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Investigation of FimH adhesin among *Enterobacter* spp. isolates and their role in biofilm formation

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Abstract:

One hundred five urine samples were collected from patients with cystitis and subjected to uriscan Cybow10 dipstick to investigate presence of leukocyte in urine (pyuria) using leukocyte esterase test (LET) and using of nitrite test (NT) to detect bacteriuria. Only samples who gave positive result for both leukocyte esterase test and nitrite test would be then subjected to culture. Twenty four *Enterobacter* isolates were recoverd from urine samples (9 isolates were *E. cloacae* (EC) and 15 isolates were *E. aerogenes* (EA). All DNA samples extracted from bacterial isolates were conducted for PCR to investigate presence of *fimH* gene among isolates. The result revealed that 18/24(75%) of isolates were positive to *fimH* gene (6 isolates among *E. cloacae* and 12 isolates among *E. aerogenes*).Phenotypic detection of biofilm formation was performed using Tissue culture plate (TCP) assay and the results demonstrate that, 17/24(70.8%) were biofilm former among which 7(29.2%) biofilm former belong to *E. cloacae* and 10(41.6%) belong to *E. aerogenes.* The results display significant positive correlation between biofilm formation and FimH adhesin expression in which fimH gene was present in 16/17(94.1%) of biofilm former isolates. Our results conclude the importance of FimH adhesin in establishment of biofilm and magnitude of biofilm formation in cystitis via antibiotics resistance and ascending infections.
Keywords: Cystitis, *Enterobacter* spp., fimH gene, Biofilm**.**

Introduction:

The genus Enterobacter belongs to the family Enterobacteriaceae. *Enterobacter* species, particularly *Enterobacter cloacae* and *Enterobacter aerogenes* are important nosocomial pathogens responsible for various infections. Wide range of Extraintestinal infections can be caused by *Enterobacter* species including bacteremia, urinary tract infections(UTIs) , lower respiratory tract infection, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections [1,2,3].

Enterobacter species can also cause various community-acquired infections, including UTIs [4,5]. Community-acquired urinary tract infection (UTI) is one of the most common infectious diseases and a frequent cause of presentation of outpatient treatment. While mortality rates are not usually high the cost to the global economy is substantial [6]. Generally UTIs are mediated by gram –negative bacteria with the most common of these being Escherichia coli and Klebsiella pneumonia, but can also include Acinetobacter and Enterobacter spp. [6,7,8].

Adhesins can also contribute to virulence, promoting colonization, invasion and replication within uroepithelial cells[9,10]. Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of uropathogen, facilitating the ability to adhere specifically to uroepithelial cells [11].Type 1 fimbriae enhance colonization and stimulate immune responses in the murine urinary tract [12]. Type 1 fimbriae confer binding to α-d-mannosylated proteins such as uroplakins, which are abundant in the uroepithelial lining of the bladder [13]. This type of fimbriae recognize their receptor targets by virtue of organelle tip-located adhesins, namely FimH [14]. Type 1 pili are highly conserved and extremely common among uropathogen isolates and have come to be considered one of the most important virulence factors involved in the establishment of a UTI[15]. Interestingly, the FimH adhesin mediates both bacterial adherence to and invasion of host cells, and contributes to the formation of intracellular bacterial biofilms by uropathogen [16,17].

Biofilm formation has a major impact on foreign bodies or devices placed in the human body. In the last decades, as part of the process of endourological development a great variety of foreign bodies have been invented, and with the steadily increasing number of biomaterial devices used in urology, biofilm formation and device infection is an issue of growing importance [18].

A bacterial mechanism of type-1 fimbriae-mediated invasion into the superficial epithelial cells apparently allows evasion of these innate defenses. Recent studies in experimental models [19,20], partly supported by observations in human UTI, suggest that bacteria initially replicate intracellularly as disorganized clusters. Subsequently, bacteria in the clusters divide without much growth presumably due to changes in genetic programs. Furthermore, the clusters become compact and organized into biofilm-like structures, termed intracellular bacterial communities (IBCs) [19,21]. Bacteria in the IBCs are held together by exopolymeric matrices, reminiscent of biofilm structures. At some point during this developmental process of IBCs, bacteria on the edges of IBCs become motile again and start to move away from IBCs [21].

