one of 60 healthcare workers (0,017%) showed MRSA, four of 60 healthcare workers (0,07%) were MSSA and 2 samples from community (0,03%) were MSSA.

Biofilm examination to seven samples consist of MRSA and MSSA was done using the microtiter plate biofilm assay method. One sample with a code of 1.20 (sample number: 12), which was an MRSA, showed as a moderate biofilm, while two MSSA samples coded as 1.51 (sample number:13) and 2.44 (sample number: 14) showed as the weak biofilm.

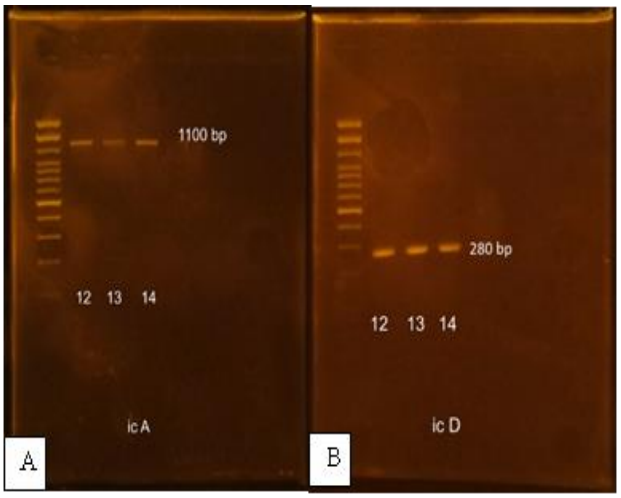

Figure 1. PCR test results of *ica AD* gene (1100 bp/280 bp) found in MRSA and MSSA. No 12 = MRSA 1.20; no 13 = MSSA 1.51; no 14 = MSSA 2.44

Tabel 1. MRSA and MSSA-Producing Biofilm and the positivity of gene *ica*AD of Healthcare worker and General Community

No.	Sampel Code	Source of Sample	Strain	Biofilm	*Ica* A/D
1.	1.9	Hospital	MSSA	Nonbiofilm	
2.	1.15	Hospital	MSSA	Nonbiofilm	
3.	1.20	Hospital	MRSA	Moderate biofilm	(+)
4.	1.50	Hospital	MSSA	Nonbiofilm	
5.	1.51	Hospital	MSSA	Weak biofilm	(+)
6.	2.13	Community	MSSA	Nonbiofilm	

One sample with a code of 1.20 (sample number: 12), which was an MRSA, showed as a moderate biofilm, while two MSSA samples coded as 1.51 (sample number :13) and 2.44 (sample number: 14) showed as the weak biofilm.

The weak biofilm and moderate biofilm bacteria then went through the identification of important gene in the biofilm formation, *ica*AD gene, by using PCR method.

Total sample for PCR examination were three samples. The sample were weak biofilm-producing MSSA (sample code 1.51 and 2.44) and moderate biofilm-producing MRSA (sample code 1.20). PCR result was obtained as seen in Figure 1. *ica*A gene was found positive at 1100 bp and *ica*D was found positive at 280 bp both for MRSA from hospital worker and MSSA from hospital worker and healthy community. MRSA and MSSA-Producing Biofilm and the positivity of gene *ica*AD of Healthcare worker and General Community are shown in Table 1.

DISCUSSION

The examination series in this study showed that out of 120 samples, 7 nasal swab samples had *S. aureus* bacteria isolate. Positive coagulase test results from the general population had a higher number compared to those of healthcare workers. This result is in line with Zhao et al. (2012) who concluded that *Community-Associated Methicillin-Resistant Staphylococcus aureus* (CA-MRSA) and *Community-Associated Methicillin Sensitive Staphylococcus aureus* (CA-MSSA) are higher in general population in relation with antibiotic susceptibility, toxin profile, and genetic factors from the strain involved [12].

The MRSA strain colonization is bigger than other groups, reaching 13,3 percent.[13] In a study by Rongpharpi et al., it was found that MRSA prevalence found in healthcare workers in the tertiary hospital around 11,43 percent. In another study,[15] MSSA strain was found in 33 samples (14.7 percent) out of 147 samples in the healthcare workers group in Pakistan. The MSSA strain was found in 24.2 percent out of 174 samples from immigrants in New York.[16] The MSSA strain found in our study was a little bit less than the study in Pakistan and New York because the coverage of our study was limited in one group of hospitals and healthcare workers, while in Pakistan and New York the studies had a wider coverage of samples.

MRSA sample which had moderate biofilm results was from a healthcare worker. This result is in line with the study of John et al., where they also had 35 (16.76 percent) MRSA samples out of 209 total samples from culture isolate and they found that those samples had moderate and strong biofilm with tissue culture plate method based on optical density values. MRSA sample also showed biofilm results in 595 nm wavelength with detailed result as following: 37 percent strong biofilm, 49 percent moderate biofilm, and 13 percent weak biofilm from nasal swab, and catheter samples, and 13 percent weak biofilm in sample taken from nasal swab, and catheter of patients hospitalized in Poland.[18] MRSA strain is related to high mortality and morbidity rate related to the multi-drug resistance phenomenon and the ability of bacteria to produce a biofilm.[17,19] Bacteria-producing biofilm can cause antibiotic resistance by the development of biofilm itself who initiated by a free moving bacteria called planktonic bacteria that attach to a surface and covered by a layer, such as proteins. The next stage is bacteria can multiplicate and causing irreversible to the surface and forming microcolonies that can produce polymer matrix. The biofilm becomes thick and causing the resistance of antibiotics.[20]

*Ica*AD gene significantly related to MRSA strain which has a higher ability to produce biofilm compared to MSSA strain. The low level of biofilm production in MSSA which is marked by weak biofilm result at ODc reading associated with the low number of MSSA strains harboring *ica*AD genes compared to that of MRSA.[18]

At Present, the standard methods available are used for AST in planktonic form and have a potential misinterpretation of the antimicrobial resistance in bacterial biofilm form. The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the conventional antibiotics to bacterial biofilm may increase up to 100-1000 times higher than planktonic form.[20] Therefore, it is important to investigate the method for measuring the antimicrobial resistance of biofilm-producing bacteria because the AST method can only be done only on planktonic bacteria. The limitation of this study was this study did not report an examination of the presence or absence of the *mec*A gene in the MRSA strain.

CONCLUSION

This study concluded that in the carriage of MSSA and MRSA strains from both healthcare workers and the health community group was found *ica*AD gene, the regulator genes of biofilm formation. Biofilm-producing bacteria can protect themselves from host immunity and antimicrobial agents. Therefore, biofilm-producing MSSA strains can also be resistant to antibiotics.

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