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Distribution Patterns of Bacteria in Dental Caries, Root Canal System and Prosthetic Restorations

Carmen Liliana Defta[1], Bianca-Mihaela Cristea[1],Cristina-Crenguța Albu[1], Ştefan-Dimitrie Albu[1], and Oana Botoacă[2]

[1] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Titu Maiorescu University, Bucharest, Romania
Corresponding Authors: Cristina-Crenguța Albu and Ştefan-Dimitrie Albu

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ABSTRACT: The oral environment is one of the key interfaces between the body and the environment, therefore it can act as an entry site for some pathogenic microorganisms, especially through the air or through the ingestion of food. Therefore, it has a multitude of complex defense strategies that include elements of the specific and non-specific immune system. As a result of eating behaviors, vicious habits or different types of intraoral lesions, the microbial flora diversifies and, most of the time, this fact acquires clinical resonance both locally and generally, influencing not only the integrity of oral health, but also the systemic one. The purpose of the study was to deduce a possible cause-effect relationship between the habits and behaviors of the patients in the study group, and the bacterial development specific to oral pathology. The study was conducted on a group of patients, with mixed oral pathology, represented by carious processes, endodontic conditions and patients wearing dental prosthesis. The collection of biological preparations took place in the dental office, and the analysis and interpretation of the results were carried out in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. The resulting conclusions indicate how we can use the obtained data, as an adjuvant in the therapeutic arsenal regarding the fight against bacterial growth and the acquisition of effective oral hygiene.

KEYWORDS: Microbiological Examination, Bacterial Distribution Patterns, Dental Caries, Root Canal System, Prosthetic Restorations.

I. INTRODUCTION

The oral cavity is the entry gate of the body from the external environment to the interior and represents one of the most complex and significant sites from a biological point of view [1, 2].

Recent studies have confirmed the fact that oral health is inextricably linked to the health of the whole body and vice versa [3, 4]. Therefore, maintaining adequate oral hygiene is of vital importance for the self-esteem and well-being of each individual [5, 6].

The oral environment is one of the key interfaces between the body and the environment, therefore it can act as an entry site for some pathogenic microorganisms, especially through the air or through the ingestion of food [7].

Therefore, it has a multitude of complex defense strategies that include elements of the specific and non-specific immune system [8, 9]. Finally, the ability of the host to recognize and trigger an immunological response against pathogens and simultaneously tolerate the resident microflora remains one of the most remarkable achievements of human evolution, and the precise mechanisms that allow this level of discrimination have not been elucidated. fully understood until now [10, 11].

As a result of eating behaviors, vicious habits or different types of intraoral lesions, the microbial flora diversifies and, most of the time, this fact acquires clinical resonance both locally and generally, influencing not only the integrity of oral health, but also the systemic one [12].

By trying to discover the variations between the bacterial plaque formed at the level of carious lesions, infected root canals and prosthetic works, compared to the commensal oral microflora, we are able to explain the current situation to patients in a way that they understand and direct them to adopt a style of balanced life [13].

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II. MATERIALS AND METHODS

Materials

The following materials were used:

- Personal protective equipment,
- Sterile degreased glass slides,
- Sterile saline or water
- Alcohol 90%,
- Sterile disposable consultation kit,
- Sterile cotton rolls,
- Oral aspirator,
- Sterile exploratory probes,
- Bacteriological loops,
- Pathological products collected from patients,
- Reagents needed for Gram staining:
- Primary Stain: Crystal Violet Staining Reagent,
- Mordant: Gram's iodine solution,
- Decolorizing Agent: Acetone/ethanol (50:50 v:v),
- Counterstain: 0.1% basic fuchsin solution,
- Water.
- Optical microscope with immersion objective (x100),
- Immersion Oil (Cedar Wood Oil),
- Bunsen burner,
- Camera of the Samsung Galaxy A40 phone.

