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Isolation and diagnosis of Streptococcus mutans and Streptococcus sorbinus from patients with tooth decay and gingivitis

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ABSTRACT

The *Streptococcus* bacteria *Streptococcus sobrinus* and *Streptococcus mutans* are one of the most common types in the mouth, where tooth decay, necrosis and gingivitis are among the health problems that the Iraqi society suffers from, as the treatment of bacteria has become a challenge due to their ability to resist many antibiotics. The study aimed to isolate and diagnose *Streptococcus mutans* and *Streptococcus sobrinus* and study their resistance to a number of antibiotics, and evaluate the susceptibility of *Streptococcus mutans* and *Streptococcus sobrinus* isolates in biofilms. Using biochemical tests and phenotypic diagnostics. The study included the collection of (275) oral swabs from patients with tooth decay from the area of caries on the surface of the tooth and from cysts for infected patients and visitors at the Medical City Hospital in Baghdad Governorate with ages ranging between (6-70) years and of both sexes for the period from 1/9/2022 to 1/11/2022. the number of isolates was 6 (2.18%) belonging to *Streptococcus mutans* and only one isolate (0.36%) of *Streptococcus sobrinus*, the sensitivity of all the isolates of the bacteria under study was tested towards 10 antibiotics, and the results showed that the isolates were resistant to most of the antibiotics used in this study, and all the isolates were chosen to test their ability to form biofilms using the microtitration plate, and the results showed that most of the isolates were strong in the formation of biofilms.

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عزل وتشخيص بكتريا *Streptococcus mutans* و*Streptococcus sorbinus* المعزولة من مرضى تسوس الاسنان والتهاب اللثة

وسن لورنس حسن , أ.م. د عبد أحمد أرديني , أ.د تغريد خضر محمد

الملخص

تعد بكتريا *Streptococcus mutans* و*Streptococcus sobrinus* من أكثر الأنواع شيوعا في الفم، إذ يعتبر تسوس الأسنان وتنخرها والتهاب اللثة من المشاكل الصحية التي يعاني منها المجتمع العراقي، إذ أصبح علاج البكتريا تحدياً بسبب قدرتها على مقاومة العديد من المضادات الحيوية . الهدف من الدراسة هو عزل وتشخيص البكتريا وأختبار مقاومتها للمضادات الحيوية و قدرتها على تكوين الأغشية الحيوية بأستخدام اختبارات بايوكميايئية وتشخيصات مظهرية، إذ إن عدد العينات الكلي كان٢٧٥ التي عينة تم جمعها من مرضى تسوس وتنخر الاسنان والتهاب اللثة من مراجعين في مستشفى مدينة الطب في محافظة بغداد وبأعمار تراوحت بين (٦-٧٠) سنة ومن كلا الجنسين للمدة من ١\٩\٢٠٢٢ الى ١\١١\٢٠٢٢ وكانت أن عدد العزلات ٦ (٢,١٨٪) التي تنتمي إلى *Streptococcus mutans* وعزلة واحدة فقط (٠,٣٦٪) تعود الى بكتريا *Streptococcus soprinus*، تم اختبار حساسية جميع عزلات البكتيريا قيد الدراسة على ١٠ مضادات حيوية ، وأظهرت النتائج أن العزلات كانت مقاومة لمعظم المضادات الحيوية المستخدمة في هذه الدراسة ، وتم اختيار جميع العزلات لاختبار قدرتها على تكوين الأغشية الحيوية باستخدام لوحة المعايرة الدقيقة Microtiter Plate ، وأظهرت النتائج أن معظم العزلات كانت قوية في تكوين الأغشية الحيوية.

