ANTIBIOTIC SENSITIVITY OF BACTERIA CAUSING DENTAL CARIES ISOLATES FROM IRAQI PATIENTS

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Abstract

This study aims to test the antibiotic resistance of *streptococcus mutants*, one of the major causes of dental caries for Iraqi patients of different ages. This study focuses on routine antibiotics used as a treatment for the patient suffering from dental caries and demonstrates whether these bacteria acquired resistance against these antibiotics or not. This study showed that *streptococcus mutants* isolates from different patients have an approximately equal ratio of resistance to the routine antibiotics used to treat dental caries, such as amoxicillin, cefixime, and azithromycin. This study is based on the disc diffusion method for diagnosing antibiotic resistance. This study targeted patients chosen randomly from different medical care centers in Baghdad, especially in Al-karkh district, to show that the minimum inhibitory concentration MIC of different isolates of *streptococcus mutants* have relative sensitivity to ceftriaxone and resistance to amoxicillin which can enhance more studies for controlling the antibiotics abused.

Keywords: MS (*Mutant streptococci).*

Introduction:

Worldwide, Dental caries is a pandemic disease affecting humans at different stages, starting from childhood to the elder age. Dental caries affect all the age groups of humans in developed and undeveloped countries. (1) Many studies indicated that approximately 90% of the residents in many countries are affected by dental caries (2). Accumulative reports showed that among mutans streptococci (MS), *Streptococcus mutans* and *Streptococcus sobrinus* were the most isolated microorganisms from most human dental caries(3). *Streptococcus mutans* is also known to cause systematic diseases such as cardiovascular and infective endocarditis. (4,5)

Streptococci have been classified into six groups: the pyogenic group, the anginosus group, the mitis group, the salivarius group, the Bovis group, and the mutans group. Among these groups, the *Streptococcus mutans* is the most prevailing species of medical importance, which live as facultative anaerobic, Gram-positive cocci bacterium, this species of bacteria produce extracellular polysaccharide layers in the form of a capsule from sucrose that aid bacteria in attachment and colonization, which causes dental caries (6).

Streptococcus mutants contribute many different surface adhesins that can attach to the salivary pellicles built on the teeth (7).

Dental caries may cause by many factors, which may depend primarily on the diversity or heterogeneity among the species of MS that represent the main factors that trigger the infection of dental caries (8). The diagnosis and identification of MS species are essential in designing a treatment

strategy (9). Most diagnoses of dental caries infection cause result that *S. sobrinus* performing a high prevalence of infecting the teeth than other species of MS (10). The most dependent diagnosis method was used to identify the species of MS by Macroscopic examination of culture, biochemical tests, Enzyme-Linked Immunosorbent Assay, and recent molecular methods, including the poly chain reaction and (PCR)-restriction fragment length polymorphism-PCR-RFLP (11).

The maximum of the species mentioned above shows α hemolysis, a green zone around growth.

S. mutans is not only the basic bacterium engaged in the development of plaque but also for the commencement of dental caries (12).

S. sobrinus attaches to the tooth superficially, breaks down sugar for energy, decreases the pH, and makes the surrounding acidic this causes demineralization of the external structures of the tooth like enamel and dentine. Sometimes to the lack of prior medication, the mechanism advances, ultimately developing dental caries (13).

Dental caries represent a primary dental infection in childhood, especially after the eruption of the first teeth. In addition, the symptoms may show deep pain and spoils and may develop into severe disease, and death, if not treated, is a universal chronic disease of childhood. It can appear in young children soon after the eruption of first teeth and sometimes can be severe. In most children, early childhood caries cause pain and spoils the status of well-being, and it develops into severe disease, hospitalization, and sometimes death. Therefore, dental caries is the leading cause of tooth loss in children and young adults.

Some specific strains of S. mutans also produce bacteriocins. The restriction of *S. mutans* bacteriocins production by oral bacteria happens to be powerful inside the biofilms.

It easily colonizes the oral cavity due of its capability to form biofilms on dental surfaces (14).

The construction of the oral biofilm is established through the basic attachment of the bacteria, S. mutans which are the former colonizers. S. mutans contribute many distinct surface adhesions that can attach to the salivary pellicles built on the teeth (15).

As a precautionary measure, antibiotics are generally prescribed by the dentists before commencing the treatment to prevent any systemic infections arising following cavity filling or tooth extraction.