Methods

Selection of the patient group

The study was conducted on a group of 27 patients, aged between 7 and 85 years, with mixed oral pathology, represented by carious processes, endodontic conditions and patients patients wearing dental prosthesis.

The collection of biological preparations took place in the dental office, where the patients presented themselves for an individualized treatment plan, and the analysis and interpretation of the results were carried out in the Microbiology Discipline, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

Collection of pathological products

For the collection of biological samples, sterile single-use consultation kits were used, and the actual sampling of the pathological product from the level of the carious processes to obtain the altered dentine and from the surface of the prosthetic works for the bacterial plaque was done with the help of exploratory probes, and in the case of samples taken from the infected root canals, a paper cone was used.

Preparation of a slide smear

The stages through which we obtained high-quality smears are highlighted in Figure 1, [14].

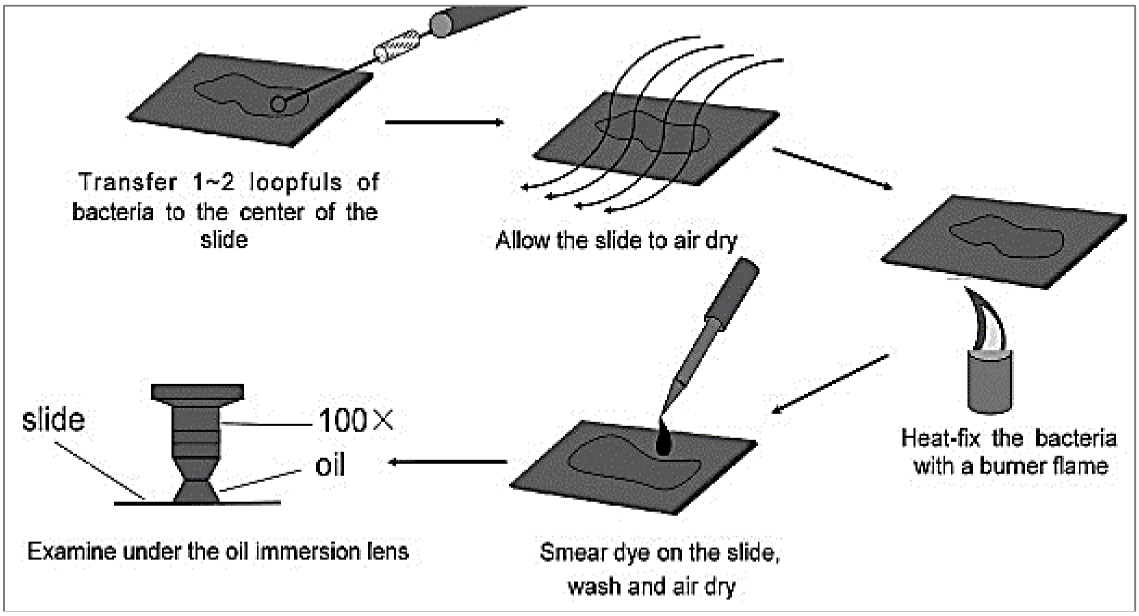

Figure 1. Performing the smear

Staining of smears was done by the Gram technique, which is a double stain in which Gram-positive bacteria are stained with gentian violet and then decolorized with alcohol-acetone mixture. The Gram stain is fundamental to the phenotypic characterization of bacteria. Gram stain procedure is highlighted in Figure 2, [15].

