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Detection of Bacterial and Fungal Pathogens Causing Dental Caries in School Children in Khartoum State, Sudan

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Abstract

Dental caries is the localized destruction of dental tissues by bacterial activity; the carious lesion is the result of demineralization of enamel-and later of dentine by acids produced by plaque microorganisms as they metabolize dietary carbohydrates. This study was conducted in two schools in Khartoum state, aimed to isolate and identify bacterial and fungal pathogens from dental caries infected school children. A total of 50 male students were enrolled in this study. The age ranging from 6 to 14 years, with mean of 10.9 + and 2.5 SD. (62%) of participants said they suffer from tooth pain and 19 (38%) of them visited the dentist before. According to eating habits majority of the students 48 (96°) drink milk, 47 (94%) eat sweets/candy, 38 (76%) eat crisps and 35 (70%) take soft drink. Samples were cultured and isolated bacterial and fungal pathogen were identified microscopically and by biochemical tests. Culture results were as follows: 41(82%) of samples showed bacterial and fungal growth, of those positive culture 34 (68%) showed bacterial growth, 1 (2%) fungal growth and 6 (12%) mixed growth (both bacterial and fungal). *Streptococcus mutans* 27(54%) was the predominant bacteria followed by *Enterococcus faecalis* 11(22%), Lactobacillus. spp 1(2%), and staphylococcus aureus 1(2%). Seven of the specimens (14%) showed growth of candida albicans. There was no statistically significant association between age/consumption of sweet food and microbial isolation. Significant association was detected between microbial growth and brush change (P. value = 0.041) but not with frequency of teeth brushing. In conclusion, *Streptococcus mutans* was the predominant bacterium isolated from caries lesions, followed by *Enterococcus faecalis*. The disease was mostly caused by bacteria, with only one type of fungus, *Candida albicans*, being isolated. No association was found between the microbial cultures and age, sugar intake, or oral hygiene. The frequency of brush change has significant association with microbial isolation.

Keyword: Dental Caries, *Streptococcus Mutans*, *Candida Albicans,* School Children, Sudan.

INTRODUCTION

Dental caries is one of the most common human diseases and affects the vast majority of individuals worldwide. It is defined as the localized destruction of dental tissues by bacterial activity; the carious lesion is the result of demineralization of enamel–and later of dentine– by acids produced by plaque microorganisms as they metabolize dietary carbohydrates [1]. These microorganisms are found as part of the oral flora and must possess certain caries-promoting characteristic, such as: the ability to rapidly transport fermentable sugars and convert them to acids, the ability to grow and metabolize in low Ph environment and the production of extracellular polysaccharides (EPS) and intracellular polysaccharides (IPS) which contribute to the biofilm matrix [2]. The first clinical sign of the disease is a white spot, which is the expression of the enamel subsurface demineralization, with symptoms of teeth pain, discomfort, eating impairment, loss of tooth and delay language development [3]. Risk for caries include physical, biological, environmental, behavioral, and lifestyle-related factors such as high numbers of cariogenic bacteria, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, and poverty [4]. In those who don't regularly brush their teeth, the prevalence of caries rises overall. Wei and Hyman claim that most children don't brush for long enough or well to fully achieve deplaquing [5]. 60 to 90% of schoolchildren are found to be affected by the disease [6], which is due to their unlimited consumption of sugary sub-stances, poor oral care practices and inadequate health service utilization [4]. Although these

factors are important in caries development, the role of microbial factors cannot be ignored.

In oral cavity, tooth as mineralized hard tissue provides non-shedding surfaces for oral microbes to colonize. Attached bacteria, embedded in polymeric matrix, form microcommunities called dental plaque, acidogenic bacteria that form these plaques are recognized as the culprit for caries initiation and progression [6]. The main acidogenic and aciduric Gram-positive bacteria involved in the instigation and progress of dental caries include *Streptococcus mutans* -as a major etiological agent of dental caries- *Actinomyces*, *Lactobacillus. spp, Bifiodobacterium. dentium, Peptostreptococcus. spp, Staphylococcus aureus, and Enterococcus fecalis.* Fungi can also cause the disease with *Candida albicans, C. tropicalis, C. glabrata* as some of the most common involved pathogens [7]. Dental caries is a major public health problem globally and is the most widespread noncommunicable disease (NCD). Almost half of the world's population is affected by dental caries, making it the most prevalent of all health conditions [7]. Untreated carious cavities especially when associated with pain may influence children's' physical and psychological development as well as school and daily life achievements. Despite the fact that Sudan is one of the countries that suffer from a high prevalence of dental caries among children, there is little available data on dental caries in Sudan and only few studies include information about socio-economic and behavioral factors. Identifying the different risk factors will help in understanding the causes of high prevalence and thus caries can be controlled and new preventive measures can be attained.