In recent years, many studies have reported the adverse effects of antibiotic abuse, which result in the high resistance to many antibiotics among the different species of bacteria. At present, adverse reactions to the antibiotics, such as bacterial resistance and the rise of multidrug-resistant bacteria, are a worldwide concern of public health (16). This study aimed to study the antibiotic sensitivity of Streptococcus mutants isolated from the patient's dental care to observe the effectiveness of the most routinely used antibiotic by the dentist in the dental care centers in Baghdad province.

Methods

Patient information

This study was conducted in microbiology laboratories at the college of health and medical techniques, Al-Bayan University, Baghdad, Iraq. The study protocol was approved by the ethics committee of Al-Bayan University with license issue No.58 on the 7th of August 2019 to the dental care centers, including the permission to collect samples from patients who have dental care that was accepted by the administrative department of the dental cares center. The dental plaque samples were collected from 40 caries-active subjects, including 27 males and 13 females in the age range 35–44 years, as referred to the World Health Organization (WHO) guidelines (17).

Isolation and cultivation of samples

The Collection of samples was performed using the tips of sterile toothpicks from carious lesions, then dipped into sterile phosphate-buffered saline (HiMedia, India) then stored at 4°C. Vortex is utilized to perform a homogenization of samples and disperse the plagues. 100-fold applied dilution in 1x Sterile PBS, then plated in Mitits Salivarius Bacitracin (MSB) agar (HiMedia), then incubated anaerobically at 37°C for 48 h (18). After the incubation period, the colonies were identified based on colony morphology. The typical colonies from each sample plate were transferred to brain–heart infusion (BHI) broth (HiMedia, India) and incubated at 37°C for 18 h. After the incubation period, the broth cultures were streaked on MSB agar and anaerobically incubated at 37°C for 48 h. The overnight bacterial cultures were stored in 80% glycerol stock at −20°C. (19). Biochemical tests were performed to determine the biotypes of the clinical isolates. A phenol red broth base (HiMedia, India) was used as the basal medium to ferment mannitol, melibiose, sorbitol, and raffinose (HiMedia, India). Arginine dihydrolase broth (HiMedia, India) was also employed in biochemical characterization. The test organisms were inoculated into the sterile broth and anaerobically incubated at 37°C for 48 h. The positive result was indicated by a color change described by Shklair and Keene (20) and Yoo *et al.* (21).

Antibiotic sensitivity

The disc diffusion method performing by adding colonies from the brain heart infusion agar to Mcferland suspension 0.5ml and shaking the suspension before streaking of suspension on Muller Hinton Agar (HiMedia), then antibiotic discs are added to each agar plate. The antibiotics added in this study were Piperacillin 100, tazobactam 10mg, Azithromycin 15 mg, Cefixime 5 mg, Amoxicillin 20 mg, 10 mg (Merseyside UK), then incubation under anaerobic conditions via sealed jar under 370c at the incubator. Bacitracin was added to Muller Hinton agar as a differentiated material to isolate *streptococcus mutants* from other normal flora in the sample.

Results

Samples were taken from patients who had dental caries. Half of the participants were younger age population, and half of them were older age population. 67% of the population were males, and the other 33% were female. The samples showed positive for *streptococcus mutans* after incubation on MSB agar by choosing the colonies with point blue colonies. The isolated colonies undergoing staining with gram stain showed a positive strep-like shape under the 100x magnification power, as shown in figure 3.

Table 1: the inhibition zone of antibiotics formed

Sample	Azithromycin 15mg	Cefixime 5mg	Amoxicillin 10mg	Ceftriaxone 30mg	Piperacillin 100/ tazobactam 10mg
1	42mm	25mm	45mm	40mm	37mm
2	41mm	25mm	30mm	39mm	36.5mm
3	42mm	25mm	44mm	41mm	37mm
4	42mm	25mm	43mm	39mm	37mm
5	42mm	25mm	44mm	39mm	37mm
6	42mm	25mm	45mm	39mm	37mm
7	42mm	25mm	44mm	39mm	37mm
8	42mm	24.5mm	44mm	39mm	37mm
9	42mm	24mm	44mm	39mm	37mm
10	42mm	25mm	44mm	39mm	37mm
11	42mm	25mm	44mm	39mm	37mm
12	42mm	25mm	44mm	39mm	37mm
13	42mm	25mm	44mm	39mm	37mm
14	42mm	25mm	44mm	39mm	37mm
15	42mm	25mm	44mm	39mm	37mm
16	42mm	25mm	44mm	39mm	37mm
17	42mm	25mm	44mm	39mm	37mm
18	42mm	25mm	44mm	39mm	37mm
19	42mm	25mm	44mm	39mm	37mm
20	42mm	25mm	44mm	39mm	37mm
Control	NA	NA	NA	NA	NA

