DANILO CÉSAR MOTA MARTINS

ANÁLISE DO PERFIL MICROBIANO E RESPOSTA IMUNE DE ABCESSOS SINTOMÁTICOS DE ORIGEM ENDODÔNTICA EM AMBIENTE HOSPITALAR

BRASÍLIA, 2024

UNIVERSIDADE DE BRASÍLIA
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Orientadora: Profª. Drª. Taia Maria Berto Rezende
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Dedico este trabalho a minha esposa e as minhas filhas.

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RESUMO

Abcessos sintomáticos de origem endodôntica podem progredir e desencadear complicações, como comprometimento das vias aéreas, septicemia e até óbito. Assim, esta tese avaliou as condições sistêmicas e locais de pacientes diagnosticados com abcesso periapical agudo, seguido da avaliação do padrão microbiano. Este foi classificado como estudo observacional transversal, envolvendo pacientes admitidos no serviço de urgência do Instituto Hospital de Base do Distrito Federal (IHBASE), no período de um ano. Foram avaliados 305 indivíduos com diagnóstico inicial de infecção odontogênica, no ano de 2019. Destes, 16% (50 indivíduos) foram diagnosticados com abscessos periapicais sintomáticos e para as análises posteriores divididos em quatro grupos: (G1) pacientes que ingressaram no IHBDF sem uso de antibiótico e sem intervenção local; (G2) pacientes que ingressaram no IHBDF utilizando antibiótico, sem intervenção local; (G3) pacientes que ingressaram no IHBDF utilizando antibiótico, sem intervenção local e necessitaram de internação por tempo menor ou igual a 7 dias; e (G4) pacientes que ingressaram no IHBDF utilizando antibiótico, sem intervenção local e necessitaram internação por tempo maior que 7 dias. Os dados clínicos e séricos (via hemograma) foram coletados por meio dos prontuários eletrônicos do IHBASE. A análise da resposta imune foi realizada por qPCR, para avaliação dos mediadores pró-inflamatórios (IL-1β, IL-6) e antiinflamatório (IL-10). Em sequência, foi realizado sequenciamento genético dos genes 16S V3-V4 e ITS1 em modo *paired end* 2x250 bp (PE250) e a execução da corrida na plataforma *MiSeq Illumina, pepline- RefSeq: NCBI*. Os resultados demonstraram que os pacientes dos grupos G2, G3 e G4 apresentaram maior percentual de sintomas graves e uma progressão significativa na evolução do processo infeccioso, comparado aos pacientes do grupo G1. Foram observados aumentos nas taxas de leucócitos e neutrófilos nos hemogramas dos pacientes dos grupos G3 e G4, em relação ao grupo G1. Não foram observadas diferenças na expressão local dos mediadores pró- e antiinflamatórios entre os grupos amostrais. O sequenciamento do gene 16S revelou um total de 780 gêneros de bactérias identificados, sendo estes principalmente representados por *Prevotella*. O sequenciamento do gene ITS resultou na identificação de 12 gêneros de fungos, liderados por *Talaromyces*. O presente estudo demonstrou a complexidade da evolução e tratamento de urgência dos abcessos periapicais agudos, com etiologia polimicrobiana e progressões clínicas importantes, permitindo reflexões sobre as condutas de urgência dos casos de abcessos periapicais sintomáticos e suas possíveis repercussões.

Palavras chave: Microbioma oral; abcesso periapical sintomático; terapia antibiótica; resposta imune.

ABSTRACT

Symptomatic abscess of endodontic origin can progress and trigger complications such as airway compromise, sepsis, and even death. Thus, this thesis evaluated the systemic and local conditions of patients diagnosed with , followed by the evaluation of the microbial pattern. This was classified as a cross-sectional observational study, involving patients admitted to the emergency service of the Instituto Hospital de Base do Distrito Federal (IHBASE), over one year. A total of 305 individuals with an initial diagnosis of odontogenic infection were evaluated in 2019. Of these, 16% (50 individuals) were diagnosed with symptomatic periapical abscesses and for subsequent analyzes divided into four groups:(G1) patients who entered the IHBDF without antibiotic use and without local intervention; (G2) patients who entered the IHBDF using antibiotics, without local intervention; (G3) patients who entered the IHBDF using antibiotics, without local intervention and required hospitalization for less than or equal to 7 days; and (G4) patients who entered the IHBDF using antibiotics, without local intervention and required hospitalization for more than 7 days. Clinical and serum data (through blood count) were collected from the electronic medical records of IHBASE. Immune response analysis was performed by qPCR to evaluate pro-inflammatories (IL-1β, IL-6) and anti-inflammatory (IL-10) mediators. Subsequently, genetic sequencing of the 16S V3-V4 and ITS1 genes was performed in paired end mode 2x250 bp (PE250) and the run was executed on the MiSeq Illumina platform, pipeline- RefSeq: NCBI. The results showed that patients in groups G2, G3, and G4 had a higher percentage of severe symptoms and significant progression in the evolution of the infectious process compared to patients in group G1. Increases in leukocyte and neutrophil counts were observed in the blood counts of patients in groups G3 and G4 compared to group G1. No differences were observed in the local expression of pro- and anti-inflammatory mediators among the sample groups. Sequencing of the 16S gene revealed a total of 780 bacterial genera identified, mainly represented by *Prevotella*. Sequencing of the ITS gene resulted in the identification of 12 fungal genera, led by *Talaromyces*. This study demonstrated the complexity of the evolution and emergency treatment of acute periapical abscesses, with polymicrobial etiology and important clinical progressions, allowing reflections on the emergency management of acute periapical abscess cases and their possible repercussions.

Keywords: Oral microbiome; symptomatic periapical abcesses; antibiotic therapy; immune response.

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LISTA DE ABREVIATURAS E SIGLAS

BPI - Biotecnologia Pesquisa e Inovação

CAPES - Coordenação de aperfeiçoamento de pessoal e nível superior

cDNA - DNA complementar (*Complementary DNA*)

CNPq – Conselho Nacional de Desenvolvimento científico e tecnológico

DNA - Ácido Desoxirribonucleico (*Deoxyribonucleic Acid*)

FAPDF – Fundação de apoio à pesquisa do Distrito Federal

GAPDH - Gliceraldeído-3-fosfato desidrogenase (*Glyceraldehyde-3-Phosphate Dehydrogenase*)

G1 - Grupo 1

G2 - Grupo 2

G3 - Grupo 3

G4 - Grupo 4

HIV - Vírus da Imunodeficiência Humana (*Human Immunodeficiency Virus*)

IHBDF – Instituto Hospital de Base do Distrito Federal

IL - Interleucina

ND – Não declarado (*Not Declared*)

PCA - Análise de Componentes Principais (*Principal Component Analysis*)

PC1 - *First principal component*

PC2 - *Second principal component*

PCR - Reação em Cadeia da Polimerase (*Polymerase Chain Reaction*)

RNA - Ácido Ribonucleico (*Ribonucleic Acid*)

SisGen - Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado

SUMÁRIO

PREFÁCIO

A motivação para a elaboração desta tese surgiu a partir da observação da quantidade e rápida progressão de pacientes com abcessos sintomáticos de origem endodôntica na emergência do Instituto Hospital de Base do Distrito Federal (IHBASE). Ao longo dos anos, o atendimento a esses pacientes revelou a necessidade de uma investigação mais aprofundada sobre os fatores microbiológicos, imunológicos e os protocolos de tratamento adotados para essa condição clínica.

Esta tese de Doutorado foi estruturada em três capítulos principais, cada um abordando um aspecto crítico da gestão e compreensão dos abscessos periapicais agudos. O primeiro capítulo, *"Urgent Management for Symptomatic Periapical Abscesses: Impact of Systemic Comorbidities"*, discute a gestão do processo de urgência dos abscessos periapicais agudos e como as comorbidades sistêmicas podem influenciar o desfecho clínico. O segundo capítulo, *"Microbial Diversity and Clinical Outcomes in Symptomatic Periapical Abscesses with Associated Systemic Comorbidities"*, explora a diversidade bacteriana presente nos abscessos periapicais e suas implicações nos resultados clínicos, especialmente em pacientes com comorbidades sistêmicas. O terceiro capítulo, *"Next-generation Sequencing Analysis of ITS1 in Symptomatic Periapical Abscesses"*, apresenta a análise detalhada do sequenciamento genético de ITS1 em amostras de pacientes, destacando a complexidade fúngica dos abscessos em perfis de pacientes com diferentes comorbidades sistêmicas.

Os abcessos sintomáticos de origem endodôntica podem progredir rapidamente e causar complicações severas a vida do paciente. Assim, esta tese teve como objetivo avaliar as condições sistêmicas e locais dos pacientes diagnosticados com abscesso periapical agudo, seguido da avaliação do padrão microbiano. Este estudo oferece uma visão abrangente sobre a complexidade dos abscessos periapicais agudos e suas implicações no tratamento de urgência, fornecendo subsídios importantes para a prática clínica e futuras pesquisas na área. Que esta tese seja recebida não apenas como uma contribuição significativa para a literatura científica, mas também como uma ferramenta valiosa para os profissionais de saúde

bucal, fornecendo reflexões sobre o manejo eficaz dos quadros de urgência de abcessos periapicais agudos.

1. INTRODUÇÃO

1.1 Etiopatogenia das infecções de origem odontogênicas

A cavidade oral abriga uma diversidade excepcionalmente alta de microrganismos, tornando-a o sítio com maior variedade microbiana do corpo humano. Análises de microbioma baseadas em DNA revelaram a presença de mais de 700 filotipos bacterianos em amostras orais, evidenciando a complexidade desse ecossistema (1,2). A formação da microbiota oral começa no nascimento do neonato, através da transmissão vertical da mãe para o filho. Este ambiente biológico possui diversos nichos, cada um proporcionando um habitat único para a colonização microbiana, o que faz com que o microbioma oral seja um dos mais bem estudados até hoje. Esses nichos incluem saliva, dentes, sulco gengival, gengiva em anexo, língua, bochecha, lábio, e palato duro e mole (3). Diferentes superfícies na boca são colonizadas, essencialmente, por bactérias orais devido à sua histocompatibilidade com os receptores complementares presentes na superfície oral (4). As diferentes superfícies na boca são colonizadas, essencialmente, pelas bactérias orais devido a sua histocompatibilidade com os receptores complementares na superfície oral (4).

Em continuação, as propriedades imunológicas que conferem estabilidade ao microbioma são importantes para a prevenção da disbiose. Entretanto, quando ocorre o comprometimento dos mecanismos de regulação da microbiota oral, derivados de alterações no pH, a disponibilidade de carboidratos e a tensão de oxigênio no ambiente bucal, ocorre a formação de um biofilme disbiótico, o que dependendo do sítio anatômico envolvido, pode originar doenças periodontais ou cárie (5). Com efeito, essas lesões iniciais são tratáveis de forma pouco invasiva, por intermédio de restaurações e/ou raspagens periodontais. Em contraste, quando essas intervenções não acontecem nos períodos iniciais dessas doenças, elas podem evoluir para quadros de infecção endodôntica e evoluírem para abcessos envolvendo diferentes espaços do complexo bucomaxilofacial (6).

Estudos revelam que a infecção odontogênica é de origem polimicrobiana, organizando-se principalmente na forma de biofilme, intra e extraradicularmente, podendo atingir espaços faciais nobres (4,15). Os estudos científicos basearam-se por muitos anos em técnicas de culturas ou em abordagens moleculares tais como hibridização *in situ* fluorescente (FISH), hibridização DNA-DNA ou PCR e suas

variações (15). Na última década, o desenvolvimento de tecnologias de sequenciamento de nova geração (*next-generation sequencing* – NGS), permitiu um alto rendimento no sequenciamento do DNA, tonando-se ferramenta fundamental no estudo da complexidade da comunidade microbiana (16).

Neste contexto, os abscessos periapicais agudos representam um desafio clínico significativo nas infecções odontogênicas de origem endodôntica, muitas vezes levando a dor intensa, inchaço e potencial envolvimento sistêmico se não forem tratados de forma rápida e eficaz (1). Essas infecções são principalmente de origem bacteriana, normalmente surgindo do tecido pulpar necrótico e estendendo-se à região periapical (1). A natureza polimicrobiana destas infecções pode complicar o seu tratamento. Dessa forma, os avanços na identificação microbiana favorecem o estabelecimento de tratamentos de melhor sucesso para casos emergenciais de abscesso periapical agudo (1).

Estudos recentes de Brito *et al.* (2020), utilizaram o *MiSeq sequencing* (Illumina) para explorar o microbioma da porção apical de infecções endodônticas. As Unidades Taxonômicas Operacionais (OTU) encontradas foram atribuídas a 10 filos bacterianos, liderados por *Bacteroidetes* (51,2%) e *Firmicutes* (27,1%). Na sequência, combinou-se a análise microbiana realizada pelo MiSeq, com o HOMINGS, que utiliza uma rápida e eficiente NGS, com um refinamento na identificação bacteriana no nível de espécie baseado nas comparações do rDNA 16S. No geral, os gêneros mais prevalentes foram *Prevotella* (17,9%) e *Bacteroidaceae G-1* (14,3%). Por sua vez, as mais numerosas espécies/filotipos foram *Bacteroidaceae* [G-1] *sp* ot 272, *P. micra*, *P. oris*, *P. endodontalis*, e *Bacteroidetes* [G-5] *sp* ot 511. Este estudo demonstrou a complexidade do microbioma dos SCR e os “denominadores comuns” das infecções dos canais radiculares, identificando taxa cuja virulência deve ser futuramente explorada, em diversos contextos clínicos.

1.2 Abcesso sintomático de origem endodôntica

À luz das evidências contemporâneas, as doenças inflamatórias perirradiculares podem ser consideradas doenças infecciosas resultantes de infecções endodônticas (13). A progressão da lesão cariosa e o estabelecimento de lesões perirradiculares podem evoluir no ambiente do canal radicular, fornecendo um

habitat seletivo para o desenvolvimento de uma microbiota mista, visivelmente dominada por bactérias anaeróbicas. Essa microbiota pode colaborar para o desenvolvimento de abscessos de origem endodôntica (13). Eles podem ser classificados de acordo com a presença ou ausência de sintomas e evolução temporal da infecção. A correta classificação desses abcessos é fundamental para determinar o tratamento adequado e prevenir complicações (35).

Os abscessos sintomáticos são caracterizados pela presença de dor, que pode variar de leve a intensa, e é frequentemente exacerbada pela mastigação ou pela pressão sobre o dente afetado. Além da dor, pode estar associada a sinais e sintomas como edema, trismo, dislalia e disfagia. Radiograficamente, pode-se observar uma área de radiolucência na região periapical, indicando a presença de uma infecção ativa (35,36).

Por outro lado, os abscessos assintomáticos não causam dor e muitas vezes são descobertos durante exames de rotina ou radiografias. Embora a ausência de dor não signifique ausência de infecção, pode haver um leve inchaço ou uma sensação de pressão sem os sinais clínicos mais evidentes dos abscessos sintomáticos. A radiografia mostra uma radiolucência periapical semelhante, indicando a presença de uma infecção que pode estar em estado latente (36,38).

Os abscessos podem ser classificados ainda como agudos ou crônicos. Os abscessos agudos se desenvolvem rapidamente e são caracterizados por dor intensa e inchaço significativo. A dor pode ser pulsátil e constante, e muitas vezes há sensibilidade ao toque e à mastigação. Além da dor e do inchaço, pode haver febre, mal-estar geral e linfadenopatia (inchaço dos gânglios linfáticos). Em alguns casos, pode haver drenagem de pus através de uma fístula ou no sulco gengival. O tratamento de emergência pode incluir drenagem do abscesso, prescrição de antibióticos e tratamento endodôntico para remover a fonte da infecção (39).

Os abscessos crônicos, por sua vez, se desenvolvem lentamente e podem ser assintomáticos ou apresentar sintomas leves, como uma sensação de pressão ou desconforto intermitente. Frequentemente, há uma fístula através da qual o pus drena, aliviando a pressão e a dor. A área ao redor do dente afetado pode apresentar uma

leve tumefação. Radiograficamente, a radiografia mostra uma área de radiolucência, indicando uma infecção crônica. A infecção pode estar presente há muito tempo sem causar sintomas significativos. O tratamento inclui tratamento endodôntico para remover a infecção da polpa e dos tecidos periapicais. Em alguns casos, pode ser necessário um procedimento cirúrgico adicional, como a apicectomia, para remover o tecido infectado (37,39).

O abcesso apical agudo é a forma mais comum de abcesso dentário sintomático, causado por infecção do canal radicular (7). Nessa perspectiva, a patogênese do abcesso apical agudo é descrita como polimicrobiana. Embora na prática clínica os profissionais tentem encontrar uma única espécie ou pelo menos um grupo das principais espécies que está associado as infecções odontogênicas agudas, estudos demonstraram que não existe um único patógeno, mas um conjunto de espécies sinérgicas, geralmente organizadas em comunidades multiespécies de biofilme, que estão envolvidas e que a causa é heterogênea (8). As espécies comumente presentes nestes casos pertencem aos gêneros *Fusobacterium, Parvimonas, Prevotella, Porphyromonas, Dialister, Streptococcus e Treponema* (7).

Com o advento das técnicas de biologia molecular e ascensão da biotecnologia associada a estudos direcionados a odontologia; as evidências científicas revelaram resultados cada vez melhores em relação a sensibilidade, especificidade e custo benefício das análises microbiológicas associadas à cavidade oral (7,9,10). Porém, diante de todas as espécies microbianas reconhecidas que habitam a cavidade oral humana, 50% permanecem ainda hoje não cultivadas (11).

Embora as bactérias sejam os principais agentes envolvidos nas infecções endodônticas, fungos, arqueas e vírus também são detectados nessas infecções (7,8,11,12). Esses microrganismos, na forma de comunidades complexas de biofilme, criam um ambiente de proteção mútua e tolerância a agentes antimicrobianos. Como relatado por ABUSREWIL *et al.* (2020), a presença de fungos, como *Candida albicans,* em infecções endodônticas polimicrobianas, permitem sua interação com várias taxas bacterianas diferentes.

Dessa maneira, a identificação e caracterização de patógenos orais torna-se algo elementar para melhorar as medidas preventivas e terapêuticas associadas ao abcesso de origem endodôntica (7,13). Em contrapartida, ferramentas e

procedimentos baseados em biologia molecular tornaram-se disponíveis para contornar as limitações da cultura e foram substancialmente melhorados para alcançar uma descrição mais realista das comunidades microbianas de diferentes ambientes sem a necessidade de cultivo (7). Um gene que tem sido amplamente utilizado para a rápida identificação de espécies bacterianas conhecidas e desconhecidas é o que codifica o rRNA 16S. Sendo assim, os avanços nas tecnologias de sequenciamento de DNA melhoraram esse conhecimento e lançaram questões sobre a etiopatogenia de modificadores permanentes ou transitórios da doença (7,14).

1.3 Perfil da resposta imune associada as lesões periapicais

A resposta imunológica periapical consiste em uma reação à infecção bacteriana presente em canais radiculares necróticos com base na expressão de citocinas pró-inflamatórias (13,17). As bactérias e seus produtos metabólicos difundem-se do canal radicular e atingem o ápice dentário estimulando primeiro a resposta imunoinflamatória inata e depois a resposta imune adaptativa, que participa da manutenção e progressão das lesões crônicas periapicais (18). Sendo assim, a resposta imune do hospedeiro que ocorre nessa área é muito complexa e envolve o recrutamento de células inflamatórias, citocinas pró-inflamatórias e imunorreguladoras e quimiocinas (19).

Nesse âmbito, a composição celular das lesões periapicais varia significativamente, dependendo do estágio de desenvolvimento da lesão (14). Sendo assim, em lesões clinicamente sintomáticas, é constatado uma proporção maior de granulócitos, e a menor proporção subsequente de linfócitos/células plasmáticas (12). Com efeito, isso se baseia no fato dos granulócitos, especialmente os neutrófilos, se destacarem na função de fagocitar patógenos e eliminar, de maneira não específica e rápida, manifestando-se com maior expressividade em etapas exsudativas das infecções (19).

Em contraste, em lesões crônicas a polarização do sistema T-*helper* é que comanda a resposta imune periapical (19,20). Os linfócitos T CD4 + são subdivididos em subconjuntos Th1 e Th2 de acordo com as citocinas que produzem. Outras células, como as células Th17 e T reguladoras (Treg), também modulam a resposta

imune periapical. A resposta Th1 é caracterizada pela produção de interferon (IFN)-γ, interleucina IL-2, IL-12 e fator de necrose tumoral (TNF), que estão envolvidos no desenvolvimento e progressão da destruição óssea perirradicular. A resposta Th2 induz a síntese e a atividade das citocinas IL-4, IL-5, IL-6, IL-1β, IL-8 e IL-13, que estão envolvidas na cicatrização e regeneração dos tecidos perirradiculares. O subtipo Th17 produz IL-17, que é uma citocina pró-inflamatória que atua em várias células envolvidas na resposta inata e é considerada uma ponte entre as respostas inata e adaptativa. As células Treg, que produzem o fator transformador de crescimento (TGF)-β e IL-10, têm um efeito inibitório na reabsorção óssea durante a formação e diferenciação dos osteoclastos e regulam a resposta imune contra a infecção (19,20).

Quando a resposta imunológica do hospedeiro não está ocorrendo de maneira adequada, sendo fundamentada na presença de sintomatologia sistêmica, a terapia antibiótica adjuvante a remoção do fator etiológico está indicada no tratamento odontológico de infecções odontogênicas (21). Sendo assim, pacientes imunocomprometidos, como transplantados, diabéticos descompensados, HIV+, podem indicar terapia com antibióticos em conjunto com o tratamento dentário, com a finalidade de controlar uma infecção em progressão e estabilizar os padrões basais celulares do sistema imunológico (21).

1.4 Perfis séricos e a influência das comorbidades sistêmicas na resposta imunológica

A resposta imune é essencial na defesa contra agentes infecciosos e atua como a principal barreira contra a disseminação de infecções. No entanto, recentes estudos têm demonstrado que, pacientes com comorbidades sistêmicas apresentam uma resposta imune insatisfatória. Nestes casos, o curso de uma infecção pode se apresentar de forma mais grave e rápida, em virtude desta resposta insatisfatória do sistema imune. Esses achados ressaltam a importância de compreender melhor as interações entre o sistema imunológico e as comorbidades para o desenvolvimento de estratégias terapêuticas mais eficazes (22).

A presença de comorbidades sistêmicas podem influenciar significativamente o curso e a gravidade de abcessos periapicais agudos. Estudos *in*

vivo de CINTRA et al. (2016) revelam um aumento nos níveis séricos de IL-6, IL-17, IL-23 e TNF-α em ratos com lesões perirradiculares bem estabelecidas, afirmando que infecções endodônticas deflagram uma resposta do sistema imune, podendo levar a repercussões sistêmicas. Portanto, sabe-se que o aumento dos níveis séricos e citocinas inflamatórias podem afetar a homeostase sanguínea, potencializando a patogênese de doenças autoimunes como diabetes, lúpus e artrite reumatoide.

Em muitas dessas situações uma reação de hipersensibilidade com resposta imune exacerbada e não modulada acontece, podendo levar a danos teciduais (22). A gravidade do dano tecidual depende da contagem bacteriana e dos fatores de virulência, bem como da resposta do hospedeiro (23). Nos abscessos, estes podem causar os danos mais significativos aos tecidos em resposta à infecção bacteriana (23).

Os anticorpos, isoladamente, são incapazes de destruir bactérias, entretanto podem neutralizar os microrganismos, impedindo a sua ligação com o tecido do hospedeiro (22). Em associação com o complemento, os anticorpos podem lisar bactérias e funcionar como opsoninas, facilitando a fagocitose (22). Os neutrófilos, eosinófilos e macrófagos exercem sua função microbicida de forma mais ampla contra vários tipos de agentes como bactérias, helmintos e protozoários ou bactérias intracelulares, respectivamente, e são células importantíssimas para a defesa do hospedeiro (22). A resposta mediada pelas células T é extremamente efetiva no mecanismo de defesa contra agentes intracelulares, como vírus, protozoários, fungos e bactérias intracelulares. As células T podem exercer sua função através da citotoxicidade mediada por células CD8+ ou através da secreção de citocinas que vão ativar os macrófagos para destruir os agentes intracelulares (23). A população de células TCD4+ (T *helper*) é heterogênea, sendo constituída de duas subpopulações: Th1 e Th2. É fundamental o entendimento de que tanto a resposta Th1 como a resposta Th2 são importantes na defesa do hospedeiro contra infecções (22). A resposta Th1 está relacionada com a defesa contra protozoários, bactérias intracelulares e vírus, enquanto a resposta Th2 é mais efetiva contra os helmintos e bactérias extracelulares (22). Contudo, essas respostas também são antagônicas, uma vez que, o IFN-γ modula negativamente a resposta Th2, e a IL-4 e a IL-10 modulam negativamente a resposta Th1, o que permite uma hemostasia no sistema

imune e uma resposta imunológica balanceada (22). Além disso, as células regulatórias da resposta imune que expressam as moléculas CD4 e CD25, e produzem IL-10 e/ou fator de crescimento transformador (TGF-β) estão envolvidas em modular a resposta imune, impedindo ou diminuindo as consequências das reações de hipersensibilidade (22,23).

