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Antibiotic effectiveness on biofilm - producing *Escherichia coli* isolated from catheterized patients

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ABSTRACT

Biofilm is one of the factors that facilitate the occurrence of catheter-associated urinary tract infection (CAUTI). *Escherichia coli* is reported as one of the most dominant bacteria that have virulence factors including biofilm formation. Uropathogenic *E. coli* (UPEC) shows increasing resistance to several antibiotics. Examination of the antibiotic sensitivity on the biofilm-producing *E. coli* and its activity on biofilm formation are important for selecting high effectiveness antibiotics which is beneficial for the management of CAUTI patients. A total of 35 *E. coli* isolates were recultured in the medium of LB agar and blood agar. The isolates were evaluated the sensitivity based on their MIC value to several antibiotics. In addition, the antibiofilm activity of the antibiotics based on their MBIC value was also evaluated. The data obtained were analyzed both descriptively and analytically. Almost the *E. coli* isolates have good sensitivity to meropenem antibiotics, amoxicillin-clavulanic acid, and Fosfomycin. However, among the evaluated antibiotics, only fosfomycin that showed antibiofilm activity. The different in terms of the resistance phenotype between the urinary isolates and the catheter isolates was observed. However, there were no significantly differences in the MIC value (pMIC=0.522) and the MBIC value (pMBIC = 0.523). In conclusion, the alternatives of antibiotic therapy for the planktonic bacteria are amoxicillin-clavulanic acid and fosfomycin, while for the biofilm bacteria is fosfomycin. A biofilm screening examination on the catheter to improve the effectiveness of therapy management for CAUTI patients is recommended.

ABSTRAK

Biofilm merupakan salah satu faktor yang mempermudah terjadinya infeksi saluran kemih akibat kateter (*catheter-associated urinary tract infection*/ CAUTI). *Escherichia coli* merupakan salah satu bakteri paling dominan yang memiliki faktor virulensi termasuk pembentukan biofilm. Uropathogenic *E. coli* (UPEC) menunjukkan peningkatan resistensi terhadap beberapa antibiotik. Pemeriksaan sensitivitas antibiotik pada *E. coli* penghasil biofilm dan aktivitasnya pada pembentukan biofilm penting untuk memilih antibiotik dengan efektivitas tinggi yang bermanfaat bagi pengelolaan pasien CAUTI. Sebanyak 35 isolat *E. coli* dikultur kembali dalam media agar LB dan agar darah. Isolat dievaluasi sensitivitasnya berdasarkan nilai MIC nya terhadap beberapa antibiotik. Selain itu, aktivitas antibiofilm antibiotik berdasarkan nilai MBIC nya juga dievaluasi. Data yang diperoleh dianalisis secara deskriptif dan analitik. Hampir semua isolat *E. coli* memiliki sensitivitas tinggi terhadap antibiotik meropenem, amoksisilin-asam klavulanat, dan fosfomisin. Namun, di antara antibiotik yang dievaluasi, hanya fosfomisin yang mempunyai aktivitas antibiofilm. Terdapat perbedaan fenotipe resistensi antara isolat urin dan isolat kateter. Namun tidak terdapat perbedaan signifikan pada nilai MIC (pMIC=0,522) dan nilai MBIC (pMBIC=0,523). Kesimpulannya, alternatif terapi antibiotik untuk bakteri planktonik adalah amoksisilin-asam klavulanat dan fosfomisin, sedangkan untuk bakteri biofilm adalah fosfomisin. Disaranakan untuk dilakukan pemeriksaan skrining biofilm pada kateter untuk meningkatkan efektivitas manajemen terapi pada pasien CAUTI.

