Uniwersytet Jagielloński
Collegium Medicum

Lek. med. Mateusz Fiema

„Mikrobiota jamy ustnej w wybranych chorobach ogólnoustrojowych”
“Oral microbiota in systemic diseases”

Rozprawa doktorska

Promotor: Dr hab. n. med. Iwona Gregorczyk Maga

Miejsce realizacji rozprawy: Szpital Uniwersytecki w Krakowie
Kierownik jednostki: mgr Marcin Jędrychowski

Kraków, 2023

Pragnę podziękować Dr Hab. Iwonie Gregorczyk-Maga,
która będąc nieocenionym przewodnikiem w świecie nauki, podzieliła się
ze mną swoją wiedzą i doświadczeniem.

Dziękuję również Dr Michałowi Kani
za profesjonalną pomoc i inspirację do działań

oraz mojej Narzeczonej Magdzie, Rodzinie i Przyjaciołom za ogromne
wsparcie na każdym etapie powstawania pracy.

Wykaz stosowanych skrótów:

ARDS - Acute Respiratory Distress Syndrome

BOAS - Beck Oral Assessment Scale

CSII - Continuous subcutaneous insulin infusion

DM1- Diabetes Miellitus type 1

DOT - Day of Treatment

GCF - Gingival Crevicular Fluid

HbA_{1C} %– odsetek hemoglobiny glikowanej

HOMD - Human Oral Microbiome Database

HMP - Human Microbiome Project

ICU – Intensive Care Unit

IP – Insulin Pump

IPA - Invasive Pulmonary Aspergillosis

MALDI-TOF MS - Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

MeSH – Medical Subject Headings

SARS-CoV2 - Severe Acute Respiratory Syndrome Coronavirus 2

WHO - World Health Organization

VAP- Ventilator-Associated Pneumonia

Spis treści

1. Wykaz publikacji stanowiących rozprawę doktorską

Niniejsza rozprawa doktorska pt. „Mikrobiota jamy ustnej w wybranych chorobach ogólnoustrojowych" powstała w oparciu o cykl trzech publikacji naukowych. Wszystkie zostały opublikowane w czasopismach naukowych z listy Journal Citation Reports.

1. I. Gregorczyk-Maga, **M. Fiema**, M. Kania, J. Kędzierska, E. Jachowicz, D. Romaniszyn, J. Wójkowska- Mach
   Cultivable oral bacteriobiota dysbiosis in mechanically ventilated COVID-19 patients
   Frontiers in Microbiology, 2022, doi: 10.3389/fmicb.2022.1013559
   (IF = 6,064, MNiSW =100)
2. **Fiema M,** Włodarczyk A, Wójkowska-Mach J, Garlicki J, Gregorczyk-Maga I. *Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review.* Microorganisms, 2022 Aug 12;10(8):1630. doi: 10.3390/microorganisms10081630
   (IF = 4,926, MNiSW =40)
3. I. Gregorczyk-Maga[#], **M. Fiema**[#], M. Kania, E. Jachowicz, D. Romaniszyn, K. Gerreth[6], T. Klupa, J. Wójkowska-Mach
   Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump
   Int. J. Environ. Res. Public Health **2023**, *20*(3), 2252;
   https://doi.org/10.3390/ijerph20032252
   (IF = 4,614 , MNiSW =140)
   #-autor równorzędny

Łączna wartość Impact Factor według JCR dla wymienionego cyklu wynosi 15,604 oraz 280 punktów według wykazu czasopism naukowych MNiSW.

2. Zarys zagadnienia i uzasadnienie podjętej tematyki

Ekosystem drobnoustrojów jamy ustnej (mikrobiota) jest bardzo złożony i odgrywa ważną rolę w utrzymaniu zdrowia człowieka. Składa się z bakterii, archeonów, pierwotniaków, grzybów i wirusów. Jama ustna jest niejednorodnym środowiskiem rozwoju i kolonizacji mikroorganizmów, które pozostają w dynamicznej równowadze z organizmem, uczestnicząc w procesach fizjologicznych, patologicznych i immunologicznych. Mikrobiota jamy ustnej znacząco różni się od ekosystemów pozostałych odcinków przewodu pokarmowego i jest drugą po mikrobiocie jelit, w organizmie człowieka, pod względem zróżnicowania oraz dynamiki [1]. Ekosystem jamy ustnej ma kilka istotnie różniących się nisz, w tym: ślinę, powierzchnie tkanek miękkich błony śluzowej, języka i kieszonki dziąsłowej oraz powierzchnie tkanek twardych zębów [2]. Każda nisza zapewnia odrębne, optymalne warunki i składniki odżywcze dla zamieszkujących ją mikroorganizmów [3, 4]. Proces kolonizacji śliny i tkanek miękkich różni się od kolonizacji płytki nazębnej, przez co profil mikrobiologiczny tych obszarów jest odmienny [5]. W konsekwencji, mikroflora z analogicznych nisz u różnych osobników jest bardziej do siebie zbliżona w porównaniu do różnych miejsc, u tego samego osobnika [6, 7]. Wybrane białka śliny powlekającej powierzchnie zębów i błonę śluzową jamy ustnej sprzyjają adhezji drobnoustrojów, w odróżnieniu do innych, które sprzyjają desorpcji, aglutynacji i usuwaniu drobnoustrojów w akcie przełykania śliny [8]. Zmniejszenie wydzielania śliny (kserostomia) sprzyja wzrostowi liczebności mikroorganizmów kwasogennych, co przyczynia się do rozwoju chorób jamy ustnej [9]. Kolonizacja drobnoustrojów na tkankach miękkich jamy ustnej jest ograniczona [10]. W porównaniu z pozostałymi obszarami błony śluzowej jamy ustnej, język jest zasiedlony przez bardziej zróżnicowane mikroorganizmy [11]. Związane jest to, m.in. z obecnością tzw. krypt na jego powierzchni, co sprzyja rozwojowi mikroflory beztlenowej [12]. Odmiennym, pod względem budowy strukturalno - czynnościowej jest mikrofilm płytki nazębnej [13]. Zasadnicza różnica występuje ze względu na jej umiejscowienie. Drobnoustroje bytujące w naddziąsłowej płytce nazębnej różnią się od mikroflory okolic poddziąsłowych. W obszarze poddziąsłowym, biofilm zawiera więcej surowicy i mniej śliny, środowisko staje się bardziej beztlenowe, a pH i temperatura są odmienne w porównaniu do okolicy naddziąsłowej [14].

Zaburzenie równowagi mikroflory jamy ustnej, określane jako dysbioza, może odgrywać znaczącą rolę w patogenezie chorób miejscowych i ogólnoustrojowych. Z drugiej strony schorzenia te, zwrotnie oddziaływują na mikrobiotę jamy ustnej. Z uwagi na rolę mikroorganizmów jamy ustnej w patogenezie chorób o często odmiennej etiologii, istotne jest utrzymanie dynamicznej równowagi mikrobioty jamy ustnej oraz zrozumienie interakcji między społecznościami drobnoustrojów.

Potencjalne oddziaływanie między mikrobiotą jamy ustnej a wirusem SARS-CoV-2 nie jest wystarczająco poznane [15, 16]. Towarzysząca infekcji dysbioza jamy ustnej została powiązana z wieloma chorobami miejscowymi oraz ogólnoustrojowymi i może potencjalnie wpływać na ciężkość przebiegu choroby COVID-19 [17]. Ponadto u pacjentów z COVID-19 zaobserwowano dużą częstość występowania zaburzeń w jamie ustnej, takich jak: kserostomia, obecność na błonie śluzowej pęcherzy, grudek a także martwicze owrzodzenia warg [18]. U pacjentów leczonych na OIT zły stan zdrowia jamy ustnej determinowany ubytkami próchnicowymi i zapaleniem przyzębia może odgrywać znaczącą rolę w rozwoju ciężkich powikłań COVID-19 [19, 20]. Podczas długotrwałej intubacji dotchawiczej dysbiotyczna mikroflora jamy ustnej może kolonizować dolne drogi oddechowe. W konsekwencji pacjenci są narażeni na wysokie ryzyko rozwoju odrespiratorowego zapalenia płuc (ventilator-associated pneumonia - VAP). Monitorowanie mikroflory oraz utrzymanie zdrowia jamy ustnej tych pacjentów na OIT może zmniejszyć częstość VAP [21, 22].

Odmienną chorobą, pod względem etiopatogenezy, przebiegu, leczenia i występowania powikłań jest cukrzyca. Cukrzyca typu 1 (DM I) to przewlekłe ogólnoustrojowe schorzenie, w którym niedobór insuliny prowadzi do hiperglikemii. W przypadku źle kontrolowanej glikemii mogą wystąpić powikłania cukrzycowe, w tym neuropatie, nefropatie i retinopatie oraz choroby secowo- naczyniowe [23]. Ciągły podskórny wlew insuliny (CSII) za pomocą osobistej pompy jest nowoczesną metodą jej podawania, umożliwiającą lepszą kontrolę metaboliczną [24]. Uważa się, że wyrównany dzięki CSII poziom glikemii czyni tę populację w mniejszym stopniu narażoną na dysbiozę jamy ustnej. Z innych doniesień wynika, że dwukierunkowy związek między zdrowiem jamy ustnej a DM I jest czynnikiem predysponującym do infekcji jamy ustnej, a to zwrotnie nasila progresję choroby ogólnoustrojowej [25]. Dotychczasowe doniesienia na temat mikrobioty jamy ustnej u dorosłych z DM I są nieliczne. Dotyczą głównie badań w populacji pacjentów w wieku rozwojowym lub pacjentów o złym stanie zdrowia jamy ustnej. Dotychczas nie podjęto prób scharakteryzowania mikroflory poszczególnych nisz

w jamie ustnej w DM I. W badaniach analizowano głównie ślinę lub wymazy z pojedynczych miejsc. Jama ustna z uwagi na duże zróżnicowanie mikrobioty nie może być traktowana jako jeden, jednorodny ekosystem mikrobiologiczny i powinna być podzielona na różne nisze skolonizowane przez odrębne mikroorganizmy [26, 27]. Współistnienie określonej grupy organizmów powoduje większą zjadliwość i większe ryzyko rozwoju chorób jamy ustnej i ogólnoustrojowych [28, 29]. Dla dokładniejszego poznania mikrobioty powstały bazy danych takie jak: Human Microbiome Project (HMP) oraz Human Oral Microbiome Database (HOMD).

Dotychczasowa wiedza na temat mikrobioty jamy ustnej i jej roli w rozwoju chorób o charakterze miejscowym i ogólnoustrojowym jest niepełna i dalece niewystarczająca. Badania precyzyjnie określające kluczową dla zdrowia i różnych schorzeń mikrobiotę jamy ustnej przyczynią się do lepszego opracowania skutecznych narzędzi do modulacji mikroflory jamy ustnej.

3. Cel rozprawy doktorskiej - ocena mikrobioty jamy ustnej w dwóch odmiennych ogólnoustrojowych stanach chorobowych.

4. Omówienie publikacji 1 i 2:

- *Cultivable oral bacteriobiota dysbiosis in mechanically ventilated COVID-19 patients,*
- *Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review.*

Cel pracy:

Charakterystyka mikrobioty wraz z oceną stanu zdrowia jamy ustnej u pacjentów z COVID-19, hospitalizowanych w Oddziale Intensywnej Terapii (OIT) z powodu ostrej niewydolności oddechowej.

Materiał i metodyka

W tym prospektywnym badaniu zrekrutowano 56 dorosłych pacjentów z COVID-19, zakwalifikowanych do wentylacji mechanicznej w tymczasowym OIT dla pacjentów z COVID-19. Kryteria włączenia do badania były następujące : zakażenie SARSCoV-2

potwierdzone metodą RT-PCR w wymazach z nosa i gardła przy przyjęciu do szpitala, pobyt na oddziale OIT, podpisana zgoda na udział w projekcie, intubacja z powodu zapalenia płuc związanego z COVID-19 w ciągu ostatnich 36 godzin od rozpoczęcia badania. Czynnikami wykluczającymi z udziału w projekcie było niespełnienie jednego z powyższych. Stan zdrowia jamy ustnej pacjentów oceniano za pomocą zmodyfikowanej skali Beck Oral Assessment Score (BOAS) [30]. Próbki do badania mikrobiologicznego pobrano z czterech nisz jamy ustnej: z błony śluzowej policzka, języka, powierzchni policzkowych zębów oraz płyn z kieszonki dziąsłowej (gingival cervical fluid - GCF). Materiały biologiczne były poddane klasycznym metodom hodowlanym, w kierunku bakterii beztlenowych, tlenowych oraz grzybów. Mikroorganizmy zidentyfikowano metodą spektrometrii mas MALDI-TOF MS. Uzyskane wyniki poddano wieloczynnikowej analizie statystycznej.

Projekt uzyskał Zgodę Komisji Bioetycznej UJCM: numer 1072.6120.333.2020; 7 Grudnia 2020 oraz numer 1072.6120.353.2020. 16 Grudnia 2020.

Wyniki

Pacjenci z COVID-19 hospitalizowani na OIT we wczesnym okresie po intubacji wykazywali jakościową i ilościową dysbiozę bakterioty jamy ustnej. W przeprowadzonym badaniu zidentyfikowano 32 rodzaje i 70 gatunków bakterii. Identyfikacja pobranego materiału wykazała ilościowe i jakościowe zaburzenia równowagi w mikrobiocie jamy ustnej. Dysbioza w postaci zmniejszenia różnorodności organizmów wraz z występowaniem patologicznych szczepów, jak *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli* i *Klebsiella pneumoniae* stanowiła cenną obserwację wśród tej grupy pacjentów. Oceniany stan jamy ustnej, wskazujący na umiarkowane i ciężkie zaburzenia (BOAS na poziomie 10-14) pozostawał w korelacji ze znaczną dysbiozą względem populacji zdrowej. *Lactobacillus spp.* stwierdzono u 57,1% badanych. Średnia liczba CFU wszystkich szczepów bakterii w materiale z powierzchni zębów wynosiła 9,3E+5 (1,4E+6), z kieszonek dziąsłowych 7,6E+5 (1,4E+6). Najwyższe liczby CFU zaobserwowano dla *Enterococcus faecalis spp*. i *Lactobacillus spp*., chociaż nie różniły się one znacząco od liczby CFU *Streptococcus spp. i Staphylococcus spp*.

W trakcie realizacji projektu badawczego odnotowano rzadki przypadek inwazyjnej aspergilozy płuc (IPA) wywołany przez *Aspergillus Niger,* zidentyfikowany w materiale z jamy ustnej pacjenta z COVID-19 wentylowanego mechanicznie.

Aspergillus niger został wykryty wcześniej w płynie kieszonki dziąsłowej (GCF) niż w popłuczynach oskrzelowych i mógł posłużyć za wczesny predyktor rozwoju inwazyjnej aspergilozy płuc. Z uwagi na nietypowość, opisano wspomniany przypadek kliniczny oraz dokonano przeglądu piśmiennictwa powiązanego tematycznie. Przeprowadzono dokładną analizę literatury w bazach danych PubMED i Cochrane pod kątem opisów przypadków i przeglądów opublikowanych w recenzowanych czasopismach anglojęzycznych, przy użyciu terminów MeSH. Zastosowanie „Aspergillus niger" i „COVID-19" jako słów kluczowych dało sześć artykułów, podczas gdy słowa kluczowe „Aspergillus niger" i „jama ustna" dały 24 artykuły. Koinfekcje między koronawirusem SARS-CoV-2 a innymi patogenami układu oddechowego wiążą się u pacjentów z COVID-19 z wysoką zachorowalnością i śmiertelnością [Lai et al. 2020; Lansbury et al. 2020]. Przed pojawieniem się COVID-19 *Aspergillus niger* uważano za bardzo rzadką przyczynę inwazyjnej aspergilozy płuc (IPA), występującej głównie u pacjentów z obniżoną odpornością [Tudesq et al. 2019]. Przeprowadzony przegląd piśmiennictwa nie ujawnił innych doniesień na temat obecności *Aspergillus niger* w płynie kieszonki dziąsłowej będącego przyczyną IPA u pacjenta z COVID-19.

5. Omówienie publikacji 3:

- Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Cele pracy:

1. Ocena mikrobioty jamy ustnej u pacjentów z DM I, leczonych za pomocą osobistych pomp insulinowych,
2. Określenie nisz mikrobiologicznych w jamie ustnej, opisanie ich różnorodności w kontekście uzyskania optymalnej identyfikacji bioty bakteryjnej i grzybowej.

Materiały i metodyka

Do badania zrekrutowano 23 dorosłych pacjentów z DM I leczonych za pomocą osobistej pompy insulinowej. Kryteria włączenia obejmowały wiek 18–35 lat, DM I zdiagnozowaną co najmniej 1 rok przed rekrutacją, leczenie ciągłym podskórnym wlewem insuliny (CSII) za pomocą osobistej pompy insulinowej (IP) przez co najmniej 6 miesięcy oraz wyrażenie świadomej zgody na udział w badaniu. Kryteriami

wykluczenia z badania były: ciąża lub karmienie piersią oraz choroby współistniejące, takie jak: zespół metaboliczny, choroby układu krążenia, nowotwory, ciężka niewydolność wątroby lub niewydolność nerek. Rozpoznanie DM I potwierdzono na podstawie kryteriów Diabetes Poland (10). Stan zdrowia jamy ustnej oceniono za pomocą narzędzia Oral Health Assessment Tool (OHAT). Próbki mikrobiologiczne pobrano z sześciu środowisk jamy ustnej: A- błony śluzowej policzka i B- podniebienia miękkiego, C- języka, Da- powierzchni podniebiennej zębów i Db-powierzchni policzkowej zębów oraz E- kieszonki dziąsłowej. Materiał z nisz A, B i C, został pobrany przy pomocy oryginalnych wymazówek ESwab. Z niszy D szczoteczkami czyszczącymi KerrHawe, a z niszy E papierowymi paskami Perio Paper Strips. Materiał biologiczny został poddany klasycznym metodom hodowli w kierunku bakterii tlenowych, beztlenowych oraz grzybów. Po izolacji, mikroorganizmy były identyfikowane metodą spektrometrii mas MAL-DI TOF MS. Uzyskane wyniki zostały poddane wieloczynnikowej analizie.

Projekt badawczy uzyskał zgodę Komisji Bioetycznej UJCM: numer 1072.61.20.10.2021.

Wyniki

Badana grupa była wyrównana metabolicznie (średnia wartość HbA1c% wyniosła 6,97 ± 0,95%), a stan zdrowia jamy ustnej w skali OHAT u 19 badanych był prawidłowy (ogólny wynik 0/16 - bez zmian). Trzech uczestników miało łagodne zmiany (łączny wynik 1/16).

Wykazano różnorodność mikrobiomu we wszystkich miejscach pobrania materiału i przedstawiono szczegółowe informacje dotyczące wszystkich zidentyfikowanych szczepów bakterii i grzybów. We wstępnej analizie, w której została porównana liczba rodzajów i gatunków oraz liczba CFU pomiędzy wszystkimi miejscami dla zidentyfikowanych szczepów oraz z podziałem na G-dodatnie, G-ujemne, gatunki Streptococci i gatunki Staphylococci - nie stwierdzono istotnych różnic w liczebności rodzajów i gatunków pomiędzy stanowiskami pobrania. Całkowite wartości CFU wszystkich szczepów w miejscu C były najwyższe i istotnie różniły się od pozostałych miejsc ($p < 0,001$ dla wszystkich porównań). W drugim etapie z analizy wykluczono miejsce C i porównano wartości CFU pomiędzy wszystkimi pozostałymi miejscami dla zidentyfikowanych szczepów z w/w podziałem. Istniały istotne różnice w ogólnej liczbie CFU wszystkich szczepów, szczepów Gram-dodatnich, paciorkowców i gronkowców

między miejscami. Analiza post-hoc wykazała, że istotne różnice ograniczały się do porównania lokalizacji błony śluzowej i powierzchni zębów. Ogólna liczba CFU wszystkich szczepów była wyższa w miejscu Db w porównaniu z A ($p = 0,011$) i miejscu Db w porównaniu z B ($p = 0,001$). Wartość CFU paciorkowców była wyższa w miejscu Db w porównaniu z B ($p = 0,007$). Następnie połączono miejsca A i B (miejsca na błonie śluzowej), a Da i Db w Da+Db (powierzchnie zębów) i porównano z miejscem E. Wystąpiły znaczące różnice w ogólnej liczbie CFU wszystkich szczepów między miejscami. Analiza post-hoc wykazała istotne różnice w połączonych powierzchniach błony śluzowej i zębów. Całkowite wartości CFU wszystkich szczepów i szczepów Gram-dodatnich były wyższe w miejscach Da+Db w porównaniu z A+B (oba $p<0,001$). Liczba CFU *S. oralis* była istotnie wyższa w Da+Db w porównaniu z E ($p = 0,013$). Próchnicogenne *S. mutans* zostały zidentyfikowane tylko w trzech próbkach od trzech badanych osób z miejsc Da i Db. *Candida* została zidentyfikowana we wszystkich miejscach z wyjątkiem miejsca E. W próbkach dominowały *C. albicans.* Stwierdzono znaczącą różnicę między całkowitą liczbą CFU wszystkich gatunków Candida między połączonymi miejscami A+B i Da+Db, ze znacznie wyższą liczbą *Candida* na powierzchniach zębów ($p = 0,015$).

6. Podsumowanie wyników rozprawy doktorskiej. Wnioski

W rozprawie doktorskiej wykazano dwukierunkowe oddziaływanie między stanem zdrowia jamy ustnej i jej mikrobiotą, a chorobą ogólnoustrojową.

Wyniki przeprowadzonych badań wskazują, że u pacjentów z SARS-CoV-2, hospitalizowanych w OIT i poddawanych mechanicznej wentylacji dochodzi do dysbiozy mikroflory jamy ustnej. Zły stan zdrowia jamy ustnej spowodowany niedostateczną jej higieną oraz mechaniczna wentylacja stwarzają drogę pasażu mikroorganizmom do dolnych dróg oddechowych, prowadząc do zapalenia płuc. Zapewnienie właściwej higieny jamy ustnej jest niezbędne w utrzymaniu myko- i mikrobiologicznej równowagi u pacjentów z ciężkim przebiegiem COVID-19. Wynik oceny mikrobioty jamy ustnej może być istotnym uzupełnieniem procesu diagnostycznego będąc predyktorem późniejszego nasilenia zmian zapalnych i rozwoju IPA.

W grupie pacjentów chorych na cukrzycę typu 1 (DM1), leczonych CSII z dobrą kontrolą metaboliczną stan zdrowia jamy ustnej był prawidłowy. Analiza myko- i mikrobiologiczna poszczególnych nisz wykazała, że jamy ustnej nie można traktować

jako jednego, jednorodnego ekosystemu. Wyodrębniono trzy odmienne i optymalne dla oceny mikrobioty jamy ustnej miejsca. Wyniki przedstawionych analiz mogą być podstawą do dalszych badań w dużych grupach pacjentów chorych na cukrzycę

7. Piśmiennictwo

1. Dong, L., Yin, J., Zhao, J., Ma, S. rui, Wang, H. rui, Wang, M., Chen, W., & Wei, W. qiang. (2018). Microbial similarity and preference for specific sites in healthy oral cavity and esophagus. Frontiers in Microbiology, 9(JUL), 1603.

2. Schwiertz A., Microbiota of the human body: implications in health and disease, Adv. Exp. Med. Biol. (2016) 45–60.

3. Aas J.A., Paster B.J., Stokes L.N., Olsen I., Dewhirst F.E., Defining the normal bacterial flora of the oral cavity, J. Clin. Microbiol. 43 (11) (2005) 5721–5732.

4. M. Avila, D.M. Ojcius, O. Yilmaz, The oral microbiota: living with a permanent guest, DNA Cell Biol. 28 (8) (2009) 405–411.

5. D.L. Mager, A.D. Haffajee, P.M. Devlin, C.M. Norris, M.R. Posner, J.M. Goodson, The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, nonrandomized study of cancer-free and oral squamous cell carcinoma subjects, J. Transl. Med. 3 (2005) 27-27.

6. J.L. Sonnenburg, M.A. Fischbach, Community health care: therapeutic opportunities in the human microbiome, Sci. Transl. Med. 3 (78) (2011).

7. D.L. Mager, L.A. Ximenez-Fyvie, A.D. Haffajee, S.S. Socransky, Distribution of selected bacterial species on intraoral surfaces, J. Clin. Periodontol. 30 (7) (2003) 644–654.

8. M. Costalonga, M.C. Herzberg, The oral microbiome and the immunobiology of periodontal disease and caries, Immunol. Lett. 162 (2) (2014) 22–38.