Our study aimed to investigate presence of fimH gene and biofilm formation among *Enterobacter* spp. local isolates recovered from urine samples of patients with cystitis and study the relationship between biofilm formation and FimH adhesin.

Material and methods

Samples:

One hundred five urine samples were collected from patients with cystitis who were admitted to Urology consultant clinic of Al-Hilla Surgical Teaching Hospital in Babylon city (Iraq) during the period from April 2011 to July 2011. All samples were subjected to uriscan10 (ten parameter urine dipstick, Cybwo/ Korea) to check presence of leukocyte in urine (pyuria) using leukocyte esterase test (LET) and using of nitrite test (NT) to detect bacteriuria. Only samples who gave a positive result for leukocyte esterase test or both leukocyte esterase test and nitrite test would be then subjected to culture.

Bacterial cultures:

Only 24 *Enterobacter* spp. isolates (9 isolates were *E. cloacae* (EC) and 15 isolates were *E. aerogenes* (EA) recovered from urine samples who processed on MacConkey and Eosin methylene blue agar and were incubated at 37°C overnight. The identification of Gram negative bacteria, purple color was performed by standard biochemical methods as follow oxidase test (to

differentiate it from non enterobacteriaceae isolates), indole test, methyl red test, Vogues – Proskauer test and citrate utilization test (to differentiate it from *E. coli* and *Citrobacter spp.*) , urease test, motility test, triple sugar iron test, kligler iron agar test, Ornithine Decarboxylase test (to differentiate it from *Klebsiella* spp.) and Arginin Decarboxylase test (to differentiate between *E. cloacae* and *E. aerogenes*) according to McFaddin, (2000)[22] and Forbes *et al*., (2007)[23].

DNA extraction form *Enterobacter* spp.:

Genomic DNA was extracted from the Enterobacter spp. isolates according to instruction provided by manufacturer using Wizard Genomic DNA purification kit supplemented by (Promega, USA).The isolated DNA was checked by 0.7% agarose gel electrophoresis and viewed using UV-transilluminator.

Detection of *fimH* by PCR:

Conventional PCR was conducted to investigate presence of FimH (type 1 fimbriae adhesin) adhesin among *Enterobacter* local isolates by targeting fimH gene. The primer used in this study was *fimH*-A: 5′-TGC AGA ACG GAT AAG CCG TGG-3′ and f*imH*-B: 5′-GCA GTC ACC TGC CCT CCG GTA-3′ as mentioned by Johnson *et. al*., (2005)[24]. Each 20 µl of PCR reaction mixture for PCR contained 3µl of upstream primer, 3µl of downstream primer, 4µl of free nuclease water, 5 µl of DNA and 5µl of master mix powered in 0.2ml thin walled PCR tube. Thermal cycler used in this study was (Clever Scientific / UK). The Thermal cycler conditions were as follows: one cycle for initial denaturation (4 min. at 95°C) and twenty five cycle for each Denaturation (1 min. at 94°C), annealing (1 min. at 63°C), Extension (3 min. at 68°C) and one cycle for final extension (7 min. at 72°C) [24].

Detection of biofilm formation and measurement of colanic acid concentration:

For biofilm detection the Tissue culture plate (TCP) assay (also called semi quantitative microtiter plate test) described by Christensen et al.,(1985)[25] was used for detection of biofilm formation with some modification as follow: Isolates from fresh agar plates were inoculated in Trypticase soy broth containing 1% glucose and incubated for 18 hour at 37C° and then diluted 1:100 with fresh TSB. Individual wells of sterile, polystyrene 96 well-flat bottom tissue culture plates wells were filled with 150µl aliquots of the diluted cultures and only broth served as control to check non-specific binding of media. Each isolate was inoculated in triplicate . The tissue culture plates were incubated for 24 hours at 37°C. After incubation content of each well was gently removed by tapping the plates. The wells were washed four times with phosphate buffer saline (PBS pH 7.2) to remove free-floating 'planktonic' bacteria. Biofilms formed by adherent 'sessile' organisms in plate were fixed by placing in oven at 37C° for 30min. All wells stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Addition of 150µl of acetone/ethanol (20:80, v/v) mixture to dissolve bounded crystal violet. Read the optical density (O.D.) at 630nm (triplicate for each samples, means three readings for each sample) and the results were interpreted according to the following table:

Table (1) Classification of bacterial adherence and biofilm formation by TCP method.

