How to Cite:
Awad, H. S., & Alzubaidi, A. F. A. (2022). Antibacterial activity effect of nano-chitosan against Streptococcus Mutans isolated from dental caries. *International Journal of Health Sciences*, *6*(S4), 10541–10547. https://doi.org/10.53730/ijhs.v6nS4.12232

Antibacterial activity effect of nano-chitosan against Streptococcus Mutans isolated from dental caries

Hiba Sattar Awad
University of Diyala / college of Education pure science

Asst. prof. Dr. Adawia Fadhel Abbas Alzubaidi
University of Diyala / college of Education pure science
Corresponding author email: adwa_a2000@yahoo.com

***Abstract*---**The study investigated the antibacterial activity Effect Of Nano-Chitosan Against *Streptococcus Mutans*, It was collected from patients with periodontal disease and dental caries, and identification of bacteria and Antibiotic susceptibility tests were performed by using the VITEK2 compact system. This retrospective study was conducted at the Ba'aqubah teaching hospital.in duration February/ 2022 - April 2022 -, Data have been collected from coming patients at Specialized Dental Centers in Diyala, A total of 50 specimens, 35 (70%) were males, and 15 (30%)were females, the growth samples appeared in 17 (34%) isolates from dental caries and *strep. mutans* appeared in 10(20 %) isolates. The highest rate of antibiotic resistance was observed in 3(6%) of *Strep. mutans*. Then the effect of Nano-chitosan on antibiotic-resistant isolates was studied by determining the value of M I C.

***Keywords*---**dental caries, bacterial oral microbiota, dental infection, Streptococcus mutans, bacterial oral infection, antibiotics, nano-chitosan.

Introduction

The oral microbiota functions as a part of the host defense by acting as a barrier, The composition varies in different sites in the oral cavity, a large and more diverse bacterial load on the dorsum of the tongue. Most of these microbes are harmless, but under certain conditions, some can cause oral infections like caries or periodontal disease.(Hayam *et al.*,2022) Dental caries is one of the most common and costly diseases in the world, and although rarely life-threatening it is a major problem for health service providers. Oral streptococci, like

Streptococcus mutans, are associated with pyogenic and other infections in various sites including the mouth, heart, joints, skin, muscle, and central nervous system (Zhang *et al.*,2019)

An increase in dietary carbohydrates, particularly sucrose, results in additional acid production that may exceed both the capacity of the saliva to remove acid end-products and the neutralizing power of the salivary/plaque buffer system and results in more frequent acidification of the plaque (Peng *et al.*,2022) . A diet containing sucrose is one of the main reasons for the high dental caries rate in developed countries (Yang *et al.*,2021) .

The primary habitats for S. mutans are the mouth, pharynx, and intestine . Several factors, such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves and the ability to synthesize extracellular polysaccharides are present in dental caries. (Ying et al.,2022) *Strep. mutans* have a central role in the etiology of dental caries because these can adhere to the enamel salivary pellicle and to other plaque bacteria (WHO , 2022). *Strep. mutans* are strong acid producers and hence cause an acidic environment creating the risk for cavities. Usually, the appearance of *Strept. mutans* in the tooth cavities is followed by caries after 6-24 months (Loesche et al.,1975)

Our ability to treat common infections is still threatened by the emergence and spread of antibiotic-resistant pathogens that devise new resistance mechanisms that give rise to antimicrobial resistance.(Tang *et al.*,2016). Many studies have shown that low molecular weight Nano-chitosan can penetrate the walls of bacterial cells, bind DNA and prevent its transcription and make it flexible. (Gregor et al.,2019) a Gram-negative bacteria show a stronger interaction with Nano- chitosan due to the negative charge on the surface of gram negative bacterial cells is higher than Gram-positive bacteria. Thus, it results in stronger antibacterial activity (Xuwen, *et al.*,2022)

Materials and Methods

Study Population

In order to obtain the most accurate assessment of the types and amounts of microorganisms present Multiple samples from several areas of the bacterial oral were collected in the proper way to avoid any possible contamination . The study was conducted in the Department of Microbiology at Diyala University, education college , biology department .

Collection of specimens

A total number of 50 swabs samples were collected from Dental caries patients admitted to the Specialized Dental Centers in Diyala, Ba'aqubah in Diyala Governorate in Iraq Hospital from (January 2022 to May 2022). samples were aseptically collected 7 days after admission to the hospital. The sample was collected from the different sites of burn, and injury, The specimen was processed according to the guidelines for the laboratory diagnosis of pathogens

Microbiological Study Dental caries patients Samples

- Plate culture method: All specimens were inoculated on nutritional agar, 5 percent Blood agar, MacConkey, and Mannitol salt agar plates by spread plate method, under aseptic conditions in a laminar airflow cabinet, fewer than 7 hours after their collection. After that, culture plates were incubated aerobically for an entire night at 37 degrees C. colony counter-based total viable count isolation of microorganisms. All types of bacteria were isolated and identified using blood agar. Ten samples showed growth, which was followed by morphological detection, gram staining, and microscopic analysis.
- Confirmative Biochemical Study: Confirmative Biochemical Study: Utilize the Vitek 2 to identify the bacteria from isolated samples utilizing techniques such as, microscopically, Gram's staining, culture, biochemical analysis, . Then, to perform an antibiotic sensitivity test, the detected bacterial species were placed into nutrient agar slant and subculture at 37 degrees C for 24 hours.