Introduction: Pathological periodontal disease is one of the most prevalent diseases in the world: dental caries, tooth decay and periodontal disease, and gingivitis [1] . Dentistry leads to the disintegration of the enamel layer or acid-mediated root surface damage, mainly due to diet, causing dental causes and other oral diseases [2] and damage to the supporting tissues of the teeth [3] . *Streptococcus mutans* is one of the most important causes of dental marketing. It is a Gram-positive, facultative anaerobe. This high profile is shown to influence biofilm formation, acid production, and salt and acid tolerance [4,5] . Citrus fruits also contain carbohydrates such as glucose and sucrose. It also contributes proteins to strep bacteria. mutans such as glycosyltransferases-GTFs, biomembrane regulatory proteins, wall-associated protein-coupling proteins, and glucan-experiment proteins, cause mutations in *Streptococcus* spp. and form caries-lesions [6,7] .In association with *Streptococcus sobrinus*, which is considered one of the types of bacteria that cause diseases of rapture, it is considered an anaerobic bacterium, spherical in shape, gram-positive, and its optimal growth temperature is at 37 C° in environments with low pH 6.3. Human oral is home to *Streptococcus* [8] . The aims of the study are to study the diagnosis and identification of *Streptococcus mutans* and *Streptococcus sobrinus* and to study their resistance to antibiotics. Also, the formation of biofilms.

Materials and methods

- **Sample collection:** The study included the collection of (275) oral swabs from patients with tooth decay from the area of caries on the surface of the tooth and from cysts for infected patients and visitors at the Medical City Hospital in Baghdad Governorate with ages ranging between (6-70) years and of both sexes for the period from 1/9/2022 to 1/11/2022. Transport Medium is a brain-heart infusion broth and the samples were then transferred directly to the laboratory for the implantation of swabs on the rich and selective culture media, and then incubated in the incubator for a period of 24 hours at a temperature of 37 C° for diagnostic tests later.
- **Culture media:**
    1. **Enrichment media) Blood agar):** was used to test the ability of bacteria to lyse blood and to know the type of haemolysis.
    2. **Simple media (Nutrient agar medium):** was used to test the ability of bacteria to grow on it.

3. **Urea agar** was used to test the ability of bacteria to produce the enzyme urease.
4. **Motility test medium** was used to study the possibility of bacteria on movement.
5. **Mitis Salivarius Bacitracin AGAR (MSBA):** It is the selective medium of *Streptococcus mutans* prepared according to [9,10].
6. **Voges-Proskauer and methyl red and Indole and Simmmons Citrate:** This medium was used to detect the complete decomposition of sugars and the production of organic acids after 24 hours (MR test) or partial decomposition of sugars and the production of acetone after embrace for 48 hours (VP Test) and the results of Indol and simmonus Citrate test were negative [11].
7. **Brain-Heart infusion broth**: It has been used as a medium to ensure that bacteria are maintained until they reach the laboratory [11].

- **Collection and Cultivation of Sample:** Samples are transplanted directly on Blood agar, and Mitis Salivarius Bacitracin agar (MSBA) and were embraced under intuitive conditions at 37 °C for 18-24 hours, and then saved at 4 C° until diagnostic tests were conducted, the diagnosis was conducted according to the standard methods used for that and contained in [12].

- **Bacterial Identification:** To distinguish between colonies of *Streptococcus mutans* and *Streptococcus sobrunis*, MSBA medium was used. Then, 10% Mannitol and 4% Triphenyl tetrazolium chloride (TTC) were added by diffusion method. and that the color of the medium changed to dark pink, which is evidence of the reduction of (TTC) and the decomposition of mannitol sugar by Mannitol-1-phosphate dehydrogenase [13,14,15] .
- **Biochemical tests:** The tests (Oxidase Test, Catalase Test, IMViC Test, Urease Test, Motily Test, Coagulase Production enzyme, and Lactose Fermentation) were conducted according to the way that each of [16,17].
- **Microscopic examination by using Gram stain.**

- **Identification by VITEK 2 [18] .**

- **Antibiotics Susceptibility Test According to CLSI**: A bacterial sensitivity test for ten antibiotics (Bioanalyse, Turkey) mentioned in Table (1) was performed where all bacterial insolation is studied against antibiotics by Disc Diffusion Method using the Kirby Bauer method [19,20] .

Table 1: Antibiotics and their concentrations

µg/disk	code	Antibiotics	
15	AK	Amikacin	-1
15	AZM	Azithromycin	-2
30	CFM	Cefixime	-3
30	CTZ	Ceftazidime	-4
30	CRO	Ceftriaxone	-5
30	CAZ	Cephalexin	-6
5	CIP	Ciprofloxacin	-7
5	IMI	Imipenem	-8
5	LEV	Levofloxacin	-9
15	TBO	Tobramycin	-10

- **Detection of biofilm formation:** Microtiter Plate (MTP) was used The optical density readings (OD) were scheduled using ELISA with a length of 630 nm [21].

