Identification Biofilm Producers *Staphylococcus aureus* Isolates and Detect their Biofilm Genes from Gingivitis Cases

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Abstract_Gingivitis is one of the most common oral disorders and is caused by the accumulation of plaque in people with poor oral hygiene. The purpose of this study is to detect *Staphylococcus aureus* biofilm producers in cases of gingivitis and detect their biofilm genes. During the months of November 2018 and February 2019, a sample of fifty patients diagnosed clinically with gingivitis were collected from the laboratory of the Faculty of Dentistry at Hawler Medical University. Using the traditional culture method and vitek 2, eleven *S. aureus* bacteria isolates were identified. Using the Congo red agar method, biofilm detection was performed to test the ability to form biofilm. The *S. aureus* isolates were put through a series of tests to see if the genes responsible for the biofilm could be found (PCR). Biofilm formation method of detection using Congo red agar showed that 10 (90.1%) of *S. aureus* were biofilm positive and one 1 (9.1%) were biofilm negative. As well as the results of molecular analysis by using PCR showed that isolated *S. aureus* were carried biofilm genes *icaC* (100%), *icaD* (100%), *cna* (90.9%) and *fnba* (100%). Gingivitis is one of the most common oral diseases in our area, and this research found that the majority of *S. aureus* isolates carry the *icaC, icaD, cna, and fnba* Biofilm genes.

I. INTRODUCTION

In the oral cavity, a complex and unique bacterial flora develops. In the oral cavity, a variety of anatomical surfaces, physical, and chemical factors promote the growth of over 300 grams of Gram-positive and Gram-negative bacteria. There are few obligate anaerobes and aerobic bacteria among these facultative anaerobes [1]. Biofilms are commonly formed by these bacteria. Plaque forms on the tooth surface when the biofilm is not washed for days [2].

Plaque bacteria have been linked to oral diseases like tooth decay, gingival inflammation, and periodontitis. Infectious disease caused by bacteria that is the most common in humans [3]. Surprisingly, oral bacteria play a role in serious systemic diseases like heart disease, pneumonia, and preterm low birth weight babies and osteomyelitis in children [4].

Antibiotics were first used in clinical practice in the 1940s. Since then, they've been essential in the treatment of bacterial infections and the prevention of infections in vulnerable patients (prophylactic action). Antibiotic resistance is proportional to the amount of antibiotics used; the more antibiotics used, the more likely resistant bacterial populations will emerge [5]. The US Centers for Disease Control and Prevention (CDC) reported an increase in morbidity and mortality due to antibiotic resistance in 2013, with more than 2 million infections and nearly 23,000 deaths each year [6]. Unfortunately, the number of antibiotic-resistant bacterial strains capable of causing infections is on the rise, with the majority of them resistant to multiple antibiotics, a condition known as multi-drug resistance (MDR). Multi-resistant strains are becoming a more serious threat to current antimicrobial therapy [7].

To identify the agents involved in the development of periodontal diseases, a bacteriological analysis of dental plaque is required. Furthermore, understanding plaque bacteriology and antibiotic resistance patterns is important in guiding antibiotic selection and appropriate therapy, which will aid health care professionals in managing local and/or systemic infections caused by plaque bacteria and avoiding complications [8]. Periodontal disease is an infectious chronic inflammatory condition that affects the tooth's protective and/or supporting periodontal tissues. The interactions between bacteria and the host, as with other infections, determine the nature of the resulting disease [9].

Some bacterial species have been linked to periodontal disease, but the evidence for this is still lacking [10]. Staphylococci, particularly *Staphylococcus epidermidis* and *S. aureus*, are among the microorganisms linked to these diseases. These species are commonly found in the skin and mucous membranes of humans [11]. They are also isolated from oral environment habitats and cases of prosthetic valve endocarditis. These microorganisms, on the other hand, are not considered resident oral bacteria, but rather transient organisms [12].

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Atypical subgingival periodontal pathogens such as Enterococcus faecalis, *S. aureus*, *S. epidermidis*, and other staphylococci may be found in high numbers in some periodontitis patients. *Streptococcus constellatus*, *Streptococcus intermedius*, and *Streptococcus anginosus*, as well as *Parvimonas micra*, *Eubacteria nodatum*, and other Eubacterium species, and each of the three anginosus streptococci group species, *S. constellatus* and *S. intermedius* [13]. In particular, periodontal pockets and gingival sulcus provide an environment for nonspecific bacterial attachment and retention of microorganisms near the host's bloodstream. Therefore, staphylococcal colonization is promoted at these sites, and these bacteria may be involved in periodontal disease [14].

S. aureus is a major cause of serious human illness. *S. aureus* is associated with alveolar infections and oral mucosal lesions in the oral cavity, and colonization of the tongue, saliva, mucosal surface, supragingival surface, and periodontal pocket has been demonstrated [15]. Despite the fact that *S. aureus* was previously thought to be a transient member of the oral microbial community, evidence from some culture studies is that *S. aureus* is healthy in children and adults, especially. It suggests that saliva, a common isolate of supraclavicular plaque from the oral cavity. And on the tongue [16].

II. Methodology

During the period 5 November 2018 to 27 February 2019, dental plaque samples were collected from 50 patients who were diagnosed clinically with gingivitis and represented both genders female and male at the Department of Periodontics at College of Dentistry/Hawler Medical University. The dentist took samples using a curette, and all of the patients had clinical signs of gingival inflammation. The sample then putted in the transport medium for the examination in the laboratory. All samples were incubated on blood agar and mannitol salt agar for 24 hours at 37 ºC. Bacteria plates of isolated colony were sent to the identification using Vitek 2 Compact system. All the identified samples were sent to laboratory for the antibiotic test.

A. Biofilm detection by congo red Agar

Tested bacteria were inoculated onto the surface of the congo red agar medium and incubated at 37°C for 24hours. Biofilm creators form black colonies on congo red agar medium while non-producers form red colonies [17].

B. Molecular Study

The *S. aureus* isolates were tested for detection of their genes responsible for the biofilm. A typical colony was cultivated in 1 ml TSB for 24 h at 37°C. The bacterial genomic DNA was extracted with a QIAGEN plasmid Minikit (Fermentas, Germany) as recommended by the manufacturer [18]. Biofilm genes determined by previously described specific primers as listed in Table 1 [19-21].

Table 1: S. aureus biofilm genes.

Genes	Nucleotide sequence of primer	Amplicon size (bp)
icaD	ATG GTC AAG CCC AGA CAG AG CGT GTT TTC AAC ATT TAA TGC AA	198
icaC	CTT GGG TAT TTG CAC GCA TT GCA ATA TCA TGC CGA CAC CT	209
fnba	GAT ACA AAC CCA GGT GGT GG TGT GCT TGA CCA TGC TCT TC	191
Cna	CGA TAA CAT CTG GGA ATA AA ATA GTC TCC ACT AGG CAA CG	716

III. Results and discussion

A. Staphylococcus aureus detection and isolation

Among 50 samples ages were ranged from 28 to 57 and gender were as (22male patients) and (28 female patients) from all patients there were 11(22%) isolate of *S. aureus***.** all samples were took from the patients by specialized dentists from the dental plaque by curette detected isolates were done by culturing the bacteria on mannitol salt agar and Gram stain of a smear was done and showed Gram-positive cocci round shaped in clusters under the microscope and for more confirmation the tested isolates were tested by Vitek2 compact system. These results were near to other study done by Jabuk *et al*., (2015) were they detected 15.4% *S. aureus* among their total isolates [22]. More over this study results are differed in comparing from another study done by Abdulaziz (2018) were the results of the study show that 7.4% of their isolates was *S. aureus* [3].

Table 2: S. aureus isolates

Isolated bacteria	N. of Patents	N. of Bacteria isolates	%
S. aureus	50	11	22

B. Biofilm detection by Congo red agar

In order to detect formation of biofilm activity from *S. aureus* isolates Congo red agar method used and the results showed that out of 11 isolates 10 (90.9%) *S. aureus* were biofilm positive which formed black colony on the agar media that indicates biofilm formation and only 1 (9.1%) isolate were biofilm negative which did not formed black colony.

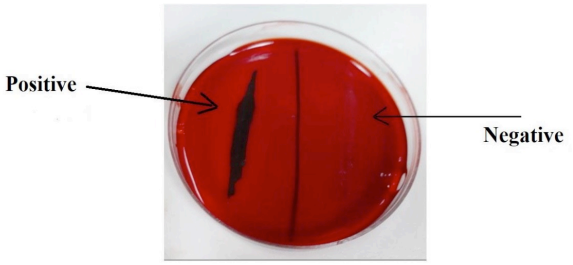

Figure 2: Biofilm test for S. aureus on congo red agar, black colony represent positive result and transparent colony represent negative colony.

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C. Molecular study:

Detection of *icaC*, *icaD*, *cna* and *fnba* biofilm genes

To detection of the Four biofilm gens of the isolated *S. aureus* bacteria the molecular based method was performed through detection of *icaC*, *icaD*, *cna* and *fnba* genes in the extracted DNA for all isolates, using the PCR technique by detection of the *icaC*, *icaD*, *cna* and *fnba* genes which are belong to the biofilm group genes of *S. aureus*. The PCR products for all 11 isolates of *S. aureus* were positive for *icaC* (100%) (209bp), *icaD* (100%) (198bp) and *fnba* (100%) (191bp) genes, but ten (90.9%) (716bp) positive for *cna* gene and these results are shown in table 2 and figure 2, 3, 4 and 5. The present results support those of a previous report, suggesting that *icaC* and *icaD* biofilm genes is ubiquitous in *S. aureus* bacteria [23]. Moreover, another study results are near current study results were they reported that 80.6% of isolated *S. aureus* harbor *icaD* gene [24]. And in another study also agreed with current study results which done by Arciola *et al*., (2005) they recorded that almost all of their isolated *S. aureus* express *fnba* gene [25]. Moreover, another study results are differed from current results were the study results show that 64.7% of the isolated *S. aureus* give positive result for *fnba* and also in the same study reported that 58.8% of the *S. aureus* isolates give positive results for *icaD* [26]. Furthermore in another study also differ with current study results were the study results recorded that 69.3% of the isolated *S.auresus* represent *icaC* gene and also 54.8% of the isolated *S. aureus* gave positive result for *icaD* gene. In addition, another study results by Karki *et al*., (2019) were near to current results the study results show that 77.2% of the isolated *S. aureus* were positive for *icaD* gene [27]. As well as another study reported results close to the current study were their results show that 82.2% of the isolated *S. aureus* had *fnba* gene [28]. Furthermore, a study by Shahmoradi *et al*., (2019) reported that 84% of their isolates were positive for *cna* gene [29]. These results provide evidence that detection of *icaC*, *icaD*, *cna* and *fnba* can be used as a simple and reliable way for identifying *S. aureus* biofilm genes [29].

Table 2: S. aureus biofilm gene detection

No of isolates	Biofilm gene	NO (%) positive	NO (%) negative
11	*icaC*	11(100)	0(0)
11	*icaD*	11(100)	0(0)
11	*fnba*	11(100)	0(0)
11	*Cna*	10(90.9)	1(9.1)

