Plant Archives Volume 20 No. 2, 2020 pp. 5987-5992 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

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

Adnan Malooh Jaber and Hasan A. Aal Owaif*

College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

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 quan-titativecount of 10^5 colony forming units ofbacteria 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 (Boroumand*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 *csgBAC* and *csgDEFG* are responsible for curli production and *csgA*gene is the major subunit in the production of Curlin (Cookson *et al.*, 2002).

Most of the UPEC isolates have the *fim*operon 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 andnon-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 thepapA-K gene operon in up to 70% of UPEC patterns. The biofilm-forming bacteria usually express *papC* gene significantly higher than non-forming

***Author for correspondence :** E-mail: dr.hasan@biotech.nahrainuniv.edu.iq

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 stabbingmethod 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 assayis the method that used 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 Owaif, 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 women owning many receptors of the type 1 fimbria (FimH) that considered one of the virulence factors of UPEC (Kolawole *et al*., 2009).

Table 2: PCR program of *csgA* gene.

Step	No. cycle	Time (M:S)	Temperature
Initial	1	05:00	95°C
Denaturation	30	01:00	95°C
Annealing		00:30	53°C
Extension		1:15	72°C
Final Extension	1	10:00	72°C

Table 3: PCR program of *fimH* gene.

Step	No. cycle	Time (M:S)	Temperature
Initial	1	05:00	95°C
Denaturation	30	01:00	95°C
Annealing		00:30	59°C
Extension		00:50	72°C
Final Extension	1	10:00	72°C

Table 4: PCR program of *papC* gene.

Step	No. cycle	Time (M:S)	Temperature
Initial	1	05:00	95°C
Denaturation	30	01:00	95°C
Annealing		00:30	51°C
Extension		00:50	72°C
Final Extension	1	10:00	72°C

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

Table 1: The sequences of forward and reverse primers.

Primers	Sequence (5'-3' direction)	Amplicon size	References
csgA-F	5'-TGCCAGTATTTCGCAAGGTG-3'	885bp	This study
csgA-R	5'-TTGCTTCGTCTGACTTTGCC-3'		
papC-F	5' -TGATATCACGCAGTCAGTAGC-3'	501 bp	(Giovanardi *et al.,* 2005)
papC-R	5' -CCGGCCATATTCACATAAC-3'		
fimH-F	5' -TGCAGAACGGATAAGCCGTGG-3'	506 bp	(Nagarjuna *et al.,* 2015)
fimH-R	5' -GCAGTCACCTGCCCTCCGGTA-3'		

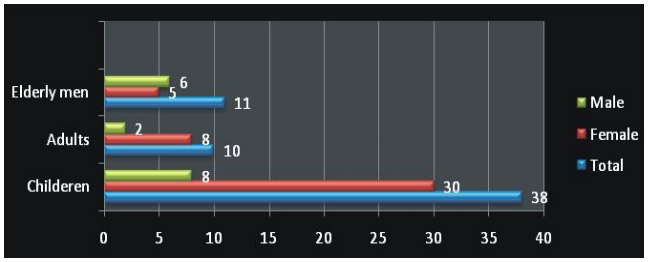

Fig. 1: Distribution of *E. coli* in patients according to the age.

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

Table 5: Biofilm formation by *E. coli* isolates.

Biofilm	No. of isolates	The percentage (%)
Strong biofilm	19	34
Moderate biofilm	25	45
Weak biofilm	12	21
Total	56	100

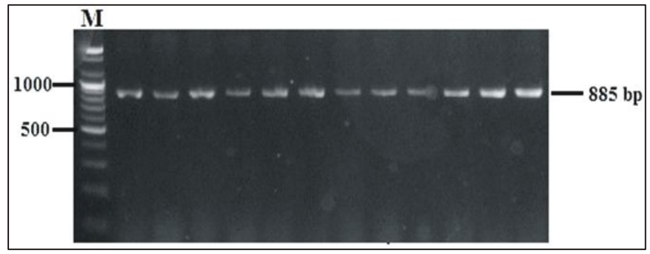

Fig. 2: Colony PCR screening of *csgA* gene. Lane (M): DNA Ladder (100-1000bp), while the other lanes represent *csgA* gene screened by *csgA-F* and *csgA-R* primers.