Isolated *streptococcus mutant* isolates were tested for the effectiveness of certain antibiotics such as penicillin and amoxicillin in eliminating these bacteria. As shown in Table 1, most samples test diagnosed as We were able to find out which antibiotic was effective in treatment versus resistant antibiotics, as shown in Table 1. Most isolated tested a sensitive versus amoxicillin while showed

broad resistance toward the cefixime and showed sensitivity against the piperacillin and tazobactam based on the inhibition zone measured in table 1 based on CLSI guidelines version 2021. All these tested antibiotics were routinely treated for dental caries remedy.

sample	piperacillin100,tazobactam10	Azithromycin 15	Cefixime 5	Amoxicllin20,10	Ceftriaxone30
1	Sensitive	sensitive	sensitive	sensitive	sensitive
2	sensitive	sensitive	resistance	resistance	resistance
3	sensitive	sensitive	resistance	sensitive	resistance
4	sensitive	sensitive	resistance	sensitive	resistance
5	sensitive	sensitive	resistance	sensitive	sensitive
6	sensitive	sensitive	resistance	sensitive	sensitive
7	sensitive	sensitive	resistance	sensitive	sensitive
8	sensitive	sensitive	resistance	sensitive	sensitive
9	sensitive	sensitive	resistance	sensitive	sensitive
10	sensitive	sensitive	resistance	sensitive	sensitive
11	sensitive	sensitive	resistance	sensitive	sensitive
12	sensitive	sensitive	resistance	sensitive	sensitive
13	sensitive	sensitive	resistance	sensitive	sensitive
14	sensitive	sensitive	resistance	sensitive	sensitive
15	sensitive	sensitive	resistance	sensitive	sensitive
16	sensitive	sensitive	resistance	sensitive	sensitive
17	sensitive	sensitive	resistance	sensitive	sensitive
18	sensitive	sensitive	resistance	sensitive	sensitive
19	sensitive	sensitive	resistance	sensitive	sensitive
20	sensitive	sensitive	resistance	sensitive	sensitive

Figure 1 : the Antibiotic Resistance for S. Mutant isolate from the Dental caries