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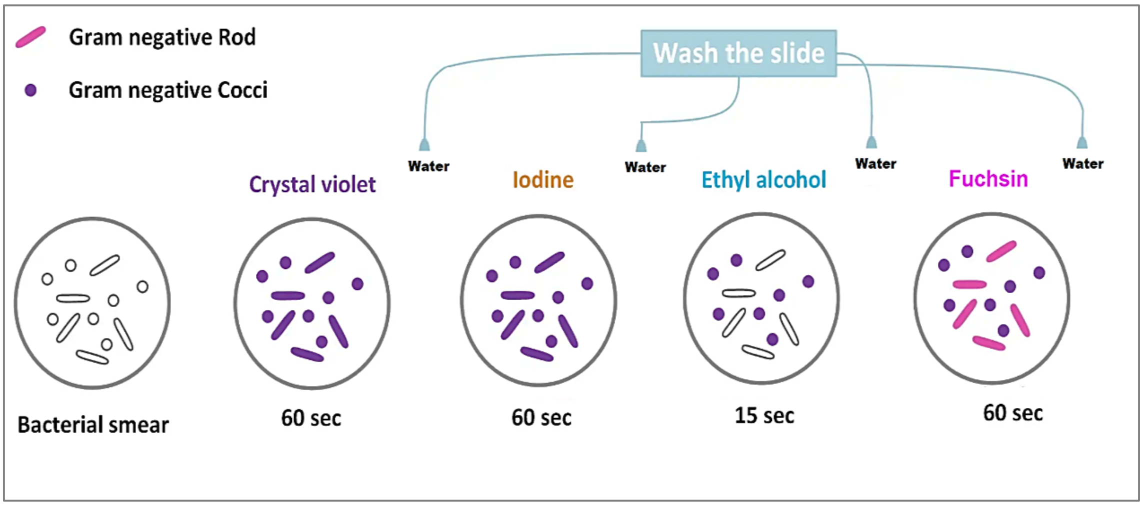

Figure 2. The Gram stain

III. RESULTS AND DISCUSSION

In patients with carious lesions, we identified the following elements: an increased percentage of cocci arranged in short chains (23.07%); thin G+ bacilli, with rounded ends, G+ cocci in diplo, in small clusters and frequent G+ cocci in diplo (15.38%); G+ cocci arranged in diplo, lanceolate, rare G+ cocci arranged in diplo, rare fusiform bacilli, and dentinal fragments (7.69%), (Figure 3).