Results

The results showed that 7 isolation and (2.54%) belong to *S. mutans* and *S. sobrinus*, as it was found that 6 isolation (2.18%) dates back to *S. mutans*, while only one isolation (0.36%) was isolated due to *S. sobrinus* (Table 2).

Table (2) percentages of bacterial isolates isolated from patients

(%) Total	(%) negative samples	positive samples on (%) bacteria	Type of bacteria
(100) 275	(97.45) 268	(2.18) 6	S. mutans
		(0.36) 1	S. sobrinus
		(2.54) 7	(%) Total

Current results showed that the percentage of bacteria is higher in females than in males, with 4 (1.45 %) females with *S. mutans*, 1 (0.36 %) female with *S. sobrinus* (Table 3). In the males, 2 (0.72 %) of *S. mutans*, and did not isolate *S. sobrinus* in males. It was also the highest rate of *S. mutans* within the age group 0-10 years, followed by the age group 11-20 years and 61-70 years, with an insulation rate of 3 (50%), 2 (33.33%) and 1 (16.66%), respectively, table (4). As for *S. sobrinus* has been isolated from an 8 -year -old girl, who was suffering from tooth decay with dental deformation and gums.

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Table (3) Percentage of bacterial isolates in both males and females suffering from caries and necrosis Teeth and gingivitis

males and females (%)	Total (%)	Negative samples in females (%)	Positive samples in females (%)	Total (%)	Negative samples in (%)males	positive samples in (%) males	Type of bacteria
٢٧٥ (١٠٠)	١٧٠ (٦١,٨١)	١٦٦ (٦٠,٣٦)	٤ (١,٤٥)	١٠٥ (٣٨,١٨)	١٠٣ (٣٧,٤٥)	٢ (٠,٧٢)	**Strep. mutans**
٢٧٥ (١٠٠)	١٧٠ (٦١,٨١)	١٦٩ (٦١,٤٥)	١ (٠,٣٦)	١٠٥ (٣٨,١٨)	١٠٥ (٣٨,١٨)	٠ (٠)	**Strep. sobrinus**

Table (4) Distribution of *Streptococcus mutans* bacteria by age groups in patients suffering from dental caries, necrosis and periodontitis

(%) Total	Isolates of Strep. mutans in females (%)	Isolates of Strep. mutans in males (%)	Age group
٣ (٥٠)	٢ (٥٠)	١ (٥٠)	**10-0**
٢ (٣٣,٣٣)	١ (٢٥)	١ (٥٠)	**20-10**
٠ (٠)	٠ (٠)	٠ (٠)	**30-21**
٠ (٠)	٠ (٠)	٠ (٠)	**40-31**
٠ (٠)	٠ (٠)	٠ (٠)	**50-41**
٠ (٠)	٠ (٠)	٠ (٠)	**60-51**
١ (١٦,٦٦)	١ (٢٥)	٠ (٠)	**70-61**
٦ (١٠٠)	٤ (١٠٠)	٢ (١٠٠)	**(%) Total**

The results of the study were somewhat compatible with local and international studies. The researcher Flayyih *et al Streptococcus mutans* isolated from primary school students and from patients with private clinics auditors in Baghdad was 15 of the totals of 109 patients with a percentage of percentage (13.79%) using the different selective and rich media to isolate *Streptococcus mutans*. The highest insulation rate for females was compared to males within different age groups, (49.3 %) in the age group of females (4-12) years, and (39.3 %) in the age group of males (4-12) years [22] .

The Identification was based on the form of bacterial isolates that were developed on Enriched, Special and Selective cultures, *S.mutaus* colonies appeared on the Nutrient agar medium in the heart and brain agar extract (BHIA) Heart and brain agar extract in a white color tending to light grey with diameters ranging between 2-1 mm, while *S. sobrinus* colonies also appeared white tending to light gray but slightly larger than *S. mutans* most of their diameters ranged between 5-2 mm (Figures 1 and 2), and the growth of both types of bacteria under anaerobic conditions (5%CO_2) was much better when incubating bacteria under aerobic conditions. This matches previous research findings[22,23,24]. On the medium of blood agar, the two species gave *S. mutans* and *S. sobrinus* is the hematolysis of the Gamma hemolysis type γ, and this is evidence of the inability of bacteria to produce haemolysin, Figure (3), and these results were consistent with what was stated in previous studies [25].