MATERIALS AND METHODS

Study Design and Population

This is a descriptive, cross-sectional study was conducted in Khartoum state. Samples were collected from students attending male schools in Khartoum. Students known with dental caries were included in this study. Students aged 6-14 years with dental caries lesions attending the mentioned schools were included. Students with upper respiratory tract infections were excluded from the study. Fifty (n=50) swab samples from infected teeth were collected for this study. Data were collected through non-self-administrated questionnaire.

Collection of Samples

Samples were collected from infected teeth of students by sterile cotton swab and were properly labeled for each patient. The swabs were transported to the Medical Microbiology Laboratory in Sudan University of Science and Technology in ice bags for culture, within 2-4 hours after collection.

Isolation of Fungi

Swabs were inoculated into well labelled Sabourauds dextrose agar (SDA) plates and incubated at room temperature until there were visible colonies. The growth was identified based on their morphological and cultural characteristics.

Isolation and Identification of Bacteria

Specimens were inoculated onto blood agar and CLED agar plates which were incubated for 24 hours at 37°C aerobically, an optochin dis was added to the well of the blood agar plates and incubated in a candle jar to provide 5-10% CO2. Emergent colonies were identified according to standard bacteriological methods.

Plates were examined at the end of incubation period for fermentation on CLED agar & hemolysis on Blood agar and the morphological characters (size, shape, color and odor) were recorded. Isolated colonies were purified and examined with Gram stain, the smear was examined microscopically, first with the 40X objective to check the staining, and then with the oil immersion objective to report the bacterial morphology (Cheesbrough, 2005). Identification was done using different biochemical tests; Catalase test, DNAse test, Litmus milk test, Bile esculin test and inoculation on Mannitol salt agar (MSA).

Identification of Fungi

Gram's stain was performed for yeast suspected colonies which revealed Gram positive yeast cells. In addition, Germ tube test (GTT) was done for yeast growth; Positive test: presence of short lateral filament (germ tube) for *C. albicans*, Negative test: yeast cell only for C. non *albicans* (Bhavan *et al*., 2010).

Data Analysis

The data collected had been analyzed by using SPSS (statistical package for social sciences) version 16. Data were presented in the form of tables and figures. A chi-square test was used to detect the correlation between variables. A *P. value* of <0.05 was considered significant for all results.

RESULTS

A total of 50 male students suffering from dental caries were enrolled in this study. The age ranging from 6 to 14 years, with mean ± SD age of 10.9 ± 2.5 years. Age groups were classified into three categories: 6-8 years represented 12(24%) of the children, 9-11 years were 12(24%) and 12-14 years were 26(52%). The maximum number of participants 26 was in the age group of (12-14 years).

(62%) of participants said they suffer from tooth pain and 20(40%) of them visited the dentist before as pointed in figure [1]. According to eating habits majority of the students 48 (96%) drink milk, 47 (94%) eat sweets/candy, 38 (76%) eat crisps and 35 (70%) take soft drink as illustrated in figure [2]. from these 25(50%) said they eat/drink the previously mentioned foods every day, 19(38%) eat/drink them several times a day and 6(12%) eat them several times a week as shown in figure (3). As for the oral hygiene habits, 26 (52%) brush their

teeth one time a day, 21 (42%) brush them two times a day, 3(6%) brush them more than two times a day, and 1(2%) brush them once in a while, as in figure (4). 28 (56%) of students take 2 minutes to brush their teeth, 16 (32%) take one minute, and 6 (12%) take more than two minutes, as pointed in figure (5). Majority of students 25 (50%) change their tooth brush every six months, 20(40%) change it every three months, 4(8%) change it as occur and 1(2%) change it every week, as shown in figure (6). 39 (78%) of respondents said they rinse their mouth with water after meals, as represented in figure [7].