Mean of OD value at 630nm	Adherence	Biofilm formation

<0.120	non	Non
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

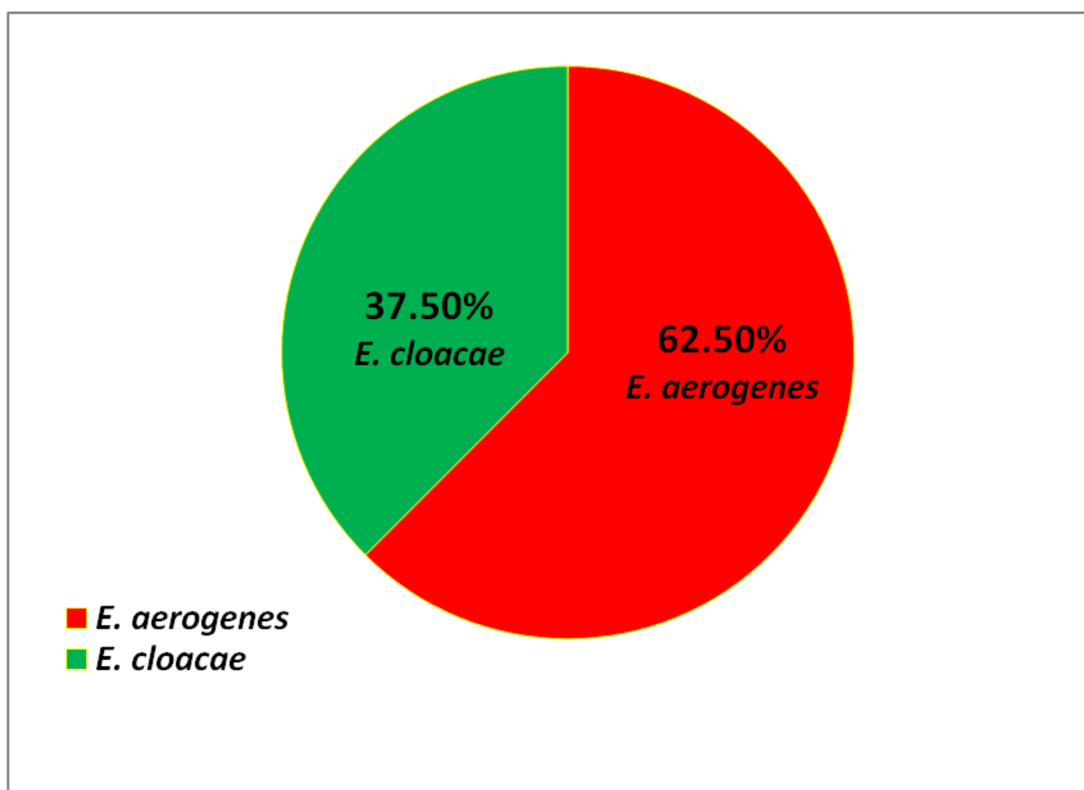
Statistical analysis:

The $\chi 2$ (Chi-square) test was used for statistical analysis. $P<0.01$ was considered to be statistically significant.

Results and Discussions:

All urine samples collected in this study were subjected to urine analysis using uriscan Cybow10 dipstick to investigate presence of leukocyte in urine (pyuria) using leukocyte esterase test (LET) and using of nitrite test (NT) to detect bacteriuria. Only samples who gave positive result for both leukocyte esterase test and nitrite test would be then subjected to culture. Many studies agreed with the results of this study and found that, rapid dipstick screen out negative samples and can save valuable time and money [26,27,28]. Dipstick tests for leukocyte esterase and nitrite test should be added into routine laboratory practices for faster diagnosis of UTI [28].

Among 105 urine samples were collected from patient with cystitis , only 24 (22.86%) isolates were positive for *Enterobacter* spp. diagnosed by standard bacteriological tests. *Enterobacter aerogenes* isolates were a largest group and compile 15/24(62.5%) and the rest, 9/24(37.5%) represent *Enterobacter cloacae* figure (1). This results was agreed with Bracq *et al.,* (2004)[29] and Nejmeddine *et al*.,(2009)[30] who found that emphysematous cystitis often occurs after an infection by anaerobic optimal germs (*Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus Mirabilis, Staphylococus aureus*, Streptococci). Other studies revealed that *E. aerogenes* consist (6.1%) among gram negative bacilli isolated from patients with UTI [31].

