

## Analysis of uropathogenic *Escherichia coli* biofilm formation under different growth conditions

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The ability to form different types of biofilm enables bacteria to survive in a harsh or toxic environment. Different structures of biofilms are related to different surfaces and environment of bacterial growth. The aim of this study was analysis of the biofilm formation of 115 clinical uropathogenic *Escherichia coli* strains under different growth conditions: surface for biofilm formation, medium composition and time of incubation. The biofilm formation after 24 h, 48 h, 72 h and 96 h was determined spectrophotometrically ( $A_{531}$ ) after crystal violet staining and it was correlated with bacterial growth ( $A_{600}$ ). The live and dead cells in biofilm structures was also observed on the glass surface by an epi-fluorescence microscope. Additionally, the presence of *rpoS*, *sdiA* and *rscA* genes was analyzed. The statistical significance was estimated by paired T-test. The observed biofilms were different for each particular strain. The biofilm formation was the highest in the rich medium (LB) after 24 h and its level hasn't changed in time. When biofilm level was compared to bacterial growth (relative biofilm) — it was higher in a minimal medium in comparison to enriched medium. These results suggest that most of the bacterial cells prefer to live in a biofilm community under the difficult environmental conditions. Moreover, biofilm formation on polyurethane surface did not correlate with biofilm formation on glass. It suggests that mechanisms of biofilm formation can be correlated with other bacterial properties. This phenomenon may explain different types of biofilm formation among one species and even one pathotype — uropathogenic *Escherichia coli*.

**Key words:** biofilm, uropathogenic *Escherichia coli*, urinary tract infections

**Received:** 30 July, 2015; revised: 21 September, 2015; accepted: 06 October, 2015; available on-line: 10 December, 2015

### INTRODUCTION

Most of bacteria usually live in a biofilm in their natural environment (Costerton *et al.* 1987; Costerton, 1999; Horn & Lackner, 2014). This specific prokaryotic community creates a diverse form characterized by different properties (Kreft, 2004; Shirliff *et al.*, 2000). It depends on bacterial species (Gram-positive and Gram-negative bacteria) and their properties to survive in various environments (Costerton *et al.* 1987; Dunne, 2002; Ganesh & Anand, 1998; Wäsche, 2002). They afford different components to bacterial growth. Environment stimulates bacteria to form a biofilm or produce specific factors that let them to survive. The mushrooms-like structures are the most frequently described in the literature (Cos-

terton, 1999; Horn & Lackner, 2014; Picioareanu *et al.*, 2007; Reisner *et al.*, 2003). Biofilm structures are highly hydrated, often composed by multi-species, characterized by the most diverse functions of the regions inside biofilm and they are not very strongly attached to the surface. Bacterial flat layers firmly adhered to the surface or specific intracellular microcolonies have been also known during last decades (Busscher *et al.*, 1995). These specific structures are common for many pathogenic bacteria and particularly important for medicine and infectious diseases (Goldberg, 2002; Stewart & Costerton, 2001). Biofilm-associated cells are characterized by increased resistance to antibiotics, detergents, and host immune defense response substances. Moreover, biofilm provides bacterial cells with high-osmolarity conditions, oxygen limitations, and high cell density. Slow growth is also an important aspect of bacterial biofilm physiology (Lewandowski *et al.*, 1991; Vieira *et al.*, 2004; Prigent-Combaret *et al.*, 1999). Uropathogenic *Escherichia coli* strains (UPEC) can create a specific biofilm — a complex intracellular bacterial community (IBC) within the superficial umbrella cells of the bladder, unlike intestinal strains (Justice *et al.*, 2004). Justice *et al.* observed this structure by using time-lapse fluorescence video-microscopy. They infected mouse bladder explants and discovered that IBCs formed by uropathogenic *E. coli* followed through four distinct developmental phases that changed with respect to growth rate, bacterial length, colony organization, motility and its eventual dispersal (Justice *et al.*, 2004; Hannan *et al.*, 2012). Those observations have given the evidence for the existence of unique uropathogenic *Escherichia coli* strains. They differ substantially from the intestinal commensals and cause recurrent urinary tract infections. This phenomenon has confirmed previous reports (Elena *et al.*, 2005; Herzer *et al.*, 1990; Pupo *et al.*, 1997), which proved that this species is quite diverse genetically and phenotypically. Analysis of the entire dataset indicated that non-pathogenic commensal *E. coli* strains B and K-12 are the most closely related, followed by enterohemorrhagic *E. coli* O157:H7. Instead, uropathogenic *E. coli* CFT073 strain is the most divergent of the strains within the *E. coli* clade. Additionally, *E. coli* B, K-12, and O157:H7 strains are more closely related to *Shigella flexneri* than to any of the uropathogenic *E. coli* strains (Elena *et al.*, 2005).

