

# Antimicrobial susceptibility and the pattern of a biofilm-forming pair of organisms from patients treated in intensive care units in Dr. Soetomo General Hospital, Indonesia



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## ABSTRACT

**Background:** Biofilm-associated microorganisms can cause diseases by attachment to individual cells or groups of cells on the medical device surface. The organisms may grow resistant to antibiotics. These microorganisms can be prokaryote or eukaryote organisms existing in one of two forms: sessile or planktonic. The treatment of device-associated infections with a systemic antimicrobial agent is usually ineffective.

**Purpose:** To find the pattern of biofilm-forming organisms and the antimicrobial susceptibility from medical devices attached to patients, so that the therapeutic management can be more accurate and useful.

**Method:** From 86 specimens that were analyzed, only 36 specimens showed organism growth and ability to form a biofilm. From 36 isolates analyzed for the ability to form a biofilm, only 30 isolates were

in planktonic and sessile form, which were then identified and tested for antimicrobial susceptibility because the density produced was greater or equal to 0,4 (cutoff  $\geq 0,4$ ).

**Result:** Planktonic *Escherichia coli* from urinary and intravenous catheters matched 100% with the sessile form on the devices, as well as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Gram-positive cocci found was *Staphylococcus aureus*. Planktonic *Staphylococcus aureus* from urinary and intravenous catheters matched 100% with the sessile form on the devices.

**Conclusion:** When the form of plankton is not found, it is not necessarily that there is no form of sessile. The antimicrobial chosen to treat the patient should match the antimicrobial susceptibility test based on the planktonic and sessile forms of biofilm.

**Keywords:** Microorganism pattern, antibiotic, susceptibility, planktonic-sessile.

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## INTRODUCTION

According to the World Health Organization, infectious diseases are the second most common cause of death in the world, causing 13 million deaths per year. The National Institutes of Health of the US estimated that over 60% of microorganism infections were associated with biofilm.<sup>1</sup>

Biofilm is a growth of cells in a matrix by optimization of existing nutrient sources and enzyme formation, where the environment and enzyme activities constantly change and eventually result in good conditions for the growth of cells. Although there are many components in biofilm, host contributions to microorganisms, such as immunologic components and physical status give impact to biofilm structure. Some environmental conditions and characteristics lead to the selection of biofilm multispecies.<sup>2</sup>

Bacterial resistance to antibiotics usually means that bacteria can grow in the presence of certain antibiotics. Tolerance, on the other hand, refers to a situation where antibiotic therapy cannot eliminate bacteria. Both resistance and tolerance can contribute to the growth of biofilm, which enables

bacteria to live in the presence of antibiotics with 1000 times the normal killing concentration.<sup>3,4,5</sup> In other words, the underlying mechanism of biofilm resistance is multifactorial. There has not been an adequate explanation of the basic mechanism of biofilm-forming resistance or tolerance to antibiotics. Limitation of penetration is possibly due to a matrix called Extracellular Polymeric Substances (EPS) which is constantly produced, creating new binding sites for antimicrobial agents.<sup>5-8</sup>

Another factor in biofilm resistance is the complex metabolic activities of cells in the biofilm. In a biofilm, there is a gradient of nutrition and oxygen, which limits the growth of most microorganisms except the cells on the biofilm surface. Usually, the target of antimicrobial agents are metabolically active cells. Thus, the cells inside the biofilm cannot be the target.<sup>6,8</sup> Antibiotic resistance can also occur because of the expression of certain genes in the biofilm.<sup>6,8,9,10</sup>

Microbes in biofilm expand. Moreover, the biofilm itself produces an extracellular polymer

which facilitates microbe attachment and provides a structural matrix. Surfaces for microbe attachment can be immobile parts of living or dead tissues. Microorganisms in a biofilm are different from planktonic (free moving) microorganisms because they can grow and survive in the presence of antibiotics, thus becoming a health problem in society. Biofilm can grow and expand inside medical devices such as soft lenses, central venous catheters (CVC), needleless connectors, endotracheal tubes, intrauterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, tympanostomy tubes, urinary catheters, and voice prostheses.<sup>11</sup>