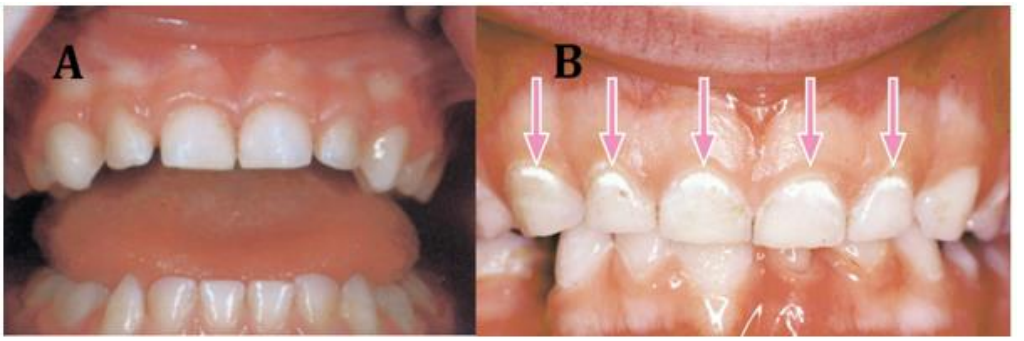
Antimicrobial sensitivity testing of dental caries Samples

The investigation of antimicrobial sensitivity testing was conducted using the common Kirby-Bauer agar disc diffusion technique. Muller Hinton agar plates were initially made. The normal saline solution tube's wall was wet and the surplus fluid was drained from the sterile swab stick before injection. By using a sterile technique bacterial cultures were taken by sterile cotton swab stick and a uniform lawn of bacterial growth was prepared on Muller Hinton agar plates. Using sterile forceps, antibiotic discs were placed equally spread apart on the surface of the medium. 5 discs were used on each plate. The plate was incubated overnight at 37 degrees C and the results were obtained no more than 24 h from incubation. The antimicrobial pattern was interpreted by the presence or absence of a clear zone around the antibiotic disc. The zone of inhibition was measured in mm by applying an ordinary ruler.

Results and Discussion

The association of Streptococcus mutans with human dental decay was investigated by using several types of samples. The results showed a significant association between plaque levels of S. mutans and caries. The strongest association was found in the dental plaque. The main reason for the increase in bacterial infections in recent times is due to the large number of infections and the spread of infection to many diseases caused by bacteria that are resistant to many antibiotics as a result of excessive and random use of antibiotics to generate bacterial resistance - in addition to the lack of health awareness or neglect in some cases. Figure 1 (Augusto *et al.,* 2020)

Figure 1: Image a depicts healthy teeth and gums and image b displays the early signs of oral clinical manifestation



A total of 50 specimens, 35 (70%) were males, and 15 (30%)were females, the growth samples appeared in 17 (34%) isolates from dental caries and strep. mutans appeared in 10(20 %) isolates. The highest rate of antibiotic resistance was observed in 3(6%) *of Strept. mutans*. Then the effect of Nano-chitosan on antibiotic-resistant isolates was studied by determining the value of M I C. Table (1).

Table (1): The clinically patients with dental caries samples

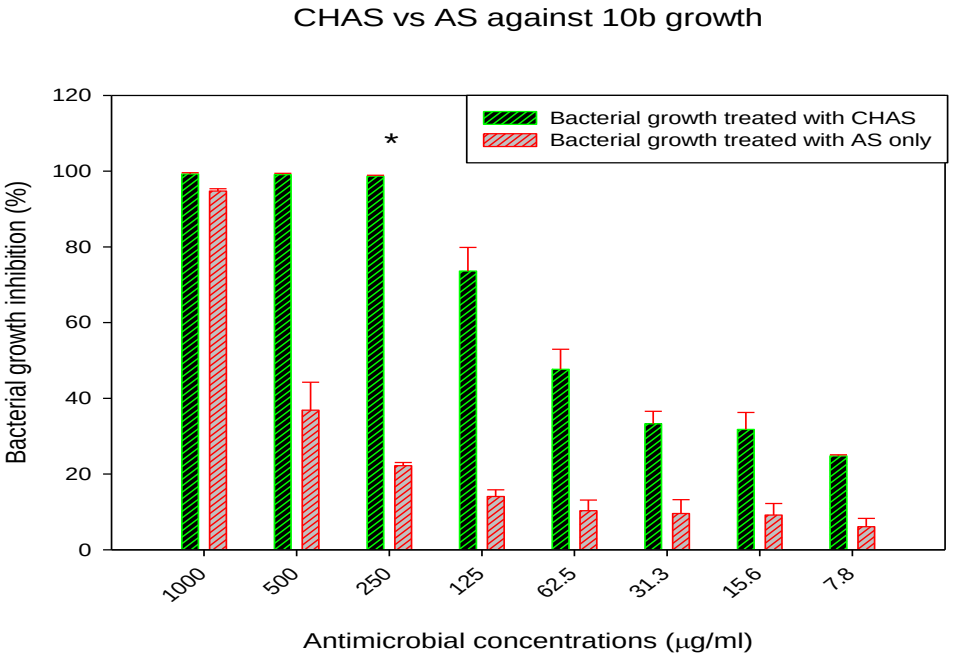
Sample Type	Total No. of sample	No. of male	No. of female	No. of grown sample .	No of *strep. mutans*	No of Resist isolates of *strep. mutans*
Infection	50	35 (70%)	15 (30%)	17(34%)	10 (20 %)	3(6%)