In this study we report the differentiations of *E. coli* strains based on their biofilm formation ability. The total of 115 *E. coli* strains from urine samples were grown

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\*The results were presented at the 6th International Weigl Conference on Microbiology, Gdańsk, Poland (8–10 July, 2015).

under different conditions as time, temperature, surface and medium contents of culture. Our results indicate that not all of the strains were able to form a biofilm, the same strains could create biofilm on the glass surface and were unable to form a biofilm on polyurethane. Furthermore, the optimal time for forming biofilm was observed to be 24 hours, and not longer.

## MATERIALS AND METHODS

**Bacterial strains.** In this study we used 115 clinical *Escherichia coli* strains isolated from the urine of patients in different wards of Military Teaching Hospital No. 2, Medical University of Lodz, Poland in the years of 2005–2007. All strains were stored at  $-80^{\circ}\text{C}$ . The strains have been previously characterized in terms of the presence of some virulence factor genes (*papG*, *sfa*, *bly*, *cnf1*, *usp*, *fimG/H*) and phylogenetic groups A, B1, B2, D (Adamus-Bialek *et al.*, 2009) and further C, E and F.

**Synthetic urine with 0.2% glucose.** Synthetic urine was prepared according to a recipe previously described by Griffith *et al.* (1976) and Torzewska and Róžalski (2015) with the composition (g/l):  $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ , 0.651;  $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ , 0.651; NaCl, 4.6;  $\text{Na}_2\text{SO}_4$ , 2.3; sodium citrate, 0.65; sodium oxalate, 0.02;  $\text{KH}_2\text{PO}_4$ , 2.8; KCl, 1.6;  $\text{NH}_4\text{Cl}$ , 1.0; urea, 25.0; creatine, 1.1. The solution was supplemented with glucose, 2. pH was adjusted to 5.8 and urine was sterilized by passing through a 0.2  $\mu\text{m}$  pore-size filter.

**Biofilm formation assay by crystal violet staining.** A fresh inoculum of bacteria was prepared in LB Broth at  $37^{\circ}\text{C}$  for 24 hours. The bacterial culture was diluted to obtain  $0.125 \pm 0.005$  OD at 600 nm using Microplate Reader TECAN Infinite 200 PRO (Tecan Group Ltd., Switzerland), corresponding to approximately  $10^3$  cfu of bacteria. The bacteria were grown in triplicate on the 96-well plates at  $37^{\circ}\text{C}$  in LB broth and synthetic urine with glucose (0.2%). A medium without bacteria incubated under the same conditions was treated as a negative control. The bacterial growth after incubation for 24, 48, 72 and 94 hours was measured at 600 nm using a Microplate Reader. The wells were carefully washed twice with PBS/water and dried in the  $37^{\circ}\text{C}$  incubator for at least 30 minutes without the lid. The crystal violet solution (0.3%) was added to each well and incubated for 5 minutes, in a well ventilated area. The wells were washed twice with PBS/water and excess water was removed with a paper towel. The biofilm stain was dissolved with acetone/ethanol solution and shaken at 200 rpm for 10 min. Biofilm formation was measured at 531 nm using a Microplate Reader. All measurements were performed in two independent experiments. A blank corrected mean absorbance value of  $<0.06$  from negative control was considered as a biofilm-negative strain. The results were normalized (Biofilm relative,  $B_{\text{Rel}}$ ).  $B_{\text{Rel}}$  was estimated

based on the proportion between the absorbance level of the formed biofilm and the absorbance level of the growth. The results were analyzed using GraphPad Prism, version 6 (San Diego, CA). Statistical analyses were performed using paired T-test. A *P* value of  $<0.05$  was considered to be significant.

