

## Investigate the spread of *Streptococcus mutans* for patients with tooth decay in Samarra city

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### Abstract

The study includes the collection of 150 samples, by using of a cotton swab from the caries area of patients with dental caries for the age group between 20-40 years for both genders, and they were collected from Samarra hospital and outpatient clinics for the period between 1-11-2021 to 1-4 -2022 for the purpose of isolation and diagnosis tooth decay-causing by bacteria *Streptococcus mutans*, all the traditional methods of implantation, microscopic and biochemical diagnosis were taken, and they were grown on Mitis Salivarius Bacitracin (MSB), the results of culture and diagnosis show that *Strep.mutans* constitutes about 33% of the rest of the bacterial species that cause dental caries, the prevalence of dental caries cases by gender is studied, which showed a discrepancy between females and males, the number of infected females was 83, which constitutes about 55%, while the number of males was 67, which constituted 45%, and this indicates that the prevalence of dental caries among females is more than males, and it was also studied according to age, and it was found that the age group between 20-30 was the most.

### Introduction

The oral cavity provides humans and other mammals with a unique environment in which there is a distinct group of microorganisms, unlike other parts of the body, teeth do not fall out or change in the long term, and therefore provide a permanent attachment point for microorganisms where the teeth are fixed in the jaw bones and are partially covered with gums and GCF (gingival crevicular fluid), and in addition the oral cavity is always washed with saliva and periodically exposed to food, Leeuwenhoek observed the first specimens microscopically in the late seventeenth century [1,2].

The mouth contains several types of bacteria, but a few of them cause tooth decay, where bacteria interfere with each other and lead to biofilm adhesion, especially the group of Viridians streptococci, the most important of which is *Streptococcus mutans*, which is the main cause of the initiation of tooth decay [3,4], and there are other bacterial species such as *Staphylococcus* and *Candida* and some species belonging to the *Enterobacteriaceae* family that can cause dental caries as well [5,6], where these can cause tooth decay, bacteria can produce

polysaccharides in large quantities from fermented carbohydrates and are firmly attached to the surface of the teeth, and are also able to withstand the acidic environment in the mouth [7,8].

Tooth decay begins as a result of an oral biological defect due to environmental factors such as eating sugars and others, and turns into a pathological condition through a series of complex chemical reactions through microbial activities associated with the biological formation on the dental plaque, which leads to the removal of minerals from the calcified tissues of the teeth and the decomposition of organic components [9,10], and dental caries is one of the most common diseases that can lead to pulpitis, tooth loss and other systemic diseases [11].

The aim of our current study is to isolate and diagnose *Streptococcus mutans* bacteria from dental caries cases of both sexes and adults only.

## **Materials and working method**

### **Sample collection**

The 150 samples of both sexes were collected from adults from Samarra hospital and some medical clinics, cotton swabs samples taken from the tooth decay area were cultured on the medium of the streptococcus bacteria called Mitis Salivarius Bacitracin (MSB), where this medium is highly selective for the green streptococcus group Viridians group Especially *Streptococcus mutans*, which contains Bacitracin, which inhibits most types of bacteria other than this group [12], and it was incubated under aerobic and anaerobic conditions for 24 hours at 37 °C, after completing the incubation process, tests were carried out, appearance and biochemistry test and antibiotic test.

### **Diagnosis of isolates**

After culturing the samples on MSB agar, diagnosed was made as follows: Form colonies on MSB agar medium recorded, form colonies under a microscope light using gram stain, and execute biochemical assays such as catalase, oxidase, urease, triglyceride fermentation test, mannitol sugar fermentation test, capsule formation test, transfer of colonies to blood agar medium and incubation for 24 hours and test bacterial breakdown of the blood.