Keywords:
antibiotics;
biofilm;
CAUTI;
resistance;
planktonic

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INTRODUCTION

Urinary tract infection (UTI) is one of the infections which often occurs most of the time and therefore this infection should be given serious attention.[1-3] Approximately 40% of nosocomial infection cases due to the UTI are related to the use of a catheter exceeding 2 x 24 h. It is well known as catheter-associated urinary tract infection (CAUTI). Around 1-4% of these cases develop into bacteremia that contributes to the rise of mortality rate.[1,2] In previous research, biofilm production was found to take place in the catheter of patients, especially among patients who used the catheter for more than five days. Furthermore, female patients are vulnerable to higher risks compared to male patients.[3]

The CAUTI-causing microorganisms commonly found is uropathogenic *Escherichia coli* (UPEC) with an occurrence percentage of 80%.[4,5] The biofilm-producing *E. coli* is the most dominant bacteria both in the urinary culture and in the catheter culture. In addition, it is also reported that the biofilm-producing *E. coli* has virulence factors that support biofilm production.[3] The presence of the virulence factors within the UPEC facilitates the occurrence of colonization on the surface of the host mucosa, destroys and invades the urinary tract tissue, and causes persistent or recurrent infection, which is in general difficult to be cured through the usual antibiotic therapy.[6,7]

Furthermore, several studies reported that UPEC shows increasing resistance to several antibiotics, especially when *E. coli* has the biofilm-producing capacity.[8-10] In fact, several experts reported that the bacteria within the biofilm have the capacity to form resistance to antibiotics around 100 – 1,000 times stronger in comparison to the free-swimming planktonic bacteria within the urine, known as the free-swimming counterparts. Such higher capacity is caused by the changes within the mutation target, the enzyme modification, and the efflux pump so that the bacteria within the biofilm have higher resistance toward the antibiotics.[11-13] Therefore, data with regards to antibiotic effectiveness on biofilm-producing *E. coli* are needed to identify the antibiotics that have the antibiofilm activity so that the antibiotics can be useful for the therapy management of CAUTI patients.

MATERIALS AND METHODS

Bacteria isolate characteristics

Thirty-five bacteria isolates (30 bacteria isolates from catheter culture and 5 bacteria isolates from urinary culture) of *E. coli* used in the study were the isolation results from the previous study (TABLE 1).[3] Among 35 bacteria isolates, 31 of them were *E. coli* with positive CRA (*E. coli* biofilm-producing), and 4 of them were *E. coli* with negative CRA (non-biofilm-producing *E. coli*).

TABLE 1. Thirty-five *E. coli* isolates used in this study

Isolate number	Origin	CRA test
C23	Urinary catheter	Positive
C26	Urinary catheter	Negative
C27	Urinary catheter	Positive
C34	Urinary catheter	Positive
C44	Urinary catheter	Positive
C48	Urinary catheter	Positive
C57	Urinary catheter	Positive
C74	Urinary catheter	Positive
C103A	Urinary catheter	Negative
C116	Urinary catheter	Negative
C122	Urinary catheter	Positive
C137	Urinary catheter	Positive
C176	Urinary catheter	Positive
C178	Urinary catheter	Positive
C179	Urinary catheter	Positive
C193	Urinary catheter	Positive
C207	Urinary catheter	Positive
C215	Urinary catheter	Positive
C216	Urinary catheter	Positive
C217	Urinary catheter	Positive
C223	Urinary catheter	Positive
C227	Urinary catheter	Positive
C230	Urinary catheter	Negative
C235	Urinary catheter	Positive
C240	Urinary catheter	Positive
C251-2	Urinary catheter	Positive
C256	Urinary catheter	Positive
C255	Urinary catheter	Positive
C275	Urinary catheter	Positive
C279	Urinary catheter	Positive
U57	Urine	Negative
U178	Urine	Positive
U207	Urine	Positive
U227	Urine	Positive
U235	Urine	Positive

Note: Isolate number means C = origin from catheter; U = origin from urine; the code number behind the C/U means patient identity (U57 and C57 are from the same patient).

Isolate preparation

The bacteria culture within the cryotube was recultured on the medium of Luria Bertani Agar and Blood Agar, then incubated at 37^0C for 18-20 h.