O mecanismo de defesa contra bactérias tem como participantes tanto as barreiras naturais contra agentes infectantes, como a imunidade inata e adaptativa (23). A participação da imunidade inata ocorre através das células fagocitárias, da ativação do sistema complemento pela via alternativa e da produção de quimiocinas e citocinas (22). Embora seja enfatizado principalmente o papel de neutrófilos e monócitos/macrófagos pela capacidade fagocítica dessas células, os basófilos e mastócitos por fatores do sistema complemento, a exemplo do C5a, C3a e C4a, liberam mediadores que, juntamente com as referidas proteínas do complemento, atraem leucócitos para o sítio de agressão e contribuem para a passagem dessas células dos vasos para os tecidos (22). Ademais, enquanto os neutrófilos são encontrados nos tecidos inflamados com uma vida curta, os macrófagos concentram-se tanto em tecidos inflamados como em tecidos sadios com uma sobrevida prolongada (22). Durante a reação inflamatória os neutrófilos produzem secreção purulenta, enquanto os macrófagos formam o granuloma (22). Ademais, a proteína C reativa (PCR), biomarcador característico da fase aguda da inflamação, produzida principalmente por células hepáticas nas infecções bacterianas, exerce a ação variada contra as bactérias (22,23).

A proteína C reativa é mensurável em pequenas quantidades em pacientes saudáveis (24). O estímulo inicial para a produção de PCR é a liberação de interleucina-1 por macrófagos próximos ao tecido afetado. Essa proteína implica no sistema imunológico inato e ativa neutrófilos, erradica antígenos e ativa o sistema complemento (24). A contagem de leucócitos no soro de pacientes saudáveis varia de 4.000 a 11.000 mm (24). Produzindo, transportando e distribuindo anticorpos como parte do sistema imunológico, o número de leucócitos aumenta em resposta aos processos de infecção e inflamação (24). Portanto, a PCR é um indicador mais preciso em relação à contagem de leucócitos e VHS, visto que, têm um rápido aumento e diminuição dos níveis séricos e ao ligar-se aos fosfolipídeos de membrana de algumas

bactérias a PCR atua como opsonina, facilitando a fagocitose por neutrófilos e estimulando a síntese de TNF-α, a qual induz a síntese de óxido nítrico e, consequentemente, a destruição de vários organismos (23,24).

Entre as citocinas que participam da defesa contra bactérias, tem sido dado destaque às citocinas pró-inflamatórias, como o fator de necrose tumoral (TNF-α), IL-1, IL-6 e IL-8 (22). Essas citocinas são produzidas nas fases iniciais da infecção e são responsáveis, por meio de sua ação no hipotálamo, pelo aparecimento de sintomas clínicos sinalizadores de infecção, como: febre, edema local, dispneia, disfagia, dislalia e trismo. A atuação dessas citocinas inibe a multiplicação bacteriana, favorecendo o processo de reparo tecidual (22). Outras citocinas produzidas nas fases iniciais da infecção interferem na resposta imune adaptativa como a IL-12, produzida por macrófagos e tem o papel de diferenciação de células Th0 para Th1, enquanto a IL-4, produzida por basófilos e mastócitos, estimula a diferenciação de células Th0 para Th2, que vão colaborar com os linfócitos B na produção de anticorpos, mais especificamente, da IgE (22).

A imunidade adaptativa, principalmente mediante os anticorpos, desempenha importante papel na defesa contra essas bactérias. Os anticorpos podem exercer suas ações de três maneiras: opsonização, ativando o sistema complemento e promovendo a neutralização de bactérias ou de seus produtos (22). Além disso, também agem como coadjuvantes na destruição de bactérias por complemento, ativando esse sistema pela via clássica (22). Por meio do mecanismo de neutralização, os anticorpos, principalmente a IgA, podem ligar-se as bactérias e, com isso, impedir que as mesmas se fixem nas mucosas, como no trato intestinal e no trato respiratório (22).

Durante a maturação da resposta imune, em infecções odontogênicas, as células apresentadoras de antígeno são responsáveis pela polarização das respostas imunológicas das células T-*helper* (Th) (25). Uma resposta imune do tipo 1, caracterizada pela produção de IFN-γ, TNF-α e IL-1, está envolvida na progressão da doença, destruição óssea e remodelações de lesões periapicais (25). A IL-17A desempenha um papel crítico na defesa e inflamação do hospedeiro e, em contrapartida, os mecanismos imunossupressores mediados por citocinas derivadas de Treg ou Th2 são responsáveis pela cura e restrição dos mecanismos imunes inflamatórios, além disso, o TGF e IL-10, inicialmente descritos como uma citocina

Th2, exibem, ambos, fortes propriedades anti-inflamatórias (25).

Embora a resposta imune em lesões periapicais e doenças periodontais seja bem conhecida, o perfil de resposta de citocinas em infecções odontogênicas graves não foi claramente definido (25). Essas infecções levam a um acúmulo de células competentes que, juntamente com outras células locais, como fibroblastos, células endoteliais e epiteliais, promovem uma resposta protetora contra a propagação microbiana para os tecidos circundantes (25). Desse modo, o conhecimento da célula hospedeira em infecções ativas, incluindo abscessos, é essencial para compreender a patogênese da doença, visto que, a propagação da infecção depende dos fatores locais e sistêmicos dos hospedeiros e da virulência do patógeno (22,25).

1.5 Manejo cirúrgico e medicamentoso de abcessos sintomáticos de origem endodôntica

Os abcessos sintomáticos de origem endodôntica podem evoluir rapidamente de uma infecção localizada para um quadro emergencial. Em vista disto, fatores anatômicos, microbianos e de resistência do hospedeiro, associados a falta de cuidados nos estágios iniciais, podem envolver processo de constituição de uma infecção odontogênica severa (7,26).

O objetivo do tratamento antibacteriano é de controlar (reduzir ou eliminar) a carga bacteriana infecciosa. Para isso, nos casos de abscessos periapicais agudos, a ação terapêutica combina desbridamento mecânico, e/ou cirurgia, e/ou antibioticoterapia sistêmica, quando apropriado (27). O primeiro passo no caso de abscesso dentário é drenar e desbridar o abscesso por meio de técnicas mecânico-cirúrgicas (27). A drenagem do trato sinusial intraoral ou extraoralmente, depende de vários fatores, como a localização do dente envolvido, a posição do ápice do dente para ligações musculares, virulência da bactéria, diminuição da imunidade do hospedeiro e menor resistência fornecida pelas estruturas subjacentes (24). Este procedimento remove exsudato infectado e tem o propósito de aliviar a pressão que foi desenvolvida nos tecidos acometidos pela infecção, aumentando o fluxo vascular, pois caso não seja liberada, essa pressão substancialmente reduz a vascularidade do tecido e previne as substâncias da defesa do hospedeiro de alcançarem as áreas onde são necessárias (28).

A infecção endodôntica crônica que é drenada através de uma comunicação intraoral para a superfície gengival é conhecida como trato do seio intraoral (24). Tratos sinusiais de origem odontogênica que ocorrem extraoralmente apresentam um desafio diagnóstico devido à sua localização distante da fonte subjacente de infecção e ausência de quaisquer sintomas dentários (24). Dessa forma, o exame clínico completo tem uma grande importância na avaliação adequada de todos os tratos sinusais de drenagem crônica da região orofacial, uma vez que, a cronicidade da lesão tem sido, muitas vezes, agravada pelo diagnóstico incorreto, que podem criar efeitos significativos na estética facial devido a tratamentos indesejáveis, como biópsias frequentes e uso de antibióticos que resultam em cicatrizes na pele (24).

Seguindo os princípios gerais do manejo cirúrgico das infecções de origem odontogênica que são a remoção da causa, incisão, drenagem, acesso ao SCR quando possível, antibioticoterapia, controle das vias aéreas e suporte médico/odontológico, assim que o tratamento começar, as decisões de gestão, como a necessidade de internação hospitalar, são com base na gravidade da doença (29). No primeiro passo, a drenagem, é realizada fazendo uma incisão na área de maior flutuação. Se o abscesso for de origem endodôntica, a drenagem pode ser realizada através dos canais radiculares (27,28). Uma terceira alternativa é a extração dentária, que fornece uma via de drenagem e elimina a via de entrada da infecção, mas é indicada apenas na fase aguda, após equilibrar esses benefícios com o risco de disseminação do inóculo bacteriano durante a cirurgia (27,28). Ressalta-se que a indicação de extração do elemento dental, também está atrelada a ausência de possibilidade de reabilitação do mesmo. O desbridamento mecânico elimina tecido necrótico e resíduos bacterianos, e consiste no desbridamento da superfície da raiz, no caso de envolvimento periodontal, ou do canal ósseo no caso de infecção pulpar (27). Técnicas mecânico-cirúrgicas têm efeito quantitativo na carga bacteriana, dando ao hospedeiro a oportunidade de recuperar a homeostase por meio da ação do sistema imunológico (27). No entanto, essas técnicas não modificam a composição do biofilme e a persistência de odontopatógenos que podem levar a recorrência ou a um estado crônico (27). Dessa maneira, técnicas adicionais surgiram para complementar o tratamento mecânico, que incluem o desbridamento químico por meio da aplicação de antissépticos e agentes antimicrobianos (27).

Recomenda-se o uso de irrigação antisséptica concomitantemente ao desbridamento mecânico, mas o uso de substâncias tópicas não é indicado na fase aguda, pois dificultam a drenagem (27). Contudo, o tratamento isolado com antimicrobianos é indicado quando a gravidade da infecção aconselha o adiamento de técnicas cirúrgicas devido ao risco de propagação da infecção durante o próprio desbridamento (27). Em infecções endodônticas, os canais radiculares podem ser preenchidos com substâncias antissépticas por um longo período de tempo, mas tais substâncias têm pouco ou nenhuma ação em nível periapical (3). Portanto, o uso tópico de antimicrobianos deve ser restrito, porque isso favorece o desenvolvimento de resistência e seu efeito clínico é limitado à superfície de aplicação, uma vez que não atuam sobre bactérias invasivas (27).

A relevância dos antimicrobianos no manejo da infecção odontogênica reside em sua utilidade clínica quando administrados sistemicamente (27). A antibioticoterapia sistêmica evita que a infecção se dissemine para órgãos nobres, atua em locais que o tratamento mecânico não pode alcançar, ou seja, sendo uma alternativa terapêutica coadjuvante ao tratamento. É prescrita frequentemente como uma tentativa de gerenciar temporariamente os sintomas angustiantes do paciente, como dor e edema intraoral (27, 30). Antibióticos sistêmicos adjuvantes não são necessários na maioria dos casos de abscessos periapicais localizados e não complicados, entretanto, analgésicos podem ser prescritos para o controle da dor (7). As ocasiões seletivas em que os antibióticos são indicados nos casos de abscessos periapicais agudos incluem: abscessos associados ao envolvimento sistêmico, contendo febre, mal estar e linfadenopatia; disseminação de infecções resultando em celulite, edema difuso progressivo e/ou trismo; e abscessos em pacientes clinicamente comprometidos que estão em maior risco de uma infecção secundária após bacteremia (7). Portanto, em casos complicados, além da drenagem cirúrgica rápida e agressiva para o tratamento, o início da terapia com antibióticos é altamente recomendado.

A seleção de antibióticos na prática clínica é baseada nos resultados dos testes de sensibilidade microbiana (7). A maioria das espécies bacterianas envolvidas com infecções endodônticas, incluindo abscessos, são suscetíveis às penicilinas, isso torna essas drogas a primeira escolha para o tratamento de infecções endodônticas

quando a alergia do paciente à penicilina é descartada (7). Uma vez que o uso de antibióticos é restrito a casos graves e infecções complicadas de abscessos, parece prudente usar amoxicilina, uma penicilina semissintética com espectro mais amplo de atividade antimicrobiana do que a penicilina (7). Além disso, a amoxicilina pode fornecer melhora mais rápida na dor ou inchaço, e a adesão do paciente ao regime prescrito pode ser melhor por causa do intervalo de dosagem mais longo de amoxicilina (7). Em casos ainda mais graves, incluindo condições de risco de vida, a associação de amoxicilina com ácido clavulânico ou metronizadol pode ser necessária para atingir efeitos antimicrobianos ideais como resultado do espectro de ação sendo estendido para incluir cepas resistentes à penicilina (7). A clindamicina tem forte atividade antimicrobiana contra anaeróbios orais e demonstra produzir bons resultado clínicos semelhantes aos da penicilina para o tratamento de abscessos dentais agudos (7). No entanto, uma taxa maior de efeitos adversos gastrointestinais e diarreia foram relatados em associação com o tratamento com clindamicina, deixando este medicamento como uma alternativa eficaz em pacientes alérgicos à penicilina ou quando o tratamento com amoxicilina resulta em falha (7). Uma outra alternativa, ainda, é a moxifloxacina, uma fluoroquinolona que surgiu como uma droga potencial para o tratamento de abscessos, dada sua boa atividade antibacteriana contra bactérias aeróbias e anaeróbias gram positivas e gram negativas isoladas de infecções odontogênicas (7).

A resistência emergente aos antibióticos comumente usados tem sido relatada para bactérias encontradas em abscesso dentais (7). Uma revisão sistemática revelou que os resultados gerais de estudos *in vitro* indicaram que nenhum antibiótico é eficaz contra todas as espécies encontradas em abcessos dentais (7). As espécies Prevotella têm sido consideradas fontes proeminentes de resistência aos agentes beta-lactâmicos na cavidade oral devido à produção de beta-lactamase (7). Outras espécies anaeróbias orais produtoras de enzimas incluem cepas das bactérias gram negativas *F. nucleatum* e *T. fosythia* e as bactérias gram positivas *Actinomyces* e *Peptostreptococcus*, essas bactérias que produzem beta-lactamases não só podem se proteger das penicilinas, mas também podem proteger outras bactérias sensíveis à penicilina presentes em uma comunidade mista (7). A farmacodinâmica antimicrobiana determina o regime de dosagem mais eficaz para atingir a erradicação

bacteriana sem estimular o desenvolvimento de resistência (27). Os antibióticos mais usados na odontologia são dependentes do tempo, o que significa que, para serem eficazes, seus níveis fisiológicos devem exceder a concentração inibitória mínima em pelo menos 40% do tempo entre as doses (27). Levando em consideração a susceptibilidade das bactérias isoladas, mecanismos de resistência e farmacodinâmica dos antibióticos, altas doses de amoxicilina com ácido clavulânico é o tratamento mais adequado para infecções odontogênicas associadas à cárie (pulpite, abscessos) e infecções periodontais quando necessário, e a clindamicina fornece uma escolha alternativa (27). A combinação de diagnóstico precoce, início de antibioticoterapia empírica e intervenção cirúrgica oportuna pode ser considerada como a tríade decisiva para o manejo bem-sucedido de complicações de abscessos dentais agudos (30).

A antibioticoterapia deve ser, fundamentalmente, baseada na microflora complexa e no perfil de sensibilidade da microbiota associada a infecção. No entanto, devido às limitações associadas a identificação de microrganismos patogênicos de forma rápida e precisa, essa terapia é feita, rotineiramente, em base empírica, isto é, sem conhecer os patógenos exatos envolvidos (26,31). Nesse contexto, a terapia pode ou não produzir resultados favoráveis devido a vários fatores, tais como especificidade microbiana e resistência aos fármacos utilizados. Sendo assim, em pacientes com comprometimento imunológico, esse regime pode levar a um agravamento do caso e deterioração das condições de saúde (31).

As penicilinas permanecem eficazes no tratamento antimicrobiano da maioria das infecções orofaciais, tendo sua atividade concentrada contra bactérias gram-positivas, cocos gram-positivos e anaeróbios não produtores de beta lactamase, motivo pelo qual muitos médicos associam o uso das penicilinas ao metronidazol (26). No trabalho de Bhagania et al. (2018), a associação da penicilina G com o metronidazol mostrou uma taxa de falha clinicamente aceitável de 4,7%. Esse acontecimento se justifica na ação complementar do metronidazol sob as bactérias anaeróbias, sendo que este foi usado apenas durante o curso de internação. Isso é baseado no fato de as bactérias anaeróbias serem destruídas por um curso de 5 dias com esses antibióticos (26,32)

De acordo com as evidências científicas, a clindamicina apresenta

características antimicrobianas satisfatórias e pode ser utilizado como alternativa terapêutica para as penicilinas (33). O espectro deste medicamento inclui estreptococos, estafilococos e pneumococos; algumas espécies de bacteroides e outros anaeróbios, gram-positivos e -negativos, também são marginalmente suscetíveis (26). No estudo de Bhagania et al. (2018), a clindamicina, como medicamento único, teve uma eficácia aceitável para o tratamento de infecções odontogênicas com uma taxa de falha de antibióticos de 3,5% (24). Esta taxa de falha da clindamicina foi menor do que a da penicilina e metronidazol, embora a clindamicina tenha um espectro antimicrobiano mais estreito. Uma possível explicação pode ser a maior prevalência de infecções por bactérias resistentes à penicilina. No geral, as taxas de falha para a clindamicina e penicilina/metronidazol foram 3,5% e 4,7%, respectivamente, o que está bem abaixo do valor crítico de 5% (26). Este estudo demonstrou a complexidade do microbioma dos SCR e os “denominadores comuns” das infecções dos canais radiculares, identificando taxa cuja virulência deve ser futuramente explorada, em diversos contextos clínicos.

Em vista disto, este trabalho avaliou as condições sistêmicas e locais de pacientes diagnosticados com abscesso periapical agudo, seguido da avaliação do padrão microbiano. As perspectivas futuras incluem a identificação de novos marcadores microbiológicos e a personalização dos tratamentos endodônticos baseados no perfil microbiológico específico de cada paciente, visando otimizar os desfechos clínicos e reduzir a recorrência das infecções.

2.OBJETIVOS

2.1 Objetivo Geral

Avaliar dados clínicos, resposta imune, perfil microbiano e protocolos de urgência para tratamento de abcessos sintomáticos de origem endodôntica que acessaram o Instituto Hospital de Base de Brasília (IHBDF).

2.2 Objetivos Específicos

- ✓ Coletar de dados clínicos de pacientes com abcesso sintomático de origem endodôntica que acessaram o IHBDF.
- ✓ Coletar dos dados relacionados aos protocolos de atendimento implementados aos pacientes com abcesso sintomático de origem endodôntica que acessaram o IHBDF.
- ✓ Analisar dos dados séricos com base no hemograma de acordo com o tempo de permanência na unidade de saúde.
- ✓ Analisar das classes de antibióticos prescritos e condutas clínicas executadas.
- ✓ Coletar exsudato de abcesso sintomático de origem endodôntica dos pacientes que necessitaram de drenagem de abcesso para determinação do microbioma e expressão de citocinas pró e antiinflamatórias.
- ✓ Analisar da expressão de citocinas pró e antiinflamatórias por qPCR.
- ✓ Analisar do microbioma local por meio do sequenciamento dos genes 16S V3-V4 e ITS1 em modo *paired end* 2x250 bp (PE250).

3.MÉTODOS

3.1 Delineamento Experimental

O presente trabalho consiste em um estudo observacional transversal. Ele apresenta um panorama microbiano local e de resposta imune local e sistêmica, de pacientes com diagnóstico de abcesso sintomático de origem endodôntica que acessaram o pronto socorro odontológico do Instituto Hospital de Base de Brasília (IHBDF), em um período determinado.

3.1.2 Coleta de dados de pacientes com infecção de origem odontogênica

O IHBDF é um hospital público que presta assistência terciária e desta forma recebe um grande número de pacientes na urgência da Odontologia. O ponto de partida deste trabalho foi sua realização sem interferência nos processos operacionais padrões da Odontologia deste hospital. Assim, foram incluídos no estudo, pacientes oriundos de demanda por conveniência que acessaram a triagem do IHBDF e direcionados para a Odontologia, apresentando quadros de infecção de origem odontogênica. Todos os pacientes incluídos neste projeto seguiram com o atendimento, conforme protocolo do hospital e o exsudato coletado foi congelado a -80 °C. Em momento posterior, o prontuário dos pacientes foram acessados e somente os pacientes com diagnóstico de abcesso sintomático de origem endodôntica foram selecionados para as análises.

3.1.2.1 Critérios de inclusão e Exclusão

Foram incluídos os pacientes (>18 anos) que acessaram a Odontologia do IHBDF, durante o período de 12 meses (Janeiro/2019 – Dezembro/2019), com diagnóstico de abcesso periapical sintomático e/ou agudo. Durante este período, foram admitidos 305 pacientes com diagnóstico de infecção odontogênica no pronto socorro. Durante acolhimento destes pacientes, em virtude do diagnóstico e dos protocolos operacionais do hospital, foi realizada drenagem de coleção purulenta de 63 pacientes, dos quais 50 pacientes foram incluídos no estudo, visto que

apresentavam diagnóstico de abcesso sintomático de origem endodôntica, além de apresentarem evolução clínica no sistema eletrônico de registro de dados e exames hematológicos ao longo do período de atendimento. Os demais pacientes (13 pacientes) que não se encontravam dentro dos critérios de inclusão, foram excluídos, uma vez que apresentavam amostras com características biológicas diferentes do objeto de estudo, dados de prontuário incompletos ou não apresentavam exames hematológicos na admissão. Os pacientes incluídos no estudo foram divididos em quatro grupos, os quais abordaram condições clínicas e condutas terapêuticas distintas (Figura 1):

- Grupo 1 (n = 8 pacientes) – Pacientes com diagnóstico de abcesso periapical sintomático que acessaram a unidade de saúde para atendimento clínico e sem o uso prévio de antibióticos e não necessitaram de internação após atendimento.

- Grupo 2 (n = 20 pacientes) – Pacientes com diagnóstico de abcesso periapical sintomático que acessaram a unidade de saúde para atendimento clínico, já em uso de antibióticos e não necessitaram de internação após atendimento.

- Grupo 3 (n = 11 pacientes) – Pacientes com diagnóstico de abcesso periapical sintomático que acessaram a unidade de saúde para atendimento clínico, já em uso de antibióticos e que necessitaram de internação por até 7 dias para tratamento da infecção.

- Grupo 4 (n = 11 pacientes) – Pacientes com diagnóstico de abcesso periapical sintomático que acessaram a unidade de saúde para atendimento clínico, já em uso de antibióticos e que necessitaram de internação por períodos maiores que 7 dias para tratamento da infecção.

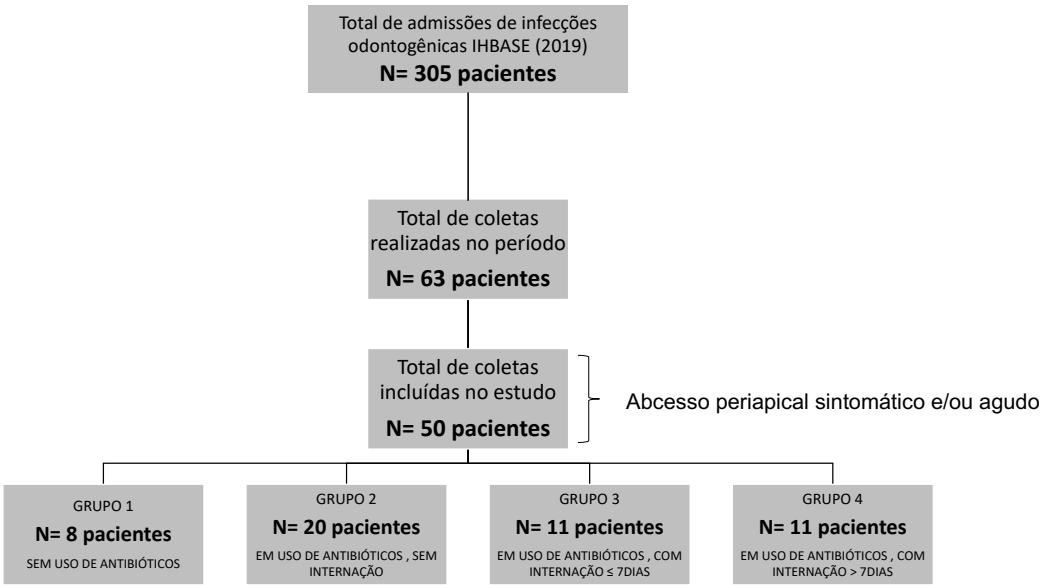


Figura 1: Fluxograma representando da distribuição amostral e condutas necessárias após admissão dos pacientes na unidade de saúde. Grupo 1: Pacientes que acessaram a unidade de saúde sem o uso prévio de antibióticos e não necessitaram de internação. Grupo 2: Pacientes acessaram a unidade de saúde com o uso prévio de antibióticos, deste grupo, pacientes não necessitaram de internação. Grupo 3: Pacientes acessaram a unidade de saúde com o uso prévio de antibióticos, deste grupo, pacientes necessitaram de internação até 7 dias. Grupo 4: Pacientes acessaram a unidade de saúde com o uso prévio de antibióticos, deste grupo, pacientes necessitaram de internação em tempo superior à 7 dias.

Foram acessados os dados clínicos, a história médica-odontológica pregressa e atual, o hemograma e as condutas clínicas implementadas de todos os pacientes incluídos nestes grupos. O exsudato destes pacientes também foi coletado após drenagem para avaliação do microbioma e de parâmetros de resposta imune local.

3.1.2.2 Considerações éticas:

Apenas os pacientes que necessitaram da realização do procedimento de drenagem e aceitaram assinar o Termo de Consentimento Livre e Esclarecido foram

submetidos a coleta de exsudato. Todos os dados dos pacientes foram mantidos em sigilo conforme termos próprios. Este projeto foi aprovado pelo comitê de ética em pesquisa humana da UCB (CAAE: 57475816.8.0000.0029) (Anexo 1), comitê de pesquisa do Instituto Hospital de Base do DF (CAAE: 57475816.8.3001.5553) (Anexo 2) e registro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen: AE5000A) (Anexo 3).

