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Effects of antibiotics on Staphylococcus aureus biofilm formation associated with Peri-implantitis

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Abstract

BACKGROUND: One hundred and twenty specimens were collected from patients with Peri-implantitis in the period from August 2022 until September 2022 from Baquba specialized dental center, in the province of Diyala. The goal of this work was to estimate the frequency of *Staphylococcus aureus* and their ability to produce biofilms associated with Peri-implantitis. Also, we aimed to assess the anti-biofilm property of Ciprofloxacin and Amoxicillin/clavulanic acid on *Staphylococcus aureus*. The result showed growth of *Staphylococcus aureus* in twenty isolates (16.6%), and 95% of the *Staphylococcus aureus* isolates had the ability to produce biofilms. The results indicate that the minimum inhibitory concentration (MIC) of Ciprofloxacin for *Staphylococcus aureus* was 32 to 512 µg/ml with the sub-MIC 16 to 256 µg/ml. The MIC for Amoxicillin/clavulanic was 8 to 512 µg/ml, and the sub-MIC 4 to 256) µg/ml. Ciprofloxacin and Amoxicillin/clavulanic acid had the ability to decrease biofilm formation and Amoxicillin/clavulanic acid was more effective than Ciprofloxacin.

KEYWORDS: *Staphylococcus aureus*; Biofilm; Peri-implantitis; Minimum Inhibitory Concentration

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Introduction

Despite the fact that dental implants have a high success rate and are a safe and predictable therapeutic option, they are not exempt from biological and iatrogenic health problems caused by inadequate treatment planning, surgical and prosthetic execution, or element rejection, as well as maintenance issues [1]. Peri-implant infections are classified as either peri-implant mucositis, which occurs when the initiated inflammation is limited to the peri-implant soft tissues, or Peri-implantitis, which occurs when the inflammation extends to the underlying bone and causes osteolysis [2]. There has been speculation that the biological side effects of peri-implant mucositis and Peri-implantitis, which can cause soft- and hard-tissue abnormalities, may be important for further marginal bone loss [3]. Poly-microbial biofilms developing on the surfaces of the implanted area are thought the primary cause of the inflammatory illness conditions peri-implant mucositis and Peri-implantitis [2]. Peri-implant mucositis and Peri-implantitis impact more than 22 percent and more than 40 percent of implants, respectively [4]. Peri-implant mucositis and Peri-implantitis

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are both "biofilm-associated pathological conditions" that affect the oral cavity. Peri-implant mucositis is characterized by inflammation in the mucosa around dental implants, while Peri-implantitis refers to its progression and subsequent progressive loss of supporting bone, and as a result, there is substantial evidence that the primary etiological cause for infections linked to dental implants is biofilm [2]. Poly-microbial biofilm development on titanium surfaces is thought to be the primary contributor to the inflammatory reactions in the tissues around implant devices that frequently result in tooth loss [5]. Because of its superior physical-chemical qualities and great bio-compatibility with host tissues, titanium has been chosen as the primary biomaterial for orthopedic and dental implant devices, promoting predictable long-term therapy [6]. But once exposed, implants are equally vulnerable to microbial adherence and biofilm development [7]. that leads to inflammation in the surrounding tissues [8]. Although there is a human inflammatory response intended to stimulate immune responses and inhibit microbial development, this mechanism can also produce a biofilm that is highly infectious and drug-tolerant [9].

Aim of the study

The existing study's objective is to look at *Staphylococcus aureus* biofilm formation around titanium dental implant in patients with Peri-implantitis, and to test approaches to prevent poly-microbial illness and lower microbial burdens on implantable devices.

Material and Methods

Specimens' collection

This cross-sectional study included specimens from 120 patients. Disposable sterile swabs were used for this purpose, and specimens were collected by taking a swab of the inflamed areas surrounding the implant. The specimens were collected from Baquba specialized dental center in province of Diyala during the time span between August 2022 until September 2022. Gram stain, catalase checking, and coagulase verification and biochemical tests were used to diagnose the clinical specimens after all specimens were cultivated on blood agar and mannitol salt agar.