**Microscopic observation of a biofilm.** An overnight inoculum of bacteria was diluted 100-fold and incubated in tubes with three coverslips inside. Bacteria were growing in LB broth at  $37^{\circ}\text{C}$ . After 24 hours, the coverslips were gently washed three times in water. The coverslips were dried and stained with a mixture of two dyes according to the manufacturer's protocol (Filmtracer™, Invitrogen). Mixtures have included two substances: SYTO® 9, a green fluorescent nucleic acid stain, and a red-fluorescent nucleic acid stain, propidium iodide. Dye solution was spread on a coverslip and incubated at room temperature in the dark for 20 min. Next, the coverslips were gently washed three times in water and placed onto slides. The biofilm formation on the glass was observed with an epi-fluorescence microscope (ZEISS Axio Scope.A1) no longer than 30 minutes after staining. The pictures were made from five randomly selected fields of the microscopic preparation. Interpretation of the microscopic observations was based on the stained cells. Dyes differ both in their spectral characteristics and ability to infiltrate healthy bacterial cells. Bacterial cells with green fluorescence are interpreted as alive, whereas bacteria with damaged membranes show red fluorescence.

**Detection of biofilm-associated genes.** Bacterial DNA was purified with the GenElute™ Bacterial Genomic DNA kit (Sigma Aldrich). PCR was used for the identification of *sdiA*, *rscA* and *rpoS* genes. The primers and their melting temperature used for PCR analysis were described in Table 1. PCRs were performed with bacterial DNA (1 ng) in a 25  $\mu\text{l}$  reaction mixture containing 12.5  $\mu\text{l}$  DreamTaq™ Green DNA Polymerase Master Mix (2x) (Thermo Scientific™) including 1U DreamTaq DNA Polymerase, optimized DreamTaq Green buffer, 2 mM  $\text{MgCl}_2$ , 0.4 mM each of dATP, dCTP, dGTP and dTTP; 10 pmol concentrations of each primer. DNA amplification was carried out in an Eppendorf AG thermocycler. Cycling conditions were as follows: denaturation at  $95^{\circ}\text{C}$  for 2 min followed by amplification for 30 cycles at  $95^{\circ}\text{C}$  for 1 min, annealing temperature (Table 1) for 1 min, and  $72^{\circ}\text{C}$  for 1 min, followed by a final extension at  $72^{\circ}\text{C}$  for 5 min.

## RESULTS AND DISCUSSION

### Growth and Biofilm formation at different time points

The 115 clinical isolates of *E. coli* were analyzed. Bacterial growth and biofilm formation were estimated

**Table 1.** Oligonucleotide primers for PCR used in this study

No.	Primer	Sequence (5' → 3')	Target sequence	PCR (bp)	$T_m$ ( $^{\circ}\text{C}$ )	References
1	sdiA1-F sdiA1-R	TCGCTATCTCTGCTGATGTC TTTAATGCTGCCAAATCGGG	sdiA	239	52	This study
2	rscA-F rscA-R	GTGATTACAGCGCCCTTCA TACTCGATTCCGGTTCGGCTC	rscA	306	54	This study
3	rpoS 768F rpoS 870R	GCAGAGCATCGTCAAATGGCTGTT ATCTCCAGTGTTCGCCCTTCGTA	rpoS	120	60	1, 2