3.1.3 Coleta de dados de protocolos de atendimento

Os dados pessoais, clínicos, séricos e de tratamento dos pacientes foram registrados durante atendimento dos pacientes, nos prontuários eletrônicos do SUS e posteriormente acessados, assim como as evoluções médico-odontológicas dos pacientes que necessitaram internação.

Todos os exames complementares foram realizados com base nos protocolos de atendimento do IHBASE. Desta forma, todos os hemogramas realizados durante o período de internação dos pacientes foram acessados. A contagem das células presentes nos hemogramas (incluindo leucócitos, eosinófilos, monócitos, basófilos, neutrófilos e linfócitos) foram utilizadas para análise de homeostase sérica, com base nos resultados dos exames desde a admissão até a alta hospitalar durante o tempo de internação dos pacientes.

Todo o protocolo terapêutico no hospital também foi coletado dos prontuários dos pacientes e estas informações foram comparadas aos dados séricos dos hemogramas dos pacientes internados no IHBASE com base na planilha de coleta de dados (ANEXO 4), e ao tempo para se atingir a homeostase sérica. Os antibióticos registrados como proposta terapêutica foram coletados e separados de acordo com a classe pertencente (Penicilinas, Carbapenêmicos, Cefalosporinas, Aminoglicosídeos, Macrolídeos, Lincosamidas e Nitromidazólicos), correlacionando-os com o quantitativo de dias de internação de cada paciente.

3.1.4 Coleta das amostras de exsudato dos pacientes

Dos pacientes que necessitaram da realização do procedimento de drenagem, o exsudato purulento foi coletado para análise da microbiota e produção de citocinas

pro e anti-inflamatória. Para tanto, inicialmente foi realizada assepsia da região acometida com uso tópico de diguclonato de clorexidina 2%, seguido de anestesia local, incisão com lâmina de bisturi e punção extraoral/intraoral da região, com agulha (40 x 12mm) e seringa de 50 mL para aspiração de exsudato das lojas faciais acometidas. O exsudato foi coletado por aspiração e imediatamente, levado ao laboratório do Programa de Pós-graduação em Ciências Genômicas e Biotecnologia, da Universidade Católica de Brasília. No laboratório, a amostra foi separada em pequenas alíquotas e estocado a temperatura de -80 °C (34).

3.1.5 Análise do perfil imunológico local do exsudato

Para análise do perfil imunológico, as concentrações dos mediadores (IL-1β, IL-6 e IL-10 foram determinadas pelo método de PCR em tempo real com *StepOnePlus™ Real-Time PCR System* (*Applied Biosystems, ThermoFisher Scientific,* Califórnia, EUA).

Método TRIzol™

Ao exsudato (100 µL) foram adicionados 1000 µL de TRIzol™ (*ThermoFisher Scientific*, Califórnia, EUA) (39). As amostras foram homogenizadas, mantidas a temperatura ambiente por 5 minutos e centrifugadas a 2.100 rpm, por 5 minutos. O sobrenadante foi transferido para um novo tubo, ao qual foram adicionados 200 µL de clorofórmio (*ThermoFisher Scientific*). As amostras foram homogenizadas e mantidas a temperatura ambiente, por 10 min, sendo em seguida centrifugadas a 4 °C, a 8.000 rpm, por 20 min. Após a centrifugação, a fase aquosa foi transferida para um novo tubo de 1,5 mL, ao qual foi adicionado 500 µL de isopropanol (*ThermoFisher Scientific*). A mistura foi homogeneizada com auxílio de aparelho vortex e incubada no gelo, por 10 minutos. Após a incubação, as amostras foram centrifugadas a 12.000 rpm por 10 min e o sobrenadante foi descartado. Então, 1 mL de etanol 70% gelado foi adicionado ao *pellet*, e o tubo foi centrifugado a 12.000 rpm, por 5 min. O sobrenadante foi novamente descartado; o *pellet* de RNA mantido a temperatura ambiente até secar e por fim, ressuspenso em 30 µL de água *RNAse free*

(*ThermoFisher Scientific*).

Quantificação de RNA

A fim de determinar o volume de RNA de cada amostra a ser utilizado na síntese de cDNA, o RNA foi quantificado no equipamento Qubit® (Invitrogen™ ThermoFisher Scientific, Califórnia, EUA) com o *Qubit® RNA Buffer* (Invitrogen™ ThermoFisher Scientific) e *Qubit® RNA Reagent* (Invitrogen™ ThermoFisher Scientific) (40). Para cada quantificação, 1 µL do fluorófilo foi adicionado e homogeneizado à 199 µL do tampão. Deste volume, 199 µL foram adicionados e homogeneizados à 1 µL da amostra, sendo estes incubados no escuro, em temperatura ambiente, por dois minutos antes da leitura no equipamento. Após a quantificação, o aparelho permite calcular a concentração final de RNA, em $\mu g.mL^{-1}$, a partir do volume inicial de amostra utilizado (1 µL).

Síntese de cDNA

O DNA complementar (cDNA) foi sintetizado a partir de 200 ng-300 ng de RNA, em um volume de 10 µL (completado com água *RNAse free*-Invitrogen™ ThermoFisher Scientific, se necessário). Ao RNA, foi adicionado 10 µL do mix preparado com o *High Capacity cDNA Reverse Transcription Kit®* (*Applied Biosystems/*ThermoFisher Scientific). Com este kit, para cada amostra a ser sintetizada em cDNA, utiliza-se: 3,2 µL de água *RNAse free* (Invitrogen™ ThermoFisher Scientific); 2 µL do *Buffer* RT 10X (Invitrogen™ ThermoFisher Scientific); 0,8 µL do mix de dNTP 25X (Invitrogen™ ThermoFisher Scientific); 2 µL de *Random Primers* RT 10X (Invitrogen™ ThermoFisher Scientific); 1 µL do Inibidor de RNAse 20 U/µL (Invitrogen™ ThermoFisher Scientific); e 1 µL de *MultiScribe™ Reverse Transcriptase* (Invitrogen™ ThermoFisher Scientific).

As amostras, em volume final de 20 µL, foram submetidas ao seguinte ciclo de temperatura em um termociclador: 10 min a 25 °C; 120 min a 37 °C; 5 min a 85 °C e 4 °C até que as amostras fossem retiradas do termociclador e armazenadas em freezer a -20 °C (41).

PCR em tempo real

A análise de expressão gênica foi realizada por PCR em tempo real com *StepOnePlus™ Real-Time PCR System* (*Applied Biosystems, ThermoFisher Scientific*), a fim de verificar a expressão de genes indicativos de processos inflamatórios e antiinflamatórios. Para essa análise serão avaliados os genes para expressão de *IL-1β, IL-6, IL-10* e GAPDH, como gene constitutivo, de acordo com os seus respectivos pares sequenciais (Tabela 1). A reação de quantificação foi preparada de acordo com as recomendações do fabricante. Cada reação apresentou um volume final de 10 µL, sendo composto por: 0,5 µL de *TaqMan® Small RNA Assay* (20X); 0,66 µL de cDNA; 5 µL de *TaqMan® Universal PCR Master Mix II* (2x) e 3,83 µL de Água *Nuclease free* com a sonda TaqMan® (*Invitrogen™ ThermoFisher Scientific - KIT q-PCR Assay).* No final desses ciclos, os resultados foram analisados para determinar diferença na expressão de miRNA nas amostras testadas (41).

Na sequência, foi produzido o DNA complementar (cDNA) sintetizado a partir de 200 ng-300 ng de DNA, em um volume de 10 µL (completado com água *RNAse free*-InvitrogenTMThermoFisher Scientific, se necessário). Ao DNA foi adicionado 10 µL do mix preparado como *High Capacity cDNA Reverse Transcription Kit®* (*Applied Biosystems/*ThermoFisher Scientific). Com este kit, para cada amostra a ser sintetizada em cDNA, utiliza-se: 3,2 µL de água *RNAse free* (InvitrogenTM ThermoFisher Scientific); 2 µL do *Buffer* RT 10X (InvitrogenTM ThermoFisher Scientific); 0,8 µL do mix de dNTP 25X (InvitrogenTM ThermoFisher Scientific); 2 µL de *Random Primers* RT 10X (InvitrogenTM ThermoFisher Scientific); 1 µL do Inibidor de RNAse 20 U/µL (InvitrogenTM ThermoFisher Scientific); e 1µL de *MultiScribeTM Reverse Transcriptase* (InvitrogenTM ThermoFisher Scientific). As amostras, em volume final de 20 µL, foram submetidas ao seguinte ciclo de temperatura em um termociclador: 10 min a 25 °C; 120 min a 37 °C; 5 min a 85 °C e 4 °C até que as amostras sejam retiradas do termociclador e armazenadas em freezer a -20 °C (41).

Os fragmentos dos genes foram amplificados via reação em cadeia da polimerase (PCR) mediadores foram determinados pelo método de PCR em tempo real com *StepOnePlusTM Real- Time PCR System* (*Applied Biosystems, ThermoFisher Scientific,* Califórnia, EUA).

GENE	SEQUÊNCIA DO PRIMER (5'-3')
Análise do perfil imunológico	
***GAPDH* (gene constitutivo)**	F: GCA CCA CCA ACT GCT TAG CA R: TGG CAG TGA TGG CAT GGA GGA
IL-6	F: ACA GCC ACT CAC CTC TTC AG R: CCA TCT TTT TCA GCC ATC TTT
IL-1β	F: TGG CAG AAA GGG AAC AGA A R: ACA ACA GGA AAG TCC AGG CTA
IL-10	F: GGT TGC CAA GCC TTG TCT GA R: TCC CCC AGG GAG TTC ACA T

Tabela 1: Genes e sequência de *primers* alvos utilizados no estudo.

3.1.6 Extração do DNA para sequenciamento do microbioma

Foi realizado extração de DNA utilizando o kit de extração QIAamp® DNA Mini Kit (QIAGEN, Hilden, Alemanha), para amplificação e sequenciamento dos genes ITS e 16S rRNA. Para tanto, o DNA microbial de cada amostra foi extraído sem acetato de amônia, conforme descrito por Yu & Morrison (35). Foi utilizado 100 μL de cada amostra de exsudato diluídos em 1000 μL de solução tampão fosfato salino (PBS 1x), conforme orientação do fabricante, seguido de centrifugação (5.180 rpm/5 minutos). Realizou-se remoção de sobrenadante e ressuspensão de todo conteúdo da amostra em180 μL de PBS 1x e adicionado 25 μL de Proteinase K e 200 μL de tampão AL, fornecidos pelo kit, para digestão de proteínas e remoção de contaminantes, atuando na inativação de nucleases, para obtenção de ácidos nucleicos. Na sequência, as amostras foram incubadas à 56 °C por 30 minutos. Feito isto, todo conteúdo foi transferido para colunas de centrifugação fornecidas pelo QIAamp® DNA Mini Kit (QIAGEN, Hilden, Alemanha). As amostras foram centrifugadas (10.000 rpm/10 minutos) e as colunas transferidas para novos tubos de coleta, fornecidos pelo kit. Adicionou-se 500 μL de tampão AW2, fornecido pelo kit, em cada coluna de extração seguidos de centrifugação (10.000 rpm/10 minutos). O fluído residual foi descartado e as colunas adicionadas em um novo tubo de coleta para diluição de DNA em 50 μL de água destilada. Foi realizado centrifugação final (10.000 rpm/10 minutos) e armazenamento do produto de ácidos nucleicos à -20 °C, conforme orienta o

fabricante (35). Em sequência, estes produtos foram quantificados via Qubit® (Life Technologies) e por Nanodrop (Thermo Scientific™) em seguida, enviados diretamente para empresa *Genotyping* Laboratório de Biotecnologia LTDA/ BPI – Biotecnologia Pesquisa e Inovação.

3.1.7 Análise do perfil microbiano do exsudato coletado

Realizada a extração de DNA, as amostras foram enviadas para a empresa *Genotyping* Laboratório de Biotecnologia LTDA/ BPI – Biotecnologia Pesquisa e Inovação para sequenciamento dos genes 16S V3-V4 e ITS1 em modo *paired end* (2 x 250 pb). O término da preparação dos amplicons foi executada como o anexo dos barcodes com o Kit Nextera® XT Index e a execução da corrida na plataforma MiSeq Illumina. Para hibridização da região conservada do gene 16S rDNA foi utilizado o par de oligonucleotídeos 341F (CCTACGGGNGGCWGCAG) e Bakt_805R (GACTACHVGGGTATCTAATCC) e (TCCGTAGGTGAACCTGCGG) para ITS1. A qualidade dos dados brutos gerados por cada uma das amostras foi verificada por meio da ferramenta FastQC, que indicou a necessidade de pré-processamento desses dados, com objetivo de remover regiões dos *reads* com bases de baixa qualidade. Assim, o programa Trimmomatic foi utilizado com os parâmetros SLIDINGWINDOW:5:20 e MINLEN:50, garantindo a permanência de apenas sequências de alta qualidade, o que foi confirmado por mais uma rodada de análises com o FastQC. Em seguida a ferramenta Kraken2 foi utilizada para a classificação taxonômica das amostras a nível de gênero e de espécie. Como base de dados para comparação, uma biblioteca de sequências 16s e ITS1 do RefSeq foi escolhida. Na etapa seguinte, os resultados gerados pelo Kraken2 foram analisados pela ferramenta online Pavian que permite visualizar os dados tabulares e fazer análises comparativas entre as amostras. Por fim, o programa ClustVis foi utilizado para fazer as análises de PCA e gerar as figuras de *heatmap* das amostras (38).

3.1.8 Análises estatística

Todos os resultados foram analisados estatisticamente por testes paramétricos e não paramétricos, de acordo com o perfil populacional e amostral.

Para análise dos resultados clínicos de estudo e caracterização do perfil populacional a normalidade da distribuição foi verificada; para grupos que apresentaram distribuição normal, foi aplicado o teste T de *student* e, para os grupos que não apresentaram normalidade na distribuição da amostra, o teste U de *Mann Whitney* foi aplicado. A normalidade das amostras foi verificada e, para os grupos em que a distribuição normal foi evidenciada, foi aplicado *one way* ANOVA. Entre os grupos em que não houve distribuição normal, o teste aplicado foi *Kruskal Wallis*. O índice de confiança assumida para estas análises foi de 95%. Os dados experimentais para avaliação de resposta imune foram submetidos ao cálculo da média e desvio padrão para cada experimento. Em seguida foi realizada a verificação de normalidade (teste de *Kolmogorov-Smirnov*) e posterior estatística paramétrica mediante a análise de variância, pelo teste *one way* ANOVA. A significância das análises clínicas populacionais foi avaliada pelos testes *Shapiro-Wilk* e de *Mann-Whitney*. Para análise dos resultados do sequenciamento do microbioma, os resultados gerados pelo Kraken2 foram analisados pela ferramenta *online Pavian* que permite visualizar os dados tabulares e fazer análises estatísticas comparativas entre as amostras. Por fim, o programa *ClustVis* foi utilizado para fazer as análises estatísticas de PCA.

4. CAPÍTULOS:

4.1. Capítulo 1: Manuscrito 1

Artigo preparado para ser submetido na revista: *Clinical Oral Investigation,* classificada como A1 no sistema Qualis da CAPES.

Urgent Management for Symptomatic Periapical Abscesses: Impact of Systemic Comorbidities

ABSTRACT

Symptomatic abscesses of endodontic origin can progress rapidly, resulting in a life-threatening risk to the patient. These infections can be exacerbated by systemic diseases, complicating management and worsening the condition's severity. This study aimed to clarify the clinical aspects and urgent therapeutic approach related to the rapid and severe progression symptomatic abscesses of endodontic origin. The study included 50 patients diagnosed with symptomatic periapical abscesses , admitted to the Instituto Hospital de Base do Distrito Federal over one-year period. These patients were divided into four groups based on prior antibiotic use and the need for hospitalization. The study analyzed clinical data, medical and dental history, blood cell counts based on hemograms, and local cytokine production by qPCR through the collection and analysis of purulent exudate. The majority of patients who required hospitalization had systemic comorbidities. The local expression of inflammatory and anti-inflammatory mediators, such as *IL-1β, IL-6*, and *IL-10*, was similar across all groups, suggesting that the local inflammatory response was not the determining factor for the different infection outcomes. Blood cell count analysis demonstrated a significant decrease in leukocyte and neutrophil levels during hospitalization ($p<0.05$), indicating the effectiveness of therapeutic interventions. The therapeutic approach included surgical drainage of the abscess and the administration of antibiotics, with penicillin being the most prescribed class. Treatment was adjusted according to the clinical evolution of the patients, highlighting the importance of combining surgical intervention and appropriate antimicrobial therapy for the successful management of

severe infections. Therefore, early identification, removal of the infectious focus, and careful management of systemic conditions are crucial to improving clinical outcomes and reducing the incidence of severe complications in patients with acute periapical abscesses. This study underscores the need for a multidisciplinary and individualized approach to effectively treat these infections.

Key words: symptomatic periapical abscesses, systemic disease, antibiotic therapy

INTRODUTION

Endodontic infection can occur due to necrosis of the dental pulp from caries, tooth trauma, or previous endodontic treatment. Once established in the root canal, microorganisms can contact periradicular tissues through apical and lateral foramina or root perforations, inducing an acute inflammatory response (1-2). In acute endodontic infections, bacteria not only reside in the root canal but also invade periradicular tissues and can spread to other anatomical spaces in the head and neck, forming cellulitis or phlegmon, a diffuse inflammatory process with pus formation (3-4). The purulent exudate formed in response to root canal infection spreads through the medullary bone, perforates the cortical bone, and drains into the submucosal or subcutaneous tissue (5).

Clinically, the patient with symptomatic periapical abscesses experiences mild to severe pain and swelling, associated with dysphagia, trismus, and dysarthria (2). Systemic manifestations may also develop, including fever, lymphadenopathy, malaise, headache, and nausea (2). Depending on the evolution of the acute periapical abscess and the areas involved, hospital care may be necessary. In these cases, according to the abscess gravity, hospitalization may be necessary for surgical drainage and antibiotic therapy. This type of treatment can generate significant costs for the state, depending on the complexity of the case and the required treatment (2,4).

Identify individuals with symptomatic periapical abscesses that are more likely to progress to serious conditions is essential for managing emergency care. These results may affect decisions about dose and treatment efficacy in certain difficult cases. Then, this study aims to elucidate the clinical aspects and urgent therapeutic approach related to the rapid and severe progression of symptomatic periapical abscesses,

providing important insights for clinical decision-making, and improving the management of urgent conditions.

MATERIALS AND METHODS

Study population

Study participants included 50-free-demand patients with symptomatic abscesses of endodontic origin diagnosis admitted to Instituto Hospital de Base do Distrito Federal (IHBDF - Brazil) emergence, during a 12-month period (January/2019 – December/2019). According to hospital protocol for symptomatic periapical abscesses, all patients were undergoing hematological tests upon admission and required an exudate drainage procedure. All included patients agreed to review the informed consent form. The exclusion criteria included patients with a different diagnosis from the aim of this study and patients whose medical records had incomplete clinical and hematological data. All patient data was kept confidential according to their own terms. This study was approved by the Universidade Católica de Brasília human ethics committee (CAAE: 57475816.8.0000.0029) and the research committee of the IHBDF (CAAE: 57475816.8.3001.5553).

Included patients were divided into four groups, which addressed different clinical conditions and therapeutic approaches:

- Group 1 (n = 8 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency without prior use of antibiotics and did not require hospitalization.
- Group 2 (n = 20 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, already using antibiotics and did not require hospitalization.
- Group 3 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, already using antibiotics and who required hospitalization for up to 7 days.
- Group 4 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, already using antibiotics and who required hospitalization for periods longer than 7 days.

Clinical data, medical and dental history, blood count and implemented clinical management were accessed in hospital records of all patients. In addition, the collected exudate during drainage was analyzed to evaluate local immune response parameters.

Collection of data from medical and dental records

All complementary exams were carried out based on hospital's care protocols, in order to provide a description of clinical data, and severity of infection upon admission of patient diagnosed with symptomatic periapical abscesses. The general characterization of sample population was based on the recorded frequency of antibiotic therapy at the time of admission and hospitalization, when necessary, as well as the main systemic diseases and number of days of hospitalization. All blood counts performed during the patients' hospitalization were accessed. Blood counts (including leukocytes, eosinophils, monocytes, basophils, neutrophils and lymphocytes) were used to make decisions regarding the need for hospitalization or to evaluate the effectiveness of antimicrobial protocols during hospitalization.

Sample exudate collection

Purulent exudate was collected for analysis of production of pro- and anti-inflammatory local cytokines. Initially asepsis of the affected region was carried out with topical use of 2% chlorhexidine digluconate, followed by local anesthesia, incision with a scalpel blade and extraoral/intraoral puncture of the region, with a needle (40 x 12mm) and a 50 mL syringe to suction the exudate from the affected facial bags. Exudate was collected by aspiration and immediately taken to the laboratory where samples were separated into small aliquots and stored at -80 °C (9).

Local immunological profile

Concentrations of local inflammatory and anti-inflammatory mediators (*IL-1β*, *IL-6* and *IL-10*) were determined by the real-time PCR method with StepOnePlus™ Real-Time PCR System. Gene expression analysis was performed by real-time PCR with StepOnePlus™ Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific), in order to verify the expression of genes indicative of inflammatory and

anti-inflammatory processes. For this analysis, the genes for expression of *IL-1β*, *IL-6*, *IL-10* and GAPDH was evaluated as a constitutive gene, according to their respective sequence pairs. Gene expression analysis was performed by real-time PCR with StepOnePlus Real-Time PCR System (ThermoFisher) using glyceraldehyde 3-phosphate dehydrogenase as the constitutive gene. Each reaction had a final volume of 10 mL, consisting of: 5 mL of SYBR Green; 0.5 mL of primers, 0.25 mL of primer for each gene (Table 1) and 2.5 mL of Nuclease free Water (ThermoFisher Scientific - KIT SYBR Green PCR Master Mix). The equipment used was StepOne (Thermo Fisher). At the end of the cycles, the results were analyzed to determine differences in gene expression in tested samples. Relative expression levels in the experimental group were calculated by Δ (Δ CT) (Δ CTControl 2 Δ CTExperiment).

Table 1 - Primers Sequence for Each Gene Used in the PCR Assay

GENE	PRIMER SEQUENCE (5'-3')
GAPDH	F: GCA CCA CCA ACT GCT TAG CA R: TGG CAG TGA TGG CAT GGA GGA
IL-6	F: ACA GCC ACT CAC CTC TTC AG R: CCA TCT TTT TCA GCC ATC TTT
IL-1β	F: TGG CAG AAA GGG AAC AGA A R: ACA ACA GGA AAG TCC AGG CTA
IL-10	F: GGT TGC CAA GCC TTG TCT GA R: TCC CCC AGG GAG TTC ACA T

Statistical analysis

Clinical analyzes were performed using IBM® SPSS® Statistics software. Normal data distribution results were analyzed by Student's T test, while non-normal data distribution was analyzed by Mann Whitney U test. The confidence level assumed for these analyzes was 95%. Statistical analysis of PCR results was performed by one-way ANOVA, in the normal distribution of data and Kruskal Wallis, in the non-normal distribution of data. The analyzes were carried out using the Graph Pad Prism 7 (CA) software and considered a significance level of 95%.

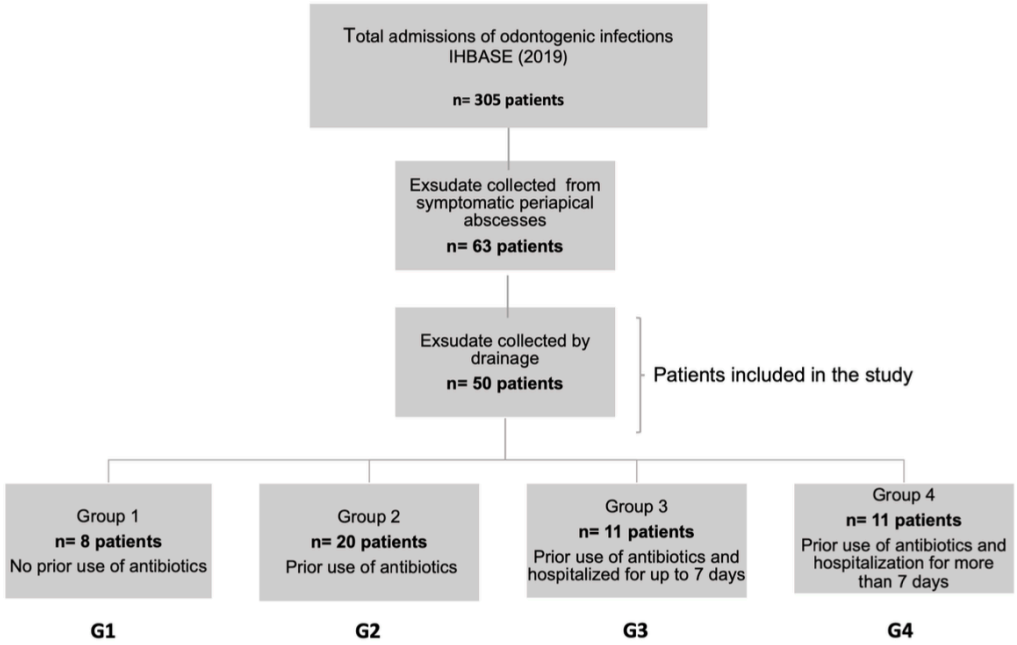
RESULTS

Patients' clinical data and infection severity upon admission

During a period of one year, 305 patients diagnosed with odontogenic infection were admitted to IHBDF emergence. Of these patients, 16% (50 patients) were diagnosed with symptomatic periapical abscesses and included in the study (Figure 1A). Among these patients, there is a greater predominance of males (76%), compared to females (24%), and variable ages: <18 years (5.3%), 19-25 years (8.2%), 26-30 years (7.1%), 31-40 (4.2%), 51-60 years (16.4%), > 60 years old (7%) and 41 to 50 years old (52.1%) (Figure 1B). All patients included in this study were divided into four groups (Figure 1A).

