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Research Article

The effect of Different concentrations of glucose solution on the growth and biofilm formation of sensitive and resistant Pseudomonas aeroginosa isolates.

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Abstract

Burns are a severely debilitating class of wound. *Pseudomonas aureugnosa* is one of most common bacteria that associated with burn infection. Hypertonic glucose solution considered as a new approach to control chronic wound infections. To study the effect of glucose solution (at different concentrations) on the growth of *P. aeruginosa* and on their biofilm formation (for both resistant and sensitive isolates), Four *P. aeruginosa* isolates were isolated from swab samples taken from different sites of burn's patients whom admitted to Al-Imam Al-Hussain Medical City. Antibiotic susceptibility patterns were determined and the effect of different concentration of glucose solution on growth and biofilm formation were studied.

The results showed that the growth of all isolates (resistant and sensitive) were reduced for more than 50% after 5 hours of incubation and this reduction increases both with the increasing of glucose concentration and with the increasing of time of exposure of the bacterial isolates to the glucose solution. The biofilm formation was increased in the presence of glucose solution with the used concentrations with the exception of the concentration of 100 mg in which the biofilm formation was inhibited in comparison to controls. Regarding Biofilm eradication assay, the formed biofilm layer was partially eradicated in the glucose concentrations of 50, 100 in most of the isolates.

In conclusion, Hypertonic glucose solution in different concentrations has inhibitory effect (both concentrations dependent and time dependent) on growth of sensitive and resistant bacteria. The inhibitory action of the glucose against *P. aeruginosa* biofilms was found in the lower concentration of glucose solution whereas higher concentration of glucose increased the biofilm formation. none of the concentrations revealed the complete destruction of biofilm.

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Introduction

Burns are considered a notorious type of wound which could have a negative effect on patient's life. The annual number of deaths due to fire, heat and hot substance is about 300,000 (1). Higher frequency of Burns that result from fire are recorded in less developing countries (2–5). Damaging of tissue at burn site leads to the loss of the biological fluids which defined as burn wound exudates (6). The microenvironment of these burn wound excaudate will provide a good environment to certain pathogens to proliferate successfully.

Infection of the wound might results in approximately 75% of cases to death. Gram-negative bacteria like *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella* spp., *Stenotrophomonas* spp., *Escherichia coli*, and *Enterobacter cloacae* are frequently isolated from wounds. These pathogens are responsible for most of the nosocomial infections and responsible for outbreaks in burn wards globally (7–9).

Pseudomonas aereugnosa is an opportunistic pathogen associated with burn infections and is responsible for a variety of acute and chronic infections including endocarditis, pneumonia, and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin, and musculoskeletal system (10,11) due to its highly resistance rates to antibiotics (12). The fast increase in drug resistance among bacteria has become a serious health problem health worldwide. To overcome this increase, broad-spectrum antibiotics have been used which leads to the selection and accumulation of resistance strains of bacteria (13,14).

Pseudomonas aereugenosa is found in different environmental due to its simple requirements for growth. Formation of biofilms in addition to the survival of this bacteria on both living and non-living surfaces are considered the main causes for burn wound complication (15,16). Biofilm is defined as aggregations of one or more than one type of bacterial species that found in an extracellular matrix which helps the bacteria to survive in their hostile environment.

There are many solutions for treatment of burns but the most effective of them is glucose solution. Dressings of the wound with hypertonic glucose solution could be used as an alternative method in wound management which enhance the auto-debridement process, increase proliferation of granulation, and reduce the patient's healing time (17,18).

Thus, the current study Aims to study the inhibitory effect of glucose solution (at different concentrations) on growth and biofilm formation of resistant and sensitive isolates of *P. aeruginosa*.

Material & Methods

Bacterial isolates

Four *P. aeruginosa* isolates were isolated from swab samples taken from different sites of burn's patients whom admitted to Al-Imam Al-Hussain Medical City during the period of sample collection (two months, from Nov. 2021 to Jan 2022) and these isolates were used in the following experiments after identification of the bacteria using VITEK 2 compact system. All isolates were sub-cultured in Blood and MacConkey agar before it used to ensure its activity and purity.

