RESEARCH ARTICLE

Comparison the Pathogenicity, Biofilm and Adhesion Activity of *Acinetobacter baumannii* Isolated from Patients using Thermoplastic Retainer with other Oral Isolates

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ABSTRACT

The study was focused on investigating the pathogenicity of *Acinetobacter baumannii* comparison with certain oral bacteria, *Streptococcus mutans, Lactobacillus acidophilus, Streptococcus sangius,* and *Enterococcus faecalis* associated with patients using a thermoplastic retainer by determination the response to Chlorhexidne (CHX), strength of biofilm formation and the adhesion ability. The results revealed that the isolation of bacteria reported by *S. mutans* was 95%, *L. acidophilu*s 85%, *S. sangius* 75%, while *A. baumannii* and *E. faecalis* 65%. Moreover, the biofilm assay revealed various strengths of biofilm formation. The strong biofilm producer was *A. baumannii*, 0. 372 ± 009, the moderate biofilm producer was *S. mutans*, 0. 320 ± 012, the weak biofilm producers, *L. Acidophilus* 0. 195 ± 0. 10 *S. sanguis* 0. 170 ± 0. 00, and *E. feacalis* 0. 154 ± 0. 23 with significant differences between isolates at ($p \leq 0.05$). Furthermore, this current study found CHX was less effective against performed biofilm with various values, but the highest value was noticed in *A. baumannii* at 0. 125 ± 0. 03 comparison with other isolates, with significant differences at ($p \leq 0.05$). *L. Acidophilus* 0. 12 ± 0. 4, *S. mutans* 0. 102 ± 0. 12 *S. sanguis* 0. 113 ± 0. 5 and *E. feacalis* 0. 1 ± 00. Finally, The adhesion ability of isolates to the thermoplastic retainer during 30, 45, 60 minutes *A. baumannii* recorded the highest value during 30,45, and 60 minutes (220 ± 0. 09, 289 ± 0. 98 0. 87 ± 1. 1), respectively. Depending on the obtained results, *A. baumannii* played an important role in oral cavity infection especially in patients using thermoplastic retainers due to their resistance to CHX and their ability to form biofilm and adhesion to materials that are used to make thermoplastic retainers.

Keywords: *A. baumannii*, Adhesion ability, Biofilm formation, Oral cavity, Pathogenicity, Thermoplastic retainer.

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INTRODUCTION

The oral cavity is the major doorway to the human body and is a complex and dynamic habitat. [1,2] Several studies have discovered approximately 1000 species from the oral cavity microbes. However, only a small fraction of them are responsible for oral illnesses such as dental caries and periodontitis. [3,4] Gram-negative enteric rods (GNRs), enterococci, and staphylococci are among the clinically relevant pathogens whose proliferation is aided by an imbalance of microbial flora. [5,6]

Oral microbiota disruptions under certain conditions might promote the growth of non-oral infections that are difficult to eliminate because of their higher antibiotic resistance, increasing the risk of treatment failure and reinfection. The presence of these bacteria in the oral cavity has been linked to various dental disorders such as periodontitis, caries, and gingivitis, as well as systemic diseases like cystic fibrosis, human immunodeficiency virus (HIV), and rheumatoid arthritis that are important in clinical medicine[7]

Fixed orthodontic appliances are a popular and effective method for treating malocclusion. However, they can have side effects like changing your microbiota and causing infections. Orthodontic appliances have a sophisticated undercut form

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that makes tooth cleaning more difficult and time-consuming. Previous research has linked the implantation of fixed orthodontic appliances with an increase in *S. mutans* bacterial levels, which is a key risk factor for tooth caries. [8]

A. baumannii, a bacterium that is rarely present in the mouth, tends to cause opportunistic infections. Although there are few reports on *A. baumannii* as a dental pathogen, the organism›s proclivity to create drug-resistant armor highlights the need for greater research into this pathogen and its role in oral infections. [9] Subgingival colonization by *A. baumannii* raises the hazard of refractory periodontitis. Adhesion, biofilm development, and iron uptake are all part of the organism›s pathogenesis. In addition, Pili, the outer membrane protein OmpA, phospholipases, and extracellular polysaccharides have also been identified as virulence factors. [10] The main aim of the study is to investigate the relationship of *A. baumannii's* pathogenicity to fixed orthodontic appliances in comparison with other bacteria isolated from patients wearing fixed orthodontic appliances.

MATERIALS AND METHODS

Collection of the Samples

A total of 20 buccal and palatal surfaces of the teeth were taken from individuals wearing a thermoplastic retainer and suspended in 1-mL phosphate buffer saline (PBS) buffer before being transported to the veterinary medicine college/ microbiology and immunology laboratory.