9. A. Almstahl, M. Wikstrom, I. Stenberg, A. Jakobsson, B. Fagerberg-Mohlin, Oral microbiota associated with hyposalivation of different origins, Oral Microbiol. Immunol. 18 (1) (2003) 1–8.].

10. J. He, Y. Li, Y. Cao, J. Xue, X. Zhou, The oral microbiome diversity and its relations to human diseases, Folia Microbiol. 60 (1) (2015) 69–80.

11. C.E. Kazor, P.M. Mitchell, A.M. Lee, L.N. Stokes, W.J. Loesche, F.E. Dewhirst, B.J. Paster, Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients, J. Clin. Microbiol. 41 (2) (2003) 558–563.

12. C. Scully, J. Greenman, Halitosis (breath odor), Periodontology 48 (2008) (2000) 66–75.
13. P.D. Marsh, Dental plaque as a biofilm and a microbial community – implications for health and disease, BMC Oral Health 6 (Suppl. 1) (2006) S14-S14.
14. B.J. Paster, S.K. Boches, J.L. Galvin, R.E. Ericson, C.N. Lau, V.A. Levanos, A. Sahasrabudhe, F.E. Dewhirst, Bacterial diversity in human subgingival plaque, J. Bacteriol. 183 (12) (2001) 3770–3783.].
15. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, Zhu F, Zhu B, Cui. Co-infection with respiratory pathogens among COVID-2019 cases, Virus Research, Volume 285, 2020,198005;
16. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020 Aug;81(2):266-275.
17. Kamel, A. H. M., Basuoni, A., Salem, Z. A., and AbuBakr, N. (2021). The impact of oral health status on COVID-19 severity, recovery period and C-reactive protein values. Br. Dent. J. 2021, 1–7.
18. Aragoneses, J., Suárez, A., Algar, J., Rodríguez, C., López-Valverde, N., and Aragoneses, J. M. (2021). Oral manifestations of COVID-19: updated systematic review with meta-analysis. Front. Med. 8:1423.
19. Hocková, B., Riad, A., Valky, J., Šulajová, Z., Stebel, A., Slávik, R., et al. (2021). Oral complications of ICU patients with COVID-19: case-series and review of two hundred ten cases. J. Clin. Med. 10, 1–13,
20. Marouf, N., Cai, W., Said, K. N., Daas, H., Diab, H., Chinta, V. R., et al. (2021). Association between periodontitis and severity of COVID-19 infection: a case– control study. J. Clin. Periodontol. 48, 483–491.
21. Bao, L., Zhang, C., Dong, J., Zhao, L., Li, Y., and Sun, J. (2020). Oral microbiome and SARS-CoV-2: beware of lung co-infection. Front. Microbiol. 11:1840.
22. Luyt, C. E., Sahnoun, T., Gautier, M., Vidal, P., Burrel, S., Pineton de Chambrun, M., et al. (2020). Ventilator-associated pneumonia in patients with SARS-CoV-2-associated acute respiratory distress syndrome requiring ECMO: a retrospective cohort study. Ann. Intensive Care 10:158.
23. DiMeglio, L. A., Evans-Molina, C., & Oram, R. A. (2018). Type 1 diabetes. The Lancet, 391(10138), 2449–2462.

24. Pease, A., Lo, C., Earnest, A., Kiriakova, V., Liew, D., & Zoungas, S. (2020). The Efficacy of Technology in Type 1 Diabetes: A Systematic Review, Network Meta-analysis, and Narrative Synthesis. Https://Home.Liebertpub.Com/Dia, 22(5), 411–421.
25. Zhang, Y., Wang, X., Li, H., Ni, C., Du, Z., & Yan, F. (2018). Human oral microbiota and its modulation for oral health. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie, 99, 883–893.
26. Verma D, Garg PK, Dubey AK. Insights into the human oral microbiome. Arch Microbiol. 2018 May;200(4):525-540. doi: 10.1007/s00203-018-1505-3.,
27. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, Franchi M. Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol. 2020 May 18;20(1):120.
28. H.F. Jenkinson, R.J. Lamont, Oral microbial communities in sickness and in health, Trends Microbiol. 13 (12) (2005) 589–595.
29. B.T. Rosier, P.D. Marsh, A. Mira, Resilience of the oral microbiota in health: mechanisms that prevent dysbiosis, J. Dent. Res. (2017) 22034517742139.
30. Haghighi, Abdullah, Vida Shafipour, Masoumeh Bagheri-Nesami, Afshin Gholipour Baradari, Jamshid Yazdani Charati. (2016). The impact of oral care on oral health status and prevention of ventilator-associated pneumonia in critically ill patients, Australian Critical Care, Volume 30, Issue 2, 69 - 73
31. Chalmers, J. M., King, P. L., Spencer, A. J., Wright, F. A. C., & Carter, K. D. (2005). The Oral Health Assessment Tool - Validity and reliability, Australian Dental Journal, 50(3), 191–199.

8. Publikacje

Publikacja nr 1

I. Gregorczyk-Maga, **M. Fiema**, M. Kania, J. Kędzierska, E. Jachowicz, D. Romaniszyn, J. Wójkowska- Mach

Cultivable oral bacteriobiota dysbiosis in mechanically ventilated COVID-19 patients

Frontiers in Microbiology, 2022, doi: 10.3389/fmicb.2022.1013559

(IF = 6,064, MEiN =100)

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 28 October 2022
DOI 10.3389/fmicb.2022.1013559

OPEN ACCESS

EDITED BY
George Grant,
University of Aberdeen,
United Kingdom

REVIEWED BY
Valerio Iebba,
University of Trieste,
Italy
Stéphane Viennot,
Université Claude Bernard Lyon 1, France

*CORRESPONDENCE
Michal Kania
mich.kania@uj.edu.pl

SPECIALTY SECTION
This article was submitted to Infectious Agents and Disease, a section of the journal Frontiers in Microbiology

RECEIVED 07 August 2022
ACCEPTED 12 October 2022
PUBLISHED 28 October 2022

CITATION
Gregorczyk-Maga I, Fiema M, Kania M, Kędzierska J, Jachowicz E, Romaniszyn D and Wójkowska-Mach J (2022) Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients. *Front. Microbiol.* 13:1013559. doi: 10.3389/fmicb.2022.1013559

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients

Iwona Gregorczyk-Maga[1], Mateusz Fiema[2], Michal Kania[3,4]*, Jolanta Kędzierska[5], Estera Jachowicz[6], Dorota Romaniszyn[6] and Jadwiga Wójkowska-Mach[6]

[1]Faculty of Medicine, Institute of Dentistry, Jagiellonian University Medical College, Krakow, Poland, [2]Department of Endocrinology, University Hospital, Krakow, Poland, [3]Doctoral School of Medicine and Health Sciences, Jagiellonian University Medical College, Krakow, Poland, [4]Chair of Metabolic Diseases, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland, [5]Microbiology Unit, University Hospital, Krakow, Poland, [6]Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

Potential interactions between the SARS-CoV-2 virus and the human oral microbiota are currently investigated widely. Patients with COVID-19 requiring mechanical ventilation in an intensive care unit (ICU) setting are at high risk of developing severe complications, including ventilator-associated pneumonia, thus making oral health management important. The aim of this study was to evaluate the oral health status and assess the dysbiosis of cultivable oral bacteriota in COVID-19 patients hospitalized in an ICU with acute respiratory distress within 36h following intubation. In this prospective cohort study, we recruited 56 adult COVID-19 patients that qualified for mechanical ventilation in the Temporary ICU for COVID-19 Patients of the University Hospital in Krakow. On admission to the ICU, oral health of patients was assessed using the modified Beck Oral Assessment Score (BOAS). Four oral habitats were sampled, namely the buccal mucosa, tongue, buccal dental surface and gingival pocket. Microorganisms were identified by MALDI/TOF mass spectrometry. The mean age of the study population was 66.5±12.7years, there were 24 (42.9%) females. All patients included in this study were intubated and ventilated in the ICU, with a corresponding high mortality rate (76.8%). On admission to ICU, 76.8% subjects scored 11–20 on the BOAS scale (median 12 [IQR 10–14]), indicating moderate or severe dysfunction of oral health. Potentially pathogenic bacteria were identified in the oral microbiota samples, including *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli and Klebsiella pneumoniae* in 23.2%, 39.3%, 17.9%, and 19.6% of patients, respectively. Lactobacillus spp. were present in 57.1% subjects. The mean CFU counts of all bacteria strains in dental brushes were 9.3E+5 (1.4E+6) and in gingival pockets 7.6E+5 (1.4E+6). The highest CFU counts were observed for *Enterococcus* spp. and, *Lactobacillus* spp., although these did not differ significantly from CFU counts of *Streptococcus* spp. and *Staphylococcus* spp. In this report we comprehensively characterized the oral health condition and cultivable oral bacteriota in COVID-19 patients hospitalized in an ICU with acute respiratory distress within 36h following intubation. The oral bacteriota showed significant qualitative and quantitative dysbiosis. Hospitalization in an

ICU and mechanical ventilation are important factors leading to oral dysbiosis in SARS-CoV-2 patients.

KEYWORDS

oral microbiota, dysbiosis, COVID-19, mechanical ventilation, ARDS

Introduction

Coronavirus disease 2019 (COVID-19), caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a global pandemic and resulting serious public health crisis (Zhu et al., 2020; World Health Organization, 2022). While most COVID-19 patients have minor symptoms, ~15% of hospitalized patients require admission to an intensive care unit (ICU; Terlecki et al., 2021). These patients exhibit respiratory failure with a systemic inflammatory reaction and multiple-organ dysfunction, requiring oxygen supplementation and, in some cases mechanical ventilation (Weiss and Murdoch, 2020).

Over 1,000 bacterial species have been reported to reside in the oral cavity (Dewhirst et al., 2010). In healthy individuals the oral bacteriota is dominated by Actinobacteria (Acctinomyces, Corynebacterium and Rothia), Bacteroides (Capnocytophaga, Porphyromonas and Prevotella), Firmicutes (Granulicatella and Streptococcus), Fusobacteria, Proteobacteria (Haemophilus and Neisseria; Zaura et al., 2001). The oral cavity can be divided into several microbiologically distinct niches, including saliva, soft tissue surfaces of the oral mucosa and tongue, and hard tissue surfaces of teeth (Xu et al., 2015; Zhang et al., 2018). Of the most common species, 54% is cultivable and identifiable, 14% is cultivable but not easily identified, and 32% cannot be cultivated, remaining in dormant state (Caselli et al., 2020). Recent research of oral microbiota resulted in two large databases: Human Microbiome Project (HMP) and Human Oral Microbiome Database (HOMD). HMP contains microbiome data from 5 main environments: the oral cavity, nasal cavity, vagina, gut and skin. Data in HOMD encompasses oral microbiota composition (Li et al., 2022).

Previous studies of changes in microbiome, including the oral microbiota, showed a significant reduction of microbial diversity in SARS-CoV-2 affected patients (Iebba et al., 2021; Wu et al., 2021; Uehara et al., 2022). These changes comprised decreased abundance of Neisseria, Corynebacterium, Aggregatibacter, Treponema, and Pseudomonas genus, and *Prevotella intermedia* in the oral cavity of COVID-19 patients. Importantly, the loss of comensal Neisseria, such as *N. subflava* and *N. mucosa*, and *Prevotella* spp. acting as a local oral probiotic, can lead to severe imbalance in the oral microbiota composition (Weyand, 2017; Rafiqul Islam et al., 2022). Enrichment of Campylobacter, Granulicatella, Veillonella and Filifactor genus was also observed, that can be of clinical significance, as those are taxa associated with periodontitis (Wu et al., 2021). *Veilonella* spp. has been also reported to induce proinflammatory responses (Haran et al., 2021). Furthermore, increased abundance of opportunistic *Hemophilus parainfluenzae* in the oral cavity can predispose patients to respiratory tract infections (Iebba et al., 2021; Wu et al., 2021). Another report revealed that COVID-19 patients had a higher abundance of *Enterococcus* spp. in the oral cavity, linking respiratory pathogens with gut microbiome abnormalities (Rafiqul Islam et al., 2022). The dysbiosis was even more pronounced in severe course of infection and long-COVID-19, suggesting that its extent can be treated as an indicator of infection severity (Haran et al., 2021; Wu et al., 2021; Fiema et al., 2022; Rafiqul Islam et al., 2022). The use of antibiotics in COVID-19 patients was also associated with independent oral and gut microbiome profiles (Wu et al., 2021).

A high prevalence of oral health problems, such as xerostomia, mucosal blistering, mouth rash and lip necrosis has been observed in patients with COVID-19 (Aragoneses et al., 2021). Several trials have correlated poor oral hygiene with hyper-inflammation (Kamel et al., 2021), and poor oral health in patients with caries and periodontitis may play a significant role in the development of severe complications of COVID-19 in patients managed in the ICU (Hocková et al., 2021; Marouf et al., 2021). Moreover, during prolonged endotracheal intubation, dysbiotic oral microbiota can colonize the lower respiratory tract. These patients are at high risk for developing bacterial ventilator-associated pneumonia (VAP). The oral management of these patients in an ICU is critical as oral care has been shown to reduce the incidence of VAP (Bao et al., 2020; Luyt et al., 2020).

The aim of this study was to evaluate the oral health status and assess the dysbiosis of cultivable oral bacteriota in COVID-19 patients hospitalized in an ICU with acute respiratory distress in the early post-intubation period.

Materials and methods

Study design and participants

In this prospective cohort study, we recruited 56 consecutive adult COVID-19 patients that qualified for mechanical ventilation in the Temporary ICU for COVID-19 Patients of the University Hospital in Krakow (UH) between January 31st and September 1st 2021. University Hospital in Krakow coordinated the care for patients with SARS-CoV-2 infection in Lesser Poland and was

responsible for the hospitalization of patients with COVID-19 requiring specialized treatment.

Patients were diagnosed with COVID-19 according to WHO and Polish guidelines with the use of RT-PCR (Diagnostic testing for SARS-CoV-2 [internet], 2022; Flisiak et al., 2022). The COVID-19 treatment algorithm in patients admitted to UH was based on constantly updated recommendations of the Polish Association of Epidemiologists and Infectiologists (Flisiak et al., 2022), including concurrent probiotic use in patients undergoing antibiotic therapy.

The inclusion criteria for this study were as follows: 1. SARS-CoV-2 infection confirmed by RT-PCR assay of nasal and pharyngeal swabs upon hospital admission, 2. Admission to the ICU, 3. Signed consent to participate in the study and 4. Intubation due to COVID-19 related pneumonia and acute respiratory distress syndrome (ARDS) within the preceding 36 h from commencement of the study procedures.

The patients included in the study were admitted from emergency wards (either at UH or non-UH) or transferred from another ward dedicated for COVID-19 patients (UH or outside UH).

Demographic and clinical data were gathered from the hospital electronic medical records. The database included information on age, sex, date of COVID-19 diagnosis (defined as the first positive result of antigen and PCR test from nasopharyngeal swab), date of admission to the hospital, institution of the patients' origin (emergency ward, hospital ward), date of discharge or death, date of admission to the ICU, date of intubation, COVID-19 severity on WHO Clinical Progression Scale (Supplementary Table S1; Marshall et al., 2020), comorbidities [previous diagnosis of diabetes, arterial hypertension, heart failure (HF), history of MI or stroke, ischemic heart disease, atrial fibrillation (AF), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD)] and pre-intubation treatment [remdesivir, antibiotic, days of antibiotic treatment (DOT) before intubation (the number of days a patient receives an antibiotic independent of dose), proton pump inhibitor]. CVD and cardiovascular risk factors were identified based on a medical history of prehospital diagnosis or treatment. Other chronic comorbidities were also diagnosed based on earlier clinical notes available in the medical records. Baseline laboratory results [C-reactive protein (CRP), procalcytonin (PCT), interleukin-6 (IL-6), D-dimer, white blood count (WBC), creatinine] were also extracted.

Oral health assessment

On admission to the ICU, oral health was assessed using a modified BOAS, consisting of five subscales, namely assessment of lips, mucosa and gingiva, tongue, teeth, and saliva. A higher score reflects dysfunction or tissue injury. BOAS scores range from 5 (no oral dysfunction) to 20 (severe dysfunction), and a score >5 is abnormal (Beck, 1979; Ames et al., 2011; Supplementary Table S2).

Oral cavity sampling methods

Four oral habitats were sampled by a trained dentist: the buccal mucosa, the tongue, buccal dental surface and gingival pocket, with the latter two only in patients with dentition. Specimens from the posterior dorsum of the tongue and buccal mucosa were collected using ESwab™ (Demuyser et al., 2018), which combines a COPAN-invented flocked swab with 1 ml of Liquid Amies in a plastic, screw cap tube. Dental plaque was collected from buccal dental surface side using Tooth Cleanic KerrHawe-KWX-OP-SZ-011, and after collection the brush was placed in 1 ml of Liquid Amies in a plastic screw cap tube. PerioPaper Strips ($n = 3$; Guentsch et al., 2011), designed to absorb or carry 0–1.2 µl of fluid, were used to collect gingival crevicular fluid (GCF) samples. The strips were placed in the gingival pocket for 30–45 s till its surface moistened. To minimize the risk of pre-analytical errors during sample collection, sterile gauze was used to remove excess saliva from the mucosa and dry the dental surfaces, preventing salivary contamination of GCF.

Microbiological cultures

The samples were immediately delivered to the microbiological laboratory, where they were inoculated by the dilution method (dilutions −1 to −6) or qualitative culture method (swabs only) on the following media: McConkey (Graso, Biotech), Columbia (Lab-Agar, Biomaxima), Scheadler (Scheadler-Agra, Biomaxima), Bile Esculine Azide (Lab-Agar, Biomaxima), MRS Agar (Oxoid), Sabouraud Agar (Biomaxima). Media were aerobically incubated at 37°C (McConkey, Columbia, Bile Esculine Azide) or anaerobically at 37°C (M.R.S and Scheadler, GENbag Atmosphere Generators [BioMérieux, France] for 48 h). After incubation, the phenotypical colonies were counted and reported, and results were presented as CFU/ml (colony forming unit). After isolation, the microorganisms were identified by MALDI TOF mass spectrometry (Vitek MS Home bioMérieux).

Multiple analyses were performed to identify factors associated with oral health status, biodiversity and composition of oral commensal and potentially pathogenic bacterial microbiota, and in-hospital mortality.

Ethics statement

The study and its protocol were approved by the Jagiellonian University Bioethics Committee, decision number 1072.6120. 333.2020; December 7, 2020. Written informed consent was obtained from each subject prior to participation.

Statistical analysis

PS Imago Pro v.6.0 and Statistica v.13 were used for all statistical analysis. The normality of continuous variable distribution was assessed using the Shapiro–Wilk test. Differences between groups were analyzed with Student's *t*-test or nonparametric tests (Mann–Whitney *U*-test, Kruskal–Wallis ANOVA) when appropriate. Paired data were analyzed using the Wilcoxon test or Friedman's ANOVA along with appropriate post-hoc tests. Continuous variables were presented as arithmetic means ($\bar{x}$) ± standard deviations (SD) or as the median with interquartile range (IQR) when the data were not normally distributed. The distribution of categorical variables was described as counts and percentages. Statistical testing was completed to compare categorical variables using an independent sample Chi-squared test or Fisher's exact test when appropriate, and dependent samples with McNemar's test and Cochran's Q ANOVA. A *p*-value of <0.05 was considered statistically significant. The Bonferroni correction was used for multiple comparisons.

Results

Demographic data and background

The study population included 56 patients admitted to an ICU ward with ARDS due to COVID-19 related pneumonia. The mean age was 66.5 ± 12.7 years, there were 24 (42.9%) females. The subjects for whom data was available were classified as obese (mean BMI 31.9 ± 5.8, data available for 35 subjects). The most prevalent comorbidities were hypertension (46.4%), diabetes (35.7%) and coronary artery disease (28.6%).

The median WHO Clinical Progression Scale score was 6 on admission to ICU, meaning patients required oxygen by NIV or high flow (Figure 1). Of the enrolled patients, 16 (28.6%) were transferred directly from the emergency department, 30 (53.6%) were transferred from another UH ward, and 10 (17.9%) were transferred from another ward outside UH. The mean time between admission to UH and intubation was 4.91 ± 5.56 days.

On admission to the hospital, inflammatory markers were increased, indicating a severe response to COVID-19 infection (Table 1). Systemic steroid therapy was used in 40 (76.9%) and antibiotics in 33 (63.5%, of whom 32 were treated with β-Lactam antibiotics) patients before admission to an ICU. Median antibiotic DOT before intubation was 8 (range 4–13 days).

Clinical outcomes

All patients were intubated and ventilated in the ICU, and a corresponding high mortality rate was observed in the recruited patients (76.8%). There were no significant differences between survivors and non-survivors with regards to demographic characteristics, laboratory findings and oral health status (Supplementary Table S3).

Furthermore, there were no clinically relevant differences in the demographic characteristics, laboratory findings and mortality between the patients with different severities of COVID-19 on the WHO Progression Scale, the ward preceding admission to ICU, or previous steroid or antibiotic treatment.

Oral health assessment

On admission to ICU, the median BOAS was 12 (IQR 10–14), and we found 76.8% subjects scored 11–20, indicating moderate or severe dysfunction of oral health (Figure 2). Furthermore, the BOAS score differed significantly between the subcategories ($p < 0.001$). Our data indicated teeth had a significantly higher BOAS score than lips and gingival/oral mucosa ($p = 0.003$, $p = 0.011$). Comparison of subjects with no or mild vs. moderate or severe dysfunction in BOAS score revealed that the latter were older (60, IQR 48–68 vs. 69.5, IQR 63–75, $p = 0.006$), had higher initial inflammatory markers (PCT 0.17, IQR 0.08–0.46 vs. 0.42, IQR 0.17–1.16, $p = 0.034$) and higher HbA1c% (5.9, IQR 5.3–6.35 vs. 6.75 IQR 6.15–8.28, $p = 0.029$). There was also a trend toward higher WHO Progression Scale score in patients with moderate or severe dysfunction as indicated by their BOAS score, but it did not reach statistical significance. Finally, there was a significant but weak positive correlation between the selected BOAS subscales and the time from COVID-19 infection detection.

Bacteriological findings

In total, 32 genera and 70 bacterial species were identified in the study subjects (Table 2, full list in Supplementary Table S4). A number of strains were identified on the genera level [*Lactobacillus acidophilus/gasseri* (39 strains), *Streptococcus mitis/oralis* (100 strains), *Streptococcus salivarius ssp thermophilus/Str.salivarius ssp salivarius/Str. Vestibularis* (38 strains), *Lactobacillus casei/paracasei/rhamosus* (26 strains)]. Furthermore, multiple, potentially pathogenic bacteria were identified in the oral microbiota samples, including *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae* in 23.2%, 39.3%, 17.9%, and 19.6% of patients, respectively (Figure 3). *Lactobacillus* spp. was present in 57.1% of patients, and Cariogenic *S. mutans* was identified in one subject.

Escherichia coli and *Streptococci* spp. were identified more frequently in patients admitted from the emergency department than in those transferred from other hospital wards (100% vs. 64.4%, $p = 0.023$ and 100% vs. 72.5%, $p < 0.001$; Figure 3). Moreover, patients in whom no *Streptococci* strains were identified had higher DOT before intubation when compared to those with *Streptococci* strains present (12, IQR 8–26.5 vs. 5, IQR 2–8.5, $p = 0.013$). *Escherichia coli* was more frequently found in patients with diabetes (70% vs. 30%, $p = 0.025$) and CAD (37.5% vs. 10%,

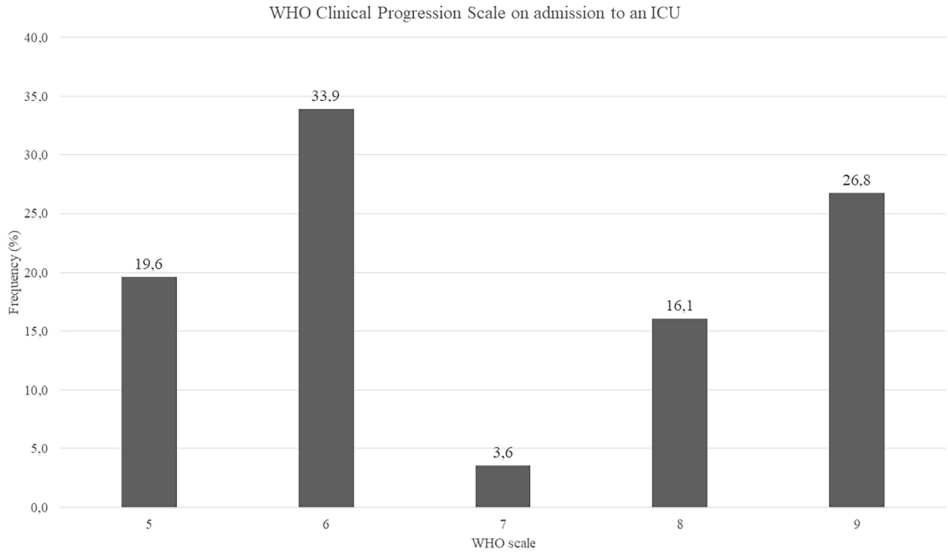


FIGURE 1
WHO Clinical Progression Scale on admission to an ICU, *N*=56, data presented as percentages.

