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Screening for *in Vitro* Biofilm Formation Ability of Locally Isolated Uropathogenic *Escherichia coli* (UPEC)

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Abstract:

Seventy-six urine specimens were collected from of patients suffering from recurrent urinary tract infections (UTIs). Specimens were bacteriologically analyzed, fifty (65.8%) of isolated bacterial strains were belonged to *E.coli*. 100% of isolated uropathogenic *E.coli* (UPEC)strains displayed a biofilm positive phenotype under optimized condition using microtiter plate assay. 21 of *E.coli* strains classified as highly positive biofilm producers (42%), and 29 (58%) as weakly positive biofilm producers.

Keywords: Uropathogenic *E.coli* (UPEC), Biofilm, Microtiter plate.

التحري عن تكوين الاغشية الحيوية خارج الجسم الحي لعزلات محلية لبكتريا *E.coli* المعزولة من اخماج المسالك البولية

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الخلاصة :

جمعت 76 عينة ادرار سريرية من مرضى يعانون من خمج المسالك البولية المتكررة، فحصت العينات بكتريولوجيا. وجد ان 50 (65.8%) تعود لبكتريا *E.coli*. أظهرت كل عزلات *E.coli* المختبرة القدرة على تكوين الأغشية الحيوية (100%) بأطباق المعايرة الدقيقة. صنفت 21 عزلة (42%) عالية الانتاجية في تكوين الغشاء الحيواني و 29 (58%) كونها ضعيفة الانتاجية.

Introduction:

Urinary tract infections (UTIs) are considered to be the most common infections in humans. Most uncomplicated UTIs are caused by *E. coli*, accounting for up to 90% of community-acquired and approximately 50% of nosocomial UTIs [1]. The origin of these strains is frequently patient's own intestinal flora. A subset of fecal *E.coli* having the virulence factors which enable them to colonize periurethral area, enter urinary tract, and cause symptomatic disease are defined as uropathogenic *E.coli*(UPEC) [2]. Virulence factors of UPEC include the ability to adhere to the uroepithelial cells and certain specific serotypes O and K antigens are resistance to phagocytosis and bactericidal action of

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normal serum. Other factors known to contribute to the virulence are the production of α hemolysins, colicins, aerobactin, cytotoxic necrotizing factor, and cell surface hydrophobicity [3].

Bacterial adherence not only contributes to colonization but also to invasion, biofilm formation, and host cell damage. The two primary fimbrial adhesins associated with UPEC strains are type 1 and P fimbriae. Type 1 fimbriae mediate adherence largely via the FimH tip adhesin, which recognizes and binds mannose moieties on biotic and abiotic surfaces. Within the host, FimH mediates UPEC binding to the bladder epithelium and is also required for proper formation of biofilm-like intracellular bacterial communities within bladder epithelial cells. [4]. approximately, 25% of patients with an episode of acute UTI later develop recurrent UTI, which is a common problem in UTIs, even in patients without anatomic abnormalities or indwelling bladder catheters. It is estimated that 30 to 40% of adult healthy human have experienced at least one UTI in their lifetime, and there is a tendency for these infections to become chronic due to a high rate of recurrence This may be related to the capacity of bacteria to form biofilms [5]. Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Biofilm can promote persistence in the urinary tract and on biomaterial surfaces by protecting bacteria from the clearing out effect of hydrodynamic forces and the killing activity of host defense mechanisms and antibiotics. Matrix of the biofilm contributes to development of resistance of pathogenic *E. coli* biofilms and lead to persistent infections [6]. Biofilm facilitates persistence leading to persistent UTI which can lead to stone formation. Virulence factors like adhesins and biofilm have been extensively studied by authors on UPEC isolated from recurrent UTI biofilm formation [7, 8]. Several surface determinants are involved in biofilm formation such as: flagella and motility, type 1 fimbriae, autotransporter proteins, curli, F conjugative pilus and exopolysaccharide production [9].

The present study was aimed the investigation of (*In vitro*) biofilm formation among the locally isolates of *E.coli* from recurrent UTI patients.

Materials and methods

Isolation and identification of *E.coli* strain

Seventy-six mid-stream urine specimens were collected aseptically from patients (age, 5 days – 86 years), 38% male and 62% female, admitted to medical city in Baghdad. specimens were transported to the laboratory and a loopful of urine specimens was inoculated on MacConkey agar, plates incubated at 37 °C for 24 hrs. Pronounced colonies, which showed large, round, and pink, (lactose fermenter) colonies, were reinoculated several times on MacConkey agar to insure the isolation of *E.coli*.

E.coli isolated strains were identified depending on morphological and microscopic examination, as well as biochemical tests [10, 11]. Identification was confirmed with using API 20 E system [12].

