Biofilm Formation and Attachment Factors Existence in Urinary Tract Infection Caused by Escherichia Coli

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Biofilm Formation and Attachment Factors Existence in Urinary Tract Infection Caused by Escherichia coli

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Abstract: *Introduction: Escherichia coli is a bacterium that is responsible for about 80% of all urinary tract infections. The formation of biofilm requires several cell surface factors such as flagella and motility, fimbriae, adhesins, autotransporter proteins, curli fimbriae, conjugative pilus, and exopolysaccharide production. Method: A total of 122 E. coli isolates were obtained from urine samples of patients with UTI. We examined the biofilm formation from the isolates in TSB, with supplemented 2% sucrose, using Congo Red Agar (CRA) method. The existence of the 2 studied genes (afa and fimA) in the isolates was evaluated using PCR assay. Result: E. coli isolates that produced and didn't produce slime using CRA method were 81 (65.9%) and 36 (29.3%) respectively. Conclusion: E. coli bacteria isolates from UTI patients were able to form biofilmin CRA method and all E. coli causing UTI strains in human studied in this research positively produced biofilm, while some of the biofilm non-producing strains can be expressed in the 2 genes studied.*

Keywords: *Biofilm, Attachment Factors, E. coli, UTI.*

1. Introduction

Urinary Tract Infections (UTIs) are the most common bacterial infection in all age classification (Tarchouna et al., 2013). The incidence rate is around 50-60% in adult

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women in their lifetime. The prevalence of UTI will increase as one gets older (Ergin et al., 2000). About 150 million people worldwide are infected with UTI each year (Makled et al., 2017). Escherichia coli is a commensal bacteria which normally grows in the human intestine, but some strains have specific virulence properties and able to adapt to new niches and cause several diseases. One of them is UTI, which is caused by Uropathogenic *E. coli* (UPEC) strain which is found around 80-90% of all UTIs in the out patient population (Oliveira et al., 2011).

Adistinct characteristic of UPEC is that it is able to form biofilm that facilitate its persistence in the urinary tract (Fattahi et al., 2015). Biofilm is a microbial community that is formed, attached to the surface, and wrapped in a matrix of exopolymer substance that display special properties which is different from their planktonic state (Senevirat). The clinical implication of biofilm-forming microbes is more difficult to handle in clinical cases, and it is physiologically more resistant to antibiotics and disinfectants. Therefore, it often lead to treatment failure or recurrent infection (Hoibi).

Various virulence factors are involved in the formation of *E. coli*'s biofilm, starting from the initial attachment stage, the formation of micro-colony, the formation of mature biofilm, and the dispersion stage. (Zhao et al., 2017). Initial attachment is the most important stage in the formation of biofilm, because it determines the viability of each microorganism in a complex environment (Berne et al., 2015). *afa* (Afimbrial adhesin) is a structural protein encoded by the AFA gene (SSSMCRI, Sri Balaji Vidhyapeeth University, Chennai and Nachammai, 2019) Previous studies have shown that there is a relationship between the expression of attachment factors such as AFA and Type 1 fimbriae with the formation of biofilm of UPEC (15_evolution). The aim of this study is to examine the formation of biofilm of UPEC using Congo Red method and their correlation with the existence of AFA and fimA genes.

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2. Research Method

This cross-sectional study was conducted in July 2017 to October 2018. All isolates were characterized by growing it in *E. coli* selective media; EMB agar (Oxoid, England) and were incubated for 24 hours at 37°C. Then, biochemical tests were conducted. A total of 114 *E. coli* isolates were isolated and collected from urine specimens of patients with UTI in the Regional General Hospital, Surabaya, Indonesia. All isolates were characterized and stored in 50% NA media at room temperature.

2.1 Biofilm formation assay

Examinationto observe the formation of *E. coli* isolates' biofilm was carried out by Congo Red Agar (CRA) method with a slight modification as described by (Freeman et al., 1989). The bacteria culture was incubated at 37°C for 24 hours. The formation of biofilm was qualitatively assessed based on the color of *E. coli* colonies growing in the media. The results were interpreted based on its form: Bordeaux red and red colonies with smooth colony surface are categorized as non-slime producers while solid textured colonies, black and reddish colony colors, coarse, dry structure, and crystal-shaped structures, are categorized as slime producers.

2.2 Detection of *afa* genes

DNA extraction of all *E. coli* isolates were carried out as described in the Cell/Network genomic DNA preparation kit (NEXprep TM). The presence of afa and fimA adhesin genes was analyzed by Polymerase Chain Reaction (PCR) method. The primers selected were base sequences available at the National Center for Biotechnology Information Bank. Genes and primary oligonucleotide sequences for PCR amplification of biofilm-related genes in an isolate of *E. coli* are presented in table 1.

PCR was consistently performed in a 20 µL reaction mixture, each reaction mixture consisted of 8.4 µL DNA templates, 0.8 µL each primer, and 10 µL PCR master mix. Amplification process was carried out using Thermal Sickle with the

thermal cycle profile as follow: initial denaturation at 95°C, (denaturation at 95°C, annealing at 65°C and extension at 72°C) for40 cycles and final extension process at 72°C. The amplified PCR products were analyzed with 2% agarose gel, 1X TAE, 80 volts, 400 mA for 20 minutes, stained with RedSafe (Intron, USA) and were visualized under UV-illuminator. *E. coli* ATCC 25923 was used as positive control and sterile distillation water was used as a negative control.

Table 1.Sequences of oligonucleotide primers for PCR amplification of biofilm associated genes in *E. coli* isolates

Gene	Forward primer	Reverse primer	Anneling temperature	Amplicon size (bp)
Afa	TTTAAGGCGAAGTA CACAGTGG	GGCGTCACAATACAG ACACAG	56°C	187 bp

3. Results and Discussion

The phenotypic determination of biofilm formation ability in CRA medium 1 of all test isolates is shown in Table I. Morphology of *E. coli* isolatescolonyin CRA media is shown in Figure 1. 80 isolates (65,6%) of E.coli from UTI patients were able to form slime in CRA method. This wasindicated by color shifting of *E. coli* colonies that grew on CRA media, i.e. the color of the colonies will shift from black to deep black. The proportion of *E. coli* that cannot form slime were 36 isolates (29,5%) in CRA media.This is indicated by color shifting of *E. coli* colonies that grow on CRA media which become red or red cherries. There were 6 (4,9%) *E. coli* isolateswhich unable to grow on CRA media.

Figure 1: The morphology of *E. coli* isolates colony from UTI patients on CRA (Congo Red Agar) media: A: black colonies, B: red colonies and C: deep black colonies

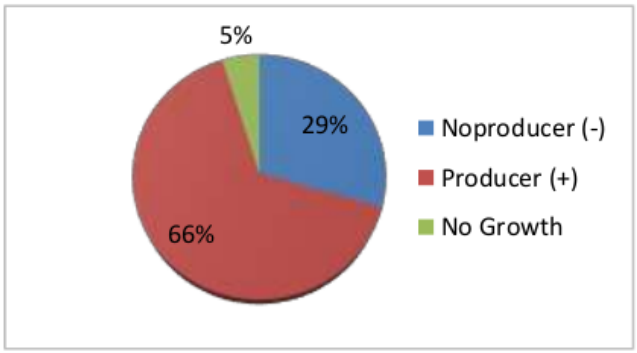

Figure 2: Morphology of *E. coli* isolates from UTI patients on CRA (Congo Red Agar) media: A: black colonies, B: red

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The ability to form biofilm in vitro and its modifications are reported in previous studies (Dadawala, 2010). The Congo Red Agar method is simple, economical, sensitive, and specific. This technique is often utilized by microbiologists in several clinical laboratories to screen bacteria strains that form slime or other substances similar to slime (Ergin et al., 2000). The results of this study denoted that a species of *E. coli* was able to form biofilm and there was a type of *E. coli* that was unable to produced biofilm, while there was phenotypic variations in biofilms. Its specific slime describes the ability of bacteria to attach properly to the host tissue, which is followed by invasive microcolony production (Dadawala, 2010). This finding describes that the formation of biofilm is highly dependent on growth condition and the use of various kinds of sugar influences biofilm formation (Kara Terki et al., 2013). This result is slightly different from previous studies where all test isolates were able to form biofilm moderately. This specific finding may occur because in this study, not all isolates types were studied, therefore only identical results were observed.

