Biofilm production as a virulence factor in Uropathogenic bacteria and yeasts

انتاج الغشاء الحيوي كعامل ضراوة في البكتيريا والخمائر الممرضه للجهاز البولي

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Abstract

This study includes isolation and identification of different uropathogenes (bacteria- yeasts) collected from catheterized patients suffering from complicated urinary tract infections. Three hundred and fifty urine samples obtained by swabs from catheterized patients were identified for the presence of uropathogenes (bacteria and yeast). 221(63.13%) samples were obtained from females and 118 (33.71%) samples from male, 339(96.85%) sample were identified by culturing as a positive result, while 11(3.14%) sample were negative result. The 339 positive isolates include 303 (89.38%) bacterial isolates and 36 (10.61%) yeast isolates. Results of biochemical tests and *Api* 20 system for bacterial and yeast isolates reveals *E.coli*, *Proteus spp*, *Klebseilla spp*, *Pseudomonas spp* and *Candida spp* represented the main causative uropathogen infect urinary system and causing a complicated type of infection. The determination of bacterial and yeasts ability to form biofilm was carried out using test tube method , 306 isolates which represented (90.26%) were capable to form biofilm with differ in the thickness of formed layer. *Pseudomonas spp* formed the thicker biofilm followed by *E.coli*, *Candida spp*, *Proteus spp*, and *Klebseilla spp*.

المستخلص

تضمنت هذه الدراسة عزل وتشخيص انواع مختلفة من الممرضات(بكتريا وخمائر) من انابيب القثطرة للمرضى الذين يعانون من التهابات مجاري بولية معقدة وبينت النتائج من بين 350 نموذج من الادرارالمزروعة وكانت 221 (63.13%)عينة ماخوذة من النساء و 118(33.711%)عينة من الرجال ووجدت (96.85%)339عينة ممثله نتيجة موجبة بينما كانت 11 (3.14%)عينة ممثله نتيجة سالبة وتضمنت العينات موجبة الزرع 303 (89.38%) عزلة بكتيرية و 36 (10.61%)عزلة خميرة و واوضحت نتائج الاختبارات الكيموحيوية و التشخيص باستعمال نظام Api 20 لعزلات البكتريا و الخمائر البالغه 339 عزلة انها تتضمن الاجناس التاليه : *Pseudomonas spp, Klebsiella spp, Escherichia coli* وتمثل المسببات الرئيسية لالتهاب المجاري البولية المعقدة . اظهرت *Proteus spp, Candida spp,* نتائج تحديد قابليه الممرضات الرئيسية المشخصة على انتاج طبقة الغشاء الحيوي باستعمال طريقة انابيب الاختباروقدره 306(90.62%) عزله على انتاج طبقة الغشاء الحيوي مع الاختلاف في سمك الغشاء المتكون و كانت العزله *Pseudomonas spp* مكونه لأسمك طبقه غشاء حيوي تليها *E.coli* و *Candida spp* ومن ثم *Proteus spp* و *Klebsiella spp* .

*البحث مستل من اطروحة ماجستير للباحث الاول

Introduction

Urinary tract infection is an extremely common clinical problem that can be defined as the presence of microorgansims in a properly collected urine samples. Factors trigger infection and symptoms which play important role to help physicians in diagnosis of UTIs and patients treatment [1].

The most common uropathogenes which responsible for properties of UTIs: members of *Enterobacteriacea*, *Staphylococcus spp.* and *Candida spp*. because they have different virulence factors enable them to invade the urinary tract system and cause infection [2].

Biofilm is a complex aggregation of microorganisms marked by the excretion and adhesive matrix which often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions and an extra cellular matrix of polymeric substances. Biofilm decrease the susceptibility to antibiotics by physical impairment of antibiotic diffusion and local alteration of microenvironment [3,4].

Urinary tract infections can be classify according to the complexity which are first: uncomplicated UTI that refer to UTIs seen in patients with normal anatomic structure and function of the urinary tract, second: Complicated UTIs are resulting from anatomic obstructions of the urinary tract or catheterization [5,6].

Aims of the study

1. Isolation and identification of most common bacteria and yeast from the catheterized patients which have urinary tract infection.
2. Detection the ability of isolates for biofilm formation as virulence factors.