$p = 0.024$) than in those without. Finally, non-survivors had lower baseline prevalence of *Lactobacillus* spp. as compared to survivors (48.8% vs. 84.6%, $p = 0.028$).

There were no associations between the sum BOAS scores and microbiological findings, although more detailed analyses revealed *Lactobacillus* spp. positive patients had lower BOAS saliva score as compared to those with no *Lactobacillus* spp. (median 2.5 vs. 3, IQR 2–3 and 2–3 respectively, $p = 0.045$). The BOAS saliva score was lower in patients using antibiotic treatment before intubation (median 2 vs. 3, IQR 2–3 and 2–3 respectively, $p = 0.05$).

The CFU counts were available for samples acquired by the dental brush and from gingival pockets, and the median CFU counts from all sites were 3.0E+5 (6.3E+4–1.0E+6). The median CFU counts of all bacterial strains in dental brushes was 4.0E+5 (1.0E+5–1.4E+6) and in gingival pockets 2.0E+5 (4.0E+4–8.0E+5), with data available for 81.2 and 68.4% samples, respectively. There were no differences in the median CFU counts between the BOAS score categories in dental brushes and gingival pockets ($p = 0.198$). Interestingly, patients with previous antibiotic use had lower CFU counts than those without (median 2.0E+5 [4.0E+4–8.8E+5] vs. 4.5E+5 [1.2E+5–1.5E+6], $p = 0.007$), while patients transferred from the emergency department had higher CFU counts than those transferred from other hospital wards (median 6.0E+5 [2.8E+4–1.5E+6] vs. 3.0E+5 [IQR 4.0E+4–1.0E+6], $p = 0.016$).

The CFU counts for Gram-positive bacteria were higher than for Gram negative (median 5.0E+5 [1.1E+5–1.5E+6] vs. 1.0E+5 [5.0E+3–3.0E+5], $p < 0.001$). Finally, the highest median CFU counts from all sites were observed for *Enterococcus* spp., *Lactobacillus* spp., *Streptococcus* spp. and *Staphylococcus* spp. (Table 3; Figure 4).

Discussion

In this report, we comprehensively characterized the oral health condition and cultivable oral bacteriota in COVID-19 patients hospitalized in an ICU with ARDS within 36 h following intubation. In this population, the oral microbiota from mucosal swabs, dental samples, and gingival pockets showed significant qualitative and quantitative dysbiosis and was distinct from healthy patients. SARS-CoV-2 infection, hospitalization in an ICU and mechanical ventilation are important factors leading to oral dysbiosis in patients.

Our study population comprised a homogenous group of patients with COVID-19 infection that were hospitalized in a temporary ICU dedicated to SARS-CoV-2-positive patients. These patients, with severe COVID-19 and ARDS, required specialist medical care, including mechanical ventilation or hemodialysis. Our data demonstrated a significantly higher mortality rate compared to normal COVID admissions, although our election criteria biased patient selection toward the most severe COVID-19

TABLE 1 Baseline characteristics of study participants and outcomes of hospitalization.

Characteristics	Data available [*N*]	Value
Age [years]	56	66.5 (12.7)
Female [*n* (%)]	56	24 (42.9%)
BMI [kg/m^2]	35	31.9 (5.8)
WHO Clinical Progression Scale on admission to an ICU	56	6 (6–9)
Source of admission [*n* (%)]	56	
Emergency ward		16 (28.6%)
Hospital ward		40 (71.4%)
Time from COVID-19 diagnosis[a] to intubation [days]	53	6.95 (6.62)
Time from hospital admission to intubation [days]	53	4.91 (5.56)
Baseline BOAS, sum score[b]	49	12 (10–14)
Baseline BOAS, lips	56	2 (2–2)
Baseline BOAS, gingival and oral mucosa[b]	49	2 (2–3)
Baseline BOAS, tongue	56	2 (2–3)
Baseline BOAS, teeth[b]	49	3 (2–4)
Baseline BOAS, saliva	56	3 (2–3)
In-hospital death [*n* (%)]	56	43 (76.8%)
Laboratory findings		
CRP, first recorded ([mg/L], normal <5[c])	55	158 (98.9)
PCT, first recorded ([ng/ ml], normal <0.5[c])	55	5.1 (19.0)
IL-6, first recorded ([pg/ml], normal <7[c])	54	141.2 (233.0)
WBC, first recorded ([10^3/mm^3], normal 4×10^3–10×10^3[c])	54	10.8 (7.5)
Comorbidities [*n* (%)]		
COPD	56	3 (5.4%)
Smoking	52	7 (13.5%)
Diabetes	56	20 (35.7%)
History of neoplasm	56	15 (26.8%)
Hypertension	56	26 (46.4%)
Coronary artery disease	56	16 (28.6%)
Heart failure	56	6 (10.7%)
CKD	56	4 (7.1%)
In-hospital pharmacotherapy before intubation [*n* (%)]	52	
Steroid therapy		40 (76.9%)
Remdesivir		15 (29.4%)
Tocilizumab		11 (21.2%)
Antibiotic		33 (63.5%)
DOT before intubation [days][d]		9.8 (10)
PPI		24 (46.2%)

data are presented mean (SD), median (Q1-Q3) or *N* [%]; BMI, body mass index; WHO, World Health Organization; ICU, intensive care unit; UH, University Hospital Cracow, Poland; CRP, C-reactive protein; PCT, procalcitonin; IL-6, interleukin 6; WBC, white blood cell count; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; PPI, proton pump inhibitor; BOAS, Beck Oral Assessment Scale; DOT, days of antibiotic therapy.

[a]Defined as first positive SARS-CoV-2 nasopharyngeal swab.

[b]Data for patients with dentition.

[c]According to local laboratory standards.

[d]Data for 33 patients undergoing antibiotic therapy.

cases. Our study population was also older, with multiple comorbidities that have confirmed deleterious effects on COVID-19 survival, such as diabetes and cardiovascular disease (Harrison et al., 2021).

Our study population presented with moderate or severe dysfunction of oral health. We believe that this resulted from first, poor oral health status in the elderly adult Polish population as presented in one recent report (Malicka et al., 2022). The authors noted that 21% of 70-year-olds were completely toothless. On average, the number of teeth was 12.97 ± 9.5., with 4.7 ± 4.8 teeth in the occlusion. 30.8% of patients wore a partial removable denture, and 25% a complete removable maxillary denture. 20% of study participants had a partial removable denture, and 22.6% had a complete removable mandibular denture. Oral dryness was observed in *ca.* one-third of the studied cohort, nearly 20% had periodontitis and ~30% required treatment for caries. They

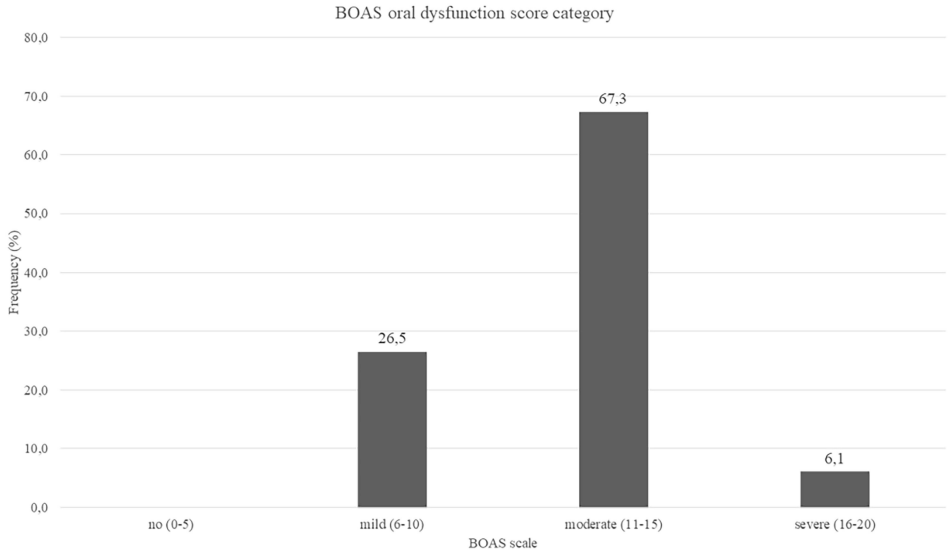


FIGURE 2
Baseline BOAS oral dysfunction score category, *N*=49, data presented as percentages.

TABLE 2 Baseline qualitative microbiological characteristics of study participants.

Characteristics	Data available [*N*]	Value	*p*
Total number of genera	56	32	–
Total number of species	56	70	–
Number of genera, all sites[a]	56	5 [4–6]	NS
Number of species, all sites[a]	56	6 [5–8]	NS
Number of patients with selected genera/species	56		
Acinetobacter baumannii		13 [23.2%]	–
Enterococcus faecalis		22 [39.3%]	–
Escherichia coli		10 [17.9%]	–
Klebsiella pneumoniae		11 [19.6%]	–
Lactobacillus spp.		32 [57.1%]	–
Streptococcus spp.		45 [80.4%]	–
Prevotella spp.		16 [28.6%]	–
Veillonella spp.		11 [19.6%]	–
Rothia spp.		6 [10.7%]	–
Neisseria spp.		5 [8.9%]	–

Data are presented as median (Q1–Q3) or *N* [%].
[a]Five sites in patients with dentition, three sites in patients without dentition.

emphasized that more than 60% of patients required professional dental prophylaxis (Malicka et al., 2022). Secondly, another report from a similar cohort (age and comorbidities) revealed patients hospitalized due to myocardial infarction presented higher plaque and periodontal indices as compared to patients with stable angina pectoris (Wozakowska-Kapłon et al., 2013). Still, this subject is under researched and further exploration is required. Finally, previous studies of ICU COVID-19 patients reported xerostomia, mucosal blistering and ulcers, rash, lip necrosis, and loss of taste and smell (Kamel et al., 2021; Eduardo et al., 2022; Yoshino et al., 2022). These can exacerbate such conditions as periodontitis, being an important risk factor for complications in patients with COVID-19 hospitalized in the ICU (Marouf et al., 2021). Pre-COVID-19 reports showed that hospitalization in an ICU together with mechanical ventilation can have a deleterious effect on oral health (Terezakis et al., 2011), including accumulation of the dental plaque and emergence of mucosal lesions. Due to the deterioration of oral health, critically ill patients in the ICU represent a group vulnerable to further complicates including VAP (Luyt et al., 2020).

Another noteworthy finding was a significant and alarming qualitative and quantitative dysbiosis of the cultivable oral bacteriota in our study population, as early as up to 36 h following intubation. Potentially pathogenic bacteria including *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae* occurred frequently with large CFU counts in our study population. While previous studies showed that these species are found in the oral cavity, their prevalence was not as high as in our patient population (Beck, 1979; Marshall et al., 2020; Diagnostic testing for SARS-CoV-2 [internet], 2022).

The CFU counts of such species as *Enterococcus faecalis* and *Acinetobacter baumannii* were as high as commensal *Streptococci*

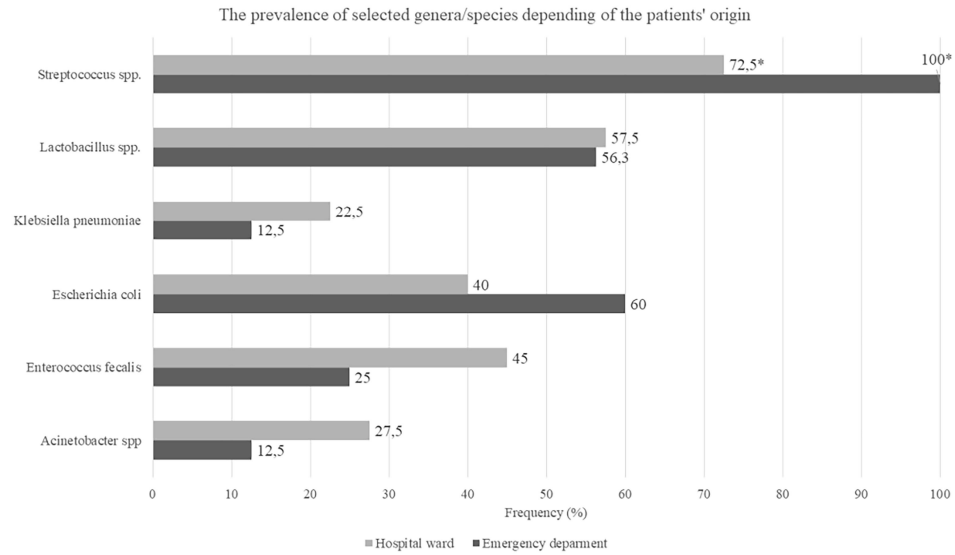


FIGURE 3
Number of patients with selected genera/species depending of the patients's origin, *N*=56, data presented as percentage.

and *Staphylococci*, indicating significant abnormalities in the oral bacteriobiota hemeostasis.

In one study, oral rinse samples from COVID-19 patients with a wide spectrum of symptoms showed a comparable extent of oral dysbiosis, with lower bacterial diversity, higher abundance of *Lactobacillus* spp., *Enterococcus* spp., *Acinetobacter baumannii*, and lower amounts of *Gemella* spp., *Fusobacterium* spp. and *Haemophilus* spp. (Soffritti et al., 2021).

The prevalence of *E. coli* was surprisingly high in our population. *Escherichia coli* is not a member of commensal oral microbiota, however it was detected in oral cavities of elderly patients with systemic diseases (Zawadzki et al., 2017). Recently, *E. coli* was reported to successfully colonize a supragingival biofilm (Pérez-Chaparro et al., 2014), so under special nutritional and environmental circumstances, *E. coli* can likely survive and even dominate this niche, especially in immunocompromised patients (Thurnheer and Belibasakis, 2014). Additionally, we found a higher prevalence of *E. coli* in patients admitted from the emergency ward than in those transferred from other hospital wards. To date, there have been no other reports on this issue, warranting further research.

Conversely, *Enterococcus faecalis* was more frequent in patients transferred from other hospital wards than those originating from the emergency department. Previous studies showed similar results, with changes in the oral bacteriobiota composition result from the exposure to hospital bacteria and each subsequent day of hospitalization increases the risk of *Enterococci* infections (Russo Fiorino et al., 2021). Moreover,

TABLE 3 Baseline quantitative microbiological characteristics of study participants.

	Number of samples with CFU count available	CFU/ml
All bacterial strains	316/881	3.0E+5 (6.2E+4–1.0E+6)
All G+ strains	79	5.0E+5 (1.1E+5–1.5E+6)
All G− strains	237	1.0E+5 (5.0E+3–3.0E+5)
Veillonella spp.	5/11	8.0E+5 (3.4E+5–2.0E+6)
Neisseria spp.	5/15	3.0E+5 (1.5E+5–5.0E+5)
Actinomyces spp.	4/9	6.8E+5 (1.1E+5–4.1E+6)
Prevotella spp.	19/55	2.0E+5 (1.0E+5–4.0E+5)
Streptococcus spp.	11/265	5.0E+5 (1.4E+5–1.5E+6)
Staphylococcus spp.	31/103	4.0E+5 (1.0E+5–1.0E+6)
Lactobacillus spp.	27/87	8.0E+5 (2.0E+5–1.5E+6)
Klebsiella spp.	19/58	5.0E+3 (3.0E+2–1.0E+5)
Escherichia coli	12/38	1.1E+4 (1.2E+3–5.9E+4)
Acinetobacter baumannii	11/50	1.0E+4 (2.0E+3–1.5E+4)
Enterococcus spp.	50/135	3.5E+5 (4.0E+4–1.0E+6)

Data are presented as median (IQR) or *N* [%].

E. coli and *E. faecalis* can employ antagonistic interactions against *S. mutans* (Thurnheer and Belibasakis, 2014), partially explaining our observations.

Our analyses revealed that the prevalence of commensal *Streptococcus* strains was lower in patients with a higher antibiotic consumption prior to intubation. We consider that antibiotics

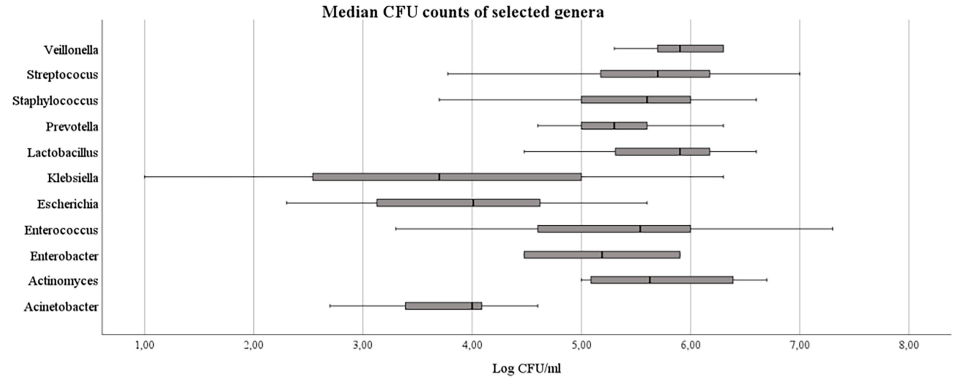


FIGURE 4
CFU counts of selected identified bacteria genera.

could be a major factor contributing to oral dysbiosis and disappearance of "healthy" commensal strains. Of all antibiotics, β-Lactam antibiotics were most commonly used in our cohort. Prospective cohort studies revealed that Shannon biodiversity index was decreased during amoxicillin treatment and was subject to further reduction in the following 6 months' time period (Menon et al., 2019; Monroy-Pérez et al., 2020; Nel Van Zyl et al., 2022). The density of Neisseria, Streptococcus and Veillonella strains in the oral cavity was also reported to decrease during treatment with amoxicillin (Larsson Wexell et al., 2016; Moraes et al., 2020). Save commonly used amoxicillin, other groups of antibiotics with various mechanisms of action can influence the oral microbiota and promote the selection of multi-drug resistant strains and their horizontal transmission (Zaura et al., 2015; Moraes et al., 2020). Other factors that can lead to oral dysbiosis include: local and systemic diseases, improper oral hygiene, unbalanced diet, smoking tobacco and immunosuppression (Li et al., 2022).

Lactobacillus was more prevalent in survivors in our study population, but there were no associations with antibiotic use, or with probiotic use according to the care standards in UH wards. One previous study reported the relative abundance of various bacterial genera, including *Lactobacillus* spp. in COVID-19 patients (Soffritti et al., 2021). These findings are notable, as *Lactobacillus* spp. may play some role in the protection against SARS-CoV-2, acting as an inhibitor of viral contamination by multiple mechanisms, including production of metabolites with antiviral activity, stimulation of mucosal immune system cells and local cytokines production (Zrelli et al., 2021).

Previous studies of COVID-19 patients tested saliva or nasopharyngeal swabs (Lloréns-Rico et al., n.d.; Miller et al., 2021). One notable strength of our study is that we investigated mucosal and dental brushes, highly representative for microbiota analysis (Zaura et al., 2001). Considering a proper and complex oral health evaluation, we used the BOAS scale. Among oral assessment tools, BOAS has been proposed as the most appropriate for ICU patients, with the mucosal-dental plaque score most applicable during observation (Ames et al., 2011).

Our study also had some limitations. First, we focused only on the SARS-CoV-2-positive patients. Moreover, in this study we used traditional methods for identification of microorganisms on the species level. As it is known, NGS it also allows obtaining information on non-cultivable microorganisms. But the method we used allowed the establishment of a microbial bank, for future studies in healthcare-associated-infections.

Conclusion

COVID-19 patients hospitalized in an ICU in the early post-intubation period presented an alarming qualitative and quantitative dysbiosis of the cultivable oral bacteriota. Abnormalities in the oral health status can trigger deterioration and dysbiosis of the oral microbiota. Poor oral hygiene, cough, increased inhalation and mainly mechanical ventilation provide a pathway for oral microorganisms to enter the lower respiratory tract, leading to pneumonia. A proper assessment of oral health can provide information on how to treat and diagnose these patients. Effective oral health care measures are necessary to reduce these infections, especially in severe COVID-19 patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by by the Jagiellonian University Bioethics Committee (Komisja Bioetyczna, Uniwersytet Jagielloński). The patients/participants provided their written informed consent to participate in this study.

Author contributions

IG-M: conceptualization, investigation, methodology, project administration, resources, supervision, and writing–review and editing. MF: data curation, methodology, investigation, writing–original draft preparation, and writing–review and editing. MK: formal analysis, methodology, visualization, writing–original draft preparation, writing–review and editing. EJ: formal analysis, methodology, validation. DR: formal analysis, methodology, validation. JW-M: methodology, formal analysis, validation, writing–review and editing, resources. All authors contributed to the article and approved the submitted version.

Funding

This publication was supported by the National Center for Research and Development CRACoV-HHS project (Model of multi-specialist hospital and non-hospital care for patients with SARS-CoV-2 infection) through the initiative "Support for specialist hospitals in fighting the spread of SARS-CoV-2 infection and in treating COVID-19" (contract number SZPITALE-JEDNOIMIENNE/18/2020). The described research was implemented by consortium of the University Hospital in Krakow and the Jagiellonian University Medical College.

Acknowledgments

The authors would like to thank all the patients who participated in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1013559/full#supplementary-material

References

Ames, N. J., Sulima, P., Yates, J. M., McCullagh, L., Gollins, S. L., Soeken, K., et al. (2011). Effects of systematic Oral Care in Critically ill Patients: a multicenter study. *Am. J. Crit. Care* 20:e103, –e114. doi: 10.4037/ajcc2011359

Aragoneses, J., Suárez, A., Algar, J., Rodríguez, C., López-Valverde, N., and Aragoneses, J. M. (2021). Oral manifestations of COVID-19: updated systematic review with meta-analysis. *Front. Med.* 8:1423. doi: 10.3389/fmed.2021.726753

Bao, L., Zhang, C., Dong, J., Zhao, L., Li, Y., and Sun, J. (2020). Oral microbiome and SARS-CoV-2: beware of lung co-infection. *Front. Microbiol.* 11:1840. doi: 10.3389/fmicb.2020.01840

Beck, S. (1979). Impact of a systematic oral care protocol on stomatitis after chemotherapy. *Cancer Nurs.* 2, 185–299. doi: 10.1097/00002820-197906000-00002

Caselli, E., Fabbri, C., D'Accolti, M., Soffritti, I., Bassi, C., Mazzacane, S., et al. (2020). Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. *BMC Microbiol.* 20, 1–19. doi: 10.1186/s12866-020-01801-y

Demuyser, T., De Geyter, D., Van Dorpe, D., Vandoorslaer, K., and Wybo, I. (2018). Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab® transport system. *J. Microbiol. Methods* 144, 73–78. doi: 10.1016/j.mimet.2017.11.009

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., et al. (2010). The human oral microbiome. *J. Bacteriol.* 192, 5002–5017. doi: 10.1128/JB.00542-10

Diagnostic testing for SARS-CoV-2 [internet]. (2022). (cited February 27, 2022).

Eduardo, F. P., Bezinelli, L. M., Gobbi, M. F., Bergamin, L. G., DLC, D. C., and Corrêa, L. (2022). Oral lesions and saliva alterations of COVID-19 patients in an intensive care unit: a retrospective study. *Spec. Care Dentist.* doi: 10.1111/scd.12705

Fiema, M., Wlodarczyk, A., Wojkowska-Mach, J., Garlicki, J., and Gregorczyk-Maga, I. (2022). Atypical presentation of aspergillus Niger infection in the oral cavity as a prediction of invasive pulmonary aspergillosis in a patient with COVID-19: case report and literature review. *Microorg* 10:1630. doi: 10.3390/microorganisms10081630

Flisiak, R., Horban, A., Jaroszewicz, J., Kozielewicz, D., Mastalerz-Migas, A., Owczuk, R., et al. (2022). Zalecenia postępowania w zakażeniach SARS-CoV-2 Polskiego Towarzystwa Epidemiologów i Lekarzy Chorób Zakaźnych, na dzień 23 lutego.

