Isolation and Identification Some of microbial causes for dental caries

Rana jalal [1] , Bari Lateef [2] , Vian Ibrahim [3]

[1] *Biology Department , College of Education for pure science , Tikrit University , Tikrit , Iraq*
[2] *Biology Department , College of Science , Kirkuk University , Kirkuk, Iraq*
[3] *Techniques department , College of Technology , Northern technical University, Medical Lab. , Kirkuk , Iraq*

Abstract

This study was conducted for the isolation of some microbial cause tooth decay and determine the risk factors related to caries. 91 sample collected from teeth (plaques or swabs from dental caries), (41) sample from children (6-14) years and (50) sample from adult (15-70) years, subdivided into (30) sample associated with risk factors and (20) sample without risk factors. A total of 61 isolates were identified in which 38 isolates (41.75%) were *S. aureus,* 16 isolates (17.58%) were *Streptococcus spp.* and 7 isolates (7.69%) were identified as *Candida albicans.*

Introduction

Destruction of calcified tissue was created by acids which are the result of carbohydrate metabolism of acidogenic bacteria ensuing to dental caries[1]. The mouth is a favorable habitat for a great variety of bacteria because of the nearness of supplement, epithelia trash and secretions. Oral bacterium includes Streptococci, Staphylococci, Lactobacilli and Corynebacteria with a large number of anaerobes. At birth the oral cavity is sterile but rapidly becomes colonized from the nature, especially from the mother in the first feeding [2]. Dental caries is a standout amongst the commonest disease around the world [3], influencing 60 – 90% of school youngsters and the vast majority of adults. It is additionally a most pervasive oral disease in a few Asian and Latin American nations, yet it seems, by all accounts, to be less common and less severe in most African nations [4]. Within most countries, geographical and sociological factors effectuates the rate of dental caries and, despite improvements, caries continues to redound the majority of children, some severely. Dental caries is the commonest dental disease in children and contributes to tooth loss in adults [5]. Caries are the localized obliteration of the tissues of the tooth by bacterial activity [6]. Bacterial plaque gathered on dental surfaces and made out of native oral flora is the essential etiologic agent of dental caries [7]. Dental plaque shapes constantly on tooth surfaces, and when exposed to fermentable carbohydrates, bacteria found in the plaque create acid. Acid brings down the pH of the mouth, and a procedure of demineralization happens on the enamel of the teeth. After some time remineralisation happens actually, be that as it may, if demineralization surpasses remineralisation, then cavities form in the teeth [8].

A simplified clarification of the clinical causes for caries includes: the nearness of plaque containing either an excessive amount of bacteria as well as an abundance of acid producing bacteria, utilization of effectively fermentable carbohydrates on a frequent basis (e.g. sugar), a low saliva production, or a diminished capacity of the saliva to act as a buffer, and a genetic make-up making the host more vulnerable to caries [9]. Mutans streptocos (*Streptococcus mutans* and *Streptococcus sobrinus*)

are the most imperative bacteria in the pathogenesis of dental caries [10], with lactobacilli and different microorganisms taking an interest in the in the disease progression [6]. It is known worldwide that some opportunistic microorganisms, such as *Staphylococcus spp* and *Candida spp*., must be mulled over as plausible pathogens, particularly in people with various systemic debilitations, e.g. diabetes mellitus, neutropenia, agranulocytosis, and AIDS [11]. Staphylococci are thought to be temporarily occupant in the oral cavity [12], however it is viewed as a putative pathogen of numerous oral illnesses, including dental caries [13]. Social propensities can bother the adjust of the oral microflora. The standard admission of dietary starches can prompt to the improvement of aciduric (corrosive tolerant) and cariogenic species, for example, mutans streptococci and lactobacilli. Concentrates on have found that patients with diabetes are more susceptible to oral tactile, periodontal, and salivary scatters, which could build their hazard for growing new and intermittent dental caries. Numerous elderly subjects take an assortment of meds, the reactions of which can diminish the stream of salivation and along these lines bother the ordinary adjust of the inhabitant oral microflora [14]. The commonness of caries increments altogether in the individuals who don't clean their teeth consistently. As per Wei and Hyman, most kids don't brush sufficiently long and with legitimate procedure to accomplish add up to deplaquing [15]. This study was carried out for the isolation of *S. aureus* from dental caries and risk factors related to caries.

Materials and Methods

Sample Collection

The study population consisted of patients attending General Al-Hawija hospital, in Kirkuk city, during the period from 11/10/2013 to 1/1/2014. A total of (91) samples were collected from age (6-70) ; Some were collected by wooden stick by rubbing the local lesion or gum edge attached to teeth, the other samples consisted of teeth pulled out by dentists in the dental clinic. Samples were collected with aseptic precautions with the assistance of dentist. The samples were placed in sterilized containers used for this purpose. Patient's name, age, date. The sample

transmitted to Microbiology laboratory in Medical laboratory techniques department in Technical College/ Kirkuk. The samples were processed immediately or (if delayed) inoculated into brain heart infusion for 24 hours.

Microbial isolation and identification:

Collected samples were enriched in brain heart infusion, then cultured on Mannitol salt agar, Blood agar and MacConkey's agar plates. All the plates were incubated at 37°C for 24 hours. After incubation, visual growth on the inoculated plates was observed and colony morphology was noted. Identification of the isolates was done by using standard microbiological techniques which involved morphological appearance of the colonies, Gram's staining, and biochemical properties including catalase test, coagulase test[4,16]

Result and Discussion

A total of (91) plaque and swab samples were included in this study; (41) sample were collected from children with age ranges from 6 - 14 years and (50) sample from adults with age ranges from 15 - 70 years, subdivided into (20) sample from adults without any risk factor, and (30) sample from adults with risk factors such as diabetes mellitus, heavy cigarette smoking, irregular teeth brushing, and increase soda pop consumption. From the (91) sample, 61 sample show growth on the cultured media, 25 from age group 6-14 years, 36 from those with age range 15-70 years (9 without risk factors and 27 with risk factors). The other 30 sample did not show any growth probably because they were anaerobes which were not included in this study (table 1).

Table 1: Microbial growth among total samples

Age groups	Microbial growth				Total samples
	Positive		Negative		
	No.	%	No.	%	
6-14 years	25	(27.47%)	16	(17.58%)	41
15-70 years (without risk factors)	9	(9.89 %)	11	(12.08%)	20
15-70 years (with risk factors)	27	(29.67 %)	3	(3.2%)	30
Total	61	(67.03%)	30	(32.96%)	91

Comparison of our findings with past studies is troublesome because of various age groups these studies utilized. In our study, the most isolated pathogens was from those with age group 15-70 years who are associated with risk factors including smoking, diabetes mellitus, irregular teeth brushing in frequency of (29.67%) followed by those with age group 6-14 years (27.47%) and (9.89%) from 15-70 years group who are not associated with risk factors. Our result disagree with Altayaar *etal* who found in their study that (55.5%) of the isolates were from children (5-15) years old and (44.5%) were from the age group (16-68) years old [19] and while Borty Sh *et al* show that the lowest prevalence (10.86%) of infection was seen in group of bellow 15 years of age [20].

A total of 61 isolates were identified in which 38 isolates (62.29%) were *Staph. aureus,*

Microscopic morphology of *S. aureus* was clarified by gram stain which appeared as Gram-positive cocci grouped in clusters. Tests for catalase and coagulase were positive [22]. On Mannitol salt agar, formed colonies surrounded by yellow zones due to fermentation of mannitol and showed beta- hemolysis on blood agar [23]. *Streptococcus spp.* showed gram-positive cocci on stained smear arranged in chains, catalase-negative, many species exhibit characteristic haemolysis on blood agar. Identification of *C. albicans* made by direct microscopy of oval Gram-positive cells, some of which showed budding or producing pseudohyphae [22] and from a positive germ tube test [23].

Table 2: General number and frequency caries isolated from dental caries

Result of the cultured samples	Number of isolates	Frequency of total isolations
S. aureus	38	62.29%
Streptococcus spp.	16	26.22%
Candida albicans	7	11.47%
Total	61	100 %

Table 3: Number and frequency of microbial caries isolated according to age range

Result of the cultured samples	From children		Adults associated with risk factors.		Adults without risk factors.	
	No.	%	No.	%	No.	%
S. aureus	11	44 %	20	74.07 %	7	77.77 %
C. albicans	0	0 %	5	18.51 %	2	22.22 %
Streptococcus spp.	14	56 %	2	7.40%	0	0 %
Total	27	100 %	9	100 %	25	100 %

Streptococcus spp. was the most isolated bacteria from children in frequency of 56% followed by *Staph. aureus* (44%) as shown in table 3. Prabill *et al* revealed in their study on children aged 5-14 that *Streptococcus spp* was the commonest organism isolated in children with dental caries followed by *C. albicans* and *Staph. aureus* [24]. Olajokun *et al* found in their study from children with age range 1-15 years that *S. mutans* was the most isolated pathogen followed by lactobacillus and then *S. aureus* [25].