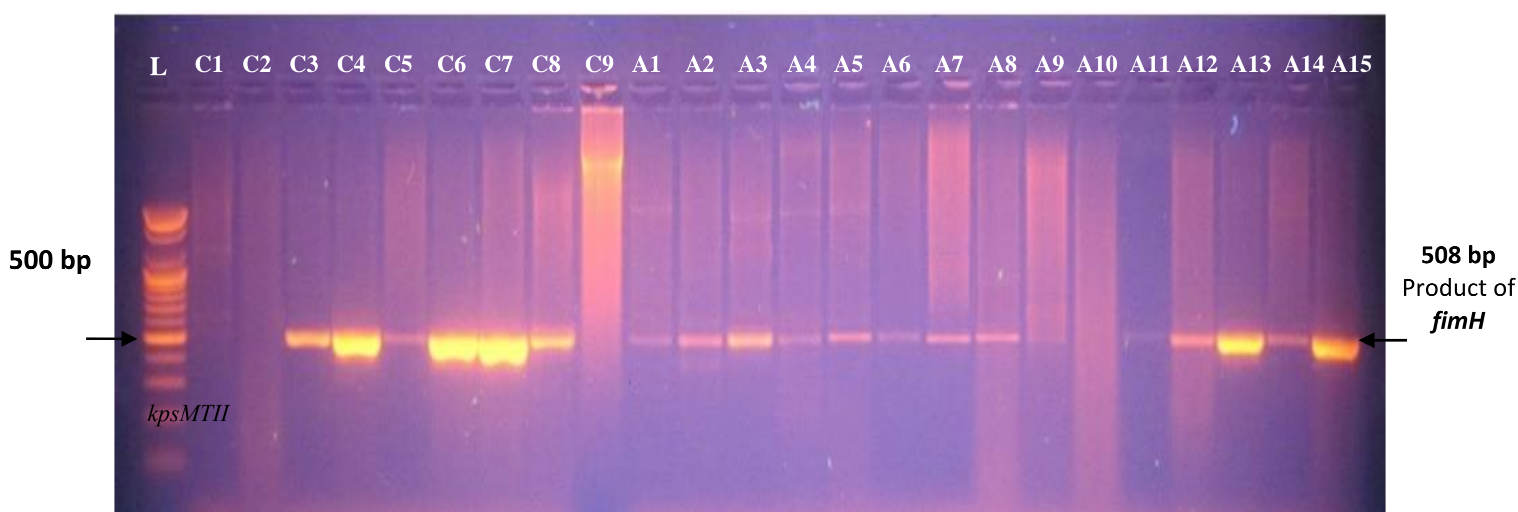


Figure(3-1) Distribution of *Enterobacter* spp. among isolates

Detection of fimH adhesins by PCR:

Type 1 fimbriae were thought to confer the ability to agglutinate erythrocytes and to attach to other cells of varying origin, while several other studies indicated a relation between adhesion and virulence for bacteria inducing infection in relation to a mucous surface. Moreover, a significant correlation was found between the presence of pili or fimbriae and the ability of the bacteria to adhere to human urinary tract epithelial cells. E. aerogenes and E. cloacae isolates were examined for presence of Type 1 fimbrial adhesin using specific primer to detect *fimH* gene.

The results showed that 18 (75.0%) of Enterobacter spp. isolates were positive for fimH gene figure (2).This result in agreement with Hornick et al., (1991)[32] who show that about 66% of E. aerogenes and 23% of E. cloacae isolates have type 1 fimbriae. Rijavec et al., (2008)[33] found that fimH was present in 95% of the E. coli isolates causing UTI. Norinder et al.,(2012)[34] reported that fimH sequences were present in 96% of the UPEC isolates.