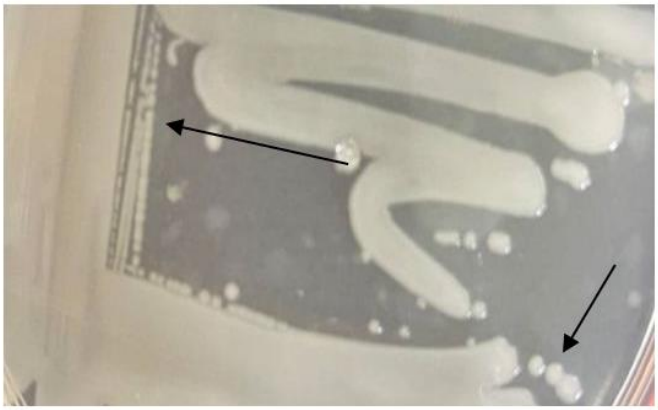

Figure 1: Bacteria colonies *S. mutans* on nutrient agar medium

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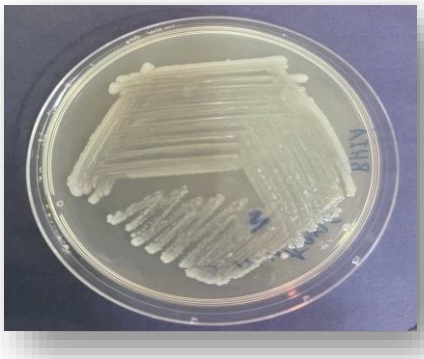


Figure 2: colonies of *S. sobrinus* on Nutrient agar medium

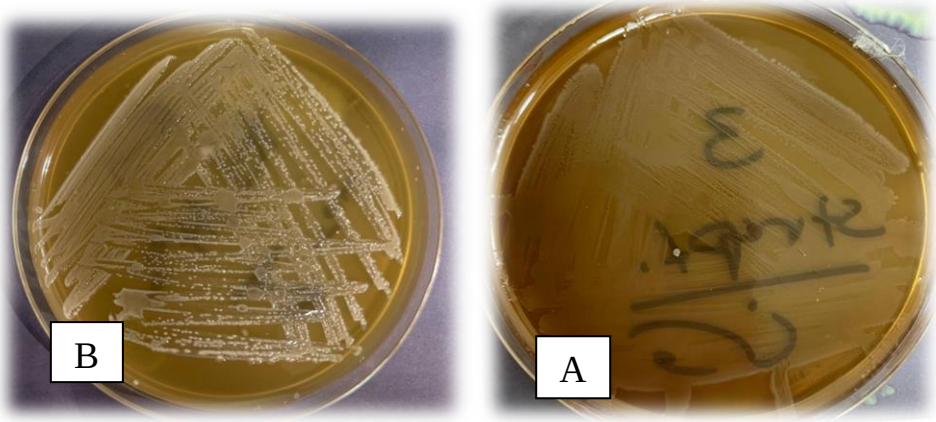
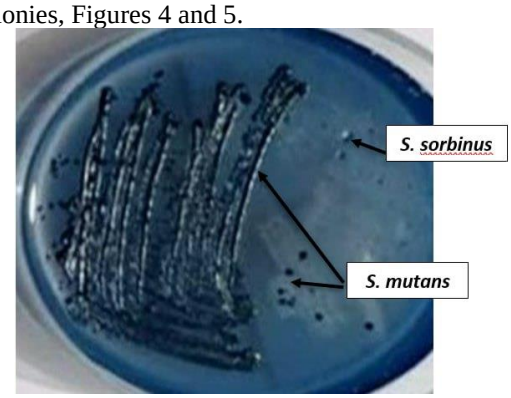


Figure (3)
A- colonies of *S .mutans* on blood agar media after 48 h incubation, γ-hemolysis.
B- colonies of *S. sobrinus* on blood agar media after 48 h incubation, gamma-γ-hemolysis.

For the growth of *Streptococcus mutans* and *Streptococcus sobrinus* on MSBA, bacterial colonies appeared dark blue to pink with light-colored edges after spraying the medium with a 4% solution of triphenyl tetrazolium chloride and aerobic incubation at 37°C for an additional hour. Only the *S. mutans* colonies. are smaller than *S. sobrinus* colonies, Figures 4 and 5.



