Article presented at 2nd International Nineveh Conference on Medical Sciences (2nd INCMS 2023)

Original Article

Phenotypic Identification of *Streptococcus mutans* and Attendant Bacteria From Dentin Caries and Necrotic Pulp, and Its Correlation with Caries Activities Tests

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Abstract

Background: Dental caries is a worldwide disease, which can affect all ages and sexes. The dominant bacteria and the first colonizer in dental plaque are *Streptococcus mutans* due to its adhesion to the hard surface, metabolizing of sugar, and forming tooth cavity if not treated will reach the root pulp, producing necrotic pulp. **Objectives:** The current study was designed to detect *S. mutans* and attendant bacteria in the oral cavity of patients with two types of caries (dentin caries and necrotic pulp), and its relation with MDFT score and Alban test. **Materials and Methods:** Two samples were collected from every 150 patients including one from dentin caries and the other from necrotic pulp, who have attended the Dental Teaching Hospital at the University of Mosul, in Mosul, Iraq with ages ranging between 6 and 76 years, and both sexes, from the beginning of March 2022 to the end of October 2022. **Results:** VITEK 2 compact system used to identify of *S. mutans* at a high number of 39 (32.5%) in dentin caries while *Enterococcus faecalis* gave a high number of 43 (35.8%) in necrotic pulp in addition to other different bacterial species were identified as *Streptococcus sanguinis, Streptococcus gordonii, Streptococcus anginosus, Lactococcus acidophilus, Staphylococcus aureus, Actinomycus* spp*., Staphylococcus hominis, Enterococcus faecium, Lactococcus lactis, Lactococcus garvieae, Lactococcus raffinolactis, Kocuria rosea, Pediococcus acidilactici, Pediococcus pentosaceus,* and the fungal *Candida albicans.* The sex had no significant correlation with other factors, while age had a significant effect at $P < 0.05$ level and pH of saliva had a significant effect at $P < 0.01$ with DMFT and lactobacillus count. The DMFT had a significant effect at $P < 0.01$ level with pH of saliva and count of *S. mutans* and Lactobacillus count at $P < 0.05$. **Conclusions:** The current study concluded that the cariogenic bacteria *S. mutans* was the most prevalent in dentin caries (carious lesion), and *E. faecalis* was the most prevalent in necrotic pulp by using VITEK 2 compact system, and the caries activities tests show pH of saliva had a significant effect with DMFT and lactobacilli colonies count and Streptococci colonies count.

Keywords: Caries activity tests, dentin caries, *Enterococcus faecalis*, necrotic pulp, *Streptococcus mutans*

Introduction

The majority of oral diseases are still caused by dental caries. The oral microbiome has a strong relationship with the development of dental caries.[1] Different tooth structures like enamel, dentin, and cement can get an infection during the caries process, these tissues may decalcify, and the organic components may dissolve. The progression of caries can result in pulpitis, which can spread to the bones and supporting structures.[2] *Streptococcus mutans* are gram-positive cocci, a non-motile facultative anaerobic micro-organism that can metabolize carbohydrates and is considered to be the principal etiological agent of dental caries.[3] The mouth and pharynx are the main environments for *S. mutans*, and they can also be found in dental caries due to the production of acidic metabolites, adhesion to enamel

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_954_23

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Submission: 10-Jul-2023 **Accepted:** 07-Dec-2023 **Published:** 21-Nov-2024

How to cite this article: Shehab EY, Abdullah BA, AlTaie AA. Phenotypic identification of *Streptococcus mutans* and attendant bacteria from dentin caries and necrotic pulp, and its correlation with caries activities tests. Med J Babylon 2024;21:S189-94.

surfaces, the ability to store glycogen and to synthesize Extracellular Polysaccharides (EPS). Acidogenic *S. mutans* can also produce EPS from fructose and glucose in addition to sucrose. *Streptococcus mutans* is the main pathogenic factor causing caries and the prevalent microbe of the tooth biofilm (plaque), so tooth brushing and flossing still are the best ways to prevent and control the plaque formation in noncariogenic commensal species at low pH environments.[4] It is rarely isolated from established root canal infections, but the fact that it is present in infected root canals and causes pulpal destruction suggests that carious lesions may be the main cause of microorganisms in endodontic infections. Although *S. mutans* involvement in dental caries may initially allow invasion of coronal tooth structure, the root canal system's hostile environment may prevent it from growing over time. A crucial step in the pathogenesis of *S. mutans* is their capacity to thrive in an acidic environment by altering the metabolic pathways for sugar and irreversible binding to teeth. In the second stage of invasion, *S. mutans* co-adheres to other microbial species, multiplies, and spreads to new areas in the oral mucosa, all of which are controlled by coordinated.[5] The aim of the current study was to detect *S. mutans* and attendant Bacteria in the oral cavity of patients with two types of caries (dentin caries and necrotic pulp), and their relation with MDFT score and Alban test.

