Profile of Biofilm-Producing *Staphylococcus epidermidis* from Intravenous Catheter Colonisation at Prof. Dr. Margono Soekarjo Hospital Purwokerto

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Info Article	ABSTRACT
	Staphylococcus epidermidis developed to be a significant human pathogen due to the ability to produce biofilm. The most significant infections of *S.epidermidis* found in the use of medical devices such as an intravenous catheter. Furthermore, the biofilm is more resistant to antibiotics up to 1000 times more than free-bacteria. This simple survey aimed to describe the profile of biofilm-producing *S. epidermidis* from intravenous catheter colonisation of some patients in surgical and internal medicine wards at the Margono Soekarjo hospital, Purwokerto, and the antibiotics resistance pattern. A vitek® 2 compact (Enseval Medika Prima) was performed to identify the bacterial species and to examine 73 antibiotics for understanding the resistance pattern automatically. The optical density (OD) representing the ability of *S. epidermidis* to produce biofilms was measured by Microtiter plate biofilm assay with crystal violet staining. A scanning electron microscopy was done to compare the thickness of the ultrastructure of biofilm-producing *S. epidermidis* visually. The present study found that 2 of 8 Gram-positive bacteria (25%) were biofilm-producing *S. epidermidis*. One of *S. epidermidis* was moderate whereas the other was high biofilm-producing bacteria. Images of SEM showed that a high biofilm-producing *S. epidermidis* has a thicker ultrastructure of biofilm than the moderate biofilm-producing, whereas a control, the weak biofilm-producing *S. epidermidis* ATCC 12228 has the least biofilm. Both of *S. epidermidis* strains were sensitive to Gentamicin, Moxifloxacin, Quinupristin/Dalfopristin, Linezolid, Vancomycin, Doxycycline, Minocycline, Tetracycline, Tigecycline, and Nitrofurantoin. Furthermore, both S. *epidermidis* strains were resistant to the other (63) antibiotics. In conclusion, two strains of *S. epidermidis* in this study have different capabilities to form the biofilm which showed that high biofilm-producing strain was thicker than moderate biofilm-producing strain by scanning electron microscopy. However, both of them were resistant to the same number of antibiotics. **Key words:** Biofilm, intravenous catheter, colonisation*, S. epidermidis*

INTRODUCTION

Medical devices-associated infection potentially cause some severe clinical problems. In general, phagocyte activity and polymorpho-nuclear leukocytes decrease in the use of invasive medical devices. For that reason, when microorganisms have a chance to reach medical devices surface, they will be attached, and biofilm formation can arise (Gottenbos *et al*., 2002). The use of invasive medical devices such as intravenous catheters is a risk factor for *Staphylococcus sp.* particularly *Staphylococcus epidermidis* in growing up in a biofilm stage (Fitzpatrick *et al.,* 2005).

MATERIAL AND METHODS
Bacterial species and antibiotics susceptibility

The bacterial strains were isolated from 39 proximal tips of the intravenous catheters from the patients in surgical and internal medicine wards of Prof. Dr Margono Soekarjo hospital, Purwokerto, on July-August, 2014. Isolates were taken from the piece of the intravenous catheter proximal tips at the catheter removal process aseptically. The proximal catheter tip is the site of catheter insertion in the blood vessels of a patient who can act as a port d'entry of infections (O'Grady *et al.,* 2011). Isolates were not taken from other parts of the intravenous catheter such as the urine bag because they were directly related to the vein and were sterile.

A vortex and viable count method were performed to measure intraluminal and extraluminal biofilm (Donlan and Costerton, 2002). Briefly, about 3 cm of the proximal tip of the intravenous catheter in the sterile aquadest was vortexed for 2min. The isolates were cultured on the blood agar (OXOID) plate at 37°C for 24h (overnight culture). A vitek® 2 compact (Enseval Medika Prima) was performed to identify the bacterial species and to examine the antibiotics resistance. Antimicrobial Susceptibility Testing (AST) done by a vitek® 2 compact (Enseval Medika Prima) be able to used to predict how pathogens will respond to particular antimicrobials automatically (Blondel-Hill *et al.*, 2006). Therefore, data obtained by using this tool are data about bacterial species and data of antibiotics sensitivity along with its MIC value**.**