Materials &methods

Sample collection

Three hundred and fifty urine samples from catheter were collected in sterile swabs containing 5 ml of normal saline from patients, 226were from female patients while 124 of the cases were from the male of (Al-Yarmouq hospital, Centeral child hospital , Al-Kathmya hospital and Al- Alwya hospital) during the period from Oct.-1-2005 to Jun.-1 -2006 and transported to the laboratory during 1 hour by using a cool box because low temperature serves to inhibit bacterial and yeast replication in the urine sample until processed to laboratory. This procedure is important because the number of bacteria in the urine sample is important in determining if there is a clinically significant bacterium in the urine; if the sample is not properly stored, small number of contaminating bacteria may multiply to large numbers and create a false impression of significant bacteriuria.

Isolation and identification of bacteria

1. The swabs were streaked on nutrient agar, blood agar, macConkey agar plates and Sabouraud dextrose agar (SDA).
2. Plates were incubated over night at 37°C.
3. Bacterial colonies were identified depending on the colony size, shape, edge, color, and odor [7]. Identification of isolates was carried out by sub-culturing representive colonies from MacConkey agar plates on api-20E microtubes

systems. This system is designed for the performance of more than 20 standard biochemical tests from a single colony on plate medium.

Isolation and identification of yeast

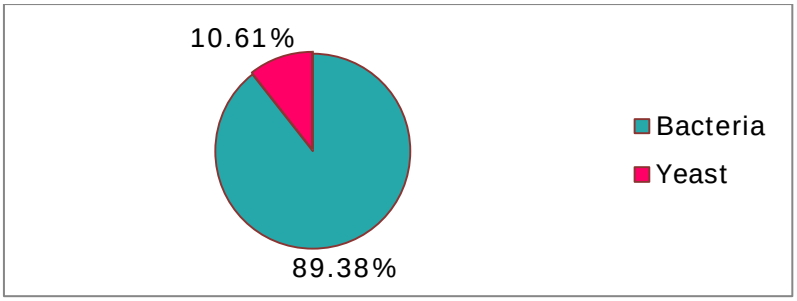
Yeast identification depends on a. surface growth b. germ tube formation test c. sugar fermentation test and chlamydospore formation test [8].

Detection the ability of bacteria and *Candida* for biofilm formation by Test tube Method

The detection of bacterial and Candida ability for biofilm formation was done according to [9].

Results and discussion

Three hundred fifty urine samples were collected from catheterized patients. After cultural examination and Gram staining of smears, results showed, 339 (96.85%) isolates 303 (89.38%) bacteria and 36 (10.61%) yeast obtained from catheterized patients Figure (1), while 11(3.14%) samples were showed no growth of microorganisms from total samples (350).



Fig(1): percentage of bacteria and yeast isolated from catheterized patients

Table (1): The percentage of different microorganisms isolated from urine of catheterized patients using biochemical tests and Api 20-E system identification

		Urine sample from catheterized patients	
	Isolates	No.of Isolates	Percentage %
1-	*E.coli*	9	2.57
2-	*Proteus mirabilis*	109	31.14
3-	*Proteus vulgaris*	23	6.57
4-	*Klebsiella pneumonia*	74	21.14
5-	*Pseudomonas aeruginosa*	39	11.14
6-	*Enterobacter spp.*	16	4.57
7-	*Staphylococcus aureus* (G+ve)	16	4.57
8-	*Citrobacter spp.*	4	1.14
9-	*Kluvyra spp.*	7	2.00
10-	*Pantoea spp.*	6	1.71
11-	*Candida spp.*(*Candida glabrata and Candida tropicalis*)	3	0.86
12-	*Candida albicans*	33	9.42
13-	No growth	11	3.14

Complicated UTIs caused by bacteria, but they occur as a result of some anatomical or structural abnormality often associated with catheter use in the hospital setting like bladder and kidney dysfunction, or kidney transplant and prostates enlargement. The common features in most complicated UTIs is the inability of the urinary tract to clear out bacteria because of a physical condition that causes obstruction to the flow of urine or problems that hinder treatment success.

Proteus and *Klebsiella* represent the main causative agent of complicated UTIs in the collected samples Table (1). This is probably because *Proteus* has a swarming ability and *Klebsiella* formed capsule, and each of them were able to produce a potent urease which acts on urea to produce ammonia, rendering the urine alkaline [10].

Although many reports were pointed that *E.coli* represent important uropathogene for complicated UTIs of catheterized patients but the result of this study showed low frequency because the samples were obtained from patients possessing catheter for (3-5 days) and the *E.coli* bacteria prefers long term catheterized urinary tract or the patients may obtained a high dose of antibiotic which inhibits *E.coli* before and after using of catheter. This result was nearly in constituent with recently Iraqi study by [11].