Isolate identification

A single colony from the Blood Agar Media was picked up and inoculated into a Vitek 2 cartridge (Vitek 2 compact, BioMerieux™, France) according to the manufacturer's instructions to identify the bacterial species.

Antibiotic test

To evaluate the effectiveness of antibiotics in biofilm eradication, the minimum inhibition concentration (MIC) test and minimum biofilm inhibitory concentration (MBIC) were performed.[14] Seven antibiotics used in this study are commonly used for treating UTIs including fosfomycin, ciprofloxacin, cefotaxime, amoxicillin-clavulanic acid, ceftriaxone, meropenem, and amikacin.

Minimum inhibition concentration test

The MIC test was performed by using the microdilution method. The colony of fresh bacteria (18-20 h) that had been cultured on the LB agar was dissolved in the physiological saline with a turbidity level equal to 0.50 according to the McFarland standards. This suspension was inoculated on the microtiter plate well with the antibiotic serial dilution (logarithmic) using the broth Mueller-Hinton medium. The final concentration of the isolates was equal to 5 x 10^5 CFU/mL. After being implanted for 18 h, the MIC assessment was conducted.[14] Then, the assessment of the sensitivity was carried out by using the 2018 CLSI standard.[15] The MIC was defined as the minimum concentration of the extract that did not allow any visible growth or turbidity of the organism in broth. MIC_{90} refers to the concentration of the test compound required to prevent the growth of 90% of organisms tested. The concentration at which all the isolates failed to grow is taken as MIC.

Minimum biofilm inhibition Concentration

The sensitivity was assessed on the polystyrene microtiter plate that had been implanted on the biofilm within the 96 wells in sequence. This initiative was conducted to minimize the occurrence of manipulation of the biofilm. Then, the fresh and sterile Brain Heart Infusion (BHI) was inserted into the plate and incubated at 37^0C for the next 24 h.[14] In the MBIC assessment, the biofilm that had grown over the microtiter plate was exposed to different antibiotic concentrations. After the microtiter plate had been exposed to the biofilm and the antibiotic compounds, the plate was washed three times using sterile PBS (pH 7.4).[14] Reading on the thickness was conducted and the results from the biofilm were compared to the control bacteria using the spectrometer with a wavelength of 595 mm. $MBIC_{50}$ was determined as the lowest concentration that causes at least 50% inhibition of the viability of formed biofilm in the presence of a biologically active agent.

Data analysis

The collected thirty-five *E. coli* isolates data were analyzed descriptively based on the MIC mean score and the MBIC mean score of each antibiotic, namely fosfomycin, ciprofloxacin, cefixime, amoxicillin-clavulanic acid, ceftriaxone, meropenem, and amikacin. The author also analyzed five samples of *E. coli* bacteria results from both urine and catheter taken from the same patients to compare the MIC and the MBIC value from each of the samples.

RESULTS

The results of MIC sensitivity on the *E. coli* still showed a high level of sensitivity toward meropenem (100%), amoxicillin-clavulanic acid (97.10%), fosfomycin (80.00%), and amikacin (77.10%). On the contrary, the sensitivity decreased on ciprofloxacin (28.60%), cefixime (17.10%), and ceftriaxone (8.60%) (TABLE 2). In comparison, the sensitivity of biofilm-producing *E. coli* (positive CRA) and the non-biofilm-producing *E. coli* (negative CRA) yields had similar sensitivity on meropenem (100% vs 100%). However, on ceftriaxone, cefixime, ciprofloxacin, and fosfomycin except for amikacin and amoxicillin-clavulanic acid, there was higher sensitivity on *E. coli* positive CRA than *E. coli* negative CRA. Based on the same results, the researchers also found that 60% of all *E. coli* isolates have the nature of extended spectrum beta lactamase (ESBL). This has been identified from the sensitivity pattern inspection using the Vitek 2 Compact (Biomeriux®) tool. Out of 21 ESBL *E. coli* isolates, 17 isolates were found to have positive CRA (81%) while 4 isolates were found to have negative CRA. This nature of ESBL does not confirm the correlation with the biofilm production capacity ($p > 0.05$).