As many as 73 antibiotics tested include Cefoxitin, Flomoxef, Latamoxef, Benzylpenicillin, Nafcillin, Amoxicillin, Ampicillin, Carbenicillin, Ticarcillin, Azlocillin, Mezlocillin, Piperacillin, Piperacillin/Tazobactam, Cloxacillin, Dicloxa-cillin, Flucloxacillin, Methicillin, Oxacillin, Cefaclor, Cefadroxil, Cefalexin, Cefalotin, Cefazolin, Cefeta-met, Cefonicid, Cefprozil, Cefradine, Cephapirin, Loracarbef, Cefamandole, Cefpodo-xime, Cefti-buten, Cefmenoxime, Cefoperazone, Cefotaxime, Ceftazidime, Ceftizoxime, Ceftriaxone, Cefepime, Cefpirome, Doripenem, Ertapenem, Faropenem, Imipenem, Meropenem, Gentamicin High Level, Streptomycin High Level, Gentamicin, Cipro-floxacin, Levofloxacin, Moxifloxacin, Inducible Clindamycin Resistance, Azithromycin, Clarithro-mycin, Erythromycin, Clindamycin, Quinupristin /Dalfopristin, Linezolid, Vancomycin, Doxy-cycline, Cefuroxime, Cefmetazole, Cefotetan, Cefoxitin, Cefdinir, Cefditoren, Cefixime, Mino-cycline, Tetracycline, Tigecycline, Nitrofurantoin, Rifampicin, Trimethoprim / Sulfamethoxazole. The isolates stored in Tryptic Soy Broth (TSB, OXOID) and 15% glycerol in - 80°C.

Biofilm assay

Biofilm optical density (OD) was measured by using microtiter plate biofilm assay with crystal violet staining, as done previously with some modification (Anjarwati *et al.,* 2017; Merritt *et al.,* 2011). The values of OD describe an index of biofilms formation. Briefly, after a dilution (1:100) in fresh Tryptic Soy Broth, the bacteria from the overnight cultures in Tryptic Soy Broth and the 20μL of cultures were inoculated into fresh Tryptic Soy Broth (200μL) in each well. After 24h of incubation at 37°C, each well was washed off three times by using phosphate buffer saline (PBS), OXOID, pH 7.3 to remove planktonic bacteria. Then the adherent bacteria stained with 100μL 1% crystal violet. After which, 5% acid Isopropanol (5% v/v 1M HCl in 2-propanol) added in each well. The absorbance at an appropriate range wavelength (630nm) was determined to measure the stained biofilms quantitatively by using a microplate reader (ELX800, Bio-Tek instruments).

Scanning electron microscopy

Scanning electron microscopy was implemented to compare the thickness of biofilm-producing *S. epidermidis* strains ultrastructure visually. Briefly, *S.epidermidis* ATCC35984 from the overnight cultures in TSB then diluted in TSB (1:100). Moreover, as much as 50μL cultures inoculated into 13mm sterile coverslips (Thermo Scientific Nunc), which laid on the bottom of 6 wells plate with TSB (5mL) in each well. Each well was washed three times with PBS to eliminate free-cells following 24h incubation (37°C). Furthermore, the cover glass was immersed in 70% methanol for 20min at room temperature for fixation; then, it was dried overnight at room temperature. Finally, the dry cover glasses were coated twice with platinum vanadium by using sputter ion. Before observed by using SEM, the cover glasses attached to the double-side tape carbon (Anjarwati *et al.,* 2017; Setiawan *et al.,* 2012)

Data analysis

Profile of biofilm-producing *S. epidermidis* in this study was analysed descriptively using the univariate method. The interpretation of the

biofilm production has used a formulation of optical density cut-off value *(ODc)* = average OD of negative control + 3 x SD of the negative control. The criteria *:* Non/weak ≤ ODc / ODc < ~ ≤ 2x ODc ; Moderate 2x ODc < ~ ≤ 4x ODc ; high > 4x ODc (Hassan *et al.,* 2011). Meanwhile, the MIC value of each antibiotic tested was obtained automatically from the vitek® 2 compact results.

Ethics

This study approved by the health research ethics committee of the medical faculty of Diponegoro University and Kariadi hospital, Semarang.

RESULTS AND DISCUSSION

In the present study, the isolates were taken from 39 proximal tips of the intravenous catheter of patients in the surgery and internal medicine wards of Prof. Dr Margono Soekarjo hospital, Purwokerto. After vortex and viable count method, species identification done by a vitek® 2 compact as well an antibiotic sensitivity test. To determine biofilms optical density (OD), a microtiter plate biofilm assay by using staining of crystal violet was performed. Then, the thickness of biofilm ultrastructure compared by using SEM visually.

