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High Prevalence of *dam* and *fimA* , Biofilm Formation, and Antibiotic Resistance in Uropathogenic *Escherichia coli*

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Background: Urinary tract infections (UTIs) are among the most common bacterial infections in women and remain a significant public health problem. Uropathogenic *E. coli* (UPEC) is the main cause of UTIs and can form biofilms, which lead to recurrent infections and antibiotic resistance. Type 1 fimbriae in UPEC, encoded by the fim operon, facilitate bladder attachment, while the dam an orphan DNA methyltransferase in *E. coli*, contributes to bacterial colonization and biofilm formation. Data on the association between antibiotic susceptibility, fimA and dam gene prevalence, and biofilm formation in UPEC isolates from UTI patients in Indonesia remain limited. This study aimed to investigate the association of the dam and fimA virulence genes with biofilm formation in UPEC causing UTIs.

Materials and methods: Fifty UPEC isolates were obtained from a clinical microbiology laboratory. Biofilm formation was assessed using the tube method. Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method with amoxicillin, ciprofloxacin, and gentamicin. The presence of the dam and fimA was determined by PCR.

Results: Seventy percent of UPEC isolates were capable of biofilm formation. High resistance rates were observed for amoxicillin (92%), ciprofloxacin (88%), and gentamicin (56%). In UPEC isolates that were positive for the dam, 62% of them had the ability to form biofilms. Meanwhile, in UPEC isolates that were positive for the fimA, 52% of them had the ability to form biofilms.

Conclusion: UPEC isolates showed a high prevalence of the *dam* and *fimA* genes, which were associated with biofilm formation and increased antibiotic resistance.

Keywords: *biofilm, antibiotic, dam, fimA, urinary tract infections*

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in humans, affecting millions of individuals annually and accounts for 40% of all nosocomial infections.[1] The incidence of UTI in the community continues to increase, indicating that UTI is a public health problem that is still difficult to overcome.[2] Uropathogenic E.

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coli (UPEC) is the most common cause of UTI.[3] Antibiotic treatment for UTI is often done empirically without urine culture or susceptibility testing. In addition, UTI also has a high recurrence rate, which leads to increased antibiotic use and resistance.[4] Antimicrobial resistance causes approximately 700,000 deaths annually worldwide and the number will increase to more than 10 million by 2050.[5] The mechanism of bacterial resistance can occur through decreased membrane permeability, mutation of target molecules, inactivation of antibiotic molecules, and approximately 60–80% of UTI-causing bacteria are able to produce biofilms.[6]

Bacterial biofilms play an important role in UTI, being responsible for the persistence of infections that result in recurrence and relapse.[7] Many bacterial adhesion factors play a role in biofilm formation. UPEC encodes a number of virulence genes associated with severe or recurrent UTIs, one of them type 1-fimbriae (fim). The type 1 fimbriae virulence factor in UPEC is able to facilitate attachment to the bladder, which is encoded by the fim operon.[8] The genes responsible for T1F synthesis are encoded in the fim gene cluster on the chromosome. The fim operon consists of seven genes involved in pilus assembly. It begins with *fimA*, which encodes the major fimbria subunit, or pilin.[9]

In addition to genetic regulation, many physiological and structural characteristics, such as biofilm formation in bacteria, are also influenced by epigenetic mechanisms.[10] The most important group of enzymes involved in epigenetic mechanisms is DNA methylase.[11] Some methyltransferases are not associated with any restriction enzymes and are called "solitary" or "orphan" methylases. The *dam* is an orphan MTase found in *E. coli* that plays a role in bacterial colonization and biofilm formation.[12]

Studies on the detection and prevalence of biofilm-coding genes have been conducted in various test pathogens. Previous study reported that *E. coli* contains the *dam* gene, a solitary or orphan methyltransferase, that plays a role in biofilm formation and is targeted by Flu-OxyR.[12] Our study highlights the significant role of DNA methylation in shaping the *S. mutans* biofilm phenotype. The absence of the dam resulted in significant changes in biofilm characteristics.[13] One study reported that 100% of UPEC isolates carried the fimH and 31.6% carried the fimA gene. Another study demonstrated that *fimA* plays a role in regulating the formation of biofilm-forming *E. coli* and also influences the distribution of L-threonine carbon.[15]