Referring to Table (2), Figure (2) it was noted that there was a significant difference in inhibiting the growth of bacteria *Strep. Mutans* . So the percentage of bacterial growth was inhibited at a concentration lower than MIC. The results of bacterial isolates varied in their resistance to chitosan solution dissolved in acetic acid, as *Strep. Mutans* bacteria showed the highest percentage of resistance compared to what was shown by Staph. aureus at a concentration of 250 µg/ml Because Nano-chitosan has the potential to inhibit bacterial growth, it has been shown that the suppression of bacterial growth and death after treating bacteria with ascorbic acid plus chitosan was higher than after using the acid alone. The results of this study agree with what was indicated by some previous studies such as (Sun et al., 2018; Khan et al., 2020; Wu et al. 2017; Badawy et al., 2019), while they differ with the results of researchers (Kuo-Shien *et al.* 2009).

Table (2): Minimum Bacterial Inhibitory Concentrations (MIC) of CHAS

Isolate, MIC of CHAS is 250 µg/ml		
Antimicrobials	Mean ± Std. Error	t-test P-value
AS	22.21 ± 0.58	0.000
CHAS	98.66 ± 0.16	

Figure (2) : Minimum Bacterial Inhibitory Concentrations (MIC) of CHAS



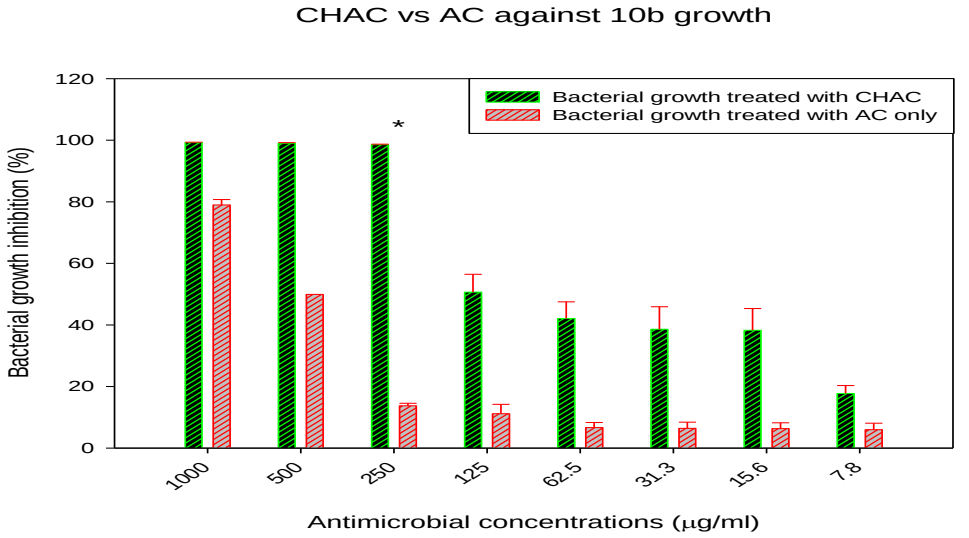
When studying the inhibitory effect of bacterial growth of chitosan solution with ascorbic acid, as shown in Table (3), Figure (3) it was found that the results of bacterial isolates and their degree of resistance varied, as showed there was a significant difference in inhibiting the growth of Strep. mutans at the rate of bacterial killing 90% MIC between the use of chitosan nanoparticles dissolved in CHAc and acetic only Ac also for the isolate at a concentration of 250 µg/ml with a value of 0.000>P value. So the percentage of bacterial growth inhibition at a concentration lower than MIC

The highest percentage of resistance to Nano- Chitosan solution with Ascorbic Acid (CHAC) , *Strep. mutans* has the lowest resistance ratio at a concentration of 250 µg/ml which means that the chitosan solution dissolved in ascorbic acid is more effective than the chitosan solution dissolved in acetic acid, perhaps due to the main difference in its composition, as acetic acid is a monobasic acid.) while citric acid is tripartite. (Tribasic), the antibacterial activity increases the lower the acidity is (Tsai *et al.* 1999) and as indicated by (Kravanja *et al.*, 2019).

Table (3): Minimum Bacterial Inhibitory Concentrations (MIC) of CHAC

Isolate, MIC of CHAC is 250 µg/ml		
Antimicrobials	Mean ± Std. Error	t-test P-value
AC	13.72 ± 0.58	0.000
CHAC	98.58± 0.08	

Figure (3) : Minimum Bacterial Inhibitory Concentrations (MIC) of CHAC



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