It was demonstrated that almost all patients included in G3 and G4 (need for hospitalization) reported previous systemic comorbidities such as hypertension/diabetes mellitus/lupus/human immunodeficiency syndrome (HIV)/chronic renal failure and rheumatoid arthritis. Among these groups, only 18.2% of patients included on group G3 reported no previous systemic diseases. All patients included in group G4 reported a history of some systemic comorbidity. Among the systemic comorbidities, hypertension stands out, presenting a higher percentage in all groups: G3 (36.4%) and G4 (27.3%). Diabetes mellitus was also a frequently observed comorbidity: type 1 diabetes representing 36.4% of patients included in G4, while type 2 diabetes represent 27.3% of patients included in G4 (Figure 1B). In addition, 84% of all included patients used penicillin prior to its admission to IHBDF. Patients hospitalized in this condition demonstrated greater severity of infection stages (edema - 43.2% and abscess - 75.2%) and a higher percentage of more severe signs and symptoms (dyspnea - 28.4%, dysphagia - 39.2% and fever - 39.6%) than patients who were already on antibiotic therapy (Supplementary Figure 1), demonstrating an infection greater evolution in these patients. Furthermore, patients that need for hospitalization also reported higher number and intensity of signs and symptoms upon patient admission (Figure 1B). All these conditions required the use of different class of antibiotics during the therapeutic approach (Penicillin / Lincosamine / Cephalosporin / Aminoglycoside / Carbapenemic) (Figure 1B).

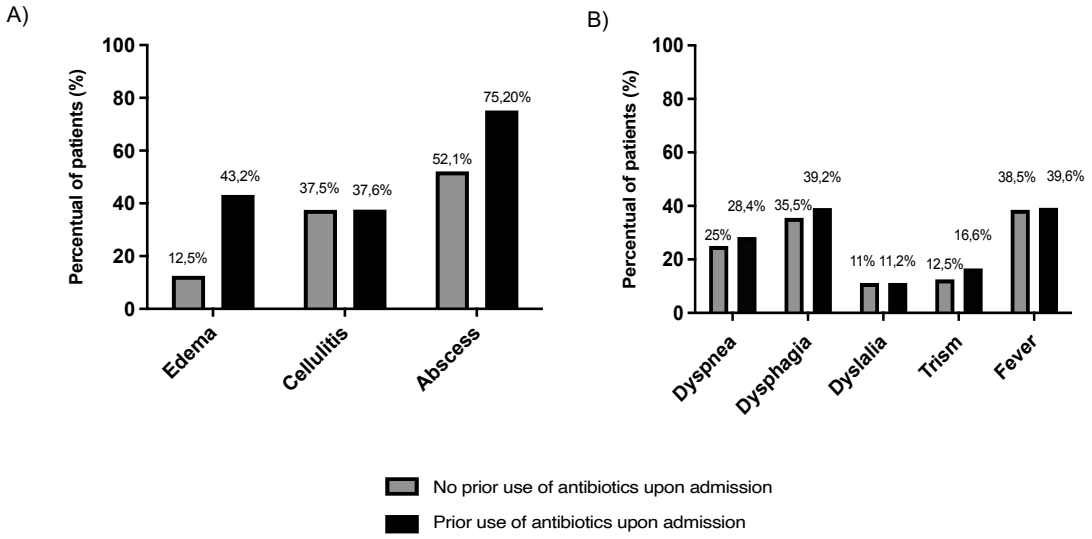
A)



B)

		G1	G2	G3	G4
	Parameters	n (%)	n (%)	n (%)	n (%)
Sex	Female	1 (87.5)	3 (15)	2 (18.2)	3 (27.3)
	Male	7 (12.5)	17 (85)	9 (81.8)	8 (72.7)
Systemic disease	Hypertension	3 (37.5)	6 (30)	4 (36.4)	3 (27.3)
	Type 1 Diabetes Mellitus	1 (12.5)	1 (5)	1 (9)	4 (36.4)
	Type 2 Diabetes Mellitus	2 (25)	1 (5)	1 (9)	3 (27.3)
	HIV	-	-	2 (18.2)	1 (9)
	Chronic kidney disease	-	-	1 (9)	2 (18.2)
	Rheumatoid arthritis	-	-	2 (18.2)	2 (18.2)
	Lupus	-	1 (5)	2 (18.2)	1 (9)
	Not declared	3 (37.5)	13 (65)	2 (18.2)	-
Clinical signs and symptoms	Dyspnea	3 (37.5)	2 (10)	6 (54.5)	3 (27.3)
	Dysphagia	-	2 (10)	8 (72.7)	4 (36.4)
	Dyslalia	1 (12.5)	6 (30)	9 (81.8)	8 (72.7)
	Fever	8 (100)	20 (100)	11 (100)	11 (100)
	Trism	1 (12.5)	9 (45)	9 (81.2)	10 (91)
Class of antibiotics	Penicilin	-	16 (80)	7 (63.6)	6 (54.5)
	Cephalosporin	-	1 (5)	2 (18.2)	3 (27.3)
	Nitromidazolic	-	-	2 (18.2)	2 (18.2)
	Licosamin	-	3 (15)	2 (18.2)	1 (9)
	Aminoglycosíde	-	-	2 (18.2)	2 (18.2)
	Carbapenemic	-	-	-	2 (18.2)

Figure 1: Characterization of patients and their distribution. A) Flowchart of patient admitted in IHBDF and their distribution. Patients were divided into 4 groups: no prior use of antibiotics (G1), prior use of antibiotics without the need for hospitalization (G2), prior use of antibiotics and need for hospitalization of up to 7 days (G3) and prior use of antibiotics and need for hospitalization for more than 7 days (G4). B) Clinical data of patients, including sex, systemic diseases, clinical signs and symptoms and class of antibiotics prescribed**.**



Supplementary figure 1: Infection severity and signs and symptoms upon patient admission, according to previous use of antibiotic. A) Percentage of patients in each stage of infections: edema, cellulitis, or abscess. B) Percentage of patients with signs and symptoms associated with the infectious process.

Expression of inflammatory and anti-inflammatory mediators in local exudate of symptomatic periapical abscesses

Given signs and symptoms of patients admitted to IHBDF, a local drainage was performed. To assess the local immune profile upon admission, local expression of inflammatory and anti-inflammatory mediators was evaluated by qPCR. The genes *IL-1β* (Figure 2A), *IL-6* (Figure 2B) and *IL-10* (Figure 2C) expression were evaluated in purulent collection, upon patient admission. All groups remained with paired expression levels, as they did not show significant statistical differences. This result suggests that the expression of mediators at the site of infection was probably not the factor responsible for the different course of infection in different patients.

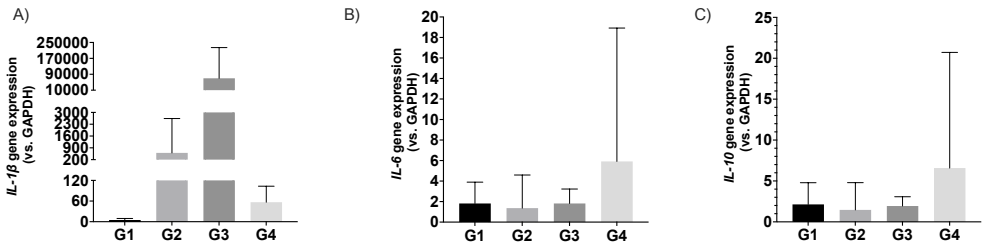


Figure 2: Production of inflammatory and anti-inflammatory mediators from patient's exudate upon admission to IHBDF by qPCR. A) Expression of *IL-1β*. B) Expression of *IL-6*. C) Expression of *IL-10*. Data were represented by mean and standard error performed in triplicate. Constitutive gene was represented by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). No statistically significant differences were observed between all groups after ANOVA and Bonferroni post-test.

Serum blood count data on admission and during hospitalization

Due to the severity of the infectious process, all hospitalized patients underwent an initial blood count. Comparing serum data, a slight increase of leukocytes, neutrophils and monocytes were observed (without statistical significance), when comparing the group G1, without prior antibiotic use with groups G2, G3, G4 that were admitted using previous antibiotics (Supplementary figure 2). Furthermore, increasing rates of leukocytes and neutrophils were observed in groups G3 and G4, which had a higher prevalence of systemic comorbidities in their medical reports (Supplementary figure 2). During the hospitalization period, blood tests were also performed to evaluate the

therapeutic measures adopted for patients included in groups G3 and G4. When the mean of initial check-in and final check-out blood counts of patients included in groups G3 and G4 were compared, there was a significant decrease in leukocytes and neutrophils rates in both groups ($p<0.05$) (Figure 3). Individual variations of neutrophil and leukocytes demonstrated that patients were discharged when these rates reached values within the normal blood range or below (Figure 3B, 3C, 3D and 3E). Therefore, discharge was determined both by evaluating serum parameters and by patients' clinical evolution.

	G1	G2	G3	G4
Leukocyte (x10^3/µL)	10,4±3,6	13,3±4,2	19,7±5,5	20,2±8,2
Neutrophil (%)	59,1±14,4	79,6±8,3	76,3±13,7	76,2±11,2
Eosinophil. (%)	1,6±1,1	0,5±0,6	3,4±3,7	2,8±3,6
Basophil (%)	1,0±0,5	0,5±0,5	0,6±0,1	1,0±1,8
Monocyte. (%)	6,2±2,6	7,0±3,6	7,1±2,4	7,3±1,7
Lymphocyte (%)	22,6±16,9	11,4±6,3	16,4±13,5	17,3±9,5

Supplementary figure 2: Mean and standard deviation of initial serum blood count data of patients upon hospital admission, included in each group.

A)

Cell Type (Unit of measurement)		G3 Initial	G3 Final	G4 Initial	G4 Final
Leukocyte	(x10³/μL)	19,7±5,5*	11,3±6,6*	20,2±8,2*	9,0±3,5*
Neutrophil	(%)	76,4±13,7*	62,1±13,3*	76,2±11,2*	61,7±9,4*
Eosinophil	(%)	3,4±3,7	3,2±3,6	2,8±3,6	1,9±1,7
Basophil	(%)	0,7±0,2	1,0±0,5	1,1±0,9	1,2±0,5
Monocyte	(%)	7,1±2,4	7,4±1,6	7,4±1,7	8,3±2,0
Lymphocyte	(%)	16,5+13,6	25,3+10,4	17,4+9,6	27,1+6,8

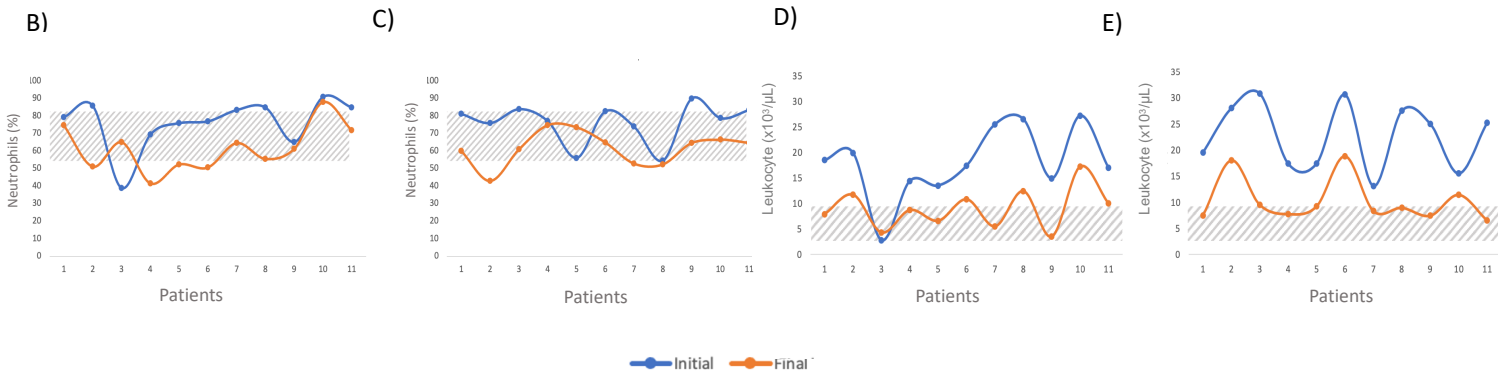


Figure 3: Cellular blood count pattern and variations of patients included in groups G3 and G3 during hospitalization. A) Media and standard deviation of initial and final serum blood count data from patients included in groups 3 and 4, during hospitalization. * $p<0.05$, after t-test, comparing initial check-in and final check-out values in each group. Individual variations in the values of neutrophils (B and C) and leukocytes (D and E) of patients included in groups G3 and G4. Blue and orange lines represent the initial and final values, respectively, and the folded area represents the normal range for each cell type.

Leukocyte patterns after emergency management

Clinical management of emergency care during hospitalization was monitored by signs and symptoms and blood count. The return to homeostasis of leukocyte levels occurred due to local drainage and antibiotic therapy during hospitalization. Thus, individually implemented drug therapy was added to leukocyte initial check-in and final check-out values, according to the class of antibiotics used (nitromidazole, lincosamine, aminoglycoside, penicillin and cephalosporin) in groups G3 and G4 (Figure 4). Based on the classes of antibiotics most often chosen during hospitalization, penicillin stands out in G3 group (63.3%) and G4 group (54.4%), followed by cephalosporin in G3 group (18.2%) and G4 (18.2%), lincosamine in G3 (18.2%) and G4 (9%), aminoglycoside in G3 (18.2%) and G4 (18.2%), and carbapenemic restricted only to the G4 group (18.2%). Antibiotic therapy has been shown to be effective when associated with surgical drainage of acute periapical abscesses, in which we highlight the homeostasis of serum standards when it reaches the normal range. Systemic comorbidities influence the process of returning to normality, as patients who did not reach the serum standard had abscesses associated with important systemic diseases. In group 3, we highlight patient number 10, who used nitromidazole and has hypertension and HIV; patient 2, who used lincosamine and has rheumatoid arthritis; and patient 8, used cephalosporin and has type 2 diabetes (Figure 4A). In addition, among patients include in group 4, we highlight patient number 2, who used penicillin and had chronic kidney disease and hypertension; patient 10, who used carbapenemic and has lupus and hypertension; and finally patient 6, who used carbapenemic and has type 2 diabetes, hypertension and chronic kidney disease (figure 4B).This last patient did not have progressive improvements and died due to very serious complications from the comorbidities presented. Penicillin antibiotic class leads the protocols of choice and was effective when associated with surgical drainage therapy.

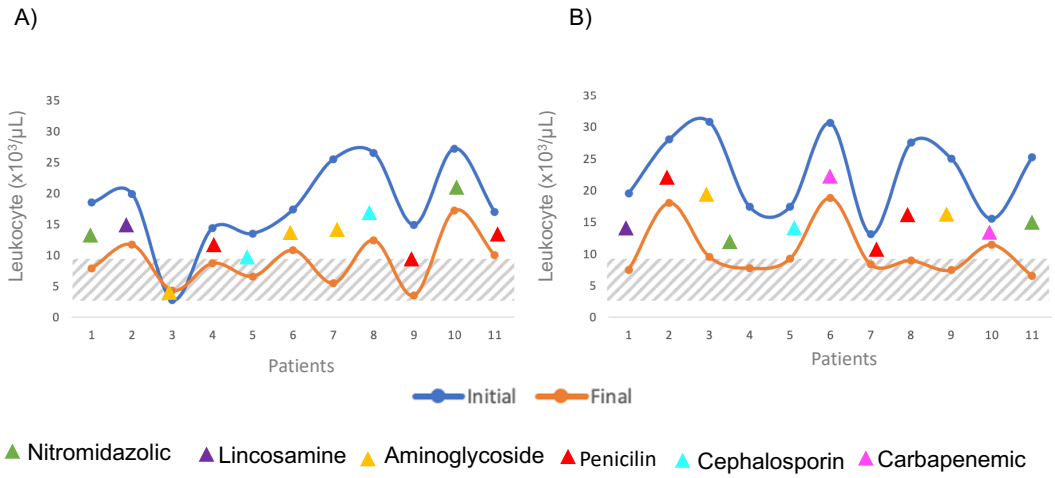


Figure 4: Leukocytes variation, based on the used antibiotic therapy. Individual variations of leukocyte values, in group 3 (A) and group 4 (B). Different triangle colors represent the used antibiotics: nitromidazole (green), lincosamine (purple), aminoglycoside (yellow), cephalosporin (teal), and carbapenemic (pink). Blue and orange lines represented initial and final values respectively and craped area represents the normal range for each cell type.

DISCUSSION

Odontogenic infections have multiple etiopathogenesis and may involve dental or supporting tissues. These infections are polymicrobial in nature and are associated with the imbalance of the oral microbiota and its interaction with the host (7). They can have many origins, including endodontic. Most patients with odontogenic infections are successfully treated in an outpatient setting. However, in some cases, the infection can progress seriously, leading to complications and reaching life-threatening levels, requiring urgent care in a hospital environment, often demanding long hospitalization periods (8).

Odontogenic infections of endodontic origin start from progressing carious lesions that compromise the pulp tissue, leading to necrosis and subsequently establishing periradicular lesions, potentially evolving into an acute periapical abscess. These infections can complicate by direct local extension or hematogenous dissemination (9). Local signs and symptoms include intense pain during vertical percussion and the sensation of a "grown" tooth. If untreated, the infection can spread beyond the cortical bone or periosteum, reaching the perioral tissues (10). Systemic symptoms may include fever, general malaise, and regional lymphadenopathy. Urgent treatment should be conducted promptly and

effectively, typically involving abscess drainage and antibiotic therapy (9-10). In more severe cases, where the infection spreads to deep neck spaces or compromises the airways, hospitalization may be necessary for a more intensive care. After the urgent intervention, it is crucial to treat the underlying cause of the infection, which may include endodontic treatment or extraction of the affected tooth (9).

This study followed cases of odontogenic infection that accessed the IHBDF emergency service for one year. A total of 305 patients were admitted with a diagnosis of odontogenic infection and of these, 50 patients were diagnosed with symptomatic abscesses of endodontic origin, representing 16% of the total number of patients admitted with odontogenic infection. All patients in this study underwent usual care at the IHBDF dental emergency, following the hospital's specific protocols, requiring drainage, use of antibiotic therapy and hospitalization, when necessary. Aiming key aspects that could be decisive in worsening the severity of infection, patients were analyzed in different groups, according to previous use of antibiotics and the need for hospitalization.

Among the signs and symptoms of symptomatic periapical abscesses pain and the establishment of edema stand out (11). The pain is related to compression of nerve endings and release of inflammatory mediators (12). Cytokines such as *IL-1β* and *IL-6* are involved in local immune activation and regulation (12), while IL-10 is an immunomodulatory cytokine with anti-inflammatory properties (12). Several studies have demonstrated the role of *IL-10* in periapical lesions and periodontal diseases (13-14) and in refractory endodontic lesions. Surprisingly, the analysis of inflammatory mediators locally produced in the exudate obtained after drainage showed similar mean values between all groups. Noting that locally the inflammatory response was not a factor that led to a bad prognosis of the infection in some patients.

However, failure to remove the infectious focus can lead to the local immune response having significant systemic repercussions, allowing its perpetuation and increase in severity, leading to edema, abscess and cellulitis (27). Factors such as upper airway obstruction, advanced age, fever upon patient admission, concurrent respiratory diseases, site of the infection and failure in first-line therapy, are associated risk points for systemic dissemination of local infection and risk of lethal complications (8-15). Most patients included in this

study were already using antibiotics, indicating a failure in primary emergency care. Patients with previous antibiotic use had a higher infection severity and a worst systemic signs and symptoms on admission. In addition, these patients also have higher mean cell values in the blood count. This severity in patients using antibiotics is related to the fact that, despite the antibiotic use, the source of the infectious process was not removed during the initial care provided to the patient. These cases require urgent dental care due to their rapid and disseminative nature (16) and accurate and early diagnosis are of vital importance for a better prognosis (16-17).

Advanced cases of symptomatic periapical abscesses may result in the spread of the purulent collection to an adjacent area and lead to bacteremia (11). In this sense, complementary tests, such as blood count and imaging exams, are of extreme importance for assessing the patient's systemic involvement in severe infections (28). Among these tests, the leukogram can aid in diagnosis, as in addition to the total white cell count, it provides numbers for each cell type and their degree of maturation. Leukocytosis indicates an active infectious process, constituting an important indicator of the patient's level of involvement (18). Furthermore, the leukogram is an important test for controlling therapy effectiveness by comparison with previously obtained values (18-19). In this study, a significant decrease in leukocyte and neutrophil levels was observed between the check-in and check-out blood counts of hospitalized patients. However, an individual analysis of patients is necessary since different records of systemic comorbidities were reported by the patients included in this study: hypertension, diabetes mellitus, HIV, chronic renal failure, and rheumatoid arthritis for example. Among the patients who required hospitalization for remission of an urgent acute periapical abscess, the majority had at least one of these comorbidities in their medical history. Highlighting the highest percentage of hypertensive patients included in all groups, and of hypertensive and type 1 diabetic patients in the group that required hospitalization for a period longer than seven days. It has been demonstrated that factors such as alcoholism, hypertension, immunosuppression, uncontrolled diabetes mellitus and several underlying medical conditions are related to a greater risk (20,21).

Successful treatment of symptomatic abscesses of endodontic origin affecting multiple spaces involves important factors such as the identification of

the source of infection, anatomical spaces, predominant microorganisms at different stages, the impact of the infectious process on the immune system, and the administration of appropriate drug therapy (20,22). Thus, the treatment of this infection at the tertiary care level is based on the removal of the etiological factor and systemic antibiotic therapy (23,24). The surgical management, performed with drainage of the infection, aims to reduce the edema and compression at the site of the infection, promoting the release of purulent secretion and necrotic contents (23,24). The incision of the abscess or cellulitis allows the removal of accumulated purulent secretion and bacteria from the underlying tissue. The evacuation of the abscess cavity dramatically reduces the bacterial load and necrotic debris. Evacuation also reduces hydrostatic pressure in the region by decompressing the tissues, which improves local blood supply and increases the release of host defenses and the levels of antibiotics to the infected area (9-25).

Siqueira et al. (2013) evidenced that systemic antibiotic therapy is a commonly employed practice in the management of patients with acute periapical abscess as an adjunctive therapy to the removal of the etiological factor of the infection (12). Therefore, in complicated cases, in addition to prompt and aggressive surgical drainage, the initiation of antibiotic therapy is highly recommended. If necessary, it can be adjusted according to the results of antibiotic sensitivity tests. The combination of early diagnosis, initiation of antibiotic therapy, and timely surgical intervention can be regarded as the decisive triad for the successful management of complications of acute dental abscesses.

It is important to highlight the adjunctive nature of this therapy, as the application of antibiotics without criterion and without the association of appropriate surgical management is associated with a worst prognosis. When local decontamination alone does not have the desired effect, and there are signs and symptoms indicating the spread of the infection, the use of antibiotics is recommended to reduce the bacterial population and thus assist the host's defense systems. In this study, all patients who had previously used antibiotics were taking penicillin. Regarding antibiotic therapy after treatment at the IHBDF, antibiotics from four different classes were prescribed, according to the evolution of the patient's health status. The selection of antibiotics in clinical practice at the initial stage is based on previously reported results of microbial sensitivity (26).

Most bacterial species involved at endodontic infections, including abscesses, are susceptible to penicillin, making these drugs the first choice for treating endodontic infections once penicillin allergy is ruled out (26).

In this study, the return to leukocyte homeostasis occurred after local drainage and antibiotic therapy. To evaluate the serum response to the use of antibiotics, we chose to analyze the leukocyte pattern, as it is a cellular group composed of neutrophils, basophils, eosinophils, lymphocytes, and monocytes (28). Assessment of the neutrophil pattern was not performed since some classes of antibiotics, such as aminoglycoside, lincosamine and cephalosporin, can cause neutropenia (27). Individual variations of neutrophils and leukocytes demonstrated that patients were discharged when these rates reached values within the normal blood range or below. Therefore, discharge was determined both by evaluating serum parameters and by patients' clinical evolution.

Systemic comorbidities can influence the success of antibiotic use (3). In this study patients who did not reach the serum standard to normal range presented periapical abscesses acute associated with significant systemic diseases. Penicillin is the class of antibiotics most prescribed and is effective when associated with surgical drainage therapy. However, bacterial resistance to antimicrobial drugs is a relevant problem for the health area, including Dentistry (25). The increasing rates of resistance to multiple antimicrobial drugs, such as macrolide, penicillin, and clindamycin, have been described for oral microorganisms. The inappropriate use of antimicrobials favors the selection of resistant bacteria and has significant repercussions on the host's ecology (25). In this study, a patient in the prolonged hospitalization group stood out, whose severe deterioration of general health resulted in death. The patient was admitted with an acute periapical abscess progressing to the airways, and had chronic renal insufficiency, hypertension, and type 2 diabetes. The patient underwent surgical drainage followed by broad-spectrum antibiotic therapies with carbapenemics, but systemic complications worsened the course of the infection, impairing the action of antibiotics.

Hematological examination is a fundamental premise for therapeutic decision-making. This is of paramount importance for clinical practice, especially in the understanding and treatment of more serious cases of symptomatic abscesses of endodontic origin. Furthermore, this study investigated the medical

and dental history and the course of follow-up and emergency treatment of severe acute periapical abscesses. The objective was to raise aspects related to the more serious and rapid evolution of abscesses and points that required greater attention for treatment of the emergency condition. In this study, previous systemic comorbidities were decisive factors for the worsening of abscesses, leading to the need for closer and daily monitoring.

CONCLUSION

Symptomatic Periapical Abscesses are serious conditions that require early diagnosis and intervention to prevent severe complications. This study demonstrated that effective treatment of these infections depends on a combination of proper surgical drainage, appropriate antibiotic therapy, and continuous monitoring, particularly in patients with systemic comorbidities. Early identification, removal of the infectious focus, and careful management of systemic conditions are crucial to improving clinical outcomes and reducing the incidence of severe complications.

ACKNOWLEDGMENTS

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4.2. Capítulo 2: Manuscrito 2:

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Microbial Diversity and Clinical Outcomes in Symptomatic Periapical Abscesses with Associated Systemic Comorbidities

ABSTRACT

Symptomatic abscesses of endodontic origin are significant clinical challenges in dental and maxillofacial infections, often leading to severe pain and potential systemic involvement if not treated promptly. These infections are mainly bacterial, resulting from necrotic pulp tissue that extends to the periapical region. This study aims to identify bacterial genera present in exudates collected from patients with symptomatic periapical abscesses using molecular techniques, evaluating the impact of systemic comorbidities on clinical outcomes. The study included 50 patients diagnosed with symptomatic periapical abscesses who accessed the emergency services at the Instituto Hospital de Base in the Distrito Federal (Brazil) over a period of one year. Patients were categorized into four groups based on prior antibiotic use and hospitalization requirements. Exudates were collected and subjected to 16S rRNA gene sequencing to identify bacterial genera. Data on systemic comorbidities, clinical signs and symptoms, and antibiotic prescriptions were analyzed. Patients with systemic comorbidities, such as diabetes, hypertension, HIV, lupus and chronic renal failure, presented more severe clinical symptoms and required longer hospitalization. *Prevotella* was the most dominant genus, representing 54.6% of the microbial population across all samples. Other significant genera included *Amniculibacterium, Fusobacterium,* and *Lancefieldella.* Prior antibiotic use influenced microbial diversity, with a notable reduction in diversity among patients who used antibiotics prior to hospital admission. Therefore, the presence of systemic comorbidities significantly impacts the clinical course of acute periapical abscesses, requiring a personalized therapeutic approach. The predominance of *Prevotella* genus must be taken into consideration when choosing antimicrobial antibiotic therapy.

Future research should focus on understanding how these comorbidities influence microbial composition and response to treatment, aiming to improve clinical management strategies for these patients.

Keywords: Symptomatic periapical abscesses, Systemic comorbidities, 16S rRNA gene sequencing, Microbial diversity, Antibiotic therapy

INTRODUCTION

Symptomatic periapical abscesses represent a significant clinical challenge in dental and maxillofacial infections, often leading to intense pain, swelling, and potential systemic involvement if not treated quickly and effectively [1]. These infections are primarily bacterial in origin, typically arising from necrotic pulp tissue and extending to the periapical region [1,2]. The polymicrobial nature of these infections can complicate their treatment. In this way, advances in microbial identification favor the establishment of better successful treatments for emergency cases of acute periapical abscess [1].

Traditional culture-based techniques have long been employed to identify microorganisms causing periapical abscesses [1,2]. However, these methods have inherent limitations, including the fastidious nature of many oral bacteria and their inability to detect uncultivable species [2]. Consequently, the advent of molecular techniques, particularly high-throughput sequencing, has revolutionized our understanding of the microbial diversity present in these infections [2]. Recent studies using 16S rRNA gene sequencing have revealed a more complex and diverse bacterial community in periapical abscesses than previously recognized [3]. This molecular approach allows for the identification of bacteria at the genus level, including those that are difficult or impossible to culture [3]. For example, next-generation sequencing has been crucial in characterizing microbial communities in apical root canals, revealing a wide range of bacterial genera involved in periapical infections [4].

A fundamental study demonstrated the complexity of microbial communities in paired root apices and periapical lesions, highlighting their

association with clinical signs in persistent apical periodontitis [5]. This research underscores the importance of using advanced molecular diagnostics to comprehensively elucidate the microbial etiology of these infections. Additionally, comprehensive reviews on the microbiology and treatment of symptomatic periapical abscesses have provided insights into the complex interaction of microbial factors in the pathogenesis and management of these infections [6,7].

Accurate identification of bacterial genera in purulent exudate from symptomatic periapical abscesses. First, it allows for the formulation of targeted antibiotic therapies, thus increasing treatment efficacy and minimizing the development of antibiotic resistance [6]. Second, the identification of specific pathogenic bacteria provides insights into their pathogenic mechanisms and potential virulence factors, which can influence the clinical progression and severity of the infection. And third, understanding the microbial diversity in these infections can aid in the development of preventive strategies and therapeutic interventions, ultimately improving patient outcomes.

Moreover, the presence of systemic diseases, such as diabetes, hypertension, HIV, lupus, and chronic kidney disease, is an extremely important factor in the context of the progression of odontogenic infections of endodontic origin [8]. These conditions can significantly influence the patient's immune response, complicating the evolution of abscesses and increasing the risk of complications. Patients with these systemic diseases may present a slower and less effective response to treatment, highlighting the need for a personalized and careful therapeutic approach.

In this study, we identified bacterial genera present in the exudate collected from patients with symptomatic periapical abscesses using cutting-edge molecular techniques. By leveraging high-throughput sequencing, we aim to provide a comprehensive view of the microbial landscape of periapical abscesses. This approach not only enhances our understanding of the microbial etiology of these infections but also lays the foundation for better clinical management and therapeutic strategies.

METODOLOGY

Study population

Study participants included 50-free-demand patients with symptomatic periapical abscesses origin diagnosis admitted to Instituto Hospital de Base do Distrito Federal (IHBDF - Brazil) emergence, during a 12-month period (January/2019 – December/2019). The exclusion criteria included patients with a different diagnosis from the aim of this study and patients whose medical records had incomplete data. All patient data were kept confidential according to their own terms. All included patients agreed to review the informed consent form. This study was approved by the Universidade Católica de Brasília human ethics committee (CAAE: 57475816.8.0000.0029), the research committee of the IHBDF (CAAE: 57475816.8.3001.5553) and registration in the National Genetic Heritage and Associated Traditional Knowledge Management System (SisGen: AE5000A).

All patients received standard emergency care for acute conditions with drainage, blood testing, antibiotic therapy, and hospitalization if necessary. The exudate collected during the initial emergency care was immediately sent for freezing at -80°C. Hospitalized patients were discharged after remission of the acute condition. Collected exudates were divided into the following groups, according to previous use of antibiotics and need for hospitalization (Figure 1):

- Group 1 (n = 8 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency without prior use of antibiotics and did not require hospitalization.
- Group 2 (n = 20 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, prior use of antibiotics and did not require hospitalization.
- Group 3 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, prior use of antibiotics and who required hospitalization for up to 7 days.
- Group 4 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, prior use of antibiotics and who required hospitalization for periods longer than 7 days.

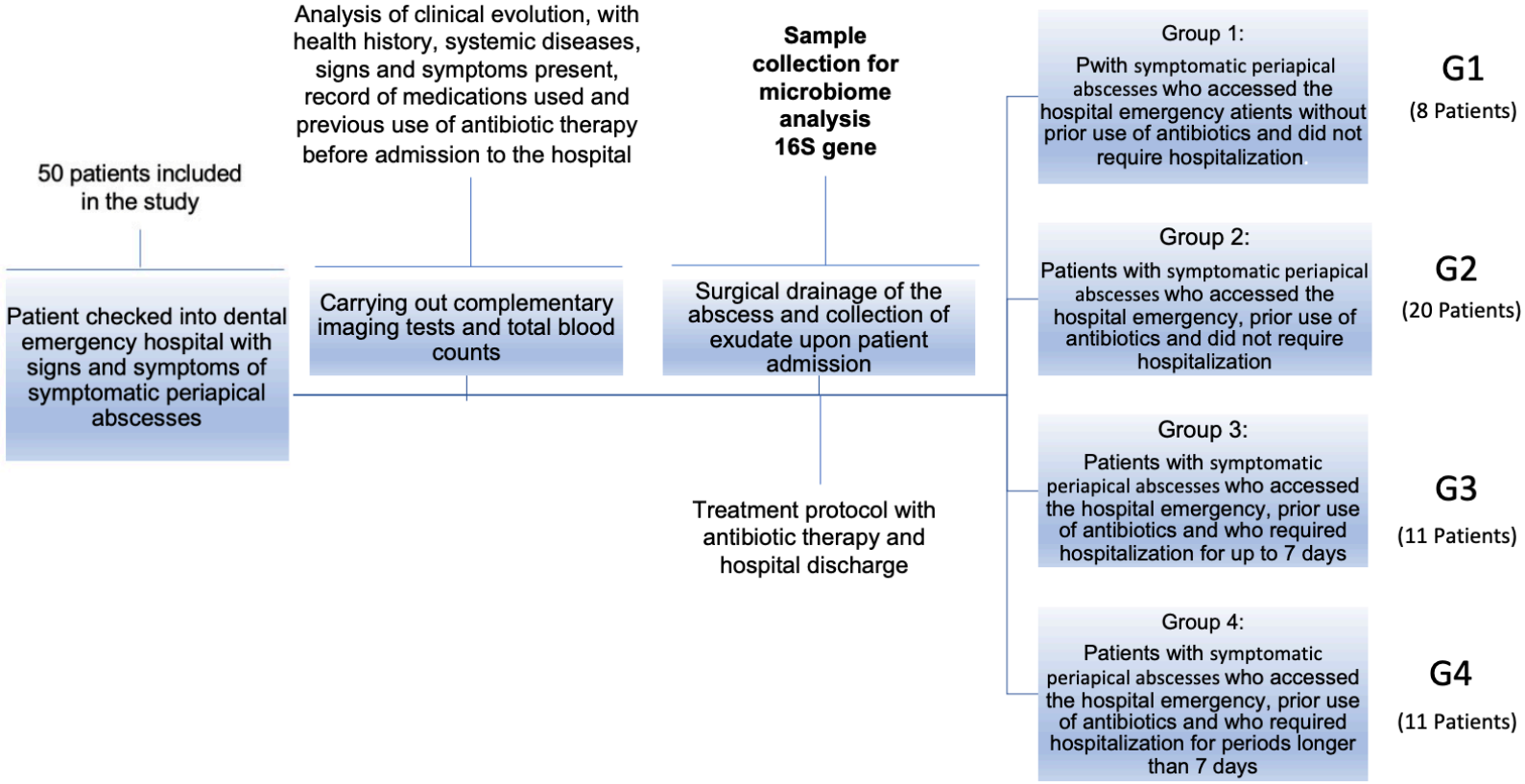


Figure 1: Flowchart of care steps for patients with symptomatic periapical abscesses and division of study sample groups.

Data Collection from Care Protocols

Personal data, anamnesis, including health history and previous medication therapy prior to admission, as well as patients' antibiotic therapy, were recorded during care in medical record and subsequently accessed. Medical and dental progress of patients who required hospitalization were also monitored. All complementary exams were conducted based on IHBASE's care protocols, from the moment of hospital admission to discharge.

Drainage and Patients Exudate Samples

Patients included in the study were admitted to the outpatient clinic and then purulent exudate was collected for microbiota analysis. Initially, the affected region was sterilized with 2% chlorhexidine digluconate (Rioquímica, São Paulo, Brazil), followed by local anesthesia, incision with a scalpel blade and extraoral/intraoral puncture of the region with a needle (40 x 12 mm) and a 50 mL syringe to aspirate exudate from the affected facial spaces. Exudate was collected by aspiration and immediately separated into small aliquots and stored at −80°C [9].

DNA Extraction

Microbiome analysis was performed through sequencing of the 16S rRNA gene in purulent exudate collected during initial emergency care, allowing the identification and characterization of the bacterial population. Therefore, DNA extraction was performed using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) for amplification and sequencing of the 16S rRNA genes. Subsequently, these products were quantified via Qubit® (Life Technologies) and Nanodrop (Thermo Scientific™) and then sent directly to Genotyping Laboratório de Biotecnologia LTDA/ BPI – Biotecnologia Pesquisa e Inovação (Brazil) [10].

16S rRNA sequencing

After DNA extraction, the samples for sequencing of the 16S (V3-V4) genes in paired-end mode (2 x 250 bp). The amplicon preparation was completed with the attachment of barcodes using the Nextera® XT Index Kit and the run was

performed on the MiSeq Illumina platform. For hybridization of the conserved region of the 16S rDNA gene, the oligonucleotide pair 341F (CCTACGGGNGGCWGCAG) was used [11]. The quality of the raw data generated by each sample was checked using the FastQC tool, which indicated the need for preprocessing of these data to remove low-quality base regions from the reads. Therefore, the Trimmomatic program was used with the parameters SLIDINGWINDOW:5:20 and MINLEN:50, ensuring the retention of only high-quality sequences, which was confirmed by another round of analysis with FastQC. Next, the Kraken2 tool was used for the taxonomic classification of the samples at the genus level. For comparison purposes, a 16S sequence library from RefSeq was chosen as the database. In the next step, the results generated by Kraken2 were analyzed using the online tool Pavian, which allows visualization of tabular data and comparative analysis between samples. Finally, the ClustVis program was used to perform PCA analyses and generate heatmap figures of the samples.

RESULTS

A total of 50 patients diagnosed with symptomatic periapical abscesses that assessed the emergency of IHBDF for one year were included in this study. Patients were analyzed according to its clinical evolution to better understand the role of microorganisms in abscess pathology (Table 1). Comorbidities varied across the groups. Notably, comorbidities such as hypertension, diabetes (type 1 and type 2), HIV, lupus, and chronic renal failure were observed among the patients. Group 4 had the highest prevalence of comorbidities corroborating the need for longer periods of hospitalization. These chronic conditions seem to complicate patient clinical evolution, requiring a careful and individualized approach to antibiotic treatment. Clinical signs and symptoms observed upon the patient's arrival at the dental emergency include dyspnea, fever, dysphagia, dyslalia, and trismus. An increase in the worsening of signs and symptoms was observed when we progressively compared all groups (Table 1). Given these conditions, antibiotic prescription was necessary for all patients.

Patients in Group 1 did not receive any antibiotics initially and, in most cases, were discharged from emergency care with a prescription for penicillin.

Similarly, Group 2 patients predominantly received penicillin, with a few cases receiving other antibiotics like lincosamide and cephalosporin. In contrast, patients in Groups 3 and 4 required adjustments to their antibiotic regimens during hospitalization. This was due to factors such as microbial complexity, worsening of symptoms, or availability of medications in the hospital. Group 3 patients were treated with penicillin, nitromidazole, cephalosporin and lincosamide throughout their hospitalization. Similarly, Group 4 patients received more aggressive and varied antibiotic treatments, including penicillin with cephalosporin, carbapenem, nitromidazole, and aminoglycoside.

Therefore, patients' clinical data revealed a worsening of signs and symptoms, leading to the need for greater variation in antibiotic prescription, when we progressively analyzed groups 1, 2, 3 and 4. This situation was also aggravated due to greater complexities of comorbidities also reported progressively in groups 1, 2, 3 and 4.

Table 1. Comorbidities, signs and symptoms and antibiotic prescription among patients with symptomatic periapical abscesses, categorized into four groups. ND – Not declared.

Group	Patient ID	Comorbidities	Signs and Symptoms	Antibiotic prescription during emergency care
1 (no prior antibiotic use)	1	Hypertension, type 2 diabetes	Dyspnea, fever	Penicillin
	5	ND	Dyspnea, fever	Penicillin
	10	ND	Fever	Penicillin
	12	Type 2 diabetes	Fever	Penicillin
	15	Hypertension	Fever	Penicillin
	16	ND	Dyspnea, fever	Penicillin
	23	Hypertension	Dyslalia, fever, trismus	Penicillin
	32	Type 1 diabetes	Fever	Penicillin
2 (prior antibiotic use and no hospitalization)	9	Hypertension	Dyslalia, fever	Penicillin
	11	ND	Fever	Penicillin
	22	ND	Dysphagia, fever	Penicillin
	29	Hypertension	Dysphagia, dyslalia, fever	Penicillin
	30	Hypertension	Dyspnea, dyslalia, fever, trismus	Penicillin
	31	ND	Fever	Penicillin
	35	ND	Dyslalia, fever, trismus	Penicillin
	36	ND	Dyspnea, fever	Lincosamide
	37	ND	Fever	Penicillin
	38	ND	Fever	Penicillin
	39	Hypertension, type 1 diabetes	Fever, trismus	Penicillin
	40	Lupus	Dyslalia, fever, trismus	Lincosamide
	41	Hypertension	Fever, trismus	Lincosamide
	44	ND	Dyslalia, fever, trismus	Cephalosporin

	45	ND	Fever, trismus	Penicillin
	47	ND	Fever, trismus	Penicillin
	48	ND	Fever	Penicillin
	49	ND	Fever	Penicillin
	50	ND	Fever	Penicillin
3 (prior antibiotic use and hospitalization ≤ 7 days)	2	HIV	Dyspnea, dysphagia, fever, dyslalia	Penicillin and nitroimidazole
	3	Lupus	Dyspnea, fever, dyslalia	Penicillin, cephalosporin and lincosamide
	4	ND	Dyspnea, dysphagia, fever, dyslalia trismus	Penicillin and aminoglycoside
	7	Rheumatoid arthritis	Fever, trismus	Penicillin throughout hospitalization
	8	ND	Fever, trismus	Penicillin and cephalosporin
	13	Hypertension, chronic renal failure, and type 2 diabetes	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin, cephalosporin and aminoglycoside
	19	Lupus	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin and aminoglycoside
	24	HIV	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin and cephalosporin
	26	Hypertension and type 2 diabetes	Dyslalia, fever, trismus	Penicillin throughout hospitalization
	27	Hypertension and type 1 diabetes	Fever, trismus	Penicillin and nitroimidazole
	28	Hypertension and arthritis	Dysphagia, dyslalia, fever, trismus	Penicillin throughout hospitalization
4 (prior antibiotic use and	6	HIV	Dyslalia, fever, trismus	Penicillin and lincosamide
	14	Type 1 diabetes	Fever	Penicillin throughout hospitalization

hospitalization > 7 days)	17	Hypertension, type 2 diabetes, chronic renal failure	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin and aminoglycoside
	18	Type 1 diabetes	Dyslalia, fever, trismus	Penicillin and nitroimidazole
	20	Rheumatoid arthritis, hypertension	Dyslalia, fever, trismus	Penicillin and cephalosporin
	21 (deceased patient)	Chronic renal failure, type 2 diabetes, hypertension	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin, cephalosporin and carbapenem
	25	Lupus	Dysphagia, fever, trismus	Penicillin throughout hospitalization
	33	Type 1 diabetes	Dyspnea, dysphagia, fever, dyslalia, trismus	Penicillin throughout hospitalization
	42	Hypertension, type 2 diabetes	Fever, trismus	Penicillin and aminoglycoside
	43	Type 2 diabetes	Dyspnea, dysphagia, fever	Penicillin and carbapenem
	46	Rheumatoid arthritis	Dyslalia, fever, trismus	Penicillin and nitroimidazole

The bacterial genetic evaluation (16S) of purulent exudate collected from all patients from all groups revealed a diverse bacterial profile (Figure 2). *Prevotella* was the most dominant bacterium genera, accounting for 54.6% of the microbial population. Other significant bacterial genera identified include *Amniculibacterium* (6.1%), *Fusobacterium* (5.7%), and *Lancefieldella* (5.4%). *Parvimonas* and *Lachnoclostridium* were also prevalent, comprising 4.8% and 4.2% of the population, respectively. *Olsenella* (3%), *Peptacetobacter* (2.4%), and *Streptococcus* (1.8%) were present in notable proportions. Additional bacteria such as *Murdochiella* (1.7%), *Dialister* (1.4%), and *Filifactor* (1%) were identified, albeit in smaller quantities. Minor constituents included Paraprevotella (0.9%), *Bacteroides* (0.9%), *Lachnoanaerobaculum* (0.8%), and *Slackia* (0.8%).

The analysis also detected *Mycoplasma* (0.6%), *Homo* (0.6%), *Clostridium* (0.3%), *Porphyromonas* (0.2%), *Acinetobacter* (0.2%), *Alistipes* (0.2%), and *Candidatus Sulcia* (0.1%). Other bacterial genera such as *Blautia, Lactobacillus, Eubacterium, Gemella, Mogibacterium,* and *Campylobacter* each accounted for 0.1% of the population. A broad array of other genera was detected, but in very low proportions, contributing 0.0% to 0.1% individually. These findings underscore the complexity and diversity of the microbial communities present in acute periapical abscesses and highlight the predominant role of anaerobic bacteria in such infections. This diverse microbiota composition suggests that targeted antibiotic therapies should be considered to effectively manage these infections and prevent the development of resistance.

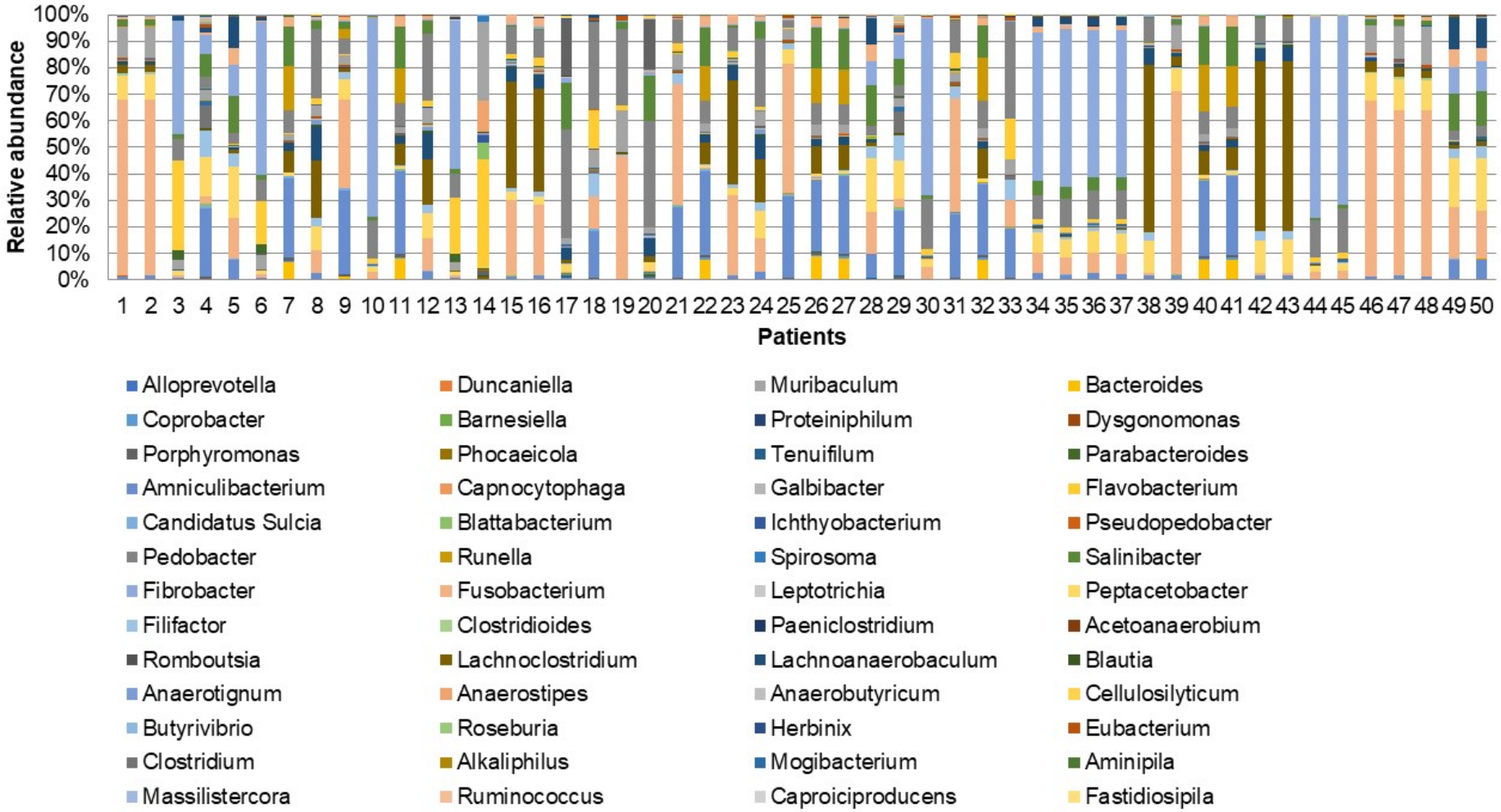


Figure 2. Relative abundance of bacterial genera in purulent exudate collected from patients with symptomatic periapical abscesses.

Each patient's microbiome was analyzed and categorized into four distinct groups according to previous antibiotic use and need for hospitalization (Figure 3). The analysis of Group 1 revealed a significant predominance of *Prevotella*, accounting for 55.9% of the relative abundance (Figure 3a). Other bacterial genera identified in smaller quantities included *Fusobacterium* (7.8%), *Parvimonas* (4.7%), *Lachnoclostridium* (9%), and *Amniculibacterium* (3.1%). The bacterial diversity observed in this group indicates a complex and varied microbiota unaffected by antibiotic selection pressure. On the other hand, a reduction in bacterial diversity was observed in group 2, with *Prevotella* remaining the most abundant genus at 54.2% (Figure 2b). Other significant genera included *Amniculibacterium* (7.1%), *Fusobacterium* (5.4%), *Parvimonas* (3.8%), and *Lachnoclostridium* (2.7%). Although same genera can be found in groups 1 and 2, *Amniculibacterium* is more evident in group 2. Subsequently, *Prevotella* (53.1%) was the most abundant bacteria genus observed in Group 3, followed by *Amniculibacterium* (7.8%), *Fusobacterium* (4.2%), *Lachnoclostridium* (2.9%), and *Parvimonas* (4.8%) (Figure 2c). The relative abundance of *Streptococcus* (1.6%) and *Parvimonas* were more evident in group 3 compared to groups 1 and 2. Finally, the analysis of Group 4 demonstrated *Prevotella* continued to be the dominant genus (55.7%), followed by *Amniculibacterium* (4.6%), *Fusobacterium* (6.3%), *Lachnoclostridium* (4.6%), and *Parvimonas* (6.6%) (Figure 2d). The high presence of *Streptococcus* (5.4%) is most evident in group 4.