Guentsch, A., Kramesberger, M., Sroka, A., Pfister, W., Potempa, J., and Eick, S. (2011). Comparison of gingival Crevicular fluid sampling methods in patients with severe chronic periodontitis. *J. Periodontol.* 82, 1051–1060. doi: 10.1902/jop.2011.100565

Haran, J. P., Bradley, E., Zeamer, A. L., Cincotta, L., Salive, M. C., Dutta, P., et al. (2021). Inflammation-type dysbiosis of the oral microbiome associates with the duration of COVID-19 symptoms and long COVID. *JCI Insight* 6:e152346. doi: 10.1172/jci.insight.152346

Harrison, S. L., Buckley, B. J. R., Rivera-Caravaca, J. M., Zhang, J., and Lip, G. Y. H. (2021). Cardiovascular risk factors, cardiovascular disease, and COVID-19: an umbrella review of systematic reviews. *Eur. Hear J. – Qual. Care Clin. Outcomes* 7, 330–339. doi: 10.1093/ehjqcco/qcab029

Hocková, B., Riad, A., Valky, J., Šulajová, Z., Stebel, A., Slávik, R., et al. (2021). Oral complications of ICU patients with COVID-19: case-series and review of two hundred ten cases. *J. Clin. Med.* 10, 1–13. doi: 10.3390/jcm10040581

Iebba, V., Zanotta, N., Campisciano, G., Zerbato, V., Di Bella, S., Cason, C., et al. (2021). Profiling of oral microbiota and cytokines in COVID-19 patients. *Front. Microbiol.* 12:1603. doi: 10.3389/fmicb.2021.671813

Kamel, A. H. M., Basuoni, A., Salem, Z. A., and AbuBakr, N. (2021). The impact of oral health status on COVID-19 severity, recovery period and C-reactive protein values. *Br. Dent. J.* 2021, 1–7. doi: 10.1038/s41415-021-2656-1

Larsson Wexell, C., Ryberg, H., Sjöberg Andersson, W. A., Blomqvist, S., Colin, P., Van Bocxlaer, J., et al. (2016). Antimicrobial effect of a single dose of amoxicillin on the oral microbiota. *Clin. Implant. Dent. Relat. Res.* 18, 699–706. doi: 10.1111/cid.12357

Li, X., Liu, Y., Yang, X., Li, C., and Song, Z. (2022). The oral microbiota: community composition, influencing factors, pathogenesis, and interventions. *Front. Microbiol.* 13:1491. doi: 10.3389/fmicb.2022.895537

Lloréns-Rico, V., Gregory, A. C., Van Weyenbergh, J., Jansen, S., Van Buyten, T., Qian, J., et al. (n.d.). Mechanical ventilation affects respiratory microbiome of COVID-19 patients and its 1 interactions with the host 2 3 chronic diseases and metabolism. *Contag. Collab.* 10:13. doi: 10.1101/2020.12.23.20248425

Luyt, C. E., Sahnoun, T., Gautier, M., Vidal, P., Burrel, S., Pineton de Chambrun, M., et al. (2020). Ventilator-associated pneumonia in patients with SARS-CoV-2-associated acute respiratory distress syndrome requiring ECMO: a retrospective cohort study. *Ann. Intensive Care* 10:158. doi: 10.1186/s13613-020-00775-4

Malicka, B., Skośkiewicz-Malinowska, K., and Kaczmarek, U. (2022). The impact of socioeconomic status, general health and oral health on health-related quality of life, oral health-related quality of life and mental health among polish older adults. *BMC Geriatr.* 22, 1–15. doi: 10.1186/s12877-021-02716-7

Marouf, N., Cai, W., Said, K. N., Daas, H., Diab, H., Chinta, V. R., et al. (2021). Association between periodontitis and severity of COVID-19 infection: a case–control study. *J. Clin. Periodontol.* 48, 483–491. doi: 10.1111/jcpe.13435

Marshall, J. C., Murthy, S., Diaz, J., Adhikari, N., Angus, D. C., Arabi, Y. M., et al. (2020). A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infectious Diseases*, 20, e192–e197. doi: 10.1016/S1473-3099(20)30483-7

Menon, R. K., Gomez, A., Brandt, B. W., Leung, Y. Y., Gopinath, D., Watt, R. M., et al. (2019). Long-term impact of oral surgery with or without amoxicillin on the oral microbiome-a prospective cohort study. *Sci. Rep.* 9, 1–10. doi: 10.1038/s41598-019-55056-3

Miller, E. H., Annavajhala, M. K., Chong, A. M., Park, H., Nobel, Y. R., Soroush, A., et al. (2021). Oral microbiome alterations and SARS-CoV-2 saliva viral load in patients with COVID-19. *Microbiol. Spectr.* 9:e0005521. doi: 10.1128/Spectrum.00055-21

Monroy-Pérez, E., Rodríguez-Bedolla, R. M., Garzón, J., Vaca-Paniagua, F., Arturo-Rojas Jiménez, E., and Paniagua-Contreras, G. L. (2020). Marked virulence and azole resistance in Candida albicans isolated from patients with periodontal disease. *Microb. Pathog.* 148:104436. doi: 10.1016/j.micpath.2020.104436

Moraes, L. C., Lang, P. M., Arcanjo, R. A., Rampelotto, P. H., Fatturi-Parolo, C. C., Ferreira, M. B. C., et al. (2020). Microbial ecology and predicted metabolic pathways in various oral environments from patients with acute endodontic infections. *Int. Endod. J.* 53, 1603–1617. doi: 10.1111/iej.13389

Nel Van Zyl, K., Matukane, S. R., Hamman, B. L., Whitelaw, A. C., and Newton-Foot, M. (2022). Effect of antibiotics on the human microbiome: a systematic review. *Int. J. Antimicrob. Agents* 59:106502. doi: 10.1016/j.ijantimicag.2021.106502

Pérez-Chaparro, P. J., Gonçalves, C., Figueiredo, L. C., Faveri, M., Lobão, E., Tamashiro, N., et al. (2014). Newly identified pathogens associated with periodontitis: a systematic review. *J. Dent. Res.* 93, 846–858. doi: 10.1177/0022034514542468

Rafiqul Islam, S. M., Foysal, M. J., Hoque, M. N., Mehedi, H. M. H., Rob, M. A., Salauddin, A., et al. (2022). Dysbiosis of oral and gut microbiomes in SARS-CoV-2 infected patients in Bangladesh: elucidating the role of opportunistic gut microbes. *Front. Med.* 9:163. doi: 10.3389/fmed.2022.821777

Russo Fiorino, G., Maniglia, M., Marchese, V., Aprea, L., Torregrossa, M. V., Campisi, F., et al. (2021). Healthcare-associated infections over an eight year period in a large university hospital in Sicily (Italy, 2011–2018). *J. Infect. Prev.* 22, 220–230. doi: 10.1177/17571774211012448

Soffritti, I., D'Accolti, M., Fabbri, C., Passaro, A., Manfredini, R., Zuliani, G., et al. (2021). Oral microbiome Dysbiosis is associated with symptoms severity and local immune/inflammatory response in COVID-19 patients: a cross-sectional study. *Front. Microbiol.* 12:1397. doi: 10.3389/fmicb.2021.687513

Terezakis, E., Needleman, I., Kumar, N., Moles, D., and Agudo, E. (2011). The impact of hospitalization on oral health: a systematic review. *J. Clin. Periodontol.* 38, 628–636. doi: 10.1111/j.1600-051X.2011.01727.x

Terlecki, M., Wojciechowska, W., Klocek, M., Olszanecka, A., Stolarz-Skrzypek, K., Grodzicki, T., et al. (2021). Association between cardiovascular disease, cardiovascular drug therapy, and in-hospital outcomes in patients with COVID-19: data from a large single-center registry in Poland. *Kardiol. Pol.* 79, 773–780. doi: 10.33963/KP.15990

Thurnheer, T., and Belibasakis, G. N. (2014). Integration of non-oral bacteria into in vitro oral biofilms. *Virulence* 6, 258–264. doi: 10.4161/21505594.2014.967608

Uehara, O., Abiko, Y., Nagasawa, T., Morikawa, T., Hiraki, D., Harada, F., et al. (2022). Alterations in the oral microbiome of individuals with a healthy oral environment following COVID-19 vaccination. *BMC Oral Health* 22, 1–9. doi: 10.1186/s12903-022-02093-6

Weiss, P., and Murdoch, D. R. (2020). Clinical course and mortality risk of severe COVID-19. *Lancet* 395, 1014–1015. doi: 10.1016/S0140-6736(20)30633-4

Weyand, N. J. (2017). Neisseria models of infection and persistence in the upper respiratory tract. *Pathog Dis.* 75. doi: 10.1093/femspd/ftx031

World Health Organization (2022). WHO COVID-19 dashboard. [online] World Health Organization. Available at: https://covid19.who.int/. [cited May 12, 2022].

Wozakowska-Kapłon, B., Włosowicz, M., Gorczyca-Michta, I., and Górska, R. (2013). Oral health status and the occurrence and clinical course of myocardial infarction in hospital phase: a case-control study. *Cardiol. J.* 20, 370–377. doi: 10.5603/CJ.2013.0095

Wu, Y., Cheng, X., Jiang, G., Tang, H., Ming, S., Tang, L., et al. (2021). Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in COVID-19 patients during hospitalization. *NPJ Biofilms Microbiomes* 7, 1–9. doi: 10.1038/s41522-021-00232-5

Xu, X., He, J., Xue, J., Wang, Y., Li, K., Zhang, K., et al. (2015). Oral cavity contains distinct niches with dynamic microbial communities. *Environ. Microbiol.* 17, 699–710. doi: 10.1111/1462-2920.12502

Yoshino, A., Nakamura, Y., Irie, Y., Kitamura, T., Takata, T., Ishikura, H., et al. (2022). Oral health of COVID-19 patients using extracorporeal membrane oxygenation: a clinical study of 19 cases. *J. Clin. Med.* 11:42. doi: 10.3390/jcm11010042

Zaura, E., Brandt, B. W., de Mattos, M. J. T., Buijs, M. J., Caspers, M. P. M., Rashid, M. U., et al. (2015). Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *MBio*. 6:e01693-15. doi: 10.1128/mBio.01693-15

Zaura, E., Pappalardo, V. Y., Buijs, M. J., Volgenant, C. M. C., and Brandt, B. W. (2001). Optimizing the quality of clinical studies on oral microbiome: a practical guide for planning, performing, and reporting. *Periodontol 2000* 85, 210–236. doi: 10.1111/prd.12359

Zawadzki, P. J., Perkowski, K., Padzik, M., Mierzwińska-Nastalska, E., Szaflik, J. P., Conn, D. B., et al. (2017). Examination of Oral microbiota diversity in adults and older adults as an approach to prevent spread of risk factors for human infections. *Biomed. Res. Int.* 2017, 1–7. doi: 10.1155/2017/8106491

Zhang, Y., Wang, X., Li, H., Ni, C., Du, Z., and Yan, F. (2018). Human oral microbiota and its modulation for oral health. *Biomed. Pharmacother.* 99, 883–893. doi: 10.1016/j.biopha.2018.01.146

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733. doi: 10.1056/NEJMoa2001017

Zrelli, S., Amairia, S., and Zrelli, M. (2021). Respiratory syndrome coronavirus-2 response: microbiota as lactobacilli could make the difference. *J. Med. Virol.* 93, 3288–3293. doi: 10.1002/jmv.26746

Publikacja nr 2

Fiema M, Włodarczyk A, Wójkowska-Mach J, Garlicki J, Gregorczyk-Maga I. *Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review.* Microorganisms, 2022 Aug 12;10(8):1630. doi: 10.3390/microorganisms10081630

(IF = 4,926, MEiN =40)

microorganisms

MDPI

Case Report

Atypical Presentation of *Aspergillus niger* Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review

Mateusz Fiema [1,†], Aleksandra Wlodarczyk [2,†], Jadwiga Wojkowska-Mach [3], Jaroslaw Garlicki [4] and Iwona Gregorczyk-Maga [5,*]

[1] University Hospital in Krakow, 30-688 Krakow, Poland
[2] Department of Angiology, Faculty of Medicine, Jagiellonian University Medical College, 30-688 Krakow, Poland
[3] Department of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, 31-121 Krakow, Poland
[4] Department of Interdisciplinary Intensive Care, Faculty of Medicine, Jagiellonian University Medical College, 30-688 Krakow, Poland
[5] Faculty of Medicine, Institute of Dentistry, Jagiellonian University Medical College, 31-155 Krakow, Poland
* Correspondence: iwona.g.maga@gmail.com; Tel.: +48-608-634-894
† These authors contributed equally to this work.

Abstract: Coinfections between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory pathogens such as *Aspergillus* have become challenging, as well as being associated with high morbidity and mortality in patients with COVID-19. *Aspergillus niger* is a common environmental mold. Before the emergence of COVID-19, it was considered a very rare cause of invasive pulmonary aspergillosis (IPA), occurring mainly in immunocompromised patients. The aim of this study was to describe a very rare case of IPA caused by *A. niger* found in the oral cavity of a mechanically ventilated COVID-19 patient. *A. niger* detected in the gingival pocket was diagnosed earlier than in the bronchial lavage, and without treatment, passed into the lungs of the patient, causing serious complications. The swab from the oral cavity of mechanically ventilated COVID-19 patients can be a predictor of the subsequent severity of inflammatory lesions and the development of suspected IPA.

Keywords: coronavirus disease 2019; severe acute respiratory syndrome coronavirus 2; invasive pulmonary aspergillosis; *Aspergillus niger*; oral cavity; gingival pocket

Citation: Fiema, M.; Wlodarczyk, A.; Wojkowska-Mach, J.; Garlicki, J.; Gregorczyk-Maga, I. Atypical Presentation of *Aspergillus niger* Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review. *Microorganisms* **2022**, *10*, 1630. https://doi.org/10.3390/microorganisms10081630

Academic Editor: Antonella d'Arminio Monforte

Received: 13 July 2022
Accepted: 10 August 2022
Published: 12 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Coinfections between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory pathogens such as *Aspergillus* have become challenging, as well as being associated with high morbidity and mortality in patients with COVID-19 [1,2]. The SARS-CoV-2 pandemic challenges clinicians with rarely co-existing fungal infections caused by *Candida* spp., *Cryptococcus* spp., *Mucorales* spp., and *Aspergillus* spp. [3,4]. More attention should be paid to Aspergillus because it can lead to severe complications such as an invasive pulmonary aspergillosis (IPA) [5]. The most pathogenic species among *Aspergilli* is *A. fumigatus*, while twenty other species may cause infection, above all *A. flavus*, *A. terreus*, *A. nidulans*, and *A. niger* [6]. According to the data from 18 Italian ICUs, the incidence of IPA was 0.2% in 2013 [7]. During the COVID-19 pandemic, several studies and case series from Europe have reported high rates of COVID-19-associated IPA, with prevalence ranging from 20% to 35%, and an increase in the percentage of IPA in patients with COVID-19 admitted to intensive care units (ICUs) was reported (15.1%) [8].

Before the emergence of COVID-19, Aspergillus was considered a rare cause of invasive pulmonary aspergillosis (IPA), occurring mainly in immunocompromised patients (0.2%) [7].

IPA is an extremely rare condition in immunocompetent patients, but also one of the most severe forms of aspergillosis. IPA occurs especially in people whose immune systems are weakened as a result of cancer chemotherapy, bone marrow transplantation, or a disease of the immune system [9]. The symptoms of IPA are non-specific, including dry cough, shortness of breath, pleuritic chest pain, hemoptysis, thrombocytopenia, hypoxia, and acute respiratory failure [5]. Patients typically present with tachypnea, tachycardia, and hypoxia, and they are often profoundly thrombocytopenic or severely ill. The condition may deteriorate over a few days with acute respiratory failure. Diagnosing IPA remains difficult and requires a high index of suspicion. The gold standard for diagnosis is via histopathological examination and culturing of a surgical lung biopsy, but due to the patient's severe condition, this is typically not feasible. Sputum or bronchoalveolar lavage (BAL) fungal stain and culture are commonly used methods of identification, but they are positive only in around 30% of cases [10]. Recently, noninvasive biochemical markers have been used in the diagnosis of IPA, including serum and BAL fungal cell wall antigens, such as galactomannan (GM), beta-D glucan, and aspergillus polymerase chain reaction (PCR) from BAL fluid and serum [11].

Imaging examinations involving X-ray and CT imaging of the lungs are not specific to IPA. The "halo" or "air crescent" symptom or the presence of cavities on CT lung images suggests IPA.

There are limited data available concerning the association between COVID-19 and IPA [3]. Therefore, we present a very rare clinical microbiological course of invasive pulmonary aspergillosis caused by *A. niger* found in the oral cavity of a previously healthy 64-year-old man with COVID-19 pneumonia.

2. Case Report

A 64-year-old man presented with symptoms of dyspnea and general weakness. The reverse transcriptase polymerase chain reaction swab (Day 0) was positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Shading with speckled and streaked thickening, partially confluent infiltrative atelectic lesions, and partially obliterated pulmonary cavities were revealed in the chest X-ray, and pneumonia was diagnosed (Figure 1A). High inflammatory parameters were found in the laboratory tests: the white blood cell (WBC) count was 13.47 $\times$ 10^3 cells per µL, IL-6 was 329.7 pg per mL, procalcitonin (PCT) was 24.30 ng per mL, thrombocytopenia with a platelet count of 65 $\times$ 10^3 per µL, as well as the features of acute renal failure (creatinine, 695 µmol per L (eGFR 7); urea, 40 mmol per L). Due to the rapidly worsening symptoms of acute respiratory failure (SpO2, 50–70%; tachypnea, 40–50 breaths/min; blood pressure, 70/40 mmHg), the patient was intubated, and mechanical ventilation in assisted/controlled mode (A/C) was applied. Due to the lack of diuresis and the diagnosis of acute renal injury, renal replacement therapy was initiated by a continuous technique in the form of continuous venous–venous hemodiafiltration (CVVHDF). After intubation, material for bacteriological tests was collected (blood, urine, bronchial washes). An additional examination involved taking a smear from the gingival pocket of the teeth in the oral cavity as part of study entitled "Influence of oral hygiene on oral cavity microbiome and lower respiratory tract infections, in patients with COVID-19 ventilated mechanically". Empirical broad-spectrum antibiotic therapy was started intravenously with meropenem (3 $\times$ 2 g) and linezolid (2 $\times$ 600 mg). In addition, dexamethasone at a dose of 8 mg/24 h was administered intravenously and continued for 14 days. Initially, the patient's condition stabilized. On Day 1, the microbiological cultures were positive for *Neisseria meningitidis* and *Hemophilus influenzae* in the BAL and *Aspergillus niger* in the gingival pocket fluid (Figure 1B). The serum antigen tests (galactomannan, mannan, and anti-candida) were negative. On Day 15 after intubation, due to the worsening of respiratory failure, microbiological diagnostics were performed again and *A. niger* was

recognized on the BAL culture. At that time, the features of an invasive fungal infection were found during computed tomography (CT) of the chest (the halo sign in the early stage, the hypodense sign, consolidation, atelectasis, bronchiectasis, and ground-glass opacities). The diagnosis of IPA was confirmed (Figure 1C). Voriconazole (2 × 400 mg p.o.) was started. Over the course of the following days, treatment with antibiotics (colistin 3 × 4 mln j. iv and cefepime 3 × 2 g iv), voriconazole, hemodialysis, and A/C ventilation was continued. Due to the prolonged need for mechanical ventilation, percutaneous tracheotomy was performed. In the following weeks of hospitalization in the ICU, the patient's condition gradually stabilized, and his circulatory and respiratory efficiency improved. On Day 53, the mechanical ventilation was completed, and on Day 57, the patient was discharged from the ICU with passive oxygen therapy through tracheostomy.

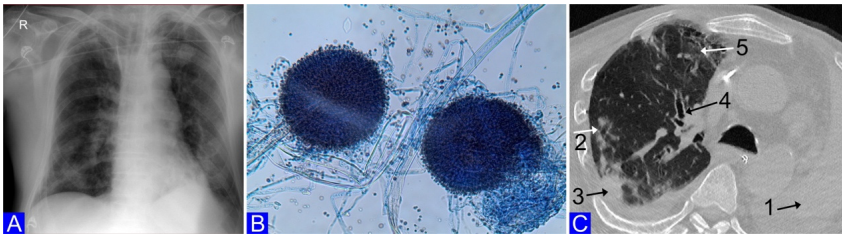


Figure 1. (**A**) Lung X-ray (Day 1). Bilateral pneumonia. (**B**) *Aspergillus niger*. Conidiophores of *Aspergillus niger* stained with lactophenol cotton blue (LCB), magnified 400×, provided by Zuzanna Tokarz MSc. (**C**) Lung HRCT (Day 15). Left lung atelectasis (1. right lung: suspected invasive pulmonary aspergillosis; 2. halo sign in early stage; 3. consolidation; 4. bronchiectasis; 5. ground-glass opacities).

3. Limitations

A limitation in this case report is that there is no evidence that the same *A. niger* strain in the oral cavity was indeed detected in BAL. However, this is very likely, as *A. niger* is not an oral mycobiote and does not routinely colonize the mucosa of mechanically ventilated patients. Genotyping techniques in microbiology, mainly bacteriology, were used, usually with a focus on outbreaks or epidemiological studies. There are limited data available concerning such methods according to *A. niger*; additionally, genotyping is costly and for this reason is not routinely performed in clinical practice [12]. Additionally, in the described case of a patient with COVID-19 in an ICU, *A. niger* genotyping was not performed, as it did not affect the therapeutic management.

4. Discussion and Literature Review

A thorough literature search was performed in the PUBMED and Cochrane databases for case reports and reviews published in English-language peer-reviewed journals using MeSH terms. A search for "*Aspergillus niger*" and "COVID-19" as keywords yielded six articles, whereas the keywords "*Aspergillus niger*" and "oral cavity" yielded 24 articles. Table 1 summarizes all studies in which *A. niger* was detected in COVID-19 patients. *Aspergillus niger*, as a ubiquitous and low-virulence mold, prefers warm, moist environments. It rarely causes infections in immunocompetent people. However, emphasis should be placed on looking for aspergillosis in immunocompetent patients, especially when the infection lasts a long time and is not treated properly. The corticosteroids promote enhanced fungal growth and the invasion of *A. fumigatus* by creating a suppressive environment affecting both epithelial as well as immune cells [13]. While various human cases of *Aspergillus* infection have been described previously in COVID-19 patients [3–6], human infection caused by *Aspergillus subsp. niger* in these patients is rare. It is often described as the etiological agent of otomycosis and cutaneous infections [6–8]. Only five cases of *A. niger* infection have

been reported previously, including four cases of invasive pulmonary aspergillosis [14–17], and a case with acute rhinosinusitis [18] (Table 1).

Table 1. Review of literature on the detection of *Aspergillus niger*.

Specimen	Diagnosis	Culture *n* (%)	PCR *n* (%)	References
BA *	IPA	1/1 (100%)	1/1 (100%)	Trovato, L.; Calvoa, M.; Migliorisia, G.; et al. [14]
TA *	IPA	1/1 (100%)	NP	Mirchin, R.; Czeresnia, J.M.; Orner, E.P.; et al. [15]
TA *	IPA	1/1 (100%)	NP	Pasula, S.; Chandrasekar, P. [16]
TA *	IPA	1/1 (100%)	1/1 (100%)	Singh, N.; Husain, S. [17]
FESS *	Acute rhinosinusitis	1/1 (100%)	NP	Tabarsi, P.; Sharifynia, S.; Pourabdollah, Toutkaboni, M.; et al. [18]

* BA, bronchoaspirate; TA, tracheal aspirate; FESS, functional endoscopic sinus surgery; IPA, invasive pulmonary aspergillosis; NP, not performed.

Laura Trovato et al. [14] described ventilator-associated pneumonia (VAP)-related pulmonary aspergillosis caused by *A. niger* in a positive COVID-19 patient. The omission of time in microbiological surveillance led to the dangerous consequence of IPA. *A. niger* proliferated and infiltrated the patient's respiratory system, and therefore, diagnosis and administration of voriconazole were not sufficient due to the critical condition of the lung epithelium. The patient with COVID-19 pneumonia described in this case report had IPA caused by *A. niger* found in the periodontal pocket in the oral cavity. Our patient, despite sufficient microbiological supervision and the detection of *A. niger* in the gingival pocket, did not receive antifungal treatment, which resulted in IPA. Although our patient was immunocompetent, the COVID-19 infection damaged the lung epithelium and resulted in the development of IPA.

The spores of *A. niger*, inhaled with air, enter the lungs and begin to germinate in immunocompromised individuals. The mycelium that develops in the lungs begins to release toxic metabolites that inhibit the immune system, which in turn allows for further efficient growth of the mycelium. Hyphae begin to penetrate the blood vessels. This results in clots forming inside the blood vessels, thus causing local infarcts in the lungs. Tinea developing in the lungs can cause extensive damage to lung tissue, causing severe hemorrhage, which may eventually kill the patient. Infection with *A. niger* in the lungs can spread through the blood to other organs. In nearly 22% of patients with invasive aspergillosis, spread of the fungal infection from the lungs to other organs is noted [19].