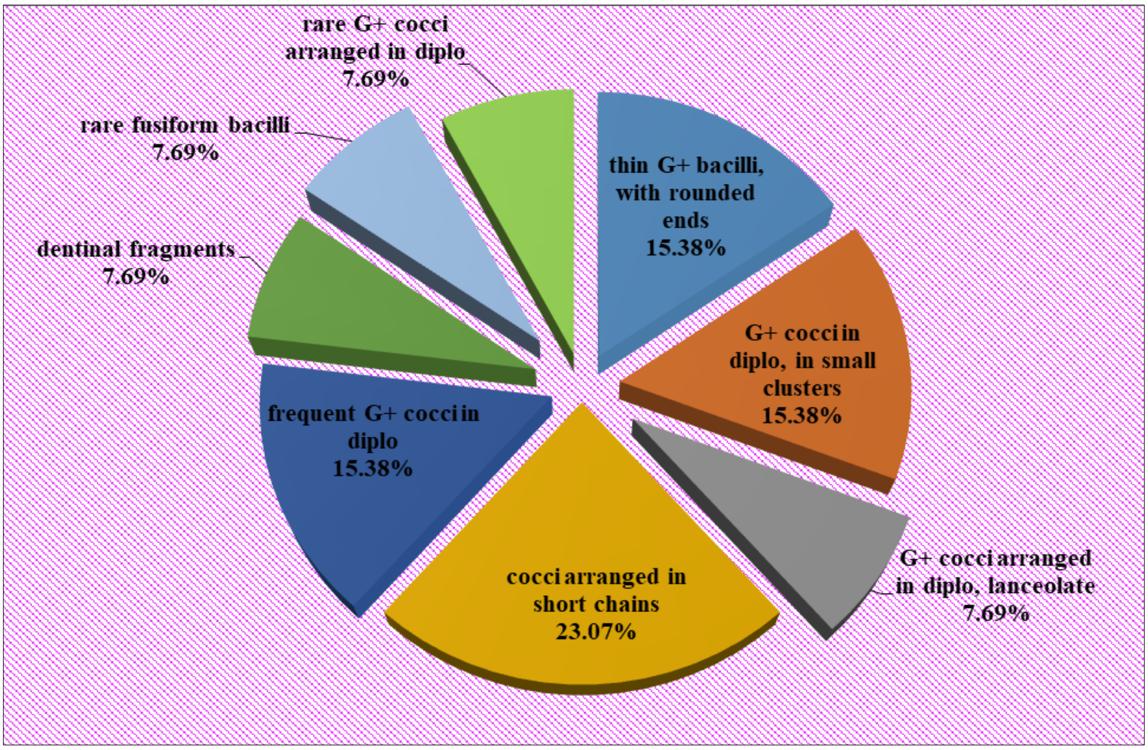

Figure 3. Distribution patterns of bacteria in patients with carious lesions

A study carried out on carious pathological products from children aged between 1.5 and 11 years, revealed a diverse and rich microflora, which includes the following elements: Scardovia wiggsiae (G+ bacillus), Slackia exigua (G+ coco-bacillus), Granulicatella elegans (coccus G+) and Firmicutes (cocobacillus G+). On the contrary, at the level of the non-carious surfaces of these individuals

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who had carious disease, taxa such as Streptococcus cristatus (coccus G+), Streptococcus gordonii (coccus G+), Streptococcus sanguinis (coccus G+), Corynebacterium matruchotii (bacillus G+) or Neisseria were highlighted flavescens (diplococci G-), [16].

Another study, based on the identification of the bacterial species incriminated in the appearance and evolution of the carious process, in elderly patients (over 60 years old), demonstrated the existence of colonies of Proteobacterium (coco-bacillus G-), Bacteroides (bacillus G-), Firmicutes (coco-bacillus G+), Fusobacterium (bacillus G-), Actinomyces (bacillus G+) and Saccharibacteria (bacillus G+), [17-19].

In patients with endodontic lesions, we identified an increased percentage of epithelial cells (27.77%), leukocytes (22.22%) and G+ cocci arranged in diplo, lanceolate (16.66%). Thin G+ bacilli, with rounded ends, cocci in short chains, G+ cocci arranged in diplo, rare fusiform bacilli, G+ cocci in diplo, in small piles, and very small fragments of epithelial tissue, were evident in a relatively low percentage (5-6%), (Figure 4).

