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Effect of Diode Laser on Antibiotic Sensitivity and Biofilm Formation by *Streptococcus* Spp. Isolated from Dental Caries

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ABSTRACT

Streptococci from dental caries were isolated and identified during this study. The samples were collected from different Dental clinics in Baghdad. Cultural, morphological and biochemical test were used to identify all isolates, the results showed that all isolates belong to *Streptococcus* spp

The susceptibility of 10 isolates toward 10 of common antibiotics, was tested, results showed that several isolates of *Streptococcus* spp. Isolated from dental caries were resistant to most of the antibiotics used. The percentages of resistance were 100% of isolates resistant to cephalothin and cefixime, 90% of isolates resistant to ampicillin, naldixic acid and erythromycin, 70% of isolates resistant to bacitracin and tetracycline, while 100% of isolates sensitive to gentamycin, ciprofloxacin and chloramphenicol.

The susceptibility to antibiotics of two isolates after exposure to diode laser was increased toward chloramphenicol, tetracycline and naldixic acid. Two isolates showed increased susceptibility to chloramphenicol after I minute from exposure to diode laser.

Also two isolates showed increased susceptibility to naldixic acid after 3 minute from exposure to diode lases. The increasing susceptibility to tetracycline begin after 10 minute for second isolate, while first isolate showed increased susceptibility to tetracycline after 5 minute from exposure to diode laser. Also the diode laser affected on viability of bacteria that increased with increased time of exposure.

Keywords- Dental caries, *Streptococcus* spp. and Diode laser.

I. INTRODUCTION

A diverse colony of bacteria colonizes the mouth cavity of humans. Several bacteria found in the mouth manifest as complex aggregation known as biofilms on the teeth's surfaces, which are referred to as "dental plaques." Streptococci are the first bacteria to invade the oral cavity, accounting for 70% of all cultured bacteria. The major odontopathogen, Streptococcus mutants, is found in supra-gingival plaque and causes dental caries, a kind of oral illness.

In the human oral cavity, One of the most frequent infectious disorders is dental caries. Acids such as lactic acid, which are formed as a by-product of carbohydrate metabolism by cariogenic bacteria in dental plaque, demineralize the enamel and dentin. Streptococci mutans has been identified as a prominent cariogenic pathogen among oral bacteria. [1]. *S. mutans* is a significant pathogen of oral cavity and initiates dental caries. The rapid growth time, ability to survive low pH, fermentation of a wide range of carbohydrates, and presence of the enzyme glucosyltransferase, which converts sucrose to glucose to improve adherence, are all characteristics that contribute to this organism's carcinogenicity. S. *mutans* is reported to cause systemic infections like intravascular and endocarditis infections [2].

Vaccines for dental caries and periodontal disease have recently been developed and tested on patients. Photodynamic therapy (PDT) is an alternative approach that has shown tremendous promise in the treatment of non-neoplastic and neoplastic disorders [3].

Tooth decay (dental caries) is the result of acids produced by decay-causing bacteria in your mouth attacking the tooth's surface, or enamel. This can result in a cavity, which is a small hole in a tooth. If left untreated, dental decay can lead to discomfort, infection, and even tooth loss [4].

Since the creation of lasers forty years ago, their applications have grown beyond pure physics to chemistry, biology, medicine, technology, and other related fields. [5].

LASER, or 'light Amplification by Stimulated Emission of Radiation,' is a scientific breakthrough from the twentieth century that has been shown to have a wide range of applications due to its unique features not present in typical light sources [6].

The distinctive property of laser light is that its light waves propagate over long distances with low divergence and maintain a stable phase relationship, which may explain why laser light has such a small band-width, is so easy to focus on a given target, and is so powerful. Another noteworthy attribute of lasers is their ability to concentrate their energy into extremely high intensities, which remain nearly constant across

extended distances due to low divergence [7]. Photodynamic inactivation of bacteria has been studied widely by Wilson et al, where low-power laser (He-Ne or diode laser) is used to kill the bacteria in the presence of a suitable photosensitizer (toluidine blue or aluminum disulphonatedphthalocyanin)[8]

II. MATERIAL AND METHODS

2.1 Collection of samples

Twenty samples were collected by swabbing the oral cavity of dental caries patients at the dental clinic in Baghdad during period between October 2019 to December 2019.

2.2 Isolation & Identification of bacteria

Before streaking on the surface of the selective media, all samples were obtained with sterilized cotton swabs. Mitis salivarius agar was then incubated in a candle jar for 24 hours at 37°C under anaerobic conditions and screened using biochemical and microscopic methods.