Isolation and Identification of Bacteria

About 100 µL of samples were inoculated into the nutrient broth for 24 hours at 37°C. The isolates were recognized using morphological and cultural properties of the colonies as well as biochemical tests,[11] lastly confirmed by the Vitek 2 compact.

CHX Susceptibility Test

This test was done by agar diffusion method, the isolates adjusted to 1. 5 10*8 CFU/mL and grown on Mullar Hinton agar using 6 mm diameter wells created with a Pasteur pipette under aseptic circumstances. Each well was filled with 0. 1 mL of Chlorhexidine Gluconate 2% w/v. [12]

Estimation of the Minimum Inhibitory Concentration (MIC) of CHX.

After adjusting the inoculums to 1. 5 10*8 CFU/mL according to 0. 5 McFarland standard, 1-mL was added to tubes having 1-mL of the selected CHX concentrations, after incubation period at 37°C for 24 hours, the amount of growth in tubes containing CHX was compared to growth-control tubes (no CHX) as a control. [13]

Screening of biofilm production in isolates

The procedure described by,[14] isolates from fresh agar plates were inoculated in tryptic soy broth and incubated for 18 hours at 37°C in stationary condition and diluted 1:100 with fresh tryptic soy broth. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates wells were filled with 0. 2 mL aliquots of the diluted cultures and only broth (without bacteria) served as control (blank) to check sterility and non-specific binding of media. The biofilm production strength was calculated as following:

OD ≤ ODC = Non-biofilm-former (NBF)
ODC < OD ≤ 2x ODC = Weak biofilm-former (WBF)
2 XC < OD ≤ 4 XC = Moderate biofilm-former (MBF)
OD > 4X ODC = Strong biofilm-former.
OD = Optical density
ODC = Optical density of control

Effect of MIC of CHX on Biofilm Formation

Biofilm formation was evaluated and modified as previously described15 MIC of CHX, prepared in 200 µL of fresh tryptic soy broth then added to wells of 96-flat bottom plates containing 20 µL of diluted (overnight culture of bacteria. After incubation at 37°C for 24 hours Furthermore, wells lacking bacteria in the same media served as blank control.

OD ≤ ODC = Non-biofilm-former (NBF)
ODC < OD ≤ 2x ODC = Weak biofilm-former (WBF)
2 XC < OD ≤ 4 XC = Moderate biofilm-former (MBF)
OD > 4X ODC = Strong biofilm-former.
OD =Optical density
ODC = Optical density of control

Effect of MIC of CHX on Preformed Biofilm

Isolates in tryptic soy broth medium added (100 µL) into the wells (96-well microtiterplate), after incubation period at 37°C for 24 hours to form a biofilm, the medium was removed gently, then the formed biofilm was washed approximately three times with PBS to remove the non-adherent cells, at that time 200 µL of MIC of CHX was added and then incubate the plate at 37°C for 24 hours, this test was renewed in absence of MIC of CHX as a control. [15] OD ≤ ODC = Non-biofilm-former (NBF)
ODC < OD ≤ 2x ODC = Weak biofilm-former (WBF)
2 XC < OD ≤ 4 XC = Moderate biofilm-former (MBF)
OD > 4X ODC = Strong biofilm-former.
OD =Optical Density
ODC = Optical Density of control

Estimation of Isolates Adhesion to Thermoplastic Retainer Surfaces

This assay has undergone some changes. In this test, a part of a thermoplastic retainer was cut into pieces (1-cm^2) and tubes filled with a 5 mL suspension of tested bacteria and then incubated the mixture for 1-hour at 37°C. Thermoplastic retainer sections were washed three times in PBS after the incubation period and then placed in 10 mL fresh PBS and sonicated for 5 min at 40 kHz to remove the adherent cells. The sonicated PBS was diluted to 1x10*^{3} serially by disseminating it (1-mL) on TSB medium through the viable colony count technique, which determines the number of adherent bacteria that indicate the degree of adhesion. [16]

Statistical Analysis

The Statistical Analysis achieved by (ANOVA), (LSD) test probability ≤0. 05.