From adults (with and without risk factors) *Staph. aureus* was the most isolated in frequency of 74.07% and 77.77% respectively, *C. albicans* isolated from both in frequency of 18.51%, 22.22% respectively. *Streptococcus spp.* isolated only from those associated with risk factors in frequency of 7.40% as shown in table 3. *Strep . aureus* and *Candida spp* are part of the microbiota of the oral cavity and cause infections in that ecosystem [11]. Individuals most susceptible to Candida infections are diabetics, those with immunodeficiency (e.g., AIDS), catheterized patients, and those individuals who are taking antimicrobial medications [23].

Number and frequency of bacterial caries isolated from adults associated with risk factor was higher from those without risk factors. In diabetic patient, the high glucose levels in the saliva expand the measure of fermentable sugars by oral

microorganisms, prompting to generation of acidic by - products that cause teeth demineralization in dental caries. Bounteous glucose in the saliva may also advance the development of cariogenic microbes and encourage the recurrence and term of acidic episodes [26]. Smoking expands the number but disables the capacity of neutrophil which deal with the principal line defense mechanism against microorganisms [27]. On the other hand, the diminished buffering impact and possible lower pH of smoker's salivation and the higher number of Lactobacilli and *Streptococcus viridnis* may show an expanded susceptibility to caries. Studies demonstrate that smoker individual not just had repulsive oral cleanliness and less primitive attitude toward wellbeing, additionally had distinctive dietary patterns, apparently expending high measure of sugar containing items like soda pops and snacks. Every day smoking was connected with expanded utilization of sugar in tea or coffee, and with more continuous liquor utilization. It is likewise observed that smokers have insufficient brushing propensities than non-smokers [28]. There are two huge dangers postured by soda pop: The sugar content unquestionably does fuel the bacteria that produce acidic by-products, which has a huge impact on the demineralization of enamel and development of caries. It also applies a significant malicious impact by washing the teeth in acid that likewise is capable of producing demineralization [29].

References

1. Chandrabhan Dh, Hemlata R, Renu B, Pradeep V (2012). Isolation of Dental Caries Bacteria from Dental Plaque and Effect of Tooth Pastes on Acidogenic Bacteria. *J. Med. Micro.*; 2: 65-69.

2. Daniyan SY, Abalaka ME (2011) . Prevalence and Susceptibility Pattern of Bacterial Isolates of Dental Caries in a Secondary Health Care Institution, Nigeria. *Shiraz E-Med. J.*; 12(3.

3. Schwendicke F, Thomson WM, Broadbent JM, and Stolpe M.(2016). Effects of Taxing Sugar-Sweetened Beverages on Caries and Treatment Costs. *J Dent Res*; 1–6.

4. Oluremi BB, Osungunna MO, Idowu OA and Adebolu OO (2010) . Evaluation of Anticaries Activity of Selected Mouthwash Marketed in Nigeria. *Tro J Pharma Res;* 9 (6): 581-586..

5. Moynihan P, and Petersen PE (2004) . Diet, nutrition and the prevention of dental diseases. *Pub Heal Nut*; 7(1A), 201–226.

6. Ozdemir D(2014) . Dental caries and preventive strategies. *J Educ & Inst St Wor*; 4 (4): 2146-7463.

7. Maripandi A, Kumar A and Al Salamah AA (2011) . Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different tooth pastes against *Streptococcus spp*. *Afr J Micro Res*; 5(14): 1778-1783..

8. Chambers S (2012) . Public Health and Dental Caries in Young Children in Deprived Communities in Scotland. *Scott Univ Med J*.

9. Salli KM and Ouwehand AC (2015). The use of in vitro model systems to study dental biofilms associated with caries: a short review. *J Oral Microbiol*; 7: 26149..

10. Al-Mudallal NHA, Al-Jumaily EFA, Muhimen NAA and Al-Shaibany A (2008) . Isolation and identification of mutan's streptococci bacteria from human dental plaque samples. *J Al-Nahrain Univ*; 11(3): 98-105.

11 Cuesta AI, Jewtuchowicz V, Brusca MI, Nastri ML, Rosa AC (2010). Prevalence of *staphylococcus spp* and *candida spp* in the oral cavity and periodontal pockets of periodontal disease patients. *Acta Odontol. Latinoam*; 23(1): 20-26.

12. Nemoto YO, Haraga H, Kimura S and Nemoto1 T K (2008). Occurrence of staphylococci in the oral cavities of healthy adults and nasal–oral trafficking of the bacteria. *J Med Micro*; 57: 95–99.

13. Petti S, Bossù M, Messano GA, Protano C, Polimeni A (2014). High salivary *Staphylococcus aureus* carriage rate among healthy paedodontic patients. *New Microbiologica*; 37: 91-96..