Although biofilm has been a challenging issue in the field of microbiology, there has not been a standardized method used to analyze biofilm formation. Some quantitative and qualitative methods have been used to evaluate certain aspects of biofilm.<sup>12</sup> Microtiter plate assay (MPA) is a quantitative test introduced by Christensen *et al.* which has become a gold standard in biofilm detection.<sup>13</sup> A micro ELISA auto reader determines the Optical Density (OD) of the microorganisms attached to the well and stained by crystal violet. The result is a change of color which indicates biofilm production.

By detecting the presence of biofilm on patients' medical devices, management of patients with biofilm will be different from those without. Through its detection, we can avoid bacterial resistance to antibiotics, especially in patients suffering from infectious diseases.

## MATERIALS AND METHODS

This research aims to detect a pattern of biofilm-forming microorganisms from medical devices of patients in intensive care wards of Dr. Soetomo General Hospital, Surabaya, Indonesia, and to test these microorganisms for antibiotic sensitivity. The study was an observational study using consecutive sampling.

The inclusion criteria are medical devices that had been detached from patients treated in the Intensive Care Unit (ICU) and the Intensive Observational Unit (IOU) in the hospital, the detachment of which occurred in June or July 2016, and the detachment having been executed complying with the recommended duration of use recorded in the hospital standard operational procedures. The exclusion criterion was medical devices detached from deceased patients.

Isolates were taken by culturing medical devices. The medical device was cut 5-7 cm and put into brain heart infusion agar (BHI) 10 ml, incubated

for 24 hours, and sent to the Clinical Microbiology Laboratory in the same hospital. The culture and the detection of biofilm-forming microorganisms were done in the Clinical Microbiology Laboratory. An OD examination was done with an ELISA reader in the Institute of Tropical Disease of Universitas Airlangga, Surabaya, Indonesia. The interpretation of the microbiological examination was made by the MPA method to assess the ability of the microorganism to form a biofilm. The MPA method includes the staining of the biofilm with crystal violet. The OD of biofilm attached to the well and stained with crystal violet was assessed with a micro ELISA auto reader. An identification and a sensitivity test were done by Biomerieux Vitek 2 System. Microorganisms isolated from medical devices were also tested for antibiotic sensitivity.

## Procedure

An isolate was immersed into tryptic soy broth (TSB) medium with the addition of 1% glucose (TSBglu) and incubated for 24 hours at 37°C. It was then diluted 1:100 with a new medium. Each sterile 96 well polystyrene was filled with 0.2 ml of the diluted culture. TSBglu without isolate was used as negative control, and TSBglu with isolate was used as positive control for the sterility test and nonspecific binding test of media.

Tissue culture was incubated for 24 hours at 37°C. After the incubation, the plate was tapped and then washed four times with 0.2 ml phosphate buffer saline (PBS) pH 7.2 to remove planktonic bacteria. Biofilm formed by microorganisms attached to the plate (sessile) will bind sodium acetate (2%) which was stained by crystal violet (0.1% w/v). The stain was then washed with ionized water and was allowed to dry. The attached cells usually formed a biofilm. The plate was washed with PBS once more and then immersed in ethanol for 15 minutes. The OD of the attached and stained microorganisms was determined by a micro ELISA auto reader (mode PR 601, quiklinger S) at 630 nm wavelength (OD 630 nm). The OD value was made based on the index of microorganism attachment on the surface and the biofilm formation.

The basic principle of MPA assessment was based on crystal violet staining which specifically assesses microorganism attachment (sessile) on the wall and the bottom of the microtiter well. The test was repeated three times to obtain an optimal result. The interpretation of biofilm production is: negative if  $\leq 0.2$ , moderate if 0.2-0.4, positive if  $\geq 0.4$ . Our study interpreted the result as positive when the MPA was moderate or positive. The isolate in each well

was examined with three tests. The purpose was to obtain the sessile and the planktonic form to be identified and tested for antibiotic sensitivity.

