Medical Science Journal
for Advance Research

Vol. 5, No.4, December 2024
e-ISSN: 2774-4892

Isolation of Dental Caries Bacteria from Dental Plaque

Ali Mohammed Hussein Abdulhadi[1], Kareem M. Alghanim[2] , Mustafa Mohsen Yassein[3], Hommam Mohammed Khadir[4] , Tiba Ahmed Abd[5], Mariam Jassim Sahib[6], and Zainab Ali Raheem[7]

[1]*Jabir Ibn Hayyan University of Medical and Pharmaceutical Sciences, Faculty of Medicine, Iraq.*

[2]*University of Al-Kafeel, Faculty of Dentistry, Iraq.*

[1,3,4,5,6,7] *Collage of Dentistry, University of AL-Kafeel, Iraq.*

***E-Mail**: ali.m.hussein@jmu.edu.iq, kareem.alghanim@alkafeel.edu.iq

ABSTRACT

Background: *Dental caries stands out as a highly prevalent and preventable condition, identified as the leading contributor to oral discomfort and tooth loss. This condition represents a significant public health challenge that obstructs the attainment and preservation of oral health across all age demographics. The WHO has indicated that the global issue of oral disease continues to exist, even with significant advancements in the oral health of populations in various countries.* ***Aim of study:*** *Isolation of Dental Caries Bacteria from Dental Plaque and detection Pattern of resistance to common antibiotics in dental clinics.* ***Methodology:*** *Specimens collection include 75 clinical swabs (45 Male and 25 Female) isolated from dental caries patients who were admitted to Al-Kafeel teaching clinic and outpatients from dentist clinic in AL-Najaf City between November 2022 to January 2023 with age group between 30-70 years. All the specimens were labeled and transported Immediately by sterile transport swabs to the laboratory for Culture and Identification. For each patient, medical records were reviewed, which including name, gender, age, address, hospitalization, and antibiotic receiving. and immediately transport to the old.* ***Results:*** *The study comprised of 75 patients with dental caries , 45 patients were male while 30 patients were female , The swabs taken from dental clinics have been showed that 64 (85.3%) of specimens were gram positive bacteria and only 11 specimens (14.7%) were gram negative bacteria. The result showed that about 64 specimens from totally 75 were gram-positive and the most common species were: Streptococcus mutans 20 (26.66%), Staphylococcus aureus 15 (20%), Lactobacilli 10 (13.33%), Staphylococcus species 8 (10.66%), Streptococcus species 5 (6.66%), Enterococcus faecalis 4(5.33%), Bacillus species 2 (2.66%) and Micrococcus species 2 (2.66%). Gram-negative bacteria that were isolated from dental caries were only 11. The bacterial species were: E. coli 3 (4%), K. pneumonia 2 (2.66%) , P. aeruginosa 2 (2.66%), Moraxella 1 (1.33%), and Acinetobacter baumannii 1 (1.33%). Tow specimens were unknown (2.66%), and the Vitek 2 system had no results.*

Keywords: Dental caries, Dental plaque, Gram-positive bacteria, Vitek 2 system.

Article Information

Received: October 5, 2024; Revised: October 29, 2024; Online: December, 2024

1. INTRODUCTION

Dental plaque is a multifaceted biofilm that forms on the hard tissues (teeth) within the oral cavity. Despite the presence of over 500 bacterial species in plaque, colonisation occurs in a systematic manner, beginning with the adherence of primary colonisers to the enamel salivary pellicle, succeeded by secondary colonisation via interbacterial adhesion. A range of adhesins and molecular interactions facilitate these adhesive contacts, contributing to plaque formation and, eventually, to conditions including dental caries and periodontal disease (A.C. Tanner, 2016).

https://doi.org/10.46966/msjar.v5i4.240

Dental caries is among the most prevalent chronic disorders globally. Dental caries result in the degradation of enamel, dentin, or cementum of teeth owing to bacterial activity. Dental caries is a significant health issue in many industrialised nations, affecting 60% to 90% of school-aged children and the majority of adults. This is mostly attributable to the rising intake of sugar and insufficient exposure to fluorides (B. J. Paster et al, 2001). The development of dental caries is linked to biofilm production, impacting a significant global population. The bacterium of the species Streptococcus mutans is considered the key etiological component contributing to this catastrophic illness. S. mutans significantly contributes to the formation of intricate, multi-dimensional structures on the oral mucosa and tooth enamel (Wang Y.,et al, 2020).

