

DOI: <https://doi.org/10.17816/dent627649>

Mycotic flora characteristics of the endontic flora in patients affected by COVID-19

Anna L. Ermolovich, Eleonora G. Borisova, Dar'ja D. Semenova

Military Medical Academy named after S.M. Kirov, Saint Petersburg, Russia

ABSTRACT

BACKGROUND: Owing to the fact that, presently, at a dental appointment, it is possible to observe a wide range of consequences arising after COVID-19, among which are observed lesions of the pulp and periodontium of the tooth, additional diagnostic methods should be introduced before choosing treatment tactics. In our previous study, we analyzed the contents of root canals of teeth in patients who had COVID-19 using bacterioscopy. Coccus flora was detected in all cases and elements of *Candida* yeast-like fungi were recorded in 89 cases (76.1%). Based on the obtained data, it was advantageous to perform microbiological examination of the endodontium in this group of patients to further study their physical and chemical properties and correct the treatment regimen in gangrenous form of chronic pulpitis and aggravated forms of periodontitis, which determined the relevance of the present study.

AIM: To reveal the characteristics of the mycotic flora of the endodontium in patients who previously had COVID-19.

MATERIALS AND METHODS: A bacteriologic study of root canal contents in patients who had previously undergone a new coronavirus infection at different times, diagnosed as "exacerbation of chronic periodontitis", "chronic gangrenous pulpitis", and "acute suppurative periodontitis", was conducted to detect fungi of the genus *Candida* in the tooth root canal system, which was obtained during mechanical treatment with a sterile endodontic instrument. The patients ($n=49$) were divided into groups according to their final diagnosis: group 1, chronic gangrenous pulpitis (27 patients); group 2, acute purulent periodontitis (9 patients); and group 3, exacerbation of chronic periodontitis (13 patients). Then, the collected material was placed in a tube with Amies transport medium and sent to the laboratory. Seeding of the material was performed in sterile Petri dishes on Sabouraud agar by rubbing with a plastic spatula. Further, Petri dishes were placed in the thermostat for 24–48 hours of incubation at $37\pm 10^\circ\text{C}$. Then, the study was carried out according to the generally accepted scheme: the obtained cultures were identified to species by the character of growth on dense medium.

RESULTS: The given bacteriological study of the patients' endontic contents confirmed the data of bacterioscopy and concretized the previously obtained results. On final examination, black colonies of *Candida albicans* were observed in 95.92% of cases. No growth of *Candida albicans* colonies was observed in two cases (4.08%).

CONCLUSION: The bacteriologic study performed after bacterioscopy of diagnosis confirmed the presence of fungi of the genus *Candida* in the root canals of teeth in patients who previously had COVID-19. The study showed that when treating similar patients, the high risk of infection of periodontal complex tissues by *Candida* fungi should be considered. It is advisable to supplement the standard protocols of endodontic treatment with physical exposure, for example, laser radiation, or additional medication with antifungal drugs, such as fluconazole.

Keywords: bacteriological examination; COVID-19; pulpitis; periodontitis; bacterioscopic examination; *Candida albicans*; endodontic treatment.

To cite this article:

Ermolovich AL, Borisova EG, Semenova DD. Mycotic flora characteristics of the endontic flora in patients affected by COVID-19. *Russian Journal of Dentistry*. 2024;28(1):47–52. DOI: <https://doi.org/10.17816/dent627649>

Received: 05.01.2024

Accepted: 14.01.2024

Published online: 12.03.2024



DOI: <https://doi.org/10.17816/dent627649>

Особенности микотической флоры эндодонта у пациентов, перенёвших COVID-19

А.Л. Ермолович, Э.Г. Борисова, Д.Д. Семёнова

Военно-медицинская академия имени С.М. Кирова, Санкт-Петербург, Россия

АННОТАЦИЯ

Обоснование. В связи с тем, что в настоящее время на стоматологическом приёме можно наблюдать широкий спектр последствий, возникших на фоне перенесённого ранее COVID-19, среди которых наблюдаются поражения пульпы и периодонта зуба, необходимо внедрение дополнительных методов диагностики до выбора тактики лечения. В нашем предыдущем исследовании с помощью метода бактериоскопии проведён анализ содержимого корневых каналов зубов у пациентов, переболевших COVID-19. Выявлено наличие кокковой флоры во всех случаях (100%), в 89 случаях (76,1%) зафиксированы элементы дрожжеподобных грибов *Candida*. На основании полученных данных определена целесообразность проведения у данной группы пациентов микробиологического исследования эндодонта с целью дальнейшего изучения физико-химических свойств и коррекции схемы лечения при гангренозной форме хронического пульпита и обострившихся формах периодонтита, что и определило актуальность настоящего исследования.