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 l, 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

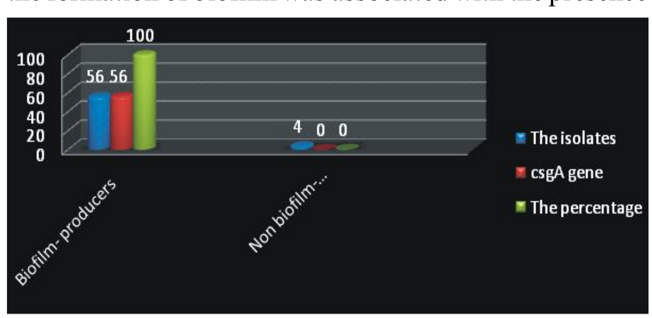

Fig. 3: The relationship between the *csgA* gene and biofilm formation.

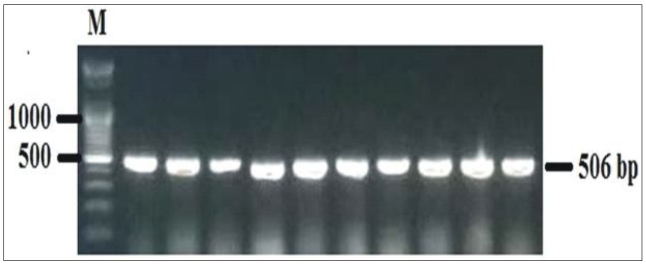

Fig. 4: Colony PCR screening of *fimH* gene. Lane (M): DNA Ladder (100-1000bp), while the other lanes represent *fimH* gene screened by *fimH-F* and *fimH-R* primers.

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 show 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

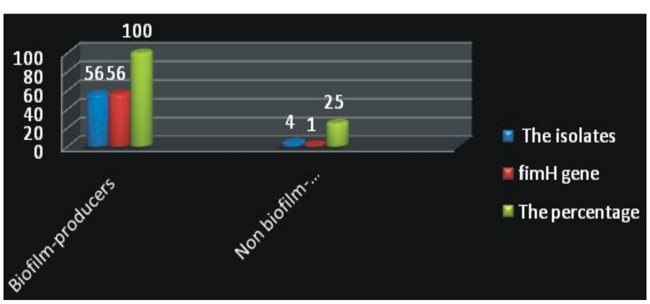

Fig. 5: The relationship between the *fimH* gene and biofilm formation.

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.

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 *pap C*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%).

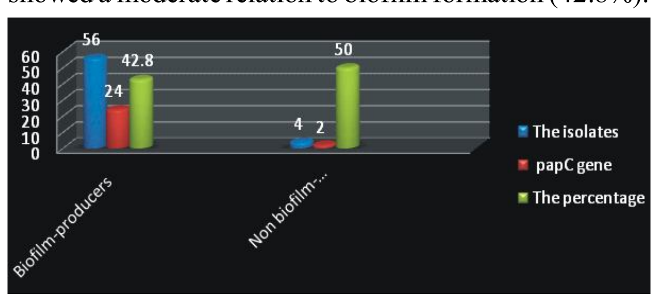

Fig. 7: The relationship between the *papC* gene and biofilm formation.

References

Aal Owaif, H.A. (2017). Regulation of Transcription of the *Escherichia coli* Group 2 Capsule Gene Clusters, Ph.D Thesis, The University of Manchester.

Aal-Owaif, H.A., A.A. Mhawesh and S.A. Abdulateef (2019). The role of *BipA* in the regulation of K1 capsular polysaccharide production of uropathogenic *E. scherichia coli*, *Ann. Trop. Med. & Public Health,* **22:** S254.