In recent years, reports of *A niger* species as a serious pathogen have become more frequent. In the past two decades, *A. niger* was rarely detected in the oral cavity or nasal cavity of healthy individuals, which the major otopathogens (*Malassezia*, *Candida*) mainly colonize [20,21]. This is mainly because the lack of oral hygiene increases oral candidal colonization.

Periodontal pockets are described as specific isolated environments, characterized by appropriate biological dynamics, with two-way interactions with the oral cavity on the one hand and the circulatory system through the gingival peripheral vessels on the other. Moreover, bacterial biofilms and the presence of viruses and fungi in the periodontal pocket are of major interest to the scientific dental community. It is more and more commonly accepted that in addition to the predisposition to developing a bacterial disease such as infective endocarditis [22], viruses from the periodontal pocket can infect distant organs and thus generate focal infections. An interesting issue is the presence of fungi in the gingival pocket, which has not been sufficiently studied. There are no case reports or studies on the presence of *A. niger* in the gingival pocket and its impact on the development of a systemic infection in patients. In our patient, *A. niger* from the gingival pocket could have connected to the oral cavity via gingival crevicular fluid (GCF) and mixed with saliva, leaving the

subgingival area. Another potential migration path for *A. niger* is through the periodontal capillary system and then the circulatory system. In our patient, the smear from the gingival pocket was positive for *A. niger* much earlier than for BAL, so we can assume that *A. niger* passed from the oral cavity to the lungs, causing invasive pulmonary aspergillosis. If the preemptive therapy antifungal treatment was started based on a swab from the gingival pocket, the IPA complication and lung damage would be avoided. Even though the cultures from the gingival pocket obtained from the patient were positive for the presence of *A. niger* and BAL remained negative, the patient developed IPA. The BAL was only positive 14 days after the detection of *A. niger* in the gingival pocket. Thus, we can assume that one of the most important hypotheses for the etiology of invasive pulmonary aspergillosis can be fungal biofilm formation in the periodontal pocket, which includes *A. niger*, among other pathogens. To establish the possibility of a previous chronic Aspergillus colonization in our patient, the detection of Aspergillus-specific antibodies was performed in the serum. Although BAL and the gingival pocket swab were positive, Aspergillus-specific antibodies remained negative. The negative results suggest that the patient was most likely infected in a hospital setting during intubation. According to recent data, *Aspergillus* spp. are recognized as a potential cause of VAP in immunocompetent hosts [23,24]. Colonization of the gingival pocket is an essential first step in the pathogenesis of IPA. However, the reason why *A. niger* is rare in clinical samples may simply be due to the inability to cultivate this fastidious organism. This organism is difficult to isolate using conventional culture methods, and the use of the gingival pocket swab method can increase the detection of this fungus from the respiratory tract.

The aforementioned swab from the oral cavity proved to be a predictor of the subsequent severity of inflammatory lesions and the development of IPA. The early and proper microbiological diagnosis of IPA is thus a prerequisite for its successful management. Our literature search revealed no other reports of *A. niger* found in the gingival pocket causing IPA in a patient with COVID-19. This case report is unique in that it describes the growth of *A. niger* in pure culture on conventional gingival pocket culture medium. We believe that the detection of *A. niger* in the gingival pocket in pure culture may be of clinical importance. Constant microbiological surveillance in the form of a swab from the gingival pocket will enable the early detection of *A. niger* and the initiation of preemptive treatment, preventing the development of invasive pulmonary aspergillosis.

Author Contributions: M.F. and I.G.-M. contributed to the conception and design of this case report. All the authors were involved in data analysis and interpretation. All the authors wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This publication was supported by the National Center for Research and Development CRACoV-HHS project "Model of multi-specialist hospital and non-hospital care for patients with SARSCoV-2 infection" through the initiative "Support for specialist hospitals in fighting the spread of SARSCoV-2 infection and in treating COVID-19" (contract number SZPITALE-JEDNOIMIENNE/18/2020). The described research was implemented by consortium of the University Hospital in Krakow and the Jagiellonian University Medical College.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Jagiellonian University Bioethics Committee (decision number 1072.6120.333.2020; 7 December 2020).

Informed Consent Statement: Written informed consent was obtained from the subject prior to participation.

Data Availability Statement: Data supporting this case report are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest regarding this case report.

References

1. Lai, C.C.; Wang, C.Y.; Wang, Y.H.; Hsueh, S.C.; Ko, W.C.; Hsueh, P.R. Global epidemiology of coronavirus disease 2019 (COVID-19): Disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. *Int. J. Antimicrob. Agents* **2020**, *55*, 105946. [CrossRef] [PubMed]
2. Lansbury, L.; Lim, B.; Baskaran, V.; Lim, W.S. Co-infections in people with COVID-19: A systematic review and meta-analysis. *J. Infect.* **2020**, *81*, 266–275. [CrossRef]
3. Zhu, X.; Ge, Y.; Wu, T.; Zhao, K.; Chen, Y.; Wu, B.; Zhu, F.; Zhu, B.; Cui, L. Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res.* **2020**, *285*, 198005. [CrossRef]
4. Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qiu, Y.; Wang, J.; Liu, Y.; Wei, Y.; et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet* **2020**, *395*, 507–513. [CrossRef]
5. El-Baba, F.; Gao, Y.; Soubani, A.O. Pulmonary aspergillosis: What the generalist needs to know. *Am. J. Med.* **2020**, *133*, 668–674. [CrossRef] [PubMed]
6. Latgé, J.P.; Chamilos, G. *Aspergillus fumigatus* and *Aspergillosis* in 2019. *Clin. Microbiol. Rev.* **2019**, *33*, e00140-18. [CrossRef]
7. Montagna, M.T.; Caggiano, G.; Lovero, G.; De Giglio, O.; Coretti, C.; Cuna, T.; Iatta, R.; Giglio, M.; Dalfino, L.; Bruno, F.; et al. Epidemiology of invasive fungal infections in the intensive care unit: Results of a multicenter Italian survey (AURORA Project). *Infection* **2014**, *42*, 141–151. [CrossRef]
8. Arastehfar, A.; Carvalho, A.; van de Veerdonk, F.L.; Jenks, J.D.; Koehler, P.; Krause, R.; Cornely, A.O.; Perlin, D.S.; Lass-Flörl, C.; Hoenigl, M. COVID-19 Associated Pulmonary Aspergillosis (CAPA)—From Immunology to Treatment. *J. Fungi* **2020**, *6*, 91. [CrossRef]
9. Tudesq, J.J.; Peyrony, O.; Lemiale, V.; Azoulay, E. Invasive pulmonary aspergillosis in nonimmunocompromised hosts. *Semin. Respir. Crit. Care Med.* **2019**, *40*, 540–547. [CrossRef] [PubMed]
10. Patterson, T.F.; Thompson, G.R.; Denning, D.W.; Fishman, J.A.; Hadley, S.; Herbrecht, R.; Kontoyiannis, D.P.; Marr, K.A.; Morrison, V.A.; Nguyen, M.H.; et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2016**, *63*, e1–e60. [CrossRef] [PubMed]
11. Dellière, S.; Dudoignon, E.; Voicu, S.; Collet, M.; Fodil, S.; Plaud, B.; Chousterman, B.; Bretagne, S.; Azoulay, E.; Mebazaa, A.; et al. Combination of Mycological Criteria: A Better Surrogate to Identify COVID-19-Associated Pulmonary Aspergillosis Patients and Evaluate Prognosis? *J. Clin. Microbiol.* **2022**, *60*, e0216921. [CrossRef]
12. van der Torre, M.H.; Shen, H.; Rautemaa-Richardson, R.; Richardson, M.D.; Novak-Frazer, L. Molecular Epidemiology of *Aspergillus fumigatus* in Chronic Pulmonary *Aspergillosis* Patients. *J. Fungi* **2021**, *7*, 152. [CrossRef] [PubMed]
13. Luvanda, M.K.; Posch, W.; Noureen, A.; Lafon, E.; Zaderer, V.; Lass-Flörl, C.; Wilflingseder, D. Dexamethasone creates a suppressive microenvironment and promotes *Aspergillus fumigatus* invasion in a human 3D epithelial/immune respiratory model. *J. Fungi* **2021**, *18*, 221. [CrossRef] [PubMed]
14. Trovato, L.; Calvoa, M.; Migliorisia, G.; Astutoc, M.; Oliveric, F.; Oliveri, S. Fatal VAP-related pulmonary aspergillosis by *Aspergillus niger* in a positive COVID-19 patient. *Respir. Med. Case Rep.* **2021**, *32*, 101367. [CrossRef] [PubMed]
15. Mirchin, R.; Czeresnia, J.M.; Orner, E.P.; Chaturvedi, S.; Murphy, K.; Nosanchuk, J.D. The Continuing Emergence of *Candida blankii* as a Pathogenic Fungus: A New Case of Fungemia in a Patient Infected with SARS-CoV-2. *J. Fungi* **2022**, *8*, 166. [CrossRef] [PubMed]
16. Pasula, S.; Chandrasekar, P. Spontaneous Hemoptysis in a Patient with COVID-19. *Chest* **2021**, *160*, e39–e44. [CrossRef]
17. Katsiari, M.; Mavroidi, A.; Palla, E.; Zourla, K.; Alonistiotis, T.; Ntorlis, K.; Nikolaou, C.; Vrioni, G.; Tsakris, A. Possible COVID-19-Associated Pulmonary Aspergillosis Due to *Aspergillus niger* in Greece. *Antibiotics* **2022**, *11*, 300. [CrossRef]
18. Tabarsi, P.; Sharifynia, S.; Toutkaboni, M.P.; Abtahian, Z.; Rahdar, M.; Mirahmadian, A.S.; Hakamifarda, A. Mixed etiology COVID-19 associated acute rhinosinusitis caused by two *Aspergillus* species. *Ann. Med. Surg.* **2022**, *75*, 103365. [CrossRef]
19. Singh, N.; Husain, S. *Aspergillus* infections after lung transplantation: Clinical differences in type of transplant and implications for management. *J. Heart Lung. Transplant.* **2003**, *22*, 258–266. [CrossRef]
20. Darwazeh, A.M.G.; Al-Dosari, A.; Al-bagieh, N.H. Oral Candida and nasal *Aspergillus* flora in a group of Saudi healthy dentate subjects. *Int. Dent. J.* **2002**, *52*, 273–277. [CrossRef]
21. Peters, B.A.; Wu, J.; Hayes, R.B.; Ahn, J. The oral fungal mycobiome: Characteristics and relation to periodontitis in a pilot study. *BMC Microbiol.* **2017**, *17*, 157. [CrossRef] [PubMed]
22. Daly, C.G.; Mitchell, D.H.; Highfield, J.E.; Grossberg, D.E.; Stewart, D. Bacteremia due to periodontal probing: A clinical and microbiological investigation. *J. Periodontol.* **2001**, *72*, 210–214. [CrossRef] [PubMed]
23. Charles, M.P.; Kali, A.; Easow, J.M.; Joseph, N.M.; Ravishankar, M.; Srinivasan, S.; Kumar, S.; Umadevi, S. Ventilator-associated pneumonia. *Australas. Med. J.* **2014**, *7*, 334–344. [CrossRef] [PubMed]
24. Torres, A.; Martin-Loeches, I. Invasive pulmonary aspergillosis in ventilator-associated pneumonia: The hidden enemy? *Am. J. Respir. Crit. Care Med.* **2020**, *202*, 1071–1073. [CrossRef] [PubMed]

Publikacja nr 3

I. Gregorczyk-Maga[#], **M. Fiema**[#], M. Kania, E. Jachowicz, D. Romaniszyn, K. Gerreth[6], T. Klupa, J. Wójkowska-Mach

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump

Int. J. Environ. Res. Public Health **2023**, *20*(3), 2252;

https://doi.org/10.3390/ijerph20032252

(IF = 4,614 , MEiN =140)

#-autor równorzędny

International Journal of
Environmental Research and Public Health

MDPI

Article

Oral Microbiota—One Habitat or Diverse Niches? A Pilot Study of Sampling and Identification of Oral Bacterial and Fungal Biota in Patients with Type I Diabetes Mellitus Treated with Insulin Pump

Iwona Gregorczyk-Maga [1,†], Mateusz Fiema [2,†], Michal Kania [3,4,*], Estera Jachowicz-Matczak [5], Dorota Romaniszyn [5], Karolina Gerreth [6], Tomasz Klupa [4] and Jadwiga Wójkowska-Mach [5]

1 Institute of Dentistry, Faculty of Medicine, Jagiellonian University Medical College, 31-155 Krakow, Poland
2 Department of Endocrinology, University Hospital, 30-688 Krakow, Poland
3 Doctoral School of Medicine and Health Sciences, Jagiellonian University Medical College, 31-008 Krakow, Poland
4 Department of Metabolic Diseases, Center of Advanced Technologies in Diabetes, Faculty of Medicine, Jagiellonian University Medical College, 30-688 Krakow, Poland
5 Department of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, 31-121 Krakow, Poland
6 Department of Risk Group Dentistry, Chair of Pediatric Dentistry, Poznan University of Medical Sciences, 60-812 Poznan, Poland
* Correspondence: mich.kania@doctoral.uj.edu.pl
† These authors contributed equally to this work.

Abstract: Objective: The oral microbiota is a very complex and dynamic microbial ecosystem. Alterations of its balance can result in oral and systemic diseases. We aimed to characterize the microbiota in particular niches of the oral cavity in adult type 1 diabetes patients treated with continuous infusion of insulin with insulin pump (IP). In addition, we aimed to determine optimal sites of oral microbiota sampling in studies of large research groups of patients with DM I. Design: In this pilot study, we sampled the buccal and soft palate mucosa, tongue, palatal and buccal dental surfaces and gingival pockets of adult DM I patients treated with IP. Results: In total, 23 patients were recruited. The oral microbiota was dominated by *Streptococcus* and *Neisseria*, with a low incidence of cariogenic *S. mutans* and *Lactobacillus*, as well as periodontal pathogens such as *Prevotella*. There were significant differences in overall CFU counts of all strains, Gram-positive, *Staphylococci*, *Streptococci* and *S. oralis* strains between mucosal and dental surface sites. The overall CFU counts of all strains and Gram-positive strains were higher in dental sites vs. mucosal sites (both $p < 0.001$). CFU counts of S. oralis were significantly higher in dental sites vs. gingival pocket sites ($p = 0.013$). Candida species were rare. The mucosal sites on the buccae presented lower diversity and bacterial counts. Conclusions: In the study group of adult DM I patients treated with IP, the microbiota in particular niches of the oral cavity was significantly different. Three distinct and optimally appropriate sampling sites for oral microflora were identified: buccal and palatal mucosa, dental surface and gingival pockets. The results of this study may be the basis for further studies of large groups of patients with DM I.

Keywords: oral dysbiosis; microbiome; sampling sites; diabetes complications; type 1 diabetes; insulin pump

check for updates

Citation: Gregorczyk-Maga, I.; Fiema, M.; Kania, M.; Jachowicz-Matczak, E.; Romaniszyn, D.; Gerreth, K.; Klupa, T.; Wójkowska-Mach, J. Oral Microbiota—One Habitat or Diverse Niches? A Pilot Study of Sampling and Identification of Oral Bacterial and Fungal Biota in Patients with Type I Diabetes Mellitus Treated with Insulin Pump. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2252. https://doi.org/10.3390/ijerph20032252

Academic Editor: Paul B. Tchounwou

Received: 12 December 2022
Revised: 22 January 2023
Accepted: 25 January 2023
Published: 27 January 2023

1. Introduction

The oral microbiota is one of the largest and most diverse microbial ecosystems, and plays an important role in maintaining human health [1]. The oral microbiota is distinctively different from the ecosystems of the remaining digestive tract sections [2], with exceptional microbiological diversity. Alterations of the oral microbiota balance can result in both

oral (e.g., periodontal diseases, caries) and systemic (e.g., cardiovascular disease, pre-term childbirth) issues [1].

Type 1 diabetes mellitus (DM I) is a chronic condition in which the pancreas produces little or no insulin, leading to hyperglycemia. Continuous subcutaneous insulin infusion with insulin pump (IP) is a modern method of insulin administration, with evidence for its superiority to traditional multiple daily injections in terms of metabolic control, lower risk of hypoglycemic events and better quality of life, especially when used with continuous glucose monitoring systems [3]. Insulin pumps have been available in Poland since the mid 1990s. When good metabolic control is not reached, diabetic complications can occur, including neuropathy, nephropathy and retinopathy, as a result of microangiopathy, as well as macro-angiopathic cardiovascular disease [4]. A bidirectional relationship between oral health and DM I has been suggested as a predisposing factor to oral infections, which, in turn, exacerbates the progression of systemic disease [5]).

Decreased secretion of aberrant saliva (of lower pH and more concentrated) can lead to xerostomia, increased caries risk and higher susceptibility to *Candida* sp. infections. The formation of advanced glycosylation end products (AGEs) and their deposition in tissues leads to microvascular damage and vascular dysfunction due to hyperglycemia. This causes immunological dysregulation, ineffective wound healing, a decrease in the regenerative potential of the mucosa, gingival resorption and periodontal diseases [6–9].

Two large databases, the Human Microbiome Project (HMP) and Human Oral Microbiome Database (HOMD), were developed through recent extensive research. The HMP encompasses microbiome data from the oral and nasal cavities, vagina, gut and skin. Data in the HOMD incorporate oral microbiota composition [10].

The oral cavity is a complex and diverse microbial ecosystem, and has to be divided into various niches colonized by distinct microorganisms [1,11]. These individual habitats can be sampled by swabbing or brushing, testing saliva or oral rinses [12].

Previous reports of oral microbiota in adult DM I are scarce, focusing rather on children or adolescents, or patients with poor oral health, with caries or periodontal disease. DM I patients with worse metabolic control of diabetes present with aberrations in the oral microbiome and its progressive dysbiosis [13,14]. In contrast, DM I patients without complications and with good metabolic control may not show oral pathology [15], but greater abundance of *Streptococcus* spp., *Actinomyces* spp. and *Rothia* spp. compared to otherwise healthy controls was observed [15]. To date, no attempts have been made to characterize the microbiota of individual niches in the oral cavity in DM I, with studies analyzing mainly the saliva or swabs from single sites.

The aim of this pilot study was to characterize the microbiota in particular niches of the oral cavity in adult DM I patients treated with IP. In addition, we aimed to determine optimal sites of oral microbiota sampling in studies of large research groups of patients with DM I.

2. Materials and Methods

2.1. Study Design and Participants

This pilot study consecutively recruited 23 adult patients with DM I treated with IP from the Outpatient Clinic of the Department of Metabolic Diseases and Diabetology of the University Hospital in Krakow, an academic referral center for diabetes in southeastern Poland. Patients who met inclusion criteria were invited to participate in the study. After acquiring written consent, the date of sampling was set, and the patients were instructed on how to prepare for the study procedures. The inclusion criteria were: patient 18–35 years old; DM I diagnosed at least 1 year before recruitment; treatment with IP for at least 6 months; informed consent to participate in the study. The exclusion criteria were: pregnancy or breastfeeding; and comorbidities such as metabolic syndrome, cardiovascular disease, cancer, severe liver failure or kidney failure. The diagnosis of DM I was confirmed based on the Diabetes Poland criteria [16]. Data on age, gender, fasting glucose levels on the day of sampling, glycated hemoglobin (HbA1c%) and DM I treatment were extracted from

medical records. HbA1c% was measured using high-performance liquid chromatography (HPLC) or an enzymatic method within 1 month prior to sampling.

The preparation for collecting microbiological samples from the oral cavity involved refraining from brushing the teeth with triclosan toothpastes and rinsing with chlorhexidine for 48 h preceding the visit. On the day of examination, the patients refrained from brushing their teeth and drinking, eating or smoking for 1 h before microbiological samples were collected. One study subject was excluded from analysis due to failing to comply with the study standards (after sampling, the subject admitted to brushing their teeth and eating breakfast within 1 h preceding the study visit).

2.2. Oral Cavity Sampling Methods

An oral assessment was performed prior to sample collection. The general condition of the oral cavity was assessed using the Oral Health Assessment Tool (OHAT) [17].

Six oral habitats were sampled: buccal (marked A) and soft palate (B) mucosa, the tongue (C), palatal (Da) and buccal (Db) dental surface, and the gingival pocket (E). The niches of each subject were sampled once. Samples were taken by an experienced dentist on an operating chair equipped with an operating light. Specimens from the posterior part of the dorsum of the tongue and soft palate were collected using an ESwab™ [18]. The ESwab combines a COPAN-invented flocked swab with 1 mL of Liquid Amies in a plastic screw cap tube. Dental plaque was collected from the buccal and palatal dental surface sides using a Tooth Cleanic KerrHawe (Dental Supplies—Dental Products | KerrDental.Com, Kloten, Switzerland [19]). After collection, the brush was placed in 1 mL of Liquid Amies in a plastic screw cap tube. A periodontal probe was used to examine the depth of the gingival pocket. Afterwards, two pieces of PerioPaper Strips [20], which are designed to absorb 0–1.2 micro-liters of fluid, were used to collect gingival crevicular fluid (GCF) samples. The strips were placed in the deepest part (1–2 mm) of the gingival pocket for 30–45 s till their surface was soaked. To minimize the risk of pre-analytical errors during sample collection, sterile gauze was used to remove excess saliva from the mucosae and dry the dental surfaces, preventing salivary contamination of the GCF.

2.3. Microbiological Cultures

The collected samples were immediately delivered to the microbiological laboratory. The samples were then inoculated using the dilution method (dilutions -1 to -6) or qualitative culture method (swabs only) on the following media: McConkey (Graso, Biotech Starogard Gdański, Poland [21]), Columbia (Lab-Agar, Biomaxima, Lublin, Poland [22]), Scheadler (Scheadler-Agra, Biomaxima, Lublin, Poland [22]), Bile Esculine Azide (Lab-Agar, Biomaxima, Lublin, Poland), MRS Agar (Oxoid, Brno, Czech Republic [23]) or Sabouraud Agar (Biomaxima, Lublin, Poland [22]). Media were aerobically incubated at 37 °C (McConkey, Columbia, Bile Esculine Azide and Sabouraud) for 24 h, or anaerobically at 37 °C (M.R.S and Scheadler) for 48 h. After incubation, the phenotypically grown colonies were counted and reported, with the results being presented as colony-forming units (CFU) per mL (CFU/mL). After isolation, the microorganisms were identified through MALDI TOF MS mass spectrometry (MALDI Biotyper, Bruker [24]).

2.4. Statistical Analysis

PS Imago Pro ver. 6.0, Statistica ver. 13 and PQStat ver. 1.8.2 were used for all statistical analysis. The normality of the continuous variable distribution was assessed using the Shapiro–Wilk test. Differences between groups were analyzed with Student's *t* test or nonparametric tests (Mann–Whitney U test, Kruskal–Wallis ANOVA), when appropriate. Paired data were analyzed using the Wilcoxon test, Friedman's ANOVA and Skellings–Mack ANOVA, along with appropriate post hoc tests. Continuous variables were presented as arithmetic means ($\overline{x}$) $\pm$ standard deviations (SD) or as the median with interquartile range (IQR) when the data were not normally distributed. The distribution of categorical variables was described as counts and percentages. Statistical testing was

completed to compare categorical variables using an independent sample chi-squared test or Fisher's exact test when appropriate, and dependent samples using McNemar's test and Cochrane's Q ANOVA. Statistical significance was set at $p < 0.05$. The Bonferroni method was used to correct for multiple comparisons.

The analyzed variables were: age, gender, fasting glucose levels on the day of sampling, glycated hemoglobin (HbA1c%), OHAT score, number of genera and species in respective sites and bacterial and fungal CFU counts.

In the first analysis, we compared mean CFU counts between all sites (A–E). Since the total number of CFUs of all strains on the back of the tongue (site C) was the highest and significantly different from the other sites, potentially distorting the results, we decided to exclude it from further analyses. In the second, we performed an analysis of sites A–E with site C excluded. Finally, based on the acquired results and previous studies suggesting treating mucosal (both palatal and buccal) and dental (buccal and lingual) surfaces as two separate habitats [12], we merged the sites A and B, and Da and Db, and compared them with site E (Figure 1).

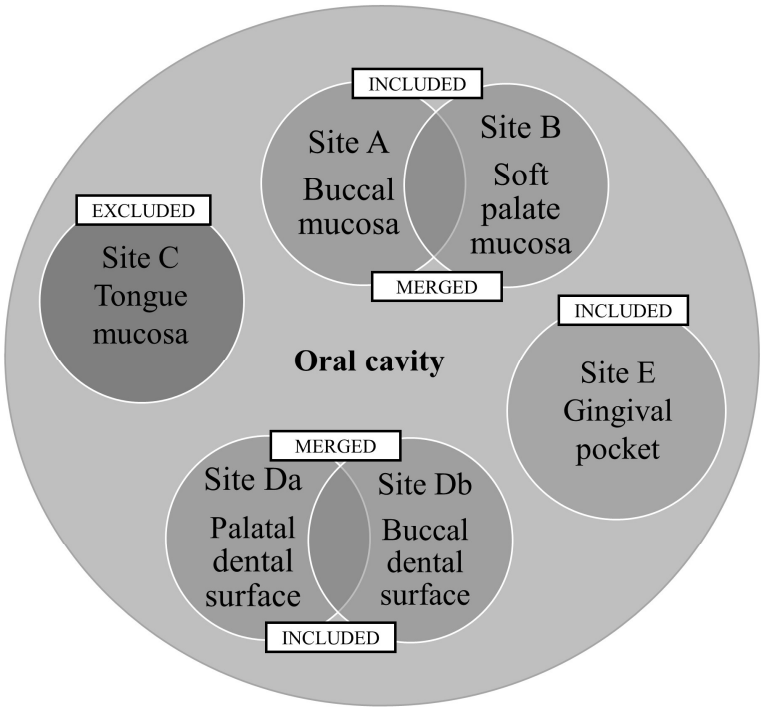


Figure 1. Flowchart of sites selected for analysis.

2.5. Ethics Statement

This study was approved by the Jagiellonian University Bioethics Committee, decision number 1072.61.20.10.2021. Written informed consent was obtained from each subject prior to participation.

3. Results

Data from 22 subjects with DM I treated with a continuous subcutaneous insulin infusion were analyzed. The mean age of the sample was 27.05 ± 5.95 years. The sample was predominantly male (n = 13; 59.1%). The mean Hba1c% was 6.97 ± 0.95%. The mean

fasting glycemia was 116.24 ± 38.29 mg/dL. The condition of the oral cavity according to the OHAT scale in 19 participants was normal (total score 0/16—no change). Three participants had benign changes (total score 1/16, due to dental plaque accumulation). All participants had gingival pocket depths of 1–2 mm, which is considered healthy. None of the participants had a history of any dental procedure in the 6-month period preceding the study procedures. The basic characteristics of the study sample are presented in Table 1.

Table 1. Characteristics of the study population.

Clinical Feature	Available Data, *N* (%)	Mean (SD), Median (Q1–Q3) or Number (%)	*p*-Value
Age	22 (100%)	27.05 (5.95) 26.5 (22–29.25)	-
Gender [male]	22 (100%)	13 (59.1%)	-
Weight [kg]	15 (68.2%)	71.78 (17.75) 66.0 (57–90.0)	-
HbA1c [%] $	22(100%)	6.97 (0.95) 6.85 (6.3–7.35	
Fasting glycemia [mg/dL]	21 (95.5%)	116.24 (38.29) 112.0 (93.0–131.5)	-
OHAT [score]	22 (100%)	0/16 (19, 86.4%) 1/16 (3, 13.6%)	-
Microbial Counts			
Number of genera			
A		2 (1.75–4)	
B		2 (2–3)	
C	22 (100%)	3 (2–4)	0.459
Da		2 (1–3)	
Db		3 (2–3)	
E		2 (2–3)	
Number of species			
A		4 (3–5)	
B		5 (4–6)	
C	22 (100%)	5 (3.75–6.25)	0.459
Da		3 (2.75–6)	
Db		5 (3–6)	
E		4 (3–5)	
Mean CFU [CFU/mL]			
All sites A–E	22 (100%)	$3.88 \times 10^7 \pm 1.88 \times 10^8$ 1.20×10^6 (3.00×10^5 –1.00×10^7)	0.018 #

OHAT—Oral Health Assessment Tool, CFU—colony-forming unit; $—HbA1c measured using high-performance liquid chromatography or enzymatic method; buccal (A) and soft palate (B) mucosa, the tongue (C), palatal (Da) and buccal (Db) dental surface, gingival pocket (E); #—in post hoc analysis, significant difference for comparison between subjects 4 and 9 ($p = 0.045$).

There were no significant differences in the number of microbial genera and species from sites A to E ($p = 0.459$). Except for one significant difference between subjects 4 and 9 (median, IQR: 15×10^7, 7×10^7–93×10^6 vs. 35×10^{14}, 35×10^{13}–275×10^4; $p = 0.045$), the patients did not differ between the sites for the overall CFU counts (Figure 2).

In the first prespecified analysis, the number of genera and species, and CFU counts between all sites for all identified strains and with division into G-positive, G-negative, streptococci species (all identified strains) and staphylococci species (all identified strains) were compared. There were no significant differences in the number of genera and species between the sites. The overall CFU counts of all strains in site C were the highest and significantly differed from the remaining sites ($p < 0.001$ for all comparisons). The detailed results are presented in Table 2. The comparison of the different sites is presented in Figure 3A–F.

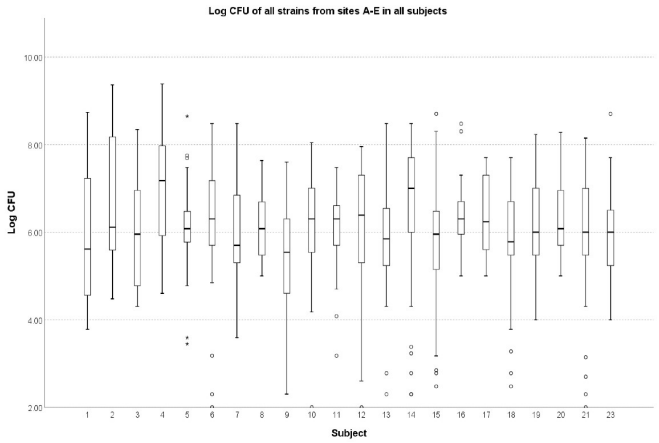


Figure 2. Log CFU of all bacterial and fungal strains from all sites A–E. * extreme values, ° outliers.

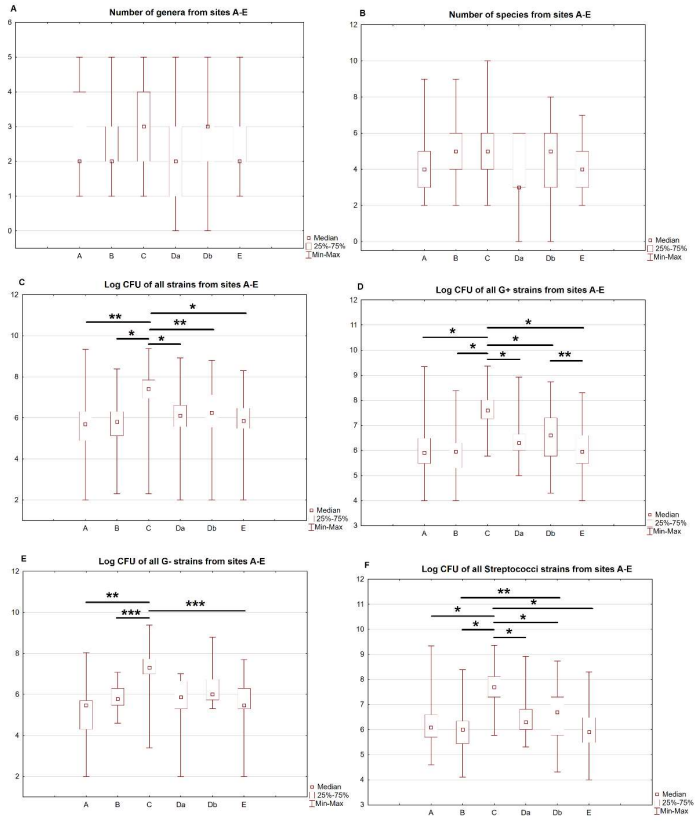


Figure 3. (**A**–**F**) Comparisons of sites A–E. Number of species (**A**) and genera (**B**) from sites A–E. Log CFU of all strains (overall, **C**), G+ (**D**), G− (**E**) and Streptococci strains (**F**) from sites A–E. * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$.

Table 2. Characteristics of sites A–E.

Site	A	B	C	Da	Db	E	*p*-Value(A–E)	*p*-Value(A–E, exl. C)
No. of genera	2 (1.75–4)	2 (2–3)	3 (2–4)	2 (1–3)	3 (2–3)	2 (2–3)	0.299	-
No. of species	4 (3–5)	5 (4–6)	5 (3.75–6.25)	3 (2.75–6)	5 (3–6)	4 (3–5)	0.124	-
				No. of samples [*N*], CFU [CFU/mL]				
Overall &	100 4.34×10^7 (2.40×10^8) 5.00×10^5 $(7.48 \times 10^4–2.00 \times 10^6)$	112 8.25×10^6 (3.56×10^7) 6.50×10^5 $(1.28 \times 10^5–2.00 \times 10^6)$	114 1.05×10^8 (3.17×10^8) 2.50×10^7 $(8.75 \times 10^6–7.00 \times 10^7)$	80 2.08×10^7 (1.09×10^8) 1.30×10^6 $(3.55 \times 10^5–4.00 \times 10^7)$	96 2.57×10^7 (8.93×10^7) 1.75×10^6 $(3.25 \times 10^5–1.43 \times 10^7)$	89 7.17×10^6 (2.43×10^7) 7.00×10^5 $(3.00 \times 10^5–3.00 \times 10^6)$	<0.001 #	0.001 *
Gram-positive &	72 5.85×10^7 (2.82×10^8) 8.00×10^5 $(3.00 \times 10^5–3.00 \times 10^6)$	82 1.09×10^7 (4.13×10^7) 9.00×10^5 $(2.00 \times 10^5–2.00 \times 10^6)$	76 1.08×10^8 (2.76×10^8) 3.90×10^7 $(1.83 \times 10^7–1.06 \times 10^8)$	52 3.12×10^7 (1.34×10^8) 2.00×10^6 $(1.00 \times 10^6–4.75 \times 10^6)$	63 2.73×10^7 (7.77×10^7) 4.00×10^6 $(6.00 \times 10^5–2.00 \times 10^7)$	67 8.20×10^6 (2.73×10^7) 9.00×10^5 $(3.00 \times 10^5–4.00 \times 10^6)$	<0.001 $	0.001 φ
Staphylococci &	4 2.03×10^5 (1.75×10^5) 1.80×10^5 $(5.50 \times 10^4–3.50 \times 10^5)$	5 2.06×10^5 (2.28×10^5) 1.00×10^5 $(2.00 \times 10^4–4.00 \times 10^5)$	7 5.00×10^7 (7.76×10^7) 2.00×10^7 $(1.00 \times 10^6–6.00 \times 10^7)$	2 1.75×10^6 (3.54×10^5) 1.75×10^6 $(1.50 \times 10^6–2.00 \times 10^6)$	4 4.25×10^7 (2.25×10^7) 4.10×10^7 $(2.50 \times 10^7–6.00 \times 10^7)$	6 2.47×10^6 (2.63×10^6) 1.70×10^6 $(4.00 \times 10^5–4.00 \times 10^6)$	0.005 φ	0.03 φ
Streptococci &	61 6.90×10^7 (3.05×10^8) 1.20×10^6 $(5.00 \times 10^5–4.00 \times 10^6)$	67 1.32×10^7 (4.54×10^7) 1.00×10^6 $(2.80 \times 10^5–2.20 \times 10^6)$	58 1.27×10^8 (3.12×10^8) 4.95×10^7 $(2.00 \times 10^7–1.30 \times 10^8)$	44 3.66×10^7 (1.46×10^8) 2.00×10^6 $(1.00 \times 10^6–6.50 \times 10^6)$	51 2.99×10^7 (1.46×10^8) 5.00×10^6 $(6.0 \times 10^5–2.00 \times 10^7)$	51 8.66×10^6 (3.06×10^7) 8.00×10^5 $(3.00 \times 10^5–3.00 \times 10^6)$	<0.001 ψ	0.001 §
Gram-negative &	18 6.64×10^6 (2.58×10^7) 3.00×10^5 $(1.88 \times 10^4–5.50 \times 10^5)$	19 1.62×10^6 (2.87×10^6) 6.00×10^5 $(3.00 \times 10^5–2.00 \times 10^6)$	19 5.01×10^7 (5.31×10^8) 2.00×10^7 $(1.00 \times 10^7–4.00 \times 10^7)$	12 2.59×10^6 (3.42×10^6) 7.50×10^5 $(2.00 \times 10^5–4.75 \times 10^6)$	20 3.71×10^7 (1.40×10^8) 1.00×10^6 $(5.25 \times 10^5–5.75 \times 10^6)$	19 4.55×10^6 (1.17×10^7) 3.00×10^5 $(2.00 \times 10^5–2.00 \times 10^6)$	<0.001%	0.054
Candida spp.	5 9.00×10^2 (8.33×10^2) 5.00×10^2 $(2.00 \times 10^2–1.80 \times 10^3)$	4 4.50×10^2 (2.38×10^2) 4.50×10^2 $(2.25 \times 10^2–6.75 \times 10^2)$	9 1.52×10^3 (1.84×10^3) 1.20×10^3 $(2.00 \times 10^3–1.95 \times 10^3)$	9 1.64×10^5 (4.28×10^5) 4200 $(2.70 \times 10^3–7.50 \times 10^4)$	9 2.86×10^4 (7.2×10^4) 2.80×10^3 $(4.50 \times 10^2–1.38 \times 10^4)$	0	0.22	0.23

Data are presented as mean (SD), median (Q1–Q3) or number. Buccal (A) and soft palate (B) mucosa, the tongue (C), palatal (Da) and buccal (Db) dental surface, gingival pocket (E). &—all identified strains. #—significant difference C vs. A. B. Da. Db. E at $p < 0.001$. $p < 0.001$. $p = 0.001$. $p = 0.026$ and $p < 0.001$, respectively. $—significant difference C vs. A. B. Da. Db. E at $p < 0.001$ in all comparisons and E vs. B. Db at $p = 0.001$ and $p = 0.003$, respectively. %—significant difference C vs. A. B. E at $p = 0.037$; $p = 0.005$ and $p = 0.038$, respectively. ψ—significant difference C vs. A. B. Da. Db. E at $p < 0.001$ in all comparisons and B vs. Db at $p = 0.009$. *—significant difference A vs. Db. $p = 0.011$; B vs. Db. $p = 0.001$. φ—no significant differences in the post hoc analysis. §—significant difference B vs. Db. $p = 0.007$.

In the second step, site C was excluded from the analysis. Then, CFU counts between all remaining sites for all identified strains and with division into G-positive, G-negative, streptococci (all identified strains) and staphylococci strains (all identified strains) were compared. There were significant differences in the overall CFU counts of all strains, Gram-positive, streptococci and staphylococci strains between the sites. Post hoc analysis showed that the only significant differences were limited to comparisons of mucosal sites and dental surfaces. Overall CFU counts of all strains were higher in site Db vs. A ($p = 0.011$) and site Db vs. B ($p = 0.001$), and CFU counts of streptococci were higher in site Db vs. B ($p = 0.007$). These results are presented in Table 2.

Finally, sites A and B were merged into category A + B (mucosal sites), and Da and Db into Da + Db (dental surfaces), and compared with site E. There were significant differences in the overall CFU counts of all strains, Gram-positive, staphylococci, streptococci and *S. oralis* strains between the sites. Post hoc analysis showed significant differences in the merged mucosal and dental surfaces. The overall CFU counts of all strains and Gram-positive strains were higher in sites Da + Db vs. A + B (both $p < 0.001$). CFU counts of *S. oralis* were significantly higher in Da + Db vs. E ($p = 0.013$). There were also some borderline significant results; CFU counts of streptococci ($p = 0.071$), *S. oralis* ($p = 0.065$) and staphyloccoci ($p = 0.083$) strains tended to be higher in Da + Db vs. A + B. Cariogenic *S. mutans* was identified in only three samples from three study subjects, and was found in sites Da (one sample, CFU 3×10^7) and Db (two samples, CFU 6×10^5 and 2×10^6). *Candida* was identified in all sites except for site E. *C. albicans* dominated the samples, with only four *C. dubliniensis* strains being identified. The only significant difference was found in the overall CFU counts of all *Candida* species between the merged sites A + B and Da + Db, with significantly higher counts of *Candida* on the dental surfaces ($p = 0.015$). In one subject, one strain of *Geotrichum* spp. was identified.

The results are presented in Table 3. The comparison of merged categories is presented in Figure 4. The detailed information concerning all identified strains of bacteria and fungi is presented in Supplementary Material Table S1.

Table 3. Characteristics of sites after merging categories A + B and Da + Db.

Site	A + B	Da + Db	E	*p*-Value
	No. of cultures [*N*], CFU counts [CFU/mL]			
Overall #	212 2.48×10^7 (1.67×10^8) 6.00×10^5 (1.20×10^5–2.00×10^6)	176 2.34×10^7 (9.85×10^7) 1.35×10^6 (3.70×10^5–7.00×10^6)	89 7.17×10^6 (2.43×10^7) 7.00×10^5 (3.00×10^5–3.00×10^6	0.006 $
Gram-positive #	154 3.32×10^7 (1.96×10^8) 9.00×10^5 (3.00×10^5–2.00×10^6)	115 2.91×10^7 (1.07×10^8) 2.00×10^6 (8.00×10^5–1.20×10^7)	67 8.20×10^6 (2.73×10^7) 9.00×10^5 (3.00×10^5–4.00×10^6)	0.002%
Gram-negative #	37 4.06×10^6 (1.80×10^7) 5.00×10^5 (1.00×10^5–8.00×10^5)	32 2.42×10^7 (1.11×10^8) 1.00×10^6 (3.20×10^5–5.00×10^6)	19 4.55×10^6 (1.17×10^7) 3.00×10^5 (2.00×10^5–2.00×10^6)	0.083
Veillonella #	9 1.02×10^6 (5.15×10^6) 5.00×10^5 (3.00×10^5–9.00×10^5)	6 8.12×10^6 (1.52×10^6) 1.45×10^6 (6.00×10^5–5.00×10^6)	4 3.38×10^6 (1.57×10^7) 1.15×10^6 (2.50×10^5–6.50×10^6)	0.42
Neisseria #	21 6.63×10^6 (2.38×10^7) 4.00×10^5 (8.00×10^4–2.00×10^6)	15 4.59×10^7 (1.62×10^8) 9.00×10^5 (3.00×10^5–4.80×10^6)	2 2.75×10^7 (3.18×10^7) 2.75×10^7 (5.00×10^6–5.00×10^7)	0.14
Staphylococci #	9 2.04×10^5 (1.94×10^5) 1.00×10^5 (5.00×10^4–4.00×10^6)	6 2.89×10^7 (2.73×10^7) 2.50×10^7 (2.00×10^5–4.00×10^6)	9 2.47×10^6 (2.63×10^6) 1.70×10^6 (4.00×10^5–5.00×10^7)	0.016 &
Actinomyces #	8 7.14×10^5 (8.17×10^5) 4.00×10^5 (1.50×10^5–1.30×10^6)	9 1.57×10^6 (1.11×10^6) 1.00×10^6 (1.00×10^6–2.00×10^6)	3 2.77×10^6 (2.36×10^6) $3.0\,0 \times 10^6$ (3.00×10^5–5.00×10^6)	0.2
Streptococci #	128 3.98×10^7 (2.14×10^8) 1.00×10^6 (3.50×10^5–3.00×10^6)	95 3.30×10^7 (1.17×10^8) 3.00×10^6 (8.00×10^5–1.20×10^7)	51 8.66×10^6 (3.06×10^7) 8.00×10^5 (3.00×10^5–3.00×10^6)	0.034 &
S. vetibularis	20 1.71×10^7 (4.57×10^7) 1.30×10^6 (4.50×10^5–3.80×10^6)	10 1.64×10^7 (4.03×10^7) 2.50×10^6 (8.00×10^5–3.00×10^6)	3 2.97×10^6 (3.52×10^6) 1.40×10^6 (5.00×10^5–7.00×10^6	0.38

Table 3. *Cont.*

Site	A + B	Da + Db	E	*p*-Value
	No. of cultures [*N*], CFU counts [CFU/mL]			
S. salivarius	30 3.43×10^7 (1.57×10^8) 9.50×10^5 (3.00×10^5–2.00×10^6)	10 7.25×10^6 (1.27×10^7) 3.00×10^6 (8.00×10^5–8.00×10^6)	7 8.37×10^5 (1.05×10^6) 8.00×10^5 (4.00×10^4–1.00×10^6)	0.22
S. parapneumonie	18 1.87×10^7 (6.10×10^7) 9.50×10^5 (5.00×10^5–2.20×10^6)	7 5.83×10^6 (5.28×10^6) 5.00×10^6 (1.40×10^6–1.00×10^7)	3 7.10×10^6 (6.85×10^6) 7.00×10^6 (3.00×10^5–$1.4\,0 \times 10^7$)	0.72
S. oralis	26 1.28×10^7 (4.36×10^8) 1.20×10^6 (7.00×10^5–4.00×10^6)	26 1.04×10^8 (1.89×10^8) 9.50×10^6 (1.30×10^6–4.00×10^7)	17 6.93×10^7 (4.83×10^7) 6.00×10^5 (5.00×10^5–1.20×10^6)	<0.001 *
S. mitis	19 1.42×10^7 (5.00×10^7) 1.10×10^6 (2.00×10^5–4.00×10^6)	3 2.87×10^6 (3.60×10^6) 1.20×10^6 (4.00×10^5–7.00×10^6)	9 4.79×10^6 (7.61×10^6) 9.00×10^5 (3.00×10^5–3.00×10^6)	0.47
Candida spp.	9 7.00×10^2 (6.52×10^2) 5.00×10^2 (2.00×10^2–7.00×10^2)	18 9.63×10^4 (3.06×10^5) 4.05×10^3 (1.50×10^3–1.8×10^4)	0	0.015

Data are presented as mean (SD), median (Q1-Q3) or number. Buccal (A) and soft palate (B) mucosa, the tongue (C), palatal (Da) and buccal (Db) dental surface, gingival pocket (E). #—all identified strains; $—significant difference A + B vs. Da + Db. $p = 0.001$; %—significant difference A + B vs. Da + Db. $p = 0.001$; &—no significant differences in the post hoc analysis; *—significant difference Da + Db vs. E. $p = 0.013$.

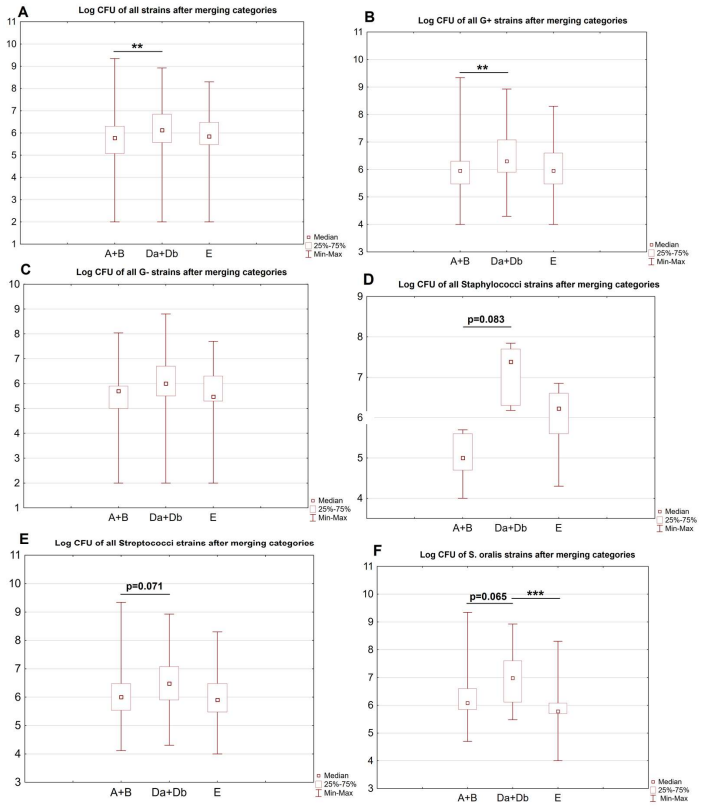


Figure 4. (**A**–**F**) Comparisons of sites A + B, Da + Db and E. Log CFU of all strains (overall, A), G+ (B), G− (**C**), *Staphylococci* (**D**), *Streptococci* (**E**) and *S. oralis* (**F**) strains after merging categories. Selected borderline significant differences are presented. ** $p < 0.01$; *** $p < 0.05$.