Although all patients demonstrated some similarity in bacterial profile, one patient died due to worsening of the acute periapical abscess and were evaluated alone. The most dominant genus was *Prevotella*, accounting for 51.2% of the bacterial composition. *Fusobacterium* was the second most prevalent genus at 21.3%. And *Amnicilibacterium* ranks third with 12.3%. While not as commonly discussed as *Prevotella* and *Fusobacterium*, its presence in significant amounts suggests it may also contribute to the microbial landscape of the infection. Microbiome of this patient also include other notable genera: *Parvimonas* (4.2%), *Dialister* (2.6%), and *Paraprevotella* (1.8%). Less abundant genera such as *Filifactor* (1.7%), *Streptococcus* (1.3%), and *Porphyromonas* (0.3%) are also present. The patient bacterial profile also lists several genera with very low relative abundances (≤ 0.3%), including *Candidatus Sulcia, Gemella, Megasphaera, Campylobacter, Eubacterium, Lachnoclostridium, Clostridium,*

Slackia, Lancefieldella, Olsenella, Erysipelothrix, and *Runella*. The low abundance of these genera does not necessarily indicate a lack of importance, as even minor components of the microbiome can play critical roles in disease processes.

Considering all groups, it is evident that *Prevotella*, *Fusobacterium, Lachnoclostridium, Amniculibacterium, Dialister, Lachnoanaerobaculum,* and *Paraprevotella* maintained their relative abundance. In contrast, other genera, including *Peptacetobacter*, *Lancefieldella, Bacteroides, Olsenella, Slackia, and Murdochiella*, demonstrated a comparative reduction. However, the genera *Streptococcus* and *Parvimonas* showed a higher relative abundance in Group 4 compared to the other groups.

These results highlight the significant impact of antibiotic use and hospitalization on the bacterial composition of periapical abscesses. The predominance of specific genera such as *Prevotella* across all groups, and the variation in other bacterial genera, underscore the importance of considering patient history in the management and treatment of these infections. Results were also demonstrated in a heatmap for all patients (Figure S1) and for groups 2, 3 and 4 (Figure S2) due to antibiotic administration. The heatmap effectively demonstrates the variation in microbial populations across different patient samples, highlighting the complexity and heterogeneity of the microbial landscape in acute periapical abscess.

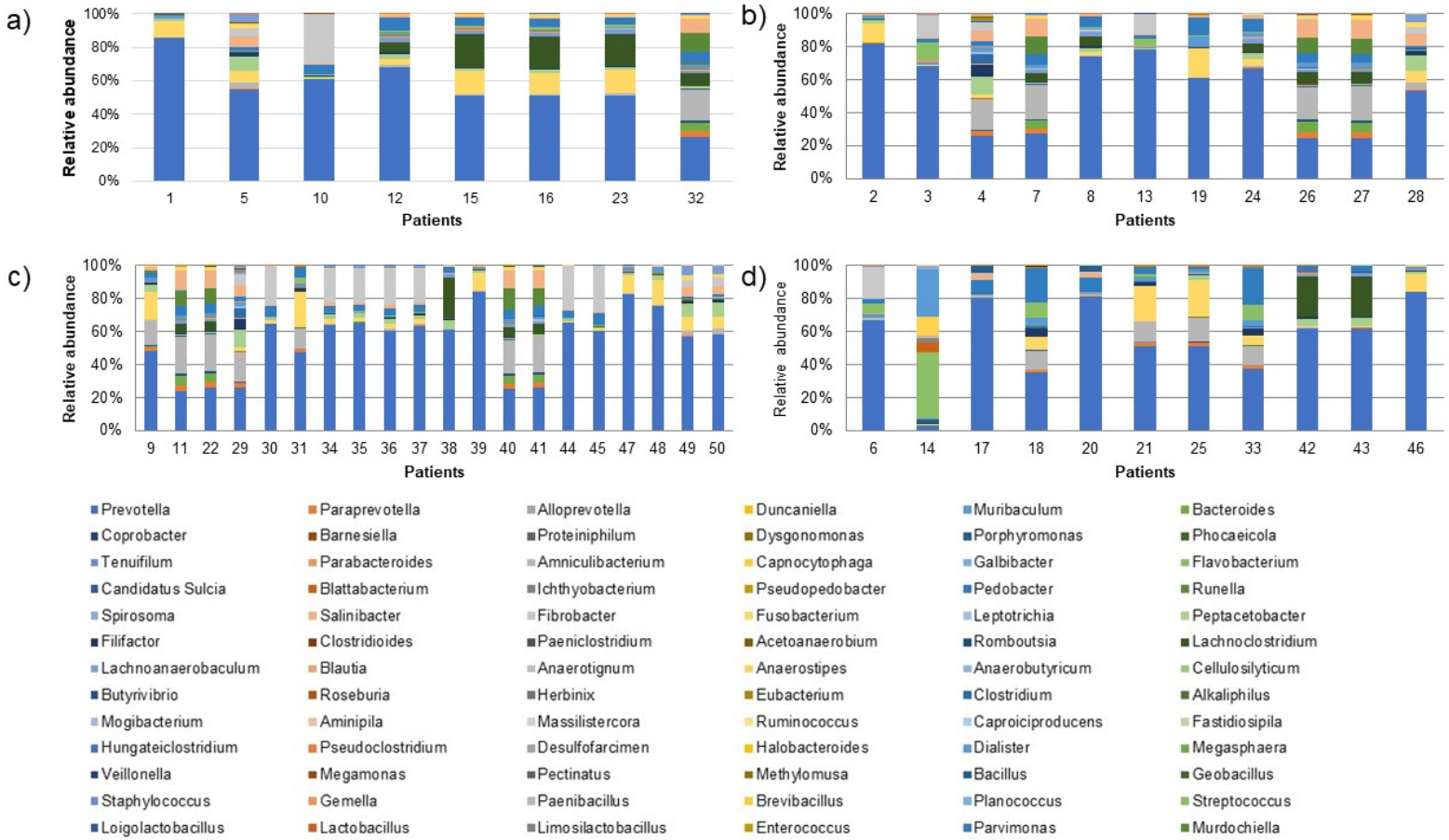


Figure 3. Relative abundance of bacterial genera in purulent exudate from patients with symptomatic periapical abscesses, divided into four groups: Group 1 (n=8): Patients with no prior use of antibiotics. Group 2 (n=20): Patients with prior use of antibiotics. Group 3 (n=11): Patients hospitalized for up to 7 days with prior use of antibiotics. Group 4 (n=11): Patients hospitalized for more than 7 days with prior use of antibiotics.

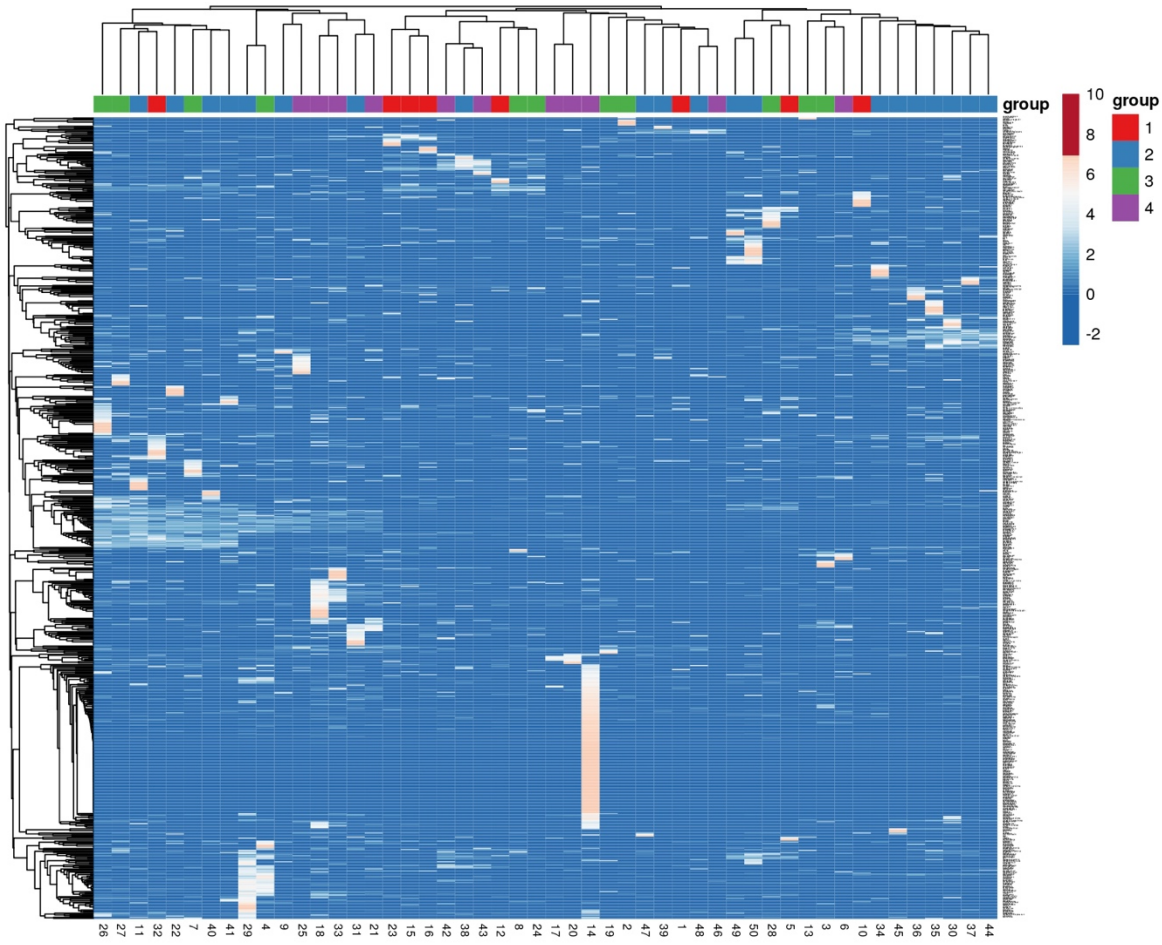


Figure S1. Heatmap of the relative abundance of bacterial genera in purulent exudate samples from patients with symptomatic periapical abscesses.

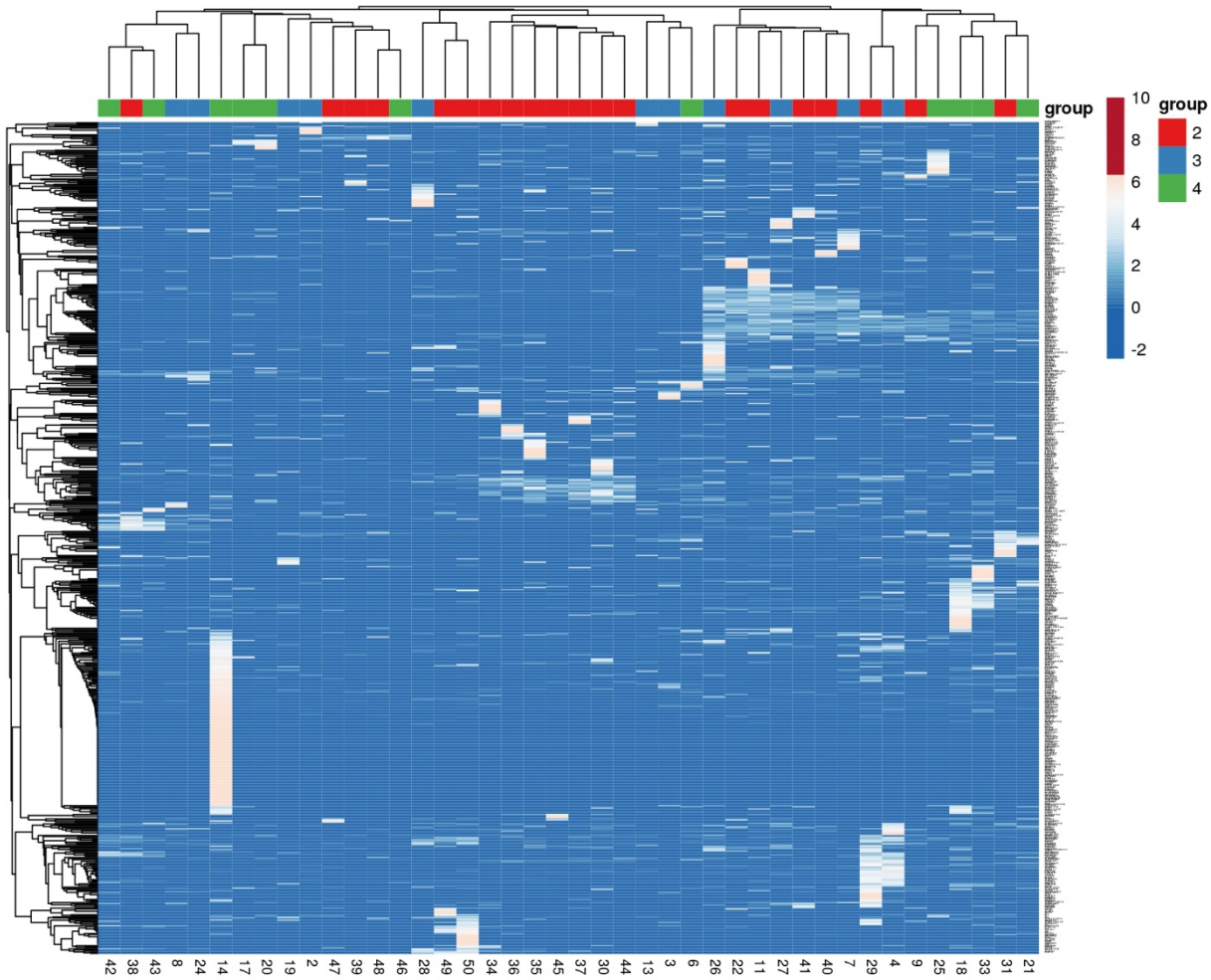
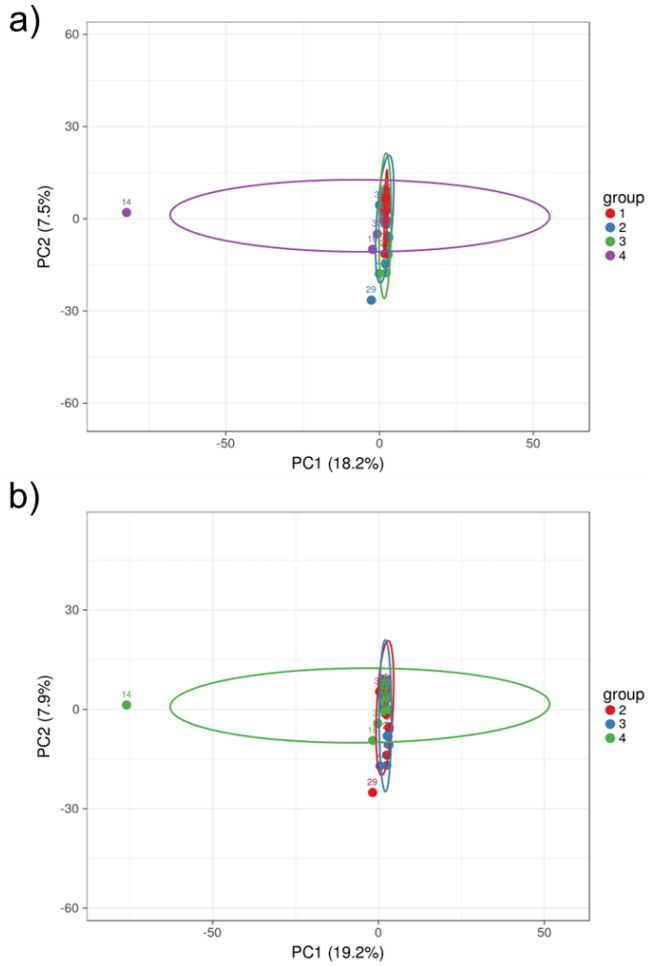


Figure S2. Heatmap of the relative abundance of bacterial genera in purulent exudate samples from groups 2, 3 and 4 (patients with symptomatic periapical abscesses and prior antibiotic administration).

The bacterial genera found in different groups were analyzed using Principal Component Analysis (PCA) to evaluate the similarity between groups (Figure 4). The first analysis illustrates the similarity across all groups (Figure 4a), while the second focuses on the similarity among groups involving prior antibiotic use (Figure 4b). Results demonstrated minimal discrepancies between the groups, indicating a high similarity in bacterial genera among the patients analyzed in the study.



- **Figure 4.** Principal Component Analysis (PCA) of bacterial genera similarity in purulent exudate samples from patients with symptomatic periapical abscesses. PCA plot compares four distinct groups based on prior antibiotic use and hospitalization status. Each point represents an individual sample, and the positioning in the two-dimensional space reflects the similarity in bacterial genera composition. The first principal component (PC1) is plotted on the x-axis, and the second principal component (PC2) is plotted on the y-axis.

DISCUSSION

The human oral cavity contains a densely populated microbial ecosystem [12, 13]. The composition of the oral microbiome in healthy adults is generally stable, with *Streptococcus*, *Neisseria*, *Veillonella*, and *Actinomyces* being the dominant genera [12]. However, microbial diversity can vary due to selective pressures, some of which include dietary modifications, systemic and local diseases, and exposure to antibiotics during treatment of infections [14].

Among the various infections that can occur in the oral cavity, symptomatic periapical abscesses is the most common form of dental abscess, originating from infection in the root canal. Normally, its location is intraoral, but in certain cases, the abscess can spread, leading to severe complications or even mortality [15]. The reasons why root canal infections become symptomatic and progress to severe disseminations, eventually resulting in potentially fatal abscesses, are not yet fully understood [14,15]. Research using culture methods and advanced molecular microbiology techniques for microbial identification in symptomatic periapical abscesses has revealed a multi-species community predominantly composed of anaerobic bacteria [13]. Commonly genera identified in these infections include *Fusobacterium*, *Parvimonas*, *Prevotella*, *Porphyromonas*, *Dialister*, *Streptococcus*, and *Treponema* [15].

Transient or permanent host-related factors can alter the course of , such as the pre-existence of systemic diseases. Thus, the presence of comorbidities and their staging can influence the course and severity of acute abscesses [15]. In this study, comorbidities such as hypertension, diabetes, HIV, lupus, and chronic renal failure seem to complicate the clinical evolution of patients, requiring a careful and individualized approach to antibiotic treatment. It has been known that dysfunctions present in diabetic patients result in greater susceptibility to serious and prolonged infections, such as acute periapical abscesses [25]. Then, personalized therapeutic approaches and strict blood glucose control are essential for the effective management of these patients [25]. Regarding lupus erythematosus, it has been known that these patients demonstrated an altered immunological response due to the dysfunction of T and B lymphocytes and the

production of autoantibodies [26]. This immune dysfunction can lead to greater susceptibility to infections, such as symptomatic periapical abscesses, delaying the inflammatory response and healing of these lesions. The immunosuppression caused by lupus and the used-medications for treatment increase the risk of infectious complications [26].

Immunosuppression is also a factor present in patients with HIV, leading to compromised immune function, mainly T cell-mediated immunity. This fact increases susceptibility to bacterial infections, including those of odontogenic origin [27]. Studies indicate that chronic kidney disease increases the prevalence of infections. The immune dysfunction associated with this disease contributes to an exacerbated inflammatory response and an increased risk of systemic complications [28]. Therefore, understanding the implications of these and other comorbidities is crucial for effective management and reduction of associated complications, ensuring safer and more effective treatment for patients with acute periapical abscess.

The presence of comorbidities was also associated with worsening signs and symptoms, such as dyspnea, fever, dysphagia, dyslalia, and trismus, which progressively worsened across the groups analyzed. Scientific evidence reveals that patients with symptomatic periapical abscesses frequently have comorbidities such as diabetes and hypertension, which can complicate treatment and increase hospitalization duration [20]. These findings emphasize the need for a careful and personalized approach in treating patients with symptomatic periapical abscesses, especially those with comorbidities. Prolonged hospitalization in these cases may be necessary to monitor and manage potential complications related to both the infection and the patient's underlying conditions, given the microbial diversity present in acute periapical abscesses.

The symptomatic periapical abscesses microbiome was performed through sequencing of the 16S rRNA gene in purulent exudate collected during initial emergency care. A complex and diverse microbial composition in acute periapical abscesses, with a significant predominance of anaerobes, especially the genus *Prevotella*, representing 54.6% of the microbial population. Studies

reveal a high prevalence of *Prevotella* in samples of acute endodontic abscesses, highlighting the transition from normal oral microbiota to acute endodontic infections, where *Prevotella* and *Fusobacterium* were the most abundant genera [20,21]. These findings underline the need to consider *Prevotella* in the choice of antibiotic therapies for acute periapical abscesses, due to its predominant role in the microbial composition of these infections.

This microbiome diversity highlights the importance of a personalized approach in prescribing antibiotics for these patients. In this study patients without prior antibiotic use demonstrated a greater microbial diversity than patients who prior used antibiotics, suggesting a direct impact of antibiotic administration on microbial composition. Irshad et al. (2021) reviewed the microbiological profile of periapical abscesses using 16S rRNA sequencing and revealed that periapical abscesses are dominated by anaerobic bacteria, such as *Prevotella*, *Fusobacterium*, and *Porphyromonas*. However, bacterial diversity can be impacted by antibiotic administration, resulting in the reduction of some species and the increase of microbial resistance [22]. Scientific studies highlight that antibiotic administration can lead to acute changes in microbial composition, enriching antibiotic-resistant organisms and significantly altering bacterial diversity [20]. These findings underline the need for treatment strategies that consider the impact of antibiotics on the microbiota of periapical abscesses. Personalized approaches and treatments that minimize antimicrobial resistance are crucial to improving clinical outcomes and maintaining the efficacy of treatments.

Most bacterial species involved with endodontic infections, including abscesses, are susceptible to penicillin [21, 22]. This makes these drugs the first choice for treating endodontic infections when the patient's allergy to penicillin is ruled out [23]. Emerging resistance to commonly used antibiotics for bacteria found in dental abscesses has been reported. A systematic review revealed that global results from laboratory studies indicate that no antibiotic is effective *in vitro* against all species found in dental abscesses [23]. *Prevotella* species have been considered prominent sources of resistance to beta-lactam agents in the oral cavity due to beta-lactamase production [24,25]. Kuriyama et al. (1998) revealed

that beta-lactamase was detected in 36% of dark-pigmented *Prevotella* species and 32% of non-pigmented *Prevotella* species isolated from exudate samples of oral abscesses [23, 24]. However, the PCA analysis demonstrated high similarity in the composition of bacterial genera among abscesses upon admission to the dental emergency service, despite the prior use of antibiotic and hospitalization need. This finding suggests that antibiotic interventions should consider the patient's prior history and comorbidities to effectively manage infections and prevent the development of resistance.

CONCLUSION

Microbiological analysis revealed a significant predominance of anaerobic bacteria, especially of the genus *Prevotella*, highlighting the importance of considering these microorganisms in the therapeutic choice of antibiotics. Microbial diversity was affected by prior antibiotic administration. Comorbidities such as diabetes, hypertension, HIV, lupus, and chronic renal failure aggravate the clinical course of periapical abscesses, resulting in more severe signs and symptoms and a longer duration of hospitalization. These conditions require personalized therapeutic approaches and careful management to minimize complications. Future research should focus on a deeper understanding of the mechanisms by which these comorbidities influence microbial composition and treatment response in patients with symptomatic periapical abscesses.

ACKNOWLEDGMENTS

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4.3. Capítulo 3: Manuscrito 3

Artigo preparado para ser submetido na revista: *International Endodontic Journal,* classificada como A1 no sistema Qualis da CAPES

Next-generation Sequencing Analysis of ITS1 in Symptomatic Periapical Abscesses

ABSTRACT

Symptomatic periapical abscesses are a significant dental condition marked by the accumulation of pus around the tooth root apex, typically caused by bacterial infections. Recent evidence highlights the role of fungi, particularly Candida species, in endodontic pathologies. This study investigates the presence and diversity of fungi in the purulent exudate of patients with acute periapical abscesses using Next-generation Sequencing Analysis of ITS1 gene. Fifty hospitalized patients with symptomatic periapical abscesses were categorized into four groups based on prior antibiotic use and hospitalization necessity. ITS sequencing was performed to identify fungal genera in the exudate samples. Patients' systemic comorbidities were also assessed. ITS sequencing revealed a diverse fungal population in purulent exudate, with *Talaromyces* being the most prevalent genus at 42.6%, followed by *Pichia* (16.2%) and *Kluyveromyces* (15.6%). Significant differences in fungal composition were observed across the groups, influenced by prior antibiotic use and hospitalization. Patients with severe comorbidities exhibited higher fungal diversity. This study highlights the diverse fungal microbiome present in acute periapical abscesses. The findings underscore the importance of considering fungal involvement in the diagnosis and treatment of periapical abscesses, particularly in patients with systemic comorbidities. A comprehensive therapeutic approach addressing both bacterial and fungal infections is crucial for improving clinical outcomes.