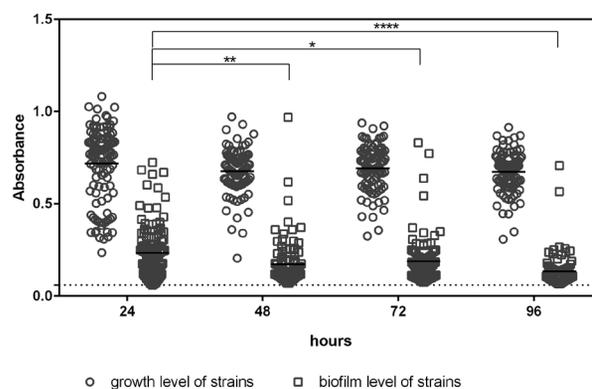
at four time points of incubation. *Escherichia coli* strains were growing differently, but the mean of optical density ( $A_{600}$ ) of bacterial growth after 24 hours of incubation was about 0.7, which represents a low value in comparison to the most of clinical strains of *Enterobacteriaceae* (Ramos *et al.*, 2012; Ulett *et al.*, 2007). The decrease in optical density of bacterial growth was not as significant at the other time-points. It means that the logarithmic phase of bacterial growth stopped before 24 hours, whereas the statistical significant decrease in bacterial biofilm was observed in the following hours of incubation (Fig. 1). This also indicates inhibition of biofilm formation by *E. coli* strains after 24 hours of incubation in the LB broth. This observation is not consistent with the beliefs that bacteria usually need more time to create a biofilm. It should be emphasized that biofilm created by uropathogenic *E. coli* strains is not typical. This pathotype usually creates a less complex biofilm in comparison to most of the typical biofilm-positive bacterial species, as *S. aureus* or *P. aeruginosa* (Ferrieres *et al.*, 2007; Reisner *et al.*, 2003; Spiers *et al.*, 2003; Vieira *et al.*, 2004; Zhang *et al.*, 2015). The mean  $A_{531}$  level (biofilm level) was 0.3, but this group of bacteria doesn't need to form an extensive and complex biofilm. UPEC form specific intracellular bacterial communities (IBC), which protect them against the host immune system. They require to penetrate the superficial umbrella cells of the bladder during a short period of time, before the influx of neutrophils (Justice *et al.*, 2004; Hannan *et al.*, 2012). The observed results may reflect this bacterial behavior.

#### Growth and Biofilm formation in different media

The following results are consistent with the conclusions mentioned above. Bacterial growth and biofilm formation were performed in synthetic urine, in comparison to optimal growth condition (LB broth). Representative 32 *E. coli* strains were selected, based on different level of formed biofilm. They were incubated for 24 hours at 37°C. A strong and significant decrease of the growth and biofilm formation was observed in synthetic urine (Fig. 2). Nevertheless, the difference between growth under different conditions was higher than differences observed between biofilm formation. Therefore, the comparison of the biofilm level independent from growth (relative biofilm) between different conditions of incubation was analyzed. It was shown that bacteria created a higher level of biofilm in synthetic urine (Fig. 3). Probably, in the minimal medium most of bacterial cells were involved in biofilm formation, in contrast to enriched medium. Uropathogenic *E. coli* strains may behave similarly in the bladder of the host causing chronic UTIs.

#### Biofilm formation on different surfaces

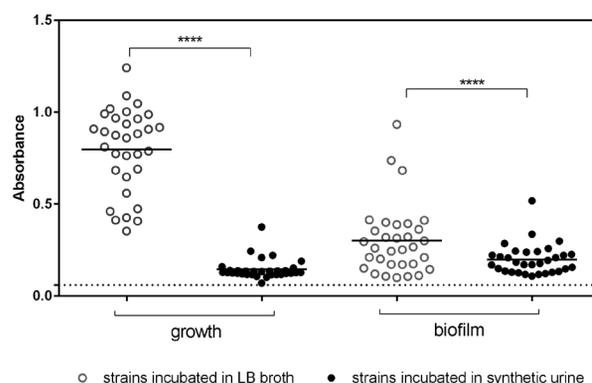
The analyses were also concerned with the microscopic observation of the biofilms formed on the glass surface (coverslip). The 115 isolates from the same collection of *E. coli* strains were incubated on the coverslips in LB broth at 37°C for 24 hours. After that, the coverslips with bacterial culture were stained and observed with an epi-fluorescence microscope. The observations revealed the live and dead bacterial cells in the microcolonies formed, which is a typical for the biofilm structure. The studied strains were divided into two groups – the strains form microcolonies were scored as biofilm-positive (44%), and the strains unable to form microcolonies were scored as biofilm-negative (56%). The biofilm-positive strains created mainly small microcolo-