TABLE 2. The pattern of *E. coli* Sensitivity on Numerous Antibiotics

Types of antibiotics	*E. coli* (n=35)				% Total Sensitivity
	Biofilm producing n* = 31[(a)]	% Sensitivity	Non-Biofilm producing n**=4[(b)]	% Sensitivity	
Ceftriaxone	3	9.70	0	0	8.60
Cefixime	6	19.40	0	0	17.10
Ciprofloxacin	10	32.20	0	0	28.60
Amikacin	23	74.20	4	100	77.10
Amoxicillin-clavulanic acid	30	96.80	4	100	97.10
Meropenem	31	100	4	100	100
Fosfomycin	25	80.60	3	75	80.00

[a]) number of drug-sensitive samples from *E. coli* producing biofilm; [b]) number of drug-sensitive samples from *E. coli* not producing biofilm

In addition, to examine the sensitivity, those antibiotics were also evaluated, in terms of biofilm-production inhibiting capacity. The results of the current study showed that the antibiotics with the lowest titer increase on $MBIC_{50}$ in comparison to MIC_{90} are ciprofloxacin and fosfomycin (without titer increase) and meropenem with a 1-fold titer increase (0.032 µg/mL to 0.064 µg/mL). On the contrary, ceftriaxone and cefixime required more than two-fold titer increasing to > 512 µg/mL. Similarly, amikacin required a three-fold titer increase to achieve $MBIC_{50}$ from 4 µg/mL to 32 µg/mL (TABLE 3).

TABLE 3. Comparison of MIC value and MBIC value on biofilm-producing *E. coli* (n = 31)

Antibiotics	MIC_{90} (µg/mL)	Min-Max (µg/mL)	MIC Sensitivity (CLSI)	$MBIC_{50}$ (µg/mL)	Min-Max (µg/mL)
Ceftriaxone	512	0.25–512	≤ 1; ≥ 4	> 512	2– > 512
Cefixime	512	0.50–512	≤ 1; ≥ 4	> 512	1– > 512
Ciprofloxacin	160	0.15–640	≤ 1; ≥ 4	160	0.32– > 640
Amikacin	4	1–16	≤ 16; ≥ 64	32	4– > 512
Amoxicillin-clavulanic acid	6	2–16	≤ 8; ≥ 32	--	--
Meropenem	0.03	0.01–0.05	≤ 1; ≥ 4	0.064	0.02– > 4
Fosfomycin	80	0.63– > 320	≤ 64; ≥ 256	80	2.50– > 320

As previously explained, the *E. coli* isolates used in the study were obtained from different sources, which were urine and catheter. The five samples from both urine and catheter were taken from the same patients to compare the MIC and the MBIC value based on the source of the isolates. Data from the MIC identification show that higher concentrations of all antibiotics were required to inhibit the isolates taken from the catheter (shown as red bars) in comparison to the isolates taken from the urine (shown as green bars) except for ciprofloxacin (FIGURE 1).

