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Research article

Biofilm formation and antibiotic susceptibility for clinical and environmental isolates of *Pseudomonas aeruginosa*

Farah Mohommed Saleh[1] and Ghadah Mohommed Saleh[2]*

ABSTRACT

Pseudomonas aeruginosa is emerging opportunistic clinical pathogens. Clinical isolates of *P. aeruginosa* resist wide spectrum of antibiotics and form biofilm. The comparison study between clinical and environmental of *P. aeruginosa* in terms of biofilm formation and antibiotic resistance is very scanty. Thus, in current study microtiter plate technique was used to measure the biofilm formation by several clinical and environmental isolates. Moreover, the antibiotic susceptibility of these bacteria was evaluated by VITIK 2 techniques. The relationship between the antibiotic susceptibility and biofilm formation was evaluated for clinical and environmental isolates. Clinical and environmental isolates of *P. aeruginosa* produced a good amount of biofilm but the clinical isolates produced higher amount of biofilm as compared to environmental isolates. Resistance to antibiotics by clinical isolates was higher than resistance to antibiotics by environmental isolates and the minimum inhibition concentration (MIC) of most antibiotics to clinical isolates were higher than MIC against environmental isolates. Little relationship was observed between the biofilm formation and antibiotic resistance in case of clinical isolates, while no relationship was seen between the antibiotic susceptibility and biofilm formation. It can be concluded that the clinical isolates produced biofilm higher than environmental isolates. The relationship was seen only between the biofilm produced by clinical isolates and antibiotic susceptibility.

Keywords: Biofilm, Clinical isolates, Environmental isolates, *Pseudomonas aeruginosa*.

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INTRODUCTION

Pseudomonas aeruginosa is an epitome of an opportunistic pathogen. It causes a wide spectrum of infections in humans such as respiratory tract infections; burn wound infections and urinary tract infections (UTIs) [1-3]. *P. aeruginosa* possesses several traits that contribute to its ability to colonize and persist in acute and chronic infections. These include high resistance to antimicrobials, a plethora of virulence products and metabolic versatility [4-5]. In addition, *P. aeruginosa* has a tendency to form biofilm

*Correspondence: ghadahalquraishi@yahoo.com
Phone number: 009647706279829
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

on the surface of urinary catheters and several medical devises leading to recurrent and chronic infections that result in significant morbidity and mortality [6-8]. Moreover, *P. aeruginosa* environmental isolates have a good ability to form biofilm on water supply tubes [9]. Biofilm bacteria form structured communities of cells embedded in an extracellular polymeric matrix [10-11]. Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms, leading to difficulty in eradication of bacteria [12-15].

Biofilms also provide an ideal niche for the exchange of extrachromosomal DNA responsible for antibiotic resistance, virulence factors and environmental survival capabilities at accelerated rates, making it a perfect milieu for the emergence of drug-resistant pathogens [16]. Previous study showed that the ability of clinical and environmental isolates of *P. aeruginosa* to resisted to different antibiotics [17].

P. aeruginosa besides of their responsibility to produce several diseases it can produce lipase and different enzyme that help to eradicate the oil and other pollutants from soil and water [18]. Previous study showed that the clinical isolates of *P. aeruginosa* had high ability to resist different kinds of antibiotics as compared with environmental isolates of this species [17].

The present study aimed to find the difference between clinical isolates of *P. aeruginosa* that isolated from patients suffering with respiratory tract infection and environmental isolates of *P*. *aeruginosa* that isolated from soil contaminated with oil products in terms of biofiom formation. This study also targeted to find the relation ship between the susceptibility to antibiotic and biofilm formation by clinical and environmental isolates of *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial isolates

Four clinical isolates of *P. aeruginosa* and 12 environmental isolates of *P. aeruginosa* were used in this study. Bacteria were preserved by lyophilization and were routinely cultured at 37ºC on Luria Bertani agar plates, Subcultures were made every 2 weeks.

Biofilm assay on polystyrene microtiter plates

Bacterial aliquots (200 µl) of standardized inoculum (10^7 c.f.u./ml) were added to the wells of sterile flat-bottom polystyrene tissue culture plates and incubated at 37°C for 24 h to check biofilm formation of *P. aeruginosa.* The medium was then discarded, and non adherent cells were removed by washing three times with sterile PBS (0.1 M, pH 7.2). Quantitatiation of adhering bacteria was performed by spectrophotometric method as previously described by Zgair and Chhibber, (2011) [19]. Slime and adherent organisms were fixed by incubating them for 30 min at 60°C and then stained with Hucker crystal violet (0.4%) for 5 min. After thorough washing with water to remove excess stain, the plates were dried for 30 min at 37°C. The extent of biofilm was determined by measuring the absorbance of stained adherent film upon treatment with acetone:ethanol (30 : 70) at a wavelength of 490 nm [19].