### **Antibiotic sensitivity test**

A sensitivity test for bacterial isolates was conducted using the disk diffusion method [13] (Disc diffusion method) as follows:

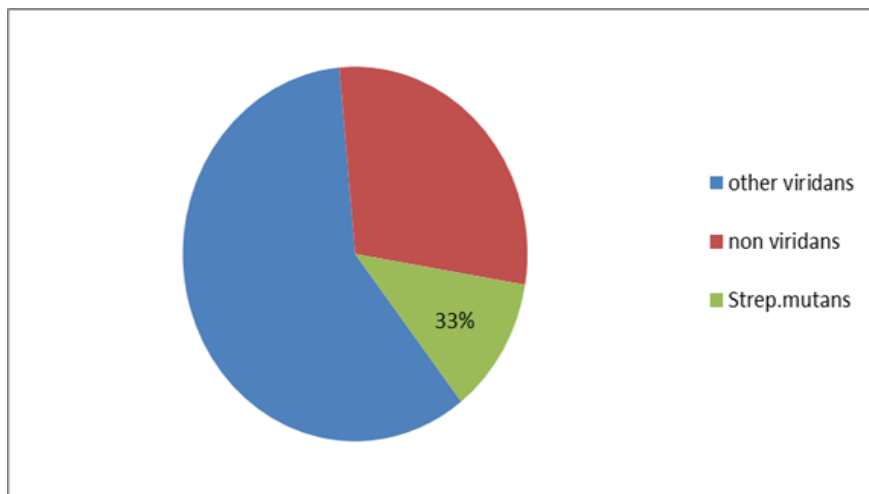
The bacterial suspension was prepared through transferring colonies of bacteria to tubes containing saline as previously prepared, the density was compared together with the standard turbidity constant solution, which gives an approximate number of cells ( $1.5 \times 10^8$  cells.ml<sup>-1</sup>), and a cotton swab sterile was inserted into the tube containing the suspension, then passed the Muller-Hinton agar medium as previously prepared for several times to be distributed evenly on all the dish, then left the dishes at room temperature for about 10 minutes to dry and then put the disc by means of sterile forceps (flame sterilized) at appropriate dimensions on the plate for the purpose of not overlapping the areas of inhibition and incubated at 37 °C for 24 hours, and after the incubation period ended, the results were

read using a graduated ruler and compared with international standard the clinical and laboratory standards institute (CLSI) [14 ].

### Results and Discussion:

The study included 150 samples of dental caries for adults of both gender whose ages ranged between 20-40 years and were collected from Samarra hospital and outpatient clinics for a period between 1/11/2021 to 1/4/2022, for the purpose of isolating and diagnosing *Strep. mutans* bacteria, all the traditional methods of culture, microscopic and biochemical diagnosis were taken and cultured on Mitis Salivarius Bacitracin agar (MSB) medium.

The results show that 98 isolates belonged to the group Viridians Streptococci, which constitutes 65%, while 52 isolates did not belong to the group of Viridians Streptococci, as they showed no growth on MSB medium, and it was found from the isolation and diagnosis at 50 isolates were *Strep. mutans* and this It constitutes 33% (Figure1).



**Figure 1:** Percentage of *Strep. mutans* bacteria isolated from dental caries

The isolates were diagnosed by microscopic assay, the isolates were shown using gram - positive series colonies arranged in chains under the light microscope with a power of 100X, and it did not produce the urease enzyme.

As for the antibiotic sensitivity test, the sensitivity of *Streptococcus mutans* isolates to eight types of commonly used antibiotics was studied according to the Kirby-Bauer method using antibiotic discs, in table 1.

**Table 1:** Resistance and sensitivity of antibiotic of *streptococcus mutans*

Antibiotic	Number Resistant isolate	percentage sensitive isolate	Number resistant isolate	Percentage sensitive isolate
Amikacin 10 (µg/disc)	15	30%	35	70%
Amoxicillin 25 (µg/disc)	50	100%	0	0%
Amoxcillin/ Calvuanic acid 30 (µg/disc)	15	30%	35	70%
Ampicillin 25 (µg/disc)	50	100%	0	0%
Cefotaxime 10 (µg/disc)	10	20%	40	80%
Erythromycin 10 (µg/disc)	48	96%	2	4%
Tetracycline 10 (µg/disc)	13	26%	37	74%
Trimethoprim/ Sulphamethaxole 25 (µg/disc)	5	10%	45	90%

The results show a clear discrepancy in the resistance and sensitivity to antibiotics, as *Strep.mutans* bacteria showed, *Strep.mutans* were 100% resistant to Ampicillin, Aoxcillin, and 96% resistant to Erythromycin.

The results of our current study are similar to what was presented by researcher Torki [15] who showed isolates of *Strep.mutans* in her study showed a sensitivity to Tetracycline by 70% and resistance to Amoxcillin and Ampecillin by 100%, She also agreed with what was presented by Al-Kaaby16 which showed in her study the sensitivity of isolates to Amikacin by 75% and to Trimethoprim/Sulphamethaxole by 93%, and it varied with it in her study showed a sensitivity to Cefotaxime by 31%, and partially agreed with the results reached by researcher Al-Mosawi17, where the isolates showed sensitivity to Tetracycline and Erythromycin by 60% and 65%, respectively.