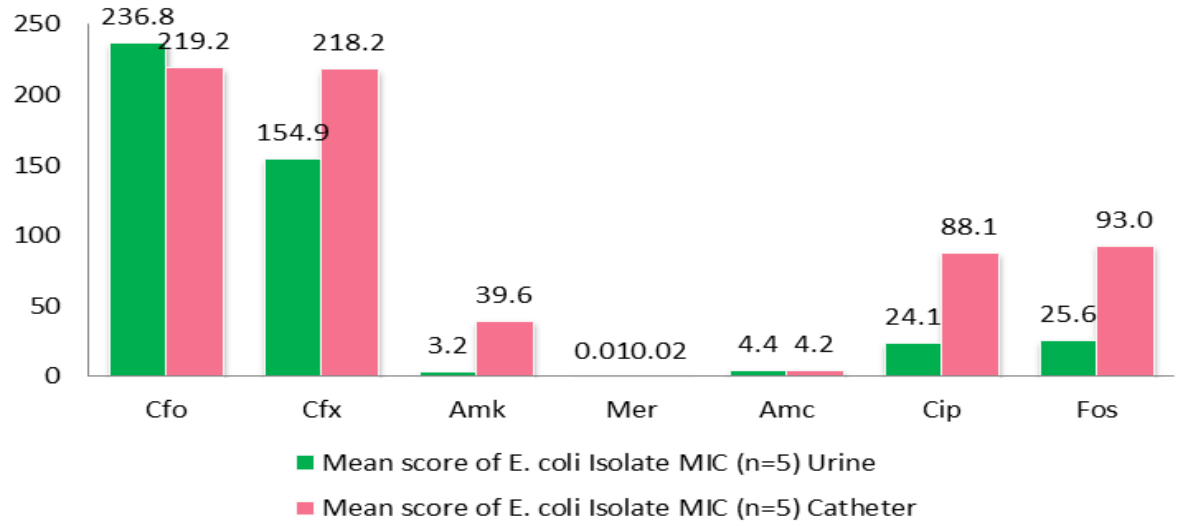

FIGURE 1. Comparison between the MIC mean score (µg/mL) of isolates obtained from the urine (n = 5) (green) and the MIC mean score (µg/mL) of isolates obtained from catheter (n = 5) (pink) CFO = ceftriaxone, CFX = cefixime, AMK = amikacin, MER = meropenem, AMC = Amoxicillin Clavulanic-Acid, CIP = ciprofloxacin, FOS = fosfomycin

On the contrary, the phenotype results of MBIC for the antibiotics on the *E. coli* taken from the urine and the catheter also showed that almost all antibiotics demand higher concentration (2-10 times) on the isolates taken from the catheter (shown in orange) than the isolates taken from the urine (shown as blue bars). These findings described the different phenotype characteristics between the isolates taken from the catheter and the isolates taken from the urine (FIGURE 2).

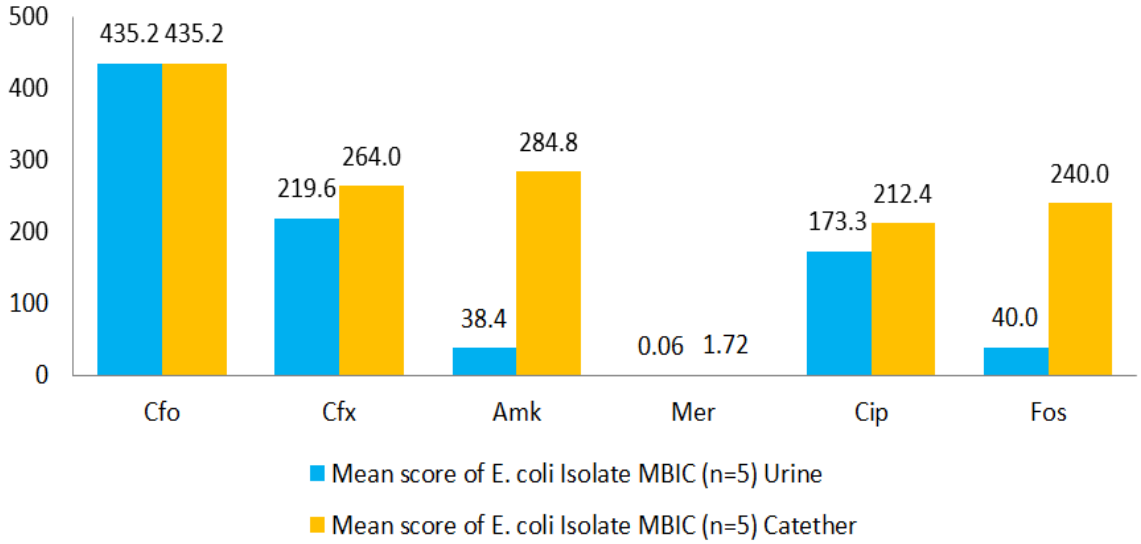

FIGURE 2. Comparison between the MBIC mean score (µg/mL) of isolates obtained from the urine (blue) and the MBIC mean score (µg/mL) of isolates obtained from the catheter (yellow). CFO = ceftriaxone, CFX = cefixime, AMK = amikacin, MER = meropenem, CIP = ciprofloxacin, FOS = fosfomycin