Although UPEC is known to be the main cause of UTI and has the ability to form biofilms that contribute to antibiotic resistance, there are still limited studies that simultaneously evaluate antibiotic sensitivity, the prevalence of the fimA gene as an adhesion factor, and the dam gene as an epigenetic regulator in biofilm-forming UPEC isolates, especially in UTI patients in Indonesia. This study aimed to determine antibiotic sensitivity, the prevalence of the *fimA* gene, which encodes type 1 fimbriae, and the *dam* gene, which encodes DNA adenine methylase, in biofilm-forming UPEC. Understanding the rate of biofilm formation, antimicrobial sensitivity, and virulence factors of UPEC will assist in proper management and initiation of appropriate antibiotics, which will help in preventing antimicrobial resistance.[16]

Materials and methods

Bacterial Isolates, Culture Conditions and Biofilm Detection

UPEC were isolated from urine samples of inpatients over a 3-month period at a public hospital in Surabaya. A disposable 1-µL inoculating loop of urine sample was streaked onto EMB agar (Merck Company, Germany) in a petri dish, then incubated at 37°C overnight. UPEC isolates were only taken from samples with significant bacteriuria, namely when bacterial colonies were more than 105 CFU/mL of organisms, and those that showed colonies with a characteristic metallic green color, fluorescence properties, has a very dark colony center (almost black), and is included in the Gram-negative bacteria.

To qualitatively detect the ability of UPEC isolates to form biofilms using the tube method.[17] A full loop of UPEC isolates was inoculated in 3 mL of trypticase soy broth supplemented with 1% glucose in a test tube. The UPEC culture in the tube was then incubated at 37˚C for 24 hours. After incubation, the solution in the tube was poured and washed with phosphate buffer saline (pH 7.3) and then air-dried. The tube was then stained with crystal violet (0.1%). Excessive crystal violet dye was washed with bidistilled water. The tube was then air-dried in an inverted position. Biofilm formation was considered positive when there was a layer stained by crystal violet along the walls and at the bottom of the tube. All procedures undertaken in this study were implemented in compliance with the ethical approval described in the Ethical Statement subsection.[18]

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Antibiotic Resistance Determination

Antibiotic resistance testing was performed on 50 UPEC isolates using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, as recommended by the Clinical Laboratory Standards Institute standard guidelines.[17] The antibiotics tested included: 5 µg amoxicillin, 5 µg ciprofloxacin, and 5 µg gentamicin were selected due to their frequent clinical use in the treatment of urinary tract infections and their representation of three different antibiotic classes with distinct mechanisms of action. The categorization of isolates as susceptible, intermediate, or resistant was conducted in accordance with the CLSI criteria based on inhibition zone diameters (mm). For amoxicillin, isolates were classified as susceptible at ≥11 mm, intermediate at 12–13 mm, and resistant at ≤14 mm. For ciprofloxacin, susceptibility was defined as ≥21 mm, intermediate as 16–20 mm, and resistance as ≤15 mm. For gentamicin, isolates were considered susceptible at ≥15 mm, intermediate at 13–14 mm, and resistant at ≤12 mm. Results were interpreted according to the CLSI zone size interpretation chart.

DNA extraction, PCR Amplification, and Virulence Factor Detection

The fifty UPEC genomic DNA was extracted with NEXprep™ Cell/Tissue Genomic DNA Preparation Kit (Cat. No. NexK-3000; Genes Laboratories, Gyeonggi-do, Republic of Korea). Amplification of the virulence genes dam and fimA was carried out by PCR using published primer pairs (Integrated DNA Technologies, Inc. USA) (Table 1). PCR amplification of the dam gene consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 40 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. Amplification of the fimA gene was performed under similar conditions, with annealing at 56 °C for 1 min for 35 cycles and a final extension at 72°C for 5 min. The amplified products were stained with a DNA-safe marker, separated on a Merck 1% agarose gel using 0.5X tris borate EDTA buffer and then and then viewed using UV transillumination imaging equipment.

Statistical Analysis

Statistical analysis was done by using SPSS software 19.0 version (IBM Corp., New York, United States). Chi-squared test and Fisher's exact test was used to analyse the relationship between biofilm and antibiotic resistance, biofilm and virulence genes (*dam* and *fimA*). The *p*-value of less than 0.05 was considered as statistically significant.

Results

Biofilm Formation Capacity

Biofilm formation was considered positive when a visible film layer coated the sides and bottom of each tube. The tubes were then examined, and the presence of biofilm was considered positive for strongly, moderately, and weakly adherent bacteria, and negative for non-adherent bacteria. The percentage of UPEC bacteria capable of producing biofilm using the tube method was 37 isolates (74%), while the percentage of UPEC bacteria that were unable to produce biofilm using the tube method was 13 isolates (26%) (Figure 1).