Enterobacter and *pseudomonas* represent the second causative agents of UTIs of catheterized patients because each of them are more frequently found in hospital acquired UTI due to their resistance to antibiotics favors their selection in hospital patients [12].

Other microorganism like fungi can cause UTIs in catheterized patients this study reveals *Candida albicans* which is due to their ability to adhere to host tissues, produce secretory aspartyl proteases and phospholipase enzymes, and transform from yeast to hyphal phase with initiated by germ tube formation, these are the major determinants of its pathogenicity [13].

Out of the 339 positive cases, 221(63.13%) were from female patients while 118 (33.71%) of the cases were from males Figure (2).

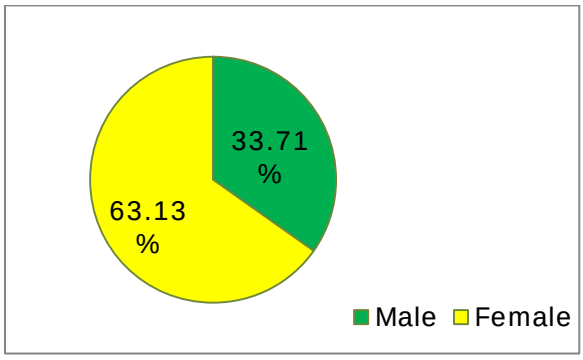


Fig (2): Prevalence of UTIs among gender of catheterized patients.

It is known that incidence of UTIs is generally higher in females than in males worldwide for several reasons like the shorter female urethra is less effective deterrent to infection than the male urethra, sexual intercourse facilitates the movement of microorganisms up the urethra particularly in female, so that the incidence of UTIs is higher among sexually active than celibate women. However the antibacterial properties of prostatic fluid may also account for increased resistance to UTI observed in men [14,15].

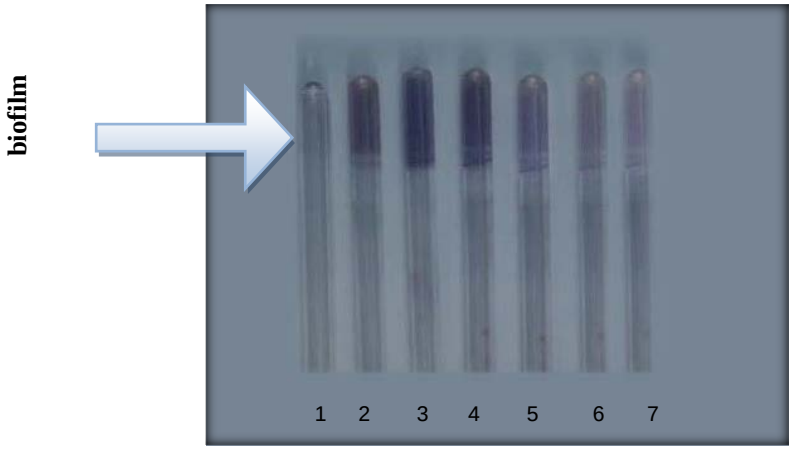


Fig (3): Biofilm formation by the isolates in test tubes method
(1)Control (nutrient broth only). (2)*P. aeruginosa* (3) *E coli* (4) *C. albicans* (5) *P. mirabilis* (6) *K. pneumonia* (7) *P. vulgaris*

Results showed that 306 isolates (90.26%) form biofilm with difference in thickness of formed layer. *P. aeruginosa* isolates were able to produce high quantity of biofilm followed by *E.coli*, *C. albicans*, *P. mirabilis*, *K. pneumonia* and *P. vulgaris* has decreased in quantities of biofilm formation Figure (3).

Biofilm formation on medical devices can negatively impact the host by causing the failure of the device and by serving as a reservoir or source for future continuing infections [16].

Biofilm can increase bacterial resistance to antimicrobial agents by some mechanisms which included impairment of antimicrobial agents diffusion, locally alteration of microenvironment that impair the activity of antimicrobial agent, reduced bacterial growth rate and finally bacterial growth within biofilm may facilitate plasmid exchange and enhance the spread of antimicrobial resistance [17,18].

Conclusion

1. Many species of uropathogenes(bacteria and yeasts) responsible of complicated UTIs with different pathogenesity rate according to their virulence factors. *Proteus sp., Klebsiella sp. and Candida sp.* represent the main causative agents of UTIs of catheterized patients suffering from complicated UTIs.

2. Most uropathogenic bacteria and yeast reflect high ability to produce biofilm specially with catheterized patients. Catheter has been considered good environment which provide bacteria and yeast with optimal conditions of biofilm formation.

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