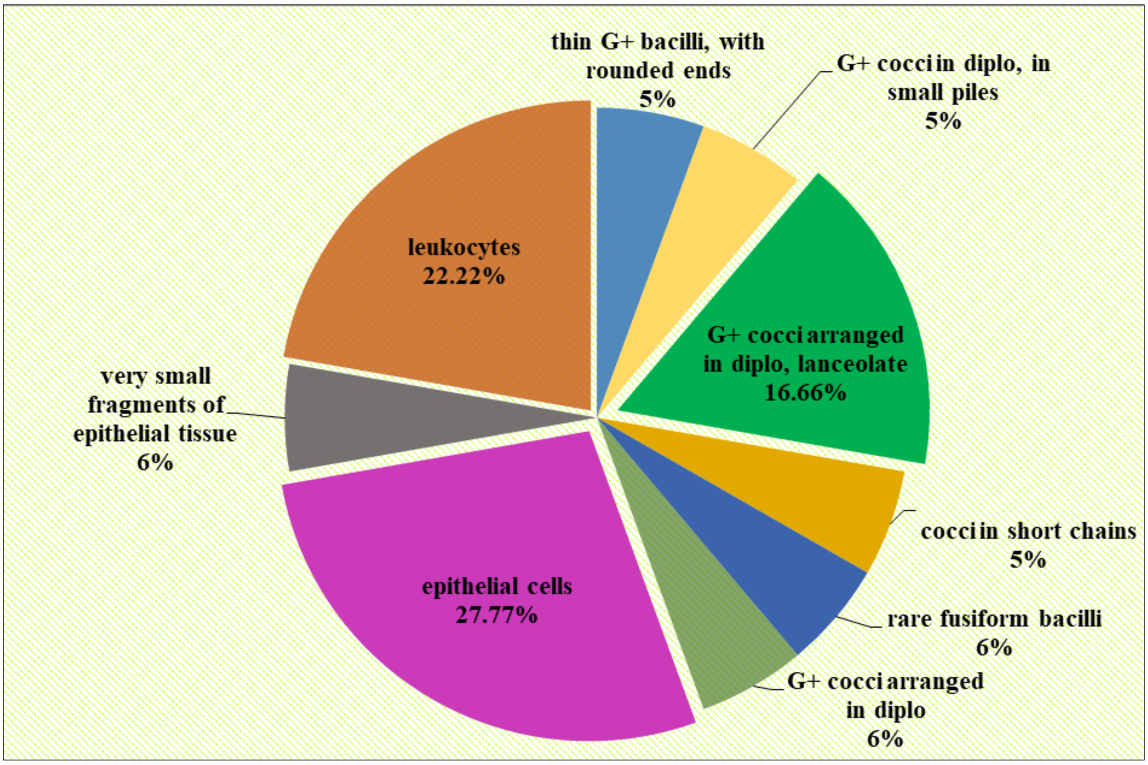

Figure 4. Distribution patterns of bacteria in patients with endodontic lesions

According to a study based on molecular methods to identify bacterial elements, several species were identified as potential pathogens incriminating in the occurrence of periapical processes, which lead to retrograde infection of the root canals [20, 21]. The most widespread microorganisms were Fusobacterium nucleatum (bacillus G-), Porphyromonas endodontalis (bacillus G-), Parvimonas micra (bacillus G+), Eikenella corrodens (bacillus G-), Olsenella uli (bacillus G+) and Streptococci. The most exciting aspect was that, in addition to the usual bacterial species found in endodontic infections, new cultures of Prevotella baroniae (bacillus G-) or Dialister invisus (bacillus G-), and even some phenotypes not yet cultured (Bacteroidetes clone X083 and Synergistes clone BA121, [22].

In patients wearing prostheses (acrylic, skeleton) and in patients with fixed prosthetic restorations, we identified the following elements: epithelial cells (19.35%); G+ cocci arranged in diplo, in small piles and G+ cocci in diplo, lanceolate 12.90%; thin G+ bacilli with rounded ends, cocci in short chain and rare polymorphonuclear (9.67%). In a smaller percentage were identified: G+ cocci in diplo, isolated (6.75%); thin G- bacilli, squamous epithelial cells, fusobacteria, rare G- cocci, rare G+ cocci arranged in diplo, and pleomorphic forms (3.22%), (Figure 5).

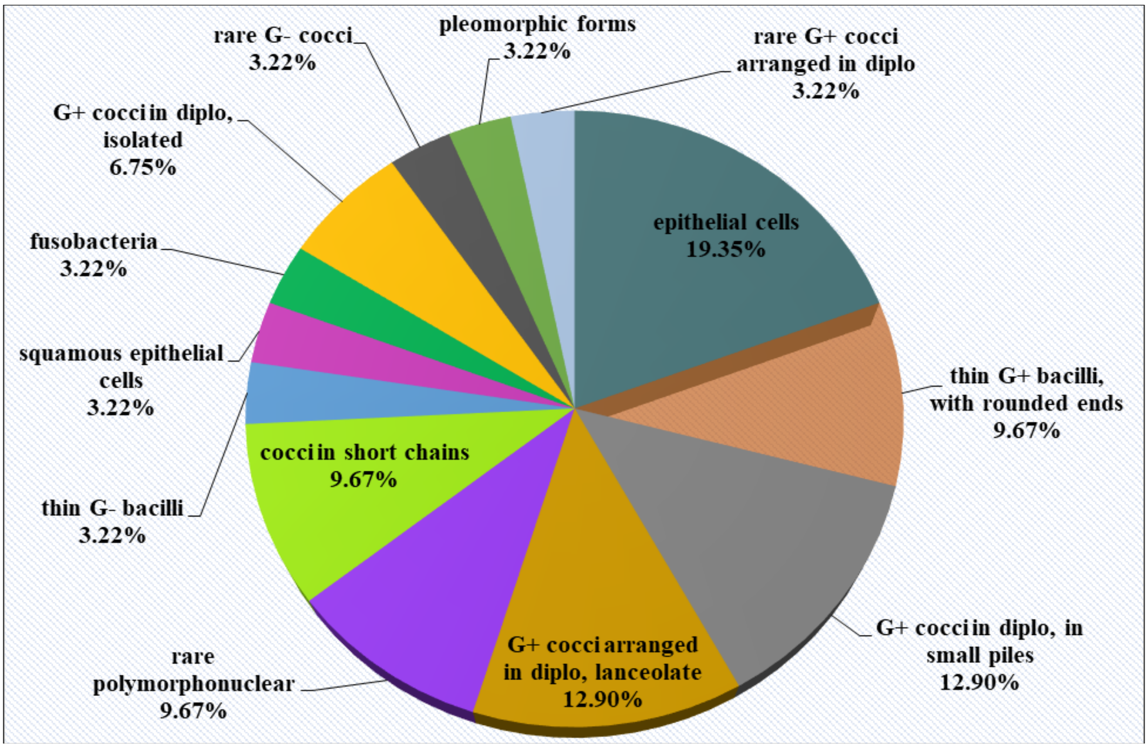

Figure 5. Distribution patterns of bacteria in patients with prosthetic restorations

A study published by Fujinami et al. (2021) for patients wearing dentures, identified the genesis of the bacterial species Streptococcus (coccus G+), Lactobacillus (bacillus G+), Rothia (coccus G+) and Corynebacterium (bacillus G+) as being much more abundant, compared to samples taken from dental plaque. Also, the bacteria predominant in the occurrence of pneumonia inhabited the surfaces of prosthetic works. Moreover, Candida albicans was closely related to three acidogenic microorganisms [23, 24].

The research submitted by O'Donnell (2015) concluded that the presence of natural teeth, in addition to artificial ones, influences the composition of the bacterial biofilm in the oral cavity, so that bacilli and actinobacteria dominated [25].

A study published by Fujinami et al. (2021) for patients wearing dentures indicated that the following bacterial species Streptococcus (coccus G+), Lactobacillus (bacillus G+), Rothia (coccus G+) and Corynebacterium (bacillus G+) were much more abundant on the surfaces of dental prostheses, compared to the samples taken from dental plaque. Also, the bacteria predominantly involved in the occurrence of pneumonia were identified on the surfaces of dental prostheses. Moreover, Candida albicans was closely related to three acidogenic microorganisms [23, 26].

Research presented by O'Donnell (2015) concluded that the presence of natural teeth, in addition to artificial ones, influences the composition of the bacterial biofilm in the oral cavity, making bacilli and actinobacteria predominant [25].

IV. CONCLUSION

The study performed on preparations taken from patients with carious lesions indicated the presence of numerous G+ cocciarranged in diplo,cocci arranged in short chains, thin G+ bacilli with rounded ends, and rare G+ cocci in diplo, fusiform bacilli and dentin fragments.

The study of preparations taken from patients with endodontic pathology indicated the presence of numerous epithelial cells and leukocytes, and rare cocci in short chains, thin G+ bacilli with rounded ends, G+ cocci in diplo and in small piles, fusiform bacilli, and very small fragments of epithelial tissue, and cocci arranged in short chains.

The study carried out on pathological preparations taken from patients with prosthetic works, recorded a relatively large number of epithelial cells and G+ cocci arranged in diplo, lanceolateand in small piles, and rare desquamated epithelial cells, fusobacteria, and pleomorphic forms.

