A Novel Approach to Overcome Adherence Property of *Candida albicans*

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Abstract

The study is aimed to introduce a procedure that mimic the adherence of microorganisms to the plastic tube and to assess the suitable solution to irrigate these microorganisms. *Candida albicans* broth was inoculated into a 30 cm plastic tube for a certain period of time to allow the adherence of microorganism to the inner surface of tube. Tubes were irrigated with distilled water, tap water or different concentrations of sodium chloride (0.1-0.9%). The results showed that the percentage of *Candida albicans* which adhered to the inner surface of tube ranged between 0.27% and 30% of the initial inoculum. Tap water was superior than distilled water in removing the adherent Candida. Sodium chloride in concentrations 0.3%, 0.6% and 0.9% completely removed the attached Candida while the other concentrations showed inconsistent results. It concludes that irrigation with water or certain sodium chloride concentrations, which interfere with the fluidity of *Candida albicans*, is the simple and best method to eliminate the adherent microorganisms.

Keywords: *Candida albicans*, adherence, medical device.

Introduction

At least half of all cases of nosocomial infections are associated with medical devices [1]. The medical consequences of device-related infections can be disastrous; they include potentially life-threatening systemic infections and device malfunction that may require device removal which often complicated by tissue destruction. An increasing proportion of device-related infections, are being caused by *Candida* species [1,2]. *Candida albicans* is one of the most common causes of intravascular catheter-associated infection in hospitalized patients[3]. Adherence of Candida yeast to implanted medical devices is a prerequisite for colonization and consequently leads to infections. Attachment of *Candida albicans* cells to extracellular matrix protein[4] is mediated by nonspecific interaction, such as hydrophobic and electrostatic forces, as well as by specific adhesin-ligand bonds [5-7]. This type of infection resulted in serious medical complications, expensive care and is noted as the most frequent factor limiting the prolonged use of certain device like central venous catheters[8]. Antifungal drugs are certainly required to treat this condition, and the infected devices generally need to be removed[9]. Disinfectants (e.g. chlorhexidine, benzalkonium chloride) were effective in reducing the adherence of *Candida albicans* to plastic surfaces but unable to prevent its adherence to extracellular matrix proteins[10]. This study is aimed to introduce in vitro model that mimic the adherence of *Candida albicans* to plastic tube and to test the ability of aqueous solutions, notably water and sodium chloride, to overcome the adherent *Candida albicans*.

Materials and Methods

This study was done at the Department of Microbiology, College of Medicine Al-Mustansiriya University, Baghdad–Iraq, during 2009. The study protocol was approved by the Scientific Committee of the College of Medicine, Al-Mustansiriya University.

In this work, *Candida albicans* ATCC 10231 strain was used. It was maintained on agar slants, and fresh streaks were made monthly from original stocks. Yeasts were grown for 48 h at 37°C on Sabouraud dextrose agar slants (Oxoid, UK) to obtain a fresh culture of synchronous stationary yeast-phase *Candida albicans* [11], a loopful of this culture was transferred to 25 ml of Sabouraud's dextrose broth to obtain a saturated culture, the culture was incubated for 24 h at 37ºC.

An autoclaved sterile plastic tube (Tygon, Type S-50-HL, Class VI) instead of any applicable medical device was used. Its dimensions were 30 cm long x 4mm inside diameter x 5mm outside diameter x 1mm wall thicknesses, fixed by stainless steel holder at

angle of 45 degree. The upper end of tube was fitted by sterile three-way device. The broth of cultured *Candida albicans* or the aqueous solutions were injected by a 10 ml sterile disposable plastic syringe (Proton sterile syringe) via three way device at a flow rate of 10 ml/min, and collected from the lower end of tube. Such model allowed the fluid to run steadily and slowly by the gravity, and it gives a chance to the yeast cells to adhere. The possibility of adhesion on the inner surface of disposable syringe or three way device cannot be excluded.

A total volume of 10 ml cultured *Candida albicans* broth was injected into the tube and allow two minutes interval before irrigating the tube either with tap water, distilled water or sodium chloride (0.1-0.9%). The absorbance (O.D.) of each treatment was recorded at λ 620 nm using UV-Visible spectrophotometer (Cecil, CE 7200, France). The difference in the optical density before and after injections of *Candida albicans* broth was used as an index of yeast cell adhesion as well as an index of the irrigating efficacy of treated solutions.

$$\%of\ \ adherence = \frac{O.D.after\ rinsing - O.D.before\ rinsing \times 100}{O.D.before\ rinsing}$$

Statistical analysis

The results were expressed as number, percentage and mean ± SD. The data were analyzed by two tailed paired "t" test taking $p \leq 0.05$ as the lowest limit of significance.

Results

Table (1) showed that *Candida albicans* cells adhered significantly to the inner surface of the plastic tubes with a great variation. The mean ± SD percent of adhered cells of the total 68 samples is 5.7 ± 5.8%. There is no significant correlation between the initial load of cells suspension and the adhesion percentage (Fig.1). The results of rinsing tubes with adherent cells showed that 0.3%, 0.6%,or 0.9% of sodium chloride showed optimum anti- adhesive effect (Table (2)). The antiadhesive effect of tap water is superior than distilled water but inferior to0.3%, 0.6%,or 0.9% of sodium chloride (Table (2)).

Table (1)
Changes in absorbance (O.D) of the broth suspended with C. albicans cells before and after running in the plastic tube of 68 samples.