Biofilm formation

We used the microtiter plate test to look for biofilm development [10]. The *Staphylococcus aureus* isolates were cultured on a nutrient broth at 37°C for 24 h. Then, two hundred microliter of *Staphylococcus aureus* from the broth had been suspended in triplicates in the micro-titer plate and incubated for 24 hours at 37°C. After three times of using distilled water to clean each well, the plate was shaken violently until it was entirely dehydrated. Two hundred µl of 100% methanol were added to fix the adhering *Staphylococcus aureus* cells. Subsequently, for 15 minutes, 200 µl of 0.5% crystal violet was used to stain each well. Based on Tang *et al*[11]. the biofilm density was calculated by measuring the OD 630 nm using an ELISA reader after removing the excessive amount of the stain by using 95% ethanol in each well.

Determination of minimum inhibitory concentration (MIC)

MIC was estimated for *Staphylococcus aureus* isolates exposed to Ciprofloxacin (CIP) and Amoxicillin/clavulanic acid (AUG) by the serial dilution method in Mueller-Hinton broth. Serial dilution of antibiotics between 2 to1024 µg/ml. Bacterial suspensions with turbidity equivalent to 0.5 McFarland was added to the tubes contained a different concentration of antibiotics. After incubation at 37°C for 18-24 hours, MIC was determined as the lowest concentration of the antibiotic that inhibits bacterial growth. Sub-minimum inhibitory concentrations (sub-MICs) represent the lowest inhibitory concentration at which bacteria can grow [12].

Antibiotics effect on the biofilm formation

Each antibiotic was tested at sub-MICs to study the change in the ability of *Staphylococcus aureus* isolates in biofilm formation. Microtiter plates were produced and then kept at 37°C for 24 hours. Control plates were prepared in a free antibiotic-microtiter plate which

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was dispensed to the wells with 200 µL of nutrient broth without antibiotics [13]. The absorbency was measured in an ELISA reader at 630 nm, according to a published protocol [11]. The absorbance of the wells was compared with the control wells, as follows: If O.D. ≤ O.D.c (Regarded as a non-biofilm creator); if O.D.c ≤ O.D. ≤ 2* O.D.c (Regarded as moderately biofilm creator); if 2* O.D.c ≤ O.D. (Regarded as strongly biofilm creator). O.D. (Display the investigated samples); O.D.c (Display control wells).

Results and Discussion

The present results displayed that *Staphylococcus aureus'* growth was positive inn twenty isolates (16.6%) from the total of 120 specimens. This result is similar to [14], who found that the percentage of *Staphylococcus aureus* that caused Peri-implantitis was 18.75% based on culture dependent methods. For the phenotypic characterization of *Staphylococcus aureus* on blood agar, Gram stain, biochemical test, and mannitol salt agar were used, followed by confirmation on a VITEK 2 system (Figure 1).

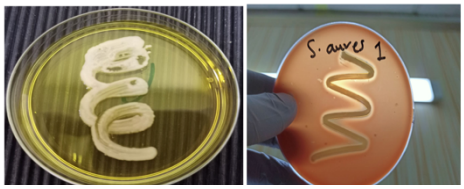

Figure 1. *Staphylococcus aureus on blood agar and Mannitol salt agar.*

The occurrence of *Staphylococcus aureus* an opportunistic pathogen in the initial phase of Peri-implantitis in patients has additionally been validated by Mombelli and Décaillet [7]. Moreover, Canullo et al. [15] indicated that if the mounting edges are not cleansed prior screwing, *Staphylococcus aureus* could be found on both the external and internal surfaces. Peri-implantitis will affect around one-third of patients overall and one-fifth of all implants [16].

