ODOVTOS
International Journal of Dental Sciences
https://revistas.ucr.ac.cr/index.php/Odontos | ISSN: 2215-3411

CLINICAL RESEARCH

DOI: 10.15517/ijds.2023.56059

Received:
21-V-2023

Accepted:
20-VII-2023

Published Online:
1-VIII-2023

Microbiota of Dental Caries in Primary Teeth of a Costa Rican Child Population

Microbiota de la caries dental en piezas primarias de una población infantil costarricense

Keysy Tenorio-Soto DDS[1]; Natalia Gutiérrez-Marín DDS, Mag[2]
Tatiana Ramírez-Mora DDS[3]; Pamela Altamirano-Silva[4]

1. General Dentist, Private Practice. Alajuela, Costa Rica. https://orcid.org/0000-0002-9819-8275
2. Faculty of Dentistry, Universidad de Costa Rica, San José, Costa Rica.
https://orcid.org/0000-0002-1801-9856
3. Faculty of Dentistry, Universidad de Costa Rica, San José, Costa Rica.
https://orcid.org/0000-0002-1837-0590
4. Faculty of Microbiology, Universidad de Costa Rica, San José, Costa Rica.
https://orcid.org/0009-0001-3940-5801

Correspondence to: Dr. Keysy Tenorio Soto - keysy.tenorio@ucr.ac.cr

ABSTRACT: The objective of this research was to identify bacteria present in the microbiota of dentinal carious lesions in primary molars of some Costa Rican pediatric patients. Data were collected from 15 children aged between 4 and 8 years old who attended the Pediatric Dentistry Clinic at the Faculty of Dentistry from the University of Costa Rica (UCR). The inclusion criteria were: infants between 4 and 8 years old who presented cavitated carious lesions in primary teeth, who were actively attended by students at the Faculty of Dentistry from the UCR, and whose parents or legal guardians signed the informed consent to participate in this research. Samples were taken using a sterile spoon, placed in storage vials, and subjected to various conventional and molecular microbial identification techniques, such as Gram stain identification, catalase tests, oxidase, TSI, API 20E, API STAPH, and VITEK 2. Of the 60 bacterial strains subjected to Gram staining, the following was obtained: 28 Gram-positive bacteria and 32 Gram-negative bacteria. The main isolated organisms were species of *Staphylococcus epidermidis*, *Pasteurella pneumotropica/Mannheimia haemolytica*, *Pantoea spp*, and *Streptococcus mutans*.

KEYWORDS: Microbiota; Dental caries; Primary teeth; Bacteria; Children.

RESUMEN: El objetivo de esta investigación fue identificar las bacterias presentes en la microbiota de lesiones cariosas dentinales en molares primarias de pacientes pediátricos costarricenses. Las muestras fueron recolectadas de 15 niños entre los 4 y 8 años que fueron atendidos en la Clínica de Odontopediatría en la Facultad de Odontología de la Universidad de Costa Rica (UCR). Los criterios de inclusión fueron: pacientes entre los 4 y los 8 años de edad que presentaran lesiones cariosas cavitadas en dientes primarios, que se encuentraran activos para su atención por estudiantes de la Facultad de Odontología de la UCR, y que los padres o encargados legales firmaran el consentimiento informado para participar en esta investigación. Las muestras se tomaron utilizando una cuchareta estéril, colocándolas en viales de almacenamiento y fueron sometidas a diversas técnicas de identificación microbiana convencionales y moleculares tales como: identificación por Tinción de Gram, pruebas catalasa, oxidasa, TSI, API 20E, API STAPH y VITEK. De las 60 cepas bacterianas sometidas a tinción de Gram se obtuvo: 28 bacterias Gram Positivas y 32 bacterias Gram Negativas. Los principales organismos aislados fueron: especies de *Staphylococcus epidermidis*, *Pasteurella pneumotropica/ Mannheimia haemolytica*, *Pantoea spp* y *Streptococcus mutans*.

PALABRAS CLAVE: Microbiota; Caries dental; Dientes primarios; Bacterias; Niños.