Compliance with ethical standards

Acknowledgments
We gratefully acknowledge the patients for giving us permission to conduct this study.

Disclosure of conflict of interest
The authors declare no conflict of interest.

Statement of informed consent
Informed consent was obtained from the patients included in the study.

Authors' contributions
Authors CLD, ICM and OB contributed to this work in conceptualization, methodology, software, and formal analysis. CCA and SDA contributed in software, formal analysis, and data curation. CLD, ICM and OB contributed in validation, supervision, project administration. All authors read and approved the final version of the manuscript.

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REFERENCES

[1]. Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL. The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontol 2000. 2021 Oct;87(1):107-131. doi: 10.1111/prd.12393. PMID: 34463991; PMCID: PMC8457218.
[2]. Homayun B, Lin X, Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics. 2019 Mar 19;11(3):129. doi: 10.3390/pharmaceutics11030129. PMID: 30893852; PMCID: PMC6471246.
[3]. Oral Health in America: Advances and Challenges [Internet]. Bethesda (MD): National Institute of Dental and Craniofacial Research(US); 2021 Dec. Section 3A, Oral Health Across the Lifespan: Working-Age Adults. Available from: https://www.ncbi.nlm.nih.gov/books/NBK578294/
[4]. Albu CC, Milicescu S, Albu SD, Ion G. Tongue Piercing: a Current Trend with High-risk Effects. Rev. Chim.[internet]. 2019 Aug;70(8):2851-2853. Available from: https://doi.org/10.37358/RC.19.8.7441
[5]. Baiju RM, Peter E, Varghese NO, Sivaram R. Oral Health and Quality of Life: Current Concepts. J ClinDiagn Res. 2017 Jun;11(6):ZE21-ZE26. doi: 10.7860/JCDR/2017/25866.10110. Epub 2017 Jun 1. PMID: 28764312; PMCID: PMC5535498.
[6]. Defta CL, Albu DF, Albu CC, Albu SD, Botoacă O. Parallelism between the efficiency of Sensodyne toothpaste and Lacalut toothpaste with hydroxyapatite in oral hygiene. World Journal of Advanced Research and Reviews. 2022; 16(03):098-106. DOI: wjarr.2022.16.3.1306
[7]. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaura E. The oral microbiome - an update for oral healthcare professionals. Br Dent J. 2016 Nov 18;221(10):657-666. doi: 10.1038/sj.bdj.2016.865. PMID: 27857087.
[8]. Aristizábal B, González Á. Innate immune system. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al., editors. Autoimmunity: From Bench to Bedside [Internet]. Bogota (Colombia): El Rosario University Press; 2013 Jul 18. Chapter 2. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459455/
[9]. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018 Dec;16(12):745-759. doi: 10.1038/s41579-018-0089-x. PMID: 30301974; PMCID: PMC6278837.
[10]. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Innate Immunity. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26846/
[11]. Nicholson LB. The immune system. Essays Biochem. 2016 Oct 31;60(3):275-301. doi: 10.1042/EBC20160017. PMID: 27784777; PMCID: PMC5091071.
[12]. Marsh P, Lewis M, Rogers H, Williams D, Wilson M. In: Alison Taylor, Veronika Watkins, editors. Oral microbiology. 5th ed. Edinburgh: Churchill Livingstone; 2009.
[13]. Hasan S, Ahmed S, Panigrahi R, Chaudhary P, Vyas V, Saeed S. Oral cavity and eating disorders: An insight to holistic health. J Family Med Prim Care. 2020 Aug

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25;9(8):3890-3897. doi: 10.4103/jfmpc.jfmpc_608_20. PMID: 33110784; PMCID: PMC7586628.