### **Epidemiology of *Strep.mutans* in patients with dental caries**

The results of the epidemiological study, which included 150 patients with dental caries, indicated that *Strep.mutans* bacteria spread in a large proportion, and the results of our current study showed that it constituted about 33% of the rest of the bacterial species that cause tooth decay, and this was very close to the percentage of its prevalence in Baghdad governorate, where it was constituted 30%18, and this was also similar to what was found in a study conducted in the city of Diwaniyah, where it constituted about 37%16, and its percentage in Thi Qar governorate was 41%19, and the lowest percentage was recorded in Diyala Governorate, where it constituted 13% [15].

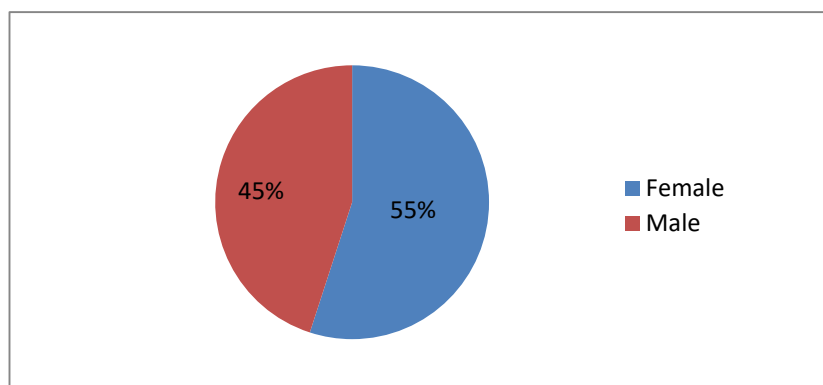
As for Egypt, it constitutes about 75 % [20], which is a large percentage compared to what was found through our study and other studies in various regions of Iraq, just as in Europe it constituted 48 % [21].

This difference in the prevalence of *Strep.mutans* bacteria is due to several reasons, including the nature of the diet of each region or country, eating foods and drinks containing sugars in different proportions will lead to an increase in their spread, and the extent of oral and dental hygiene care in terms of interest in brushing the teeth twice a day the use of oral solutions that help maintain the pH of the mouth is one of the important reasons for these different ratios, in addition to the nature of the environment itself and the difference in the level of health awareness.

### **Distribution of dental caries cases by sex**

The results of our current study show that the percentage of females affected by dental caries is higher than that of males, where the percentage of females was 55% and the percentage of males was 45%, as shown in figure 2, the hormonal nature and the changes that occur as a result of pregnancy and childbirth affect tooth decay, as the level of calcium in the body of women decreases during pregnancy, and therefore the level of vitamin D3 which is associated with tooth decay decreases, and women in general have a deficiency of vitamin D3 due to their lack of exposure to the sun as a result of their lack of going out, Outside the house [22], this is due to the nature of the eastern culture and the customs that exist in our regions and the Middle East in general.

The results of our current study agreed with Al-Dulaimi [23], where he explained in his results that the percentage of dental caries in females is often higher than in males, and also agreed with the findings of Khamis [24] ,where it was found that the incidence of dental caries in females is higher than Males in the permanent teeth, and as agreed with what was found by Torki [15] that the rate of dental caries for females is higher than males, but the results were inconsistent with what was found by Al-Mosawi [17] where he stated that the percentage of dental caries for males was 64 % and thus It is higher than the percentage of females, which was 36 %.