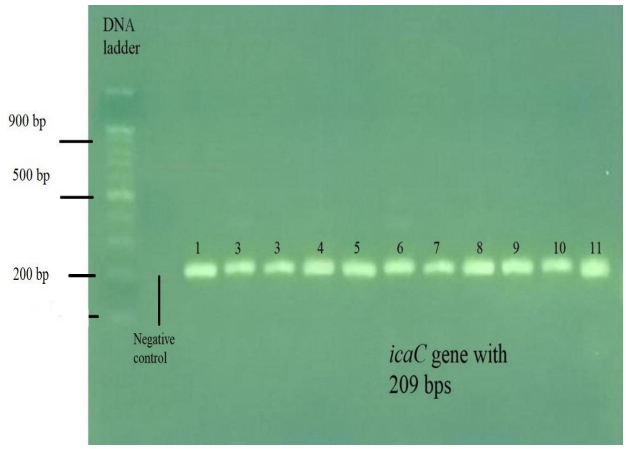

Figure 2: Polymerase chain reaction products on gel electrophoresis for icaC gene. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 Amplified PCR product of icaC gene (209bp) for S. aureus isolates.

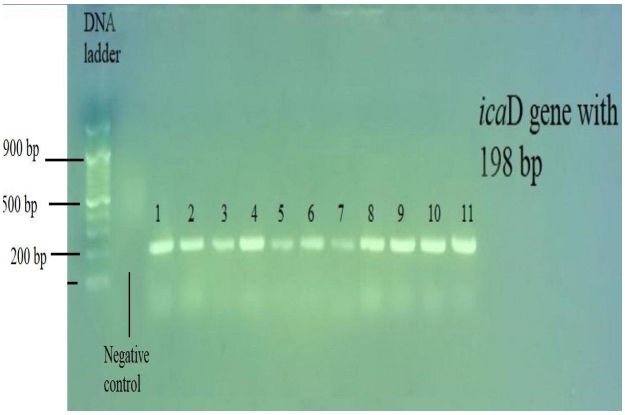

Figure 3: Polymerase chain reaction products on gel electrophoresis for icaD gene. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 Amplified PCR product of icaD gene (198bp) for S. aureus isolates.

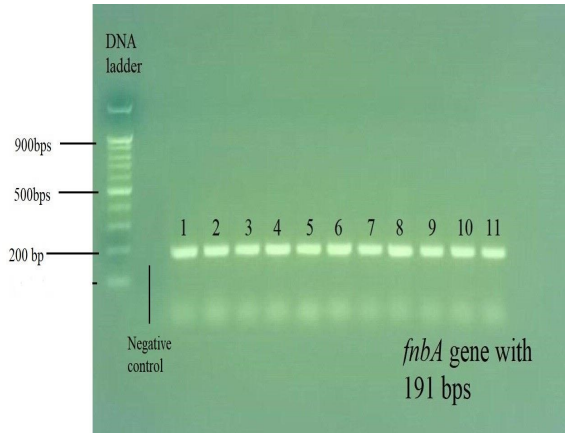

Figure 4: Polymerase chain reaction products on gel electrophoresis for fnba gene. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 Amplified PCR product of fnba gene (191bp) for S. aureus isolates.

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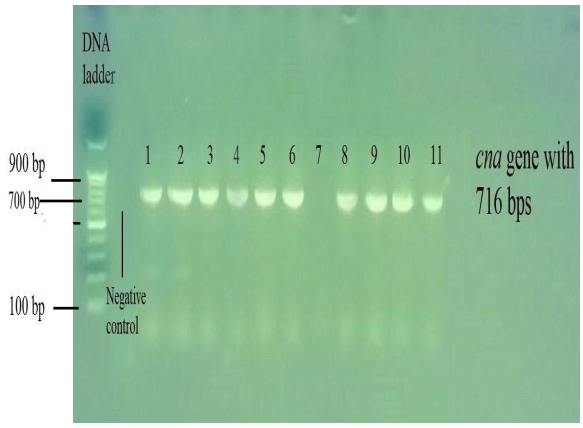

Figure 5: Polymerase chain reaction products on gel electrophoresis for cna gene. Lanes 1, 2, 3, 4, 5, 6, 8, 9, 10 and 11 Amplified PCR product of cna gene (716 bp) for S. aureus isolates. Lanes 7: negative for cna gene.

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