Antibiotic Susceptibility patterns

Susceptibility patterns of the isolated bacteria was done using VITEK2 system for gram negative bacteria. Four isolates were selected based on susceptibility test recovered from VITEK2 system, two isolates were sensitive and two other isolates were resistant to most of the tested antibiotics. MIC of the antimicrobial agents were tested within VITEK2 system including Amoxicillin, Amoxicillin-Clavulanic acid, Ampicillin/Sulbactam, Ticarcillin, Ticarcillin/Clavulanic Acid, levofloxacin, Amikacin, Gentamicin, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Ofloxacin, Tobramycin, colistin, Piperacillin, Ciprofloxacin, Cefepime, Tetracycline.

Glucose solution

Seven concentrations of glucose solution (D-(+)-glucose, Anhui Herrman Impex Co., Ltd) were prepared (50 ,100 ,200 ,300 ,400 ,500, 600 mg/ml) to be used.

The effect of hypertonic glucose solution on growth of *P. aeruginosa*

The effect of hypertonic glucose solution was done using the method prescribed previously (19). Briefly, 50ul of overnight bacterial suspension (10^8) were added to 4950ul (Final volume is 5 ml) nutrient broth media to prepare 1% (v/v) dilution for each isolate. One hundred microliter of the bacterial solution were added to each well in the microtiter plate. Then, 100ul of glucose solution (each concentration was repeated twice) was added to each well. A hundred microliter of nutrient broth was added as a negative control. After 5 h, 8 h, and 24 h of incubation at 37ºC, the absorbance were measured by ELISA reader (PKL PPC 142) at wave length 630 nm.

Biofilm Inhibition test

Biofilm formation inhibition test was done using the method prescribed previously (19). Briefly, 100ul of overnight bacterial solution with nutrient broth (50ul of bacteria suspension (10^8 CFU) was mixed with 4950ul nutrient broth media to prepare 1% v/v dilution) and 100ul of different concentration of glucose were added into two wells for each concentration and for each isolate. Then, the plate were incubated for 24h to allow the bacteria to grow and form Biofilm. The following day, the mixture was removed from the wells and the attached bacteria was washed. One percent Crystal Violate (CV) solution (200ul) was added to each well for 20 min followed by washing step. The absorbed CV dye were eluted using absolute Ethanol after addition of 200ul to each well and the absorbance were read immediately by using ELISA reader (OD = 630nm).

Biofilm Eradication test

Biofilm formation inhibition test was assessed using method described previously (19). Briefly, 100ul of overnight bacterial solution with nutrient broth (1% v/v) was added to each well and incubated at 37 ºc for 24 hours. The following day, 100ul of glucose solution was added to each well (each concentration repeated twice) and 100ul distilled water was added to control wells. After incubation for 24h, The mixture was removed from the wells and the attached cells was rinsed. Crystal Violate (200ul of 1% CV) solution was added to each well for 20 min followed by washing step. The absorbed dye was eluted using absolute Ethanol (200ul were added to each well) and the absorbance were read immediately by using ELISA reader (OD = 630nm).

Ethical statement:

The current study was approved by the scientific committee in Department of Clinical Laboratories in College of Applied Medical Sciences, Kerbala University. The study design didn't include any patients and is based on laboratory work only on bacterial isolates, thus , No ethical consent was required.

Statistical Analysis:

The mean of the absorbance reading was calculated and No other statistical analysis was used. Figures were constructed using Excel program.

Results:

In the current study, four *Pseudomonas aeruginosa,* were isolated from wound samples taken from different sites of burn's patients. The identification and antibiotic susceptibility testing were performed using VITEK2 compact system. The selection of these isolates were done based on the results

of susceptibility testing. Thus, two isolates which are sensitive and two other isolates which are resistant to most of the tested antibiotics were enrolled.

The effect of hypertonic solution on bacterial growth.

The results showed that the growth of all isolates (resistant and sensitive) were reduced for more than 50% after 5 hours of incubation and this reduction increases both with the increasing of glucose concentration and with the increasing of time of exposure of the bacterial isolates to the glucose solution(after 8hrs and24hrs),as demonstrated in figures1-4.