S. epidermidis found was 2 of 8 Gram-positive bacteria (25%). The type of all bacteria has published as a poster in a previous 9th National Symposium of IARW and annual scientific meeting of Indonesian Society for Clinical Microbiology. The other bacteria were *S. haemolyticus* (1), *S. hominis* (2), *E.faecium* (1), *K. rosea* (1), *L mesenterdfghgdfhoides* (1) (Anjarwati *et al.,* 2014). This paper present mainly the potency of *S. epidermidis* to produce biofilm. Both *S. epidermidis* were biofilm-producing bacteria, one of them was moderate (Se2) whereas the other was a high (Se1) biofilm-producing bacteria. The ODc value in this study was 0.12*.* A moderate biofilm-producing bacteria has the optical density range of 0.2422 <...≤ 0.4844. Meanwhile, a high biofilm-producing bacteria has an optical density of more than 0.4844. This study found differences in optical density between Se1 and Se2 biofilms (Figure 1).

Furthermore, SEM showed the different thickness of the biofilm ultrastructure (Figure 2). High biofilm-producing *S. epidermidis* consist of more layers biofilm than moderate biofilm. Meanwhile, the control bacteria, *S. epidermidis*

ATCC 12228, a weak biofilm-producing bacteria showed only a small number of biofilm.

In this simple survey by using vitek® 2 compact automatically reported that both *S. epidermidis* (100%) sensitive to Gentamicin, Moxifloxacin, Quinupristin / Dalfopristin, Linezolid, Vancomycin, Doxycycline, Minocycline, Tetracycline, Tigecycline, and Nitrofurantoin (Figure 3). Whereas both of *S. epidermidis* (100%) was resistance to the other 86.3% (63 of 73) antibiotics.

This study presents the profile of biofilm-producing *S. epidermidis* (Figures 1 dan 2). The results indicated that in this study biofilm-producing *S. epidermidis* were found as colonisation on the intravenous catheter. Colonisation is a critical point of medical devices associated with infection. Additionally, the fragile steadiness of colonisation and infection is frequently sustained for months (Fux *et al.,* 2009)*. As a biofilm-producing bacteria, S. epidermidis* then can adhere to a surface in the initiation phase, followed by formation of microcolonies, which grow into a mature biofilm structure to disperse phase (Agarwal *et al.,* 2010).

There are no standardised procedures thus far used regularly to define the antibiotic sensitivity of biofilms-producing bacteria (Estela and Alejandro, 2012). In some cases, antibiograms showed susceptibility against some microorganisms from medical devices, but that medical devices associated infection fail to be eliminated by the same antibiotics (Fux *et al.,* 2005). In this study, the antibiotic sensitivity test done by a vitek® 2 Compact. The sensitivity test and identification of species carried out automatically at the same time. The advantages of this tool are practical and useful in detecting species. However, sensitivity test data obtained for species that are not pathogenic was incomplete, such as data of MIC. Both *S. epidermidis* strains, either it produces a moderate or a high biofilm, are resistant to the same antibiotics.