Antibiotic susceptibility

The standard method of Mazzariol et al (2008) was followed to test the susceptibility of *P. aeruginosa* to the several antibiotics [Ampicillin (AMP), Amoxicillin/ Clavulanic acid (AMC), ampicillin/ Sulbactam (SAM), Piperacillin/Tazobactam (TZP), cefazolin (CFZ), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), imipenem (IPM), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LVX), nitrofur-antoin (NIT), trimethoprim/ sulfamethoxazole (SXT), tricarcillin (TIC), amikacin (AMK)]. VITEK 2 DensiCheck instrument (biome ´rieux) was used to check the supportability of clinical and environmental isolated of *P. aeruginosa* [20].

Statistical analysis

All data represent mean values and standard error of at least three replicas of independent experiments. The differences between two groups were analyzed by using student's *t* test. Pearson coefficient test was used to estimate the correlation between biofilm formation and MIC. Origin 8.0 version software was employed to perform these calculations and plot graphs. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Biofilm formation

Fig. 1 showed the biofilm formation in terms of optical density by 4 clinical isolates of *P. aeruginosa* and 12 environmental isolates. It was observed clearly that all clinical and environmental isolates showed high ability to form biofilm on polystyrene microtiter plates. Clinical isolates, PAC1 showed the highest level of biofilm followed by PAC4, while the lowest biofilm formation was found in case of PAC2. When ability of environmental isolates of *P. aeruginosa* to form biofilm was checked, the highest biofilm formation was formed by PAE17 followed by PAE16, while PAE12 showed the lowest ability to form biofilm on polystyrene microtiter plate.

The results of present study showed that the ability of clinical isolates to form biofilm was significantly ($P < 0.05$) higher than the ability of environmental isolates of *P. aeruginosa* (**fig. 2**).