2.3 Detecting bacteria's ability to produce biofilms (test tube method)[9]

The ability of Streptococcus spp. isolates to form biofilms was determined by inoculating 5ml of TSB with these isolates and incubating for 48 hours at 37°C. After that, the contents of the tubes were carefully discarded, and the crystal violet stain 1 percent was added to each tube for fifteen minutes (15 min), then rinsed and allowed to dry at room temperature (20-25°C). The result was determined by observing the formation of biofilm as a layer on the internal wall of tubes with the naked eye and comparing it to the negative control (the tube containing TSB without inoculation). The color of the biofilm layer and its thickness were used to determine the bacterial ability to form biofilm.

2.4 Antibiotic susceptibility test [10]

Antibiotic susceptibility test was applied by using Kirby Bauer's disc diffusion method according to the Manual on Antimicrobial Susceptibility Testing (MAST). The results were compared with (NCCLs, 2007).

2.5 Irradiation the bacteria with Diode laser [11]

It is considered a continuous laser. That has the following parameters: It emits the light at a wavelength gamma corresponding to 623nm, the spot size is about 2cm, was maintained at 1000Hz. The exposure time in this study varied with each peak power where the following times were used in this study for each peak power (1, 2, 5, and 10) minutes to get different energy densities. *Streptococcus spp*. was cultivated in Nutrient broth at 37℃ for 18hrs, followed by centrifuging 5ml at 7000 rpm for 5min. The cell pellet was diluted to a concentration of 106 CFU/ml in sterile phosphate buffer saline at PH7. Dilute bacterial suspension was placed in tubes and exposed to laser light for various periods of time (1, 2, 5, 10) minutes, with the exception of one tube containing bacterial suspension that was not exposed to laser light to serve as a control. Irradiated suspension and non-irradiated suspension were grown on MSA for 24hrs at 37℃.

III. RESULTS AND DISSCUSION

3.1 Streptococcus spp. isolation and identification

Oral cavity samples were streaked on selective agar medium. Because this medium contains crystal violet and potassium tellurite, which inhibit most Gram negative (G-ve) and Gram positive (G+ve) bacteria except streptococci, it promotes the development of Streptococcus spp. and inhibits the growth of other bacterial species [12].

3.2 Identification of bacterial isolates

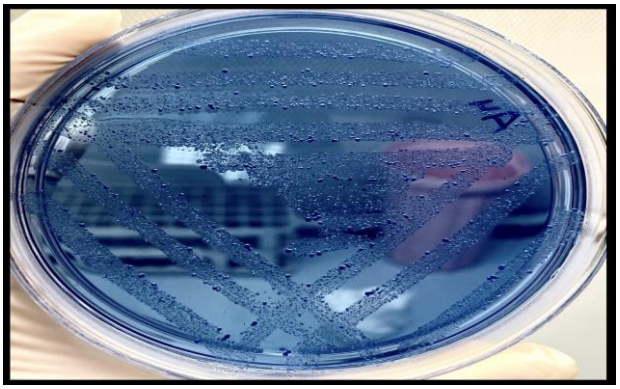

Figure (3-1): Streptococci on mitis salvaris agar media

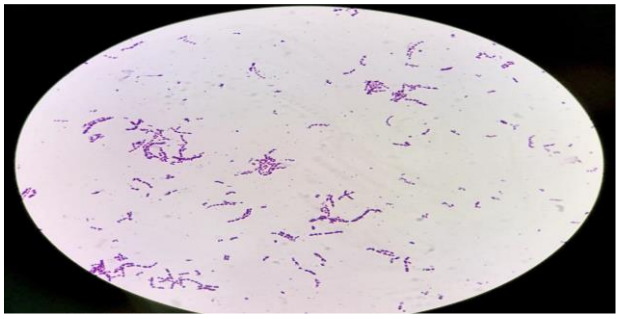

Figure (3.2): Gram stain of Streptococci under light microscope

3.3 Biochemical tests [13]

The biochemical properties of bacterial isolates suspected of belonging to the genus Streptococcus were examined. The results revealed that these isolates were gram positive, catalase negative, and unable to make hemolysin with gamma. Ten isolates were identified as S. mutans based on the findings [13].

3.4 Antibiotics Sensitivity

Results showed that 70% from isolates resistant to bacitracin and 90% resistant to two antibiotics (Naldixic acid and erythromycin) and 70% from isolates resistant to tetracycline. Antibiotics can be resisted by bacteria through the following mechanisms: the creation

of enzymes that render antibiotic compounds inactive (e.g., lactamases and aminoglycoside-modifying enzymes) hyperproduction of efflux pumps or the development of new efflux pumps, as well as other alterations in the cell wall [14]. The determination of the resistance or sensitivity of bacterial isolates is based on the measurement of the diameter of the inhibition zone. Antibiotic susceptibility pattern of the ten isolates against different antibiotics was studied. The establishment of a new erythromycin resistance-efflux mechanism. This section is dedicated to the mechanisms underlying macrolide and related antibiotic resistance in Streptococcus spp. [15].

