

Ability of Cellulose Polysaccharide and Curli Pili Production in Uropathogenic Escherichia Coli and its Association with Biofilm Formation Intensit

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ABSTRACT

Background and Objective: the Formation of urinary infection by uropathogenic *E.coli* needs numerous virulence factors and biofilm formation is among these factors. Bacteria that form biofilms express phenotype traits that appear according to the bacteria type. Cellulose is an important compound on the outside of *E.coli* causing bacterial cell-cell reactions and connection to nonliving surfaces. Curli pili cause the reaction between cell-cell and surface-cell in biofilms and lead to bacteria aggregation. Microorganisms' ability to form biofilm on a surface depends on the surface nature and its conditions. This study aimed at determining the production ability of cellulose polysaccharide and curli pili in UPEC strains, and its correlation with formation and intensity of biofilm.

Methods: In this study carried out to compare the ability of cellulose and pili curli production ability in 40 uropathogenic *E.coli* isolates ,by morphotype method in Congo Red medium (CR), each isolate was incubated at 37 °C, for 24 hours. After 24 hours, all colonies' morphology characteristics were studied

Results: It was shown that 67.5% of strains produced cellulose and 72.5% produced curli pili. In addition, 92.6% and 89% of isolates that produce cellulose and curli, respectively, had a moderate to strong biofilm. Moreover, it was shown that there is a significant correlation between cellulose and / or curli pili production with biofilm intensity.

Conclusion: About 70% of *E.coli* isolates from patients' urine are able to produce cellulose or curli pili; therefore, it can be concluded that the production of these two combinations is effective in amount and intensity of biofilm formation.

Keywords: *Escherichia coli*; Cellulose Polysaccharide; Curli Pili; Biofilm.

INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) have various pathogenic factors such as biofilm formation and are the primary cause of urinary tract infection (UTI) in developed countries (1). Biofilms formed by pathogenic *Escherichia coli* strains can cause serious problem by increasing bacterial stability and colonization in the host's body (2, 3). Cellulose is an important compound on the outside of *E.coli* produced as an extracellular polysaccharide that causes bacterial cell-cell interactions and attachment to abiotic surfaces (4). Curli is a filamentous protein that causes more induced pro-inflammatory cytokines, facilitates cell-cell and/or cell-surface interactions and results in bacterial aggregation (5). There are several factors involved in *E.coli* biofilm formation, such as adhesine, fimbriae, flagel and exopolysaccharide (cellulose, Curli pili, colonic acid) (6). Curli fimbriae and/or cellulose are expressed by *E. coli*, *Salmonella spp.* and other *Enterobacteriaceae* (7). The red, dry and rough morphotype system (rdar) is used to describe *salmonella typhimurium* and *E.coli* Colonies morphology in Congo red medium. Congo red medium has non-covalent bonds that have tendency to cellulose and/or curli pili. When the color is added to agar cultivation medium, the color of the growing colonies in the plate changes according to expression of cellulose and/or curli pili (7). The existence of curli pili is associated with brown, dry and rough morphotype (bdar) and red, dry and rough morphotype (rdar), while pink, dry and rough morphotype (pdr) is associated with cellulose. Rdar morphotypes: cellulose (+), curli (+); pdr morphotype: cellulose (+), curli (-); bdar: cellulose (-), curli (+); saw (smooth and white): cellulose (-), curli (-), (figure 2). The more imperceptible expression of cellulose and curli pili results in the production of soft morphotypes of bas, ras, pas, respectively (7-8). The high level of cellulose expression may also hide the low expression of curli pili and as results in synchronous expression of curli pili and cellulose ends in the production of more powerful biofilms. Generally, the strains with rdr or ras morphotypes have moderate or powerful biofilms, while pdr and bdr morphotypes have less biofilm formation

ability compared to rdr. Also, saw morphotype has either weak or no biofilm (9). This study aimed at determining the production ability of cellulose polysaccharide and curli pili in UPEC strains, and its correlation with formation and intensity of biofilm.

MATERIAL AND METHODS

isolates of *E.coli* were collected in Gorgan medical faculty from patients with urinary infection symptoms, confirmed by customary methods as *E.coli* bacteria. Different cultivation media (TS1, Broth VP_MR, SIM, EMB) (Merk, Germany) have been used for revival, purification, movement specification and biofilm formation of *E.coli* species (10, 11) based on Marhova method (8), saltless Loria Bertani medium, containing 40 µg/mL of Congo red and 20 µg/mL of Kumasi blue, was made. The medium was transferred to plates and then were put in for 15 minutes at 121°C under the pressure of 15 lbs autoclave. Each isolate was infused to this medium and incubated at 37 °C, for 24 hours.

After 24 hours, all colonies morphology characteristics were studied and the clones were put in four group of rdr, bdar, pdr and saw, based on cellulose and/or curli pili production (figure1). The Isolates with and without cellulose and/or curli pili genes (ATCC1399) were used as positive and negative controls, respectively. In the previous study, the isolates were specified by PCR method, considering having or not having cellulose and curli pili (11-12).

All processes were repeated 3 times to increase the accuracy. Biofilm production study on urinary catheters of the 40 isolates was considered in previous study (12).

RESULTS

It was found that 27 (67.5%) of the 40 tested isolates produced cellulose and 29 (72.5%) produced curli pili. In addition, 22 isolates (55%) produced both (rdar) synchronously, six isolates (15%) produced none (saw), seven isolates (17.5%) produced only curli pili (bdar) and five isolates (12.5%) produced only cellulose (pdr).

The correlation between cellulose and curli pili production in 40 *E.coli* isolates and biofilm formation on urinary catheters was studied. The Biofilm formation was classified into two

groups: moderate to strong and weak to biofilmles (without biofilm). It was observed that 27 isolates (67.5%) produced cellulose, 29 (72.5%) produced curli pili, 22 (55%) produced both of them synchronously, six (15%) produced none, seven (17.5%) produced only curli pili and five of them (12.5%) produced only cellulose. All of 27 isolates expressing cellulose produced biofilms while 25 (92.6%) had strong to moderate biofilm and two (7.4%) had weak biofilms, and this difference was found to be statistically significant ($p=0.001$). Among the 29 isolates producing curli pili, 26 (89.7%) had strong

to moderate biofilm, and three (10.3%) had either weak or no biofilm, which was also statistically significant ($p=0.003$), (table1). Among 22 isolates expressing both genes all of them formed strong to moderate biofilms. Of seven isolates expressing curli pili only, four (54.1%) had strong to moderate biofilms; of five isolates producing cellulose only, three (60%) produced strong to moderate biofilms; of six isolates producing none of them, four (66.7%) neither formed biofilm nor had weak biofilm, all of which were statistically significant ($p=0.001$), (table1).

Figur 1- E.coli morphotype system in Congo red media: (a): bdar: cellulose(-), curli(+); (b): pdar morphotype: cellulose(+), curli(-); (c): Rdar morphotype: cellulose(+), curli(+); (e):saw(smooth and white): cellulose(-), curli(-),

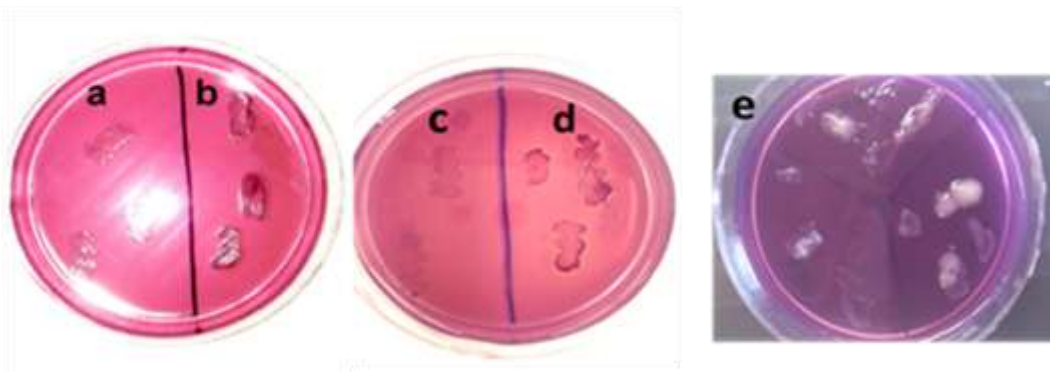


Table 1- Comparing biofilms' ability to produce cellulose and curli pili

Gene names		Without biofilm	With biofilm	With biofilm		P value
				Hard to moderate	Weak/no biofilm	
Curli pili	Yes	(3/4%) 1	(96/6%) 28	(89/7%) 26	(10/%3) 3	0.003
	no	(18/2%) 2	(81/8%) 9	(45/5%) 5	(54/5%) 6	
cellulose	Yes	0	(100%) 27	(92/6%) 25	(7/4%) 2	0.001
	no	(23/1%) 3	(76/9%)10	(46/%2) 6	(53/8%) 7	

DISCUSSION

Studying cellulose and curli pili production specified that 27 isolates (67.5%) produced cellulose polysaccharides and 29 (72.5%) produced curli pili. Also, 22 of them (55%) expressed both genes synchronously (rdar). Moreover, the investigation of biofilm formation ability of *E.coli* isolates in broth BHI medium, containing %1 saccharose, in the previous study (12) showed that among 40 isolates, 38 (95.5%) had the ability to form, 19 isolates (47.5%) showed strong biofilm, 12 (30%) moderate biofilm and seven (17.5%) weak biofilm, while only two isolates (5%) didn't have the ability to form biofilm on urinary catheter. Comparing production of cellulose and curli pili with biofilm formation, in UPEC strains, showed that all cellulose producing isolates formed biofilm, 90% of which had strong to moderate biofilms, while, 96% of curli pili producing isolates formed biofilms and 90% had strong to moderate biofilms. On the other hand, 70% of isolates lacking cellulose and curli pili were either unable to form biofilm or formed very insignificant biofilm. This may indicate the important role of cellulose and curli pili production in biofilm formation. The synchronous expression of the two genes have broad important effects on biofilm intensity so that all the isolates producing cellulose and curli pili synchronously formed moderate to strong biofilms. According to our finding, it seems that cellulose has more impact on the intensity of formed biofilms. Mihaylova and his colleagues by this method in 2012 specified that 50% of isolates were able to produce cellulose, similar to our results (13). Generally, the curli pili producing isolates in this study are more than those producing cellulose polysaccharides. This conclusion is similar to the study results obtained by Bokranz and his colleagues in 2005, on *E. coli* bacteria isolated from urine by phenotype method. Furthermore, similar to our study results, Bokranz showed that there was a considerable correlation between biofilm formation ability and cellulose polysaccharides (1). The results showed us that the

synchronous production of cellulose and curli pili has tremendous effect on biofilm formation intensity, since all the isolates producing cellulose and curli pili synchronously have formed strong to moderate biofilm. These results are close to the study results obtained by Saldana and his colleagues in 2009, who studied the common role of cellulose and curli pili in attachment efficacy. They showed that cellulose polysaccharide and curli pili cause colonization in the host, affect biofilm formation and survive in different media (14). In another study conducted by Marhova in 2010, by phenotype method, it was shown that cellulose is produced in 76% of strains, which is greater than cellulose produced in the present study (and even more than the amount of curli pili production) (8). In our study cellulose and curli pili synchronous production was seen in 55% of isolates, which is approximately close to Norinder observation results in 2011, done on Congo red and calcofluor plates, showing 41% of isolates expressed cellulose and curli pili synchronously (15).

CONCLUSION

About 70% of *E.coli* isolates, obtained from patients' urine in the city of Gorgan, are able to produce cellulose or curli pili and more than half of them express these two combinations synchronously. By comparing cellulose polysaccharide and/or curli pili production with biofilm intensity (in the previous study), it may be concluded that the production of this combination is effective in amount and intensity of biofilm formation

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CONFLICT OF INTEREST

There are no conflicts of interest.

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