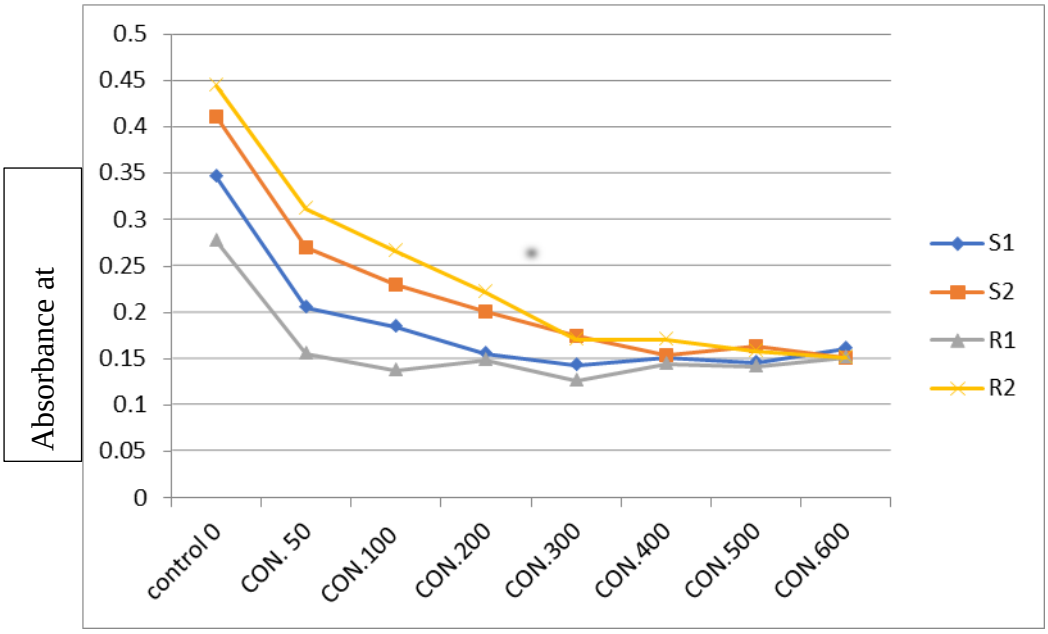

Figure 1. Growth of bacterial isolates in different concentrations of glucose solution after 5 hours of incubation

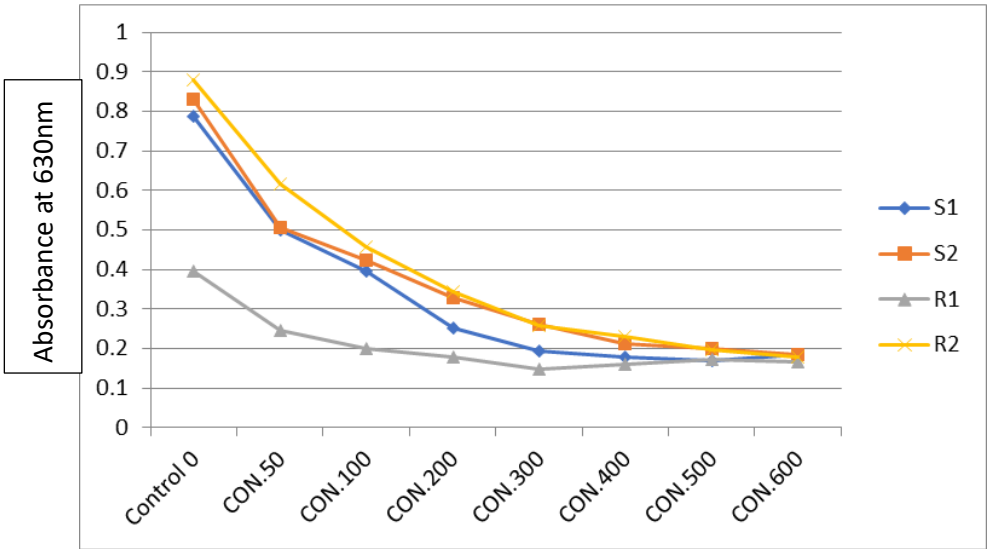

Figure 2. Growth of bacterial isolates in different concentrations of glucose solution after 8 hours of incubation