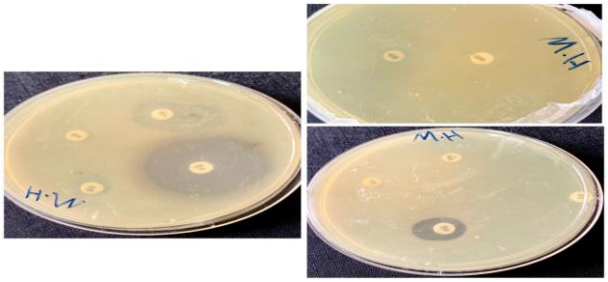

Figure (3.3): Antibiotic susceptibility test for Streptococcus isolate.

Results showed that change in susceptibility to antibiotic of *Streptococcus* spp. after exposure to diode laser, These result agreed with Al- Rassam (2010)[11] who discovered that the diode laser effect reduced MIC values while increasing susceptibility.

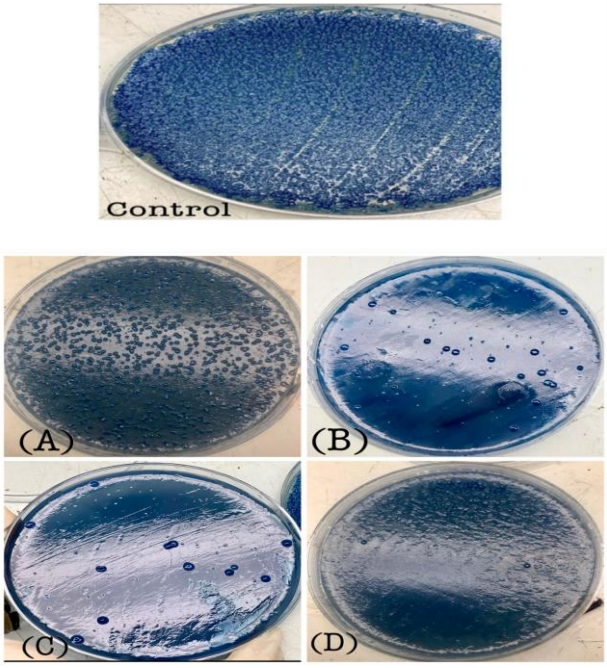

Figure (3.4): Streptococcus isolate after exposed to diode laser at different times
(A)Colonies after 1min from exposure to diode laser.
(B) Colonies after 3min from exposure to diode laser.
(C) Colonies after 5min from exposure to diode laser.
(D) Colonies after 10min from exposure to diode laser.

Laser parameters (wavelength, exposure time, energy output, laser irradiation emission modality) and optical features of the laced tissues influence the amount of these modifications (hydration condition, presence of chromophores, absorption coefficient) [16]. A diode laser irradiation system is used to treat biological tissue without exposing it to harmful heat effects [17].

Bacterial sensitivity to antibiotics can be caused by changes in the bacterial pumping mechanism (efflux pump), which is primarily responsible for bacterial resistance to medicines like penicillin (B- lactams, aminoglycoside). Bacterial sensitivity to antibiotics may be increased if bacteria fail to develop certain enzymes that chemically alter specific drugs [18].

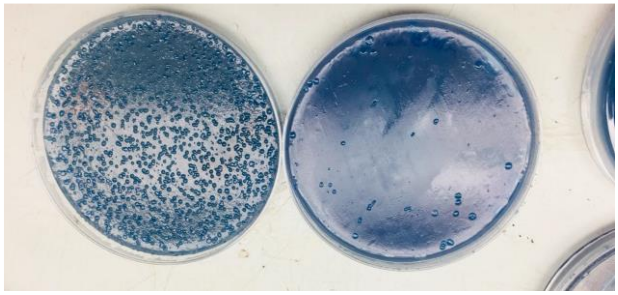

Figure (3-5): Effect of diode laser on viability of *Streptococcus* spp. After 10 min from exposure.

The results in the figure (3-5) indicated the effected of diode laser on viability of *Streptococcus* spp., the ability to diode laser to kill bacteria increased with increased time of exposure.

IV. CONCLUSION

1- *Streptococcus mutans* was one of the major micro-flora in dental carries infections.
2- *Streptococcus spp*. isolated from dental caries was sensitive antibiotics (gentamicin, ciprofloxacin, naldixic acid andchloramphenicol), while they were resistant to (cefixime, cephalothin, ampicillin, bacitracin and tetracycline).
3- Diode laser affected on viability of bacteria with increased time of exposure.
4- Diode laser affected on antibiotic sensitivity of *Streptococcus spp*. isolated from dental caries.

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