Figure(4) colonies *S. mutans* and *S. sobrinus* on MSBA medium prior to spraying of Tetrazolium chloride (aerobic incubation at 37°C, for an additional hour)

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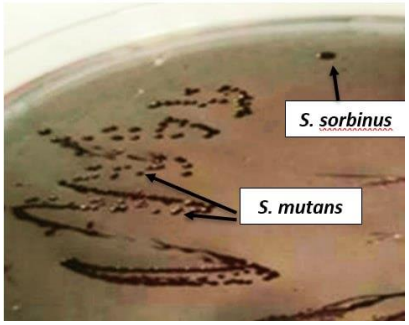

Figure 5: colonies *S. mutant* and *S. sobrinus* on MSBA medium

Microscopy using the Gram stain shows that all bacterial isolates were in the form of cocci tending to the oval positive for the Cram stain, resulting in the form of short chains or pairs (Figure 6). These results are consistent with previous local and international studies [26,27].

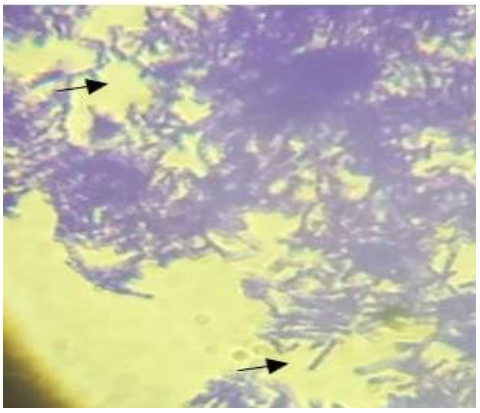

Figure (6) *Streptococcus mutans* positive bacteria under a normal light microscope using an oil lens at full magnification (X1000).

Diagnosis of isolates using biochemical Tests: As for the IMVIC test, which included the Indole test, the Methyl red (MR) test, the Vogus-Proskauer (VR) test and the consumption of citrate, the results for the *S. mutans* and *S. sobrinus* isolates showed that they were negative for the Indole, MR and citrate consumption. While a positive result for the VR test gave an indication that the bacteria produced acetoin after the fermentation of glucose sugar Table (5) and Figure (7). After biochemical tests, it was found that all *S. mutans* and *S. sobrinus* isolates had given a negative result for the Catalase test Figure(8). The isolates also showed a negative result for the Oxidase test, [28,29].

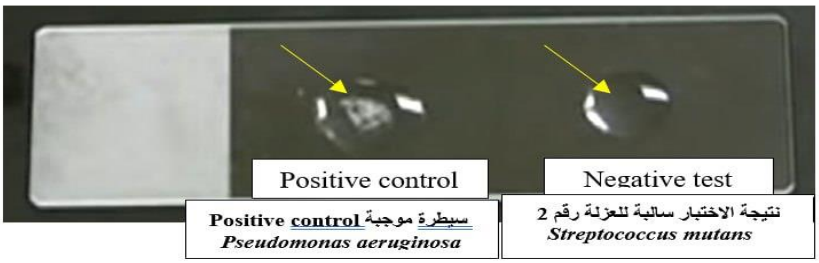

Figure (8) negative result of Isolate No. 2 of *S. mutans* and *S. sobrinus* in the catalase test

As for the production of bacterial isolates of the enzyme urease, the results showed that they were unable to produce the enzyme urease, so gave a negative result for this test. To confirm the diagnosis of isolates as *S. mutans a*nd *Sterp. sobrinus*, they were also examined by the VITEK2 system (Table 6) The results showed that the isolates *of Strepr. mutans* gave positive results for the test of TyrA, LAC, dSOR, dMAN, SAL, AGAL, dMNE, SAC, dMAL, dGAL, Dtre BACl. AGLU, LeuA, AlaA, NOVO, dRAF, OPTO, for *S. sobrinus.* The same results as *S. mutans* isolates except that dSOR had a negative result (Table 6). The results of Abo Baker *et al.* showed that *S. mutans* isolated from patients in the Arab Republic of Egypt with tooth decay and decay had the ability to ferment four main sugars used in their diagnosis: mannitol, sucrose, sorbitol and inulin [30], as well as the two types of bacteria *S. mutans* and *S. sobrinus* have the ability to ferment and consume lactose, mannose

and raffinose, and the production of dextran of sucrose has an effective role in the pathogenesis of bacteria and the cause of decay. While it does not have the ability to consume [31,32].