Fig(2):Gel electrophoresis of PCR of *fimH* amplicon product. L lane (2000 bp ladder). All isolates were positive for *fimH* except C1, C2, C9 and A9, A10, A11 were negative for *fimH* . C= denote for *E. cloacae* and A=denote for *E. aerogenes*

gene. Krogfelt *et al*.,(1990)[36] found that FimH was later found to be the gene responsible for the mannose-specific or receptor-specific adhesin of the type 1 fimbriae. Very recently studies involving molecular evolutionary dynamics have shown that there is evidence for strong selection in the type 1 fimbrial adhesin fimH, a consequence of which resulted in increased binding of fimH to monomannose-containing receptors previously shown to be adaptive for Uropathogenic E. coli, and which also correlates with increased adhesion to vaginal epithelial cells [37]. Some studies indicate that type 1 fimbriae are more important for colonization of the bladder than for colonization of the kidney [38].

In this study, we confirmed the prevalence of *fimH* among *Enterobacter* spp. isolates like its presence in UPEC [9,39]. This result demonstrated that type 1 fimbriae is an important and relevant virulence factor and that it can also contribute to virulence in *Enterobacter* spp. isolates. The high prevalence of type 1 fimbriae is in accordance with previous results from studies conducted by other investigators as Ruiz et al., (2002)[40] who have found a high prevalence of type 1 fimbriae among uropathogenic E coli strains. Some studies indicate that type 1 fimbriae are more important for colonization of the bladder than for colonization of the kidney [40].

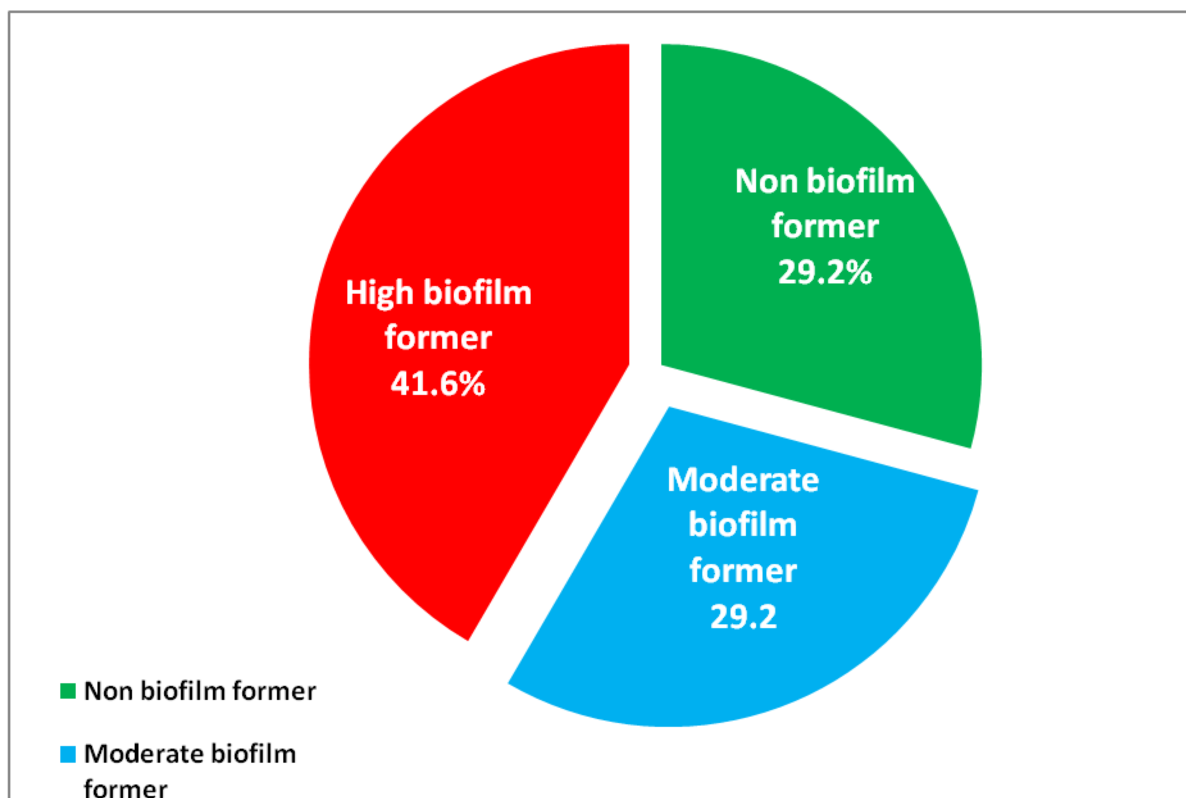
Type 1-mediated adherence has been proposed to play a role in the induction of inflammation, enhancing *Enterobacter* spp. virulence for the urinary tract [35]. Connell et al., (1996)[12] reported that mice infected with a type 1-positive 01:K1:H7 *E. coli* isolate showed a higher urinary neutrophil influx into the urine than type 1- negative isolates. Moreover FimH adhesin mediates both bacterial adherence to and invasion of host cells, and contributes to the formation of intracellular bacterial biofilms by uropathogen [16,17].