Test 1. After dilution and incubation, the liquid in the well was not discarded. Instead, it was aspirated and isolated on Mueller Hinton agar. It was then identified and tested for antibiotic sensitivity. This isolate was considered as planktonic.

Test 2. The liquid in the well was not discarded. Before it was stained with crystal violet, the wall and the bottom of the well were swabbed. The swab was then inoculated on Mueller Hinton agar to isolate microorganisms, which would be identified and tested for antibiotic sensitivity. This isolate was considered as sessile.

Test 3: The last method was to assess OD by an ELISA auto reader.

## The Identification and Antimicrobial Sensitivity Test

The identification and sensitivity test were done with the BioMérieux Vitek 2 System. The wall and the bottom of a microtiter 96 well were swabbed and then inoculated by streaking the swab on Mueller Hinton agar and incubated for 24 hours. A loopful of the colony was then standardized and diluted to be put in a cassette. The sample number was registered into a computer through a barcode reader. The Vitek 2 system read the barcode number which represented patient ID. A cassette was inserted in the filler module. The instrument performed the identification and sensitivity test.

## RESULTS

There were 86 collected specimens. Only 36 out of 86 grew microorganisms and were further tested for biofilm formation by the microtiter plate assay method. Only 30 planktonic and sessile forms out of 36 isolates tested for biofilm formation gave an optical density over 0.4 (cutoff point  $\geq 0.4$ ). These isolates were then identified and tested for antibiotic sensitivity.

The patient age ranged from 1 month to 79 years old. The specimens were taken from an equal number of males and females (18 patients each).

Based on device type distribution, the most common devices acquired from Intensive Observation Unit (IOU) and ICU were 25 IV lines (69.4%), 7 urinary catheters (19.4%), 2 CVCs (5.6%) and 2 ETTs (5.6%). The distribution of device types which grew microorganisms can be seen in [Table 1](#).

[Table 2](#) shows that the duration of attachment on patient depends on the device type. IV line is the most common device found. As many as 20 IV lines were used for 1 to 3 days, 4 for 4 to 7 days, and one for more than 10 days. A urinary catheter was the second most common device used in IOU and ICU. There was one urinary catheter which was used for 1 to 5 days, 4 were used for 6 to 10 days, 2 for 11 to 15 days. Only 2 CVCs were obtained, one was used for 10 days, and the other was used for 14 days. There were 2 ETTs found, both used for 7 days.

The distribution of specimens which grew microorganisms based on the type of ward can be seen in [Table 3](#).

Specimens were taken from patients with different diagnoses. The diagnosis of patients whose devices grew microorganisms after detachment can be seen in [Table 4](#).

Results of microorganism density performed by an ELISA auto reader showed 30 isolates that produce biofilm. These isolates were tested for identification and antibiotic sensitivity. Using the microtiter plate assay method, 9 isolates of

**Table 1** Distribution of examined medical device

Type of Device	Frequency	%
Intravenous (IV) line	25	69.4%
Urinary catheter	7	19.4%
CVC	2	5.6%
ETT	2	5.6%
Total	36	100.0%

**Table 2** The duration of medical device usage

Type of Device	Duration	Frequency
Intravenous line	1 - 3 days	20
	4 - 7 days	4
	8 - 10 days	0
	> 10 days	1
Urinary catheter	1 - 5 days	1
	6 - 10 days	4
	11 - 15 days	2
CVC	10 days	1
	14 days	1
ETT	7 days	2