Цель исследования — выявить особенности микотической флоры эндодонта у пациентов, ранее перенёвших COVID-19.

Материалы и методы. Проведено бактериологическое исследование содержимого корневых каналов у пациентов, ранее переболевших в разные сроки новой коронавирусной инфекцией, с диагнозом «обострение хронического периодонтита», «хронический гангренозный пульпит», «острый гнойный периодонтит». Выявляли грибы рода *Candida* в системе корневых каналов зуба, содержимое которых брали во время механической обработки каналов стерильным эндодонтическим инструментом. Пациентов ($n=49$) разделили на группы в соответствии с окончательным диагнозом: первая группа — «хронический гангренозный пульпит» ($n=27$); вторая группа — «острый гнойный периодонтит» ($n=9$); третья группа — «обострение хронического периодонтита» ($n=13$). Далее собранный материал помещали в пробирку с транспортной средой Amies и отправляли в лабораторию. Посев материала проводили в стерильных чашках Петри на агар Сабуро путём втирания пластмассовым шпателем. Затем чашки Петри помещали в термостат на 24–48 ч инкубации при температуре 37 ± 1 °C. Далее исследование выполняли по общепринятой схеме: полученные культуры идентифицировали до вида по характеру роста на плотной среде.

Результаты. Бактериологическое исследование не только подтвердило данные бактериоскопии, но и конкретизировало ранее полученные результаты. При окончательном исследовании чёрные колонии *Candida albicans* наблюдались в 95,92% случаев. В 2 случаях в (4,08%) роста колоний не наблюдалось.

Заключение. Бактериологическое исследование, проводимое после бактериоскопического метода диагностики, подтвердило наличие грибов рода *Candida* в содержимом корневых каналов зубов у пациентов, ранее переболевших COVID-19. Установлено, что при лечении аналогичных пациентов целесообразно учитывать высокий риск инфицирования тканей периодонтального комплекса грибами рода *Candida*. Целесообразно дополнить стандартные протоколы эндодонтического лечения физическим воздействием, например лазерным излучением, или дополнительным медикаментозным воздействием противогрибковыми препаратами, например Флуконазолом.

Ключевые слова: бактериологическое исследование; COVID-19; пульпит; периодонтит; бактериоскопическое исследование; *Candida albicans*; эндодонтическое лечение.

Как цитировать:

Ермолович А.Л., Борисова Э.Г., Семёнова Д.Д. Особенности микотической флоры эндодонта у пациентов, перенёвших COVID-19 // Российский стоматологический журнал. 2024. Т. 28, № 1. С. 47–52. DOI: <https://doi.org/10.17816/dent627649>

BACKGROUND

Although on May 5, 2023 the World Health Organization declared that COVID-19 was no longer a global public health emergency and the epidemic's emergency phase ended, many individuals continue to suffer from "post-COVID syndrome" and exacerbations of chronic conditions triggered by viral infection or medications administered during acute treatment. In any case, all these factors contribute to an imbalance in the human immune system [1, 2].

In dental practice, clinicians now observe a broad spectrum of oral mucosal manifestations following SARS-CoV-2 infection. These include herpes simplex virus lesions, oral candidiasis, geographic tongue, hemorrhagic and necrotic ulcerations, petechiae, and pustular enanthema [3–5, 7]. There is also frequent exacerbation of chronic pulp and periapical diseases, the emergence of acute pulpitis, accelerated progression of dental hard tissue destruction, and periodontal disease [1–3]. Poor oral hygiene is commonly present in these patients and may be a compounding factor. It remains unclear whether these manifestations result directly from viral infection, reflect systemic health decline, or are adverse effects of treatment [2].