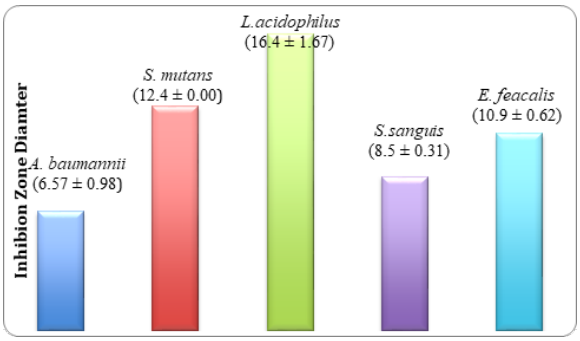

Figure 1: The CHX susceptibility

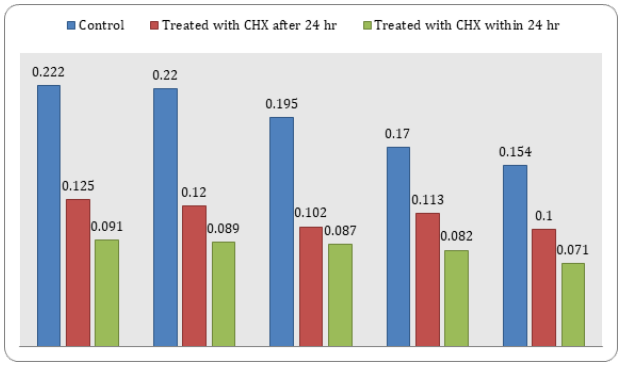

Figure 2: Biofilm assay of selected isolated treated with different periods with CHX.

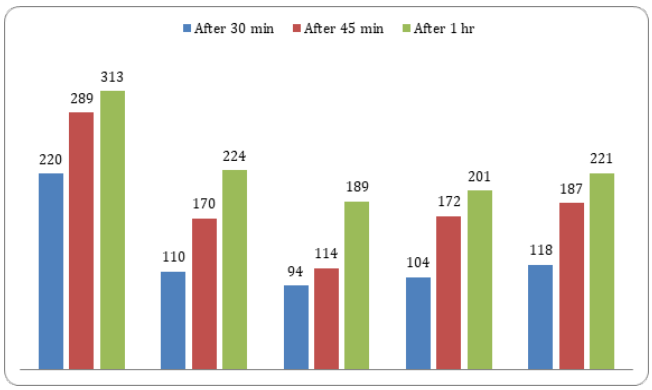

Figure 3: Adhesion assay of selected isolates on the thermoplastic retainer segments during different period.

RESULTS

The isolation of bacteria showed 20 patients with dental caries carried *S. mutans* 95%, *L. acidophilus* 85%, *S. sangius* 75%, while *A. baumannii* and *E. feacalis* were 65%. The results of susceptibility to CHX based on inhibition zone diameter (mm) revealed that *A. baumannii* had the lowest susceptibility (6. 57 ± 0. 98), while *L. acidophilus* had the highest (16. 4 ± 0. 00), followed by *S. mutans* (16. 4 ± 1. 67), *E. feacalis* (10. 9 ± 0. 62), and *S. sanguis* (8. 5 ± 0. 31) (Figure 1).

Furthermore, the MIC of CHX for isolates' results recorded various values between isolates, as shown in Table 1.

However, the biofilm assay revealed various strengths of biofilm formation. The strong biofilm producer was *A. baumannii,* 0. 372 ± 009, the moderate biofilm producer was *S. mutans,* 0. 320 ± 012, the weak biofilm producers, *L. Acidophilus* 0. 195 ± 0. 10 *S. sanguis* 0. 170 ± 0. 00, and *E. feacalis* 0. 154 ± 0. 23 with significant differences between isolates at ($p \leq 0.05$). Furthermore, this current study found CHX was less effective against performed biofilm with various values, but the highest value was noticed in *A. baumannii* at 0. 125 ± 0. 03 comparison with other isolates, with significant differences at ($p \leq 0.05$). *L. Acidophilus* 0. 12 ± 0. 4, *S. mutans* 0. 102 ± 0. 12, *S. sanguis* 0. 113 ± 0. 5 and *E. feacalis* 0. 1 ± 00 (Figure 2).

Table 1: The minimum inhibitory concentration of CHX

Isolates	*MIC of CHX (µg/mL)*
A. baumannii	≤ 5
L. Acidophilus	≤ 3. 5
S. mutans	≤ 4. 5
S. sangius	4
E. feacalis	≤ 4

On the other hand, CHX was more effective against the biofilm formation within 24 hours *A. baumannii* 0. 091 ± 0. 11, *L. Acidophilus* 0. 089 ± 0. 54, *S. mutans* 0. 087 ± 0. 87, *S. sanguis* 0. 082 ± 0. 63, *E. feacalis* 0. 071 ± 0. 01.