	Before rinse	*After rinse*	*% adhesion*	*Probability*
Distilled water (n=7)	0.711±0.090	0.702±0.090	1.3	0.002
Tap Water (n=8)	0.788±0.061	0.739±0.078	6.2	0.021
0.1% NaCl (n=5)	0.526±0.01	0.510±0.006	3.0	0.022
0.2% NaCl (n=5)	0.535±0.014	0.431±0.063	19.4	0.016
0.3% NaCl (n=7)	0.763±0.016	0.748±0.009	2.0	0.007
0.4% NaCl (n=8)	0.802±0.026	0.696±0.108	13.2	0.011
0.5% NaCl (n=6)	0.458±0.014	0.421±0.037	8.1	0.041
0.6% NaCl (n=6)	0.884±0.005	0.853±0.025	3.5	0.043
0.7% NaCl (n=5)	0.834±0.015	0.809±0.014	3.0	0.002
0.8% NaCl (n=6)	0.845±0.023	0.828±0.023	2.0	0.008
0.9% NaCl (n=5)	0.835±0.010	0.826±0.012	1.1	0.013

Table (2)
The antiadhesive effect of several treatment modalities.

	Antiadhesive	*No effect*	*Total*
Distilled water	3	4	7
Tap water	5	3	8
0.1% NaCl	4	1	5
0.2% NaCl	0	5	5
0.3% NaCl	7	0	7
0.4% NaCl	2	6	8
0.5% NaCl	5	1	6
0.6% NaCl	6	0	6
0.7% NaCl	1	4	5
0.8% NaCl	3	3	6
0.9% NaCl	5	0	5

The results were expressed as numbers of experiments.

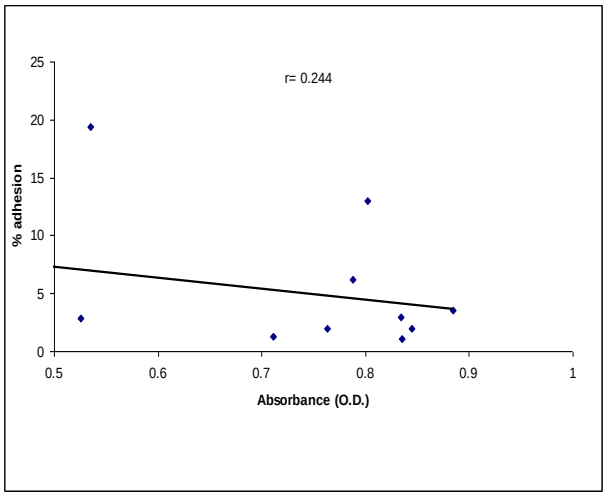

Fig. (1) The relationship between initial cell suspension adhesion percent.

Discussion

The results show that simple irrigation of plastic tubes with tap water or sodium chloride solution can overcome the adhered yeast cells. The significance of these results is not only related to the adhered yeast cells but it extended to other microorganisms. In one study, it was found that the adherence of *P. aeruginosa* to the urinary catheter was enhanced by *Candida albicans*[12]. Therefore simple irrigation of catheters with tap water may prevent the adherence of such pathogenic microorganisms. Moreover, the use of tap water or sodium chloride solution can substitute the use of antiseptics like chlorhexidine and gentian violet in reducing the adherence of *Candida albicans* to the catheters [10,13]. Also, the effectiveness of sodium chloride solution (0.9%) in this study highlights the significant use of this solution instead of antimicrobial coated central venous catheter, as well as its effect is comparable to the irrigation with antimicrobials[14]. The effect of certain concentrations of sodium chloride solutions may be related to the interaction of chloride solution with the materials of catheter. In one study, it was found that the latex catheter facilitated adherence of *Candida albicans* more than the silicon catheter[15]. Limitations of this study include the use of reference antimicrobials for comparison with the solutions used in this study and to look for the effectiveness of these solutions over long period of time adherence. It concludes that irrigation with water or certain sodium chloride concentrations, which interfere with the fluidity of *Candida albicans*, is the simple and best method to eliminate the adherent microorganisms.

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الخلاصة

الهدف: هدفت الدراسة الى ايجاد طريقه تماثل التصاق فطر المبيضات على بطانة الانابيب البلاستيكية وايجاد افضل المحاليل لازالة التصاق هذه المبيضات.

طريقة العمل : تم تمرير مرق فطر المبيضات في انبوب بلاستيكي بطول 30 سنتمتر لمده معينة من الزمن سمح لهذه المبيضات الالتصاق على جدران بطانة الأنبوب. تم غسل الانابيب بالماء المقطر و ماء الحنفيه وبتراكيز مختلفه من كلوريد الصوديوم بنسب تراوحت بين 0،1% و0،9%. تم مراقبة الكثافة البصرية لمرق فطر المبيضات وك ذلك محاليل سقي الأنبوب بوساطة مقياس الطيف الضوئي عند طول موجي 620 نانوميتر.

النتائج: اظهرت النتائج ان نسبة المبيضات التي التصقت على بطانة الانبوب البلاستيكي تراوحت بين 0.27 و 30% من الجرعة الملقحة. كان سقي ماء الحنفيه افضل من الماء المقطر في ازالة المبيضات الملتصقة، كما لوحظ ان سقي كلوريد الصوديو (تركيز 0.3 % و 0.6% و 0.9 %) أزال المبيضات الملتصقة في جميع النما ذج بينما اظهرت التراكيز الاخرى نتائج متباينة. كما واظهرت النتائج انه لا توجد علاقة بين قابليه سقي المحاليل في ازالة التصاق المبيضات وبين جرعة المبيضات الملقحة.

الاستنتاج: نستنتج من هذه الدراسه ان طريقة السقي بالماء أو بتراكيز معينة من كلوريد الصوديوم الذي يتداخل مع سيولة المبيضات هي افضل وابسط الطرق لازالة التصاق فطر المبيضات.

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