Culture results were as follows: 41(82%) of samples showed bacterial and fungal growth, while 9 (18%) were negative. Of those positive culture 34 (68%) showed bacterial growth, 1 (2%) fungal growth and 6 (12%) mixed growth (both bacterial and fungal) as in table (1). Streptococcus mutans 27(54%) was the predominant bacteria followed by *enterococcus faecalis* 11(22%), *lactobacillus. spp* 1(2%), and *staphylococcus aureus* 1(2%) table (2). Seven of the specimens (14%) showed growth of *candida albicans* table (3). Regarding age group 10(20%) of the children with positive microbial growth were in the age group (6-8) , 8 (16%) were in (9_11), and 23(46%) were in [12-14], with statisial insignificant difference between microbial isolation and age as in table (4). Fourty seven of children eat sweets/candy, 38/47 of them had positive result and 9/47 were negative, with statisial insignificant association between consuming candy and microbial growth table (5). The frequency of eating snacks also showed no statistically significant association with microbial growth Table (6).

For oral hygiene habits, 24(48%) of children with positive microbial growth change their brush every 3 months, 14(28%) change them every 6 months and 2(4%) change them as occur with statistically significant association between brush change and microbial growth (P. value = 0.041) as in table (7). 23(46%) of children with positive culture brush their teeth once a day, 16(32%) brush them twice a day and 2(4%) brush them more times, with statistical insignificant association between frequency of brushing teeth and microbial growth table (8).

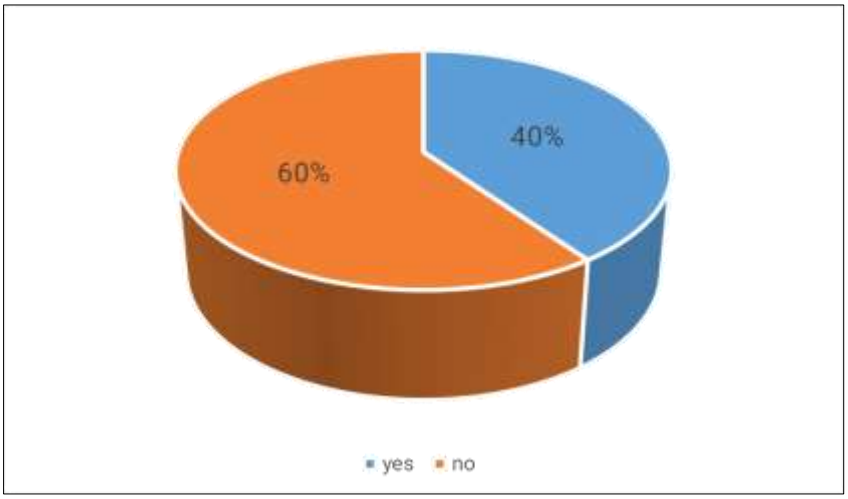

Figure 1: Distribution of study population according to visiting the dentist. (60%) of participants said they suffer from tooth pain and 20(40%) of them visited the dentist before

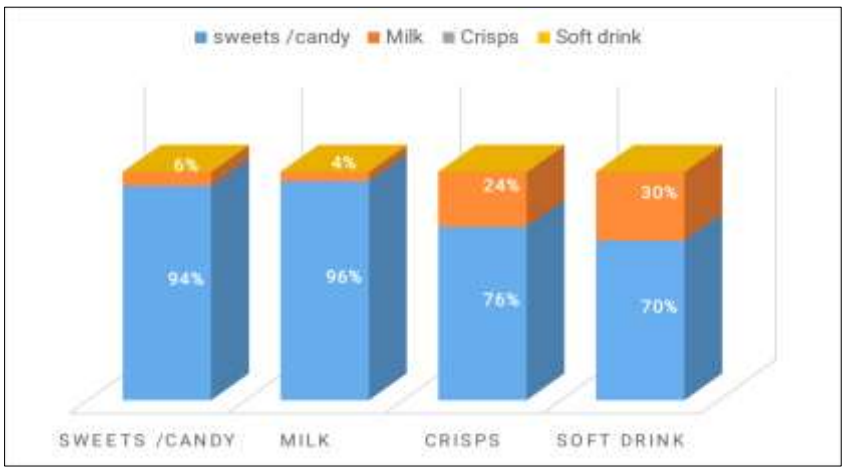

Figure 2: Distribution of the study population according to eating/drinking habits