Oral microbiota contains the microorganisms that are present in the human oral cavity which is considered the second-largest microbial community in the human body after the gut (GaoL., et al, 2018), (Zaura E., et al 2014). The genera Streptococcus and Actinomyces which are facultative anaerobes are acquired at birth and the hours after (Dzidic M ., et al, 2018). The oral cavity harbours a very diversified normal microbiota that performs several beneficial activities. Approximately 1000 distinct types of microorganisms have been identified in the oral cavity, with one individual harbouring about 50 to 100 species. Streptococci constitute approximately 50% of the oral microbiome (Chavan N., et al, 2015). Streptococci species inhabit the human mouth cavity as their native environment and are classified within the Viridans group streptococci (VGS). The viridans group streptococci are facultative anaerobic, Gram-positive, spherical or ovoid, non-spore-forming, non-motile bacteria that digest carbohydrates, producing acid without gas. They are the primary contributors to plaque formation, which constitutes a very intricate ecosystem including several bacterial kinds (Heng CC,2016). The mutans streptococci group (Streptococcus mutans and S. sobrinus) is regarded as the primary causative agents of dental caries. These bacteria are the predominant pathogens identified in human dental disorders, as documented in epidemiological research (Aas JA.,et al, 2008).

2. METHODOLOGY

2.1 Specimens Collection

The specimen collection involves 75 clinical swabs (45 from males and 25 from females) obtained from dental caries patients admitted to Al-Kafeel Teaching Clinic and those attending an outpatient dentist clinic in Al-Najaf City, between November 2022 and January 2023, between the age range of 30 to 70 years. All specimens were labeled and promptly transferred using sterile swabs to the laboratory for culture and identification. Medical data for each patient were inspected, including name, gender, age, address, hospitalization, and antibiotic administration, and were promptly transported to the previous location.

2.2 Specimens Culture

The samples obtained specimens were mostly put into two types of culture medium: blood agar and mannitol salt agar (MSA), which are regarded as prominent enrichment, selective, and differential media for the isolation, purification, and identification of various bacterial species. The plates were incubated at 37˚C for a duration of 24 hours.

2.3 Identification of Bacteria

A solitary colony was extracted from each primary positive culture on blood agar, followed by repeated growth to obtain a pure culture. Identification was conducted based on morphological and cultural characteristics, including blood haemolysis, lactose fermentation, and mannitol fermentation, as well as colony shape, size, colour, borders, and texture. Subsequently, a smear was prepared from the pure colony on a clean slide and stained with Gram's stain for microscopic examination of bacterial arrangement and staining reaction (MacFaddin, 2000). The specimen collection also identified by using Vitek-2 automated system were GP, GN identification card have been used for identification Gram positive and Gram negative bacteria. The time between preparation of the inoculum and the card filling was always less than 30 min.

3. RESULTS

3.1 Distribution of Specimens according types of bacteria

Swabs collected from dental clinics indicated that 64 specimens (85.3%) were gram-positive bacteria, whereas only 11 specimens (14.7%) were gram-negative bacteria, **Figure (1)**.

The results indicated that approximately 64 out of 75 specimens were gram-positive, with the predominant species being: Streptococcus mutans 20 (26.66%), Staphylococcus aureus 15 (20%), Lactobacilli 10 (13.33%), Staphylococcus species 8 (10.66%), Streptococcus species 5 (6.66%), Enterococcus faecalis 4 (5.33%), Bacillus species 2 (2.66%), and Micrococcus species 2 (2.66%). Only 11 gram-negative bacteria were identified from dental cavities. The bacterial species identified were: E. coli 3 (4%), K. pneumoniae 2 (2.66%), P. aeruginosa 2 (2.66%), Moraxella 1 (1.33%), and Acinetobacter baumannii 1 (1.33%). Two specimens were unidentified (2.66%); the Vitek 2 system yielded no findings., **(Table 1).**

The data collected during this investigation reveal that three species of gram-positive bacteria are prevalent: Streptococcus mutans, Staphylococcus aureus, and Lactobacilli (60%), **Figure (2,3).**

3.2 Distribution of Patients according to age

The average age of patients in this research ranged from 11 to 70 years. The results indicated a greater prevalence of dental caries in those over the age of 50 (45%). The remaining age group constituted 55%, with 10 patients aged 10-20 years, 15 patients aged 20-30 years, 5 patients aged 30-40 years, and 16 patients aged 40-50 years.

3.3 Distribution of Patients according to history of disease

Patients with dental caries are categorized into two categories based on their medical history. Thirteen patients (17.3%) had chronic conditions, including diabetes and heart disease. Among them, eight were female, four were male, and one patient was an adolescent (16 years old)., **Figure (4).**

3.4 Distribution of specimens according to antibiotics resistance

The present investigation employed two categories of antibiotics on two strains of bacteria obtained from individuals with dental caries. Amoxicillin-clavulanate and cefixime were utilised against Streptococcus mutans and Staphylococcus aureus. Clear variance in resistance has been seen, with most isolates exhibiting resistance to one or more of these antibiotics, as evaluated by the Vitek 2 method (AST card). The results showed that 9 (45%) specimens of *S. mutans* from totally 20 were resistance to (Amoxicillin – culvanate) and (cefixime) , while in other hand , S. aureus appeared a high resistance to (Amoxicillin – culvanate) and (cefixime) where 8 specimens (53.3%) from totally 15 were resistance to both antibiotics , **Table (2) and Table (3).**

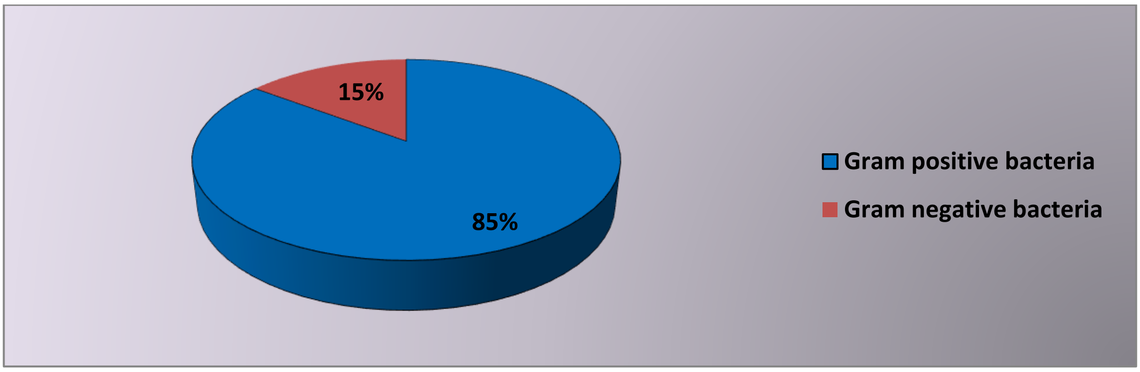

Figure (1): Distribution of Specimens according types of bacteria.

Table (1): Types of bacteria that isolated from dental caries patients.

Types of bacteria	Name of bacteria	Number of specimens	Percentage %
Gram positive bacteria			
	Streptococcus mutans	**20**	**26.66%**
	Staphylococcus aureus	**15**	**20%**
	Lactobacilli	**10**	**13.33%**
	***Staphylococcus* species**	**8**	**10.66%**
	***Streptococcus* species**	**5**	**6.66%**
	Enterococcus faecalis	**4**	**5.33%**
	***Bacillus* species**	**2**	**2.66%**
	***Micrococcus* species**	**2**	**2.66%**
Gram negative bacteria			
	E .coli	**3**	**4%**
	K. pneumonia	**2**	**2.66%**
	P. aeruginosa	**2**	**2.66%**
	Moraxella	**1**	**1.33%**
	Acinetobacter baumannii	**1**	**1.33%**
	Unknown Bacteria	**2**	**2.66%**
Total		**75**	**100%**

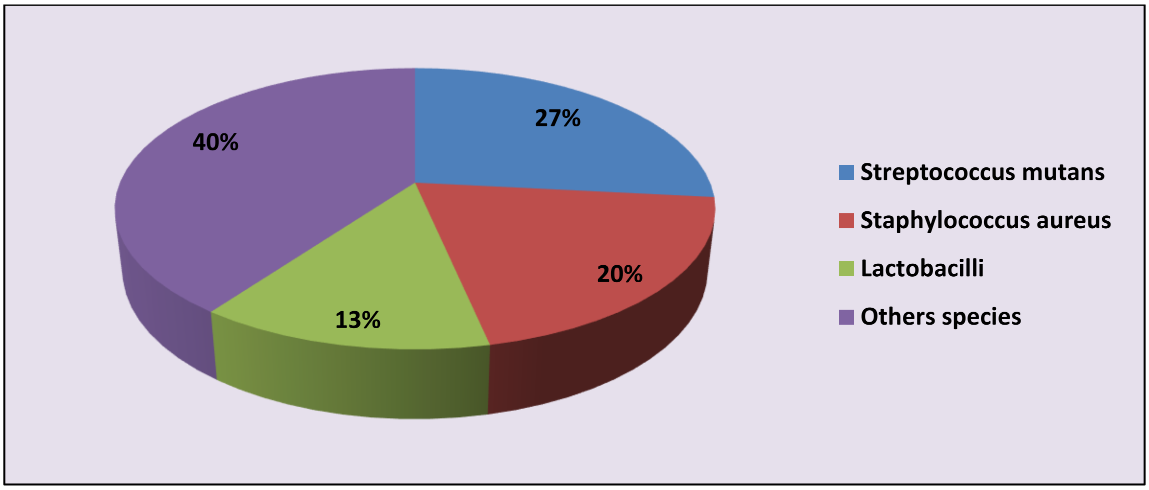

Figure (2) : The percent of gram positive bacteria isolate from dental caries.

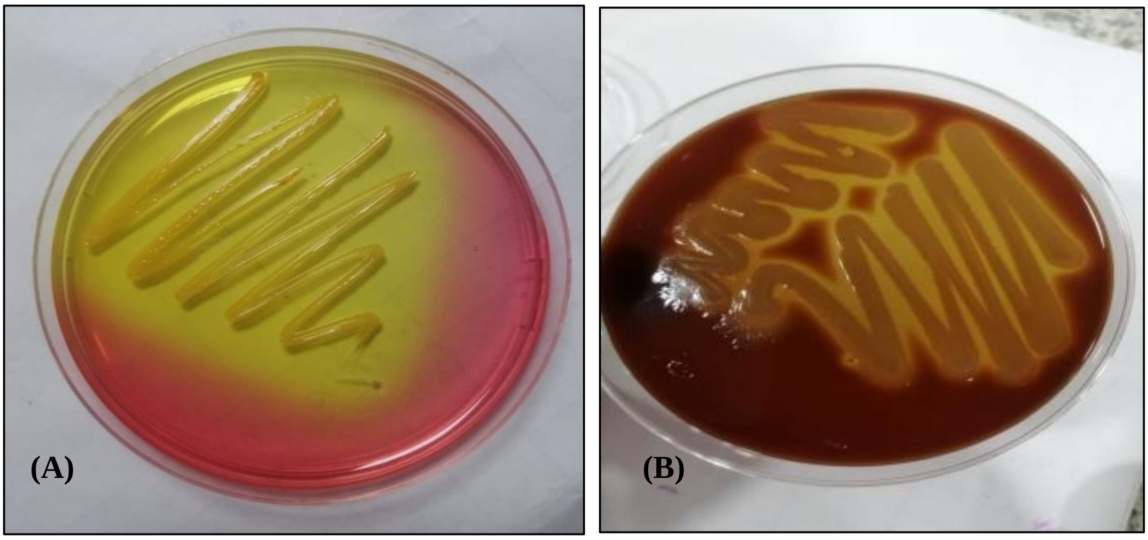

Figure (3) A : *S. aureus* on mannitol slat agar , B: *Bacillus* spp on blood agar.

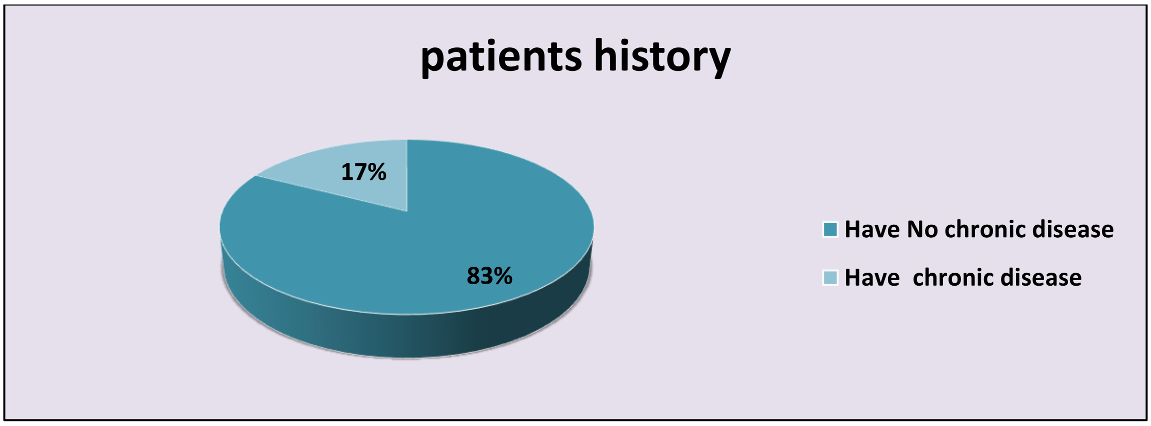

Figure (4): Distribution of Patients according to history disease.

Table (2): Antibiotics sensitivity test of S. mutans isolated from dental caries .

Number	Name of bacteria	AMC	CFX
1	Strep.8	**Resistance**	**Resistance**
2	Strep.34	**Resistance**	**Resistance**
3	Strep.33	Sensitive	Sensitive
4	Strep.15	Sensitive	Sensitive
5	Strep.10	Sensitive	**Resistance**
6	Strep.14	**Resistance**	**Resistance**
7	Strep.41	Sensitive	Sensitive
8	Strep.24	**Resistance**	**Resistance**
9	Strep.39	Sensitive	Sensitive
10	Strep.43	**Resistance**	**Resistance**
11	Strep.35	**Resistance**	**Resistance**
12	Strep.23	Sensitive	Sensitive
13	Strep.38	**Resistance**	**Resistance**
14	Strep. 44	Sensitive	Sensitive
15	Strep. 45	Sensitive	Sensitive
16	Strep. 48	**Resistance**	Sensitive
17	Srep.53	Sensitive	Sensitive
18	Strep. 55	Sensitive	Sensitive
19	Strep. 62	Sensitive	Sensitive
20	Strep. 68	**Resistance**	**Resistance**

Table (3): Antibiotics sensitivity test of *S. aureus* isolated from dental caries.

Number	Name of bacteria	AMC	CFX
1	Staph.4	**Resistance**	**Resistance**
2	Staph.7	**Resistance**	**Resistance**
3	Staph.11	**Resistance**	**Resistance**
4	Staph.13	**Resistance**	**Resistance**
5	Staph.16	**Resistance**	**Resistance**
6	Staph.17	**Resistance**	**Resistance**
7	Staph.22	Sensitive	Sensitive
8	Staph.29	Sensitive	Sensitive
9	Staph.33	Sensitive	Sensitive
10	Staph.37	Sensitive	Sensitive
11	Staph.40	**Resistance**	**Resistance**
12	Staph.41	Sensitive	Sensitive
13	Staph.47	Sensitive	Sensitive
14	Staph.52	Sensitive	**Resistance**
15	Staph.60	**Resistance**	Sensitive

4. DISCUSSION

Dental caries is a multifactorial condition and the most often diagnosed chronic illness in both children and adults. It continues to be a significant public health issue in the majority of industrialised nations. Despite its extensive global presence and detrimental effects on individuals, it remains a largely overlooked public health concern(Pitts.,et al , 2017). Inadequate oral hygiene and poor dietary practices are the primary contributors to the initiation and progression of tooth decay; however, factors such as low socioeconomic status, limited educational attainment, and orthodontic treatment have also been identified as significant predictors of caries prevalence (Contaldo., et al,2021). Risk factor identification should occur at an early age because of the immediate and long-term detrimental effects of dental caries. Upon installation, the progression is rapid, with suitable interventions varying from minimally invasive techniques to procedures conducted under general anaesthesia (Pecci-Lloret ., et al 2021), (Moca., et al,2020). The impact of age on the prevalence of dental caries encompasses many changes that occur throughout time, and it can also serve as a direct predictor of the condition. Age assessment might rely on the evaluation of chronological age, dental age, or skeletal age. The determination of dental age is straightforward through the analysis of the patient's panoramic radiograph (Lopez., et al, 2017). In this study, the quantity of male patients with dental caries exceeded that of female patients. This may be associated with several factors, including diet, smoking, inherited influences, and others. A comprehensive assessment of representative research on sex differences in caries, tooth loss, and periodontal disease is conducted. Surveys undertaken in Hungary, India, and a distinct traditional Brazilian population offer further evidence of a notable sex bias in dental caries, particularly among older individuals.

The disparity in oral health between sexes in India seems to be influenced by hormonal and reproductive variables, as well as social and religious elements such as son preference, ceremonial fasting, and nutritional limitations during pregnancy. The disparity in tooth loss between genders indicates that women experience more tooth loss than males, which has been associated with caries and parity. Genome-wide association studies have identified loci associated with caries susceptibility and protection that alter variations in taste, saliva, and enamel proteins, hence influencing the oral environment and the microstructure of enamel (Ito Y., et al,2020). Type 2 diabetes has emerged as one of the most prevalent chronic systemic disorders. It is primarily defined by chronic hyperglycemia and disrupted metabolism of glucose, lipids, and proteins. The global incidence rates are perpetually rising, resulting in significant medical, social, and economic challenges. Numerous research has established a strong correlation between oral disorders (e.g., caries, periodontal disease, mucosal diseases) and type 2 diabetes. They provide as reciprocal feedback.

The interplay of oral microbiota is intricately linked to both oral and systemic health and illness. Alongside its inherent symptoms, oral manifestations, including alveolar bone loss and tooth loss, have emerged as a consequence of diabetes (J.L. Ebersole ., et al 2008). Oral microorganisms constitute the primary constituents of the oral microbiota. Prevalent oral bacteria encompass Streptococcus mutans, Porphyromonas gingivalis, Staphylococcus, and Lactobacillus. S. mutans constitutes a main element of the oral microbiota and is a significant component of dental plaque (Dzidic M ., et al , 2018). It is the primary pathogen responsible for caries, a bacterial infectious illness affecting the hard tissues of the teeth and exhibiting the greatest prevalence among oral disorders. Bacteria are the primary pathogens, and symptoms encompass the loss of hard dental tissue (S. Suwannakul ., et al, 2010).

The prevalence of dental caries is significant, and the condition can manifest at any stage of human life. Dental caries occurs more frequently in children than in adults and is directly associated with oral microbiota. A prior study identified a high frequency of consuming sweets before bedtime as a risk factor for dental caries in Chinese children (X. Cao ., et al, 2017).

The current study examines the antibiotic resistance of S. mutans and S. aureus to amoxicillin-clavulanate and cefixime. Staphylococcus aureus acquires antibiotic resistance through many routes. These methods encompass the restriction of drug absorption, alteration of the drug target, enzymatic degradation of the drug, and active expulsion of the drug. The bacteria may employ one or more resistance pathways, dependent upon the specific antibiotic implicated. The positioning of resistance genes on transferable genetic elements, such as plasmids and transposons, enables horizontal gene transmission of resistance among bacteria (Van Hoek ., et al, 2011). Acquired resistance in S. aureus may arise through many mechanisms. Resistance to β-lactam antibiotics arose due to the formation of β-lactamases, such as penicillinase, which facilitates the hydrolysis of the β-lactam ring, resulting in the inactivation of the molecule's antibacterial properties (Matias, C., & Oliveira, M. (2015)). Additional virulence factors contributing to resistance in S. aureus include cell wall-associated components such as the capsule, peptidoglycan, teichoic acids, and protein A. S. aureus produces toxins include staphylococcal enterotoxins, exfoliative toxins, and cytolytic membrane-damaging toxins, such as haemolysins and leukocidins, as well as biofilm formation and a bacterial quorum-sensing system.

5. CONCLUSION

Cases of dental caries varied by gender, age, and several factors that contribute to infections and chronic conditions, such as diabetes and systemic diseases associated with the development of dental caries. The prevalence of Gram-positive bacteria isolated from patients with dental caries was greater than that of Gram-negative bacteria. S. mutans and S. aureus exhibited greater prevalence compared to other bacterial species, demonstrating contrasting patterns of antibiotic resistance.

Acknowledgment

Thanks and appreciation to everyone who contributed to the completion of this work, especially those working in Al-Kafeel Dental Clinics and Al-Amin Research Center in Najaf.

REFERENCES

- A.C. Tanner, C.A. Kressirer, L.L. Faller, Understanding caries from the oralmicrobiome perspective, J. Calif. Dent. Assoc. 44 (7) (2016) 437–446.
- B. J. Paster, S. K. Boches, J. L. Galvin, R. E. Ericson, C. N. Lau and V. A. Levanos, “Bacterial Diversity in Human Subgingival Plaque,” Journal of Bacteriology, Vol. 183, No. 12, 2001, pp. 3770-3783.
- Wang Y, Shen X, Ma S, Guo Q, Zhang W, Cheng L, Ding L, Xu Z, Jianga J, Gao L. Oral biofilm elimination by combining iron-based nanozymes and hydrogen peroxide-producing bacteria. Biomater Sci. 2020;8(9):2447–2458.
- GaoL,XuT,HuangG,JiangS,GuY, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein & cell. 2018;9(5):488-500.
- Zaura E, Nicu EA, Krom BP, Keijser BJ. Acquiring and maintaining a normal oral microbiome: current perspective. Frontiers in cellular and infection microbiology. 2014;4:85.
- Dzidic M, Collado MC, Abrahamsson T, Artacho A, Stensson M, Jenmalm MC, et al. Oral microbiome development during childhood: an ecological succession influenced by postnatal factors and associated with tooth decay. The ISME journal. 2018;12(9):2292-2306.
- Chavan N, Phadtare R, Chavan T. Effect of aqueous extracts of different medicinal plants on control of Streptococcus mutans. Int J Curr Microbiol App Sci. 2015;4(4):1072-1081.
- Heng CC. Tooth Decay Is the Most Prevalent Disease. Federal Practitioner. 2016;33(10):31.
- Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. Journal of clinical microbiology. 2008;46(4):1407-1417.
- Pitts, N.B.; Zero, D.T.; Marsh, P.D.; Ekstrand, K.; Weintraub, J.A.; Ramos-Gomez, F.; Tagami, J.; Twetman, S.; Tsakos, G.; Ismail, A. Dental caries. Nat. Rev. Dis. Primers 2017, 3, 17030.
- Contaldo, M.; Lucchese, A.; Lajolo, C.; Rupe, C.; Di Stasio, D.; Romano, A.; Petruzzi, M.; Serpico, R. The Oral Microbiota Changes in Orthodontic Patients and Effects on Oral Health: An Overview. J. Clin. Med. 2021, 10, 780.
- Pecci-Lloret, M.P.; Guerrero-Gironés, J.; López-González, B.; Rodríguez-Lozano,

F.J.; Oñate-Cabrerizo, D.; Oñate-Sánchez, R.E.; Pecci-Lloret, M.R. Dental Treatments under General Anesthesia on Children with Special Health Care Needs Enrolled in the Spanish Dental Care Program. J. Clin. Med. 2021, 10, 182.

- Moca, A.E.; Pelea, D.C.; Negrut, iu, B.M.; Burta, O.L.; Vaida, L.L.; Halit, chi, G. In-vitro assessment of silver diamine fluoride´s effects on a dental plaque specimen. Int. J. Med Dent. 2020, 24, 375–380.
- Lopez, R.; Smith, P.C.; Göstemeyer, G.; Schwendicke, F. Ageing, dental caries and periodontal diseases. J. Clin. Periodontol. 2017, 44 (Suppl. 18), S145–S152.
- Ito Y, Ito T, Yamashiro K, Mineshiba F, Hirai K, Omori K, Yamamoto T, Takashiba S. Antimicrobial and antibiofilm effects of abietic acid on cariogenic Streptococcus mutans. J Odontology. 2020;108(1):57–65
- J.L. Ebersole, S.C. Holt, R. Hansard, M.J. Novak, Microbiologic and immunologiccharacteristics of periodontal disease in Hispanic americans with type 2diabetes, J. Periodontol. 79 (4) (2008) 637–646.
- Dzidic M, Collado MC, Abrahamsson T, Artacho A, Stensson M, Jenmalm MC, et al. Oral microbiome development during childhood: an ecological succession influenced by postnatal factors and associated with tooth decay. The ISME journal. 2018;12(9):2292-2306.
- S. Suwannakul, G.P. Stafford, S.A. Whawell, C.W. Douglas, Identification ofbistable populations of Porphyromonas gingivalis that differ in epithelial cellinvasion, Microbiology 156 (Pt. 10) (2010) 3052–3064.
- X. Cao, D. Wang, J. Zhou, H. Yuan, Z. Chen, Relationship between dental cariesand metabolic syndrome among 13 998 middle-aged urban Chinese, J.Diabetes 9 (4) (2017) 378–385.
- Van Hoek, Angela HAM, et al. "Acquired antibiotic resistance genes: an overview." Frontiers in microbiology 2 (2011): 203.
- Matias, C., & Oliveira, M. (2015). Quorum-sensing system of Staphylococcus aureus isolates from diabetic foot ulcers. Foot.