**Figure 1. Growth and biofilm level of uropathogenic *Escherichia coli* strains at different time-points of incubation.**

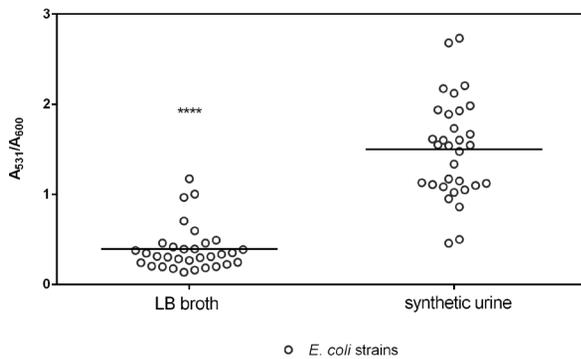
Bacterial isolates (n=115) were grown in triplicate, in 96-well plates in LB broth at 37°C. Growth and biofilm level were measured spectrophotometrically at 600 nm and 531 nm, respectively, for each time-point of incubation (24, 48, 72 and 96 hours). The absorbance value <0.06 represents a biofilm-negative strain. The experiment was performed twice. Statistical significance of the differences was assessed by the Paired *T*-test, and *P* values <0.05 were considered as significant, where \*<0.05, \*\*<0.01, \*\*\*\*<0.0001.

nies (Fig. 4A, B) but a few strains covered almost whole coverslips (Fig. 4C). These modest biofilms confirm the above findings of biofilms formed by UPEC strains. Filamentous cells were also observed just after the cell division (Fig. 4D). This phenomenon was observed by other authors (Justice *et al.*, 2004), but we identified it only for one strains in the analyzed collection of *E. coli*, probably because of the *in vitro* culture conditions. An interesting observation was revealed by comparing of the biofilm results made by various techniques (Table 2). The strains were divided into strong biofilm-forming strains ( $A_{531} \geq 0.3$ ), moderate biofilm-forming strains ( $A_{531} < 0.3$ ;  $A_{531} \geq 0.1$ ) and weak biofilm-forming strains ( $A_{531} < 0.1$ ;  $A_{531} \geq 0.06$ ). The biofilm created on the polyurethane did not correlate with biofilm created on the glass surface. All of the bacteria adhered to the polyurethane (strongly or weakly), but only 44% of them were able to develop



**Figure 2. Growth and biofilm level of uropathogenic *Escherichia coli* strains in different media.**

Bacterial isolates (n=32) were grown in triplicate, in 96-well plates, in LB broth and synthetic urine at 37°C for 24 hours. The growth and biofilm level was measured spectrophotometrically at 600 nm and 531 nm, respectively. The absorbance value <0.06 represents a biofilm-negative strain. The experiment was performed twice. Statistical significance of the differences was assessed by the Paired *T*-test, and *P* values <0.05 were considered as significant, where \*\*\*\*<0.0001.