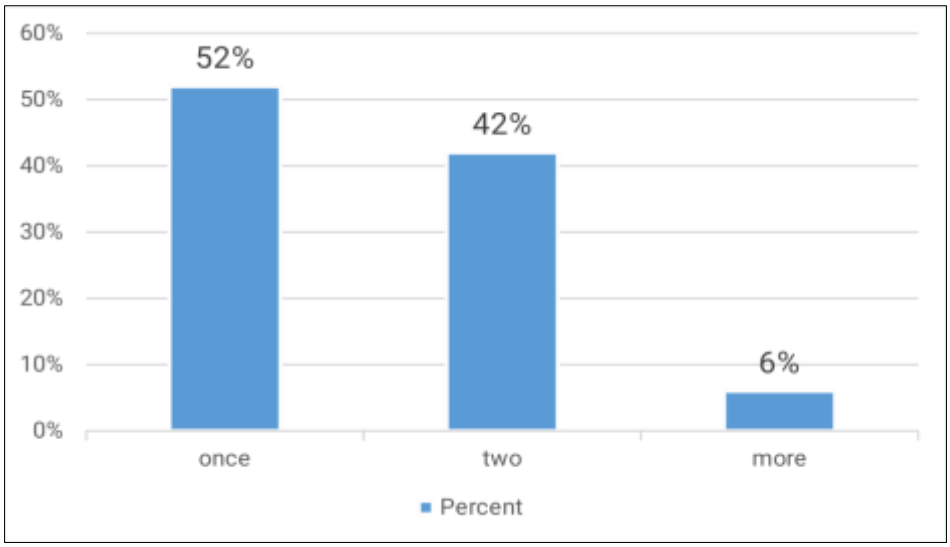

Figure 3: Distribution of study group according to eating/drinking snacks

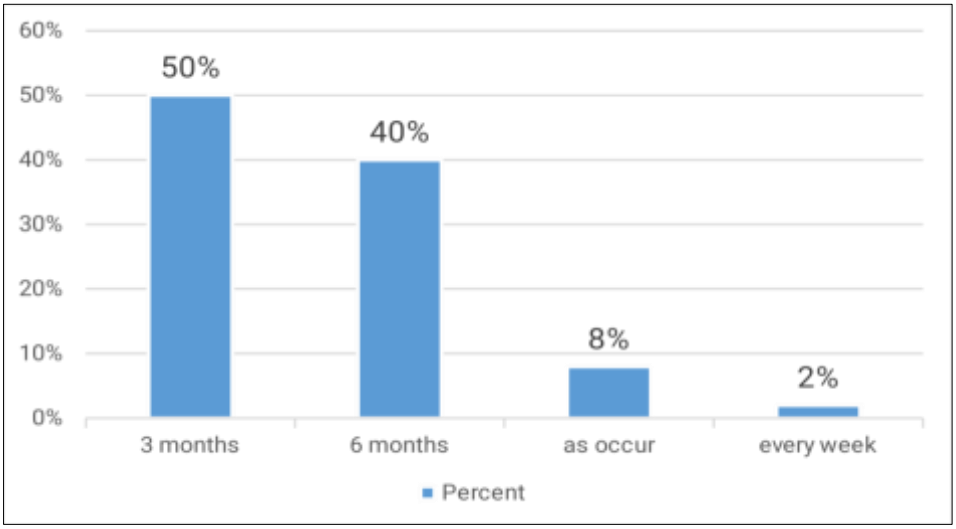

Figure 4: Percentage of participants according to frequency of brushing teeth per day

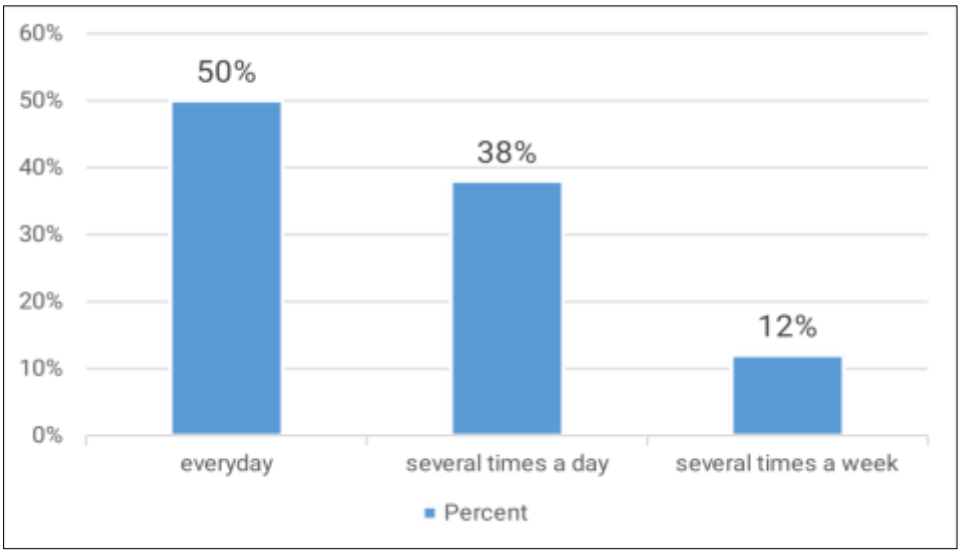

Figure 5: Percentage of participants according to the time they take to brush their teeth per minutes

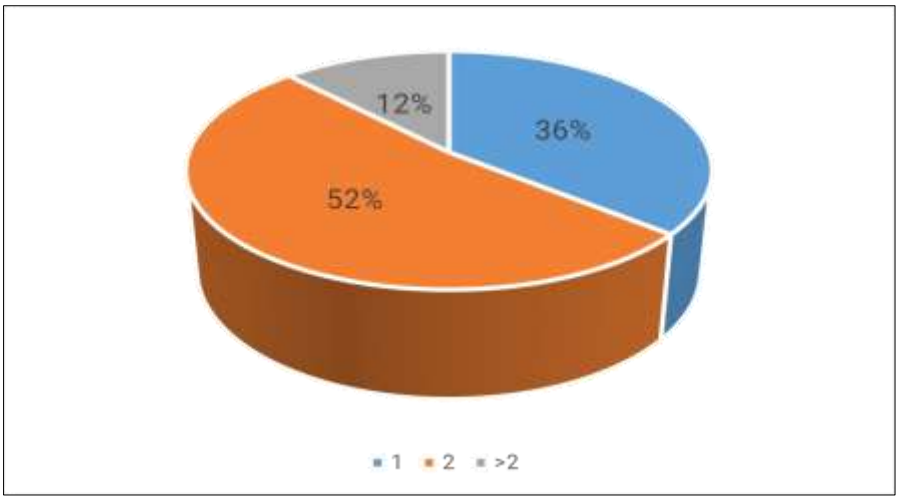

Figure 6: Distribution of toothbrush change among participants

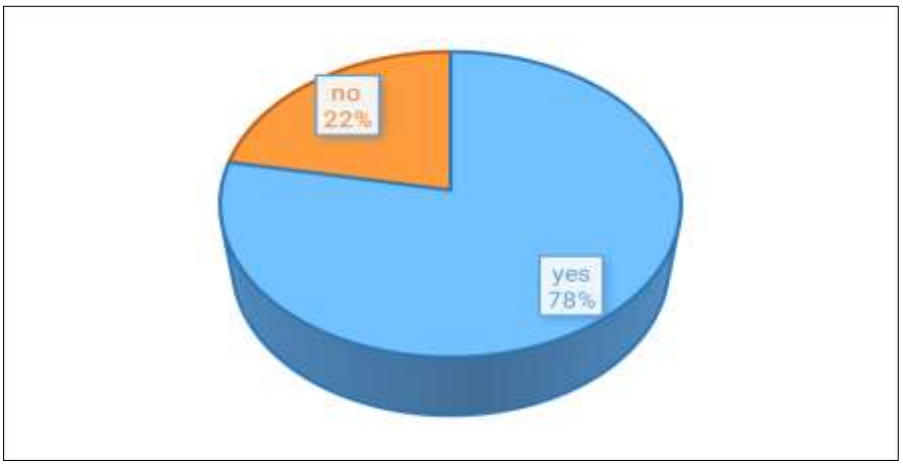

Figure 7: Distribution of participants rinsing their mouths with water after meals

Table 1: The frequency of the bacterial and fungal growth

Culture Result	Frequency (%)
Bacteria	34(68%)
Fungi	1(2%)
Mixed Result (Bacteria & Fungi)	6(12%)
Not Detected (Negative)	9(18%)
Total	**100(100%)**

Table 2: Frequency of the bacterial isolates

Isolated bacteria	Frequency	Percent
S.mutans	27	54%
E.faecalis	11	22%
lactobacillus	1	2%
S.aureus	1	2%
no growth	10	20%
Total	**50**	**100%**

Table 3: Frequency of fungal isolates

The isolated fungi	Frequency	Percent (%)
no growth	43	86%
Candida albicans	7	14%
Total	**50**	**100%**

Table 4: Association between Microbial growth and Age

age group	Result			P value
	growth	No growth	Total	
6-8	10	2	12	0.264
9-11	8	4	12	
12-14	23	3	26	
Total	**41**	**9**	**50**	