Detection of biofilm production by UPEC isolates

All the *E.coli* isolates were assessed for biofilm formation by microtiter plate assay as described by Amaral et al [13]. Briefly, overnight cultures of UPEC isolates were grown in brain heart infusion supplemented with 1 % (w/v) glucose. Bacterial cultures were diluted and adjusted in comparison to MacFarland tube 0.5 and aliquots 200 μ l from the each culture were deposited in the wells of a 96 - well polystyrene microtiter plate and incubated under constant condition 37 °C for 24hr. After incubation, contents of wells were removed from each well by aspiration, wells were gently washed twice with normal saline, then dried and fixed at 65 °C for 1 hr. Subsequently, the wells were stained with 0.1 % (w/v) methylene blue for 10 min, gently washed twice and the quantitative analysis of biofilm production was performed by adding 200 μ l of 95 % ethanol for 10 min. Finally, microtiter plate reader (Elisa reader) measured the absorbance of the methylene blue present in the destaining solution (ethanol) at 630 nm. Control were performed with methylene blue binding to the wells exposed only to the culture medium without bacteria. This assay was performed by triplicate and the mean biofilm absorbance value was determined. Biofilm degree was calculated as follows:

Biofilm degree = Mean OD₆₃₀ of tested bacteria - Mean OD₆₃₀ of control

The data obtained were used to classify the strains as high producers OD higher than 0.24; weak produce between 0.125 -0.250 while non-producer less than 0.120 [14].

Results and discussion.

Bacterial isolates from urine specimens

Seventy-six urine specimens from UTI patients were bacteriologically analyzed for the presence of UPEC on MacConkey agar plates. From total positive samples 50 isolates (65.8%) were *E. coli*. Identification of isolates was carried out by biochemical testing [15]. And diagnosis was confirmed by use of API- 20E micro tubes system table 1.

Table 1-Biochemical Characteristics of *E. coli* Isolates

Test	Result
Catalase	+
Oxidase	-
Indole Production	+
Methyl Red	+
Voges Proskauer	-
Citrate Utilization	-
Hemolysin production	±
Lactose fermentation	+
Gas production	+
Motility	+
Nitrate reduction	+
H ₂ S Production	-

Biofilm production

A total of 50 UPEC isolates were tested for production of biofilm by microtiter plate assay. Biofilm productions were detected in all isolates, with different potential capacity to form biofilm under same experimental conditions figure 1. UPEC tested isolates are confined between two groups, strong biofilm producers (21 isolate, 42%) and weak producers (29 isolates, 58%). The UPEC isolates; E 1, E 17, E 24 and E 47 produced the thickest biofilm; 0.862, 0.425, 0.523, and 0.436, respectively. Obviously, the isolate E 1 was achieved the maximum biofilm thickness, while minimum biofilm producer was the isolate 49, with OD value 0.120.

UPEC isolates showed a remarkable high capability to form biofilm in this study, as 100% of tested isolates were showed biofilm phenotype positive. This is higher than that reported in previous studies [17], even though previously reported the high incidence (74%) of biofilm producing *E. coli* in repeated UTIs [16]. This may explain why *E. coli* is more prevalence in UTIs than other associated microorganisms, and this shows that the biofilm formation is mostly companied with repeated UTIs.

The difference in biofilm thickness resulted from different reasons such as; differences in isolates capacity to form biofilm The potential biofilm capacity in *E. coli* are depends on several surface determinants[18].which are profoundly induce biofilm formation on the population level such as, expression of curli , type I fimbriae , flagella and motility, autotransporter proteins, F conjugative pilus, and exopolysaccharids production [19]. The primary number of cells that succeeded in adherence and the differences of quality and quantity of autoinducers (quorum sensing signaling molecules) that produced from each from each isolate play an essential as well as important role [16].

The results of [20] mentioned the thickness of UPEC biofilm differs according to the nature of producing bacteria, environmental conditions like, temperature and pH, and the type of UTIs, in that UPEC isolates from prostates give high ability to produce biofilm with large thickness more than other isolates from pyelonephritis and cystitis.

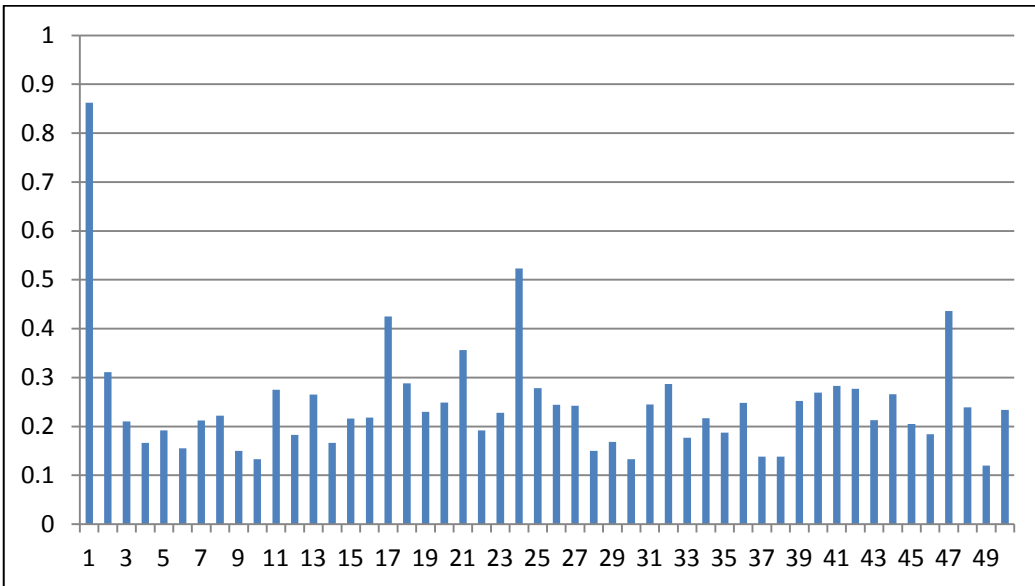


Figure 1-Biofilm Production Capacity (OD₆₃₀) of UPEC isolates

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