**Figure 3. Relative biofilm of uropathogenic *Escherichia coli* strains in LB broth and synthetic urine.**

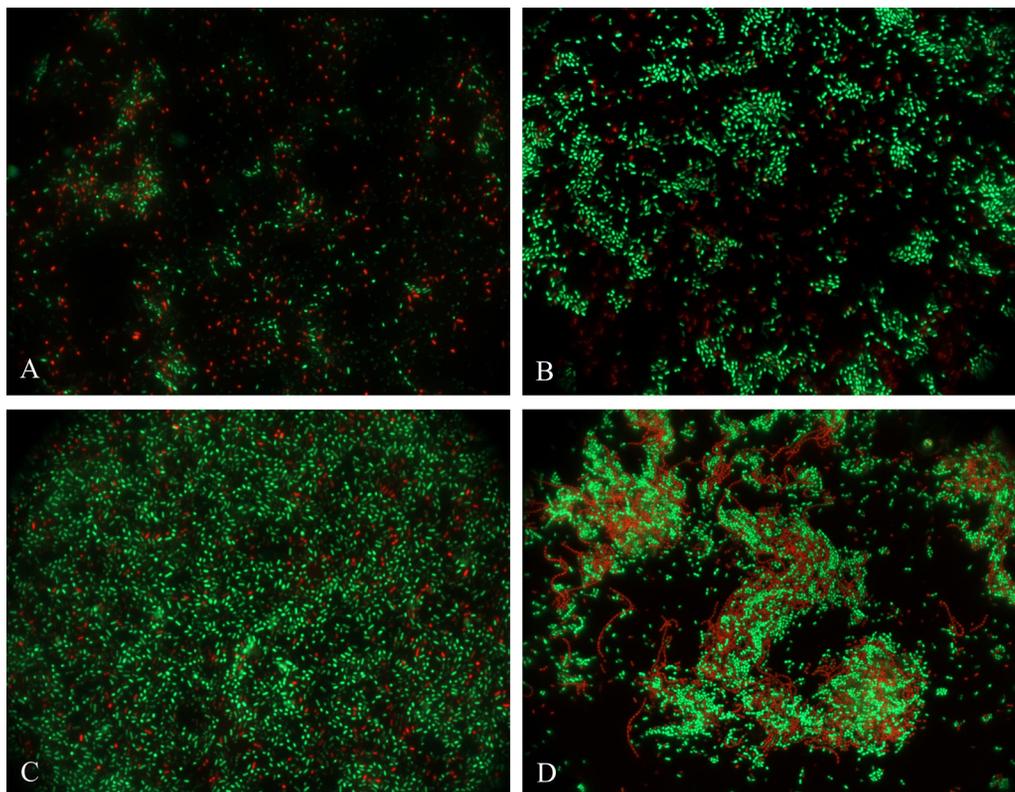
Relative biofilm was estimated based on the proportion between the absorbance value of the biofilm ( $A_{531}$ ) and the absorbance value of the growth ( $A_{600}$ ) for the individual strains ( $n=32$ ). Statistical significance of differences was assessed by the Paired *T*-test, and *P* values  $<0.05$  were considered as significant, where  $****<0.0001$ .

the microcolonies on a glass surface. This disagreement can be related with the different stages of biofilm observed by different techniques. The biofilm test with crystal violet allows mainly to check the bacterial adhesion ability. On the other hand, it is difficult to indicate which  $A_{531}$  value of adhesion determines which bacterial cultures would form microcolonies. In this study, usually the bacterial strains which created microcolonies on the glass surface were characterized by a different level of adhesion to polyurethane. The mechanism of biofilm formation may be connected together features of the bacterial strains. The relationship between biofilm formation and surface (polyurethane, glass) can be associated with

the hydrophobicity of the environment surface and outer membrane of a particular strain (Krasowska & Sigler, 2014; Liu *et al.*, 2004). Probably, hydrophobic bacteria will attach better to a hydrophobic polyurethane and *vice versa*. Our initial studies have found that the analyzed *E. coli* strains were characterized by different hydrophobicity of their surface, but it was difficult to correlate it with their biofilm formation ability (data not shown).