**Table 3** The distribution based on the type of ward from which the specimens came

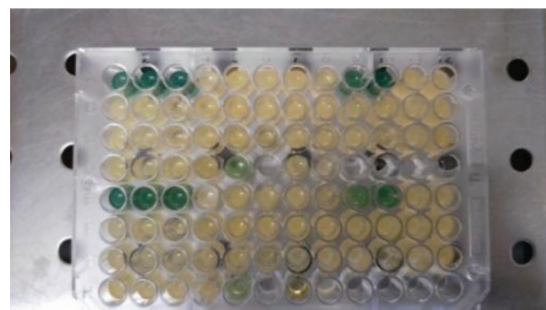
Type of Ward	Frequency	%
Intensive Observation Unit	24	66.7%
Intensive Care Unit	12	33.3%
Total	36	100.0%

**Table 4** The diagnosis of patients whose detached devices grew microorganisms

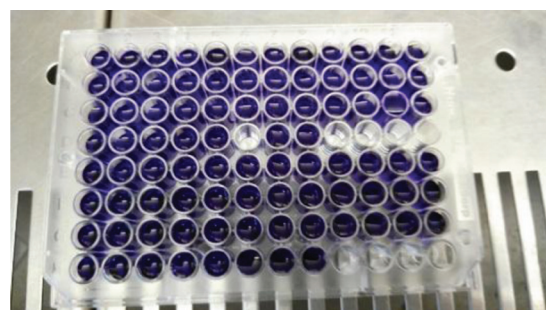
Diagnosis	Frequency	%
Acute lymphoblastic lymphoma	1	2.7%
Acute peritonitis + sepsis	1	2.7%
Atherosclerotic heart disease	2	5.5%
Bronchopneumonia + ASD + congenital mitral stenosis	1	2.7%
Cerebral infarction	1	2.7%
Combustion grade II AB + septic shock	1	2.7%
Congenital heart disease	1	2.7%
Crush injury of thumb and other fingers	1	2.7%
Diagnosis of the intercondylar femur	1	2.7%
Fracture of a bone (open or closed fracture)	4	11.1%
Heart failure	2	5.5%
Hypertensive renal disease with renal failure + peritonitis + sepsis	1	2.7%
Intracerebral hemorrhage	2	5.5%
Liver and gallbladder injury	1	2.7%
Malignant neoplasm of rectosigmoid junction	1	2.7%
Meningoencephalitis	1	2.7%
Mild brain contusion + EDH + fracture of maxilla	1	2.7%
Mitral stenosis	4	11.1%
Other unspecified injury of cervical spinal cord + pneumonia	2	5.5%
Post procedural disorder of nervous system	1	2.7%
Pregnancy with complications	2	5.5%
Pulmonary edema + pneumonia + ischemic cardiomyopathy	1	2.7%
Status epilepticus	1	2.7%
Traumatic subdural hemorrhage	1	2.7%
Unspecified abdominal hernia with obstruction + HIV disease resulting in unspecified infectious disease and parasitic disease	1	2.7%

**Table 5** Biofilm-forming microorganism pattern

Species	Frequency	%
<i>Acinobacter baumannii</i>	1	3.3%
<i>Alcaligenes faecalis ssp faecalis</i>	1	3.3%
<i>Citrobacter farmeri</i>	1	3.3%
<i>Citrobacter freundii</i>	3	10.0%
<i>Escherichia coli</i>	9	30.0%
<i>Klebsiella pneumoniae (ESBL +)</i>	2	6.7%
<i>Klebsiella pneumoniae ssp pneumoniae</i>	1	3.3%
<i>Pseudomonas aeruginosa</i>	2	6.7%
<i>Raoultella ornithinolytica</i>	1	3.3%
<i>Serratia marcescens</i>	1	3.3%
<i>Staphylococcus aureus</i>	6	20.0%
<i>Staphylococcus epidermidis</i>	1	3.3%
<i>Staphylococcus haemolyticus</i>	1	3.3%
Total	30	100.0%



**Picture 1** Microtiter plate assay after incubation



**Picture 2** Microtiter plate assay after adding crystal violet



**Picture 3** Identification and sensitivity test process



**Picture 4** Identification and sensitivity test process

*Escherichia coli* and 6 isolates of *Staphylococcus aureus* were identified. Details of biofilm-forming microorganisms isolated in this research can be seen in [Table 5](#).