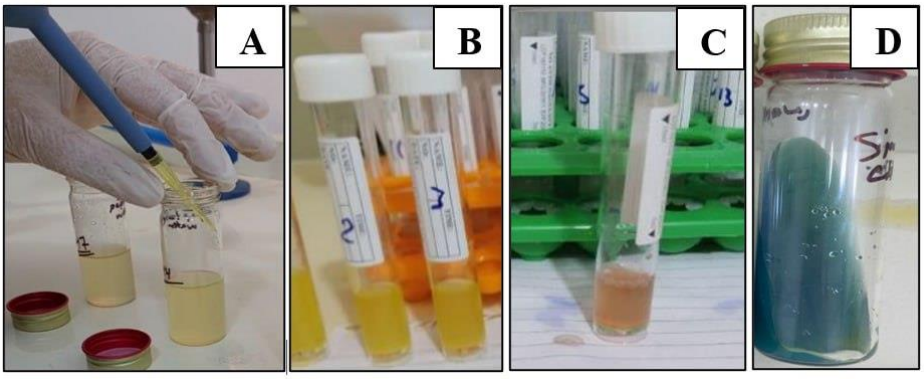

Figure (7) IMVIC biochemical tests
(Negative result of A: indole, B: methyl red, D: citrate consumption), C: positive result of Voges-Proskauer test

Table (6) Phenotypic and biochemical tests for *S. sobrinus* and *S. mutans*

S.sobrinus	S.mutans	Tests	
Spherical, Gram-positive cocci	Spherical, Gram-positive cocci	Gram stain	-١
-	-	Catalase test	-٢
-	-	Oxidase test	-3
-	-	Indole	-4
-	-	Methyl Red	-٥
+	+	Voges-Proskauer	-٦
-	-	citrate consumption	-7
-	-	Urease	-٨
+	+	(LeuA) Leucine arylamidase	-9
+	+	(AGLU) Alfa-glucosidase	-10
+	+	(ALaA)	-11
+	+	(NOVO) Novobiocin Resistance	-12
+	+	(dRAF) D-Raffinose	-13
+	+	(OPTO) Optochin Resistance	-14
+	+	(TyrA) Tyrosine arylamialase	-15
_	+	(dSOR) D-Sorbitol	-16
+	+	(LAC) Lactose	-17
+	+	(dMAN) D-Mannitol	-18
+	+	(SAL) Salicin	-19
+	+	(AGAL) Alpha-galactosidase	-20
+	+	(dMNE) D-Mannose	-21
+	+	(SAC) Saccharose /Sucrose	-22
+	+	(dMAL) D-Maltos	-23
+	+	(dTRE) D-Treahlose	-24
+	+	(dGAL) D-Galactose	-25
+	+	(BACL) Bacitracin Resistance	-26
-	-	Motility	-٢٧
-	-	Coagulase	-28

susceptibility of *S. mutans* and *S. sobrinus* to antibiotics

The bacterial sensitivity test for antibiotics was performed using Muller-Hinton Agar medium and ten types of antibiotics. The current study showed that bacterial isolates show a difference in their resistance and sensitivity to the antibiotics under study. Fig. 10 and Appendix 3 showed that the *S. sobrinus* isolate was moderately sensitive

to Imipenem and sensitive to Amikacin, Tobramycin, Ciprofloxacin, Azithromycin, and Levofloxacin, while it was resistant to Ceftazidime, Cephaloxin, Cefixime and Ciprofloxacin. With regard to Strep.mutans, the results showed that (6) isolates have high resistance to Ceftazidime, Cefixime, Ceftriaxone and Cephaloxin by (100%), followed by Tobromycin 5 (83.33%), Figure (9) Table (7) . While bacterial isolates recorded the highest percentage of sensitivity to Amikacin and Ciprofloxacin (100%), followed by Imipenem (83.33%), then Azithromycin and Levofloxacin (66.66%) respectively.

