DETECTION OF GENES INVOLVED IN BIOFILMS FORMATION BY ESCHERICHIA COLI ISOLATED FROM PATIENTS SUFFERING OF URINARY TRACT INFECTIONS

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Abstract

A hundred samples of urine were collected from patients of different ages and genders, who have symptoms of Urinary Tract Infection (UTI) from three hospitals in Baghdad, especially Ibn Al-Baladi Hospital during the period from September 2019 to December 2019. After culturing the urine samples, the isolates were identified by morphological and biochemical characteristics as well as API 20E and VITEK 2 system. The results showed that 60 of 100 isolates (60%) were *E. coli*. This study demonstrated that (77%) of the *E. coli* isolates were from females and only (23%) were from males, also it showed that (65%) were from children (1-10) years. The present study shows that (93%) of the *E. coli* isolates have the ability to form biofilms and they were divided into three groups: strong biofilm-producers (19 isolates, 34%), moderate biofilm-producers (25 isolates, 45%) and weak biofilm-producers (12 isolates, 21%). It showed that (93.3%), (95%) and (43.3%) of the *E. coli* isolates have *csgA*, *fimH* and *papC* genes, respectively. While, the relationship between the biofilm formation and the presence of *csgA*, *fimH* and *papC* genes were (100%), (100%) and (42.8%), respectively.

Key words: *E. coli*, UTI, Biofilm, *csgA*, *fimH*, *papC*.

Introduction

Urinary tract infection (UTI) is generally defined as infection caused by bacteria that occurs more commonly in all parts of the urinary tract. The concept of a symptomatic UTI broadly requires the occurrence of urinary tract-specific symptoms in the setting of significant bacteriuria with a quantitive count of $10^5$ colony forming units of bacteria per milliliter (CFU/ml) in one urine specimen (Rowe and Juthani-Mehta, 2014).

A broad range of species causes UTI, including *Escherichia coli*, which accounts for the majority of uncomplicated UTI isolates. Others are *Staphylococcus saprophyticus*, *Klebsiella* spp, *Proteus* spp, *Enterococcus* spp and *Enterobacter* spp (Masinde et al., 2009; King et al., 2015; AalOwaif et al., 2019). *E. coli* has been isolated more than other bacteria that cause UTI in women in Iraq (Hindi et al., 2013).

Biofilm formation is the pathogenic process that enables *E. coli* to be maintained in the urinary system and prevents the loss of bacteria. Biofilms minimize the exposure of bacteria to antibiotics and contribute to the transfer of nutrition, as well as the exchange of genetic materials such as plasmid from one bacterium to another and the exchange of plasmids contributes to the production of antimicrobial resistance (Boroumandet et al., 2019). The first step in the formation of biofilm is the production of curli protein, which promotes the adhesion of bacterial cells to the solid surface (Gawad et al., 2018). The operons of *csgBC* and *csgDEFG* are responsible for curli production and *csgAgene* is the major subunit in the production of Curlin (Cookson et al., 2002).

Most of the UPEC isolates have the *fimperon* that encodes the type 1 fimbria (T1F) (Parvez and Rahman, 2018, Stærk et al., 2016). The FimH protein is the major virulence factor responsible for biofilm formation (Bishop et al., 2007; Tajbakhsh et al., 2016). Studies have shown that the FimH promote the formation of biofilms on biological and non-biological surfaces in the early stages of biofilm formation (Makled et al., 2017). Also, some strains of pathogenic *E.coli* have the fimbriae that called P fimbriae (Godaly et al., 2002). P fimbriae are encoded by 11 genes within the *papA-K* gene operon in up to 70% of UPEC patterns. The biofilm-forming bacteria usually express *papC* gene significantly higher than non-forming
bacteria (Fattahi et al., 2015).

**Materials and Methods**

**Collection of samples**

A total of 100 urine samples were collected from three hospitals in Baghdad, especially Ibn Al-Baladi Hospital during the period from September 2019 to December 2019 from patients who have symptoms of urinary tract infections and then transferred directly to the laboratory under chilled conditions for the purpose of transplantation and diagnosis. The samples were collected from both females and males of different ages.

**Isolation of bacteria**

Urine samples were cultured immediately onto MacConkey agar, blood and nutrient agar by stabbing method and then incubated at 37°C for 24 hours and the growing colonies were subjected to other tests such as biochemical and morphological tests, API 20E and VITEK 2 system.

**Biofilm formation (quantitative method)**

Microtiter plate assay to determine the ability of bacteria to produce biofilm and according to (K1rmusaolu, 2019).

**Polymerase chain reaction (PCR)**

The primers used in this study are listed in table 1. A single colony of bacterial isolate was taken from the Nutrient agar plate and was added to the PCR mixture instead of purified template DNA (Aal Owaf, 2017). The PCR tubes were transferred to the thermal cycler to start the amplification reaction according to specific program for pair of primers as in tables (2, 3, 4).

**Results and Discussion**

**Isolation and Identification of E. coli**

A hundred samples of urine were collected from patients of different ages and genders, who have symptoms of Urinary Tract Infection (UTI) from three hospitals in Baghdad, especially Ibn Al-Baladi Hospital during the period from September 2019 to December 2019. The urine samples were plated on MacConkey agar and blood agar and incubated at 37°C for 24h followed by microscopical examination via Gram stain, biochemical tests, API 20 and Vitek2 system. The results showed that only 60 isolates (60%) have morphological and biochemical characteristics of E. coli.

In this study, the number of E. coli isolates from females was 47 isolates (77%), while it was 13 isolates (23%) from males of a total of 60 E. coli isolates. The ages of patients for both genders in this study were ranged between 1-80 years. This results is agreed with a study reported by Neamati et al., (2015) who showed that 78% of the E. coli isolates were from females and 22% were from males of a total 150 urine samples collected from Beheshti Hospital, Kashan, Iran and the ages of the patients were was ranged between 1-95 years. Another study in Baghdad by Ali and Khudhair, (2018) mentioned the number that 329 females and 58 males have UTIs of a total 450 urine samples. The high incidence of UTIs in women increases with age due to several factors including birth control pills, aging of the urinary tract and other factors, which make the urinary tract more susceptible to infections.

**Table 1:** The sequences of forward and reverse primers.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’-3’ direction)</th>
<th>Amplicon size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>csgA-F</td>
<td>5’-TGCCAGTATTTGCCAAGGTG-3’</td>
<td>885bp</td>
<td>This study</td>
</tr>
<tr>
<td>csgA-R</td>
<td>5’-TTGCTTCGTCTGACATTTGCC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>papC-F</td>
<td>5’-TGATATACCGCAGTCGATAGC-3’</td>
<td>501 bp</td>
<td>(Giovanardi et al., 2005)</td>
</tr>
<tr>
<td>papC-R</td>
<td>5’-CCGGCCATATTCACATAC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fimH-F</td>
<td>5’-TGCAGAACGGA TAAGCCGTGG-3’</td>
<td>506 bp</td>
<td>(Nagarjuna et al., 2015)</td>
</tr>
<tr>
<td>fimH-R</td>
<td>5’-GCAGTACCTGGCCCTCGGA-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The highest percentage of infection was in children between 1-10 years 65%, that showed 38 isolates of a total of 60 isolates, where the percentage of girls was high than boys owning many receptors of the type 1 fimbria (FimH) that considered one of the virulence factors of UPEC (Kolawole et al., 2009).

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76.9% and 23% respectively. In adults, the percentage of infection was 16.3% and it was 18.3% in elderly as shown in fig. 1. These results agreed with the results of Naji and Abbas, (2010) who showed in children (1-10 years) the girls were predominant over boys in UTIs, also agreed with Dormanesh *et al*., (2014) who reported that the infection in girls (75%) was higher than in boys (25%). This is due to several reasons, the most important of which is the incomplete growth of the child’s immune system and the weak body structure (Cavagnero, 2005).

Urinary tract infection usually occurs in older men who have an enlarged prostate and who use catheters to drain the urine from the bladder that leads to the spread and colonization of bacteria in the urinary tract (Chamberlain, 2009).

Biofilm formation

The 96-well polystyrene microtiter a plate was used to detect the ability of *E.coli* isolates to form the biofilm (Atshan *et al*., 2012). The results revealed that 56 of 60 isolates (93%) have the ability to form the biofilm in different quantities under same experimental conditions. These 56 isolates were divided into three groups, strong biofilm producers (19 isolates, 34%), moderate biofilm producers (25 isolates, 45%) and weak producers (12 isolates, 21%) as in the table 5. These results were consistent with the results achieved in Iran by Fattahi *et al*., (2015) who demonstrated that 92% of the UTIs isolates were biofilm positive. Another study in Iraq showed that 90% of the isolates have the ability to produce biofilm (Makia *et al*., 2013). Studies submitted by Al-Taai *et al*., (2018), Umamageswari and Priya, (2019) and Gawad *et al*., (2018), reported that the biofilm-producing isolates were 100%, 96% and 76.5%, respectively.