These observations underscore the potential impact of such factors on alterations in the activity of oral microbiota. The species composition of oral microbiota is highly diverse, encompassing numerous aerobic, obligate anaerobic, and facultative anaerobic microorganisms [5–7]. Immune dysregulation induced by SARS-CoV-2 (or other factors) can activate normally harmless members of the oral microbiome, including *Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella intermedia*, *Peptostreptococcus micros*, *Fusobacterium* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *Candida* spp., etc. More recently, interest has sharply increased in the incidence of candidiasis among patients recovering from COVID-19, with *Candida albicans* being the most common causative agent of this fungal disease [4, 6, 7].

In our previous studies [5, 7], we documented high prevalence of endodontic pathology among affected patients: chronic gangrenous pulpitis (59%), exacerbation of chronic periapical periodontitis (18%), and acute suppurative periapical periodontitis (23%). In the root canal ecosystem, various virulent bacterial species—including fastidious and nonculturable microorganisms—can be found. According to our investigations, microscopic examination of 117 pulp and root canal samples collected from patients with a history of COVID-19 revealed yeast-like fungal elements (*Candida* spp.) in 98 cases (83.8%) [5, 7]. This finding is most likely a consequence of prior SARS-CoV-2 infection, which, as reported by many researchers, results in immune system dysregulation [8]. The activation of opportunistic microorganisms, which

are typically nonpathogenic in the oral cavity, may trigger various pathologies, particularly of the dental pulp and periodontium. These pathologies often present atypically in patients who had COVID-19 at various time intervals. This underscores the clinical rationale for expanding the standard dental evaluation in such patients to include additional diagnostic methods, such as bacterioscopic and bacteriological examination, prior to initiating endodontic treatment. In such clinical scenarios, dentists can select a more targeted and effective therapeutic approach aimed at eradicating specific pathogens, thereby minimizing the risk of delayed complications after endodontic treatment [5, 7].

This study aimed to reveal the characteristics of the mycotic flora of the endodontium in patients who previously had COVID-19.

MATERIALS AND METHODS

In our previous study [5, 7], bacterioscopic method was used to analyze the contents of root canals in patients who had recovered from COVID-19.

In the present investigation, bacteriological examination was performed in 49 patients with a history of COVID-19 (at various intervals post-infection) who were diagnosed with either acute suppurative periapical periodontitis, chronic gangrenous pulpitis, or exacerbation of chronic periapical periodontitis. The primary aim was to identify the presence of *Candida* spp. within the root canal system. The patients were preliminarily stratified into three groups based on their final diagnoses established using standard clinical examination and adjunctive diagnostic methods, including electric pulp testing, radiographic evaluation, and cold pulp testing. Group 1 comprised 27 patients (55.1%) diagnosed with chronic gangrenous pulpitis. Group 2 included 9 patients (18.37%) diagnosed with acute suppurative periapical periodontitis. Group 3 consisted of 13 patients (26.53%) with an exacerbation of chronic periapical periodontitis.

Root canal contents were sampled using sterile endodontic instruments. The collected material was thinly smeared onto glass slides, air-dried, and stained with 1% aqueous solutions of hematoxylin and eosin for 15–30 seconds. The slides were rinsed with running water, dried, and examined microscopically. The presence of fungal elements and coccal flora was assessed based on their quantity in the field of view and categorized as isolated elements, isolated clusters, abundant in the field of view, or diffusely covering the entire field.

The root canal contents served as the material for microbiological culture and were collected during mechanical instrumentation using sterile endodontic instruments. The samples were then transferred into tubes containing Amies transport medium (COPAN, Italy). This medium, a modified version of Stuart's basic transport

medium, maintains the viability of microorganisms such as *Neisseria* spp., *Haemophilus* spp., *Corynebacterium*, *Streptococcus* spp., *Enterobacteriaceae*, and *Candida* spp. for up to 3 days. However, to ensure optimal culture yield, inoculation was performed within the first 24 hours.

The collected samples were transported to the laboratory for culture on growth media. The samples were inoculated on Sabouraud dextrose agar in sterile Petri dishes by streaking the sample using a plastic spatula. The plates were incubated at $37 \pm 1^\circ\text{C}$ for 24–48 hours. Cultures were identified based on colony morphology and growth characteristics on solid media.