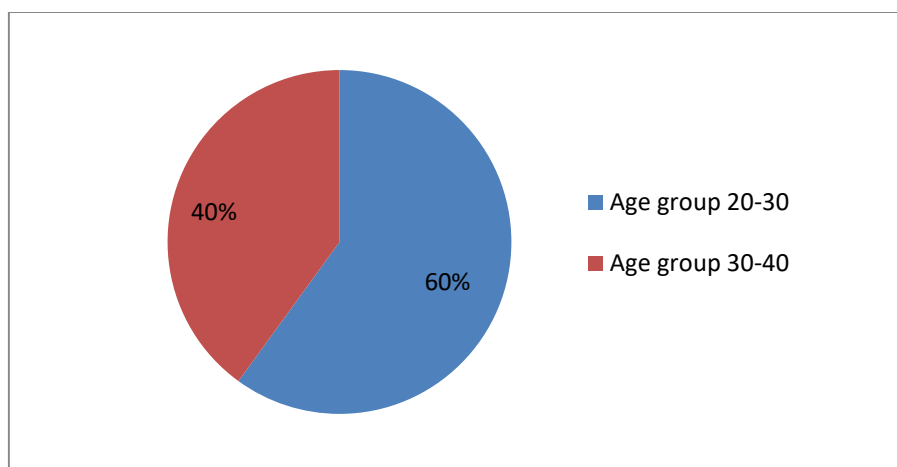


**Figure 2:** Percentage of males and females with dental caries

### **Distribution of dental caries cases by age**

As for age, our current study found that the rate of infection in the age group between 20-30 was 60 %, and thus it is higher than the age group between 40-30, which was 40 % as shown in Figure 3, and these results agreed with what It was found by Torki [15] and the

reason for this may be attributed to the nature of food that includes fast food and soft drinks and this came in line with Yan *et al.*, [25] who found the effect of the relationship of vegetarian and non-vegetarian food on tooth decay, and most likely this is due to the increased consumption of foods Marine fluoride-rich, which may lead to a lower prevalence of dental caries in the non-vegetarian population, moreover, it is well established that the greater the exposure of the teeth to acidic components, the greater the tooth decay, which was confirmed by Lu *et al.*, [26] where it was stated that saliva is responsible for Neutralize acids produced by microorganisms operating in the presence of sugar, but when fermentable carbohydrates are not present in saliva, putrefaction will replace fermentation and alkalinity will replace acidity and no calcium removal is usually observed, as demonstrated Mahboobi *et al.* [27] having a relationship there is a strong correlation between the type of diet and caries, as it was mentioned that the consumption of foods and drinks containing sugar leads to a significant increase in tooth decay, so people who follow a sugar-free diet are less likely to have tooth decay.



**Figure 3:** Percentage of age groups of people with dental caries

## Conclusions

Our current study shows that *Streptococcus mutans* bacteria have a wide causes spread among other causes of tooth decay for infected people in the local area (Samarra), and the prevalence of dental caries disease in the age group between 20-30 was the most, and females were more infected than males, and *Streptococcus mutans* has a clear diversity in terms of resistance and sensitivity to antibiotics.

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## التحري عن مدى انتشار بكتريا *Streptococcus mutans* للمصابين بتسوس الاسنان في مدينة سامراء

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البحث مستل من رسالة ماجستير الباحث الاول

### الخلاصة:

تضمنت الدراسة جمع 150 عينة بوساطة المسحة القطنية الناقلة من منطقة التسوس للمصابين بتسوس الاسنان للفئة العمرية ما بين 20-40 سنة لكلا الجنسين، وجمعت من مستشفى سامراء والعيادات الخارجية ولمدة ما بين 1/11/2021 لغاية 1/4/2022 لغرض عزل وتشخيص بكتريا *Streptococcus mutans* المسببة لتسوس الاسنان وقد اتخذت جميع الطرق التقليدية للزرع والتشخيص المجهرى والكيموحيوي وزرعت على الوسط الخاص *Mitis Salivarius Bacitracin* (MSB)، وقد اظهرت نتائج الزرع والتشخيص ان بكتريا *Strep.mutans* كانت تشكل حوالي نسبة 33% من بين باقي الانواع البكتيرية المسببة لتسوس الاسنان، وتم دراسة انتشار حالات تسوس الاسنان حسب الجنس التي اظهرت تباينا بين الاناث والذكور فقد كان عدد الاناث المصابين 83 وهذا ما يشكل حوالي نسبة 55% اما الذكور فقد كان عددهم 67 إذ يشكلون نسبة 45% وهذا يشير الى ان انتشار تسوس الاسنان بين الاناث اكثر من الذكور، وقد درست حسب العمر ايضا وتبين ان الفئة العمرية ما بين 20-30 كانت هي الاكثر.

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### الكلمات المفتاحية:

تسوس الاسنان، الذكور، الاناث

*Streptococcus mutans*،

### معلومات المؤلف

الايمل:

الموبايل: