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# Root canal microbiota as an augmented reservoir of antimicrobial resistance genes in type 2 diabetes mellitus patients

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## Abstract

Antimicrobial resistance is a global public health problem. Root canal microbiota associated with apical periodontitis represents a well-known reservoir of antimicrobial resistance genes (ARGs). However, the effect of type 2 diabetes mellitus (T2DM) in this reservoir is unknown. This study aimed to establish if root canal microbiota associated with apical periodontitis in T2DM patients is an augmented reservoir by identifying the prevalence of nine common ARGs and comparing it with the prevalence in nondiabetic patients. Methodology: This cross-sectional study included two groups: A T2DM group conformed of 20 patients with at least ten years of living with T2DM and a control group of 30 nondiabetic participants. Premolar or molar teeth with pulp necrosis and apical periodontitis were included. A sample was collected from each root canal before endodontic treatment. DNA was extracted, and ARGs were identified by polymerase chain reaction. Results: *tetW* and *tetM* genes were the most frequent (93.3 and 91.6%, respectively), while *ermA* was the least frequent (8.3%) in the total population. The distribution of the ARGs was similar in both groups, but a significant difference ($p<0.005$) was present in *ermB*, *ermC*, *cfxA*, and *tetQ* genes, being more frequent in the T2DM group. A total of eighty percent of the T2DM patients presented a minimum of four ARGs, while 76.6% of the control group presented a maximum of three. Conclusions: Root canal microbiota associated with apical periodontitis in T2DM patients carries more ARGs. Therefore, this pathological niche could be considered an augmented reservoir.

**Keywords:** Root canal. Apical periodontitis. Type 2 diabetes mellitus. Antibiotic resistance genes.

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## Introduction

Apical periodontitis is a common oral disease[1] characterized by an inflammatory response of the host immune system to microorganisms from the oral microenvironment that have infected the tooth root canal,[2] mainly due to caries. It is considered a biofilm-induced pathology[3,4] that can be solved by extracting the tooth or by eliminating and entombing microorganisms by cleaning and filling the root canal. Therefore, while the tooth is not extracted or the root canal endodontically treated, it remains as a pathological niche harboring a well-established multispecies biofilm.[5]

Chronic hyperglycemic states, whether for defects in insulin secretion, insulin action, or both, impair the host's immune response, making the individual susceptible to different, recurrent, and severe infections, including processes in the oral cavity, which may worsen glycemic control inducing a vicious circle. Then, the use of antimicrobials becomes necessary and more frequent in these individuals, thus promoting greater antimicrobial resistance and negatively impacting infection control.[6,7] Type 2 diabetes mellitus (T2DM) and antimicrobial resistance are two leading global public health problems, whose prevalence constantly increases worldwide. The high risk of presenting difficulty to treat potentially deadly infections indicates the synergistic coexistence of both conditions.[6,8]

Different mechanisms of bacterial resistance are conducted and transferred by antimicrobial resistance genes (ARGs). Their propagation in commensal and pathogenic microorganisms benefits from establishing multispecies biofilms on specific niches, which provides an ideal environment for horizontal gene transfer via plasmids, bacteriophages, or transposons[9,10] from bacterial cells of the same and other species, even from bacteria to yeast.[11,12] Previous studies estimate that 40% of the horizontal gene transfer occurs between bacteria of the same niche (intra-niche), while the mechanism is still unknown in the remaining 60%. However, one theory establishes that it occurs inter-niche, i.e., from one body site to another, either during disease states or throughout the individual's life.[12] This established interconnection of multispecies biofilms can produce new genetic combinations.[11]

Horizontal gene transfer can become clinically relevant when occurring between niches, which happens mainly among the two body sites with the most significant number of microorganisms, that is, the oral cavity and the gastrointestinal tract. The genetic propagation in these niches is expected due to their anatomical relation. However, a connection between them and systemic circulation suggests that genetic information could move directly to the human circulatory system and then to other locations,[12] as in Figure 1. Thus the need to investigate ARGs presentation patterns in distinct niches, not only because of the possibility of local resistant infections, but also because of the possible association with complex resistant infections in locations other than the oral cavity.

Therefore, this study aimed to establish if root canal (oral pathological niche) microbiota, associated with pulp necrosis and apical periodontitis, is an augmented ARGs reservoir in T2DM patients by identifying the prevalence of nine ARGs and comparing it with the present prevalence in nondiabetic patients.

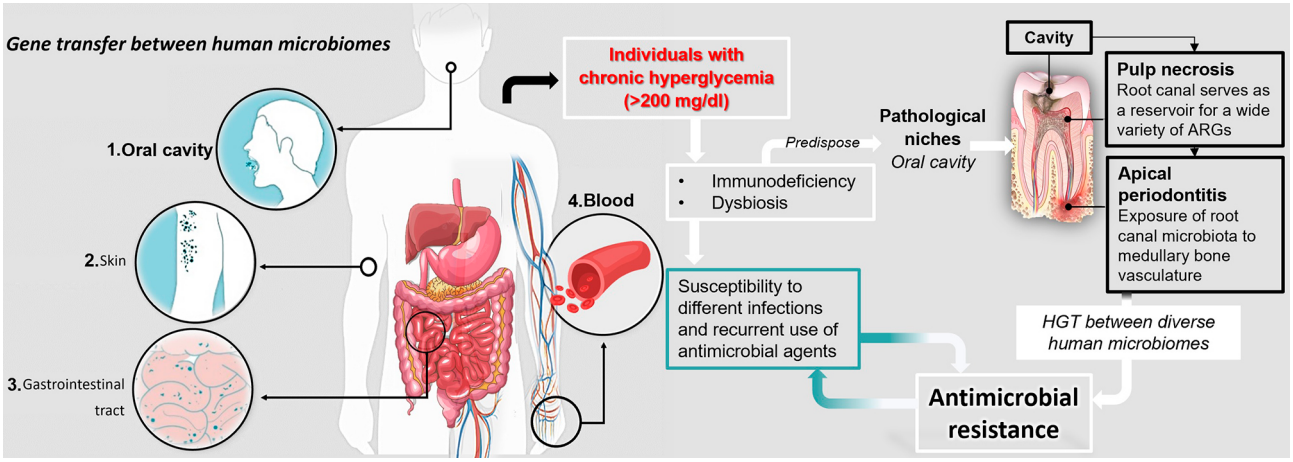


**Figure 1-** Influence of pathogenic root canal biofilms in individuals with poorly controlled type 2 diabetes mellitus on human antimicrobial resistance. ARGs: antimicrobial resistance genes; HGT: horizontal gene transfer

# Methodology

## Patient selection and clinical evaluation

This cross-sectional study recruited patients who attended the Clinic of Endodontics of the Faculty of Medicine at the Autonomous University of Querétaro (FMUAQ) who strictly met the selection criteria. Before clinical examination and sample collection, informed consent was obtained, according to the ethical criteria described by the Declaration of Helsinki. This study was approved by the Ethics Committee of the Dentistry Department of the FMUAQ.

A total of 50 participants were divided into two groups: a T2DM group with 20 patients with at least ten years of living with T2DM and glucose at a maximum of 300mg/dL at the time of sampling, and a control group of 30 nondiabetic participants with glucose from 70 to 126 mg/dL. Smokers, those who have taken antimicrobials in the last three months, and pregnant or lactating women were excluded. The teeth inclusion criteria were premolar or molar tooth with a diagnosis of pulp necrosis and apical periodontitis following the American Association of Endodontists guideline, and a periapical radiolucency classified ≥ 3 according to the periapical index (PAI) score.[13] On the other hand, teeth with a gingival abscess, endo-periodontal disease, and non-restorable were excluded.

Clinical (including height, weight, and blood glucose concentration) and radiographic examinations, as well as all the procedures, were done by a resident of the endodontic specialization program, supervised by an expert.

## Sample collection

After anesthesia and rubber dam isolation, disinfection of the tooth was done using 2.5% sodium hypochlorite (NaOCl). Restauration, caries, and weak tissues were removed, and the endodontic access was prepared with a high-speed N°4 carbide bur. The operative field was cleaned with 3% hydrogen peroxide and a 2.5% NaOCl solution. A number 3 Gates-Glidden drill was used for coronal flaring with copious irrigation of 2.5% NaOCl solution, then neutralized with 5% sodium thiosulfate solution and final irrigation with distilled sterile water. To take the root canal sample associated with apical periodontitis, a new sterile, size 15 stainless-steel hand file, was introduced into the canal to determine a tentative working length with a Root ZX II electronic apex locator (J. Morita USA., Irvine, CA). The file was then introduced into a microtube containing sterile phosphate-buffered saline and vortexed. Two sterile absorbent paper tips were consecutively introduced into the canal to soak up the fluid and then were transferred to the same microtube.[14,15] The root canal treatment was routinely continued, and the sample was kept at − 80°C until DNA extraction.

## DNA extraction and polymerase chain reaction (PCR)

DNA was extracted by phenol-chloroform purification and isopropanol precipitation method. Specific oligonucleotides were selected to identify nine common ARGs in oral bacteria. PCR assays were conducted in 25μL reactions using the parameters reported in Table 1. PCR products were analyzed by electrophoresis in a 2% agarose gel and a 100-bp DNA ladder marker. Each gel was stained and examined in ultraviolet light.[16]

## Statistical analyses

Quantitative data were analyzed using Student's *t*-test. For qualitative variables, Fisher's exact test was applied. GraphPad Prism V8.0 (GraphPad Software, San Diego, CA) was used. The statistical significance adopted was $p<0.05$. Statistical power was estimated with PS: Power and Sample size calculation program (HyLown Consulting, Atlanta, GA).

# Results

Table 2 shows the clinical characteristics of the patients. No significant difference was observed in the distribution by age, sex, or type of affected tooth, except for a large body mass index (BMI) in the T2DM group ($p<0.001$). The mean age of the study population was 46 years, and the patients were predominantly female (73.3%).

Regarding ARGs distribution in the total population, *tetW* and *tetM* were the most frequent (93.3 and 91.6%, respectively), while *ermA* was the least frequent (8.3%). ARGs distribution was similar in both groups, but a significant difference ($p<0.005$) was present in *ermB*, *ermC*, *cfxA*, and *tetQ,* being more frequent in the T2DM group (Table 3). Among T2DM patients, 80% presented a minimum of four ARGs

**Table 1-** Oligonucleotides employed to detect the nine antimicrobial resistance genes (ARGs)

| ARG | Oligonucleotide sequence (5´- 3´) | Annealing temperature (ºC) | Amplicon size (bp) |
|---|---|---|---|
| ***bla-TEM*** | 5'CCAATGCTTAATCAGTGAGG3'<br>5'ATGAGTATTCAACATTTCCG3' | 50 | 858 |
| ***ermC*** | 5'AATC GGCTCAGGAAAAGG3'<br>5'ATCGTC AATTCCTGCATG3' | 50 | 562 |
| ***cfxA*** | 5'GCGCAAATCCTCCTTTAACAA3'<br>5'ACCGCCACACCAATTTCG3' | 55 | 802 |
| ***tetM*** | 5'GTGGACAAAGGTAC AACGAG3'<br>5'CGGTAAAGTTCGTCACACAC 3' | 55 | 406 |
| ***tetW*** | 5'GAGAGCCTGCTATATGCCAGC3'<br>5'GGGCGTATCCACAATGTTAAC3' | 55 | 168 |
| ***tetQ*** | 5'TTATACTTCCTCCGGC ATCG3'<br>5'ATCGGTTCGAGAATGTCCAC3' | 55 | 904 |
| ***ermA*** | 5'AACACCCTGAACCCAAGGGACG3'<br>5'CTTCACATCCGGATTCGCTCGA3' | 50 | 420 |
| ***ermB*** | 5'GAAAAGGTACTCAACCAAATA3'<br>5'AGTAACGGTACTTAAATTGTTTAC3' | 55 | 639 |
| ***mecA*** | 5'-TAGGTAAAATGTCTGGACATG3'<br>5' GCATCAASTGTATTGGATAGC3' | 55 | 533 |

*The references from where each sequence originates are described by Rôças and Siqueira[14] (2012).

**Table 2-** Clinical characteristics of the included subjects and teeth in both groups

| Group | Control (n=30) | T2DM (n=20) | P-value |
|---|---|---|---|
| | Mean ± S.D. (Range) | | |
| Age (Years) | 46.73 ± 11.02 (28-67) | 43.8 ± 10.51 (29-60) | 0.3527[a] |
| BMI (Kg/m$^2$) | 28.08 ± 3.10 (21.5-32.3) | 31.44 ± 2.73 (24.7-35.2) | 0.0003[a*] |
| | Frequency (%) | | |
| Female | 22 (73.3) | 15 (80) | 1.000[b] |
| Male | 8 (26.7) | 5 (20) | |
| Premolars | 12 (40) | 7 (35) | |
| Molars | 18 (60) | 13 (65) | 0.7737[b] |

T2DM: Type 2 diabetes mellitus; SD: Standard deviation. aStudent's t-test, bFisher´s exact test. *Statistically significant

(p<0.01), while 76.6% of the control group presented a maximum of three (p<0.0001) (Table 4).

## Discussion

Oral microbiota is considered a reservoir for several ARGs.[17,18,19] Root canals as pathological niches in individuals without systemic diseases have been especially reported as reservoirs for ARGs that encode resistance mainly to tetracyclines, beta-lactams, and macrolides.[16,20] However, no reports from this pathological niche in immune-compromised individuals who tend to consume more significant quantities and types of antimicrobials than patients diagnosed with T2DM have been found.

T2DM is one of the most prevalent diseases worldwide. Individuals living with it are considered a risk group for local infections and are more prone to developing severe systemic conditions.[6] Hence, their antimicrobial consumption is more remarkable.

The few reports of ARGs in T2DM individuals mostly address urinary tract infections, sepsis, pneumonia, and diabetic foot wounds.[6] Nevertheless, T2DM patients present a higher prevalence of apical periodontitis,[21,22] and considering the connection that may exist between niches, we should investigate this niche too. This study presents novel information on the presentation pattern of some common ARGs in root canal microbiota associated with apical periodontitis in patients with T2DM.

**Table 3-** Distribution of the nine antimicrobial resistance genes (ARGs) in both groups

| ARG | Control (n=30) | T2DM (n=20) | P-value |
|---|---|---|---|
| | | Frequency (%) | |
| *tetW* | 26 (86.6) | 20 (100) | 0.1400 |
| *tetM* | 25 (83.3) | 20 (100) | 0.0746 |
| *blaTEM* | 24 (80) | 19 (95) | 0.2192 |
| *ermB* | 3 (10) | 9 (45) | 0.0070* |
| *cfxA* | 2 (6.6) | 8 (40) | 0.0088* |
| *ermC* | 2 (6.6) | 7 (35) | 0.0207* |
| *tetQ* | 2 (6.6) | 7 (35) | 0.0207* |
| *mecA* | 1 (3.3) | 3 (15) | 0.2885 |
| *ermA* | 2 (6.6) | 2 (10) | 1.000 |

ARG: Antimicrobial resistance gene; T2DM: Type 2 diabetes mellitus; Fisher´s exact test; *Statistically significant

**Table 4-** Frequency of positive samples to a different number of assessed genes

| Number of ARG | Control (n=30) | T2DM (n=20) | P-value |
|---|---|---|---|
| | | Frequency (%) | |
| Eight or nine | 0 | 0 | -- |
| Seven | 0 | 2 (10) | 0.1551 |
| Six | 1 (3.3) | 1 (5) | 1.000 |
| Five | 0 | 1 (5) | 0.4082 |
| Four | 6 (20) | 12 (60) | 0.0065* |
| Three | 20 (66.6) | 0 | < 0.0001* |
| Two | 1 (3.3) | 4 (20) | 0.1432 |
| One | 2 (6.6) | 0 | 0.5102 |
| No ARGs | 0 | 0 | -- |

ARG: Antimicrobial resistance gene; T2DM: Type 2 diabetes mellitus; Fisher´s exact test. *Statistically significant

Although the limitation of this study is the small number of participants, they met strict selection criteria. Both groups presented homogeneous distributions regarding age and sex. BMI was the only different variable, which was expected since obesity is frequently related to T2DM.[23] The distribution in both groups was also similar regarding the type of affected teeth: All of them had the same diagnosis, that is, pulp necrosis and apical periodontitis with a forthright periapical radiolucency classified by the PAI index,[13] which is especially relevant because of the necessary time for apical periodontitis to develop and become radiographically visible. Thus, the included teeth would represent a long-standing pathologic process caused by a well-established intraradicular biofilm infection.

Outstandingly, 80% of the T2DM group presented more than four ARGs in their root canal microbiota, while 76.6% of the control group presented a maximum of three. Such result surely relates to selective pressure due to a higher intake of antimicrobials by T2DM patients than in healthy ones, because more frequent infections are associated with them. This could be partially sustained by the information obtained from the patients during the clinical interview (data not shown). We found that 100% of both groups ingested at least one antimicrobial from the beta-lactam group in the last five years. However, the use of tetracyclines or macrolides was reported in the T2DM group by 17 (85%) and 12 (60%) patients, respectively, but only in six (20%) and five (16.6%) patients in the control group.

A total of five of the nine ARGs presented a similar distribution in both groups. However, in the T2DM group, a significantly high presentation was observed in four of them: *ermB, ermC, cfxA*, and *tetQ.* They occurred in at least 35% of the T2DM samples but only in a maximum of 10% of the nondiabetic samples. Although the small number of participants is a limitation of our study, when we performed the statistical power analysis, we observed, at least for

these four ARGs, which are mainly responsible for the difference between the proportions of ARGs in healthy versus T2DM patients, an 80% power with a confidence level of 95%.

The *tet* genes that encode for tetracyclines are found in diverse niches of the oral cavity,[18] *tetW* and *tetM* were the most prevalent, agreeing with a previous report in which *tetW* was present in 93.5% and *tetM* in 83.9% of root canal samples with primary infection of a similar nondiabetic Mexican population.[16] In comparison, *tetQ* was less present in the nondiabetic group samples (6.6%), which agrees with previous reports in which *tetQ* was not even found,[14,16] thus explaining the remarkableness of such an event, that is, its frequency in the T2DM group (35%), which had not been reported before.

The *erm* genes that encode rRNA methylases were present in both groups in distinct frequencies, and the gene *ermA* was the least frequent. This could agree with previous investigations in isolated species from primarily infected root canals of nondiabetic patients in which *ermA* was undetected.[14,24] The *ermB* and *ermC* genes were less detected in the nondiabetic group (10 and 6.6%, respectively) and moderately found (45 and 35%, respectively) in the T2DM group. The presence of *ermB* in root canals had not been previously reported.[14,24] Interestingly, the T2DM group presented it in almost half of the group. The *ermC* gene has been previously reported with varying frequencies depending on the study: 10%,[14] 24%,[19] 3.2, and 51.5% in primary infection or post-treatment infection, respectively,[16] the last being a more similar presentation percentage to the current and probably due to it, as previously mentioned, to better-established and overexposed to antimicrobials microbiota.

The *blaTEM* gene was the third most prevalent in both groups (87.5% on average), agreeing with a previous report from four years ago in patients from the same municipality (87.1%),[16] confirming a significant difference from what was reported in US[20] (43%) and Brazilian[19] (24%) populations about ten years ago. The *cfxA* gene was also relevant in the T2DM group since it was consistently found in only 6.6% of nondiabetic individuals, similar to the 2% found in root canal isolates[14] and the 0% reported in samples of the root canal,[19] especially in a similar population.[16] However, *cfxA* was notably found in 40% of the T2DM samples. The *mecA* gene was detected in one nondiabetic individual (3.3%). Although its prevalence is low, this is the first report on the oral microbiota of systemically healthy individuals.[14,25] It is thus remarkable that *mecA* was detected in three T2DM patients (15%). This higher frequency partially agrees with a recent study that included oral mucosa of diabetic individuals in Brazil[26] in which *mecA* was present in 4.8% of the individuals. However, the presence of *mecA* in these oral cavities was underestimated because it was detected only in isolated *Staphylococcus aureus*, excluding all other bacteria. Therefore, they did not evaluate the contribution of distinct species in the complete microbiota. The performance of this evaluation was a strength of our study. The direct screening of clinical samples for the presence of ARGs is more appropriate for this purpose.

Antimicrobial resistance is a severe problem. Public health strategies have been implemented in many countries after the presentation of the action plan by the World Health Organization in 2015.[27] It is well known that antimicrobial resistance results from an evolutionary process whose frequency is influenced by human, animal, and agricultural consumption of antimicrobials, sanitation, and water reuse, among others.

This study evidence that the oral health of an individual not only benefits them directly since microorganisms from a pathological oral niche could spread to aponeurotic planes of the head and neck and could cause severe septic conditions such as descending mediastinitis, Lemierre's syndrome, cervical necrotizing fasciitis, orbital abscess, cavernous sinus thrombosis, cerebral abscess, and osteomyelitis[28] which would be difficult to treat with antimicrobials. In addition, oral health plays an essential role in antimicrobial resistance, since maintaining pathological niches in the mouth further promotes the reservoir and spread of ARGs. Therefore, eliminating these niches, especially in individuals with systemic conditions, such as T2DM, in which the ARGs reservoir is more significant, is essential to help controlling this global problem, since bacteria and their ARGs can be shared between humans or even with their pets.[29] Therefore, strategies to reduce the appearance and spread of resistant and multiresistant pathogens should also consider preventive Dentistry to avoid the appearance of pathological niches, and when this has failed, the intervention of a dentist is crucial to eliminate these

niches. From a general health perspective, the results shown here for individuals with T2DM that constantly display recurrent and difficult-to-treat infections could not only be explained by a malfunction of the immune system, but also by a high prevalence of antimicrobial resistance from oral bacteria. Therefore, future investigations should investigate the presence of ARGs in other niches of the human body, such as the gastrointestinal, respiratory, urinary tracts, blood, or skin, which would help to confirm the transference between niches.

Considering the limitations of this study, we conclude that root canals with pulp necrosis and apical periodontitis are common pathological niches in the human body in which the transference of ARGs is favored and acts as a reservoir. This condition in patients with T2DM would represent an augmented presence of a wide variety of ARG. Therefore, eliminating these pathological niches is of utmost importance, especially in patients with T2DM, not only to avoid complications in their infection management, but also to avoid the spread of ARGs in different niches and among individuals in the community.

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## Conflicts of interest

The authors declare no conflicts of interest.

## Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

## Authors' contributions

**Vázquez-Ramos, Víctor Rafael:** Conceptualization (Equal); Investigation (Lead); Methodology (Equal); Writing – original draft (Equal); Writing – review & editing (Equal). **Pérez-Serrano, Rosa Martha:** Methodology (Equal); Supervision (Equal); Visualization (Equal); Writing – review & editing (Equal). **García-Solís, Pablo:** Methodology (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal). **Solís-Sainz, Juan Carlos:** Formal analysis (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal). **Espinosa-Cristóbal, Léon Francisco:** Validation (Supporting); Visualization (Equal). **Castro-Ruíz, Jesús Eduardo:** Validation (Supporting); Visualization (Equal). **Domínguez-Pérez, Rubén Abraham:** Conceptualization (Lead); Data curation (Lead); Formal analysis (Lead); Funding acquisition (Lead); Investigation (Equal); Methodology (Equal); Project administration (Lead); Resources (Lead); Writing – original draft (Equal); Writing – review & editing (Equal).

# References

1- Tibúrcio-Machado CS, Michelon C, Zanatta FB, Gomes MS, Marin JA, Bier CA. The global prevalence of apical periodontitis: a systematic review and meta-analysis. Int Endod J. 2021;54(5):712-35. doi: 10.1111/iej.13467

2- Stashenko P, Teles R, D'Souza R. Periapical Inflammatory responses and their modulation. Crit Rev Oral Biol Med. 1998;9(4):498-521. doi: 10.1177/10454411980090040701

3- Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318-22. doi: 10.1126/science.284.5418.1318

4- Carr GB, Schwartz RS, Schaudinn C, Gorur A, Costerton JW. Ultrastructural examination of failed molar retreatment with secondary apical periodontitis: an examination of endodontic biofilms in an endodontic retreatment failure. J Endod. 2009;35(9):1303-9. doi: 10.1016/j.joen.2009.05.035

5- Amaral RR, Braga T, Siqueira JF, Rôças IN, Costa Rachid CT, Guimarães Oliveira AG, et al. Root canal microbiome associated with asymptomatic apical periodontitis as determined by high-throughput sequencing. J Endod. 2022;4(48):487-95. doi: 10.1016/j.joen.2022.01.012

6- Boyanova L, Mitov I. Antibiotic resistance rates in causative agents of infections in diabetic patients: rising concerns. Expert Rev Anti Infect Ther. 2013;11(4):411-20. doi: 10.1586/eri.13.19

7- Akash MSH, Rehman K, Fiayyaz F, Sabir S, Khurshid M. Diabetes-associated infections: development of antimicrobial resistance and possible treatment strategies. Arch Microbiol. 2020;202(5):953-65. doi: 10.1007/s00203-020-01818-x

8- Núñez SA, Lacal V, Núñez J, Serruto G, Zárate MS, Verón MT. Antibiotic resistance in community-acquired intra-abdominal infections: diabetes mellitus as a risk factor. Surg Infect (Larchmt). 2020;21(1):62-8. doi: 10.1089/sur.2019.032

9- Baron SA, Diene SM, Rolain JM. Human microbiomes and antibiotic resistance. Hum Microbiome J. 2018;10(sp. Issue):43-52. doi: 10.1016/j.humic.2018.08.005

10- Wheatley R, Diaz Caballero J, Kapel N, Winter FH, Jangir P, Quinn A, et al. Rapid evolution and host immunity drive the rise and fall of carbapenem resistance during an acute *Pseudomonas aeruginosa* infection. Nat Commun. 2021;12(1):1-12. doi: 10.1038/s41467-021-22814-9

11- Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. FEMS Immunol Med Microbiol. 2012;65(2):183-95. doi: 10.1111/j.1574-695X.2012.00960.x

12- Jeong H, Arif B, Caetano-Anollés G, Kim KM, Nasir A. Horizontal gene transfer in human-associated microorganisms inferred by phylogenetic reconstruction and reconciliation. Sci Rep. 2019;9(1):1-18. doi: 10.1038/s41598-019-42227-5

13- Ørstavik D, Kerekes K, Eriksen HM. The periapical index: a scoring system for radiographic assessment of apical periodontitis. Dent Traumatol. 1986;2(1):20-34. doi: 10.1111/j.1600-9657.1986.tb00119.x

14- Rôças IN, Siqueira JF Jr. Antibiotic resistance genes in anaerobic bacteria isolated from primary dental root canal infections. Anaerobe. 2012;18(6):576-80. doi: 10.1016/j.anaerobe.2012.10.001

15- De la Torre-Luna R, Domínguez-Pérez RA, Guillén-Nepita AL, Ayala-Herrera JL, Martínez-Martínez RE, Romero-Ayala ME, et al. Prevalence of *Candida albicans* in primary endodontic infections associated with a higher frequency of apical periodontitis in type two diabetes mellitus patients. Eur J Clin Microbiol Infect Dis. 2020;39(1):131-8. doi: 10.1007/s10096-019-03702-z