Sabouraud agar consists of enzymatic peptone digest, glucose, casein, and agar. Owing to its high glucose concentration and low pH, this medium exhibits selective properties favoring fungal growth. Additionally, potassium tellurite is included in the medium, which imparts a characteristic black pigmentation to *Candida albicans* colonies, in contrast to their usual white appearance.

The principle of the bacteriological method lies in the visual detection of microbial growth on culture media after inoculation of clinical specimens.

RESULTS AND DISCUSSION

The success of endodontic treatment for pulpitis and periapical periodontitis largely depends on the effectiveness of eradicating microbial and fungal agents. A key criterion for successful endodontic therapy is thorough chemo-mechanical debridement of the carious lesion and root canal system, aimed at complete elimination of bacteria, fungi, and their associated toxins.

According to our previous findings [5, 7], in patients with a history of COVID-19, bacterioscopic examination of

dental pulp samples revealed yeast-like *Candida* fungi in 83.8% of cases and coccal flora in all cases. Bacterioscopy was selected as a diagnostic method because of its simplicity and accessibility for detecting fungal elements in pathological specimens.

One illustrative case involved a 47-year-old patient who had recovered from COVID-19 less than a year prior to the dental appointment. *Candida* elements were detected in the specimen, and the diagnosis of chronic gangrenous pulpitis of tooth 46 (FDI/ISO notation) was established. A periapical radiograph of tooth 46 (see Fig. 1) and a microphotograph of the canal contents (see Fig. 2) are provided.

To determine the fungal species, a microbiological analysis of root canal contents was conducted in 49 patients, which not only confirmed the bacterioscopic findings but also allowed identifying the fungal species.

The first observation was performed in 48 hours, and final culture characterization was conducted on day 5.

In group 1 (chronic gangrenous pulpitis, $n = 27$), the initial observation revealed smooth, raised black colonies with regular margins and hemispherical shape measuring approximately 1.0–2.0 mm in diameter in 21 of 27 cases (77.8%) on Sabouraud agar (see Fig. 3). Final analysis confirmed that *Candida albicans* colonies were present in 100% of cases.

In group 2 (acute suppurative periapical periodontitis, $n = 9$), yeast-like colonies of *Candida albicans* were observed in all samples (100%) during the first observation. By day 5, the colonies exhibited more pronounced elevation.

In group 3 (exacerbation of chronic periapical periodontitis, $n = 13$), extensive growth of *Candida albicans* colonies was observed in 11 cases (84.6%)



Fig. 1. Intraoral periapical radiograph of tooth 46.



Fig. 2. Bacterioscopic examination of the root canal contents of tooth 46. *Candida* spp. identified.

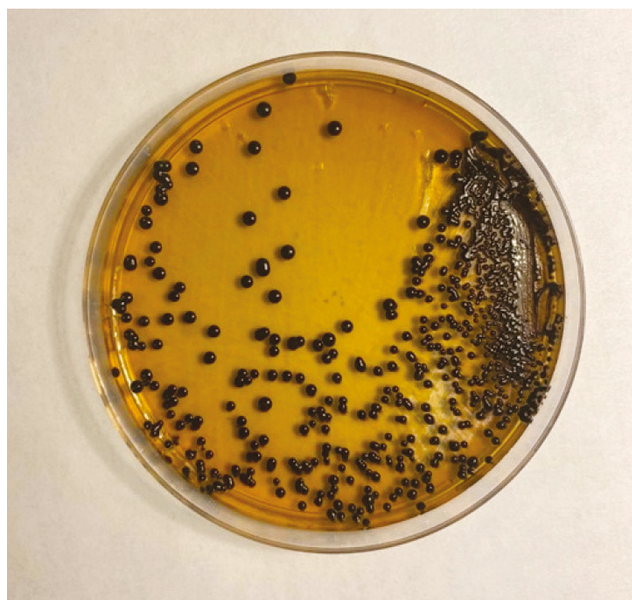


Fig. 3. *Candida albicans* colonies cultured on Sabouraud agar.

during the first examination, while no colony growth was noted in 2 cases (15.4%).

CONCLUSION

Bacteriological examination of root canal contents in patients with a history of COVID-19—regardless of the time elapsed since infection—revealed the presence of *Candida* spp. fungi. Bacteriological analysis confirmed the microscopic findings and identified the cultured colonies in Petri dishes as *Candida albicans*. Black colonies of *Candida albicans* were observed in 95.92% of cases, with only 2 negative samples (4.08%), potentially

because of errors in sampling, transport, or incubation conditions.

Despite the wide array of endodontic treatment techniques and antimicrobial agents targeting root canal microbiota, patients with a prior history of COVID-19 often present treatment challenges and experience complications. These outcomes may be associated with the activity of unidentified microorganisms within the root canal system. This study demonstrated that, in the treatment of patients with a prior history of COVID-19, it is advisable to consider the elevated risk of infection of the periapical tissues by *Candida* spp., which necessitates additional diagnostic methods for assessing root canal contents in order to prevent complications during the management of deep carious lesions with pulpal or periapical involvement.

ADDITIONAL INFORMATION

Funding sources: This work was not supported by any external sources.

Conflict of interests: The authors declare no explicit or potential conflicts of interests associated with the study and publication of this article.

Author contributions: All authors affirm their compliance with the international ICMJE criteria (all authors made substantial contributions to the conceptualization, research, and manuscript preparation, and reviewed and approved the final version prior to publication).

The largest contribution is distributed as follows: E.G. Borisova—significant contribution to the conception and design of the study, preparation of the article and its critical revision in terms of significant intellectual content; A.L. Ermolovich—data collection and analysis, text writing; D.D. Semenova—data collection and analysis, text writing.

REFERENCES

1. Hüpsch-Marzec H, Dziedzic A, Skaba D, Tanasiewicz M. The spectrum of non-characteristic oral manifestations in COVID-19 — a scoping brief commentary. *Med Pr.* 2021;72(6):685–692. doi: 10.13075/mp.5893.01135
2. Mitronin AV, Apresian NA, Ostanina DA, Yurtseva ED. Correlation between oral health and severity of respiratory coronavirus infection COVID-19. *Endodontics Today.* 2021;19(1):18–22. EDN: YDFBKO doi: 10.36377/1683-2981-2021-19-1-18-22
3. Brandini DA, Takamiya AS, Thakkar P, et al. Covid-19 and oral diseases: crosstalk, synergy or association? *Rev Med Virol.* 2021;31(6):1–15. doi: 10.1002/rmv.2226
4. Tsarev VN, Mitronin AV, Podporin MS, et al. Combined endodontic treatment: microbiological aspects by using scanning electronical microscopy. *Endodontics Today.* 2021;19(1):11–17. EDN: XRTLQU doi: 10.36377/1683-2981-2021-19-1-11-17
5. Ermolovich AL, Borisova EG, Zheleznyak VA, Khrustaleva JuA. The significance of bacterioscopy in the complex of diagnostics of complicated forms of dental caries of patients who previously have COVID-19. *Applied and It Research in Medicine.* 2023;26(3):41–46. EDN: EPYSIB doi: 10.18499/2070-9277-2023-26-3-41-46
6. Razumova SN, Brago AS, Barakat HB, et al. Microbiological study of the efficiency of root canal treatment with Er:yAG laser. *Biomedical Photonics.* 2019;8(4):11–16. EDN: OGIPMC doi: 10.24931/2413-9432-2019-8-4-11-16
7. Borisova EG, Ermolovich AL. Diagnosis and treatment of chronic gangrenous pulpitis in the detection of mycotic flora after a coronavirus infection. *Medical & Pharmaceutical journal Pulse.* 2023;25(2):11–16. EDN: QIBXWH doi: 10.26787/nydha-2686-6838-2023-25-2-11-16
8. Soldatov IK, Juravleva LN, Tegza NV, et al. Scientometric analysis of dissertations on pediatric dentistry in the Russian Federation. *Russian Journal of Dentistry.* 2023;27(6):571–580. EDN: QINWXB doi: 10.17816/dent624942