14. Marsh Ph D, Martin M V (2009). Oral Microbiology .5th ed. Churchil livingstone Elsevier.

15. Munjal V, Jindal R, Dua R, Garewal RS, Bathla P (2014). Correlating dental caries with tooth brushing frequency, type of toothpaste and dentalvisits. *Den J Adv St*; 2(I): 9-15..

16. Maal1 KB, Bouzari1 M and Zavareh FA (2010) . Identification of *Streptococcus salivarius* bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy. *Af J Microbiol Res*; 4(20): 2127-2132..

17. Yadav Kh, Prakash S (2015). Antibiogram profiles against polymicrobial pathogens among dental caries patients at Janaki Medical College teaching hospital, Nepal. *Inter J App Dent Sci*; 1(4): 156-162..

18. Elfaki NK, Elgarrai ASE, Shwail AI, Brair SL and Alsheikh MA (2014) . Prevalence of Dental Caries among Primary School Attendees in Najran-Saudi Arabia. *IOSR J Den Med Sci*; 13 (11): 37-41.

19. Altayyar IA, Abdalla AM, Alfellani MA, Abdullah OO (2015) . Determination of Aerobic Bacterial Composition of Dental Plaque Biofilms and Their Role in Oral health. *Emer Life Sci Res*; 1(1): 8-12..

20. Borty ShCh, Bin Hafiz KhM, Ali MdM, Begum Kh, Ahammed T, Monir MdSh and Islam MdA (2015) . Isolation, identification and antibiogram profile of bacteria isolated from dental caries patients of Mymensingh district of Bangladesh. *Asian J Med Biol Res*; 1 (2): 244-253..

21. Saini S, Gupta N, Mahajan A and Arora DR (2003). Microbial Flora in Orodental Infections. *Indian J. Med Microbiol*; 21: 111-114..

22. Elliott T, Casey A, Lambert P and Sandoe J (2011) . Medical microbiology and infection.5th ed. Wiley Blackwell. UK.

23. Leboffe MJ, Pierce BE (2011). A Photographic Atlas for the Microbiology laboratory. 4th ed. Morton Publishing Company. USA..

24. Prabil1A and Dalal P (2016). Oral Microbial Pattern in Children with Decayed Teeth. *Int. J. Curr. Microbiol. App. Sci* :5(6): 762-766..

25. Hassan-Olajokun RE, Folarin AA, Olaniran O, Umo AN (2008). The prevalent bacterial isolates of dental caries in school age children attending the dental clinic of oauthc, Ile-ife. *Afr J Cln Exper Microbiol*: 9 (2): 103 – 108..

26. Abayon M (2009). Diabetes and Dental Caries Prevalence: Is There an Association?. M.Sc.Thesis. University of Rochester..

27. Kentala J (2007) . Smoking prevention in oral health care. Academic dissertation. University of Tampere.

28. Vellappally S, Fiala Z, Šmejkalová1 J, Jacob V, Shriharsha P(2007) . Influence of tobacco use in dental caries development. *Cent Eur J Pub Health*; 15 (3): 116–121..

29. Kaplowitz GJ, Florman M, Aaronson SA (2004) . The dangers of soda pop. The Academy of Dental Therapeutics and Stomatology is an ADA CERP Recognized Provider.

عزل وتشخيص بعض المسببات الجرثومية لتسوس الاسنن

رنا جلال[1] ، بري لطيف[2] ، فيان ابراهيم[3]

[1]قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق

[2]قسم علوم الحياة ، كلية العلوم ، جامعة كركوك ، كركوك ، العراق

[3]قسم تقنيات التحليلات المرضية ، الكلية التقنية/كركوك ، الجامعة التقنية الشمالية ، كركوك ، العراق

الملخص

أجريت هذه الدراسة لعزل بعض المسببات الجرثومية التي تسبب تسوس الأسنان وتحديد بعض عوامل الخطورة الم vjf'I للاصابة بها. تم جمع 91 عينة من الأسنان (لويحات أو مسحات من الأسنان المتسوسة) توزعت الى (41) عينة من الأطفال (6-14) سنة و (50) عينة من البالغين (15-70) سنة مقسمة إلى (30) عينة مرتبطة بعوامل الخطورة بلغ عدد العزلات الموجبة النمو (61) عزلة بنسبة 67% شخصت منها 38 عزلة (62.29٪) عائدة لبكتريا المكورات العنقودية الذهبية ، 16 عزلة (26.22٪) عائدة لبكتريا المكورات العقدية و 7 عزلات (11.47٪) تم تشخيصها من المبيضات البيض.