#### Presence of genes associated with biofilm formation

During the course of this study, we tried to find a direct link between presence of particular genes and bacterial ability to form a biofilm. The phylogenetic groups of strains were characterized previously (Adamus-Bialek *et al.*, 2009) and later supplemented based on a protocol described by Clermont *et al.* (2013). We observed, that the strains belonging to phylogenetic group D, and also E and F, formed a weaker biofilm on the polyurethane, than the strains from the other groups (data not shown). The results were not significant because of the low number of strains. Results present in the literature indicate that *E. coli* belonging to group D diverged first, and B2, A and B1 are related groups that separated later (Sims & Kim, 2011). This can be connected with weaker primary adhesion to the surface or some specific features of bacterial outer membrane. Nonetheless, these observations need to be further investigated. Next, a correlation between the virulence factors gene (*papG*, *fimG/H*, *sfa*, *cnf1*, *hly*, *usp*) and biofilm-positive strains was studied. The presence of the mentioned above virulence factor genes was also characterized previously (Adamus-Bialek *et al.*, 2009) for the same collection of bacteria. It is often described in the literature that Type 1 pili, encoded by the *fim* gene cluster, are extremely common among



**Figure 4. Microcolonies of uropathogenic *Escherichia coli* strains.**

(A) *E. coli* No.27, (B) *E. coli* No. 59, (C) *E. coli* No. 64, (D) *E. coli* No. 108. Biofilm was grown on the coverslips for 24 hours at 37°C, and then was observed with an epi-fluorescence microscope with 1000-fold magnification (Nikon Eclipse). Live (green) and dead (red) cells were stained by Filmtracer™, Invitrogen.

Table 2. Characteristics of 115 uropathogenic *Escherichia coli* strains based on biofilm formation

		UPEC strains	% of total strains	UPEC strains	%*
Biofilm on polyurethane (A <sub>531</sub> )	≥0,3	2, 10, 13, 23, 26, 30, 35, 46, 58, 60, 64, 69, 73, 78, 79, 80, 82, 84, 91, 94, 97, 107, 108, 122, 125	22	yes	35, 58, 60, 64, 69, 79, 91, 94, 107, 108, 122, 125, 48
				no	2, 10, 13, 23, 26, 30, 46, 73, 78, 80, 82, 84, 97 52
	<0,3; ≥0,1	3, 4, 5, 6, 7, 9, 11, 14, 15, 16, 18, 21, 24, 27, 29, 33, 34, 38, 39, 41, 42, 43, 45, 47, 48, 50, 51, 52, 59, 61, 62, 65, 67, 70, 72, 74, 75, 76, 81, 82, 83, 85, 86, 87, 88, 90, 93, 95, 96, 98, 99, 102, 103, 105, 106, 109, 110, 111, 112, 114, 116, 119, 120, 121, 126, 127, 129, 130	59	yes	3, 4, 5, 14, 16, 21, 24, 27, 29, 33, 38, 41, 42, 45, 51, 52, 59, 65, 67, 70, 81, 83, 86, 96, 99, 102, 105, 110, 111, 121, 126 46
				no	6, 7, 9, 11, 15, 18, 34, 39, 43, 47, 48, 50, 61, 62, 72, 74, 75, 76, 82, 85, 87, 88, 90, 93, 95, 98, 99, 103, 106, 109, 112, 114, 116, 119, 120, 127, 129, 130 54
				yes	31, 40, 49, 54, 56, 63, 92, 104, 36
				no	8, 17, 19, 22, 25, 44, 53, 55, 71, 77, 89, 101, 118, 124 64
<0,1; ≥0,06	8, 17, 19, 22, 25, 31, 40, 44, 49, 53, 54, 55, 56, 63, 71, 77, 89, 92, 101, 104, 118, 124	19			

\*% of compatibility with biofilm on polyurethane (A<sub>531</sub>)

*E. coli* isolates (Wiles *et al.*, 2009). Type 1 fimbriae, as well as P (*pap*) and S (*sfa*), are considered to be the most important virulence factors of UPEC involved in the induction of an UTI and formation of biofilm (Bergsten *et al.*, 2005; Johnson 1991). We observed the correlation between the *papG* positive and/or *sfa* positive strains and biofilm formation (Fig. 5). It should be also noted that the absence of *fimG/H*, *papG* or *sfa* did not definitely limit the biofilm formation of the studied *E. coli* strains, which is consistent with the results presented by Miyazaki (2002). Therefore, the presence of other genes encoding adhesins is highly possible. In this study, we have analyzed the occurrence of three additional genes (*sdhA*, *rscA* and *rpoS*) to check this relationship. A lot of results presented in the literatures have shown that *rpoS*, *sdhA*