[14]. Techniques for oral microbiology [Internet]. Atlas of Oral Microbiology. Academic Press; 2015 [cited 2022Dec23]. Available from: https://www.sciencedirect.com/science/article/pii/B9780128022344000021

[15]. Gram staining : Principle, procedure, results and interpretation [Internet]. Gram staining : A Complete Guide. [cited 2022Dec23]. Available from: https://microbiologie-clinique.com/gram-stain-principle-steps-interpretation.html

[16]. Fakhruddin KS, Ngo HC, Samaranayake LP. Cariogenic microbiome and microbiota of the early primary dentition: A contemporary overview. Oral Dis. 2019 May;25(4):982-995. doi: 10.1111/odi.12932. Epub 2018 Sep 19. PMID: 29969843.

[17]. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2013; 380(9859):2224-2260.

[18]. Albu ŞD, Pavlovici RC, Imre M, Ion G, Ţâncu AMC, Albu CC. Phenotypic heterogeneity of non-syndromic supernumerary teeth: genetic study. Rom J MorpholEmbryol. 2020 Jul-Sep;61(3):853-861. doi: 10.47162/RJME.61.3.23. PMID: 33817726; PMCID: PMC8112786.

[19]. Albu CC, Pavlovici RC, Imre M, Ţâncu AMC, Stanciu IA, Vasilache A, Milicescu Ş, Ion G, Albu ŞD, Tănase M. Research algorithm for the detection of genetic patterns and phenotypic variety of non-syndromic dental agenesis. Rom J MorpholEmbryol. 2021 Jan-Mar;62(1):53-62. doi: 10.47162/RJME.62.1.05. PMID: 34609408; PMCID: PMC8597362

[20]. Vasilache A, Popa M, Albu CC, Dragomirescu AO, Vasilache A, Bencze MA, Suciu I, Ionescu E. Evaluation of the biocompatibility of laser irradiated plant extracts used as adjuvants in irrigation and sanitization of root canals. Farmacia. 2021;69(5):934-940.DOI: 10.31925/farmacia.2021.5.16.

[21]. Vasilache A, Popa M, Albu CC, Dragomirescu AO, Vasilache A, Suciu I, Chirilă M, Ionescu E. Evaluation of the antimicrobial effect of herbal extracts used as an adjuvant in the cleaning of root canals by laser beam irradiation. Romanian Journal of Oral Rehabilitation, 2021;13(2):18-26.

[22]. Siqueira JF Jr, Rôças IN. The microbiota of acute apical abscesses. J Dent Res. 2009 Jan;88(1):61-5. doi: 10.1177/0022034508328124. PMID: 19131319.

[23]. Fujinami W, Nishikawa K, Ozawa S, Hasegawa Y, Takebe J. Correlation between the relative abundance of oral bacteria and Candida albicans in denture and dental plaques. J Oral Biosci. 2021 Jun;63(2):175-183.

[24]. Albu DF, Onofriescu M, Nada ES, Ion G, Milicescu S, Albu SD, Albu CC. The importance of customized biometric correlations in the prevention of growth and development disorders - a determining factor in the social integration of children and adolescents with mental disabilities. Rev. de Cercet. siInterv. Soc. 2021;72(1):324-337. DOI: 10.33788/rcis.72.20.

[25]. O'Donnell LE, Robertson D, Nile CJ, Cross LJ, Riggio M, Sherriff A, Bradshaw D, Lambert M, Malcolm J, Buijs MJ, Zaura E, Crielaard W, Brandt BW, Ramage G. The Oral Microbiome of Denture Wearers Is Influenced by Levels of Natural Dentition. PLoS One. 2015 Sep 14;10(9):e0137717. doi: 10.1371/journal.pone.0137717. PMID: 26368937; PMCID: PMC4569385.

[26]. Şaramet V, Meleşcanu-Imre M, Ţâncu AMC, Albu CC, Ripszky-Totan A, Pantea M. Molecular Interactions between Saliva and Dental Composites Resins: A Way Forward. Materials (Basel). 2021 May 13;14(10):2537. doi: 10.3390/ma14102537. PMID: 34068320; PMCID: PMC8153278.