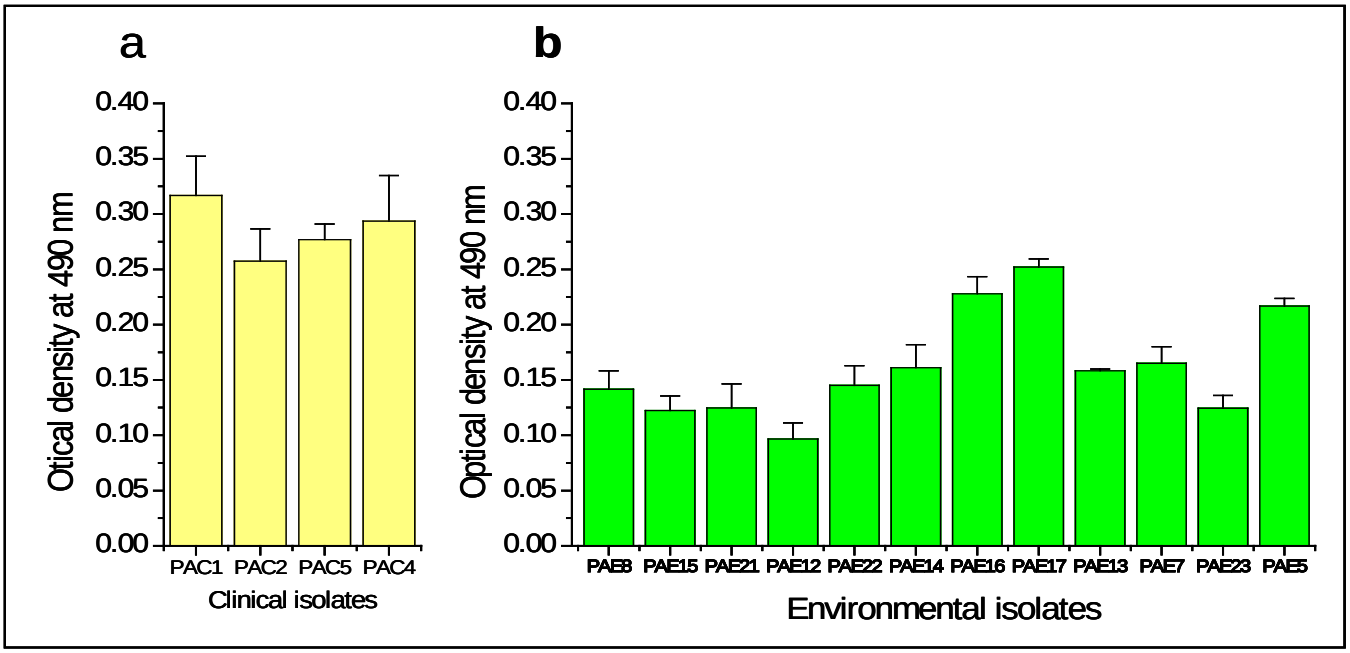

Fig. 1 Biofilm formation of clinical isolates of *P. aeruginosa* (a) (PAC1, PAC2, PAC5, PAC4) and environmental isolates of *P. aeruginosa* (b) (PAE8, PAE15, PAE21, PAE12, PAE22, PAE14, PAE16, PAE6, PAE13, PAE7, PAE23, PAE5) on polystyrene microtiter plates. Spectrophotometric method was used to perform biofilm formation by *P. aeruginosa*.

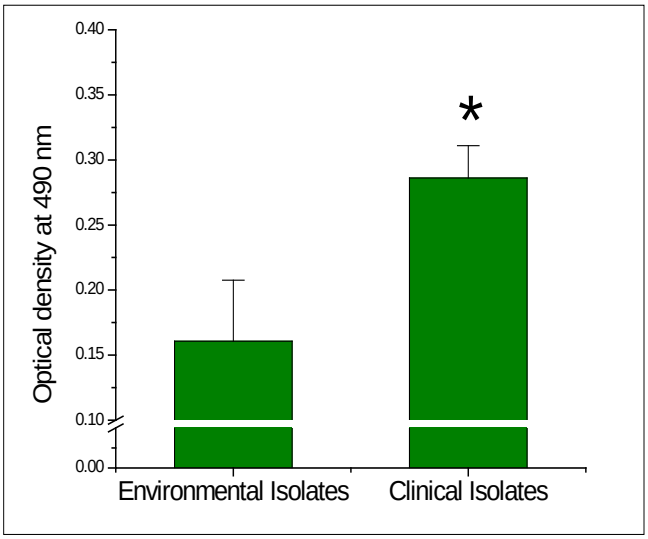

Fig. 2 Mean of biofilm formation of environmental isolates and clinical isolates of *P. aeruginosa*. Asterisk represents the significant difference from environmental isolates.

The relationship between antibiotic susceptibility and biofilm formation

In present study, the effect of biofilm formation on antibiotic susceptibility was studies. **Fig. 3** showed the relationship between biofilm formation and antibiotic susceptibility in terms of MIC. The result showed the low positive relationship between the ability of clinical isolate to form biofilm formation and the value of MIC (**fig. 3a**) but this correlation was not significant (P= 0.058). **Fig. 3b** showed no significant correlation between biofilm formation of environmental isolates of *P. aeruginosa* and antibiotic susceptibility (MIC).

DISCUSSIONS

P. aeruginosa has become the model organism for bacterial biofilm, and much work has been done in order to identify the biofilm formation by this bacterial species and role of the types of surface on the bacterial adhesion and biofilm formation [9, 21]. Other studies focused on the role of bacterial appendages on the ability of bacteria to adhere and biofilm formation [22-25]. In present study, the ability of clinical and environmental isolates of *P. aeruginosa* to form biofilm was evaluated and relationship between the antibiotic susceptibility and biofilm formation. The current study proved the ability of clinical and environmental isolates of *P. aeruginosa* to form biofilm but the highest ability to form biofilm was found in clinical isolates. Moreover, weak positive relationship was fund between antibiotic resistance and biofilm formation in clinical isolates but no relationship was observed between the biofilm formation by environmental isolates and antibiotic resistance.

The creased frequency of strains resistant to many antibiotics and chemotherapeutics is responsible for a substantial number of infections in hospital environments. The resident hospital microflora is relatively dynamic and susceptibility and resistance patterns of microorganisms isolated from clinical environmental samples can vary significantly [26]. Several studies focused on the ability of clinical isolates that isolated from hospital to form high level of biofilm [22, 23, 27]. Thus the positive relationship ould be seen between the antibiotic resistance and biofilm formation in case of clinical isolates.