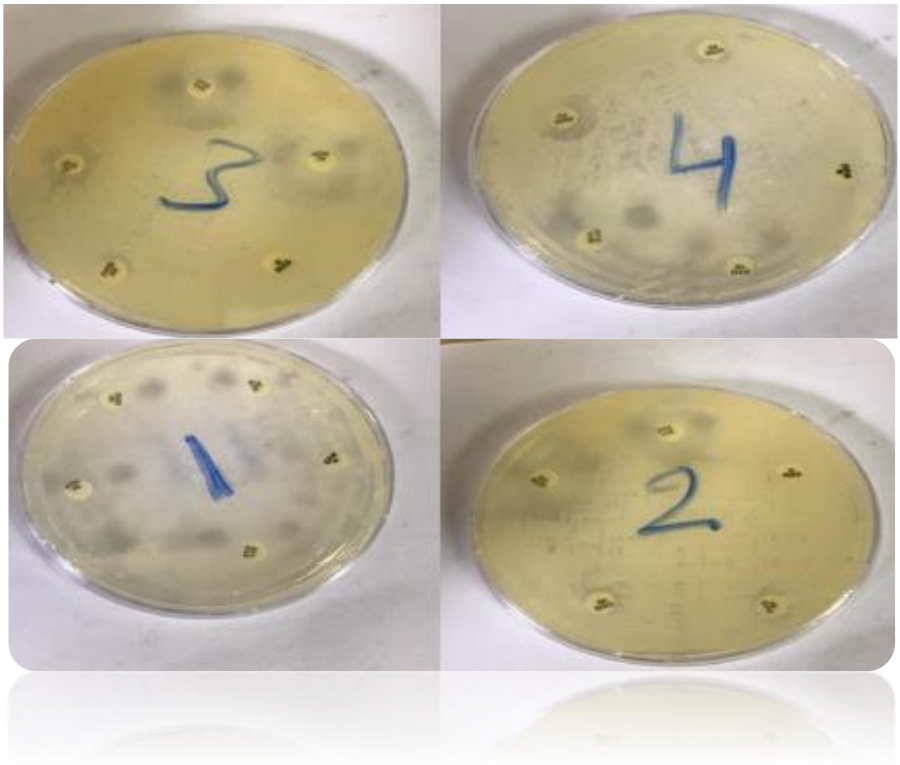

Figure 2 :the disc test for antibiotic resistance method

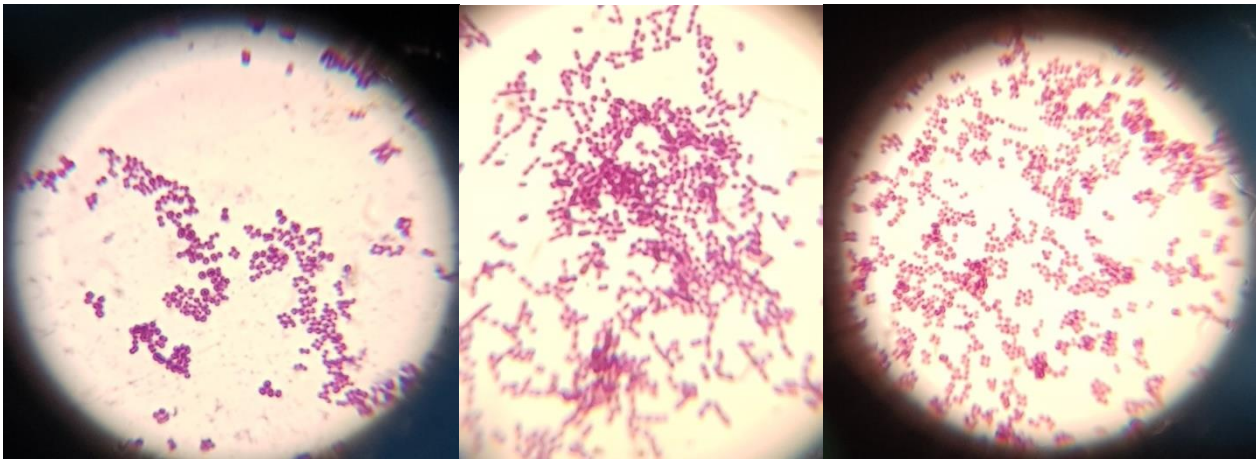

Figure 3: Streptococcus mutants under 100x Magnification power

Discussion

Streptococcus mutans is the leading cause of tooth decay globally and is the most significant cause of streptococcus orally. Dentists commonly prescribe most of the antibiotics employed in this study. Most people misusing the antibiotics or undergoing antibiotics misuse developed a number of oral resistant *mutants, streptococcus*. The most common antibiotics prescribed are beta-lactam antibiotics, so more streptococcus developed a more resistance against the beta-lactam group of antibiotics (22). The resistance to penicillin by bacteria is the most health issue globally, making the prevalence for test another possible drug of choice for dentists to test their effectiveness on the oral bacteria. Twenty samples were collected from Al-Bayaa health center, benefiting from the proliferation of nodal components. The current research evaluates the effectiveness of 5 antibacterial drugs available in Iraq that were conducted against *Streptococcus mutans* using the spreading technology well and defining the drug that dentists should prescribe with a minimum of side effects and a maximum of inhibitory activity. It was found that when using the drug piperacillin, 100mg tazobactam 10mg showed its sensitivity to bacteria by 100% in the samples collected from people of different ages and both sexes. Also, the antibiotic Azithromycin 15mg showed a sensitivity of 100% for these bacteria. In comparison, the antibiotic cefixime 5mg showed its resistance by 98%, And the antibiotic Amoxicillin 20mg showed 10% resistance to this bacterium. In contrast, the ceftriaxone antibiotic 30mg showed a 3% resistance to this bacterium Studies recommend the importance of isolation, laboratory diagnosis, knowledge of virulence factors, the pattern of allergies, and antibiotic resistance, which are due to localization of the appropriate antibiotic to treat cases with *streptococcus mutans* in a scientifically accurate way to avoid the antibiotic abuse by the patient.

Conclusion

Our study demonstrates a significant resistance to amoxicillin and cefixime in S.mutants clinical isolates in dental caries patients. Also, isolates were more susceptible to azithromycin and piperacillin than other tested antibiotics. Further study is required to know the Minimum inhibitory concentration of beta-lactam and non-beta-lactam antibiotics with sensitivity to the azithromycin. An alternative antibiotic such as herbal extract is most likely preferable for the coming years to avoid the upcoming bacterial resistance to the antibiotics. In addition, the rise in the rate of antibiotic resistance in S.

mutans clinical isolates suggests that taking extra precautions while prescribing antibiotics will maintain the bacteria with less resistance.

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