This study revealed that two strain of *S. epidermidis* (100%) sensitive to Gentamicin (aminoglycoside), Moxifloxacin (quinolone), Quinupristin/ Dalfopristin (streptogramin), Linezolid (oxazolidinone), Vancomycin (glycopeptide), Tigecycline (glycylcycline), Nitrofurantoin and three kinds of tetracycline (Doxycycline, Minocycline, Tetracycline), but resistant to other 63 of 73 (86.3%) antibiotics.
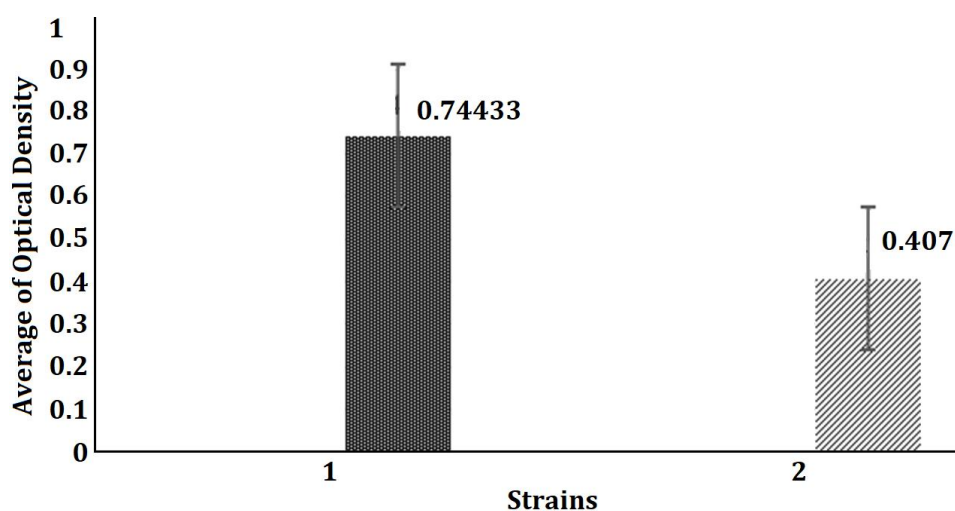
Figure 1. Biofilm-producing *S. epidermidis* (1= strain 1 of *S. epidermidis* (Se1), High biofilm-producing *S. epidermidis,* 2 = Strain 2 of *S. epidermidis* (Se2), moderate biofilm-producing *S. epidermidis*) ODc = 0,12 (Moderat : 0.2422 < .... ≤ 0.4844; High : > 0.4844).
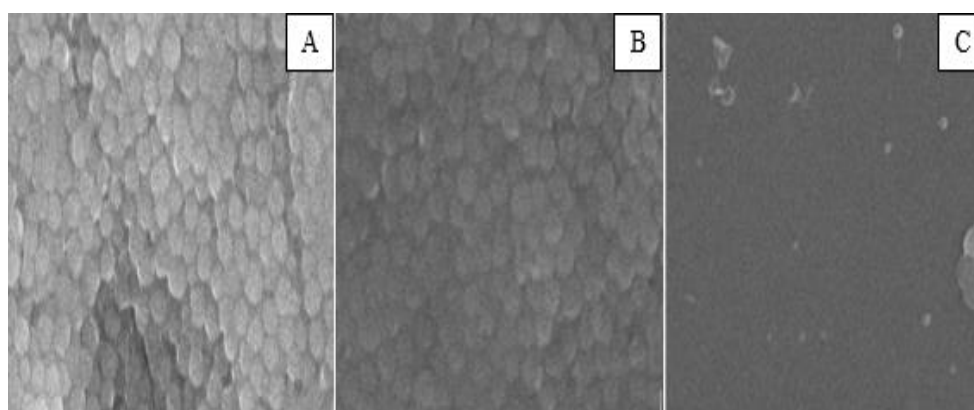

Figure 2. Images of SEM. A. High biofilm-producing *S. epidermidis* (Se1); B. Moderate biofilm-producing *S. epidermidis* (Se2); C. Weak biofilm-producing *S. epidermidis* ATCC 12228. Magnification: 20.000 X.

The antibiotics resistance mechanisms of biofilms and planktonic bacteria are different. The antibiotics resistance mechanisms in planktonic bacteria consist of efflux pumps, modifying enzymes, target mutations outer membrane impermeability (Estela and Alejandro, 2012; Poole, 2002). Meanwhile, biofilm-producing bacteria has been presented to have their intrinsic mechanisms of resistance. Multi mechanisms suggested to account for antibiotic resistance of biofilm bacteria such as the low or incomplete penetration of the antibiotics into biofilm, a chemical micro environment alteration within the biofilm, counter balancing enzymatic, functional hetero-geneity, slow growth and persistent cells and the modification of biofilm phenotype due to adaptive mechanisms to stress (such as efflux pumps and alterations in the membrane). Consequently, these mechanisms should be measured to explain therapeutic failure in the management of patients for whom laboratory outcomes show proper sensitivity patterns (Estela and Alejandro, 2012).

Data of infections caused by biofilm-producing bacteria particularly in the use of invasive medical devices have not been found widely in Indonesia. Studies and publications associated with these infections nationally

Table I. Proper sensitive antibiotics to both biofilm-producing *S. epidermidis* strain

Antibiotics	Sensitivity	MIC
		S. epiermidis
Gentamicin	S	≤0.5
Moxifloxacin	S	2
Quinupristin/Dalfopristin	S	≤0,25
Linezolid	S	1
Vancomycin	S	1
Doxycyclin	S	-
Minocycline	S	-
Tetracycline	S	2
Tigecycline	S	≤0.12
Nitrofurantoin	S	≤16

S = sensitive; The table performs 10/73 (13.7%) antibiotics which have proper sensitivity to both of biofilm-producing *S. epidermidis. Meanwhile, both strains* were 100% (2/2) resistant to others (63 antibiotics) ; MIC value obtained automatically from vitek® 2 compact.

need to be improved. The difference between the resistance mechanisms of planktonic bacteria and biofilm bacteria is an interesting point for researchers to investigate this phenomenon.

CONCLUSION

Two clinical strains of *S. epidermidis* from the patients in surgical and internal medicine wards at Prof. Dr Margono Soekarjo hospital, Purwokerto have different capabilities to form the biofilm which showed that high biofilm-producing strain was thicker than moderate biofilm-producing strain by scanning electron microscopy. However, both of them were resistant to the same number of antibiotics.

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