Keywords: Symptomatic periapical abscesses, systemic comorbidities, fungi, ITS1

INTRODUCTION

Symptomatic periapical abscesses are a common and significant dental condition characterized by the accumulation of pus within the tissues surrounding the apex of a tooth root, often resulting from bacterial infection [1]. These infections can cause severe pain, swelling, and systemic complications, making timely and accurate diagnosis crucial [1,2]. Traditional diagnostic methods primarily focus on the identification of bacterial pathogens; however, emerging evidence suggests a role of fungi in the pathogenesis of endodontic and periapical pathologies [3,6].

Fungi, particularly Candida species, have been increasingly recognized as opportunistic pathogens in various oral and systemic infections [6]. The identification of fungal involvement in endodontic infections has important implications for treatment strategies due to the necessity of reducing both bacterial and fungal infections. Despite this, the prevalence, and specific roles of fungi in periapical pathology remain poorly explored, necessitating further investigation using advanced molecular techniques [8].

The Internal Transcribed Spacer (ITS) region of the ribosomal RNA gene complex is widely accepted as a robust molecular marker for fungal identification due to its high variability among species [4]. ITS sequencing has proven effective in detecting and differentiating fungal species in clinical samples, providing a more comprehensive understanding of the microbial landscape in infections [5]. Application of ITS-based genetic analysis to periapical abscesses can reveal the presence and diversity of fungal species, thereby improving our understanding of etiology and definition of treatment protocols.

Additionally, systemic comorbidities such as diabetes, HIV, lupus, and chronic renal failure can significantly influence the progression and outcome of periapical abscesses [8,9]. These conditions often impair the immune system, making individuals more susceptible to infections and potentially altering the microbial profile of the abscesses. For instance, diabetic patients are more prone

to severe infections and may exhibit a different spectrum of pathogens, including a higher prevalence of fungal organisms [8,9]. Understanding the interplay between systemic health and periapical infections is crucial for developing effective treatment strategies tailored to patients with systemic comorbidities, ultimately aiming to improve clinical outcomes and reduce complications [9,10]**.** Then, this study aims to evaluate the presence and diversity of fungi in the purulent exudate collected from patients with acute periapical abscesses using ITS sequencing. By elucidating the fungal profile in infections, we hope to contribute to a better understanding of the etiopathogenesis allowing the design of more assertive strategies for therapeutic proposals for future.

METODOLOGY

Study population

This study included 50 patients diagnosed with symptomatic periapical abscesses, admitted to the emergency department of the Instituto Hospital de Base do Distrito Federal (IHBDF - Brazil) over a 12-month period (January 2019 to December 2019). Patients with diagnoses not aligned with the study's objectives and those with incomplete medical records were excluded. All patient data were kept confidential according to agreed terms. All participants signed the informed consent form. The study was approved by the ethics committee of the Universidade Católica de Brasília (CAAE: 57475816.8.0000.0029), the research committee of IHBDF (CAAE: 57475816.8.3001.5553) and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen: AE5000A). All patients received standard emergency care for acute conditions, including drainage, blood tests, antibiotic therapy, and hospitalization if necessary. The exudate collected during the initial emergency care was immediately frozen at -80°C. Hospitalized patients were discharged after the acute condition had resolved. The collected exudates were categorized into the following groups based on prior antibiotic use and the need for hospitalization (Figure 1):

- Group 1 (n = 8 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency without prior use of antibiotics and did not require hospitalization.
- Group 2 (n = 20 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, with prior use of antibiotics, and did not require hospitalization.
- Group 3 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, with prior use of antibiotics, and required hospitalization for up to 7 days.
- Group 4 (n = 11 patients) – Patients with symptomatic periapical abscesses who accessed the hospital emergency, with prior use of antibiotics, and required hospitalization for periods longer than 7 days.

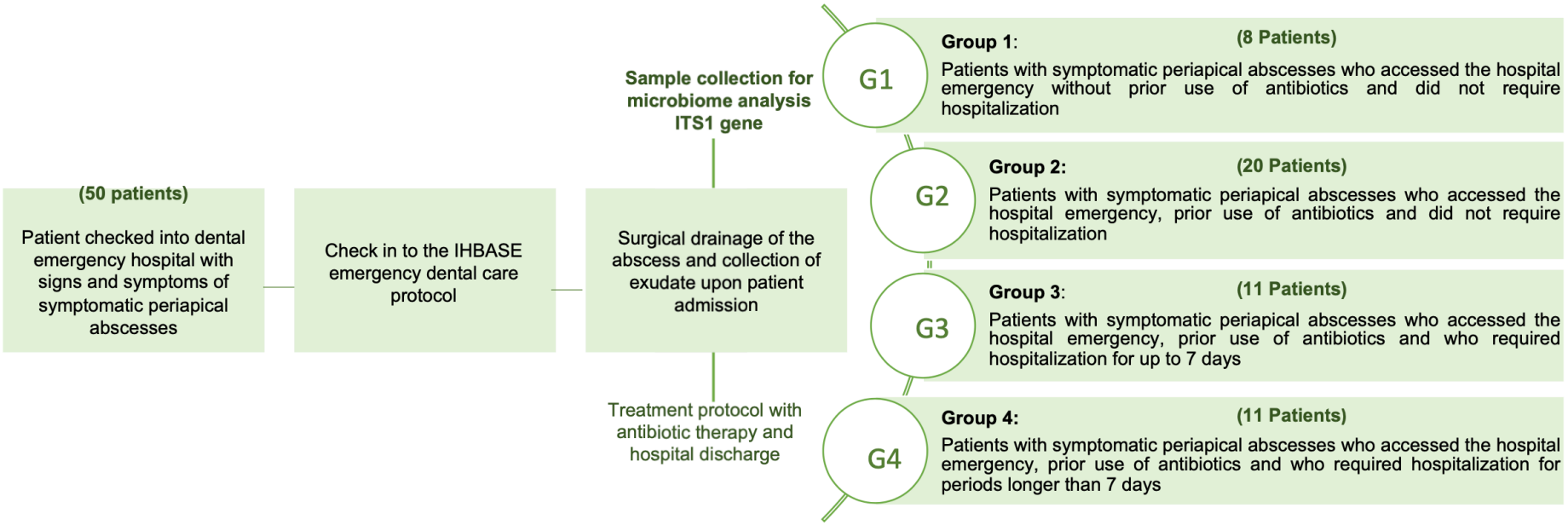

Figure 1: Flowchart of care steps for patients with symptomatic periapical abscesses and division of study sample groups.

Data Collection from Care Protocols

Personal information, anamnesis, including health history and previous medication therapy prior to admission, as well as patients' antibiotic therapy, were documented in the medical record during care and accessed later. Medical and dental progress of patients who required hospitalization was also monitored. All complementary exams were performed according to IHBASE's care protocols, from the moment of hospital admission to discharge.

Drainage and Patients Exudate Samples

Patients participating in the study were admitted to the outpatient clinic, where purulent exudate was collected for fungal microbiota analysis. First, the affected area was disinfected with 2% chlorhexidine digluconate (Rioquímica, São Paulo, Brazil). Local anesthesia was then administered, followed by an incision with a scalpel blade and an extraoral/intraoral puncture of the region using a needle (40 x 12 mm) and a 50 mL syringe to aspirate exudate from the affected facial spaces. The exudate was collected through aspiration, immediately separated into small aliquots, and stored at −80°C (11).

DNA Extraction

Fungal microbiome analysis was performed through sequencing of the ITS rRNA gene in purulent exudate collected during initial emergency care, allowing the identification and characterization of the bacterial population. Therefore, DNA extraction was performed using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) for amplification and sequencing of the ITS1 rRNA genes. Subsequently, these products were quantified via Qubit® (Life Technologies) and Nanodrop (Thermo Scientific™) and then sent directly to Genotyping Laboratório de Biotecnologia LTDA/ BPI – Biotecnologia Pesquisa e Inovação (Brazil) (12).

ITS1 rRNA sequencing

After DNA extraction, the samples for sequencing of the ITS1 genes in paired-end mode (2 x 250 bp). The amplicon preparation was completed with the attachment of barcodes using the Nextera® XT Index Kit and the run was

performed on the MiSeq Illumina platform. For hybridization of the conserved region of the ITS1 rDNA gene (TCCGTAGGTGAACCTGCGG) was used (13). The quality of the raw data generated by each sample was checked using the FastQC tool, which indicated the need for preprocessing of these data to remove low-quality base regions from the reads. Therefore, the Trimmomatic program was used with the parameters SLIDINGWINDOW:5:20 and MINLEN:50, ensuring the retention of only high-quality sequences, which was confirmed by another round of analysis with FastQC. Next, the Kraken2 tool was used for the taxonomic classification of the samples at the genus level. For comparison purposes, a ITS1 sequence library from RefSeq was chosen as the database. In the next step, the results generated by Kraken2 were analyzed using the online tool Pavian, which allows visualization of tabular data and comparative analysis between samples. Finally, the ClustVis program was used to perform PCA analyses and generate heatmap figures of the samples.

RESULTS

This study evaluated 50 hospitalized patients diagnosed with symptomatic periapical abscesses, categorized into four groups based on prior antibiotic use and hospitalization necessity. Table 1 presents the distribution of comorbidities among the patients in different groups who were admitted with a diagnosis of acute periapical abscess. ITS genetic sequencing was performed to access the fungal microbiome present in the purulent exudate upon patient admission to the dental emergency room.

Previous comorbidities reported by patients were verified in dental records (Table 1). Hypertension and type 2 diabetes were prevalent comorbidities reported by patients include in Group 1. While a significant number of patients did not declare any comorbidities, suggesting a relatively healthier baseline or underreporting. Group 2 patients reported a higher prevalence of hypertension, lupus, and type 1 diabetes. The high number of patients not declaring comorbidities could indicate either a lack of awareness or a tendency to overlook less severe conditions. However, patients included in Group 3 reported a notable increase of more severe comorbidities such as HIV, lupus, rheumatoid arthritis, and chronic renal failure. This group had a higher burden of chronic illnesses,

which might have contributed to the necessity of hospitalization even with prior antibiotic use. In addition, patients include in Group 4 had complex comorbidities that affect kidney, heart and associated metabolic functions. The prolonged hospitalization in this group highlights the impact of multiple and severe comorbidities on the clinical course and recovery from symptomatic periapical abscesses.

Table 1: Distribution of patients between different groups and their systemic comorbidities.

Group	Patient ID	Comorbidities
1 (no prior antibiotic use)	1	Hypertension, type 2 diabetes
	5	Not declared
	10	Not declared
	12	Type 2 diabetes
	15	Hypertension
	16	Not declared
	23	Hypertension
	32	Type 1 diabetes
2 (prior antibiotic use and no hospitalization)	9	Hypertension
	11	Not declared
	22	Not declared
	29	Hypertension
	30	Hypertension
	31	Not declared
	35	Not declared
	36	Not declared
	37	Not declared
	38	Not declared
	39	Hypertension, type 1 diabetes
	40	Lupus
	41	Hypertension
	44	Not declared
	45	Not declared
	47	Not declared
	48	Not declared
	49	Not declared
	50	Not declared
3 (prior antibiotic use and hospitalization - 7 days)	2	HIV
	3	Lupus
	4	Not declared
	7	Rheumatoid arthritis
	8	Not declared
	13	Hypertension, chronic renal failure, type 2 diabetes
	19	Lupus

	24	HIV
	26	Hypertension, type 2 diabetes
	27	Hypertension, type 1 diabetes
	28	Hypertension, arthritis
4 (prior antibiotic use and hospitalization < 7 days)	6	HIV
	14	Type 1 diabetes
	17	Hypertension, type 2 diabetes, chronic renal failure
	18	Type 1 diabetes
	20	Rheumatoid arthritis, hypertension
	21	Chronic renal failure, type 2 diabetes, hypertension
	25	Lupus

The genetic analysis of the purulent exudate samples from 50 patients, focusing on the ITS conserved region, revealed the average relative abundance of various fungal genera (Figure 2). The most prevalent genus was *Talaromyces*, with an average relative abundance of 42.6%, followed by *Pichia* and *Kluyveromyces*, which had abundances of 16.2% and 15.6%, respectively. *Sugiyamaella* was also notable, present at 8.8%, while *Botrytis* was detected at 4.7%. Moderate amounts of *Candida* and *Malassezia* were found, with relative abundances of 3.4% and 3%, respectively. Additionally, *Eremothecium* and *Brettanomyces* were present at 2.5% and 1.3%. Lesser amounts of *Torulaspora* and *Saccharomyces* were observed, at 1.2% and 0.6%, and *Zygotorulaspora* was poorly detected in any samples. These findings highlight a diverse fungal population, with *Talaromyces* being the dominant genus in the samples.

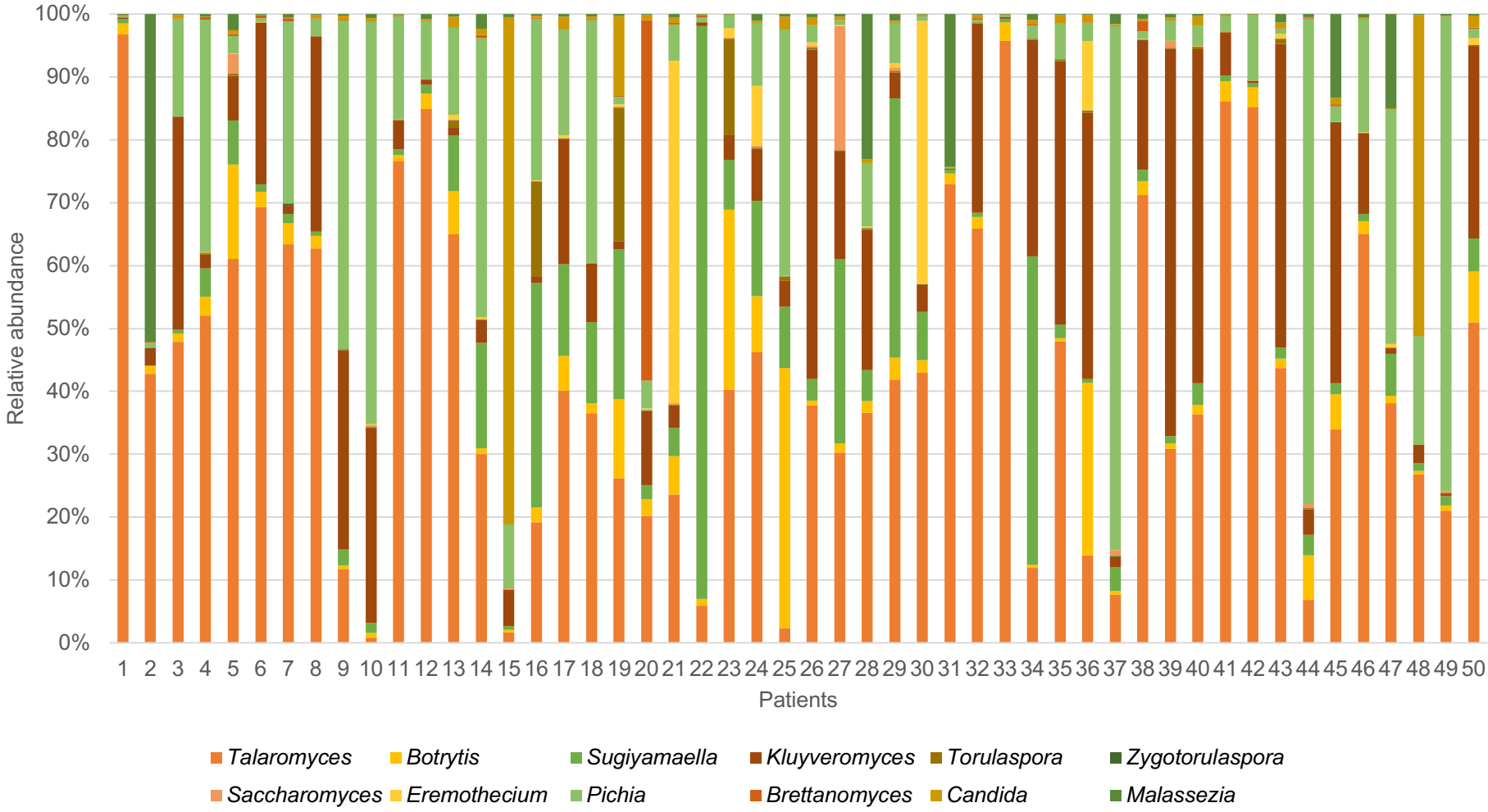

Figure 2. Relative average abundance of fungal genera in purulent exudate samples of patients with symptomatic periapical abscesses upon arrival at the emergency department.

Separate analyses of fungal genera were conducted according to prior use of antibiotics and need for hospitalization (Figure3). *Talaromyces* was the most prevalent, with a relative abundance of 46.3%, followed by *Pichia* at 14.3%, and *Candida* at 10.4% in patients without prior use of antibiotics (Group 1). *Kluyveromyces* and *Sugiyamaella* were present at 10% and 6.9%, respectively, while *Botrytis*, *Torulaspora* and *Malassezia* showed relative abundances of 6.6%, 3.9%, and 0.6%. Lesser amounts were observed for *Saccharomyces* (0.5%), *Eremothecium* (0.3%), and *Brettanomyces* (0.1%).

A few differences were found in Group 2 (G2). *Talaromyces* abundance decreased to 36.8%, while *Pichia* increased to 19.6%. *Kluyveromyces* and *Sugiyamaella* also increased to 19.4% and 11.3%, respectively. *Botrytis* decreased to 3.5%, and *Candida* to 3.1%. *Malassezia* increased significantly to 3%. *Eremothecium* rose to 2.8%, while *Torulaspora* decreased to 0.1%, and *Brettanomyces* remained at 0.2%. *Saccharomyces* fell to 0.2%, and *Zygotorulaspora* was poorly detected.

However, Group 3 (G3) showed a resurgence in *Talaromyces* to 46.4%, while *Pichia* decreased to 11.2%. *Kluyveromyces* remained steady at 15.8%, and *Sugiyamaella* slightly decreased to 8.4%. *Botrytis* further decreased to 3.9%, and *Candida* fell to 1.7%. *Malassezia* saw a significant increase to 7.2%. *Eremothecium* remained low at 1.1%, while *Torulaspora* increased to 2.1%, and *Brettanomyces* remained at 0.2%. *Saccharomyces* increased to 1.9%, and *Zygotorulaspora* remained low at 0.1%.

Finally, it was possible to found in Group 4 (G4) that *Talaromyces* maintained its prevalence at 46.5%. *Pichia* increased slightly to 16.3%, while *Kluyveromyces* decreased to 12.7%. *Sugiyamaella* fell to 6%, and *Botrytis* remained steady at 6.4%. *Candida* showed a significant decrease to 0.8%, while *Malassezia* remained low at 0.6%. *Eremothecium* increased to 5.1%, and *Torulaspora* remained low at 0.2%. *Brettanomyces* increased to 5.3%, *Saccharomyces* decreased to 0.1%, and *Zygotorulaspora* was poorly detected in samples.

Genera results was also demonstrated by a heatmap (Figure S1). This heatmap highlights the dynamic and complex nature of the fungal populations across different patient groups, with certain genera maintaining consistent levels while others fluctuate significantly.

Analyzing the fungal pattern between the different groups, some points are highlighted. *Candida* shows a dramatic decrease in relative abundance when comparing group 1 with groups 2, 3 and 4, suggesting that both antibiotic use and the length of hospitalization significantly impact its prevalence. The marked reduction from Group 1 (10.4%) to Group 4 (0.8%) indicates that *Candida* is highly sensitive to these factors. Another intriguing fungal pattern was related to the *Malassezia* genus, which showed a significant increase in Group 3 (patients with antibiotic use and a hospital stay of up to 7 days). This spike suggests that the combination of antibiotics and a relatively short hospital stay may favor the growth of *Malassezia*. On the other hand, *Brettanomyces* shows a notable increase in Group 4, indicating that extended hospital stays with prior antibiotic use significantly favor its growth. This is particularly interesting as *Brettanomyces* is almost nonexistent in the other groups.

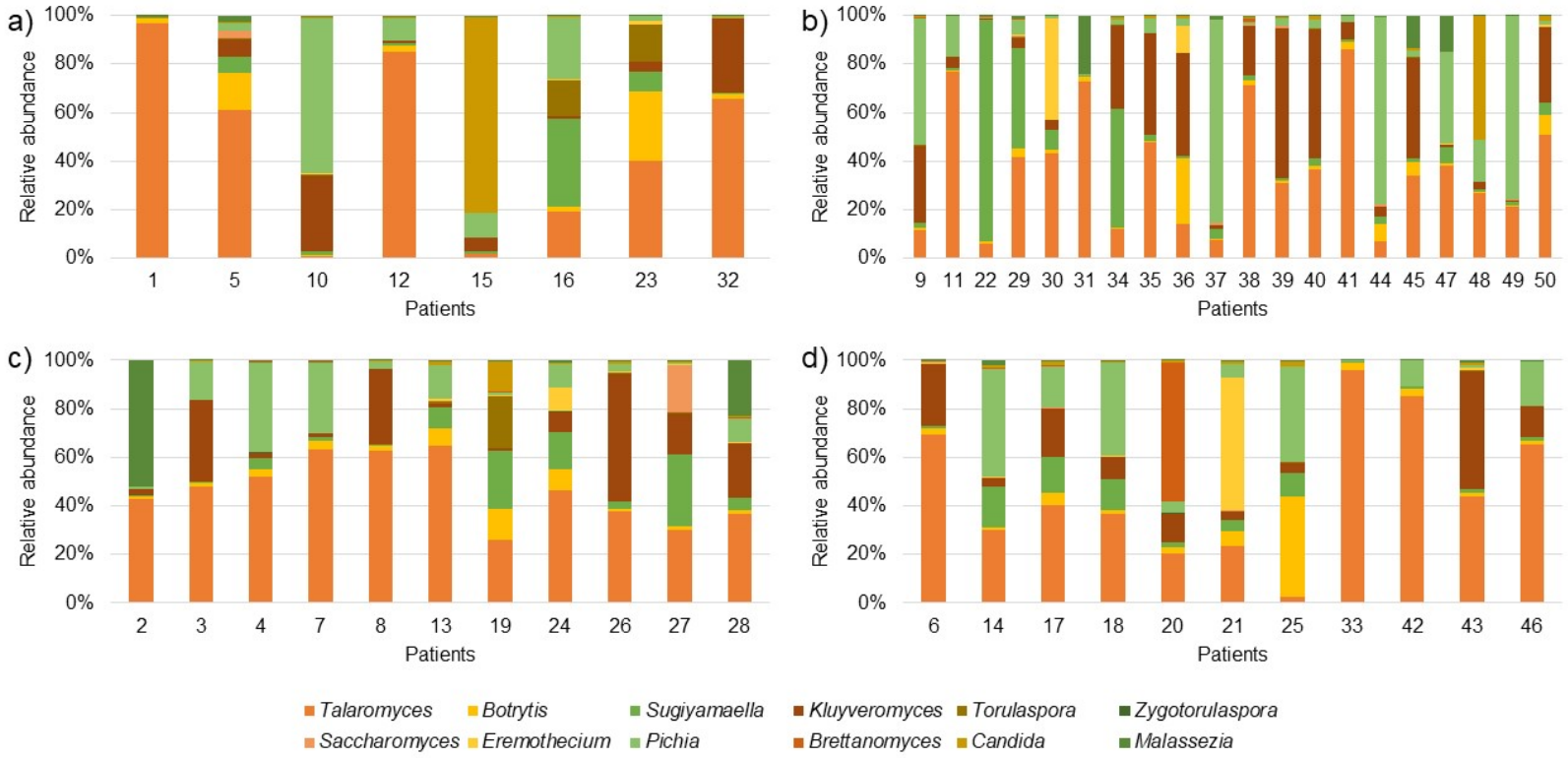

Figure 3. Relative abundance of fungal genera in purulent exudate from patients with symptomatic periapical abscesses, divided into four groups: Group 1 (n=8): Patients with no prior use of antibiotics. Group 2 (n=20): Patients with prior use of antibiotics. Group 3 (n=11): Patients hospitalized for up to 7 days with prior use of antibiotics. Group 4 (n=11): Patients hospitalized for more than 7 days with prior use of antibiotics.