The integral membrane glycoprotein uroplakin 1a, which is abundantly expressed on the apical surface of the bladder, appears to be a key receptor for the FimH adhesin, although FimH can also bind many other host proteins [41,42]. FimH selectively recognized uroplakin Ia, without detectable binding to its structurally related uroplakin Ib. The realization that uroplakin Ia is the unique bacterial receptor has major implications for the mechanisms of bacterial invasion [42]. In addition to promoting bacterial–host interactions, recent studies have demonstrated that some FimH variants can also mediate interbacterial contacts, stimulating bacterial autoaggregation and biofilm formation [43,44,45]. The role of FimH in these processes is not yet clear, but they do not seem to necessarily depend on the mannose-binding capacity of the adhesin. FimH-mediated autoaggregation and biofilm formation may enable uropathogen to better withstand antibiotic

treatments and host antibacterial defenses within the urinary tract. In addition, type 1 pilus-mediated biofilm formation may facilitate bacterial colonization of urinary catheters and other medical implants, an unfortunately common problem for hospitalized individuals [10].

Biofilm formation and adhesins expression :

Biofilm formation on polymeric surfaces was tested in the semi quantitative microtiter plate test (biofilm assay) using Trypticase Soy Broth supplemented with 1% glucose. This assay was repeated as triplicate to increase the accuracy of assy. According to mean of OD value at 630nm the results were interpreted as non, moderate and high biofilm former when the mean of OD value were (<0.120 , 0.120-0.240, and >0.240) respectively. The results revealed that 7/24(29.2%) of all Enterobacter isolates were non biofilm former (two isolates among E. cloacae and five isolates among E. aerogenes). The moderate biofilm former were account for 7/24(29.2%) in which only one isolates belong to E. cloacae and the rest belong to E. aerogenes while isolates that express high biofilm formation mode were 10/24(41.6%), six of which belong to E .cloacae and 4 isolates for E. aerogenes figure (3). As a total (moderate and high biofilm former) the biofilm formation



Fig(3):Distribution of *Enterobacter spp.* isolates according to biofilm formation

The crystal violet microtiter plate test was described in the literature as a simple and rapid method to quantify biofilm formation of different bacterial strains [46,47]. Crystal violet is a basic dye known to bind to negatively charged molecules on the cell surface as well as nucleic acids and polysaccharides, and therefore gives an overall measure of the whole biofilm. It has been used as a standard technique for rapidly accessing cell attachment and biofilm formation in a range of Gram positive [48] and Gram-negative [49] bacteria as well as yeasts [50].

Biofilm facilitates the adherence of microorganisms to biomedical surfaces and protect them from host immune response and antimicrobial therapy [51]. In addition the production of biofilm my promote the colonization and lead to increased rate of UTI's and such infections may be difficult to treated as they exhibit multi drug resistance [52].

Joseph *et al*.,(2001)[53] and Tompkin (2002)[54] found that Cells in a biofilm have been shown to be significantly more resistant to disinfectants than planktonic cells. These sessile communities pose serious problems for human health and are of concern in medical, environmental and industrial settings. For example, many persistent and chronic bacterial infections, including periodontitis, otitis media, biliary tract infection and endocarditis, are now believed to be linked to the formation of biofilms. In the urinary tract, infections associated with biofilms include chronic cystitis, prostatitis, and catheter- and stent-associated infections [55].

Moreover, biofilm formation seems to be a trait associated with cystitis and asymptomatic bacteriuria isolates rather than pyelonephritis isolates, indicating that biofilm might be important for persistent colonization of the urinary tract [56,57]. Virtually all medical implants are prone to colonization by pathogenic bacteria, and these biofilms often serve as a source of recurrent infections. Bacterial biofilm infections are particularly problematic because sessile bacteria can withstand host immune responses and are drastically more resistant to antibiotics (up to 1000-fold), biocides and hydrodynamic shear forces than their planktonic counterparts [58].