INTRODUCTION

Dental caries is one of the most prevalent diseases around the world, according to the World Health Organization about 2000 million people suffer from it (1), and over 530 million children suffer from caries in the primary dentition (2). In our country, the prevalence of dental caries in children has been hardly investigated; however, in 1999 an Oral health survey was conducted; it showed that the prevalence of caries in primary dentition was 75.2% in infants between 6 and 8 years old (3,4).

The progression of carious lesions is closely related to sugar consumption, oral hygiene habits, systemic conditions, and oral microbiota (5). According to Tanner *et al*. (6), this oral microbiota is an ecological community of commensal or symbiotic microorganisms and pathogens that share the same place in our body; it colonizes the dental surfaces naturally forming the biofilm.

The oral microbiota is composed of hundreds of bacterial species, where environmental factors (such as diet, type of calving, geographic location, and host genetics) play an important role in shaping the community structure of the microbiome and determining the pathogenic species associated with dental caries (7).

Traditionally, *Streptococcus mutans* was accredited as the main cause of dental caries; nonetheless, since the 2000s, there have been studies related to cultures strictly of carious lesions that have evidenced the presence of Gram-positive bacteria such as *Lactobacillus*, *Streptococcus*, *Propionibacterium*, *Bifidobacterium*, *Actinomyces*, *Eubacterium*, *Rothia*, *Arachnia*, *Micromonas*, *Pseudoramibacterium*; and Gram-negative bacteria like *Prevotella*, *Porphyromona* and *Selenomones* (8-14).

Recent research focused on pediatric population confirm this heterogeneity in the microbiota; e.g. in Columbus, Ohio, USA, Aas *et al*. (15) identi-

fied bacterial groups in carious lesions in infants and adolescents; some of them were *Streptococcus mutans*, *Atopobium*, *Propionibacterium*, *Lactobacillus*, *Actinomyces spp*. and *Atopobium spp*. In Canada, Agnello *et al*. (16) recognized a greater number of microbial communities like *Veillonella*, *Porphyromonas*, *Streptococcus gordonii*, and *Streptococcus sanguinis*. On the other hand, in European and African populations, Yang *et al*. (17) determined the presence of *Actinobacteria* and *Firmicutes*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, and *Filifactor Alocis*.

Due to the fact that the cariogenic microbiota constantly changes (based on the race or origin place of each person), and that in Central America, specifically Costa Rica, no studies have been performed regarding this field, the objective of this research was to identify the bacteria present in the microbiota of dentinal carious lesions in primary molars in Costa Rican pediatric patients.

METHODOLOGY

A convenience sample of 15 children aged between 4 and 8 years with deep carious lesions in primary molar dentin was used. The inclusion criteria were: infants who were actively attended by students at the Faculty of Dentistry (FD) from the UCR, and whose parents or legal guardians signed the informed consent to participate in this research. The exclusion criteria were: uncooperative patients for sample collection and children who are under chronic pharmacological treatments or during the last 3 months.

The sample collection was done as follow; the molar with advanced caries was selected in each patient; the operator applied an anesthetic technique based on that molar, and absolute isolation was placed with rubber dam sheet. Then, using a sterile spoon, the researcher removed a portion of the decayed dentin and deposited it directly in a sterile centrifuge tube with 2cc of trypticase soy broth (TSB). Finally, it was transported to the laboratory. After taking the sample, the operator continued with the restorative treatment of the decayed molar.

In less than 3 hours, this sample was transferred to the microbiology laboratory from the UCR, where it was placed in TSB for 24 hours in agitation, with a temperature of 37°C to guarantee a homogeneous liquid. After incubation, the broth was sown in Blood Agar; the plates were stored for 24 and 48 hours in the Carbon Dioxide (CO_2) chamber (ThermoFisher Scientific, Waltham, Massachusetts, Estados Unidos) at 37°C to promote bacterial growth. Once this time had elapsed, the macroscopic characterization of the different bacterial colonies was carried out to determine their shape, size, and color. Each bacterial strain was isolated in a new plate of Blood Agar and stored for 24 hours in the CO_2 chamber (ThermoFisher Scientific, Waltham, Massachusetts, Estados Unidos) at 37°C as in Elbing´s and Brend´s study (18).