Materials and Methods

Collection of samples

Two samples were collected from each 150 patient first from dentin caries and second from necrotic pulp who attended the Dental Teaching Hospital at the University of Mosul, in Mosul, Iraq with ages ranging between 6 and 76 years, from the beginning of March 2022 to the end of October 2022.

Isolation of *Streptococcus mutans*

The samples were dipped in sterile tubes containing brain heart infusion broth and immediately cultured in the laboratory on mitis salivarius medium for oral Streptococci and M Enterococcus agar for isolation of *E. faecalis* and Rogosa SL agar for isolation of *lactobacillus* spp. The facultative anaerobic incubation method was used for 24–48 h. Subsequently, colony characteristics were demonstrated using traditional methods including Gram stain, biochemical tests, catalase, and sugar fermentation in brain heart infusion broth supplemented with 0.02% of phenol red and 10% of each carbohydrate (mannitol, sorbitol, raffinose, and sucrose). Bacterial identification was then confirmed using VITEK 2 compact system.[6]

Caries activities tests

(1) Lactobacillus colony count test, which estimates the number of acidogenic and aciduric in the patient's saliva by counting the number of colonies on Rogosa agar after incubation with a sample of saliva.[7]
(2) *Streptococcus mutans* level in saliva: by incubating mitis salivarius agar (MSA) with the addition of a high concentration of sucrose (20%) and (0.2) U bacitracin to inhibit the growth of the majority of non-*S. mutans* colonies, the number of *S. mutans* Colony Forming Units (CFU/mL) per volume of saliva can be determined.[8]
(3) Albans test: It is a simplified substitute for the Snyder test uses unstimulated saliva and the change of color from bluish green (pH 5) to definite yellow (pH 4 or below) and the daily results are collected for 4 days and recorded on the patient's chart [Table 1].[9]
(4) pH of saliva has a pH normal range of 6.2–7.6 and it decreased in dental caries and lower to the acidic pH.
(5) DMFT index is the sum of the number of decayed, missing due to caries, and Filled teeth in the permanent teeth? The mean number of DMFT is the sum of individual DMFT values divided by the sum of the population, Note: If a tooth has both a caries lesion and a filling it is calculated as D only.[10]

Statistical analysis

The Statistical Analysis System (SAS, 2018) A program was utilized to determine how various circumstances affected the study's parameters. To meaningfully compare between means, the Analysis of Variation (ANOVA) method known as the LSD test was employed. The chi-square test was employed to compare percentages (0.05 and 0.01 likelihood) in the study significantly.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by a local

Table 1: Alban test scores

Color change	Score
No change in color	Negative
beginning of the color shift (from medium top to bottom)	+
One-half color change (from top down)	++
Three-fourths color change (from top down)	+++
All the tube color changes (from top down)	++++

ethics committee according to document number 4189 (on February 1, 2022) to get this approval.

Results

In the current study, 150 oral samples were collected from dentin caries and necrotic pulp, and were primarily cultured on three agar media; mitis salivarius bacitracin agar, M enterococcus agar, and Rogosa agar. Out of 150 samples, only 120 (80%) were given positive culture, whereas 30 (20%) samples showed no bacterial growth. The dentin caries gave positive culture at 66 (55%) while necrotic pulp gave 54 (45%) and the negative culture was 9 (30%) and from necrotic pulp dentin caries were at 21 (70%), which may be attributed to antibiotics administration or infection by another microbial causative agent, that might need a special technique for their detection such as viruses, fungi, and others as in Table 2.

The current study indicated by VITEK 2 compact system the different bacterial species that were isolated from dentin caries and necrotic pulp as *S. mutans* that dominant at a high number in dentin caries while *E. faecalis* was dominant in necrotic pulp in addition to different bacterial species like *S. sanguinis, S. gordonii, S. anginosus, L. acidophilus, S. aureus, Actinomycus* spp., *S. hominis, E. faecium, L. lactis, L. garvieae, L. raffinolactis, K. rosea, P. acidilactici, P. pentosaceus,* and the fungal *Candida albicans* as shown in Table 3.