The adhesion ability of isolates to the thermoplastic retainer during 30, 45, 60 minutes *A. baumannii* recorded the highest value during 30, 45, and 60 minutes (220 ± 0. 09, 289 ± 0. 98 0. 87 ± 1. 1) respectively, followed by *L. acidophilus* (224 ± 0. 12, 170 ± 0. 00, 110 ± 0. 08) respectively, *S. mutans* (94 ± 0. 45, 114 ± 0. 14, 189 ± 0. 34) respectively, *S. sanguis* (104 ± 0. 76, 172 ± 0. 14,201 ± 0. 01) respectively, *E. feacalis* (118 ± 0. 38, 187 ± 0. 22, 221 ± 0. 02) respectively but there was no significant differences at ($p \leq 0.05$) between isolates (Figure 3).

DISCUSSION

A. baumannii is one of the possible nosocomial pathogens that are related to additional pathogens into the oral cavity in dental care and periodontitis, Because oral bacteria are heterogeneous and complicated, the main cause of problems with *A. baumannii* is the presence of resistance genes. As a result, *A. baumannii* poses a substantial risk of infection in the oral cavity. The result showed the isolation of *A. baumannii* bacteria from 20 patients where it was 65%, while *S.sangius* was 75%, *L. acidophilus* was 85% and *S. mutans* was 95%. *E. feacalis* was 65% too. This result agreed.[17] The sources of contamination in commercial laboratories (dental laboratories) are pathogenic bacteria such as Pseudomonas spp, Acinetobacter sp, Micrococcus spp, Moraxella spp,

Souto et al.[18] detected the genes of bacteria found in the oral cavity such as *Acinetobacter spp.* and *P. aeruginosa* and revealed the oral cavity is considered a reservoir for those bacteria as well as connected with periodontal infections. However, *Acinetobacter spp* was discovered in patients who had been wearing a removable orthodontic device for 2 to 4 months 19, also it has been reported as the most common respiratory pathogen linked to nosocomial infections.[20]

Acinetobacter bacteria can be transmitted through contaminated food, water, person-to-person contact, and medical supplies.[21]

CHX's antibacterial activity is dependent on the release of cytoplasmic materials caused by the bacterial cytoplasmic membrane being damaged.[22] However, resistance to CHX is ascribed to alterations in the cell membrane of bacteria.[23] On the other hand, as seen in *A.baumannii* isolates, long-term use of antibacterial drugs promotes the emergence of new strains of bacteria with high resistance characteristics.[24] Also, antibiotic-resistant bacteria are more capable of forming biofilms when exposed to specific antibiotics.[25] Furthermore, the antibiotics might boost induced gene regulation and/or fitness benefits for resistant strains, culminating in biofilm development.[26]

A. baumannii was identified as a significant biofilm producer in our study. *A. baumannii* produces biofilms on six typical hospital materials: glass, porcelain, stainless steel, rubber, polycarbonate plastic, and polypropylene plastic. It was also reported that *A. baumannii* forms biofilms on polycarbonate, followed by stainless steel.[26] In any case, biofilm development is crucial in A.baumannii's interaction with the host, and it aids in the majority of medical-device-associated illnesses.[27] The processes of biofilm development in *A. baumannii* depend on a variety of microbiological and physicochemical variables such as adhesins, surface appendages, capsular polysaccharides, virulence, and resistance factors, all of which aid in the creation and maintenance of *A. baumannii* biofilms. Biofilm production in *A. baumannii* is linked to the blaPER-1gene.[28] The variations in surface roughness and porosity, ionic charge, and hydrophobicity may all contribute to variances in biofilm development across different material types.[29]

CHX has lower effectiveness. This was due to the biofilm previously developed by isolates. The function of bacterial biofilm is usually to avoid drugs, ingestion by phagocytosis, and other antimicrobial agents[30], antibiotic activity of CHX showed more effectiveness against biofilm formation over 24 hours this is due to the antibacterial action against free bacterial isolates and no biofilm formed.[31] The ability of isolates to adhere to the thermoplastic retainer was measured across periods of 30, 45, and 60 minutes. The greatest levels of Acinetobacter were found at 30, 45, and 60 minutes (220±0.09, 289 ±0.98, 0.87±1.1) respectively.

CONCLUSION

Based on the results of this study, it was found that *A. baumannii* plays an important role in oral infections due to their ability to resist antibiotics, their ability to form a biofilm, and their ability to stick to materials that are used to treat dental problems, particularly in patients using thermoplastic retainer comparison with other normal oral cavity isolates.

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