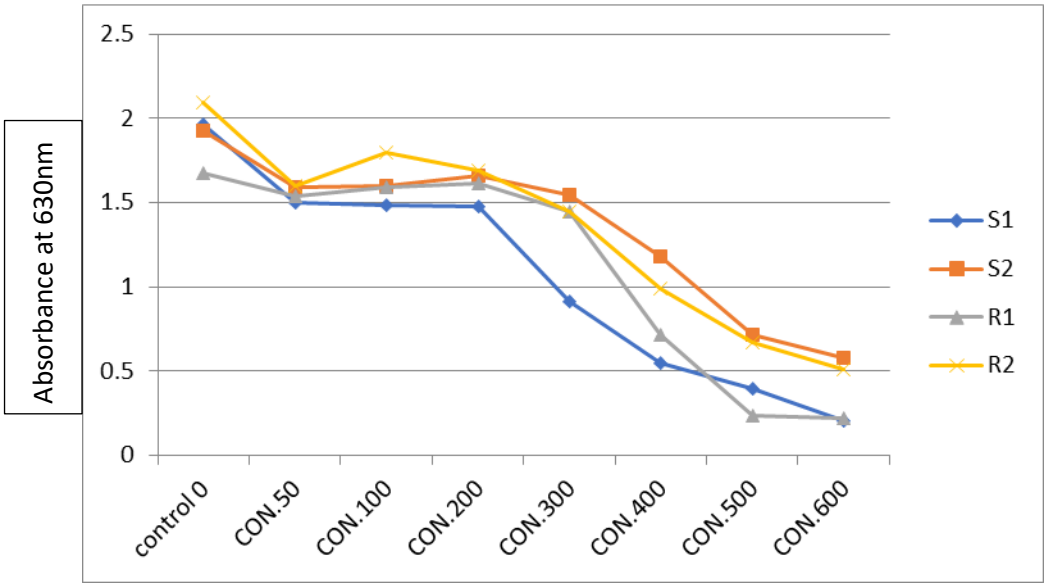

Figure 3. Growth of bacterial isolates in different concentrations of glucose solution after 24 hours of incubation

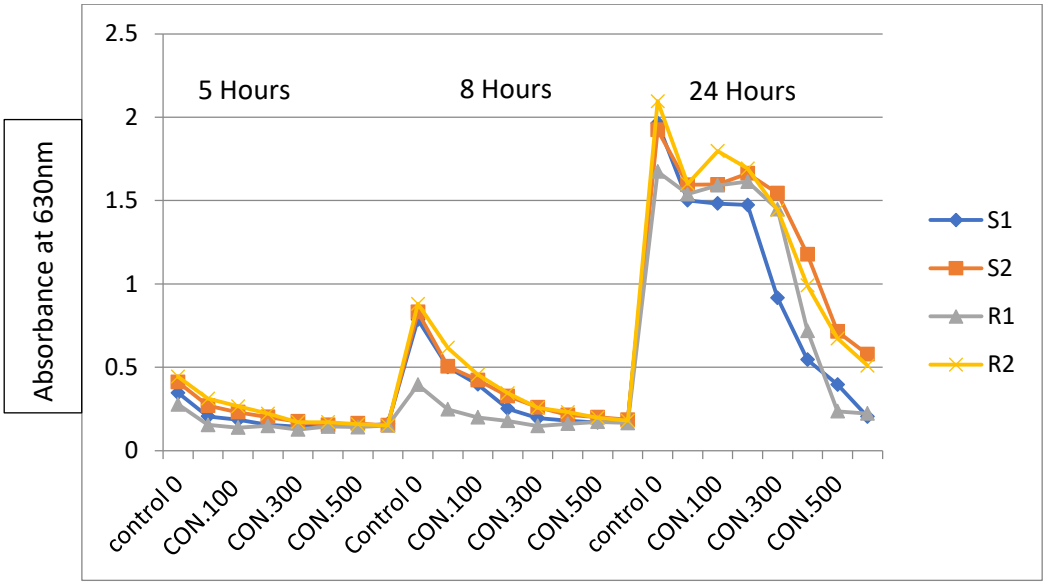

Figure 4: Growth of *Pseudomonas aerogenosa*. With the different glucose concentration for 5, 8, 24 hours of incubation.

Effect of hypertonic solution on formation and eradication of Biofilm

The antibiofilm activity of glucose solution were measured by using crystal violet assay. The current study found that the biofilm formation was increased in the presence of glucose solution with the used concentrations with the exception of the concentration of 100 mg in which the biofilm formation was inhibited in comparison to controls, as shown in figure 5. Regarding Biofilm eradication assay, the formed biofilm layer were eradicated in the glucose concentrations of 50, 100, and 600 mg as shown in Figure 6.