Caries activity tests (CATs) are a group of tests used for expected caries in an individuals and age in our study appeared increased of caries in females 60% and in males 40% as shown in Figure 1 whereas age group is classified into three groups; first under 20 years old, second between 21 and 40 years old, and third above 41 years old, and the result shows increased caries in second group in 51% as show in Figure 2. And the common method used to identify caries-susceptible people is estimating the increased number of cariogenic bacteria such as lactobacilli and mutans Streptococci in saliva, and in the current study we focus on the correlation between acid production in saliva from cariogenic bacteria and dental caries, the acids will increase the pH of saliva and in our result the high percentage about 37.5% in the group of 6.1–7, which is around a normal range of saliva about 6.2–7.6 as shown in Figure 3.

Alban CAT (modified Snyder test) for assessment of caries activity used to detect the severity of caries and four plus referred to high caries which is formed from acid accumulation in the medium from cariogenic bacteria

Table 2: Numbers and percentages of bacterial isolates

Type of caries	Positive culture		Negative culture	
	Dentin caries	Necrotic pulp	Dentin caries	Necrotic pulp
No. (%)	66 (55)	54 (45)	9 (30)	21 (70)
No. (%)	120 (100)		30 (100)	
Total	150			
P value = 0.001				

Table 3: Numbers and percentages of *S. mutans* and other bacteria that were isolated from the dentin caries and necrotic pulp

Bacterial species	Dentin caries	%*	Necrotic pulp	%*
Streptococcus mutans	**39**	32.5	20	16.7
Enterococcus faecalis	14	11.7	**43**	35.8
Streptococcus sanguinis	12	10	2	1.7
Streptococcus gordonii	10	8.3	1	0.8
Streptococcus anginosus	15	12.5	0	0
Lactococcus acidophilus	32	26.7	5	4.2
Candida albicans	10	8.3	19	15.8
Staphylococcus aureus	16	13.3	8	6.7
Actinomyces spp.	14	11.7	1	0.8
Staphylococcus hominins	3	2.5	0	0
Enterococcus faecium	3	2.5	18	15
Lactococcus lactis	3	2.5	0	0
Lactococcus garvieae	4	3.3	0	0
Lactococcus raffinolactis	2	1.7	0	0
Kocuria rosea	2	1.7	0	0
Pediococcus acidilactici	2	1.7	0	0
Pediococcus pentosaceus	2	1.7	0	0
Total number of isolates	183	—	117	—

*Percentage was calculated from total isolated bacteria (183 for sort caries and 117 for necrotic pulp)