Abass, S.K., R.A. Munim and S.H. Authman (2014). Isolation of Multi Antibiotics Resistance Escherichia coli from urinary tract infection and the Detection of *PapC* and *fimH* virulence genes by Polymerase chain reaction Technique. *Diyala Jour. for Pure Science*, **10(1):** 112-127.

Abdul-Ghaffar, S.N. and R.A. Abu-Risha (2017). Virulence Genes Profile of Escherichia coli Isolated from Urinary Catheterized and Non-Catheterized Patients. *Iraqi Journal of Science*, **58(2B):** 820-835.

Ali, M.R. and A.M. Khudhair (2018). Detection of Colony Adhesion Factors and Genetic Background of Adhesion Genes Among Multidrug-Resistant Uropathogenic Escherichia coli Isolated in Iraq. *J. Pure Appl. Microbiol.*, **12(4):** 2017-2025.

Aljebory, I.S. and K.A. Mohammad (2019). *Molecular Detection of Some Virulence Genes of Escherichia coli Isolated from UTI Patients in Kirkuk City, Iraq*.

Al-Taai, H.R.R., Z.A.S. Al-Jebouri, B.H. Khalaf and Y.Q. Mohammed (2018). Antibiotic resistance patterns and adhesion ability of uropathogenic Escherichia coli in children. *Iraqi Journal of Biotechnology*, **17(1):** 18-26.

Anderson, B.N., A.M. Ding, L.M. Nilsson, K. Kusuma, V. Tchesnokova, V. Vogel and W.E. Thomas (2007). Weak rolling adhesion enhances bacterial surface colonization. *Journal of Bacteriology*, **189(5):** 1794-1802.

Atshan, S.S., M.N. Shamsudin, Z. Sekawi, L. Than, T. Lung, R.A. Hamat and C.P. Pei (2012). *Prevalence of Adhesion and Regulation of Biofilm-Related Genes in Different Clones of Staphylococcus aureus*. 2012.

Bishop, B.L., M.J. Duncan, J. Song, G. Li, D. Zaas and S.N. Abraham (2007). Cyclic AMP-regulated exocytosis of Escherichia coli from infected bladder epithelial cells. *Nature Medicine*, **13(5):** 625-630.

Boroumand, M., A. Sharifi, L. Manzouri, S.S. Khoramrooz and A. Khosravani (2019). Evaluation of pap and sfa Genes Relative Frequency P and S Fimbriae Encoding of Uropathogenic Escherichia coli Isolated from Hospitals and Medical Laboratories; Yasuj City, Southwest Iran. *Iranian Red Crescent Medical Journal*, (In Press).

Cavagnaro, F. (2005). Urinary tract infection in childhood. *Clin. Microb*., **18:** 417-422.

Chamberlain, N.R. (2009). *The Big Picture: Medical Microbiology*. McGraw-Hill Medical.

Cookson, A.L., W.A. Cooley and M.J. Woodward (2002). The role of type 1 and curli fimbriae of Shiga toxin-producing Escherichia coli in adherence to abiotic surfaces. *International Journal of Medical Microbiology*, **292(3-4):** 195-205.

Dormanesh, B., F.S. Dehkordi, S. Hosseini, H. Momtaz, R. Mirnejad, M.J. Hoseini and E.K. Darian (2014). Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian pediatric patients. *Iranian Red Crescent Medical Journal*, **16(2)**.

Fattahi, S., H.S. Kafil, M.S. Nahai, M. Asgharzadeh, M. Aghazadeh and R. Nori (2015). Relationship of biofilm formation and different virulence genes in uropathogenic Escherichia coli isolates from Northwest Iran Zusammenhang zwischen Biofilmbildung and unterschiedlichen. *G.M.S. Hygiene and Infection Control*, **10:** 1-7.

Frömmel, U., W. Lehmann, S. Rödiger, A. Böhm, J. Nitschke, J. Weinreich and H. Ansorge (2013). Adhesion of human and animal Escherichia coli strains in association with their virulence-associated genes and phylogenetic origins. *Appl. Environ. Microbiol.*, **79(19):** 5814-5829.