СПИСОК ЛИТЕРАТУРЫ

1. Hüpsch-Marzec H., Dziedzic A., Skaba D., Tanasiewicz M. The spectrum of non-characteristic oral manifestations in COVID-19 — a scoping brief commentary // *Med Pr.* 2021. Vol. 72, N 6. P. 685–692. doi: 10.13075/mp.5893.01135
2. Митронин А.В., Апресян Н.А., Останина Д.А., Юрцева Е.Д. Взаимосвязь стоматологического статуса пациентов с тяжестью течения респираторной коронавирусной инфекции COVID-19 // *Эндодонтия Today.* 2021. Т. 19, № 1. С. 18–22. EDN: YDFBKO doi: 10.36377/1683-2981-2021-19-1-18-22
3. Brandini D.A., Takamiya A.S., Thakkar P., et al. Covid-19 and oral diseases: crosstalk, synergy or association? // *Rev Med Virol.* 2021. Vol. 31, N 6. P. 1–15. doi: 10.1002/rmv.2226
4. Царев В.Н., Митронин А.В., Подпорин М.С., и др. Комбинированное эндодонтическое лечение: микробиологические аспекты с использованием сканирующей электронной микроскопии // *Эндодонтия Today.* 2021. Т. 19, №1. С. 11–17. EDN: XRTLQU doi: 10.36377/1683-2981-2021-19-1-11-17
5. Ермолович А.Л., Борисова Э.Г., Железняк В.А., Хрустаева Ю.А. Значение бактериоскопии в комплексе диагно-
- стики осложненных форм кариеса зубов у пациентов, ранее переболевших COVID-19 // *Прикладные информационные аспекты медицины.* 2023. Т. 26, № 3. С. 41–46. EDN: EPYSIB doi: 10.18499/2070-9277-2023-26-3-41-46
6. Разумова С.Н., Браго А.С., Баракат Х.Б., и др. Микробиологическое исследование эффективности обработки корневого канала эрбиевым лазером // *Biomedical Photonics.* 2019. Т. 8, № 4. С. 11–16. EDN: OGIPMC doi: 10.24931/2413-9432-2019-8-4-11-16
7. Борисова Э.Г., Ермолович А.Л. Диагностика и лечение хронического гангренозного пульпита при выявлении микотической флоры после перенесенной коронавирусной инфекции // *Медико-Фармацевтический журнал «Пульс».* 2023. Т. 25, № 2. С. 11–16. EDN: QIBXWH doi: 10.26787/nydha-2686-6838-2023-25-2-11-16
8. Солдатов И.К., Журавлёва Л.Н., Тегза Н.В., и др. НаукOMETрический анализ диссертационных работ по детской стоматологии в Российской Федерации // *Российский стоматологический журнал.* 2023. Т. 27, № 6. С. 571–580. EDN: QINWXB doi: 10.17816/dent624942

ОБ АВТОРАХ

* Ермолович Анна Леонидовна;

адрес: Россия, 194044, Санкт-Петербург, ул. Академика Лебедева, д. 6;

ORCID: 0000-0001-5885-0559;

eLibrary SPIN: 8073-3050;

e-mail: anya.ermolovich@mail.ru

Борисова Элеонора Геннадиевна, д-р мед. наук, профессор;

ORCID: 0000-0003-2288-9456;

eLibrary SPIN: 3918-3090;

e-mail: pobedaest@mail.ru

Семёнова Дарья Дмитриевна, канд. мед. наук;

ORCID: 0000-0003-1467-3321;

eLibrary SPIN: 7676-7360;

e-mail: malyshewa.dasha@mail.ru

AUTHORS' INFO

* Anna L. Ermolovich;

address: 6 Academician Lebedev street, 194044 Saint Petersburg, Russia;

ORCID: 0000-0001-5885-0559;

eLibrary SPIN: 8073-3050;

e-mail: anya.ermolovich@mail.ru

Eleonora G. Borisova, MD, Dr. Sci. (Medicine), Professor;

ORCID: 0000-0003-2288-9456;

eLibrary SPIN: 3918-3090;

e-mail: pobedaest@mail.ru

Dar'ja D. Semenova, MD, Cand. Sci. (Medicine);

ORCID: 0000-0003-1467-3321;

eLibrary SPIN: 7676-7360;

e-mail: malyshewa.dasha@mail.ru

* Corresponding author / Автор, ответственный за переписку