Antimicrobial Susceptibility Profile

The highest percentage of resistance in UPEC was found in the antibiotic amoxicillin (92%), followed by ciprofloxacin (88%) and gentamicin (56%) (Table 2). However, resistance to the three antibiotics was not significantly related to their biofilm formation ability.

Table 1. Sequences of oligonucleotide primers for PCR amplification *dam* and *fimA* associated genes of UPEC.

Gene	Forward Primer (5'- 3')	Reverse Primer (3'- 5')
dam	CTTTTGTAGGTGCCGGGTCG	CCAGCAGTTCGTCCACCT
fimA	TTCAGGGTGGTTTGTGCACT	GCTCTGTCCCTCAGTTCCAC

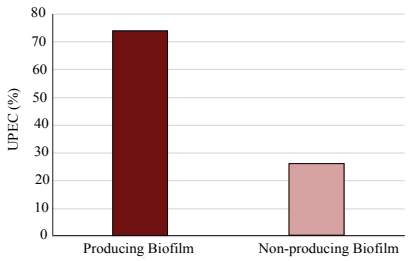

Figure 1. The percentage of biofilm production in UPEC was carried out using the tube method.

The Majority of The Isolates Demonstrated A High Prevalence of dam and fimA Genes

the detection of genes responsible for biofilm formation was carried out, and a PCR assay was used to detect genes (*dam* and *fimA*) that contribute to UPEC biofilm formation from UTI patients. The dam gene was detected at 696 bp, while the *fimA* gene was detected at 407 bp through electrophoresis results (Figure 2).

The relationship between the dam and fimA genes, and the biofilm formation capacity using the tube method in 50 different UPEC isolates associated with UTI. The majority of UPEC isolates showed positivity for the dam (88%) and the fimA gene (68 %) (Table 3). In UPEC isolates that were positive for the dam gene, 62% of them had the ability to form biofilms. Meanwhile, in UPEC isolates that were positive for the fimA gene, 52% of them had the ability to form biofilms. However, the presence of virulence genes (dam and fimA) in UPEC was not significantly related to their biofilm formation ability.

Discussion

The tube method showed that most UPEC isolates could form biofilms. Qualitative extracellular polymeric substances (EPS) or biofilm-formation was observed visually; the appearance of visible biofilm lining at the bottom and on wall of the glass tubes were considered positive for biofilm-production.[19] This method requires minimal setup but is limited by poor quantification, reproducibility, and sensitivity.[20] Although now largely obsolete, it played an important role in early biofilm detection. The tube method provides qualitative assessment through visual observation of biofilm formation on the tube walls and bottom.[21] In this study, 74% of UPEC isolates were capable of biofilm formation as determined by the tube method. This finding is consistent with previous studies reporting a high prevalence of biofilm-producing UPEC, with reported rates ranging from approximately 60% to over 80% depending on the detection method and isolate source.[22] Variability in the sensitivity and specificity of the Tube Method and Congo Red Agar arises from subjective interpretation and media differences; however, the Tube Method demonstrates superior sensitivity and specificity compared to Congo Red Agar.[23] Nonetheless, the high proportion observed in this study reinforces the notion that biofilm formation is a common trait among UPEC and contributes to their persistence in urinary tract infections.

Several previous studies have shown differences in the prevalence of biofilm formation in UPEC.[24,25] These differences may be due to methodological differences, such as environmental conditions and experimental setup, differences in geographic region, time of study, or source of sample isolation.[26] In this study, it was found that UPEC resistance occurred to the three antibiotics tested, where the highest resistance rate was in amoxicillin (92%). Several previous studies have demonstrated findings consistent with those of the present study. The majority of UPEC were resistant to commonly used antibiotics, namely amoxicillin, co-trimoxazole, tetracycline, and gentamicin. Most isolates were sensitive to nalidixic acid, ciprofloxacin, nitrofurantoin, and ceftazidime.[27] Antibiotic resistance in gram-negative bacteria, such as *E. coli*, is highest against ampicillin, ceftriaxone, ceftazidine, and cefotaxime. High antibiotic resistance may be due to self-medication practices or a lack of medication management guidelines

Table 2. Comparison of antibiotic resistance against biofilm-forming and non-biofilm-forming UPEC (n=50).