DISCUSSION

The results showed that the bacteria's capacity for forming the biofilm does not influence its sensitivity to antibiotics. In general, the effectiveness of antibiotics was good in inhibiting the development of biofilm-producing *E. coli*, except for several antibiotics such as ciprofloxacin (28.60%), cefixime (17.10%), and ceftriaxone (8.60%) (TABLE 2). The results of this study are similar to those of research conducted in Nepal. This study found that the biofilm-producing *E. coli* shows resistance to the antibiotics like amoxicillin (10.60%), ciprofloxacin (39.40%), and cefixime (24.50%).[16] Resistance to fluoroquinolone (ciprofloxacin) and ceftriaxone was also found in the results of other studies.[17] The results of this study align with those of Neupane *et al.*[16] suggesting that the sensitivity of *E. coli* to amoxicillin is low (10.60%), while the sensitivity of *E. coli* to amoxicillin clavulanic acid is high (97.10%). The difference showed that amoxicillin combined with the lactamase beta inhibitor (amoxicillin-clavulanic acid) has higher effectiveness in inhibiting the development of the biofilm-producing *E. coli* compared to amoxicillin itself.[16,18,19]

Thus, this finding indicated that the effectiveness of the antibiotics used in the present study is not influenced by the capacity of *E. coli* to produce biofilm. On the other hand, the results of a study by Kobir *et al.*[20] showed that the resistant pattern correlates with the biofilm-producing capacity of the uropathogenic *E. coli*. Similarly, the results of a study by Cho *et al.*[21] also showed that the biofilm-producing *Pseudomonas aeruginosa* has a higher resistance level in comparison to the non-biofilm-producing *P. aeruginosa* on amikacin, ceftazidime, and cefepime. Such a difference implies that the antibiotic effectiveness (amikacin) can be different in different species although the bacteria are equally able to produce the biofilm. The different results between this study with other studies might be caused by the small samples of non-biofilm producing *E. coli* (n=4) compared to biofilm producing *E. coli*.

Furthermore, in this study 60% of the *E. coli* taken from the urinary isolates and the biofilm were ESBL.

This figure aligns with the surveillance results in Mexico from 2009 until 2015 for the cases of UTIs, which resulted from nosocomial infection or catheter use.[22] In other words, this finding shows that the presence of bacteria within the biofilm can lead to the occurrence of CAUTI. Based on the high resistance of the biofilm-producing *E. coli* to several antibiotics such as quinolone, amoxicillin, cotrimoxazole, and ceftriaxone in the case of CAUTI, biofilm-screening examination on the catheter should be performed so that the infection management within the CAUTI patients can be conducted effectively.

The effectiveness of antibiotics as antibiofilm has been assessed based on their capacity in inhibiting biofilm production by *E. coli*. To assess this capacity, the MBIC was conducted under the rate $MBIC_{50}$. $MBIC_{50}$ is the lowest antibiotic rate in inhibiting 50% of biofilm production by *E. coli*.[14] At the same time, the results of the current study also show that ciprofloxacin and fosfomycin do not need higher antibiotic concentrations both in the planktonic form (MIC_{90}) and in the biofilm form ($MBIC_{50}$) (TABLE 3). These findings are similar to the results of a study by Gonzales *et al*.[23] which showed that ciprofloxacin can inhibit biofilm production. However, the only difference shown by the results of the current study is the concentration of ciprofloxacin used (160 µg/mL) is very high compared to the range value suggested by CLSI (TABLE 3). This implies that the antibiotic considered the most effective one for inhibiting biofilm production is fosfomycin (80 µg/mL). The results of previous research also showed that fosfomycin can serve as an alternative therapy that can be used as an antibiofilm, especially in combination with gentamicin.[24]

The biofilm resistance to high-dose antibiotics is multifactorial and depends on the class of the antibiotics used, including numerous mechanisms that can be different from one another, such as poor antibiotic diffusion, antibiotic use negligence, and biofilm genetical expression variants.[25] The extracellular matrix of the biofilm is considered responsible for biofilm resistance. Then, consistent with the statement, it had been mentioned previously by Parrino *et al*.[26] that the mechanical and the physiochemical nature of the biofilm matrix can reduce or inhibit several compounds, including antibiotics and antiseptics. The chemical structure of the biofilm matrix is crucial, such as the different types of exopolysaccharide (EPS) and the dependency on the surrounding environment of the biofilm.[10,26] However, the decreasing antibiotic penetration cannot fully explain the biofilm resistance toward antibiotics.[25]

The antibiotics such as fluoroquinolone, rifampin, and ampicillin have quite good penetration toward the matrix although the penetration cannot eradicate 100% biofilm bacteria. For example, in the case of *P. aeruginosa* and *E. coli* during the 24 h *in vitro* experiment, the biofilm bacteria cannot be eradicated by the 24 h therapy using fosfomycin and ciprofloxacin, whereas the two antibiotics have reached 50% maximum concentration within 6 h.[25] In addition, the mean score of the antibiotics' MIC and MBIC is higher on the isolates taken from the catheter in comparison to the isolates taken from the urine except for ceftriaxone (both FIGURE 1 and FIGURE 2 are consistent with the results of other studies, which state that the bacteria in the form of biofilm have 100-1,000 times stronger resistance capacity on the antibiotics compared to bacteria in the form of free-swimming counterparts planktonic).[11-13] The antibiotic effectiveness toward *E. coli* is not influenced by biofilm-producing capacity; instead, since *E. coli* becomes part of the biofilm, the bacteria

will undergo changes in mutation target, enzyme modification, and efflux pump. Consequently, the bacteria will have higher resistance to antibiotics.[11-13]

However, the results of statistical analysis using the Mann-Whitney procedures did not show significant differences between the MIC mean score and the MBIC mean score of the antibiotics on the isolates taken from the catheter compared to the isolates taken from the urine (pMIC = 0.522, pMBIC = 0.523). It showed that the characteristics of *E. coli* found in the catheter are similar to those found in the urine. Consequently, there is a possibility that the bacteria within the urine come from the bacteria colonization found in the catheter and vice versa. This incident further indicated that the presence of colonization or biofilm within the catheter can cause infection in the urinary tract. Such an indication is similar to the results of the previous studies, which stated that the presence of biofilm increases the risk of CAUTI occurrence.[3]

The only limitation found in the study were in the planktonic bacteria sensitivity test (MIC), and the biofilm sensitivity test (MBIC) conducted *in vitro*. In the last several years, the management of biofilm-associated infections has been a challenge because generally the studies are conducted *in vitro*; consequently, the results of these studies are not close to the clinical (*in vivo* studies) manner. Therefore, there should be another approach in addition to relying on the use of antibiotics that have antibiofilm characteristics. The approach that should be developed is the other biofilm therapy target, such as QS inhibition, adhesion-inhibiting-type bacteria, anti-virulence factor, and exopolysaccharide matrix degradation, to overcome antibiotic resistance.[26-28]

CONCLUSION

The alternatives of antibiotic therapy for the biofilm-producing planktonic bacteria are amoxicillin-clavulanic acid and fosfomycin, while the antibiotic that has the antibiofilm characteristics is fosfomycin. There should be a biofilm screening examination on the catheter to improve the effectiveness of therapy management for CAUTI patients.

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