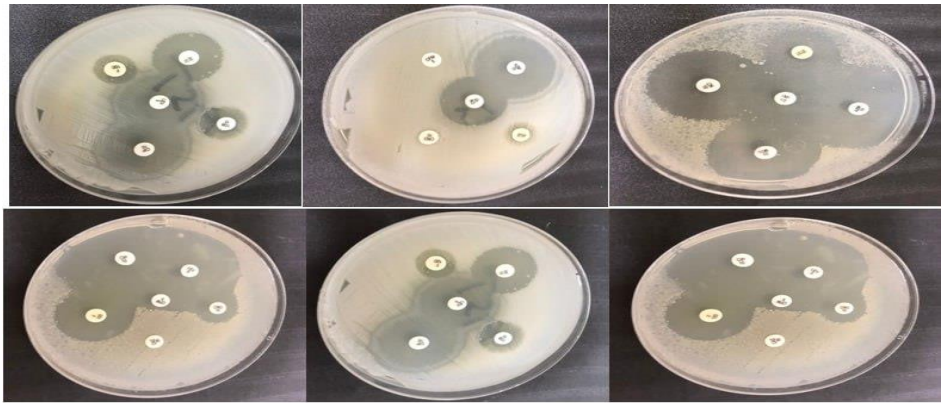

Figure (9) Resistance sensitivity of some isolates of *S. mutans* and *S. sobrinus* to a number of antibiotics (On MHA at 37 for 24 hrs)

Table (7) Susceptibility of (6) isolates of *S. mutans* to antibiotics

Sensitive isolates (S)		**Intermediate isolates (I)**		**Resistant isolates (R)**		**Cod**	**Antibiotics**
Percentage %	Number	Percentage %	Number	percentage %	Number		
١٠٠	٦	٠	٠	**0**	**0**	AK	Amikacin
٦٦,٦٦	٤	١٦,٦٦	١	**16.66**	**1**	AZM	Azithromycin
0	**0**	٠	٠	**100**	**6**	CFM	Cefixime
0	**0**	٠	٠	**100**	**6**	CAZ	Ceftazidime
0	**0**	٠	٠	**100**	**6**	CRO	Ceftriaxone
100	**6**	٠	٠	**0**	**0**	CIP	Ciprofloxacin
83.33	**5**	١٦,٦٦	١	**0**	**0**	IMI	Imipenem
66.66	**4**	١٦,٦٦	١	**16.66**	**1**	LEV	Levofloxacin
0	**0**	٠	٠	**100**	**6**	CN	Cephalexin
16.66	**1**	٠	٠	**83.33**	**5**	TOB	Tobramycin

R: Resistance, I: Intermediate, S: Sensitive

It was observed that the sensitivity of the isolates *S. mutans* and *S. sobrinus* is sensitive to some antibiotics under study, and these results are close to the results of some other studies carried out by a number of researchers. The results of the current study are consistent with previous studies, including the local results reached by Saleh with other researchers in 2023, where they showed isolates of *S. mutans* isolated from Baghdad patients were sensitive to Ciprofloxacin 100%). It was resistant to the anti-Cefixime by (100%) [33] and these results were identical to the results of the current study, and the results reached by the researchers Saleh and Abdel-Rahman in 2017 were consistent with the current study, where it appeared that the isolated *S. mutans* isolates from patients in Thi Qar Governorate were sensitive to anti-Imipenem by (78.3%) and Ciprofloxacin by (60.9%) and the study did not agree with the Ceftriaxone because its results were sensitive to bacteria (60.9%) While it was resistant by (100%) in the current study, the reason may be due to the size of the sample and the number of positive samples, as well as for the antibody Amikacin, which showed resistance, while in the current study, the bacteria were sensitive to it by (100%), the reason may be due to the indiscriminate use of antibiotics [34] The current study agreed with the results of a study in Argentina carried out in 2021 by researcher Bachmeier *et al*., that the bacterial isolates of *S. mutans* were sensitive to Amikacin (83.30%) and Ciprofloxacin (77.75%) [35] . As for *S. sobrinus*, the results showed that that isolation, which was only one isolation that was isolated during the study, had resistance to the antibodies Ceftazidime, Cephalexin, Cefixime and Ceftriaxone, while Amikacin, Tobramycin, Azithromycin and Levofloxacin were

sensitive and were moderately sensitive to Imipenem, and because of the rarity of their isolation, they are present in small proportions in patients with caries and tooth decay, so studies on them are rather few, and the researcher Salman pointed out in 2015 in a study conducted on the sensitivity of *S. sobrinus* as being sensitive to ampicillin and Penicillin antagonists and less sensitive to chloramphenicol and Cefazolin [36] One of the main reasons for bacterial resistance to antibiotics is the horizontal transfer of genes, as it is the reason for the development of the ability of bacteria to resist pesticides and antibiotics through the mechanism of gene transfer from bacteria to other bacteria through plasmids or through viruses known as phages or through sexual filaments [37] .