The production of biofilms by microorganisms is one of the important virulence factors for the species that produce them. Bacterial adhesion to the surfaces of epithelial cells is the first step towards the formation of biofilm, especially for patients with implanted medical devices and among other diseases (Anderson *et al*., 2007). The biofilm capacity produced in *E.coli* depends on many surface determinants that deeply stimulate the biofilm formation like the expression of curli, type 1, S, P fimbriae, F Pilus, motility and flagella and production of exopolysaccharides (Schembri and Klemm, 2001).

Molecular study

Specific primers were used to detect the presence of the *csgA*, *fimH* and *papC* virulence genes in order to demonstrate the relationship between the presence of these genes and the biofilm formation in the *E. coli* isolates. Colony PCR was used in detection of these genes.

- Detection of *csgA* gene and its relation to biofilm formation:

The results showed that 56 out of the 60 isolates (93.3%) have *csgA* gene (curli gene) as shown in fig. 2. This result is consistent with the result of Cordeiro *et al*., (2016) who reported that 100% of the UPEC isolates carry the *csgA* gene.

The results showed that 100% of the biofilm-producing isolates were positive for *csgA* gene, while the non biofilm-producing isolates were negative for *csgA* gene as shown in fig. 3. A study by Schiebel *et al*., (2017) revealed that the formation of biofilm was associated with the presence
of $csgA$ gene at 99.5%. Another Study showed that biofilm formation is correlated with $csgA$ gene at 90.5% (Frömmel et al., 2013).

CsgA is the major subunit protein of the Curli fimbria and responsible for the synthesis of biofilm and considered one of the distinguishing features between the Biofilm-producing bacteria and planktonic bacteria (Uhlich et al., 2006).

• Detection of $fimH$ gene and its relation to biofilm formation

The results in this study show that 57 out of 60 isolates (95%) have $fimH$ gene as shown in fig. 4. The present results agreed with Merza, (2017), who detected that 94.5% of the isolates, were positive for the $fimH$ gene. Other studies submitted by Hojati et al., (2015); Al-Taai et al., (2018); Salih et al., (2015) and Abass et al., (2014) demonstrated that 92.2%, 100%, 91% and 71% respectively of the isolates were positive for $fimH$ gene.

This study revealed that 100% of the biofilm-producing isolates were positive for $fimH$ gene, while the non biofilm-producing were negative for $fimH$ gene except one was positive as shown in the fig. 5. These results agreed with Mahmood and Abdullah, (2015) and Muhammad and Ghareb, (2019) who found that all the biofilm-producing isolates were positive for $fimH$ gene. Other studies submitted by Zamani and Salehzadeh, (2018) and Makled et al., (2017) showed that 86% and 81.3% respectively was positive for $fimH$ gene. The $FimH$ helps bacteria to attach to the epithelial cells in the urinary tract that considered the first step of infection and biofilm formation (Orndorff et al., 2004).

• Detection of $papC$ gene and its relation to biofilm formation

The results presented here show that 26 out of 60 isolates (43.3%) have the $papC$ gene. The $papC-F$ and $papC-R$ primers are used in detection of $papC$ gene and the size of the fragment was 501 bp as shown in fig. 6. This result is consistent with the results submitted by Fattahi et al., (2015); Aljebory and Mohammad, (2019) and Ali and Khudhair, (2018) who reported that 43%, 45% and 55.4% respectively of the UPEC isolates carry the $papC$ gene. Other studies by Abdul-Ghaffar and Abu-Risha, (2017) and Abass et al., (2014) showed that 72% and 79% respectively were positive for $papC$ gene.

The results demonstrate that 42.8% of the biofilm-producing isolates were positive for $papC$ gene, while 2 out of 4 of non biofilm-producers were positive for $papC$ gene as shown in fig. 7. This result is similar to the result by Naves et al., (2008) who found that 53% of the biofilm-producing isolates were positive for $papC$ gene. Other study submitted by Schiebel et al., (2017) showed that only 33.7% were positive for $papC$ gene, while the results by Fattahi et al., (2015) showed that 100% were positive for $papC$ gene.

The results in this paper indicate a strong relationship between the virulence genes ($csgA$ and $fimH$) and biofilm formation (100%), while the virulence gene $papC$ showed a moderate relation to biofilm formation (42.8%).

Fig. 4: Colony PCR screening of $fimH$ gene. Lane (M): DNA Ladder (100-1000bp), while the other lanes represent $fimH$ gene screened by $fimH-F$ and $fimH-R$ primers.

Fig. 5: The relationship between the $fimH$ gene and biofilm formation.

Fig. 6: Colony PCR screening of $papC$ gene. Lane (M): DNA Ladder (100-1000bp), the other lanes represent $papC$ gene screened by $papC-F$ and $papC-R$ primers.

Fig. 7: The relationship between the $papC$ gene and biofilm formation.
References