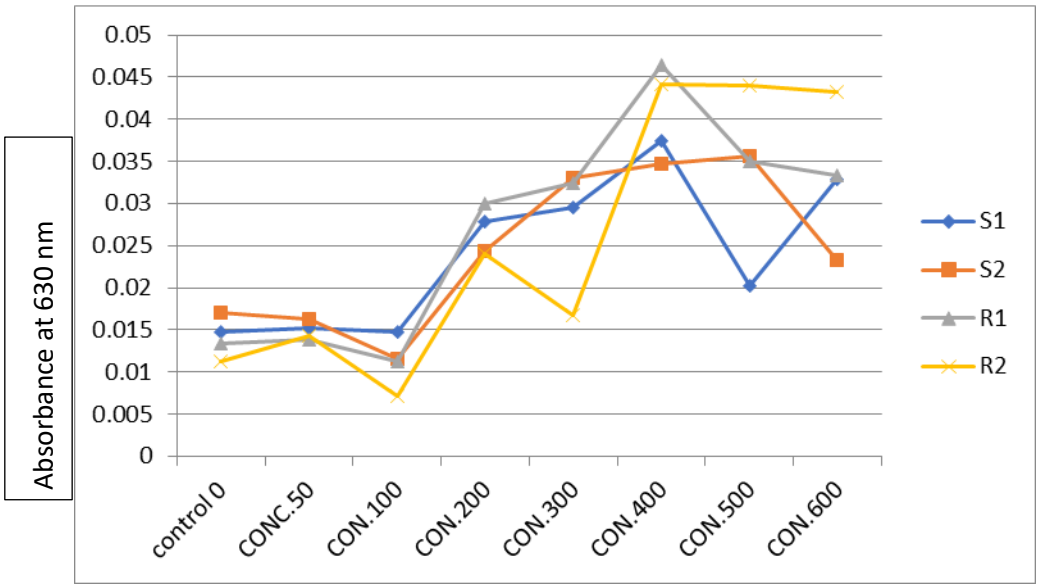

Figure 5. Biofilm formation inhibition Assay

Figure 6. Biofilm Eradication assay

Discussion

It has been documented that burns considered as sever forms of trauma that need special medical care. The microenvironment of burn wounds are good environment for bacterial growth and proliferation (20,21).

Pseudomonas aeruginosa, is one of the most commonly isolated pathogen from infected burns with high resistance rate to most of antibiotics and subsequently alternative methods for burn management is required (22). Additionally, this bacteria characterized by formation of biofilm which considered as an obstacle for treatment of wound infection because bacteria within biofilm are protected from immune response and antibiotics (23).

Although treatment of burn wounds with glucose solution has been used previously (24), the inhibitory effect of this solution on bacterial growth and biofilm formation ability has not documented. This study investigated the inhibitory action on bacterial growth and biofilm formation on resistant and sensitive bacterial isolates.

The current study revealed that hypertonic glucose solution in different concentrations has inhibitory effect (both concentrations dependent and time dependent) on growth of sensitive and resistant bacteria. Similar findings were documented by previous study (25).

It has been documented that Pseudomonas has the ability to form biofilm on different medical surfaces. Bacteria inside biofilm are much more resistant to antimicrobial agents than planktonic forms since bacteria that are unresisting to antimicrobial agents in any way can turn resistant after forming a biofilm(26). This biological development protects the pathogen from host immunity and contributes to its antimicrobial resistance. It is estimated that about 80% of infectious diseases are due to biofilm formation. Biofilm-forming ability and antimicrobial resistance of this pathogen lead to many persistent and chronic bacterial infections) (27,28).

It is the most commonly isolated species from chronic wounds and is considered a potent biofilm producer since they act as a barrier in wound healing and exhibits high resistance to antimicrobial therapy (29,30). Thus, We further explored the effect of glucose solution on biofilm for this bacteria. To illustrate this aim, the antibiofilm activity of various concentration of glucose were tested crystal violet staining technique. The results revealed that The inhibitory effects of the glucose towards *P. aeruginosa* biofilms was found in the lower concentration of glucose solution for 75% of the isolates. However, higher concentration of glucose result in higher level of biofilm formation. Similar finding was documented by Wang *et al*., who reported that glucose induce biofilm formation (24).

Concerning Eradication assay, the used concentrations didn't reflect complete eradication of biofilm. However, glucose concentration of 100 mg/mL considered the best concentration which might possibly reflect the inhibitory action of glucose

solution on expression of the genes involved in the biofilm formation. Similar findings was reported by Chen *et al*., (25).

Conclusion:

Hypertonic glucose solution in different concentrations has inhibitory effect (both concentrations dependent and time dependent) on growth of sensitive and resistant bacteria. Lower concentration of glucose solution is required for biofilm formation inhibition and higher concentration of glucose increased the biofilm formation. Eradication of biofilm formation does not occur with all concentration.

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