Gawad, W.E., O.M. Helmy, W.M. Tawakkol and A.M. Hashem (2018). Antimicrobial Resistance, Biofilm Formation and Phylogenetic Grouping of Uropathogenic Escherichia coli Isolates in Egypt: The Role of Efflux Pump-Mediated Resistance. *Jundishapur Journal of Microbiology*, **11(2)**.

Giovanardi, D., E. Campagnari, L.S. Ruffoni, P. Pesente, G. Ortali and V. Furlattini (2005). Avian pathogenic Escherichia coli transmission from broiler breeders to their progeny in an integrated poultry production chain. *Avian Pathology*, **34(4):** 313-318.

Godaly, G., G. Bergsten, B. Frendéus, L. Hang, M. Hedlund, D. Karpman and B. Wullt (2002). Innate defences and resistance to gram negative mucosal infection. In *Genes and Proteins Underlying Microbial Urinary Tract Virulence* (9-24). Springer.

Hindi, N.K.K., S.O. Hasson and S.K.K. Hindi (2013). Bacteriological study of urinary tract infections with antibiotics susceptibility to bacterial isolates among honeymoon women in Al-Qassim Hospital, Babylon Province, Iraq. *Biotechnology Jour. International*, 332-340.

Hojati, Z., B. Zamanzad, M. Hashemzadeh, R. Molaie and A. Gholipour (2015). Detection of fimh gene in uropathogenic escherichia coli strains isolated from patients with urinary tract infection. *Jundishapur Journal of Microbiology*, **8(2):** 12-15.

King, J.E. *et al*., (2015). Phenotypic Heterogeneity in Expression of the K1 Polysaccharide Capsule of Uropathogenic *Escherichia coli* and Downregulation of the capsule genes during growth in urine.*Infection and Immunity,* **83(7):** 2605-2613.

K1rmusaolu, S. (2019). The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents, Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods. IntechOpen, UK.

Kolawole, A.S., O.M. Kolawole, Y.T. Kandaki-Olukemi, S.K. Babatunde, K.A. Durowade and C.F. Kolawole (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. *International Journal of Medicine and Medical Sciences*, **1(5):** 163-167.

Mahmood, M.T. and B.A. Abdullah (2015). The relationship between biofilm formation and presence of *fimH* and *mrkD* genes among *E. coli* and *K. pneumoniae* isolated from patients in Mosul. *Mosul Journal of Nursing*, **3(1):** 34-42.

Makia, R.S., A.M.A. Fadhil and M.C. Ismail (2013). Biofilm production as a virulence factor in Uropathogenic bacteria and yeasts. *Journal of Biotechnology Research Center*, **7(1):** 29-34.

Markled, A.F., E.H. Salem and A.M. Elbrolosy (2017). Biofilm formation and antimicrobial resistance pattern of uropathogenic E. coli: comparison of phenotypic and molecular methods. *The Egyptian Journal of Medical Microbiology*, **38(5781):** 1-9.

Masinde, A., B. Gumodoka, A. Kilonzo and S.E. Mshana (2009). Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania journal of health research*, **11(3)**.

Merza, N.S. (2017). Prevalence and Molecular Characterization of *Fim H* Gene in *Escherichia Coli* Isolates Recovered From Patients With Utis. *Medical Journal of Babylon*, **14(3):** 470–477.

Muhammad, I.A. and D.J. Ghareb (2019). Biofilm Forming Capability, Multidrug Resistance and Detection of Associated Genes in Uropathogenic *Escherichia coli* isolated from Catheterized Patients. *Zanco Journal of Pure and Applied Sciences*, **31(4)**.

Nagarjuna, D., G. Mittal, R.S. Dhanda, P.K. Verma, R. Gaind and M. Yadav (2015). Faecal Escherichia coli isolates show potential to cause endogenous infection in patients admitted to the ICU in a tertiary care hospital. *New Microbes and New Infections*, **7:** 57-66.