Antibiotics	Biofilm		Total resistant-UPEC n (%)	p-value	OR
	Producers n (%)	Non-producers n (%)			
Amoxicillin			46 (92)		05.08
Resistant	35 (70%)	11 (22%)		0.254*	
Sensitive	2 (4%)	2 (4%)			
Ciprofloxacin			44 (88)		02.05
Resistant	32 (64%)	11 (22%)		0.662*	
Sensitive	5 (10%)	2 (4%)			
Gentamicin			28 (56)		00.03
Resistant	19 (38%)	9 (18%)		0.264*	
Sensitive	18 (36%)	4 (8%)			

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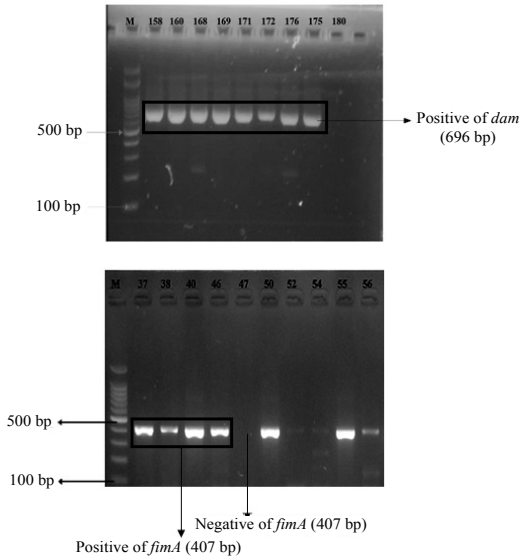

Figure 2. Genes involved in the development of biofilms can be found using PCR amplification (*dam* and *fimA*) isolated UPEC bacteria in UTI patients. The numerical codes shown above indicate the sample codes.

regarding drug selection.[28] Similar results with previous study, where the highest resistance rates were observed to amoxicillin (69.8%), ampicillin (62.3%), cefazolin (39.6%), trimethoprim (37.7%), ceftriaxone (34.9%), and tetracycline (33.0%).[29] Amoxicillin has high resistance in UPEC due to the misuse and overuse of antibiotics. In addition, previous exposure to amoxicillin is a significant risk factor for developing resistance, and resistance genes can spread rapidly through mobile genetic elements such as plasmids.[30] The increasing resistance to antimicrobials by *E. coli* is thought to be mediated by antimicrobial resistance determinants contained in genetic components such as plasmids, transposons, and integrons.[31]

Similar to the results shown by previous study, there was no significant relationship between biofilm production levels and antibiotic resistance patterns because all isolates studied were biofilm producers.[32] Present study found no significant differences or significant relationships. This could be explained by the fact that microorganisms adapt their virulence factor expression only in critical survival situations, or perhaps indicates that our in vitro methodology was not sophisticated enough to detect such relationships.[33] Investigating the correlation between biofilm formation and antibiotic resistance in UPEC is essential due to its contribution to persistent and recurrent urinary tract infections. Biofilm formation enhances bacterial survival by restricting antibiotic penetration, inducing metabolic heterogeneity, and facilitating the emergence of persister cells with reduced antibiotic susceptibility. Additionally, the biofilm environment promotes horizontal gene transfer, thereby accelerating the dissemination of antibiotic resistance determinants.

This study shows that the presence of the dam gene has a fairly high percentage of approximately 88% in UPEC. The dam gene is a gene that codes for the formation of DNA methyltransferase enzymes. This enzyme plays a role in the process of DNA methylation. DNA methylation can affect the expression of virulence factors and, thus, the pathogenicity of some bacteria.[12] The *dam* gene is widely found in bacteria such as Escherichia coli, Salmonella, and Yersinia. The *dam* gene is required for efficient biofilm production by *Salmonella enteridis*, and its ability to form biofilms is closely related to bacterial virulence.[34] In *E. coli*, DNA adenine methyltransferase (dam) mediates the generation of N6-methyladenine (6mA). DNA methylation (6 mA or 5 mC) plays a crucial role in regulating many biological processes, including restriction-modification systems, replication initiation, mismatch repair, virulence persistence, and global gene regulation.[34]

This study shows that the presence of the fimA gene has a fairly high percentage of approximately 68% in UPEC. Fimbrial adhesion is an extracellular protein structure that allows UPEC to adhere to bladder cells and prevent its excretion from the urinary system through urine flow. Population genomic analysis of UPEC has reported a prevalence of type 1 fimbriae (T1F) between 86–100%. T1F is encoded by the fim operon found in most UPEC.[35]

Table 2. Analysis of *dam* and *fimA* gene variations and biofilm formation ability in UPEC.