and *rscA* are important for bacterial ability to carry on biofilm formation (Corona-Izquierdo & Membrillo-Hernandez, 2002; Collet *et al.*, 2007; Ito *et al.*, 2007; Ranjit & Young, 2013; Wei *et al.*, 2001). RpoS is an alternative sigma transcription factor that controls the expression of a large number of genes involved in the cellular response to stress (Adams & McLean, 1999; Corona-Izquierdo & Membrillo-Hernandez, 2002). Similarly, *rscA* is a regulatory gene belong to the complex Rcs system which controls cell wall integrity, cell division, stationary phase sigma factor activity, motility, and virulence. It was also shown the effect of this gene on modulating *tolQRA* and *csgBA* expression which also plays a role in adhesion to eukaryotic cells using curli. Instead, *sdhA* gene of *E. coli* is homologous to *luxR* in other bacteria. This is the activator of quorum sensing in biofilm and one of the most important elements of bacterial communication in the biofilm (Bassler, 1999; Miller, 2001; Wei *et al.*, 2001). These three genes have not proven to differentiate strains for their ability to form biofilms. All of the studied *E. coli* strains possess them, so the presence of those genes does not determine the biofilm formation. The lack of the association between those bacterial properties may result from complex mechanisms involved in the biofilm formation. The most important role plays the expression of particular genes at the subsequent stages of biofilm formation (Schembri *et al.*, 2003).

In summary, it is an interesting observation that one species' (*Escherichia coli*) isolates from the same environment (urine) can differently adhere to different surfaces and create different biofilms. Biofilm formed by the tested species matures in first 24h, and prolonged cultivation leads to reduction in biofilm biomass. Biofilm formation in minimal medium provides higher amounts of biofilm, which is probably caused by cell starvation which triggers biofilm formation. Differences in adherence and biofilm formation of uropathogenic *E. coli* on two types of surfaces: considered as hydrophilic — glass,

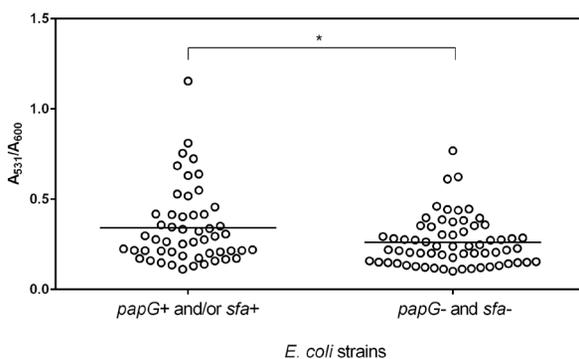


Figure 5. Relative biofilm of uropathogenic *Escherichia coli* strains correlated with the presence of fimbriae genes (*papG* and/or *sfa*).

Relative biofilm was estimated based on the proportion between the absorbance value of the biofilm (A<sub>531</sub>) and the absorbance value of the growth (A<sub>600</sub>) for the individual strains (n=115) with or without particular genes (*papS* and/or *sfa*). Statistical significance of differences was assessed by the unpaired, nonparametric T-test, and P values <0.05 were considered as significant

and hydrophobic — polyurethane, suggest that cell surface hydrophobicity of the tested bacterial strains may differ, although all strains were isolated from the same type of infection, where conditions were similar. All tested strains possess *sdhA*, *rscA* and *rpoS* genes, shown to be engaged in biofilm formation process, and their presence does not determine differences in biofilm formation of the tested strains. Determination of the ability to create biofilm is not simple even for a defined bacterial group, such as uropathogenic *Escherichia coli*.

### Acknowledgements

We would like to thank Dorota Derlatka and Mariusz Świercz for excellent technical support. This study was supported by National Science Centre research grant NCN 2011/01/D/NZ7/00107.

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