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# Sub-minimum Inhibition Dose of Cefotaxime Diminishes Biofilm Arrangement by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro.

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## ABSTRACT

The phenomenon of biofilm is the most important challenges for physicians. Biofilm contributes to increasing the resistance of pathogenic bacteria to antibiotics. In the current study, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from infected wounds. The capacity of these two isolates to form a biofilm in vitro was checked. A standard microtiter plate was used for this. The effect of non-inhibiting dose of cefotaxime (0.5 x MIC) on the ability of these two isolates to form biofilm was also determined. The study showed the great ability of these bacteria to form the biofilm. The study also reported that the treatment of these two isolates with a non-inhibiting dose (0.5 x MIC) of cefotaxime that corresponding to treat S. aureus with 64.5 µg/ml and P. aeruginosa with 125 µg/ml greatly contributed to reduce the formation of biofilm these two isolates. Finaly, concluded from this study that the use of 0.5 x MIC of cefotaximereduces biofilm of *S. aureus* and *P. aeruginosa* in vitro and that finding very important in the treatment of infection with low dose of antibiotics.

**Keywords:** biofilm, antibiotic, bacteria.

## INTRODUCTION

Cefotaxime (CTX) is a beta-lactam antibiotic classified as a third-generation cephalosporin, it was first manufactured in 1976 and is FDA approved for the treatment of different species of aerobic and anaerobic bacteria. Its broad-spectrum antibacterial activity is important in treating the susceptible strains that cause pneumonia, urinary tract infection, central nervous system infection and joint infections, it also use for treating skin infections and septicemia. (12).*Staphylococcus aureus* represents a significant proportion among the bacteria responsible for serious infections. Moreover, they are observed in multiple clinical situations, both in community and nosocomial pathologies. In addition, staphylococci are predominant pathogens of postoperative infections (14). *Pseudomonas aeruginosa* is a ubiquitous organism that is a known opportunistic pathogen, causing both chronic and acute infections in susceptible populations, including individuals with cystic fibrosis or burn wounds, or Intensive Care Unit patients. *P. aeruginosa* is among many bacteria that grow as a biofilm during infection (19, 11,8). Biofilm is considered as a major natural factor responsible for a assortment of persistent contaminations. *S. aureus* and *P. aeruginosa* have picked up impressive consideration in clinical settings owing

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tothe arrangement of unmanageable and long-lasting biofilm in restorative gadget (9, 13).

The aims of study ability of clinical isolates o *S. aureus* and *P. aeruginosa* to form biofilm and role of sub-inhibition dose of CTX in reducing of biofilm formation.

## MATERIALS AND METHODS

### Specimen collection

In current study, 20 samples were collected from 20 patients suffering from contaminated burns. Samples were collected under sterile conditions using sterile swabs. The samples were immediately transported to the laboratory to be implanted in the appropriate media. The age of patients was 37.5 ± 5.7 years. The number of males was 12 and the number of females 8. The study was conducted after obtaining ethical approvals from the ethics committee in the College of Science, Department of Biology, University of Baghdad as well after obtaining the patients 'consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours.

### Isolation and identification

The collected samples were cultured on MacConkey agar (HiMedia, Mumbia, india) and blood agar (HiMedia, Mumbia, india) under aerobic and sterile conditions (37 $^{o}$C for 18 h) to identify the isolated bacteria the select colonies were re-cultured on mannitol salt agar (HiMedia, Mumbia, india), nutrient agar (HiMedia, Mumbia, india). For further identification of isolated bacteria catalase test, oxidase test and Gram stain were used to identify the pure isolated bacteria.The VITEK 2 was used to identify bacterial isolatesandtest the susceptibility to the several antibiotics (6).

### Preservation of bacterial isolates

Clinical isolates of *S. aureus* and p. *aeruginosa* used in this study preserved for long term by glycerol at -20 $^{o}$C. These isolates were routinely cultured at 37$^{o}$ C on N.Aplates and vials and the sub-cultured were kept at 4 $^{o}$C for weekly use.

### Cefotaxime susceptibility and MICs

The antibiotic discs technique was applied estimate susceptibility of *S. aureus* (Sa1) ,*P. aeruginosa* (P.a1) to Cefotaxime.Cefotaximepowder was purchased from Wockhardt UK Ltd, UK. MICs (Cefotaxime) for these bacteriawere estimated using a brothmicro-dilution technique. The standard method of Clinicaland Laboratory Standards Institute guidelines (CLSI,2006) was followed. Results were reported post-overnightincubation at 37$^{o}$C, with the MIC (20).