This research uses the microtiter plate assay method in which the density value indicates biofilm-forming microorganisms. We obtained 30 isolates of biofilm-forming microorganisms in both planktonic and sessile forms. Gram staining before



**Table 6** Biofilm-forming microorganism pattern in planktonic and sessile forms and Gram stain

Biofilm Form	Gram Stain Result	Species
Planktonic	Gram-Negative Rod	<i>Klebsiella pneumoniae</i> (ESBL +)
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Negative Rod	<i>Klebsiella pneumoniae</i> (ESBL +)
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Negative Rod	<i>Serratia marcescens</i>
Sessile	Gram-Negative Rod	<i>Klebsiella pneumoniae ssp pneumoniae</i>
Planktonic	Sterile	sterile
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Positive Coccus	<i>Staphylococcus haemolyticus</i>
Sessile	Gram-Negative Rod	<i>Citrobacter freundii</i>
Planktonic	Gram Positive Coccus	<i>Staphylococcus epidermidis</i>
Sessile	Gram-Negative Rod	<i>Citrobacter freundii</i>
Planktonic	Gram-Negative Rod	<i>Acinetobacter baumannii</i>
Sessile	Gram-Negative Rod	<i>Raoultella ornithinolytica</i>
Planktonic	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Sessile	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Planktonic	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Sessile	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Planktonic	Gram-Negative Rod	<i>Escherichia coli</i>
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Negative Rod	<i>Escherichia coli</i>
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Negative Rod	<i>Pseudomonas aeruginosa</i>
Sessile	Gram-Negative Rod	<i>Pseudomonas aeruginosa</i>
Planktonic	Gram-Negative Rod	<i>Citrobacter farmeri</i>
Sessile	Gram-Negative Rod	<i>Citrobacter freundii</i>
Planktonic	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Sessile	Gram-Positive Coccus	<i>Staphylococcus aureus</i>
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>
Planktonic	Gram-Negative Rod	<i>Alcaligenes faecalis ssp faecalis</i>
Sessile	Gram-Negative Rod	<i>Escherichia coli</i>

**Table 7** The pattern of antibiotic sensitivity based on biofilm form or Gram stain result

Antibiotic	Planktonic			Sessile			Gram-Negative Rod			Gram-Positive Coccus		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin	9	-	1	11	-	1	20	-	2	-	-	-
Tobramycin	5	1	4	9	0	3	14	1	7	-	-	-
Gentamicin	7	1	6	10	1	4	15	0	7	2	2	3
Astreonom	1	-	3	-	-	-	1	-	3	-	-	-
Amoxicillin-Clavulanic Acid	5	0	5	6	1	5	11	1	10	-	-	-
Ampicillin	4	1	7	5	0	7	9	1	13	0	0	1