Susceptibility of bacteria to Biofilm formation

In Figure (10) it was noted that 4 isolates (1, 3, 4, 5) have a high ability to form biofilms, while isolates (2, 6) have a moderate ability to form membranes, as for the isolation of *S. sorbinus* showed the ability to form biofilms strongly, Table (9) showed that the total number of isolates 7 (100%) of bacterial isolates showed the ability to form membranes at varying rates, the results of the examination of the microplate plate of researchers Zayed *et al*. in 2021) showed that (80) isolates were isolated from *S. mutans* from dental plaque samples have the ability to form higher amounts of strong biofilm patterns compared to saliva samples, evidenced by the strong production of biofilm by 35 isolated isolates of dental plaque compared to only 26 isolated from saliva. Also, 4 (5%) isolated isolates of dental plaque showed an ability to produce moderate biofilm versus 12 (15%) isolates isolated from saliva samples. and one isolation (1.25%) isolated from dental plaque showed impairment in the ability to produce biofilms compared to two isolates (2.5%) of isolated isolates from the saliva sample. Comparing the data obtained from isolated samples from saliva and dental plaque samples, it was observed that isolated isolates from dental plaque have the ability to form a stronger biofilm than those isolated from saliva samples [38] . The findings of Alhasani *et al*. in 2020 were somewhat similar to those of the current study, as 261 isolates of bacteria were tested *S. mutans* to see how capable they are to form biofilms, where the results showed 31 isolates (2). (12%) had the ability to form strong biofilms, 46 isolates (18%) had an average biofilm formation capacity, and 184 isolates (72.2%) had a poor biofilm formation capacity [40] .

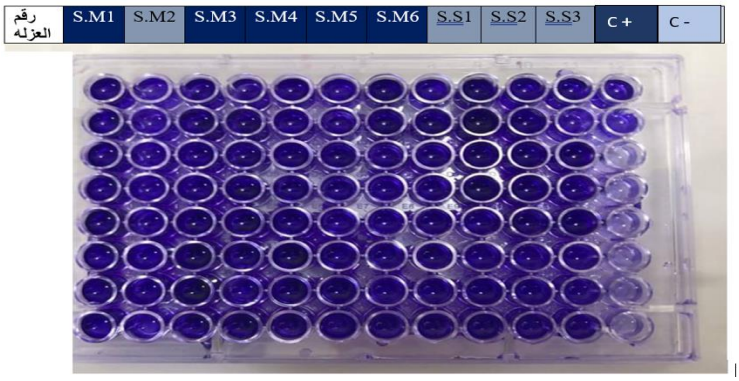

Figure (10): Isolates of *S. mutans* (5, 4, 3, 1) have a high ability to form biofilms, while isolates (2, 6) have a medium ability to form membranes, while isolates of *S. sorbinus* was shown to have the ability to form biofilms in a strong way. C-: Negative Control, C+: Positive Control.

Table (9): Number of *S .mutans* and *S. sorbinus* and the percentages of its ability to form biofilms

Percentage(%)	Number of isolates (N)	susceptibility of bacterial isolates Formation of biofilms
٧١,٤٢	5	Strong
٢٨,٥٧	2	Moderate
0	0	Weak
0	0	No Biofilm
100	7	Total

Conclusion

The results of the current study showed that the percentage of isolation of S. mutans and S.sobrinus isolated from the mouth 2.54%. and Isolates are characterized by their antibiotic constituent (Cefixime, Ceftazidime, Ceftriaxone, Cephaloxin, Tobramycin). and less resistant (Levofloxacin and Azithromycin). And The current study showed that all isolates had the ability to form biofilms differently, where 71.42% had the ability to form strong biofilms and 28.57% formed medium-strength biofilms.

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