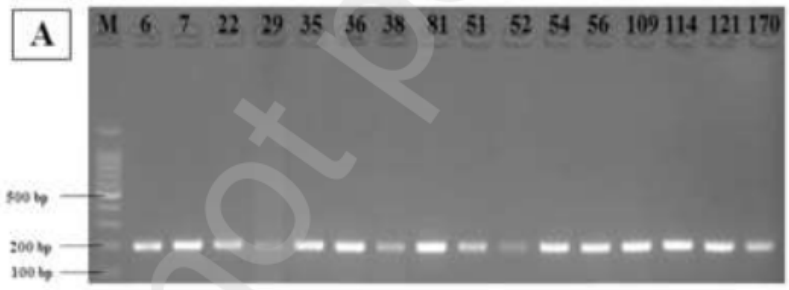

Figure 3: PCR amplicon of afa fimH gene

Table 2.Sequences of oligonucleotide primers for PCR amplification of biofilm associated genes in *E. coli* isolates

Virulence factors	Number of isolates N= 116	Biofilm formation n= 80	Biofilm formation (%)	P-value
afa gene				0,339
Positive	114 (98,27%)	78	68,42 %	
Negative	2	2	100 %	

Clinically, biofilm is the cause of chronic, nosocomial and medical device-related infections (Khatoon et al., 2018). Bacterial adherence to the surface accelerates the

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expression of genes which responsible for the production of matrix exopolysaccharide (EPS) or "slime" and biofilm maturation. EPS or "slime" acts as a barrier or protector that protects bacteria against the host's endogenous defense system or external agents such as antibiotics (Khatoon et al., 2018).The formation of biofilm can be described in three stages including adhesion, maturation and dispersion. The attachment stage can be further divided into 2 processes: Reversible and irreversible initial adhesion. Irreversible attachment can tolerate harsh physical or chemical movemen (Rabin et al., 2015). Biofilm is one of the virulence factors in UPEC which has been reported in various studies ((Eberly et al., 2017); (Zamani and Salehzadeh, nd); (Soto et al., 2011); (Terlizzi et al., 2017); (Pompilio et al., 2018))

Several different virulence factors are needed by the bacterial population to cause infection. Based on the data obtained from NCBI,the afa gene is detected in Escherichia coli (UPEC 26-1) and produces a protein in the form of Afimbrial adhesin afa-I. The fimH gene produces a protein in the form of Type I fimbriae. Purification of afa-1 protein shows that there is a structure of afimbrial adhesins on the surface of bacteria. The afa protein is formed from the afa gene cluster which consists of afaA, afaE, afaD, afaB, and afaC. afaE-I is a type of adhesin (Bien et al., 2012). Adhesin afais abundant in the isolates from patients with cystitis (26-65%) compared with pyelonephritis (6-36%) and ABU (6%) (Baby, 2018). Adhesin afa is encoded by five members of the gene operon including afaA, afaE, afaD, afaB and afaC. afaI and afaIII protein are known as the member of Drfamily. Previous studies have shown that some Dr adhesin and afa have similarities with the chaperone-usher adhesion pathway (Behzadi, 2018).

In conclusion, there is no significant relationship between the existence of these genes and biofilm production. (Rijavec et al., 2008) reported that no relationship was observed between the virulence genes of usp, papC, and sfa/foc with biofilm production in the pathogenic *E. coli*. This is caused by the frequency of papC, papG, sfa / foc, focG, hlyA and cnf1 genes was higher in resistant strains of *E. coli* biofilms coli producer. This report is consistent with the observation of (Naves et al., 2008). Finally, considering the influence of various environmental factors in biofilm production process such as bacterial species, the diversity of attachment factors in UPEC entailed with a high level of genetic similarity between non-pathogenic and pathogenic extraintestinal *E. coli* isolates, causing it to be difficult to determine specific attachment factors that determine the ability producing biofilm (Moori Bakhtiari and Javadmakoei, 2017). Various results in some studies might be caused by differences in hygiene status in each region and increased resistance cases of antibiotics (Fattahi et al., 2015).

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4. Conclusion, Implication and Limitation

4.1. Conclusion

All *E. coli* strains that cause UTIs in the human studies that produce positive and negative biofilm can be related genes observed. The absence of correlation between the ability to produce biofilm and the existence of the genes being investigated is also followed by the highest proportion of the gene expressed in this study..

4.2. Implication and Limitation

This study is able to provide information related to the genes that play a role in the attachment of *E. coli* isolates that cause UTIs in humans and the relationship of these genes in biofilm formation. The limitations of this study are: 1. The number of genes evaluated in the virulence and biofilm formation are fewer than that of the other studies and 2. The target communities in this study were only from one particular geographical area. These might explain the differentresultof this study compared to the other.

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