Regarding to role of colanic acid concentration in biofilm formation our result demonstrate the linear or positive relationship between colanic acid concentration and biofilm formation . Biofilm former colanic acid concentration ranged from 12.1- 52.6 µg/ml table (2). This results in agreement with Valle *et al*.,(2006)[59]who suggested that biofilm structure is largely determined by the concentration of substrate. Polysaccharides such as colanic acid polymer had been identified as part of the extracellular matrix, which plays an important positive role in building the mature biofilm structure [59]. Only recently it has been discovered that colanic acid is not necessarily a primary biofilm former, but plays an important role in the development of the complex three-dimensional structure of a biofilm [60].

The exopolysaccharides (EPS) synthesized by microbial cells vary greatly in their composition and hence in their chemical and physical properties. Some are neutral macromolecules, but the majority are polyanionic due to the presence of either uronic acids (d-glucuronic acid being the commonest, although d-galacturonic an dmannuronic acids are also found) or ketal-linked Pyruvate. The slow bacterial growth observed in most biofilms would also be expected to enhance EPS production as occurred in colanic-acid-producing E. coli [61]. Some reports do suggest the role of EPS (colanic acid) in biofilms that, it may interact with antimicrobial agents and protect the cells, either by preventing access of the compounds or by effectively reducing their concentration. However, the protective effects are probably limited. By maintaining a highly hydrated layer surrounding the biofilm, the EPS will prevent lethal desiccation in some natural biofilms and may thus protect against diurnal variations in humidity [62].

Regarding to the relationship between biofilm formation and adhesin expression our results date that ,there was strong positive correlation between biofilm former and fimH+ (Type 1 fimbriae) isolates in which 16/17 (94.1%) of biofilm former had fimH gene while it was absent in only one isolates (5.9%) table (3). Type 1 fimbriae have been strongly linked to many aspects of

bladder infection [63]. This results in accordance with Schembri and Klemm (2001)[44] who display that, the FimH adhesin has been shown to be instrumental in biofilm formation by E. coli K-12 under both static and hydrodynamic growth condition in vitro. This suggested that the Type 1 fimbriae may confer on UPEC the ability to form biofilm that opposes bacterial clearance from bladder [64].

In perfused environments, microbial communities are established via a process known as ‘‘self-immobilization’’ [65]. Sessile biofilm are formed as bacteria embed themselves in an endogenously formed matrix. This compact community, consisting of organisms’ adherent to each other and/or a surface, provides extraordinary resistance to hydrodynamic flow shear forces. Sung *et al*.,(2006)[66] demonstrate that deletion of either the curli-, colanic acid-, or type I pilus-related genes or the combined deletion of two of these three gene clusters was effective in decreasing the adherence ability of UPEC and eliminate biofilm formation. In E. coli K-12, several cell-surface factors, including type 1 fimbriae [67,68] and flagella [43], have been implicated in biofilm formation. It is likely that many of these factors also contribute to catheter-associated UTIs caused by UPEC. Motility has been suggested to be involved in biofilm formation in several cases via the role of flagellum itself in adhesion [69,70,71,72]. Microarray data gathered from Schembri *et al*.(2003) [68] revealed that FimH were expressed in all biofilms. In the case of type 1 fimbriae, the biofilm expression levels were similar to those observed in exponential phase, but increased almost 6.5-fold when compared with a stationary phase culture. Melican *et al*.,(2011)[73] suggest that UPEC’s attachment organelles, P and Type 1 fimbriae, act synergistically to facilitate bacterial colonization in the face of challenges such as renal filtrate flow. P fimbriae provides a fitness advantage in vivo, aiding bacterium in withstanding the filtrate flow and enhancing colonization during the first hours of infection and establishment of biofilm. On the contrary Type 1 fimbriae facilitate inter-bacterial adhesion and biofilm formation, allowing bacteria to maintain themselves within the bladder [73].

Table (2) Relationship between biofilm formation and colanic acid concentration among *Enterobacter spp*. isolates.