Virulence factors	Number of isolates n= 50 (%)	Biofilm formation n= 50 (%)	*p*-value	OR
dam gene				00.00
Positive	44 (88)	31 (62)	0.122*	
Negative	6 (12)	6 (12)		
fimA gene				01.08
Positive	34 (68)	26 (52)	0.562*	
Negative	16 (32)	11 (22)		

Fimbriae 1 or type 1 pili are considered to play a major role in the initial steps of *E. coli* biofilm formation. Fimbriae 1 is encoded by the fimAICDFGH operon. FimA is the main subunit in type 1 fimbria.[36] Bacterial pathogenicity is largely expressed by virulence factors encoded by genes. These virulence factor genes can enhance biofilm formation in bacteria.[37]

An important implication of this research is that understanding the specific genes involved in the pathogenicity of biofilm-forming UPEC will help elucidate the mechanisms of UTI infection, from initial colonization to more severe complications. Furthermore, it could lead to the development of better treatments for UTIs, such as antivirulence drugs or vaccines that specifically inhibit virulence factors without killing the bacteria, which could help reduce antibiotic resistance. It could also help monitor trends in UTI pathogenicity and prevalence, especially in specific patient populations such as hospitalized patients or children.

This study has several limitations that should be considered when interpreting the findings. The relatively small sample size and the use of isolates from a single clinical microbiology laboratory may limit the generalizability of the results to broader UTI populations in Indonesia. In addition, biofilm formation was assessed using the qualitative tube method, which may be less sensitive and reproducible than quantitative assays, and the analysis focused only on the presence of dam and fimA genes without evaluating their expression levels or other virulence determinants. Future studies should involve larger, multicenter cohorts, apply quantitative biofilm assays and gene expression analyses, and include a wider panel of virulence and resistance genes, as well as minimum inhibitory concentration (MIC) testing, to provide a more comprehensive understanding of the molecular mechanisms linking biofilm formation and antibiotic resistance in UPEC.

The high prevalence of the dam and fimA genes in uropathogenic *E. coli* (UPEC) and their association with biofilm formation and antibiotic resistance have important practical implications for healthcare settings. The *fimA* gene plays a critical role in bacterial adhesion to uroepithelial cells and medical device surfaces, while the dam gene contributes to the regulation of virulence and genomic stability, collectively enhancing UPEC persistence and resistance to antimicrobial therapy through biofilm formation. These findings highlight the need for improved infection prevention strategies, particularly in patients with long-term urinary catheterization. Furthermore, detection of *dam* and *fimA* genes has potential utility as molecular markers for identifying high-risk UPEC strains associated with persistent infection and multidrug resistance, thereby supporting more targeted and rational antibiotic selection. At the institutional level, this study underscores the importance of strengthening antimicrobial stewardship programs, infection control measures, and the development of anti-biofilm–based interventions to reduce UPEC-related treatment failure and healthcare-associated urinary tract infections.

Conclusion

Of the 50 UPEC isolates, 37 UPEC isolates (74%) were found to be able to form biofilms through testing using the tube method. UPEC bacteria showed resistance to amoxicillin, ciprofloxacin, and gentamicin with percentages of 92, 88, and 56%. UPEC bacteria showed a high positive frequency for the dam gene (88%), while the positive frequency for the fimA gene was slightly lower (68%). The high prevalence of dam and fimA genes associated with the ability to form biofilms can be used as diagnostic markers for biofilms or as vaccine targets.

Authors' Contributions

TK, DA, YH, and IA were involved in conceptualizing and planning the research. TK performed data acquisition and collection, and calculated the experimental data. TK also designed the figures. TK, DA, YH, and IA analyzed the data, drafted the manuscript, interpreted the results, and contributed to the critical revision of the manuscript.

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Authors' Contributions

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RP contributed to conceptualization, data curation, formal analysis, and methodology. SLU was responsible for validation, visualization, and writing the original draft, as well as review and editing. AFL contributed to conceptualization, investigation, methodology, validation, and writing the original draft, review, and editing.

Ethical Statement

The study was approved by the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya, under approval number 0341/KEPK/VII/2018.

Conflict of Interest

The authors declare no conflict of interest.

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