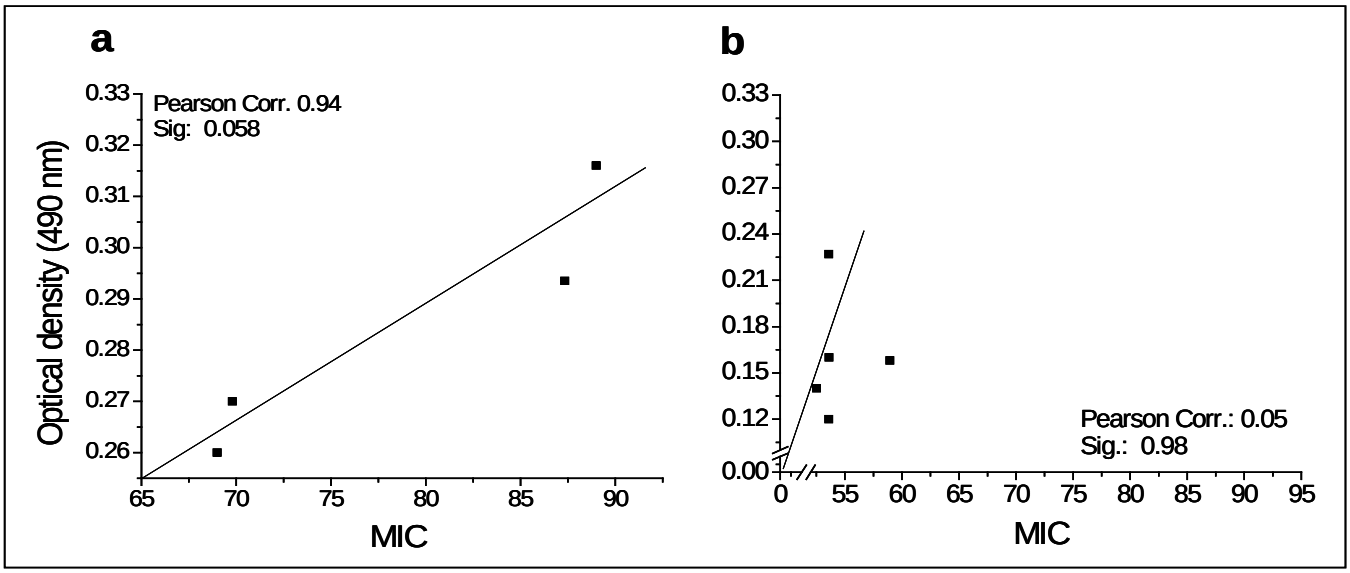

Fig. 3 Relationship between biofilm formation in terms of optical density and antibiotic susceptibility (MIC). a, relationship (correlation coefficient, Pearson correlation) between biofilm formation of clinical isolates and antibiotic susceptibility of these isolates; b, relationship (correlation coefficient, Pearson correlation) between biofilm formation of environmental isolates and antibiotic susceptibility.

P. aeruginosa infections generally persist despite the use of long-term antibiotic therapy. This has been explained by postulating that *P. aeruginosa* forms an antibiotic-resistant biofilm consisting of bacterial communities embedded in an exopolysaccharide matrix. Alternatively, it has been proposed that resistant *P. aeruginosa* variants may be selected in the Cystic fibrosis respiratory tract by antimicrobial therapy itself. Drenkard and Ausube, (2002) reported that both above explanations were correct, and were interrelated. They found that antibiotic-resistant phenotypic variants of *P. aeruginosa* with enhanced ability to form biofilms arise at high frequency both in vitro and in the lungs of CF patients. They also identified a regulatory protein (PvrR) that controls the conversion between antibiotic-resistant and antibiotic-susceptible forms [28]. Coban et al. (2009) showed that the little positive relationship between the ability of *P. aeruginosa* to form biofilm and resistance to particular antibiotics such as piperacillin/tazobactam [29], this finding going on with the results of current study. The present study is the pioneer study that highlighted the relationship between biofilm formation and antibiotic susceptibility in case of environmental isolates and provided the good comparison with clinical isolates. The present study showed that clinical and environmental isolates of *P. aeruginosa* form biofilm with high efficiency and that was correlated with resistance to antibiotic only in case of clinical isolates.

Conflict of interest

The authors declare that they have no conflict of interests.

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Author affiliation:

1. *Department of Medical Lab Sciences, Al-Yarmouk University College, Baghdad, Iraq.*
2. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*