Table 5: Association between consumption of candy and microbial growth

Consumption of candy	Result			P value
	growth	No growth	Total	
yes	38	9	47	0.403
no	3	0	3	
Total	**41**	**9**	**50**	

Table 6: Association between frequency of eating snacks and microbial growth

Eating times	Result			P value
	growth	No growth	Total	
everyday	19	6	25	0.161
several times a day	18	1	19	
several times a week	4	2	6	
Total	**41**	**9**	**50**	

Table 7: Association between brush change and microbial growth

change brush	Result			P value
	growth	No growth	Total	
3 months	24	1	25	0.041
6 months	14	6	20	
as occur	2	2	4	
every week	1	0	1	
Total	**41**	**9**	**50**	

Table 8: Association between frequency of brushing teeth and microbial growth

Brushing times	Result			P value
	growth	No growth	Total	
once	23	3	26	0.429
twice	16	5	21	
more	2	1	3	
Total	**41**	**9**	**50**	

DISCUSSION

Fifty samples were enrolled in our study, from which 82% showed microbial growth and 18% were negative, this finding was comparable to Al-mudallal *et al*., [8], who had the same sample size, this is probably because the bacteria were anaerobes which were not included in this study. The age of participants was divided into 3 groups; 6-8 years, 9-11 years and 12-14 years. Although the highest frequency of infection was seen in the third group (52%), there was no significant association between age and presence of microbial growth (P value = 0.264) Findings of Kiros *et al*., [9], study Also agreed with our results. Other studies such as Mulu *et al*., [3], showed significant association between age and dental caries infection. Although the majority of the participants with positive cultures consumed sweets 38/41, and soft drinks 30/41, there was no significant association between consumption of sweets/soft drinks and microbial growth (P value = 0.403 and 0.094) respectively. These findings were in line with Kiros *et al.,* [9], and Bahar *et al.,* [10], studies, but unmatched with studies of Maru *et al*., [11], Mulu *et al.,* [3], and Zhang *et al*., [12], and various other studies across different regions which showed that the consumption of sweet foods plays an important role in developing of caries. The lower sample size in the present study and variations in age groups might be accounted for the difference.

Regarding oral hygiene, all of the study participants had a habit of teeth brushing 50(100%) and out of this, 26(52%) of them had a habit of teeth brushing once per day. Although the statistical association between frequency of brush change and microbial growth was significant (P value = 0.041), No significant association was found between the frequency of teeth

brushing and dental caries (P value = 0.429). This finding was in agreement with Kiros *et al.,* [9], study but disagreed with findings of Mohammed *et al.,* [13], and Zhang *et al.,* [12], studies. The reason for this disparity could be the types of material for teeth brushing, time for teeth brushing, and way of teeth brushing. The most common isolated bacterial pathogen in this study was *Streptococcus mutans* 54%, This finding was in line with other studies like Khan *et al*., [14] 47.1% and Borty *et al*., [15]. 28.8%, which showed *Streptococcus mutans* as the predominant bacteria isolated from caries lesions. The results of Jalal *et al.,* [6], study disagreed with this finding with *S.aureus* 62.29% being the predominant bacteria isolated. *E.faecalis* was the second most isolated organism (22%) which was consistent with the findings of Abed [16], study in Iraq. *Staphylococcus aureus* 2%, and *Lactobacillus.spp* 2%, being the least isolated organisms, findings of Borty *et al*., [15], and Khan *et al*., [14], studies showed these two organisms to be isolated in higher frequencies, which disagreed with our findings. *Candida albicans* was the only isolated fungi 7(14%), which was in agreement with Jala *et al.,* [6], study in Iraq. The variation of these results among researchers could be due to several factors such as source of sampling, geographical origin, sensitivity of identification methods and sample size can affect the outcomes.

CONCLUSION

In conclusion, *Streptococcus mutans* was the predominant bacterium isolated from caries lesions, followed by *Enterococcus faecalis*. The disease was mostly caused by bacteria, with only one type of fungus, *Candida albicans*, being isolated. No association was found between the microbial cultures and age, sugar intake, or oral hygiene. The frequency of brush change has significant association with microbial isolation.

Conflict of Interest: None declared

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Ethical Approval: N/A

Author Contributions

Hewida A. M, Rayan. A.b methodology, and conducted the study, Mutaz Fathelrhman Saad supervised the study, data analysis conceptualized, visualized, designed, interpretation of the results, and wrote the drafted paper. Thuwaiba A. A data analysis and check the methodology, editing and approved the manuscript critically for remarkable intellectual contents. All authors read and approved the final manuscript.

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