4. Discussion

Patients with DM I are at risk of developing numerous oral-related pathologies, such as mucosal disorders, caries, and periodontal disease [6–8,25]. The microvascular damage and vascular dysfunction evident in patients with DM I result in higher susceptibility to oral lesions, impaired wound healing, abnormal bleeding during dental procedures and a higher prevalence of mucosal disorders such as candidiasis [7,26,27]. Most studies to date have investigated patients with DM II, but this sample differs from our patients, who were all diagnosed with DM I. Previous reports of oral microbiota in adults with DM I are scarce, focusing rather on children or adolescents, or patients with poor oral health and those with caries or periodontal diseases [26,28,29]. Our study sample included homogenous, relatively young patients with DM I, without diabetic complications or excess cardiovascular burden. The sample was already described in another study [30], showing exceptional metabolic control. Our study population had good oral health, with no significant signs of periodontal pathology.

In this report, for the first time to the best of our knowledge, we thoroughly characterized the oral bacterio- and mycobiota with traditional culture-based methods, with MALDI-TOF species identification in adult patients with DM I, treated with IP and achieving satisfactory metabolic control. In these patients, the characteristics of the oral microbiota were comparable to previously reported data from otherwise healthy controls. Despite the health and financial burden imposed by DM I, a portion of IP-treated patients with good metabolic control may not present significant abnormalities in oral health and microbiota, confirming our initial hypothesis.

Most studies have mainly analyzed saliva or swabs obtained from single sites, with a paucity of available data regarding the diversity of microbiota in individual niches in the oral cavity of patients with DM I. In this pilot study, we also confirmed our hypothesis that the oral cavity consists of several separate microbiologically distinct habitats. We defined the three most appropriate sites for sampling the oral microbiota for future studies: The buccal and palatal mucosa, dental surfaces and gingival pockets.

In the general population, the oral microflora consists mainly of Firmicutes (*Streptococcus*, *Veillonella*, *Granulicatella*), Proteobacteria (*Neisseria*, *Haemophilus*), Actinobacteria (*Corynebacterium*, *Rothia*, *Actinomyces*), Bacteroidetes (*Prevotella*, Capnocytophaga, *Porphyromonas*) and Fusobacteria (*Fusobacterium*) [3,11,31,32]. In our sample, the oral microbiota was dominated by *Streptococus* and *Neisseria* taxa, with a low incidence of cariogenic *S. mutans* and *Lactobacillus*, as well as periodontal pathogens such as Porphyromonas, Prevotella and *Treponema*.

Our results were similar to reports of previous studies including patients with DM I. In one study of adults with DM I without complications and with good metabolic control (HbA1c < 10%), the participants had a greater abundance of *Streptococcus* spp., *Actinomyces* spp. and *Rothia* spp. than otherwise healthy controls. However, DM I was not associated with oral pathology. Similarly, there were no significant correlations between oral microbiota and glycemic control [15]. Another study, which included children with DM I, reported *Streptococcus* as one of the largest groups of isolated microorganisms [33]. Finally, a recent study of children with DM I that used similar traditional methods of bacteria culture and identification reported significantly higher numbers of bacteria from the *Streptococcus* genus in the group of children with well-controlled DM I than otherwise healthy controls [26]. Another study reported higher abundance of *S. mitis* and lower abundance of *S. salivarius* in DM I individuals, linking this with inter-microbial competition [34]. In contrast, patients with poor glycemic control exhibited worse oral health status with higher frequency of caries and gingivitis [35]. Maintaining metabolic control may partially ameliorate oral microbiota dysbiosis in DM I patients [36]. Considering the plaque microbiota, worse glycemic control was associated with more complexity and richness, with increasing HbA1c levels [13,37].

Candida species were also rare in our samples, dominated by common commensal *C. albicans* strains. Few studies reported the relationships of DM I with mycobiota. Fungi,

including selected *Candida* species, *Aspergillus*, *Fusarium* and *Saccharomyces* species, have been associated with healthy oral microbiota [38]. However, some *Candida* species have been identified as a risk factor for oral pathology, such as periodontal disease [39,40]. In contrast to previous studies, we showed a higher load of *Candida* on the oral mucosa in patients with DM I [9]. Interestingly, one identified strain of *Geotrichum* was previously reported to be used as a cheesemaking culture [41], which confirms, on the one hand, the importance of patient preparation for the examination in such a study and, on the other hand, how variable and dependent on the environment or patient behavior the oral microbiota is.

The data concerning adults with DM I treated with IP are extremely limited, thus making our findings noteworthy. Patients in this study had no history of oral cavity pathology and showed good oral health, which, however, was not the intention of the authors—the good or bad condition of the oral cavity was not a qualification criterion. In addition, the fact that our subjects had exceptional metabolic control resulted in the healthy characteristics of the oral microbiota.

The oral cavity cannot be treated as one large, homogenous microbial ecosystem. It is extremely complex and diverse, and has to be divided into various niches colonized by distinct microorganisms [1,11]. Shedding (mucosae) and non-shedding (dental surfaces) surfaces form two main, compositionally separate communities [12]. Dental sites can be divided into supra- and sub-gingival (gingival pocket, gingival crevicular fluid). Epithelial surfaces are covered with non-keratinized covering mucosa (oral floor, buccae, labiae, soft palate), keratinized masticatory mucosa (gingiva and hard palate) and papillary mucosa (tongue dorsum) [42]. Finally, saliva includes bacteria originating from various niches, bearing some resemblance to the tongue surface [12,42].

In our study, we compared multiple niches and sample types to facilitate the selection of appropriate ones for investigating the oral microbiota of DM I. The dorsal surface (mucosal swab) of the tongue was the richest in microorganisms, and showed shared biodiversity with the remaining oral sites. These findings are in keeping with previous studies that reported a significant abundance of bacteria accumulated from various oral niches [12,43], thus making it is less useful for site-specific research. The mucosal sites on the buccae (mucosal swab) presented lower taxonomical diversity and lower bacterial counts. Some studies reported that mucosal swabs of the buccae and palate, though easily obtainable, show low bacterial diversity and are usually contaminated by microorganisms from other surfaces such as the tongue or teeth [12,44]. Finally, we identified the dental surfaces (by brush) as distinctive to mucosal sites, with higher bacterial counts. Supragingival dental plaque, formed by the biofilm covering the dental surfaces, represents a specific dental surface, and allows distinction between caries lesions and healthy surfaces. However, it requires a trained professional and clinical setting for sampling [12,45,46]. The gingival pockets did not show any significant quantitative differences from the remaining sites. We believe that this may have resulted from the good oral health of our subjects, who had no history of periodontal disease, and the fact that some subgingival-specific strains can be undetected with traditional identification methods [2]. Interestingly, one study showed buccal samples, as compared to subgingival plaque, provided better distinction between patients with periodontitis and otherwise healthy controls [47]. Nevertheless, subgingival plaque is highly relevant to oral health, but requires professionals to perform the procedure [12].

As shown in previous studies, each of these habitats can be sampled with different method and equipment, including swabbing and brushing with dedicated equipment, testing saliva or oral rinses [12]. Each method has its own advantages and disadvantages, such as the possibility of being performed by non-professionals or no requirements for special equipment. However, these advantages come with a cost of imprecision [12]. Currently, the techniques based on next-generation sequencing of the 16 rRNA remain the gold standard for diagnostics in microbiome research. The conventional culture-dependent

techniques can still provide useful information on the bacterial diversity on the subspecies level, and identify non-bacterial organisms, such as *Candida* [2,12].

To sum up, we identified three distinct and most appropriate sites for sampling the oral microbiota in DM I patients: buccal and palatal mucosa, dental surfaces and gingival pockets. This finding may facilitate the choice of adequate methods for similar future studies. Nevertheless, the aim of an individual study and its design should inform the choice of methods and sites used to sample the oral cavity. To investigate gross oral microbiota, especially in larger cohorts, sampling saliva can be appropriate [12], but may fail to discriminate between clinically relevant differences [48]. When researching total oral biodiversity, the optimal approach combines multiple samples from different niches [12]. In studies of site-specific pathologies, such as periodontal diseases or caries, site-specific sampling is recommended [12]. Swabbing or brushing are considered the most reliable methods [12], while saliva and oral rinses, though non-invasive and easy to obtain, are merely a proxy for oral microbiota, not representing any specific niche [49,50].

Our study has some limitations. Since this was a pilot study aimed at the initial assessment of the oral microbiota and the selection of optimal niches for the collection of microbiological samples, it was decided to include only patients with DM I in the first stage and compare them narratively with healthy controls described in previous studies (Verma et al., 2018; Zaura et al., 2009). We used the basic methods of identifying oral microorganisms as a starting point for further, more advanced diagnostic methods. The assessment of the microbiota will be extended with metagenomic analyses. Funding for this study was granted by the Polish Ministry of Science and Higher Education (grant number NdS/545131/2022/202). The subjects were all in relatively good health, with good metabolic control, no diabetic complications and no oral pathologies, so our findings are only applicable to a similar subpopulation of DM I patients. Another limitation was associated with the nature of microbial count data. Since the data were, in most cases, non-normal, paired and unbalanced, they required specific methods of statistical analysis that are usually more conservative than tests for parametric or balanced data. Therefore, some statistical power was lost; however, importantly, the type I error probability was decreased.

5. Conclusions

The oral cavity cannot be treated as one large, homogenous microbial ecosystem. In the study group of adult patients with type 1 diabetes treated with continuous infusion of insulin with insulin pump, the microbiota in particular niches of the oral cavity was significantly different. Three distinct and optimally appropriate sampling sites for oral microbiota have been identified: buccal and palatal mucosa, dental surfaces and gingival pockets. The results of this study may be the basis for further studies of large groups of patients with DM I.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph20032252/s1, Table S1. Microbiome diversity in sites A–E.

Author Contributions: Conceptualization, I.G.-M.; Data curation, M.F.; Formal analysis, M.K., E.J.-M., D.R. and J.W.-M.; Investigation, I.G.-M. and M.F.; Methodology, I.G.-M., M.F., E.J.-M., D.R., T.K. and J.W.-M.; Project administration, I.G.-M.; Resources, I.G.-M., T.K. and J.W.-M.; Supervision, I.G.-M., K.G., T.K. and J.W.-M.; Validation, E.J.-M., D.R. and J.W.-M.; Visualization, M.K.; Writing—original draft, M.F., M.K. and K.G.; Writing—review & editing, I.G.-M., M.F., M.K., T.K. and J.W.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board—Jagiellonian University Bioethics Committee, decision number 1072.61.20.10.2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank all the patients who participated in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Caselli, E.; Fabbri, C.; D'Accolti, M.; Soffritti, I.; Bassi, C.; Mazzacane, S.; Franchi, M. Defining the oral microbiome by whole-genome sequencing and resistome analysis: The complexity of the healthy picture. *BMC Microbiol.* **2020**, *20*, 120. [CrossRef] [PubMed]
2. Dong, L.; Yin, J.; Zhao, J.; Ma, S.-R.; Wang, H.-R.; Wang, M.; Chen, W.; Wei, W.-Q. Microbial Similarity and Preference for Specific Sites in Healthy Oral Cavity and Esophagus. *Front. Microbiol.* **2018**, *9*, 1603. [CrossRef] [PubMed]
3. Pease, A.J.; Lo, C.; Earnest, A.; Kiriakova, V.; Liew, D.; Zoungas, S. The Efficacy of Technology in Type 1 Diabetes: A Systematic Review, Network Meta-analysis, and Narrative Synthesis. *Diabetes Technol. Ther.* **2020**, *22*, 411–421. [CrossRef] [PubMed]
4. A DiMeglio, L.; Evans-Molina, C.; A Oram, R. Type 1 diabetes. *Lancet* **2018**, *391*, 2449–2462. [CrossRef] [PubMed]
5. Zhang, Y.; Wang, X.; Li, H.; Ni, C.; Du, Z.; Yan, F. Human oral microbiota and its modulation for oral health. *Biomed. Pharmacother.* **2018**, *99*, 883–893. [CrossRef]
6. Dicembrini, I.; Serni, L.; Monami, M.; Caliri, M.; Barbato, L.; Cairo, F.; Mannucci, E. Type 1 diabetes and periodontitis: Prevalence and periodontal destruction—A systematic review. *Acta Diabetol.* **2020**, *57*, 1405–1412. [CrossRef]
7. Mauri-Obradors, E.; Estrugo-Devesa, A.; Jane-Salas, E.; Vinas, M.; Lopez-Lopez, J. Oral manifestations of Diabetes Mellitus. A systematic review. *Med. Oral Patol. Oral Cir. Bucal.* **2017**, *22*, e586–e594. [CrossRef]
8. Ferizi, L.; Dragidella, F.; Spahiu, L.; Begzati, A.; Kotori, V. The Influence of Type 1 Diabetes Mellitus on Dental Caries and Salivary Composition. *Int. J. Dent.* **2018**, *2018*, 1–7. [CrossRef]
9. Rodrigues, C.; Rodrigues, M.; Henriques, M. *Candida* sp. Infections in Patients with Diabetes Mellitus. *J. Clin. Med.* **2019**, *8*, 76. [CrossRef]
10. Li, X.; Liu, Y.; Yang, X.; Li, C.; Song, Z. The Oral Microbiota: Community Composition, Influencing Factors, Pathogenesis, and Interventions. *Front. Microbiol.* **2022**, *13*. [CrossRef]
11. Verma, D.; Garg, P.K.; Dubey, A.K. Insights into the human oral microbiome. *Arch. Microbiol.* **2018**, *200*, 525–540. [CrossRef] [PubMed]
12. Zaura, E.; Pappalardo, V.Y.; Buijs, M.J.; Volgenant, C.M.C.; Brandt, B.W. Optimizing the quality of clinical studies on oral microbiome: A practical guide for planning, performing, and reporting. *Periodontology* **2021**, *85*, 210–236. [CrossRef]
13. Jensen, E.D.; Selway, C.A.; Allen, G.; Bednarz, J.; Weyrich, L.S.; Gue, S.; Peña, A.S.; Couper, J. Early markers of periodontal disease and altered oral microbiota are associated with glycemic control in children with type 1 diabetes. *Pediatr. Diabetes* **2021**, *22*, 474–481. [CrossRef] [PubMed]
14. van Heck, J.I.; Gacesa, R.; Stienstra, R.; Fu, J.; Zhernakova, A.; Harmsen, H.J.; Weersma, R.K.; Joosten, L.A.; Tack, C.J. The Gut Microbiome Composition Is Altered in Long-standing Type 1 Diabetes and Associates with Glycemic Control and Disease-Related Complications. *Diabetes Care* **2022**, *45*, 2084–2094. [CrossRef] [PubMed]
15. de Groot, P.F.; Belzer, C.; Aydin, Ö.; Levin, E.; Levels, J.H.; Aalvink, S.; Boot, F.; Holleman, F.; van Raalte, D.H.; Scheithauer, T.P.; et al. Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study. *PLoS ONE* **2017**, *12*, e0188475. [CrossRef] [PubMed]
16. Araszkiewicz, A.; Bandurska-Stankiewicz, E.; Borys, S.; Budzyński, A.; Cyganek, K.; Cypryk, K.; Czech, A.; Czupryniak, L.; Drzewoski, J.; Dzida, G.; et al. 2021 Guidelines on the management of patients with diabetes. A position of Diabetes Poland. *Clin. Diabetol.* **2021**, *10*, 1–113. [CrossRef]
17. Chalmers, J.; King, P.; Spencer, A.; Wright, F.; Carter, K. The Oral Health Assessment Tool—Validity and reliability. *Aust. Dent. J.* **2005**, *50*, 191–199. [CrossRef]
18. Demuyser, T.; De Geyter, D.; Van Dorpe, D.; Vandoorslaer, K.; Wybo, I. Extensive evaluation of fastidious anaerobic bacteria recovery from the Copan eSwab®transport system. *J. Microbiol. Methods* **2018**, *144*, 73–78. [CrossRef]
19. Dental Supplies—Dental Products | KerrDental.com. (n.d.) Available online: https://www.kerrdental.com/ (accessed on 22 January 2023).
20. Guentsch, A.; Kramesberger, M.; Sroka, A.; Pfister, W.; Potempa, J.; Eick, S. Comparison of Gingival Crevicular Fluid Sampling Methods in Patients With Severe Chronic Periodontitis. *J. Periodontol.* **2011**, *82*, 1051–1060. [CrossRef]
21. Produkty—GRASO Biotech. (n.d.) Available online: https://grasobiotech.pl/produkty/ (accessed on 22 January 2023).
22. Biomaxima. (n.d.) Available online: https://biomaxima.com/ (accessed on 22 January 2023).
23. Thermo Fisher Scientific—PL. (n.d.) Available online: https://www.thermofisher.com/pl/en/home.html (accessed on 22 January 2023).
24. Jang, K.-S.; Kim, Y.H. Rapid and robust MALDI-TOF MS techniques for microbial identification: A brief overview of their diverse applications. *J. Microbiol.* **2018**, *56*, 209–216. [CrossRef]
25. Mosler, B.; Twardawa, H.; Trzcionka, A.; Korkosz, R.; Rahnama, M.; Tanasiewicz, M. Oral Cavity Status of Type 1 Diabetic Patients Who Underwent an Oral Hygiene Tuition. *Healthcare* **2022**, *10*, 606. [CrossRef] [PubMed]
26. Pachoński, M.; Koczor-Rozmus, A.; Mocny-Pachońska, K.; Łanowy, P.; Mertas, A.; Jarosz-Chobot, P. Oral microbiota in children with type 1 diabetes mellitus. *Pediatr. Endocrinol. Diabetes Metab.* **2021**, *27*. [CrossRef]

27. Verhulst, M.J.L.; Loos, B.G.; Gerdes, V.E.A.; Teeuw, W.J. Evaluating All Potential Oral Complications of Diabetes Mellitus. *Front. Endocrinol.* **2019**, *10*, 56. [CrossRef]
28. Latti, B.R.; Kalburge, J.V.; Birajdar, S.B.; Latti, R.G. Evaluation of relationship between dental caries, diabetes mellitus and oral microbiota in diabetics. *J. Oral Maxillofac. Pathol.* **2018**, *22*, 282. [CrossRef] [PubMed]
29. Matsha, T.; Prince, Y.; Davids, S.; Chikte, U.; Erasmus, R.; Kengne, A.; Davison, G. Oral Microbiome Signatures in Diabetes Mellitus and Periodontal Disease. *J. Dent. Res.* **2020**, *99*, 658–665. [CrossRef] [PubMed]
30. Surowiec, P.; Matejko, B.; Kopka, M.; Filemonowicz-Skoczek, A.; Klupa, T.; Cyganek, K.; Romanowska-Dixon, B.; Malecki, M.T. Low prevalence of diabetic retinopathy in patients with long-term type 1 diabetes and current good glycemic control - one-center retrospective assessment. *Endocrine* **2021**, *75*, 427–436. [CrossRef]
31. Deo, P.N.; Deshmukh, R. Oral microbiome: Unveiling the fundamentals. *J. Oral Maxillofac. Pathol.* **2019**, *23*, 122–128. [CrossRef]
32. Zaura, E.; Keijser, B.J.F.; Huse, S.M.; Crielaard, W. Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiol.* **2009**, *9*, 259. [CrossRef]
33. Moskovitz, M.; Nassar, M.; Moriel, N.; Cher, A.; Faibis, S.; Ram, D.; Zangen, D.; Yassour, M.; Steinberg, D. Characterization of the Oral Microbiome Among Children with Type 1 Diabetes Compared with Healthy Children. *Front. Microbiol.* **2021**, *12*. [CrossRef]
34. Kunath, B.J.; Hickl, O.; Queirós, P.; Martin-Gallausiaux, C.; Lebrun, L.A.; Halder, R.; Laczny, C.C.; Schmidt, T.S.B.; Hayward, M.R.; Becher, D.; et al. Alterations of oral microbiota and impact on the gut microbiome in type 1 diabetes mellitus revealed by integrated multi-omic analyses. *Microbiome* **2022**, *10*, 243. [CrossRef]
35. Carneiro, V.L.; Fraiz, F.C.; Ferreira, F.D.M.; Pintarelli, T.P.; Oliveira, A.C.B.; Boguszewski, M.C.D.S. The influence of glycemic control on the oral health of children and adolescents with diabetes mellitus type 1. *Arq. Bras. Endocrinol. Metabol.* **2015**, *59*, 535–540. [CrossRef]
36. Yuan, X.; Wu, J.; Chen, R.; Chen, Z.; Su, Z.; Ni, J.; Zhang, M.; Sun, C.; Zhang, F.; Liu, Y.; et al. Characterization of the oral microbiome of children with type 1 diabetes in the acute and chronic phases. *J. Oral Microbiol.* **2022**, *14*. [CrossRef]
37. Mrozinska, S.; Kapusta, P.; Gosiewski, T.; Sroka-Oleksiak, A.; Ludwig-Słomczyńska, A.H.; Matejko, B.; Kiec-Wilk, B.; Bulanda, M.; Malecki, M.T.; Wolkow, P.P.; et al. The Gut Microbiota Profile According to Glycemic Control in Type 1 Diabetes Patients Treated with Personal Insulin Pumps. *Microorganisms* **2021**, *9*, 155. [CrossRef] [PubMed]
38. Ghannoum, M.A.; Jurevic, R.J.; Mukherjee, P.K.; Cui, F.; Sikaroodi, M.; Naqvi, A.; Gillevet, P.M. Characterization of the Oral Fungal Microbiome (Mycobiome) in Healthy Individuals. *PLoS Pathog.* **2010**, *6*, e1000713. [CrossRef]
39. Dehghan, P.; Mohammadi, F.; Javaheri, M.R.; Nekoeian, S. Identification of Candida species in the oral cavity of diabetic patients. *Curr. Med Mycol.* **2016**, *2*, 1–7. [CrossRef]
40. Unniachan, A.S.; Jayakumari, N.K.; Sethuraman, S. Association between Candida species and periodontal disease: A systematic review. *Curr. Med Mycol.* **2020**, *6*, 63–68. [CrossRef]
41. Eliskases-Lechner, F.; Guéguen, M.; Panoff, J. Yeasts and Molds | Geotrichum candidum. *Encycl. Dairy Sci. Second. Ed.* **2011**, *11*, 765–771. [CrossRef]
42. Yamashita, Y.; Takeshita, T. The oral microbiome and human health. *J. Oral Sci.* **2017**, *59*, 201–206. [CrossRef]
43. Marsh, P. In Sickness and in Health—What Does the Oral Microbiome Mean to Us? An Ecological Perspective. *Adv. Dent. Res.* **2018**, *29*, 60–65. [CrossRef]
44. Zhou, Y.; Gao, H.; Mihindukulasuriya, K.A.; La Rosa, P.S.; Wylie, K.M.; Vishnivetskaya, T.; Podar, M.; Warner, B.; Tarr, P.I.; Nelson, D.E.; et al. Biogeography of the ecosystems of the healthy human body. *Genome Biol.* **2013**, *14*, R1. [CrossRef]
45. Hall, M.W.; Singh, N.; Ng, K.F.; Lam, D.K.; Goldberg, M.B.; Tenenbaum, H.C.; Neufeld, J.D.; Beiko, R.G.; Senadheera, D.B. Inter-personal diversity and temporal dynamics of dental, tongue, and salivary microbiota in the healthy oral cavity. *Npj Biofilms Microbiomes* **2017**, *3*, 1–7. [CrossRef] [PubMed]
46. Richards, V.P.; Alvarez, A.J.; Luce, A.R.; Bedenbaugh, M.; Mitchell, M.L.; Burne, R.A.; Nascimento, M.M. Microbiomes of Site-Specific Dental Plaques from Children with Different Caries Status. *Infect. Immun.* **2017**, *85*, e00106-17. [CrossRef] [PubMed]
47. Wei, Y.; Shi, M.; Zhen, M.; Wang, C.; Hu, W.; Nie, Y.; Wu, X. Comparison of Subgingival and Buccal Mucosa Microbiome in Chronic and Aggressive Periodontitis: A Pilot Study. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 53. [CrossRef] [PubMed]
48. Foxman, B.; Luo, T.; Srinivasan, U.; Ramadugu, K.; Wen, A.; Goldberg, D.; Shedden, K.; Crout, R.; McNeil, D.W.; Weyant, R.; et al. The effects of family, dentition, and dental caries on the salivary microbiome. *Ann. Epidemiol.* **2016**, *26*, 348–354. [CrossRef]
49. Faust, K.; Sathirapongsasuti, J.F.; Izard, J.; Segata, N.; Gevers, D.; Raes, J.; Huttenhower, C. Microbial Co-occurrence Relationships in the Human Microbiome. *PLoS Comput. Biol.* **2012**, *8*, e1002606. [CrossRef]
50. Yu, G.; Phillips, S.; Gail, M.H.; Goedert, J.J.; Humphrys, M.; Ravel, J.; Ren, Y.; Caporaso, N.E. Evaluation of Buccal Cell Samples for Studies of Oral Microbiota. *Cancer Epidemiol. Biomark. Prev.* **2017**, *26*, 249–253. [CrossRef]

10. Streszczenie w języku polskim

Wprowadzenie

Mikrobiota jamy ustnej jest drugą pod względem zróżnicowania oraz dynamiki w organiźmie człowieka. Odgrywa istotną rolę w utrzymaniu jego zdrowia [27]. Z jednej strony zaburzenie równowagi mikroflory jamy ustnej (określane jako dysbioza) może odgrywać znaczącą rolę w patogenezie chorób miejscowych i ogólnoustrojowych. Z drugiej strony schorzenia te oddziaływują na mikrobiotę jamy ustnej.