After 24 hours, Gram stain were performed following the procedure implemented by Coico (19) to confirm the purity of each strain. At the end of the Gram stain drying, the sample was observed under the microscope (Olympus Life Science®, Waltham, Massachusetts, Estados Unidos) (20).

The bacterial strains identified as Gram-positive were examined with the catalase test as Taylor in 1972 mentioned (21). If the result was negative, the VITEK 2 (VITEK® bioMérieux, Ciudad de México, México) test described by Vargas *et al*. was performed (22). When the result of the catalase test was positive, a rapid effervescence with bubble detachment was formed; then, the bacterial identification test API STAPH (API® bioMérieux, Ciudad de México, México) (mentioned by Holmes *et al*. and bioMérieux) was applied (22-24).

In the case of the bacterial strains identified as Gram-negative, the oxidase test was performed, according to the method described by Bou (25), through strips of paper impregnated with the reagent para-amino-N-dimethyl-aniline (Bioser® S.A, Barcelona, España), and also the Triple Sugar Iron Agar test (T.S.I Agar), as González (26) applied it in his study to determine the fermentation capacity of bacteria with lactose, glucose or sucrose. When the oxidase test result was negative, bacterial identification was performed, specifically, the API 20E (API® bioMérieux, Ciudad de México, México) tests mentioned by Holmes *et al*. and bioMérieux (23,24). However, when the oxidase test was positive or there was no more than an 85% probability of identification, the VITEK 2 (VITEK® bioMérieux, Ciudad de México, México) bacterial identification test described by Vargas *et al*. was applied (22).

ETHICAL CONSIDERATIONS

This research was approved by the Scientific Ethics Committee of the UCR (CEC-311-2022) and by the National Health Council (CONIS-390-2022).

RESULTS

Of the 15 samples of carious lesions in primary teeth, 60 pure bacterial strains were isolated through striatum in Agar plates. In each sample, a different number of strains was obtained, not only after 24 hours the growing was different but also after 48 hours because there are bacteria that take a longer time to cultivate.

Of the 60 bacterial strains subjected to the Gram test, 28 Gram-positive and 32 Gram-negative strains were obtained. Of the Gram-positive strains, 11 were negative for the catalase test, so they were examined with VITEK 2 (VITEK® bioMérieux, Ciudad de México, México). In addition, the other 17 positive strains were analyzed with the API STAPH (API® bioMérieux, Ciudad de México, México), test for their bacterial identification. On the other hand, all 32 Gram-negative bacterial strains were tested with TSI, and the results were the following: only 1 non-fermenter (it did not change its color); 18 are only glucose fermenters; 13 fermented to glucose, sucrose or lactose. Also, the oxidase test was applied to these 32 stains, in which 31 were negatives, so the API 20E (API® bioMérieux, Ciudad de México, México), test was used, and the only positive strain of oxidase (Bioser® S.A, Barcelona, España), was identified through the VITEK 2 test (VITEK® bioMérieux, Ciudad de México, México) (Figure 1).

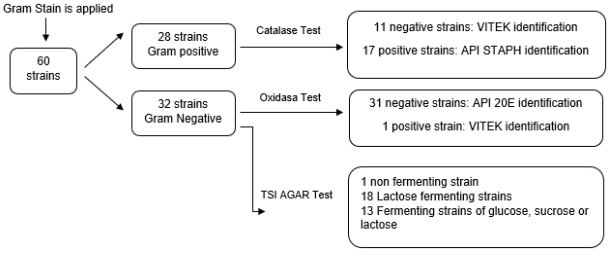

Figure 1. Laboratory analysis sequence.

After obtaining the preliminary bacterial identification tests, the microorganisms present in the dental caries samples were determined through the biochemical methods: the quantity of bacterial strains identified with API STAPH test (API® bioMérieux, Ciudad de México, Mexico) was 12: 6 *Staphylococcus epidermis*, 2 *Staphylococcus sciuri*, 2 *Staphylococcus xylosus* and 2 *Staphylococcus aereus*. With API 20E test (API® bioMérieux, Ciudad de México, Mexico) it was obtained 22 strains: 6 *PasteurellaPneumotropical/Mannheimia haemolytica*, 1 *Photobacterium damselae*, 2 *Serratia marcescen*, 6 *Pantoea spp*, 1 *Klebsiella pneumoniae spp azaenae*, 1 *Pseudomonas fluorescens putida* and 1 *Shigella spp*. Finally with VITEK 2 (VITEK® bioMérieux, Ciudad de México, Mexico) a total of 24 strains: 5 *Streptococcus mutans*, 2 *Streptococcus sanguinis*, 2 *Streptococcus thoraltensis*, 2 *Streptococcus sobrinus*, 2 *Streptococcus salivarius spp salivarius*, 2 *Streptococcus mitis Streptococcus oralis*, 1 *Vibrio alginolyticus*, 1 *Enterococcus fecalis*, 5 *Sphingomonas paucimobillis*, 1 *Rothia mucilaginosa* and 1

Dermacoccus nishinomiyaensis/*Kytococcus sedentarius*. The results in percentages can be seen in Figure 2, Figure 3 and Figure 4.

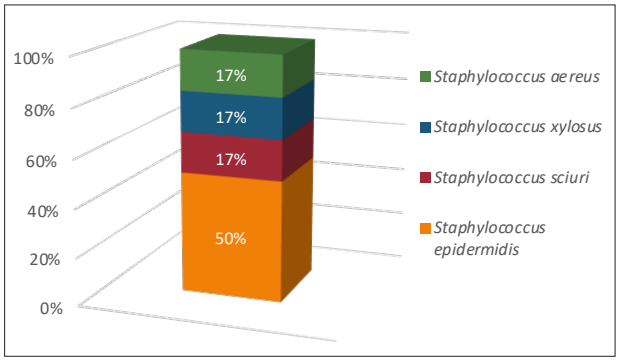

Figure 2. Percent of bacteria found according to the API STAPH bioMérieux test used.

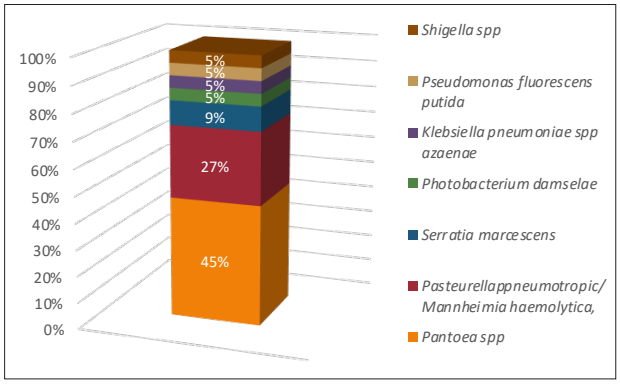

Figure 3. Percent of bacteria found according to the API 20E bioMérieux test used.

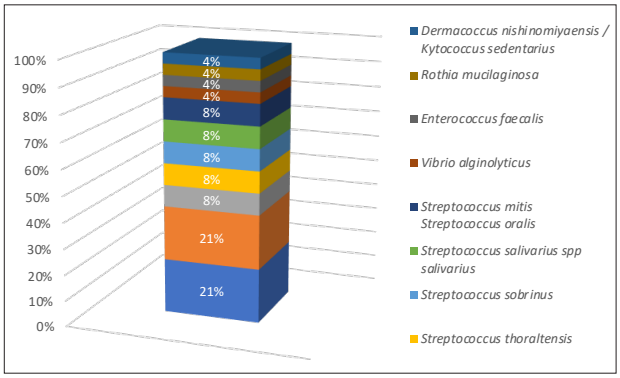

Figure 4. Percent of bacteria found according to the VITEK 2 bioMérieux test used.

DISCUSSION

The diversity of the bacterial microbiota is a very relevant issue (16,27,28). This research strengthens the evidence not only the *Streptococcus mutans* are isolated in advanced caries lesions, but also there are different bacterial strains that act together to destroy dental tissues.

Gram-positive bacteria were found in this research, whereby *Streptococcus mutans* previously identified as specific bacteria of dentinal caries in primary pieces (29) was present in some samples. This result agreed with the study made by Quidemat *et al*. (30), in which they reported that some subjects with active caries had detectable levels of *Streptococcus mutans* bacteria. However, the researchers also pointed out a high level of anaerobic bacteria such as *Leptotrichia shahii* and *Prevotella melaninogenica*, but this situation did not occur in the present study because only aerobic bacteria were identified. Other *Streptococcus* found were: *Streptococcus sanguinis* and *Streptococcus salivarius*, situation also presented in Bansal´s research (31).

Regarding the *Staphylococcus*, the main subvariant presented was the *Staphylococcus epidermidis*, similar to the research of Devang *et al*. (32); they indicated that this microorganism has a high adaptive capacity, which allows it to obtain, lose or regulate genetic elements providing better environments for colonization in the host. That is why, it is so prevalent in dental caries.

An important finding in this research was the presence of *Enterococcus faecalis* which is a Gram-positive; facultative anaerobic coccus bacterium that possesses numerous virulence factors, according to Carrero *et al*. (33). It may

be found transiently in oral cavity and soft tissue lesions, principally in populations exposed to hospital environments or in nosocomial infections; For this reason, it could be assumed that children might be exposed to these contexts prior to the sampling appointment.

Another Gram-positive bacterium found in a small number of strains in this study was *Rothia mucilaginosa*; is part of respiratory tract and oral cavity, but also has the ability to produce several infections and dental caries progression combined with other microorganisms (34,35). *Rothia mucilaginosa* that was not observed in the research of De Jesus *et al*. (36), who instead reported the presence of bacteria like *Mycosphaerella*, *Cyberlindnera*, and *Trichosporon*, in samples of deep carious lesions. Other studies also reinforce the heterogeneity of the oral microbiota; even though they did not report the finding of *Rothia*, they identified *Veillonella* and *Actinomyces*. These studies were carried out in the United States and Germany, in several age populations, so they did not share either geographical location or age range (37,38). *Rothia mucilaginosa* has been classified an opportunistic pathogen, it has the ability to be found in symbiosis and dysbiosis, which is why it was able to detect in deep caries in this investigation (34).

Regarding Gram-negative bacteria, *Pantoea* was identified. This is an enterobacterium frequently isolated in plants, fruits, and vegetables, according to Segado *et al*. (39), so the presence of this bacterium could be due to the fact that some of the subjects (before receiving dental care) did not perform proper oral hygiene; therefore, it is possible that food residues are lodged in an open cavity of caries.

They also identified the strains *Sphingomonas paucimobilli* and *Pasteurella pnuemotropica*, both Gram-negative. It is a relevant finding to highlight in this research because *Sphingomonas paucimobilli* is a bacterium located in aqueous media and is rare to find in humans; in addition, its persistence is related to infections and fever in pediatric patients (40). On the other hand, Pasteurella pnuemotropic is an opportunistic pathogen presented in the oral cavity of rodents, dogs, and cats. In fact, there are cases of mortality due to septicemia and long stays in hospitals that have been reported (41-43). In other words, the results in this research could be considered an isolated event, and not directly related to carious lesions in dentin; however, it can be assumed that children have been exposed to environments with any of the aforementioned animals; therefore, the presence of this bacterium.

The bacterial heterogeneity identified in this research accorded with studies which have established that the microbiota varies based on its location in different dental structures. It also indicates that the destruction in tissues from enamel to dentin involves different microorganisms in each area. *Streptococcus*, *Veillonella* and *Actinomyces* have been identified in carious enamel lesions. While the destruction increases and caries is harbored in dentin, other bacteria such as *Prevotela denticolens* and *Lactobacillus can* be detected (29,37,44) .

One of the benefits of this study is that it was the first research which identified the microbiota of carious lesions in dentin of primary teeth of Costa Rican children establishing similarities and differences with others, in several countries (29,31,38). However, one of the limitations was that the same identification methods were not used. In Umea Sweden, the detection was performed by Polymerase Chain Reaction (PCR) in samples obtained in 13 children, and the main species associated with caries were *Actinobaculum*, *Atopobium*, *Aggregatibacter* and *Streptococcus mutans* (38). In addition, Ribeiro *et al*. (45) applied 16S and n rRNA metagenomic identification in 13 children from Rio de

Janeiro, Brazil; they found *Streptococcus*, *Corynebacterium matruchotii*, *Actinomyces viscosus Prevotella nigrescens* and *Dialister micraerophilus*.

One of the forward-looking statements of this research is to continue with the characterization of the microbiota using molecular identification techniques, and to relate the presence of different types of bacteria with other variables such as diet type and oral hygiene.

CONCLUSION

The bacteria of the oral microbiota of Costa Rican pediatric patients, with advanced stages of dentinal carious lesions in primary dentition, which were presented in a greater number of strains, were *Staphylococcus epidermidis*, *Pasteurella ppneumotropic/Mannheimia haemolytica*, *Pantoea spp*, and *Streptococcus mutans*.

AUTHOR'S CONTRIBUTION STATEMENT

Conceptualization and design: K.T.S., N.G.M., T.R.M. and P.A.S.
Literature review: K.T.S.
Methodology and validation: K.T.S., N.G.M., T.R.M. and P.A.S.
Formal analysis: N.G.M., T.R.M. and P.A.S.
Research and data collection: K.T.S.
Resources: K.T.S. and P.A.S.
Data analysis and interpretation: K.T.S., N.G.M., T.R.M. and P.A.S.
Writing and preparation of the original draft: K.T.S. and N.G.M.
Writing, proofreading and editing: K.T.S., N.G.M., T.R.M. and P.A.S.
Supervision: N.G.M., T.R.M. and P.A.S.
Project management: .T.S. and N.G.M.
Acquisition of funds: Not applicable for this study.

REFERENCES

1. World Health Organization. Global oral health status report: towards universal health coverage for oral health by 2030. Dental Abstracts. Geneva: World Health Organization; 2022; 57 p.2.
2. OMS. Salud bucodental. Salud Bucodental. 2020. Vol. 2: p. 119-22.
3. Salas M., Solorzano I., Chavarría P. Encuesta Nacional De Salud Oral, 1999 Caries Dental. Tres Ríos; 1999. 1 Edición. Tres Ríos, Costa Rica: Inciensa 2001-03- 04, 110 p.
4. Montero O., Ulate J., Rodríguez A., Méndez Monge Docentes Odontopediatras CL, Elías A. Prevalence of dental caries on scholar children of 12 years old in Costa Rica. Rev Científica Odontológica. 2011; 7 (2): 1-10.
5. Conrads G., About I. Pathophysiology of Dental Caries. Monogr Oral Sci. 2018; 27: 1-10.
6. Tanner A.C.R., Kressirer C.A., Rothmiller S., Johansson I., Chalmers N.I. The Caries Microbiome: Implications for Reversing Dysbiosis. Adv Dent Res. 2018 Feb 22; 29 (1): 78-85.
7. Premaraj T.S., Vella R., Chung J., Lin Q., Hunter P., Underwood K., et al. Ethnic variation of oral microbiota in children. Sci Rep. 2020 Sep 8; 10 (1): 14788.
8. Huis in't Veld J.H.J., van Palenstein Helderman W.H., Backer Dirks O. Streptococcus mutans and dental caries in humans: a bacteriological and immunological study. Antonie Van Leeuwenhoek. 1979 Mar; 45 (1): 25-33.
9. Kohler B., Pettersson B.-M., Bratthall D. Streptococcus mutans in plaque and saliva and the development of caries. Eur J Oral Sci. 1981 Feb; 89 (1): 19-25.

10. Simón-Soro A., Mira A. Solving the etiology of dental caries. Trends Microbiol. 2015 Feb; 23 (2): 76-82.
11. Li Y., Ge Y., Saxena D., Caufield P.W. Genetic Profiling of the Oral Microbiota Associated with Severe Early-Childhood Caries. J Clin Microbiol. 2007 Jan; 45 (1): 81-7.
12. Struzycka I. The oral microbiome in dental caries. Polish J Microbiol. 2014; 63 (2): 127-35.
13. Obata J., Takeshita T., Shibata Y., Yamanaka W., Unemori M., Akamine A., et al. Identification of the Microbiota in Carious Dentin Lesions Using 16S rRNA Gene Sequencing. Ratner AJ, editor. PLoS One. 2014 Aug 1; 9 (8): e103712.
14. Liu G., Wu C., Abrams W.R., Li Y. Structural and Functional Characteristics of the Microbiome in Deep-Dentin Caries. J Dent Res. 2020 Jun 20; 99 (6): 713-20.
15. Aas J.A., Griffen A.L., Dardis S.R., Lee A.M., Olsen I., Dewhirst F.E., et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008 Apr; 46 (4): 1407-17.
16. Agnello M., Marques J., Cen L., Mittermuller B., Huang A., Chaichanasakul Tran N., et al. Microbiome Associated with Severe Caries in Canadian First Nations Children. J Dent Res. 2017 Nov 14; 96 (12): 1378-85.
17. Yang Y., Zheng W., Cai Q., Shrubsole M.J., Pei Z., Brucker R., Steinwandel M., Bordenstein S.R., Li Z., Blot W.J., Shu X.-O., Long J. 2019. Racial differences in the oral microbiome: data from low-income populations of African ancestry and European ancestry. mSystems 4: e00639-19. https://doi.org/10.1128/ mSystems.00639-19
18. Elbing K.L., Brent R. Growth in Liquid or Solid Media. In: Current Protocols in Protein Science. Hoboken, N.J., USA: John Wiley & Sons, Inc.; 1998. p. A.4B.1-A.4B.3.
19. Coico R. Gram Staining. Curr Protoc Microbiol. 2006 Feb 15; 00 (1): 3-4.
20. Casasola M. La importancia de realizar una correcta tinción de Gram en la identificación bacteriana. Rev Col Microbiol y Química Clínica Costa Rica. 2022; 27 (2): 89-98.
21. Taylor W.I., Achanzar D. Catalase Test as an Aid to the Identification of Enterobacteriaceae. Appl Microbiol. 1972; 24 (1): 58-61.
22. Vargas L.J., Vila A., Lanza A., Bonvehi P., Nazar J., Mikietuk A., et al. Utilidad del sistema VITEK en la identificación bacteriana y estudios de sensibilidad antimicrobiana. Acta Bioquimica Clinica Latinoamericana. 2005 Mar; 39 (1): 19-25.
23. Holmes B., Willcox W.R., Lapage S.P. Identification of Enterobacteriaceae by the API 20E system. J Clin Pathol. 1978 Jan 1; 31 (1): 22-30.
24. BioMérieux. Sistemas Miniaturizados API®. ApiWeb. 2010. p. 5.
25. Bou G., Fernández-Olmos A., García C., Sáez-Nieto J.A., Valdezate S. Métodos de identificación bacteriana en el laboratorio de microbiología. Enferm Infecc Microbiol Clin. 2011 Oct; 29 (8): 601-8.
26. González J. Reacciones En El Agar Hierro-Triple Azucar. Universidad Central De Venezuela. 2011. p. 1-3.
27. Wade W.G. The oral microbiome in health and disease. Pharmacol Res. 2013 Mar; 69 (1): 137-43.
28. Fakhruddin K.S., Ngo H.C., Samaranayake L.P. Cariogenic microbiome and microbiota of the early primary dentition: A contemporary overview. Oral Dis. 2019 May 19; 25 (4): 982-95.
29. Zhang J.S., Chu C.-H., Yu O.Y. Oral Microbiome and Dental Caries Development. Dent J. 2022 Sep 30; 10 (10): 184.
30. Qudeimat M.A., Alyahya A., Karched M., Behbehani J., Salako N.O. Dental plaque microbiota profiles of children with caries-

free and caries-active dentition. J Den. 2021 Jan; 104 (November 2020): 103539.
31. Bansal K., Chaudhary R., Mathur V., Tewari N. Comparison of oral micro-flora in caries active and caries free Indian children using culture techniques and PCR analysis. Indian J Dent Res. 2020; 31 (3): 420.
32. Devang Divakar D., Muzaheed, Aldeyab S.S., Alfawaz S.A., AlKheraif A.A., Ahmed Khan A. High proportions of Staphylococcus epidermidis in dental caries harbor multiple classes of antibiotics resistance, significantly increase inflammatory interleukins in dental pulps. Microb Pathog. 2017 Aug; 109: 29-34.
33. Carrero Martínez C., Cristina M., Gilbert G., Alexandra M., Lapiolo M., Serna Varona F., et al. Baja Frecuencia de Enterococcus faecalis en Mucosa Oral de Sujetos que Acuden a Consulta Odontológica. Rev Fac Odontol Univ Antioq. 2015; 26 (2): 261-70.
34. Tsuzukibashi O., Uchibori S., Kobayashi T., Umezawa K., Mashimo C., Nambu T., et al. Isolation and identification methods of Rothia species in oral cavities. J Microbiol Methods. 2017; 134: 21-6.
35. AlEraky D.M., Madi M., El Tantawi M., AlHumaid J., Fita S., AbdulAzeez S., et al. Predominance of non-Streptococcus mutans bacteria in dental biofilm and its relation to caries progression. Saudi J Biol Sci. 2021; 28 (12): 7390-5.
36. de Jesus V.C., Shikder R., Oryniak D., Mann K., Alamri A., Mittermuller B., et al. Sex-Based Diverse Plaque Microbiota in Children with Severe Caries. J Dent Res. 2020 Jun 28; 99 (6): 703-12.
37. Grier A., Myers J.A., O'Connor T.G., Quivey R.G., Gill S.R., Kopycka-Kedzierawski D.T. Oral Microbiota Composition Predicts Early Childhood Caries Onset. J Dent Res. 2021 Jun 24; 100 (6): 599-607.
38. Lif Holgerson P., Öhman C., Rönnlund A., Johansson I. Maturation of Oral Microbiota in Children with or without Dental Caries. Wen Z, editor. PLoS One. 2015 May 28; 10 (5): e0128534.
39. Segado Arenas A. Pantoea agglomerans: ¿un nuevo patógeno en la unidad de cuidados intensivos neonatales? Arch Argent Pediatr. 2012 Aug 1; 110 (4): e77-9.
40. Benevides G.N., Hein N., Lo D.S., Ferronato A.E., Ragazzi S.L.B., Yoshioka C.R.M., et al. Otomastoiditis caused by Sphingomonas paucimobilis: case report and literature review. Autops Case Reports. 2014; 4 (3): 13-20.
41. Scharmann W., Heller A. Survival and transmissibility of Pasteurella pneumotropica. Lab Anim. 2001 Apr 1; 35 (2): 163-6.
42. Nimri L.F. Bacteremia in Children: Etiologic Agents, Focal Sites, and Risk Factors. J Trop Pediatr. 2001 Dec 1; 47 (6): 356-60.
43. Porter R.S., Hay C.M. Pasteurella Endocarditis: A Case Report and Statistical Analysis of the Literature. Case Rep Infect Dis. 2020 Jul 20; 1-10. doi:10.1155/2020/8890211
44. García-Castro L., Tello-Guerrero G., Álvaro-Ordoñez L., Perona-Miguel de Priego G.A. Caries dental y microbiota. Revisión. Rev Cient Odontol. 2017; 5 (1): 668-78.
45. Ribeiro A.A., Azcarate-Peril M.A., Cadenas M.B., Butz N, Paster B.J., Chen T., et al. The oral bacterial microbiome of occlusal surfaces in children and its association with diet and caries. Nascimento M, editor. PLoS One. 2017 Jul 5; 12 (7): e0180621.