16- Domínguez-Pérez RA, De la Torre-Luna R, Ahumada-Cantillano M, Vázquez-Garcidueñas MS, Pérez-Serrano RM, Martínez-Martínez RE, et al. Detection of the antimicrobial resistance genes blaTEM-1, cfxA, tetQ, tetM, tetW and ermC in endodontic infections of a Mexican population. J Glob Antimicrob Resist. 2018;15(2018):20-4. doi: 10.1016/j.jgar.2018.05.011

17- Roberts AP, Mullany P. Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance. Expert Rev Anti Infect Ther. 2010;8(12):1441-50. doi: 10.1586/eri.10.106

18- Moraes LC, Só MV, Silva Dal Pizzol T, Ferreira MB, Montagner F. Distribution of genes related to antimicrobial resistance in different oral environments: a systematic review. J Endod. 2015;41(4):434-41. doi: 10.1016/j.joen.2014.12.018

19- Rôças IN, Siqueira JF Jr. Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment. Arch Oral Biol. 2013;58(9):1123-8. doi: 10.1016/j.archoralbio.2013.03.010

20- Jungermann GB, Burns K, Nandakumar R, Tolba M, Venezia RA, Fouad AF. Antibiotic resistance in primary and persistent endodontic infections. J Endod. 2011;37(10):1337-44. doi: 10.1016/j.joen.2011.06.028

21- Yip N, Liu C, Wu D, Fouad AF. The association of apical periodontitis and type 2 *diabetes mellitus*: a large hospital network cross-sectional case-controlled study. J Am Dent Assoc. 2021;152(6):434-43. doi: 10.1016/j.adaj.2021.01.005

22- Sisli SN. Evaluation of the relationship between type ii diabetes mellitus and the prevalence of apical periodontitis in root-filled teeth using cone beam computed tomography: an observational cross-sectional study. Med Princ Pract. 2019;28(6):533-8. doi: 10.1159/000500472

23- American Diabetes Association Professional Practice Committee; 2. Classification and diagnosis of diabetes: standards of medical care in diabetes - 2022. Diabetes Care. 2021;45(Supplement_1):S17-S38. doi: 10.2337/dc22-s002

24- Lins RX, Oliveira Andrade A, Hirata R Junior, Wilson MJ, Lewis MA, Williams DW, et al. Antimicrobial resistance and virulence traits of *Enterococcus faecalis* from primary endodontic infections. J Dent. 2013;41(9):779-86. doi: 10.1016/j.jdent.2013.07.004

25- Koukos G, Sakellari D, Arsenakis M, Tsalikis L, Slini T, Konstantinidis A. Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in the oral cavity. Arch Oral Biol. 2015;60(9):1410-5. doi: 10.1016/j.archoralbio.2015.06.009

26- Teixeira NB, Fortaleza CB, Magno C, Souza MC, Monteiro Pereira TA, Camargo Colenci BP, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* among insulin-dependent diabetic individuals in Brazil. Ann Clin Microbiol Antimicrob. 2021;20(1):1-12. doi: 10.1186/s12941-020-00401-y

27- Silva JB, Espinal M, Ramón-Pardo P. Antimicrobial resistance: time for action. Rev Panam Salud Publica/Pan Am J Public Heal. 2020;44:1-2. doi: 10.26633/RPSP.2020.131

28- Bali R, Sharma P, Gaba S, Kaur A, Ghanghas P. A review of complications of odontogenic infections. Natl J Maxillofac Surg. 2015;6(2):136. doi: 10.4103/0975-5950.183867

29- Pérez-Serrano RM, Domínguez-Pérez RA, Ayala-Herrera JL, Luna-Jaramillo AE, Zaldivar-Lelo de Larrea G, Solís-Sainz JC, et al. Dental plaque microbiota of pet owners and their dogs as a shared source and reservoir of antimicrobial resistance genes. J Glob Antimicrob Resist. 2020;21:285-90. doi: 10.1016/j.jgar.2020.03.025