Table 7 Continue

Antibiotic	Planktonic			Sessile			Gram-Negative Rod			Gram-Positive Coccus		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin-Sulbactam	-	-	6	-	-	-	-	-	5	-	-	1
Piperacillin-Tazobactam	6	-	3	10	-	2	16	-	5	-	-	-
Oxacillin	-	-	5	-	-	3	-	-	1	-	-	7
Cephazolin	-	-	4	-	-	-	-	-	4	-	-	-
Ceftazidime	4	2	4	5	1	6	9	3	10	-	-	-
Cefotaxime	4	1	5	5	0	7	9	1	12	-	-	-
Ceftriaxone	4	-	5	5	-	6	9	-	11	-	-	-
Cefoperazone-Sulbactam	7	1	2	9	0	3	16	1	5	-	-	-
Cotrimoxazole	3	-	3	3	-	0	0	-	3	6	-	0
Tetracycline	1	-	8	1	-	2	0	-	5	2	-	5
Tygecycline	1	-	-	1	-	-	-	-	-	2	-	-
Imipenem	8	-	2	9	-	3	17	-	5	-	-	-
Meropenem	8	-	2	8	-	4	16	-	6	-	-	-
Ertapenem	3	-	1	-	-	-	3	-	1	-	-	-
Doripenem	5	-	1	9	-	3	14	-	4	-	-	-
Chloramphenicol	1	2	3	-	-	-	1	1	3	0	1	0
Ciprofloxacin	2	-	4	1	-	2	1	-	2	2	-	4
Levofloxacin	6	-	5	9	-	4	15	-	7	0	-	2
Nitrofurantoin	2	1	3	2	0	1	0	0	2	4	1	2
Erythromycin	1	-	4	1	-	2	0	-	1	2	-	5
Clindamycin	1	-	4	1	-	2	0	-	1	2	-	5
Fosfomycin	2	1	1	-	-	-	2	1	1	-	-	-
Penicillin G	-	-	2	-	-	-	-	-	1	-	-	1
Daptomycin	-	-	2	-	-	-	1	-	-	1	-	-
Moxifloxacin	-	-	2	-	-	1	-	-	1	-	-	2
Doxycycline	4	-	2	5	-	7	9	-	9	-	-	-
Quinopristin-dalfopristin	1	-	-	1	-	-	-	-	-	2	-	-
Linezolid	1	-	-	1	-	-	-	-	-	2	-	-
Vancomycin	1	-	-	1	-	-	-	-	-	2	-	-

identification and the sensitivity test revealed Gram-negative rods and Gram-positive cocci. Gram-negative rods were more frequently found compared to Gram-positive cocci. The pattern of biofilm-forming microorganisms in both planktonic and sessile forms can be seen in Table 6.

The pattern of the antibiotic sensitivity test based on biofilm forms (planktonic and sessile) or based on Gram stain result can be seen in Table 7.

There were 22 isolates of Gram-negative rods and 8 isolates of Gram-positive cocci. Based on

Gram staining, there were 22 Gram-negative rods and 7 Gram-positive cocci of biofilm-forming microorganisms. As presented in Table 8, planktonic Gram-negative rods were found more frequently than planktonic Gram-positive cocci (10 vs. 5 isolates). The sessile form of Gram-negative rods was also more frequently found than the sessile form of Gram-positive cocci (12 vs. 3 isolates). Table 9 describes the frequency of bacteria found in devices and duration of device usage in patients.

**Table 8** Number of biofilm-forming planktonic and sessile forms based on the Gram stain result

	Planktonic	Sessile	Total
Gram-negative rod	10	12	22
Gram-positive coccus	5	3	8
Total	15	15	30

**Table 9** Bacteria in planktonic and sessile form relations to medical devices and the usage duration

No.	Planktonic	f	Sessile	f	Device	duration
1	<i>Escherichia coli</i>	3	<i>Escherichia coli</i>	3	catheter	10
					catheter	5
					iv line	2
2	<i>Klebsiella pneumoniae</i> (ESBL +)	2	<i>Escherichia coli</i>	2	iv line	3
					catheter	12
3	<i>Serratia marcescens</i>	1	<i>Klebsiella pneumoniae ssp pneumoniae</i>	1	CVC	14
4	<i>Citrobacter farmeri</i>	1	<i>Citrobacter freundii</i>	1	catheter	6
5	<i>Pseudomonas aeruginosa</i>	1	<i>Pseudomonas aeruginosa</i>	1	iv line	3
6	<i>Acinobacter baumannii</i>	1	<i>Raoultella ornithinolytica</i>	1	iv line	5
7	<i>Alcaligenes faecalis ssp faecalis</i>	1	<i>Escherichia coli</i>	1	iv line	2
8	Sterile	1	<i>Escherichia coli</i>	1	iv line	3
9	<i>Staphylococcus aureus</i>	3	<i>Staphylococcus aureus</i>	3	iv line	3
					catheter	10
					iv line	2
10	<i>Staphylococcus epidermidis</i>	1	<i>Citrobacter freundii</i>	1	iv line	2
11	<i>Staphylococcus haemolyticus</i>	1	<i>Citrobacter freundii</i>	1	iv line	4

## DISCUSSION

Another health center had conducted a similar study such as ours to detect biofilm in clinical isolates.<sup>14</sup> Unfortunately, the study did not assess the difference in the planktonic and sessile form of biofilm-forming bacteria. Therefore, we cannot compare the pattern of antibiotic sensitivity between the research results.