Naji, S.A. and A.F. Abbas (2010). Determination of the Bacterial Types that cause urinary tract infection in Diyala Proven. *Diyala Journal For Pure Science*, **6(2):** 274-283.

Naves, P., G. Del-Prado, L. Huelves, M. Gracia, V. Ruiz, J. Blanco and F. Soriano (2008). Correlation between virulence factors and *in vitro* biofilm formation by *Escherichia coli* strains. *Microbial Pathogenesis*, **45(2):** 86-91.

Neamati, F., F. Firoozeh, M. Saffari and M. Zibaei (2015). Virulence genes and antimicrobial resistance pattern in uropathogenic *Escherichia coli* isolated from hospitalized patients in Kashan, Iran. *Jundishapur Journal of Microbiology*, **8(2)**.

Orndorff, P.E., A. Devapali, S. Palestrant, A. Wyse, M. Lou Everett, R.R. Bollinger and W. Parker (2004). Immunoglobulin-mediated agglutination of and biofilm formation by *Escherichia coli* K-12 require the type 1 pilus fiber. *Infection and Immunity*, **72(4):** 1929-1938.

Parvez, S.A. and D. Rahman (2018). Virulence Factors of Uropathogenic *E. coli*. In *Microbiology of Urinary Tract Infections-Microbial Agents and Predisposing Factors*. Intech Open.

Rowe, T.A. and M. Juthani-Mehta (2014). Diagnosis and management of urinary tract infection in older adults. *Infectious disease clinics of North America*, **28(1):** 75.

Salih, E.G.M., M.I. Nader and M.N. Rasheed (2015). *Rapid Detection of Uropathogenic Escherichia Coli virulence factors in Iraqi patients by multiplex polymerase chain reaction*.

Schembri, M.A. and P. Klemm (2001). Biofilm formation in a hydrodynamic environment by novel *FimH* variants and ramifications for virulence. *Infection and Immunity*, **69(3):** 1322-1328.

Schiebel, J., A. Böhm, J. Nitschke, M. Burdukiewicz, J. Weinreich, A. Ali and P. Schierack (2017a). Genotypic and phenotypic characteristics associated with biofilm formation by human clinical *Escherichia coli* isolates of different pathotypes. *Applied and Environmental Microbiology*, **83(24)**.

Schiebel, J., A. Böhm, J. Nitschke, M. Burdukiewicz, J. Weinreich, A. Ali and P. Schierack (2017b). Genotypic and phenotypic characteristics in association with biofilm formation in different pathotypes of human clinical *Escherichia coli* isolates. *Applied and Environmental Microbiology*, AEM-01660.

Stærk, K., S. Khandige, H.J. Kolmos, J. Møller-Jensen and T.E. Andersen (2016). Uropathogenic Escherichia coli express type 1 fimbriae only in surface adherent populations under physiological growth conditions. *The Journal of Infectious Diseases*, **213(3):** 386-394.

Tajbakhsh, E., P. Ahmadi, E. Abedpour-Dehkordi, N. Arbab-Soleimani and F. Khamesipour (2016). Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrobial Resistance & Infection Control*, **5(1):** 11.

Uhlich, G.A., P.H. Cooke and E.B. Solomon (2006). Analyses of the red-dry-rough phenotype of an Escherichia coli O157: H7 strain and its role in biofilm formation and resistance to antibacterial agents. *Appl. Environ. Microbiol.*, **72(4):** 2564-2572.

Umamageswari, S.S.M. and M.M. Priya (2019). Biofilm formation by Uropathogenic Escherichia Coli and its Antibiotics resistance patients in Tertiary care center. *International Journal of Scientific Research*, **8(10)**.

Zamani, H. and A. Salehzadeh (2018). Biofilm formation in uropathogenic *Escherichia coli*: association with adhesion factor genes. *Turkish Jour. of Medical Sci.*, **48(1):** 162-167.