Bold values indicate the highest isolates in two types of caries

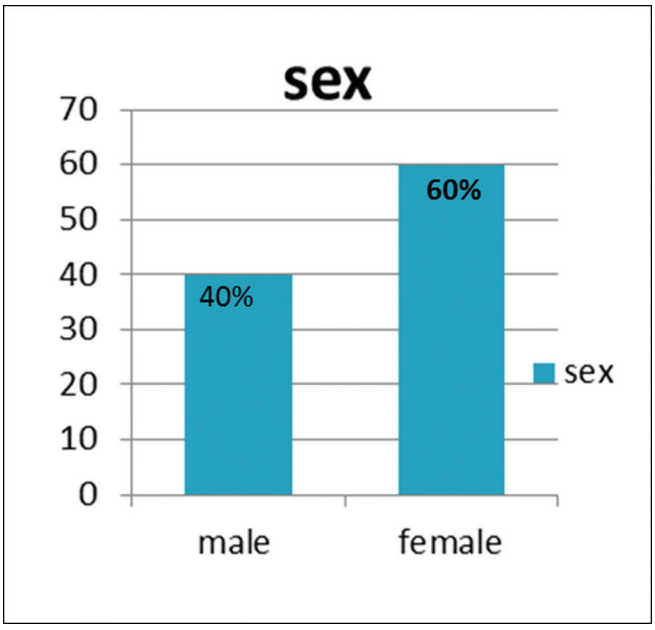

Figure 1: Frequency of sex in all patients

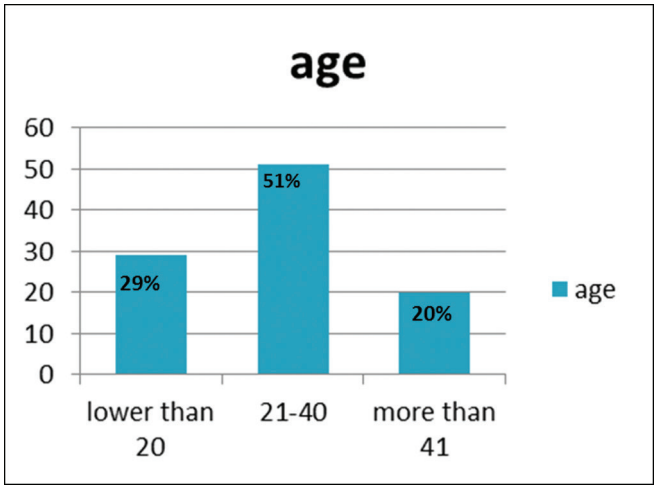

Figure 2: Frequency of age in all patients

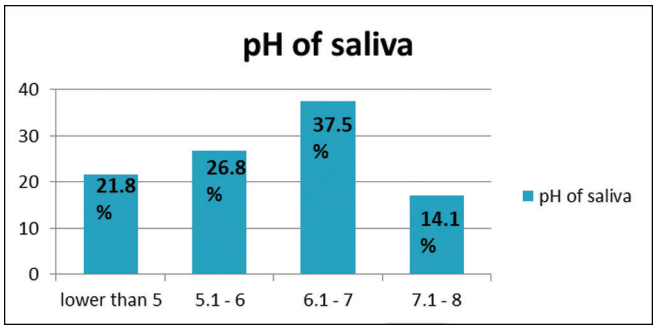

Figure 3: Frequency of pH of saliva in all patients

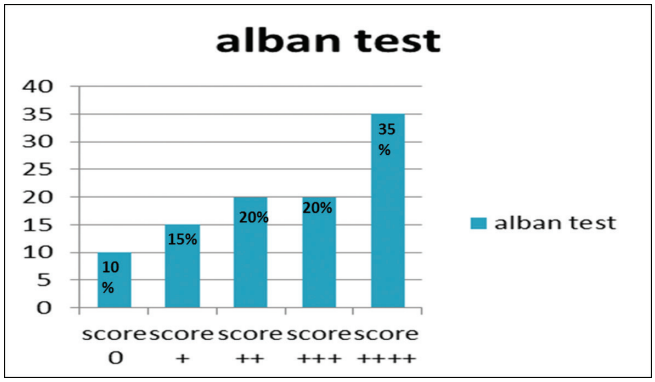

Figure 4: Scores of the Alban test

especially *S. mutans* and *Lactobacillus* spp. and in this study 35% of samples show high Alban test (++++) as in Figure 4.

The correlation between factors of CATs is represented in Table 4 and appears the sex had no significant correlation with other factors while the pH of saliva had a significant effect at $P < 0.01$ with DMFT and lactobacillus count, and the age factor had significant at $P < 0.05$ level with lactobacillus count. The DMFT had significance at $P < 0.01$ level with pH of saliva and *Streptococcus mutans* count and had significance at $P < 0.05$ with lactobacillus count.

Discussion

The prevalence of microbial diseases has dramatically increased over the past few decades. The superbug that is, producing dental caries are microorganisms. *Streptococcus mutans* is the most significant etiological agent of dental caries, however many facultative anaerobic bacteria predominate the microbial community of dental caries. Researchers and scientists are still looking into how many elements, including genetic and environmental factors, contribute to the pathogenesis of this multifactorial disease.[11,12]

The high percentage of *S. mutans* in this study (32.5%) is the most common bacteria prevalent in dentin caries (carious lesion) with a high score of DMFT 15.8 which can be approved with other studies[13] found 52% of *S. mutans* in adult dental caries which is related to a high score of DMFT 16.5. and[3] found *S. mutans* strains were isolated from a total of 140 clinical samples with a frequency of 75.7%. and this result also agrees with.[14] Patients with tooth pain who visited a dentist revealed that dental pulp was involved as a result of deep caries, 10% of caries patients had endodontic operations because they obtained care late.[15] Showed that untreated dental decay or dental caries reach the pulp and become an endodontic problem requiring treatment to reserve their teeth. In this study, the prevalence of *E. faecalis* in necrotic pulp was about 35.8% and this was approved with research of the study.[16] *Enterococcus faecalis* was detected in 10.2% and 79.5% of samples by culture and PCR.[17] The present study focuses on two factors that may affect dental caries, age, and sex, and also focuses on caries activities tests, *S. mutans* count, *Lactobacillus* spp. count, pH of saliva, MDFT score, and Alban test. The results of bacterial counting appeared highly significant cumulatively increasing in CFU (colony forming unit) with the severe dental caries by counting the bacteria in saliva and these results agree with the study by Katiyar *et al.*[18] According to sex, the present study indicated a high percentage of females compared to males at 60% and 40%, respectively, as in Figure 1, and this result accordance with many studies.[14,19-21] The prevalence of dental caries was low among adolescent males whereas a study[22] reported a high prevalence in male tobacco chewers and tobacco smokers, as a predisposing factor of dental caries and also there are many factors induce caries in females like hormonal change in puberty or pregnancy. Whereas the higher percentage in the middle age group (21–40) was 51% as shown in Figure 2, this result may be attributed to various factors such as puberty changes, high amount of sugar intake, bad oral hygiene, increased consumption of fast food, and ignorance of dentist visits. A study by Lin *et al.*[23] reported the prevalence of caries in school children between 6 and 12 years old, and a study by Hoch[24] reported the high prevalence in children between 3 and 6 years old. Whereas a study by