### Bacterial treatment with Cefotaxime (sub-MICs)

After overnight incubation, the colonies of S.aureua (Sa 1) and P. aeruginosa (Pa1) that grown onto Mueller-Hinton agar were suspended in nutrient broth (NB; Himedia, Mumbia, India). Thebacterial number wasadjusted to 1 x $10^{7}$with MuellerHinton broth (Himedia, Mumbia, India) and then treatedfor 18 h at 37$^{o}$C with 0.5 x MIC of Cefotaxime.Bacterial suspension that was not treated withCefotaximewas considered as a control. Bacteria werethen washed three times with PBS (10000g, 5min) toeliminate the antibiotic and then bacteria were resuspendedin NB for adhesion method (6).

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**Biofilm formation**

Overnight cultures of *S. aureua* (Sa1) and *P. aeruginosa* (Pa1) in three ml of media (NB) (Himedia) washed 3times with fresh NB, bacterialcount adjusted to $10^7$c.f.u/ml. Two hundred microliter of standardized inoculums were included to the wells of sterile flat-bottom polystyrene tissue culture platesand incubated at 37°C for 24 h in a closed and humidified plastic container. The medium was at that point disposed of, and non disciple cells were expelled by washing three times with sterile PBS (0.1 M, pH 7.2). Spectrophotometric strategy with minor adjustments was utilized to amount biofilm. Disciple bacterial development were settled by h atching them for 30 min at 60°C and after that recolored with Hucker precious stone violet (0.4%) for 5 min. After careful washing with water to evacuate overabundance recolor, the plates were dried for 30 min at 37°C. The degree of biofilm was decided by measuring the absorbance of recolored disciple film upon treatment with acetone:ethanol (30 : 70) at a wavelength of 492 nm (20,16). The comes about were compared with control negative (comparative strategy was taken after without bacterial development).

**Statistical analysis**

All values were calculated as the implies ± SD. Contrasts between test and control bunches were analyzed utilizing Student's t-test. Contrasts among different bunches were analyzed by applying the Tukey truly noteworthy contrasts test to one-way ANOVA Utilizing Beginning form 8.0 program. A esteem of $p < 0.05$ was considered measurably noteworthy.

## RESULT AND DISSCUTION

**Bacterial isolation and identification**

In the current study, 20 samples were collected from 20 patients suffering from infected burn wounds with bacteria. From these samples, one isolate of *S. aureus*(Sa1) and one isolate P. *aeruginosa*(pa1) wereidentified according the biochemical tests and VITIK technology. Susceptibility of these bacteria to antibiotics was shown in table 1

**Table 1.**Minimuminhibition concentration in µg/ml of antibiotics against clinical isolates belong to *S. aureus* and *P. aeruginosa* that isolated from infected wounds of indoor patients.

| No | Antibiotic | *S. aureus* | | Antibiotic | *P. aeruginosa* | |
|---|---|---|---|---|---|---|
| | | MIC | Interpretation | | MIC | Interpretation |
| 1 | Benzylpencillin | ≥ 0.5 | R | Ticarcilin | ≥ 128 | R |
| 2 | Oxacillin | ≥ 4 | R | Ticarcilin/ Clavulanic acid | ≥ 128 | R |
| 3 | Imipenem | 4 | R | Piperacilin | ≥ 128 | R |
| 4 | Gentamicin | ≤ 0.5 | S | Piperacilin/ Tazobactam | ≥ 128 | R |
| 5 | Ciprofloxacin | 1 | S | Ceftazidime | ≥ 64 | R |
| 6 | Moxifloxacin | o.5 | S | Cefepime | ≥ 64 | R |
| 7 | Linezolid | 4 | S | Imipenem | 2 | S |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | Teicoplanin | 4 | S | Meropenem | 1 | S |
| 9 | Vancomycin | 2 | S | Amikacin | ≥ 2 | S |
| 10 | Tigecycline | 0.25 | S | Gentamicin | ≥ 1 | S |
| 11 | Tetracycline | ≥ 16 | R | Tobramycin | ≥ 1 | S |
| 12 | Fusidic acid | ≤ 0.5 | S | Ciprofloxacin | 0.5 | S |
| 13 | Rifampicin | ≤ 0.5 | S | | | |
| 14 | Trimethoprim/ sulfamethoxazole | 20 | R | | | |

**Cefotaxime susceptibility and MICs**

In present study, antibiotic discs was appliedto estimate the susceptibility of S. *aureus* (Sa1) ,*P. aeruginosa* (P.a1) to Cefotaxime. The results howed that both bacterai were resistant to Cefotaxime with MIC 250 µg/ml and 125 µg/ml against P. aeruginosa (Pa1) and S. aureus (Sa10 in respectively.

**Biofilm formation**

Figure 1 shows capacity of clinical isolates to form biofilm in vitro. The results showed clearly that the significant biofilm formation (P<0.01) of both isolates as compared with control negative (without bacteria). The results approve capacity of both isolates to form biofilm in vitro.

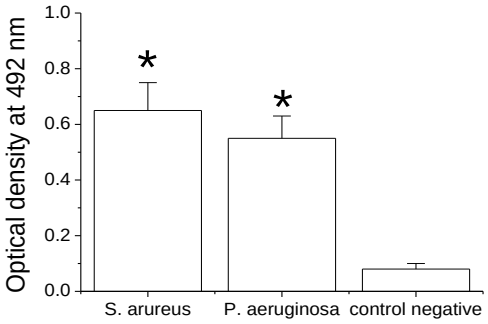


**Figure 1.**Biofilmformation of clinicalisolates of *S. aureus* and *P. aeruginosa*. Negative control represents the wells of microtiter plates without bacterial growth.Asterisksrepresent the significant difference from negative control (P < 0.01).

**Effect of 0.5 x MIC on biofilm formation**

In the current study, a concentration of 0.5 x MIC of cefotaxime was used to demonstrate its effect on thebiofilm of clinical isolates of *staphylococcus* and*Pseudomonas*. Both clinical isolates were pre-treated with 0.5 x MIC of Cefotaxime that corresponding to 125 µg/ml in case of *P. aeruginosa* and 64.5 in case of *S. aureus* before measuring the biofilm formation of both isolates. The results showed that the both concentrations of cefotaximereduced the biofilm formation in both isolates, and the reduction was significant (P<0.05) when comparing the results with the results of control group that representing the biofilm formation of both isolates, butbeforetreated with a 0.5 x MIC of cefotaxime(Figure 2).

Previous studies demonstrated the ability of *S. aureus* and P. aeruginosa to adhere on a number of surfaces, especially medical equipment and devices (10). The ability to these species of bacteria to form biofilmonmedicaldevices is one of the most importantchallenges facing doctors, as the

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presence of the biofilm of these types of bacteria on the surfaces of a number of medical equipment and devices leads to many medical problems, including the production of toxins in addition to the blockage of some tubes of some medical devices, and this is considered one of the threats to the lives of patients (7,2).

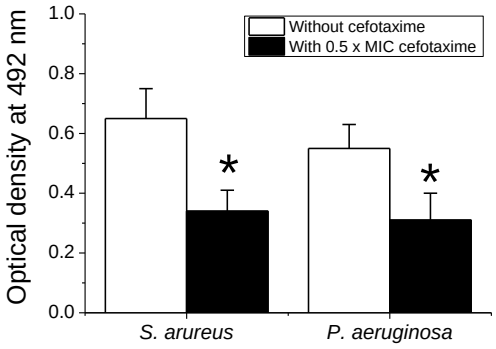


**Figure 2.** Biofilm formation of clinical isolates post treate with0.5 x MIC of cefotaxime that represent to 125 µg/ml in case of *P. aeruginosa* and 64.5 in case of *S. aureus.* The control represents biofilm formation of both clinical isolates but not treated with antibiotic. Asterisks represent significant deference from control group (P<0.05).

In addition, the formation of the biofilm will provide the bacteria with great resistance to antibiotics, making them very difficult to get rid of (4,3).In this study, the ability of *S. aureus* and P. *aeruginosa* that isolated from wound infection to form a biofilm in vitro was estimated. Thestandard method of microtiter plate was used for that. It was found that both bacterial isolates have a highcapacityto form biofilm on polystyrene microtiter plate. Thestudy also showed that the use of a sub-inhibitory concentration of cefotaximewas contributed in a qualitative reducing biofilm formation of both isolates. The formation of the biofilm for any bacteria requires passing through several steps, the most important of which is the initial attachment of the bacteria to surfaces (5, 2). Bacterial adhesion and biofilm is the most important steps necessary for causing bacterial infection (15, 1), and if this phenomenon is reduced, it will have a positive effect by reducing the ability of bacteria to causing infection. The interesting thing is that the current study showed that the use of a non-lethal dose of the cefotaxime (0.5 x MIC) had greatly contributed to reducing the ability of two of the most important bacterial isolates that cause a large percentage of infected wounds to form the biofilm, and this makes us suggest that it is possible to use a dose of antibiotics are less than lethal doses of bacteria in preventing and/or limitation of bacterial infection.

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