W pracy przedstawiono ocenę mikrobioty jamy ustnej w dwóch odmiennych stanach chorobowych: u pacjentów leczonych w Oddziale Intensywnej Terapii z powodu infekcji SARS-CoV-2 oraz u chorych z cukrzycą typu 1 (DM I) .

Potencjalne interakcje między wirusem SARS-CoV-2 a ludzką mikrobiotą jamy ustnej nie są wystarczająco poznane [15, 16]. Pacjenci z COVID-19 wymagający wentylacji mechanicznej są narażeni na wysokie ryzyko wystąpienia ciężkich powikłań ogólnoustrojowych, w tym zapalenia płuc związanego z respiratorem. To sprawia, że ważne jest utrzymanie i monitorowanie zdrowia jamy ustnej.

Odmienną chorobą, pod względem etiopatogenezy, przebiegu, leczenia, występowania powikłań jest cukrzyca typu 1 (DM I). Ciągły podskórny wlew insuliny (CSII) za pomocą osobistej pompy jest nowoczesną metodą jej podawania, umożliwiającą lepszą kontrolę metaboliczną [24]. Z doniesień wynika, że dwukierunkowy związek między zdrowiem jamy ustnej a DM I jest czynnikiem predysponującym do infekcji jamy ustnej, a to zwrotnie nasila progresję choroby ogólnoustrojowej [25].

Dotychczas nie podjęto prób scharakteryzowania mikroflory poszczególnych nisz w jamie ustnej w DM I.

Cele rozprawy doktorskiej

1. Ocena mikrobioty jamy ustnej w dwóch odmiennych ogólnoustrojowych stanach chorobowych, tj. u pacjentów z COVID-19 hospitalizowanych w OIT oraz u pacjentów z DM I leczonych CSII.
2. Określenie nisz mikrobiologicznych w jamie ustnej, opisanie ich różnorodności w kontekście uzyskania optymalnej identyfikacji bioty bakteryjnej i grzybowej.

Materiały i metody

W pierwszej części badania zrekrutowano 56 dorosłych pacjentów z COVID-19, zakwalifikowanych do wentylacji mechanicznej w tymczasowym OIT z powodu zapalenia płuc. Stan zdrowia jamy ustnej pacjentów oceniano za pomocą zmodyfikowanej skali Beck Oral Assessment Score (BOAS) [30]. Próbki do badania mikrobiologicznego pobrano z czterech nisz jamy ustnej: z błony śluzowej policzka, języka, powierzchni policzkowych zębów i płyn z kieszonki dziąsłowej (gingival cervical fluid- GCF). Materiały biologiczne były poddane klasycznym metodom hodowlanym, w kierunku bakterii beztlenowych, tlenowych oraz grzybów. Mikroorganizmy zidentyfikowano metodą spektrometrii mas MAL-DI TOF MS.

Druga część badania została przeprowadzona u 23 dorosłych pacjentów z DM I w wieku 18–35 lat, leczonych za pomocą osobistej pompy insulinowej. Stan zdrowia jamy ustnej oceniono za pomocą narzędzia Oral Health Assessment Tool (OHAT) [31]. Próbki mikrobiologiczne pobrano z sześciu środowisk jamy ustnej: A- błony śluzowej policzkowej i B podniebienia miękkiego, C- języka, Da- powierzchni podniebiennej zębów i Db-powierzchni policzkowej zębów oraz E- kieszonki dziąsłowej. Materiał z niszy (A,B,C,) został pobrany przy pomocy oryginalnych wymazówek ESwab, z niszy (D) szczoteczkami czyszczącymi KerrHawe, a z niszy (E) papierowymi paskami Perio Paper Strips. Materiał biologiczny został poddany klasycznym metodom hodowli w kierunku bakterii tlenowych, beztlenowych oraz grzybów. Po izolacji, mikroorganizmy były identyfikowane metodą spektrometrii mas MALDI-TOF MS.

Podsumowanie wyników i wnioski

Identyfikacja materiału pobranego u pacjentów z COVID-19 wykazała ilościowe i jakościowe zaburzenia równowagi w mikrobiocie jamy ustnej. Dysbioza w postaci zmniejszenia różnorodności organizmów wraz z występowaniem patologicznych szczepów, jak *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli* i *Klebsiella pneumoniae* stanowiła cenną obserwację wśród tej grupy pacjentów. Oceniany stan jamy ustnej, wskazujący na umiarkowane i ciężkie zaburzenia (BOAS na poziomie 10-14) pozostawał w korelacji ze znaczną dysbiozą. Średnia liczba CFU wszystkich szczepów bakterii w materiale z powierzchni zębów wynosiła 9,3E+5 (1,4E+6), z kieszonek dziąsłowych 7,6E+5 (1,4E+6). Najwyższe liczby CFU zaobserwowano dla *Enterococcus faecalis spp*. i *Lactobacillus spp*.W trakcie realizacji

pierwszej części projektu odnotowano rzadki przypadek inwazyjnej aspergilozy płuc (IPA) wywołany przez *Aspergillus niger* zidentyfikowany w materiale z jamy ustnej pacjenta z COVID-19, wentylowanego mechanicznie. *Aspergillus niger* został wykryty wcześniej w płynie kieszonki dziąsłowej (GCF) niż w popłuczynach oskrzelowych.

Badania przeprowadzone w grupie pacjentów z DM I wykazały, iż byli oni wyrównani metabolicznie (średnia wartość HbA1c% wyniosła 6,97 ± 0,95%). Stan zdrowia jamy ustnej w skali OHAT u badanych był prawidłowy. Wykazano różnorodność mikrobiomu we wszystkich miejscach pobrania materiału i przedstawiono szczegółowe informacje dotyczące wszystkich zidentyfikowanych szczepów bakterii i grzybów.

W rozprawie doktorskiej wykazano dwukierunkowe oddziaływanie między stanem zdrowia jamy ustnej i jej mikrobiotą, a chorobą ogólnoustrojową.

Wyniki przeprowadzonych badań wskazują, że u pacjentów z SARS-CoV-2, hospitalizowanych w OIT zły stan zdrowia jamy ustnej spowodowany niedostateczną jej higieną oraz mechaniczna wentylacja stwarzają drogę pasażu mikroorganizmom do dolnych dróg oddechowych, prowadząc do zapalenia płuc. Zapewnienie właściwej higieny jamy ustnej jest niezbędne w utrzymaniu myko- i mikrobiologicznej równowagi u pacjentów z ciężkim przebiegiem COVID-19. Wynik oceny mikrobioty jamy ustnej może być istotnym uzupełnieniem procesu diagnostycznego będąc predyktorem późniejszego nasilenia zmian zapalnych i rozwoju IPA.

Analiza mikrobioty poszczególnych nisz u pacjentów chorych na cukrzycę typu 1 (DM1), leczonych CSII, z dobrą kontrolą metaboliczną wykazała, że jamy ustnej nie można traktować jako jednego, jednorodnego ekosystemu. Wyodrębniono trzy odmienne i optymalne dla oceny mikrobioty jamy ustnej miejsca. Wyniki przedstawionych analiz mogą być podstawą do dalszych badań w dużych grupach pacjentów z cukrzycą.

11. Streszczenie w języku angielskim

Introduction

The oral microbiota is the second most diverse and dynamic microbial ecosystem in the human body. It plays essential role in maintaining human health [27]. On the one hand, the imbalance of the oral microbiota (referred to as dysbiosis) may play a significant role in the pathogenesis of local and systemic disorders. On the other hand, such disorders can also affect the oral micriobiota.

This work presents the assessment of the oral microbiota in two different disease states - in patients treated in the Intensive Care Unit (ICU) due to SARS-CoV-2 infection and in patients with type 1 diabetes (DM I).

Potential interactions between the SARS-CoV-2 virus and the human oral microbiota are not sufficiently understood [15, 16]. Patients with COVID-19 requiring mechanical ventilation in an intensive care unit (ICU) are at high risk of developing severe systemic complications, including ventilator-associated pneumonia, this maks its important to maintain and monitor oral health.

Type 1 diabetes is a disease with different etiopathogenesis, clinical course, treatment and complications. Continuous subcutaneous insulin infusion (CSII) with personal insulin pump (IP) is a modern method of insulin administration, with evidence for its superiority to traditional multiple daily injections in metabolic control. A bidirectional relationship between oral health and DM I has been reported as a predisposing factor to oral infections, which in turn exacerbates the progression of systemic diseases. To date, no attempts have been made to characterize the microbiota of particular niches in the oral cavity in DM I patients.

Objectives of the work

1. Assessment of the oral microbiota in two different systemic disease states, that is in patients with COVID-19 hospitalized in an intensive care unit and patients with type 1 diabetes treated with CSII.

2. The determination of optimal sites of oral microbiota sampling concerning bacterio- and mycobiota and characterisation of the biodiversity of the identified niches.

Material and methods

In the first part of the study, 56 adult COVID-19 patients qualified for mechanical ventilation in a Temporary ICU due to pneumonia. Patients oral health assessed using a modified Beck Oral Assessment Score (BOAS) [30] . Four oral habitats were sampled: the buccal mucosa, the tongue, buccal dental surface and gingival cervical fluid (GCF). The samples were cultured with the use of traditional methods for aerobic and anaerobic bacteria and fungi. After isolation, the microorganisms were identified by MALDI-TOF MS (Vitek MS Home bioMérieux).

The second part of the study conducted in 23 adult patients with DM I, aged 18-35 years old, treated with IP. The general condition of the oral cavity was assessed using the Oral Health Assessment Tool (OHAT) [31]. Six oral habitats were sampled: buccal (marked A) and soft palate (B) mucosa, the tongue (site marked C), palatal (Da) and buccal (Db) dental surface, and the gingival pocket (E). Material from sites A, B and C was collected using ESwab™, from site D with Tooth Cleanic KerrHawe, and from site E with PerioPaper Strips. The samples were cultured with the use of traditional methods for aerobic and anaerobic bacteria and funghi. After isolation, the microorganisms were identified by MALDI-TOF MS (Vitek MS Home bioMérieux).

Results summary and conclusions

Analysis of material from COVID-19 patients revealed significant qualitative and quantitative dysbiosis in the oral microbiota. The dysbiosis involved decreased species diversity and abundance of pathogenic strains of *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae,* comprising noteworthy findings among this group of patients. Moderate or severe disorders of oral health (BOAS score 10-14) was correlated with the increased level of dysbiosis. The median CFU counts of all bacterial strains in buccal dental surface was 4.0E+5 (1.0E+5 –1.4E+6) and in gingival crevicular fluid 2.0E+5 (4.0E+4 – 8.0E+5). The highest median CFU counts were observed for *Enterococcus* spp. and *Lactobacillus* spp. During the the first part of the study, a very rare case of invasive pulmonary aspergillosis (IPA) caused by *A. niger* from in the oral cavity of a mechanically ventilated COVID-19 patient was identified. *A. niger* was detected in the gingival pocket was diagnosed earlier than in the bronchial lavage fluid..

Analysis of patients with DMI showed that they had good metabolic control of diabetes (mean HbA1c% 6,97 ± 0,95%). The oral health status in this group of patients

was good as indicated by the OHAT scores. The oral microbiota in this group of patients was diverse across all investigated sampling sites and characteristics of each niche with detailed information on all identified bacteria and fungi species were presented.

In this doctoral thesis a bidirectional relationship between the oral health status and oral microbiota with systemic diseases was demonstrated.

The acquired results suggest, that in patients with a severe course of COVID-19 who are hospitalized in an ICU, improper oral hygiene leading to poor oral health status and mechanical ventilation, can facilitate the passage of microorganisms from the oral cavity to lower respiratory tract, leading to pneumonia. Ensuring effective oral hygiene is crucial in sustaining the balance of oral bacterio- and mycobiota in patients with severe COVID-19. The results of oral microbiota analysis can supplement the diagnostic process, being a predictor of the subsequent exacerbation of inflammation and the emergence of IPA.

The analysis of microbiota from distinct oral niches in patients with DM I treated with CSII and with good metabolic control of diabetes showed that the oral cavity cannot be treated as one homogenous microbial ecosystem. Three different and optimal sampling sites for the assessment of the oral microbiota were identified. The results of the presented analysis may be the basis for further studies of large groups of patients with DM I.

Dr hab. n. med. Iwona Gregorczyk Maga
Zakładu Stomatologii Dziecięcej
Instytut Stomatologii, Szpital Uniwersytecki w Krakowie
ul. Montelupich 4 Kraków 31-155

Kraków, dnia 1.02.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- nadzór merytoryczny
- opracowanie i interpretacja wyników badań
- krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Dr hab.n.med. Iwona Gregorczyk-Maga
specjalista stomatologii zachowawczej
z endodoncją 1633221

/podpis współautora/

Dr hab. n. med. Iwona Gregorczyk Maga
Zakładu Stomatologii Dziecięcej
Instytut Stomatologii, Szpital Uniwersytecki w Krakowie
ul. Montelupich 4 Kraków 31-155

Kraków, dnia 1.02.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients, Front. Microbiol., 28 October 2022Sec. Infectious Agents and Disease Volume 13 - 2022 | https://doi.org/10.3389/fmicb.2022.1013559

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- opracowanie i interpretacja wyników badań
- nadzór merytoryczny
- pisanie, edycja oraz krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Dr hab.n.med. Iwona Gregorczyk-Maga
specjalista stomatologii zachowawczej
z endodoncją 1633221

/podpis współautora/

Dr hab. n. med. Iwona Gregorczyk Maga
Zakładu Stomatologii Dziecięcej
Instytut Stomatologii, Szpital Uniwersytecki w Krakowie
ul. Montelupich 4 Kraków 31-155

Kraków, dnia 1.02.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji
- opracowanie i interpretacja wyników badań
- pisanie i opracowanie manuskryptu
- krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, napisaniu i opracowaniu manuskryptu.

Dr hab. n. med. Iwona Gregorczyk-Maga
specjalistka stomatologii zachowawczej
z endodoncją

/podpis współautora/

Lek. Michał Kania
Oddział Kliniczny Chorób Metabolicznych i Diabetologii
Szpital Uniwersytecki w Krakowie
ul. Jakubowskiego 2 Kraków 30-688

Kraków, dnia 1.02.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie i interpretacja wyników badań
- analiza statystyczna danych
- pisanie i opracowanie manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Michał Kania
LEKARZ
3599[illegible]

.....

/podpis współautora/

Lek. Michał Kania
Oddział Kliniczny Chorób Metabolicznych i Diabetologii
Szpital Uniwersytecki w Krakowie
ul. Jakubowskiego 2 Kraków 30-688

Kraków, dnia 1.02.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients, Front. Microbiol., 28 October 2022Sec. Infectious Agents and Disease Volume 13 - 2022 | https://doi.org/10.3389/fmicb.2022.1013559

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie i interpretacja wyników badań
- analiza statystyczna danych
- pisanie i opracowanie manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Michał Kania
LEKARZ
8599314

.....

/podpis współautora/

Kraków, dnia 25.01.2023

Dr. n.med. Jarosław Garlicki
Oddział Kliniczny Anestezjologii
i Intensywnej Terapii
Szpital Uniwersytecki w Krakowie

OŚWIADCZENIE

Jako współautor pracy pt.

Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- rekrutacja pacjenta
- krytycznea ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, napisaniu i opracowaniu manuskryptu.

Dr med. Jarosław Garlicki
lekarz specjalista
anestezjologii i intensywnej terapii
5170604

.....

/podpis współautora/

Kraków, dnia 31 stycznia 2023

prof. dr hab. Jadwiga Wójkowska-Mach

Zakład Kontroli Zakażeń i Mykologii

Katedra Mikrobiologii

Czysta 18, 31-121 Kraków

OŚWIADCZENIE

Jako współautor pracy

Gregorczyk-Maga I, Fiema M, Kania M, Kędzierska J, Jachowicz E, Romaniszyn D, Wójkowska-Mach J. Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients. Front Microbiol. 2022;13:1013559. doi: 10.3389/fmicb.2022.1013559.

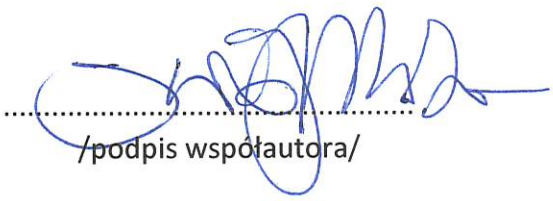
Oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

– opracowanie koncepcji oraz metodologii

– opracowanie i interpretacja wyników badań

– pisanie, edycja oraz krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.



.....

/podpis współautora/

Kraków, dnia 31 stycznia 2023

prof. dr hab. Jadwiga Wójkowska-Mach

Zakład Kontroli Zakażeń i Mykologii

Katedra Mikrobiologii

Czysta 18, 31-121 Kraków

OŚWIADCZENIE

Jako współautor pracy pt.

> *Iwona Gregorczyk-Maga; Mateusz Fiema; Michal Kania; Estera Jachowicz-Matczak; Dorota Romaniszyn; Karolina Gerreth; Tomasz Klupa; Jadwiga Wójkowska-Mach Oral Microbiota—One Habitat or Diverse Niches? A Pilot Study of Sampling and Identification of Oral Bacterial and Fungal Biota in Patients with Type I Diabetes Mellitus Treated with Insulin Pump Int. J. Environ. Res. Public Health 2023;20(3):2252 doi.org/10.3390/ijerph20032252*

Oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

– opracowanie koncepcji oraz metodologii

– opracowanie i interpretacja wyników badań

– pisanie, edycja oraz krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

.....

/podpis współautora/

Kraków, dnia 31 stycznia 2023

prof. dr hab. Jadwiga Wójkowska-Mach

Zakład Kontroli Zakażeń i Mykologii

Katedra Mikrobiologii

Czysta 18, 31-121 Kraków

OŚWIADCZENIE

Jako współautor pracy

> *Fiema M, Wlodarczyk A, Wojkowska-Mach J, Garlicki J, Gregorczyk-Maga I. Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review. Microorganisms. 2022;10(8):1630. doi: 10.3390/microorganisms10081630*

Oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

– opracowanie koncepcji oraz metodologii

– opracowanie i interpretacja wyników badań

– pisanie, edycja oraz krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

.....

/podpis współautora/

Kraków, dnia 31.01.2023

Dr n. med. Estera Jachowicz-Matczak
Zakład Kontroli Zakażeń i Mykologii
Katedra Mikrobiologii
Czysta 18, 31-121 Kraków

OŚWIADCZENIE

Jako współautor pracy pt.

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients, Front. Microbiol., 28 October 2022Sec. Infectious Agents and Disease Volume 13 - 2022 | https://doi.org/10.3389/fmicb.2022.1013559

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- opracowanie i interpretacja wyników badań

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Estera Jachowicz-Matczak
.....
/podpis współautora/

Dr n. med. Estera Jachowicz-Matczak
Zakład Kontroli Zakażeń i Mykologii
Katedra Mikrobiologii
Czysta 18, 31-121 Kraków

Kraków 31.01.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- opracowanie i interpretacja wyników badań

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Estera Jachowicz-Matczak

/podpis współautora/

Kraków, dnia 30.01.2023

Lek. Aleksandra Włodarczyk
Oddział Kliniczny Angiologii
Szpital Uniwersytecki w Krakowie
ul. Jakubowskiego 2 Kraków 30-688

OŚWIADCZENIE

Jako współautor pracy pt.

Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie i interpretacja wyników badań
- napisanie i opracowanie manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, napisaniu i opracowaniu manuskryptu.

Aleksandra Włodarczyk

/podpis współautora/

Kraków, dnia 30.01.2023

Prof. dr hab. n.med. Tomasz Klupa
Oddział Kliniczny Chorób Metabolicznych i Diabetologii
Szpital Uniwersytecki w Krakowie
ul. M. Jakubowskiego 2, 30-688 Kraków

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- nadzór merytoryczny
- krytyczna ewaluacja manuskryptu

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Prof. dr hab. Tomasz Klupa
Specjalista Chorób Wewnętrznych i Diabetologii
305651
Kraków, Do Wilgi 22/44

.....
/podpis współautora/

Prof. dr hab. n. med. Karolina Gerreth
Zakład Stomatologii Grup Ryzyka
Katedry Stomatologii Dziecięcej
Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu
Katedra Stomatologii Dziecięcej
ul. Bukowska 70, 60-812 Poznań

Poznań, 27.01.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- nadzór merytoryczny,
- pisanie, opracowanie oraz krytyczna ewaluacja manuskryptu.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

Kierownik Katedry
Stomatologii Dziecięcej
Prof. dr hab. n. med. Karolina Gerreth

.....

/podpis współautora/

Dr n. med. Dorota Romaniszyn
Zakład Kontroli Zakażeń i Mykologii
Katedra Mikrobiologii
Czysta 18, 31-121 Kraków

Kraków 31.01.2023

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.

Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- opracowanie i interpretacja wyników badań

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

.....

/podpis współautora/

Kraków, dnia 31.01.2023

Dr n. med. Dorota Romaniszyn
Zakład Kontroli Zakażeń i Mykologii
Katedra Mikrobiologii
Czysta 18, 31-121 Kraków

OŚWIADCZENIE

Jako współautor pracy pt.

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients, Front. Microbiol., 28 October 2022Sec. Infectious Agents and Disease Volume 13 - 2022 | https://doi.org/10.3389/fmicb.2022.1013559

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji oraz metodologii
- opracowanie i interpretacja wyników badań

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Mateusza Fiema przy opracowywaniu koncepcji, wykonywaniu części badawczej, opracowaniu i interpretacji wyników badań, napisaniu i opracowaniu manuskryptu.

.....

/podpis współautora/

Kraków, dnia 7.02.2023

Lek. med. Mateusz Fiema
Oddział Kliniczny Endokrynologii,
Endokrynologii Onkologicznej i Medycyny Nuklearnej
Szpital Uniwersytecki w Krakowie
Ul. Jakubowskiego 2, Kraków 30-688

OŚWIADCZENIE

Jako współautor pracy pt.

Atypical Presentation of Aspergillus niger Infection in the Oral Cavity as a Prediction of Invasive Pulmonary Aspergillosis in a Patient with COVID-19: Case Report and Literature Review

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji
- wykonanie części badawczej
- opracowanie i interpretacja wyników badań
- pisanie i opracowanie manuskryptu

.....

/podpis współautora/

Mateusz Fiema
LEKARZ
specjalista chorób wewnętrznych
3245088

Kraków, dnia 7.02.2023

Lek. med. Mateusz Fiema
Oddział Kliniczny Endokrynologii,
Endokrynologii Onkologicznej i Medycyny Nuklearnej
Szpital Uniwersytecki w Krakowie
Ul. Jakubowskiego 2, Kraków 30-688

OŚWIADCZENIE

Jako współautor pracy pt.

Cultivable oral bacteriota dysbiosis in mechanically ventilated COVID-19 patients, Front. Microbiol., 28 October 2022Sec. Infectious Agents and Disease Volume 13 - 2022 | https://doi.org/10.3389/fmicb.2022.1013559

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji
- wykonanie części badawczej
- opracowanie i interpretacja wyników badań
- pisanie i opracowanie manuskryptu

.....

/podpis współautora/

Mateusz Fiema
LEKARZ
specjalista chorób wewnętrznych
3245088

Kraków, dnia 7.02.2023

Lek. med. Mateusz Fiema
Oddział Kliniczny Endokrynologii,
Endokrynologii Onkologicznej i Medycyny Nuklearnej
Szpital Uniwersytecki w Krakowie
Ul. Jakubowskiego 2, Kraków 30-688

OŚWIADCZENIE

Jako współautor pracy pt.

Oral microbiota - one habitat or diverse niches? A pilot study of sampling and identification of oral bacterial and fungal biota in patients with type I diabetes mellitus treated with insulin pump.
Int. J. Environ. Res. Public Health 2023

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

- opracowanie koncepcji
- wykonanie części badawczej
- opracowanie i interpretacja wyników badań
- pisanie i opracowanie manuskryptu

.....

/podpis współautora/

Mateusz Fiema
LEKARZ
specjalista chorób wewnętrznych
3240093