The MIC and Sub-MIC were determined for two antibiotics, Ciprofloxacin and Amoxicillin-clavulanic acid based on the Clinical and Laboratory Standards Institute (CLSI-2020) guidelines breakpoint. MIC values were estimated for each isolate by selecting the lowest concentrations in which no growth by the serial dilution method on Mueller-Hinton broth while the sub MIC values were determined by selecting the lowest inhibitory concentration at which the bacteria could grow, the findings of the existing study indicated that the MIC of Ciprofloxacin for *Staphylococcus aureus* was 32 to 512 µg/ml and the sub-MIC 16 to 256 µg/ml while the MIC for Amoxicillin-clavulanic was 8 to 512) µg/ml and the sub-MIC 4 to 256) µg/ml as mentioned in Tables 1 and 2.

The outcomes of the present study showed that 19 out of 20 isolates (95%) had the capability to create biofilm, 18 isolates were strong creators of biofilm and one isolate was a moderate biofilm producer as showed in Table 3.

The present results revealed that Ciprofloxacin decreased the density of biofilm formation in 14 isolates after incubation for 24 hours, and only six isolates were not affected. Amoxicillin/clavulanic acid decreased the density of biofilm formation in all the twenty isolates of *Staphylococcus aureus*. The experiment on the antibiotic free samples showed that the *Staphylococcus aureus* has the ability to form biofilm (p=0.001).

Generally, antibiotics reduce biofilm formation. However, several studies have shown that antibiotics can significantly induce biofilm formation depending on the antibiotic class and the bacterial strain [17]. Peri-implant illnesses have appeared as a side effect of antibiotic therapy [2].

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Table 1. MIC and sub-MIC of Ciprofloxacin for *Staphylococcus aureus.*

No.	MIC	Sub-MIC	No.	MIC	Sub-MIC
1	512	256		256	128
2	256	128		64	32
3	256	128		128	64
4	64	32		512	256
5	512	256		32	16
6	128	64		512	128
7	128	64		128	64
8	64	32		64	32
9	256	128		512	256
10	32	16		128	64

Table 2. MIC and sub-MIC of Amoxicillin/clavulanic for *Staphylococcus aureus.*

No.	MIC	Sub-MIC	No.	MIC	Sub-MIC
1	256	512	11	256	128
2	512	128	12	32	16
3	64	32	13	128	64
4	512	128	14	512	128
5	64	32	15	64	32
6	32	16	16	128	64
7	8	4	17	32	16
8	128	64	18	128	64
9	256	128	19	512	128
10	16	8	20	64	32

Table 3. Biofilm formation before and after treatment.

No	Absorbance before treatment	Absorbance after treatment (CIP)	Absorbance after treatment (AUG)
1	0.173 (S)	0.140 (S)	0.034 (N)
2	0.142 (S)	0.102 (M)	0.042 (N)
3	0.157 (S)	0.144 (S)	0.051 (N)
4	0.121 (S)	0.103 (M)	0.044 (N)
5	0.159 (S)	0.117 (M)	0.040 (N)
6	0.052 (N)	0.051 (N)	0.046 (N)
7	0.219 (S)	0.162 (S)	0.051 (N)
8	0.112 (M)	0.109 (M)	0.044 (N)
9	0.186 (S)	0.145 (S)	0.041 (N)
10	0.190 (S)	0.154 (S)	0.045 (N)
11	0.184 (S)	0.161 (S)	0.048 (N)
12	0.197 (S)	0.170 (S)	0.044 (N)
13	0.152 (S)	0.112 (M)	0.041 (N)
14	0.169 (S)	0.120 (M)	0.047 (N)

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15	0.231 (S)	0.229 (S)	0.045 (N)
16	0.147 (S)	0.118 (M)	0.050 (N)
17	0.157 (S)	0.157 (S)	0.043 (N)
18	0.222 (S)	0.219 (S)	0.051 (N)
19	0.215 (S)	0.164 (S)	0.049 (N)
20	0.097 (M)	0.095 (M)	0.053 (N)

OD of the control 0.060, S (Strong biofilm producer), M (Moderate biofilm producer), N (Non-biofilm producer).

Conclusion

Staphylococcus aureus have a robust ability to produce biofilm on titanium which can affect the integrity of a dental implant. Amoxicillin/clavulanic acid has decreased the density of biofilm formation by *Staphylococcus aureus* and at a lower rate for Ciprofloxacin.

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