Our study acquired more Gram-negative bacteria (22 isolates) than Gram-positive bacteria (8 isolates), in planktonic or in sessile form. Based on their sensitivity patterns, for aminoglycosides, the best choice of antibiotic in this class was Amikacin, followed by gentamicin and tobramycin. The best choice of  $\beta$ -lactam penicillin antibiotic was piperacillin-tazobactam, followed by amoxicillin-clavulanic acid. Ampicillin had developed most resistance and oxacillin could not be used due to high resistance. The most effective  $\beta$ -lactam cephalosporin was cefoperazone-sulbactam. Resistance was increasing for ceftazidime, cefoperazone, and ceftriaxone. Cephazolin could not be used

because all isolates were resistant to it. Sensitivity to Cotrimoxazole was still acceptable. Thus the drug can still be used. Tigecycline was better than tetracycline. Sensitivity to Chloramphenicol was lower than its resistance.

For quinolone, levofloxacin was better than ciprofloxacin. The best choice of carbapenem was imipenem, followed by meropenem, doripenem, and lastly ertapenem. Nitrofurantoin had the same proportion of sensitivity and resistance. For macrolides, erythromycin and clindamycin had low sensitivity level. Vancomycin, linezolid, quinupristin-dalfopristin, daptomycin had a reasonable level of sensitivity. Fosfomycin and doxycycline had the same proportion of sensitivity and resistance.

When planktonic *Escherichia coli* is found in blood or urine, the sessile form of *Escherichia coli* had 50% chance to be also present in the device (iv line or urinary catheter) of the patient. There was 100% possibility that when the planktonic *Klebsiella pneumoniae* is found in blood or urine,

the sessile form of *Escherichia coli* is also present in the medical device. When there is a planktonic *Pseudomonas aeruginosa*, the sessile form of *Pseudomonas aeruginosa* is also present. When the planktonic *Staphylococcus aureus* is found in blood or urine, the sessile form of *Staphylococcus aureus* is also present. When the planktonic *Staphylococcus epidermidis* or *Staphylococcus haemolyticus* is found, there is a possibility that the sessile form of *Citrobacter freundii* present in the device of the patient. A sterile blood culture does not always mean there is no biofilm formation. In our study, the sessile form of *Escherichia coli* biofilm was present in the iv line despite the absence of its planktonic form.

Planktonic and sessile pairs of Gram-negative microorganism have some identical pattern of sensitivity to amikacin (88.88%), cefoperazone sulbactam (83.33%), imipenem (83.33%), and piperacillin-tazobactam (66,66%). The planktonic and sessile pairs of Gram-positive microorganism have less sensitivity pattern to cotrimoxazole (100%). Tobramycin and levofloxacin are not recommended as the therapeutic drug of choice to planktonic and sessile pairs due to low sensitivity levels. Planktonic and sessile pairs with discordant results of Gram staining (Gram positive and Gram negative) can be treated with a combination of antibiotics according to each bacteria sensitivity test results.

## CONCLUSION

The biofilm-forming microorganisms isolated from medical devices are varied. The antimicrobial sensitivity test based on the planktonic and the sessile pair showed the best result for amikacin, followed by gentamicin, imipenem, piperacillin-tazobactam, cefoperazone-sulbactam, and levofloxacin.

## STUDY LIMITATION

Further research is needed, especially the ones with a larger sample size to ensure the accuracy of microorganism pattern and antibiotic sensitivity pattern of biofilm-forming microorganisms. Further explorations are needed in each biofilm-harbored in medical devices to ensure the accuracy of microorganism pattern and antibiotic sensitivity pattern of biofilm-forming microorganisms.

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