Isolate name	Colanic acid concen. µg/ml	Adherence force	Biofilm formation
EC1	4.1	Non	-

EC2	9.7	Non	-
EC3	30.1	Strong	+ (high)
EC4	47.4	Strong	+ (high)
EC5	29.3	Strong	+ (high)
EC6	49.1	Strong	+ (high)
EC7	31.9	Strong	+ (high)
EC8	42.7	Strong	+ (high)
EC9	12.3	Moderately	+ (Moderate)
EA1	15.1	Moderately	+ (Moderate)
EA2	34.7	Strong	+ (high)
EA3	52.6	Strong	+ (high)
EA4	12.1	Moderately	+ (Moderate)
EA5	20.3	Moderately	+ (Moderate)
EA6	20.3	Moderately	+ (Moderate)
EA7	33.9	Strong	+ (high)
EA8	28.4	Strong	+ (high)
EA9	11.3	Non	-
EA10	10.9	Non	-
EA11	6.1	Non	-
EA12	16.1	Moderately	+ (Moderate)
EA13	19.9	Moderately	+ (Moderate)
EA14	11.4	Non	-
EA15	4.2	Non	-

Table (3) Relationship between biofilm formation and presence of FimH adhesin among *Enterobacter spp*. isolates. There was significant positive correlation between biofilm and fimH expression at P<0.005.

Bacteria	Biofilm former	*fimH +ve*		*fimH –ve*	
		No	%	No	%
E. cloacae	7	6	35.3	1	5.9
E. aerogenes	10	10	58.8	0	0.0
Total	17	16	94.1%	1	5.9%

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التحري عن عامل الالتصاق FimH ضمن العزلات المحلية لبكتريا *Enterobacter* spp. ودوره في تكون الأغشية الحيوية

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كلمات مفتاحيه: التهاب المثانه، *Enterobacter* spp.، fimH gene ، الاغشيه الحيويه.

الخلاصه:

تم خلال هذه الدراسه جمع 105 عينة ادارار اخذت من المرضى الذين يعانون من التهاب المثانه حيث اجري لكل هذه العينات فحص الادراا العام باستخدام الاشرطة الجاهزة نوع Cybow10 dipstick للتحري عن وجود كريات الدم البيض في الادرار(Pyuria) باستخدام الاختبار استريز الكريات البيض(leukocyte esterase test (LET) وكذلك تم التحري عن تجرثم الادرار(Bacteriuria) باستخدام فحص النتريت (Nitrite test (NT). تم اجراء الزرع البكتري فقط لعينات الادرار التي اعطت نتيجه موجبه لاختباري LET و NT حيث تم عزل 24 عزله من عزلات بكتريا*Enterobacter* spp. منه 9 عزلات عائده للنوع *E. cloacae* و 15 عزله عائدة للنوع *E. aerogenes* بعد ذلك تم استخلاص الحمض النووي الريبي منقوص الاوكسجين (DNA) من هذه العزلات واجريت عملية تفاعل البلمره المتسلسل (PCR) باستخدام بوادئ (Primer pairs) متخصصه للتحري عن الجين*fimH* المشفر لعامل الالتصاقFimH . وكانت النتيجه موجبه لوجود هذا الجين في 75% من العزلات (18 عزله من اصل 24عزله 6 عزلات منها عائدة للنوع E. cloacae و12 عزله عائده للنوع *E. aerogenes*). اجري التحري المظهري عن تكوين الاغشية الحيويه باستخدام اختبار (Tissue culture plate (TCP) assay) واوضحت النتائج ان 70.8% من العزلات كانت منتجه للاغشية الحيويه (17عزله من اصل 24 منها 7 عزلات عائده للنوع E. cloacae و 10 عزلات للنوع E. aerogenes). بينت النتائج ان هنالك علاقه موجبه بين تكوين الاغشيه الحيويه وامتلاك هذه العزلالت المكونه للاغشيه على عامل الالتصاقFimH حيث وجدا ان 94.1% من العزلات المكونه للاغشيه الحيويه تمتلك عامل الالتصاقFimH. مماتقدم يمكن الاستنتاج ان عامل الالتصاقFimH مهم جدا في تأسيس وتكوين الاغشيه الحيويه ودور هذه الاغشيه في التهاب المثانه عبر مقاومة المضادات الحياتيه وحصول Ascending Infections.