Table 4: Correlations between *Streptococcus mutans, Lactobacillus acidophilus* count with different factors: Sex, age (years), pH of saliva, and DMFT

Factors	Significant	Sex	pH	Age (years)	DMFT	*Streptococcus mutans* count	*Lactobacillus acidophilus* count
Sex	Pearson correlation	1	0.090	0.175	0.061	0.052	0.126
	Sig. (2-tailed)		0.328	0.056	0.505	0.573	0.170
	N	120	120	120	120	120	120
pH	Pearson correlation	0.090	1	−0.038	−0.623**	158	−0.356**
	Sig. (2-tailed)	0.328		0.683	0.000	0.085	0.000
	N	120	120	120	120	120	120
Age	Pearson correlation	0.175	−0.038-	1	0.080	−0.114-	0.230*
	Sig. (2-tailed)	0.056	0.683		0.386	0.215	0.011
	N	120	120	120	120	120	120
DMFT	Pearson correlation	0.061	−0.623**	0.080	1	0.074**	0.225*
	Sig. (2-tailed)	0.505	0.000	0.386		0.423	0.013
	N	120	120	120	120	120	120
Strept	Pearson correlation	0.052	−0.158	−0.114	0.074**	1	0.017
	Sig. (2-tailed)	0.573	0.085	0.215	0.423		0.857
	N	120	120	120	120	120	120
Lacto	Pearson correlation	0.126	−0.356**	0.230*	0.225*	0.017	1
	Sig. (2-tailed)	0.170	0.000	0.011	0.013	0.857	
	N	120	120	120	120	120	120

*Correlation is significant at the $P < 0.05$ level (2-tailed)

**Correlation is significant at the $P < 0.01$ level (2-tailed)

Borg-Bartolo *et al.*[25] reported the highest prevalence in persons aged 65–74 years. The present study points out some of the CATs with pH being one of the caries tests which decreased saliva to acidic and even reached under 5 pH and it was by study done by Piroozmand *et al.,*[26] who have reported decreased salivary pH and increased dental caries and this result is scientific because caries play a role in the fermentation of sugar and producing acid which accumulates and decreases of pH of saliva as in Figure 3. Alban test in this study was given about 35% for score (++++) and it is one of the CATs used to check the acid production ability in the oral cavity by measuring the degree of occurrence of bacteria in the saliva and oral biofilm by colorimetric method and is a microbiological parameter and the different color results were interoperated into a different score which was tabulated and analyzed statistically. This result was similar to that of Thakur *et al.*[9] as shown in Figure 4.

Correlations results were reported above with many researchers[27] clarify that age, and DMFT are risk factors significantly associated with dental caries prevalence amongst Egyptian adults while no significant correlation between gender and caries has been. As study by Hong-Ying *et al.*[28] shows the significant correlation between sex and age and shows a high prevalence of caries in females.[29] A review article from 77 articles in 14 countries from 2000 to 2019 shows dental caries was found to be high among children in many of the studies. The key determinants of dental caries were found to include factors related to child characteristics, family background, and oral hygiene. The high dental caries prevalence emphasizes the need to address the prevailing modifiable socio behavioral and socioeconomic determinants by translating them into effective oral health prevention policies and programs. Also, another review article[30] from India shows gender proved to be a risk factor for oral diseases in India and male high prevalence and had a significant correlation with age (65–74 years and root caries were 3.9%) and DMFT and pH of saliva and had significant correlation in bacteria count either in *S. mutans* or lactobacillus species. Dental caries are associated with many types of bacteria gram-positive and negative bacteria like *Sphingomonas paucimobilis* which play a role in carious lesions and open another line for the detection of different genera of bacteria.[31] Also, *E. faecalis* is a major factor in the development of chronic endodontic infections.[32]

Conclusion

The current study concluded that the cariogenic bacteria *S. mutans* was the most prevalent in dentin caries (carious lesion), and *E. faecalis* was the most prevalent in necrotic pulp by using VITEK 2 compact system, and the caries activities tests showed pH of saliva had a significant effect with DMFT and lactobacilli colonies count and Streptococci colonies count.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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