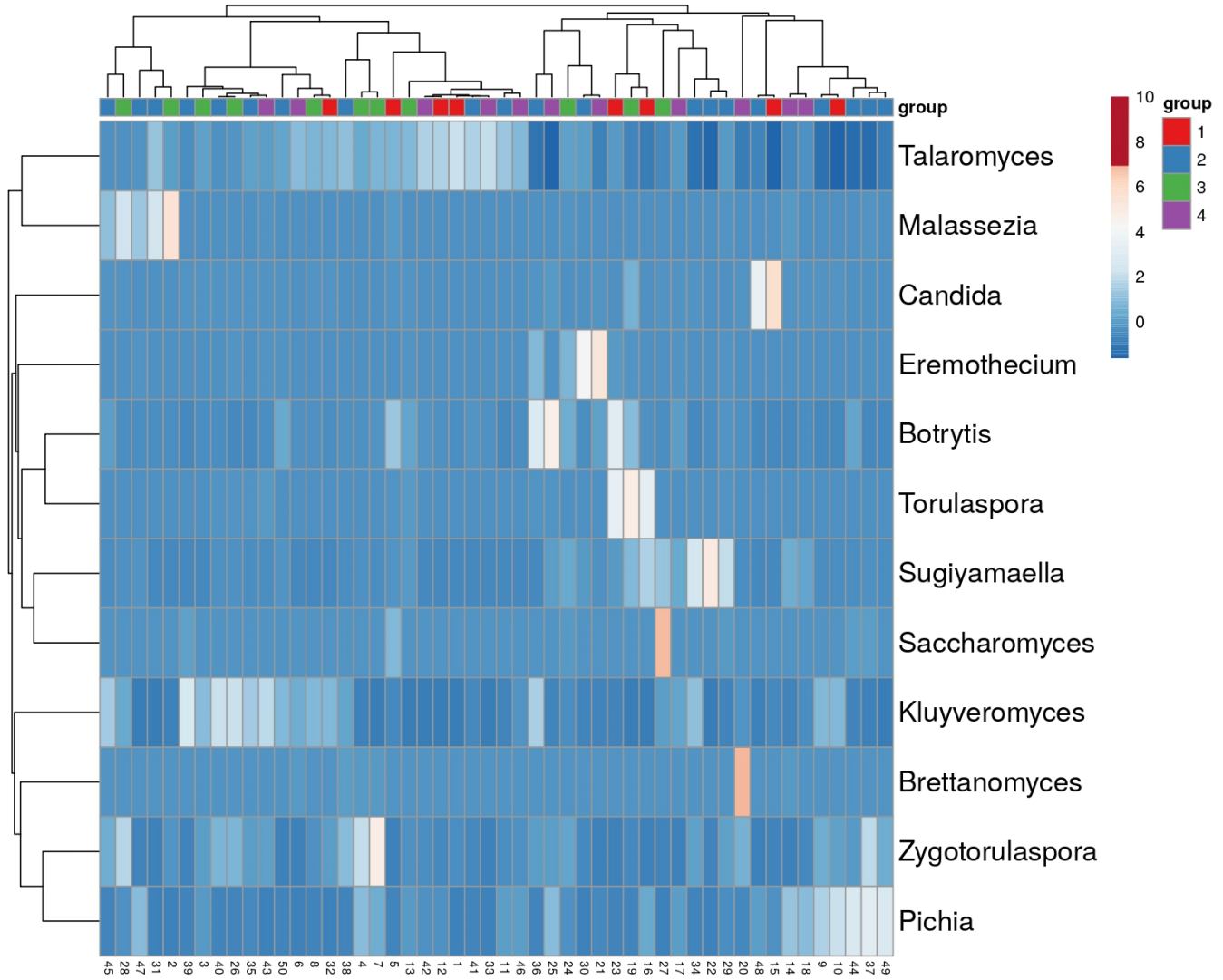

Figure S1. Heatmap of relative abundance of various fungal genera across all patients. Color intensity represents the abundance levels, with darker shades indicating higher abundance.

Fungi genera was also evaluated by PCA analysis to represents a combination of variables that capture the most significant variance within the data. This analysis helps to identify patterns and similarities between all groups (Figure 4a) and different groups (Figure 4b). PCA analysis can demonstrate that Groups 1 and 2 share more similarities in their microbial genera compared to Groups 3 and 4. It is also important to see that 3 groups (Groups 2, 3 and 4) are similar although group 3 is more variable (Figure 3b).

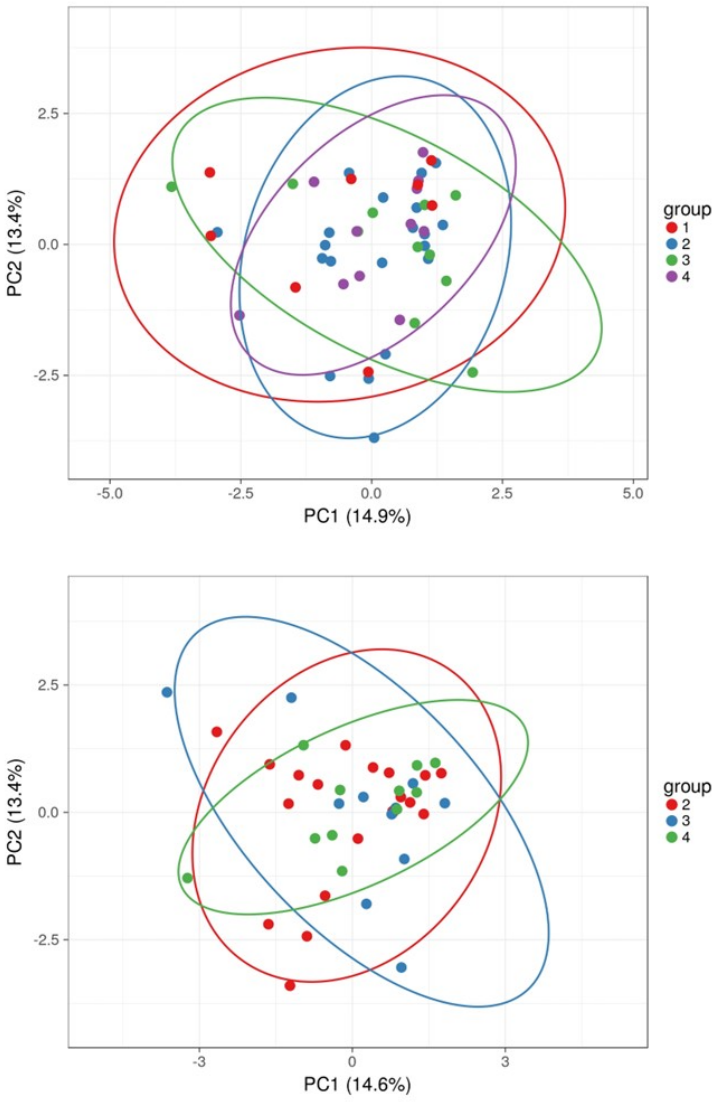

Figure 4. PCA Analysis for ITS – Genera. PCA plot displays the distribution of samples from all groups (a) and groups 2, 3, and 4 (b) based on the ITS region data for various genera.

DISCUSSION

The oral microbiota contributes to health and disease, and its disruption can influence the course of oral diseases [14]. Recent studies using advanced molecular biology techniques such as next-generation sequencing (NGS) have revealed a greater diversity of microorganisms in the oral cavity than was previously known [14]. Different microorganisms coexist in the oral microbiome, including bacteria, fungi, viruses, and archaea [15]. These microorganisms establish different relationships with each other in the microbiome, including additive and synergistic relationships [14,15]. Cells and components of the immune system act to maintain the balance between the microbiome and the host's defenses [15].

Microbial imbalance may trigger situations of oral diseases such as cavities, pulp necrosis and periodontal disease that can evolve into more serious conditions [15]. When pulp necrosis sets in, pulp defenses no longer exist, and the infection progresses to the periapical space [16]. Depending on microbial diversity and virulence and host defenses, acute or chronic conditions can be established in the periapical space [16]. Among acute conditions, acute periapical abscess deserves attention as it can lead to major repercussions [15,16].

Symptomatic periapical abscesses is characterized as purulent infections located in the periapical region of teeth, primarily resulting from bacterial infections that reach the dental pulp [17,18]. The evolution of a periapical abscess can vary, but it generally follows an acute inflammatory course with intense pain and swelling, potentially progressing to more severe complications such as osteomyelitis or systemic dissemination of the infection, requiring hospital care and even hospitalization, in some cases [17,18].

Bacteria play a central role in the formation and progression of abscesses [19,20,21]. However, recent studies have highlighted the importance of the presence of fungi in the microbial community of abscesses [17,24]. The coexistence of fungi and bacteria can influence the pathogenicity of the infection and the host's inflammatory response [22,23,25]. The role of fungi in periapical abscesses and endodontic infections has been largely unexplored until recently. Studies suggest that fungi may contribute to treatment resistance and the chronicity of endodontic infections. The ability of fungi to form biofilms, resist antimicrobial agents, and interact with pathogenic bacteria can complicate the complete eradication of the infection [23].

Fungi can form biofilms in the root canal system, similar to bacterial biofilms, which provide them with protection against antifungal treatments and host immune responses [21,23]. The biofilms created by fungi like *Candida* spp. are often more resilient and can interact synergistically with bacterial biofilms, enhancing the overall pathogenicity of the infection. The coexistence bacteria-fungi complicates the eradication of the infection and often leads to treatment failures [23,26]. Furthermore, the use of antibiotics and the hospital environment can lead to oral dysbiosis, promoting a cascade of effects that impact oral health [14,15]. In addition, fungal dysbiosis can significantly increase the risk of secondary bacterial infections [23,24].

In our work, we detected several different fungal genera, with *Talaromyce* being the most abundant. This genus includes approximately 110 approved species divided into seven sections: *Bacillispori, Helici, Islandici, Purpurei, Subinflati, Talaromyces, and Trachyspermi* [28]. *Talaromyces marneffei* is an emerging pathogen causing systemic mycosis in immunocompromised patients in Southeast Asia, India, and China. Despite the rarity of *Talaromyces* infections, Guevara-Suarez *et al*. (2017) introduced four novel species from clinical sources, highlighting their potential pathogenic roles [28,29,31]. These species were isolated from various clinical samples, suggesting possible involvement in respiratory, joint, and otological infections [29].

The second most abundant genus was *Pichia*. Although *Pichia* infections are rare, certain species like *Pichia kudriavzevii* can cause infections, particularly in immunocompromised patients, and are sometimes resistant to antifungal treatments like fluconazole. Research by Opulente *et al*. (2019) highlighted the ecological distribution of *Pichia kudriavzevii* in non-clinical environments, showing its significant presence in diverse ecological niches and potential as an opportunistic pathogen [30,33].

Kluyveromyces, the third most expressive genus, is widely recognized for its industrial and biotechnological applications, particularly in the production of dairy products, bioethanol, and various enzymes. *The genus Sugiyamaella* is primarily found in natural environments like decaying wood and insects. *Candida genus* showed significant differences across various groups. Candida can cause infections like oral candidiasis when the immune system is weakened, or the natural microbiota is disrupted. The immune response plays a crucial role in maintaining the balance between Candida as a commensal organism and its pathogenic state. Disruptions in

immune responses can lead to infections, especially in immunocompromised individuals [23,34].

The genus *Malassezia* also presented a substantial difference in Group 3 in compare with the other groups. *Malassezia*, notably differing in prevalence among groups, this includes lipophilic yeasts that are part of the normal skin microbiota. These fungi are associated with various skin conditions such as pityriasis versicolor, seborrheic dermatitis, and atopic dermatitis. They metabolize skin lipids, producing irritant compounds that trigger inflammatory responses [23,35].

Understanding the roles and interactions of these fungal genera in the oral microbiome is crucial. While many are not typically associated with infections, their presence can significantly alter the oral microbiome's balance, potentially affecting immune responses and leading to secondary bacterial complications. This delicate balance of microorganisms is essential for maintaining oral health and preventing broader health issues, such as systemic complications [27,36].

Moreover, immunocompromised patients, such as those with diabetes, HIV, lupus, and chronic renal failure, may be more susceptible to fungal infections, increasing the complexity of treating periapical abscesses in these individuals [17,24]. Among these conditions, our study highlights diabetes, HIV, lupus, and chronic renal failure. It has been observed that auxiliary therapies for treating oral infections often do not include specific treatment for fungi, focusing predominantly on bacterial antibiotics. However, the presence of fungi in the oral cavity and their contribution to oral diseases are issues that need to be better understood and addressed. Studies such as Mukherjee *et al.* (2014) show that fungal colonization is complex and can have significant interactions with the bacterial microbiota and the host’s immune system.

Fungi can transition from being commensal organisms to pathogenic agents, particularly in immunocompromised individuals. Conditions such as HIV/AIDS, diabetes, cancer, and the use of immunosuppressive drugs can disrupt the body's natural defenses, making it more susceptible to opportunistic fungal infections. *Candida albicans*, for example, is a commensal yeast that can cause systemic candidiasis when the host's immune system is weakened [41,42,45].

Similarly, *Aspergillus* species can cause invasive aspergillosis, particularly in patients undergoing chemotherapy or organ transplantation. This condition can lead to severe respiratory issues and can disseminate to other organs if not treated effectively [42,43]. Other fungi, such as *Cryptococcus neoformans*, are known to cause

cryptococcal meningitis, particularly in HIV-positive individuals, further illustrating the impact of fungal infections on systemic health [44,45].

Despite these advancements, the role of fungi in systemic diseases and their specific treatment protocols remain underexplored. There is a pressing need for more research to understand the pathogenic mechanisms of fungi and to develop targeted therapies that can effectively manage fungal infections [41,43,44]. After all, we know that the oral cavity is a vital entry point for several microorganisms, including bacteria, fungi, and viruses. It maintains a delicate balance of these microorganisms to preserve oral health. The oral cavity represents complex ecosystem where a delicate balance of microorganisms is essential for maintaining oral health [14,15]. While many fungal genera are not typically associated with infections, their presence can significantly alter the oral microbiome's balance, potentially affecting the immune response and leading to secondary bacterial complications [14,15].

CONCLUSION

This study provided a comprehensive insight into the fungal diversity in purulent exudates from patients with symptomatic periapical abscesses, highlighting the predominant presence of genera such as *Talaromyces, Pichia*, and *Kluyveromyces.* Our findings suggest that the diversity and abundance of fungi are significantly influenced by prior antibiotic use and the necessity of hospitalization, with notable changes in the fungal composition among different patient groups. These results underscore the need to consider the presence of fungi in the diagnostic and therapeutic approach to symptomatic periapical abscesses, especially in patients with severe comorbidities. Additionally, they emphasize the importance of a therapeutic management that accounts for not only bacterial infections but also fungal colonization, to improve clinical outcomes and reduce the rate of treatment failures.

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5. CONSIDERAÇÕES FINAIS

A partir das conclusões apresentadas, podemos inferir que os abcessos sintomáticos de origem endodôntica representam um desafio clínico significativo, especialmente quando associados a comorbidades sistêmicas. A identificação precoce e o tratamento adequado dessas infecções são essenciais para evitar complicações severas e melhora dos resultados clínicos. A abordagem terapêutica deve ser abrangente, incluindo drenagem cirúrgica eficaz, antibioticoterapia apropriada e monitoramento contínuo das condições sistêmicas dos pacientes.

A predominância de bactérias anaeróbias, como *Prevotella*, destaca a necessidade de um entendimento aprofundado da microbiologia dos abscessos periapicais para a seleção de terapias antimicrobianas eficazes. Além disso, a administração prévia de antibióticos, sem a contínua atenção ao atendimento da urgência, demonstrou um impacto significativo na diversidade microbiana. Desta forma, é recomendado que o uso de antibióticos deve ser cuidadosamente considerado e orientado em conjunto com planejamento do restante do tratamento.

A presença de comorbidades como diabetes, hipertensão, HIV, lúpus e insuficiência renal crônica foi identificada como um fator agravante no curso clínico dos abscessos periapicais. Estes pacientes exibiram sinais e sintomas mais severos e necessitando de hospitalizações mais prolongadas, o que sublinha a importância de abordagens terapêuticas personalizadas. Futuros estudos devem se concentrar em desvendar os mecanismos pelos quais essas comorbidades influenciam a composição microbiana e a resposta ao tratamento.

Por fim, este estudo revelou uma diversidade fúngica significativa nos exsudatos purulentos de abcessos sintomáticos de origem endodôntica, sugerindo que fungos como *Talaromyces*, *Pichia* e *Kluyveromyces* desempenham um papel relevante na patogênese dessas infecções. A inclusão da avaliação fúngica na abordagem diagnóstica e terapêutica é crucial, especialmente em pacientes com comorbidades graves. O manejo terapêutico deve considerar tanto infecções bacterianas, quanto fúngicas para melhorar os desfechos clínicos e reduzir a incidência de falhas terapêuticas.

Estas considerações ressaltam a complexidade do manejo de urgência dos abcessos sintomáticos de origem endodôntica e a necessidade de abordagens terapêuticas multifacetadas e personalizadas para otimizar os resultados clínicos e minimizar complicações. Além disso, a continuidade da pesquisa é imperativa. Estudos adicionais são necessários para aprofundar nosso entendimento sobre os mecanismos de interação entre comorbidades e microbiota, bem como para desenvolver novas estratégias terapêuticas que considerem a diversidade microbiana e fúngica. Investigações futuras devem explorar novas terapias e protocolos de tratamento que integrem esses conhecimentos para aprimorar a eficácia dos tratamentos e reduzir significativamente a taxa de complicações e recidivas nesses pacientes.

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ANEXOS

ANEXO 1: Parecer consubstanciado de aprovação do projeto no CEP/UCB:

UNIVERSIDADE CATÓLICA DE BRASÍLIA - UCB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE MICROBIOMA E PROTOCOLOS DE TRATAMENTO DE INFECÇÕES DE ORIGEM ODONTOGÊNICA NO HOSPITAL DE BASE DO DISTRITO FEDERAL

Pesquisador: Taia Maria Berto Rezende

Área Temática:

Versão: 2

CAAE: 57475816.8.0000.0029

Instituição Proponente: Stricto Sensu em Ciências Genômicas e Biotecnologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.739.996

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_747915.pdf	06/09/2016 12:04:42		Aceito
Outros	Resposta.pdf	06/09/2016 12:03:47	VANESSA REINALDO CARVALHO DE	Aceito
Outros	Carta.pdf	06/09/2016 12:00:21	VANESSA REINALDO CARVALHO DE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	06/09/2016 11:50:00	VANESSA REINALDO CARVALHO DE CASTRO	Aceito
Outros	Termodeconcordanciahbdftaia.pdf	29/07/2016 12:19:44	Cintia do Couto Mascarenhas	Aceito
Outros	Termodeanuenciadainstituicaocoparticipantehbdftaia.pdf	29/07/2016 12:19:22	Cintia do Couto Mascarenhas	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.docx	30/06/2016 13:24:24	Taia Maria Berto Rezende	Aceito
Folha de Rosto	Protocolo_013521.pdf	30/06/2016 10:22:41	Taia Maria Berto Rezende	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ANEXO 2: Parecer consubstanciado de aprovação do projeto FEPECS/SES:

SECRETARIA DE ESTADO DE SAÚDE DO DISTRITO FEDERAL - FEPECS/ SES/ DF

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE MICROBIOMA E PROTOCOLOS DE TRATAMENTO DE INFECÇÕES DE ORIGEM ODONTOGÊNICA NO HOSPITAL DE BASE DO DISTRITO FEDERAL

Pesquisador: Taia Maria Berto Rezende

Área Temática:

Versão: 1

CAAE: 57475816.8.3001.5553

Instituição Proponente: Stricto Sensu em Ciências Genômicas e Biotecnologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_747915.pdf	06/09/2016 12:04:42		Aceito
Outros	Resposta.pdf	06/09/2016 12:03:47	VANESSA REINALDO CARVALHO DE	Aceito
Outros	Carta.pdf	06/09/2016 12:00:21	VANESSA REINALDO	Aceito
Outros	Carta.pdf	06/09/2016 12:00:21	CASTRO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	06/09/2016 11:50:00	VANESSA REINALDO CARVALHO DE CASTRO	Aceito
Outros	Termodeconcordanciahbdftaia.pdf	29/07/2016 12:19:44	Cintia do Couto Mascarenhas	Aceito
Outros	Termodeanuenciadainstituicaocoparticipantehbdftaia.pdf	29/07/2016 12:19:22	Cintia do Couto Mascarenhas	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_747915.pdf	30/06/2016 13:24:46		Aceito
Projeto Detalhado / Brochura Investigador	Projeto.docx	30/06/2016 13:24:24	Taia Maria Berto Rezende	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	30/06/2016 10:23:00	Taia Maria Berto Rezende	Aceito
Folha de Rosto	Protocolo_013521.pdf	30/06/2016 10:22:41	Taia Maria Berto Rezende	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ANEXO 3: Registro de cadastro na plataforma Sisgen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado):

SisGen

Número do Cadastro: **AE5000A**
Usuário: **Taia Maria Berto Rezende**
CPF/CNPJ: **76844919115**
Objeto do Acesso: **Patrimônio Genético**
Data: **03/05/2024**

ANEXO 4: Planilha para preenchimento e plotagem dos dados coletados (clínicos, hematológicos e de tratamento):

NOME DO PACIENTE	Nº PRONTUÁRIO	SEXO	IDADE	ADMISSÃO E COLETA	DIAGNÓSTICO INICIAL	HEMOGRAMA INICIAL/FINAL	TEMPO DE INTERNAÇÃO	TERAPIA MEDICAMENTOSA	DATA COLETA DE EXSUDATO	GRUPOS (1: COM ATB/ 2:SEM ATB

LEUCÓCITOS INICIAIS	LEUCÓCITOS FINAIS	NEUTRÓFILOS INICIAIS	NEUTÓRFILOS FINAIS	EOSINÓFILOS INICIAIS	EOSINÓFILOS FINAIS	BASÓFILOS INICIAIS	BASÓFILOS FINAL	MONÓCITOS INICIAIS	MONÓCITOS FINAIS	LINFÓCITOS INICIAIS	LINFÓCITOS FINAIS

ANEXO 5: Artigo preparado durante período do doutorado e que foi aceito para publicação na *Australian Endodontic Journal*; Previsão de publicação: Julho/2024

ARTIGO: Martins DCM, Sousa MGC, Silva PAO, Aguiar LR, Andrade RV, Silva-Carvalho AE, Saldanha-Araújo F, Franco OL, Rezende TMB. Association between host defense peptide IDR-1002 and ciprofloxacin: effects on human dental pulp cells. Aust Endod J. 2024

ORIGINAL RESEARCH

Association between host defence peptide IDR-1002 and ciprofloxacin: Effects on human dental pulp cells

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior : 409196/2018-5 : 88887.724458/2022-00
Conselho Nacional de Desenvolvimento Científico e Tecnológico : 305242/2022-9

Received Date: 28 December 2023 | Revised Date: 15 April 2024 | Accepted Date: 08 June 2024

ANEXO 6: Colaborações em demais artigos durante o período do doutorado:

ARTIGO 1: Lima SMF, Freire MS, Cantuária APC, Martins DCM, Amorim IA, Dantas EMGL, Farias JO, Castro MB, Silva JS, Barriviera FA, Barriviera M, Almeida JA, Uehara IA, Silva MJB, Oliveira APL, Silva ON, Hancock REW, Franco OL, Rezende TMB. The use of host defense peptides in root canal therapy in rats. Clin Oral Investig. 2021 Jun;25(6):3623-3632. doi: 10.1007/s00784-020-03684-9. Epub 2020 Nov 16. PMID: 33200281.

Clinical Oral Investigations
https://doi.org/10.1007/s00784-020-03684-9

ORIGINAL ARTICLE

The use of host defense peptides in root canal therapy in rats

Stella M. F. Lima[1,2] · Mirna S. Freire[2,3,4] · Ana Paula C. Cantuária[2,5] · Danilo C. M. Martins[1,2,5] · Ingrid A. Amorim[2] · Elaine M. G. L. Dantas[1,2] · Jade O. Farias[1,2] · Márcio B. Castro[6] · Jackson S. Silva[7,8] · Fernando A. Barriviera[8] · Maurício Barriviera[8] · Jeeser A. Almeida[9] · Isadora A. Uehara[10] · Marcelo J. B. Silva[10] · Ana Paula L. Oliveira[11] · Osmar N. Silva[12] · Robert E. W. Hancock[13] · Octávio L. Franco[2,3,12] · Taia M. B. Rezende[1,2,5]

Received: 20 August 2019 / Accepted: 6 November 2020

Abstract

Objectives In order to evaluate host defense peptides (HDPs) HHC-10 and synoeca-MP activity in in vitro osteoclastogenesis process and in vivo induced apical periodontitis, testing the effect of molecules in the inflammatory response and in apical periodontitis size/volume after root canal treatment.

Materials and methods In vitro osteoclastogenesis was assessed on bone marrow cell cultures extracted from mice, while in vivo endodontic treatment involved rats treated with $Ca(OH)_2$ or HDPs. In vitro osteoclasts were subjected to TRAP staining, and in vivo samples were evaluated by radiographic and tomographic exams, as well as histologic analysis.

Results None of the substances downregulated the in vitro osteoclastogenesis. Nevertheless, all treatments affected the average of apical periodontitis size in rats, although only teeth treated with HDPs demonstrated lower levels of the inflammatory process. These results demonstrated the in vivo potential of HDPs. Radiographic analysis suggested that HHC-10 and synoeca-MP-treated animals presented a similar lesion size than $Ca(OH)_2$-treated animals after 7-day of endodontic treatment. However, tomography analysis demonstrated smaller lesion volume in synoeca-MP-treated animals than HHC-10 and $Ca(OH)_2$-treated animals, after 7 days.

Conclusions These molecules demonstrated an auxiliary effect in endodontic treatment that might be related to its immunomodulatory ability, broad-spectrum antimicrobial activity, and possible induction of tissue repair at low concentrations. These results can encourage further investigations on the specific mechanisms of action in animal models to clarify the commercial applicability of these biomolecules for endodontic treatment.

Clinical significance HDPs have the potential to be adjuvant substances in endodontic therapy due to its potential to reduce inflammation in apical periodontitis.

Keywords Host defense peptides · Synoeca-MP · HHC-10 · Apical periodontitis · Antimicrobial peptide

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Published online: 16 November 2020

ARTIGO 2: Silva PAO, Lima SMF, Martins DCM, Amorim IA, Lacorte C, de Almeida JA, Franco OL, Rezende TMB. Concentrated MTA Repair HP reduced biofilm and can cause reparative action at a distance. Int Endod J. 2021 Oct;54(10):1925-1936. doi: 10.1111/iej.13592. Epub 2021 Jul 28. PMID: 34164821.

Received: 11 June 2020 | Accepted: 22 June 2021

DOI: 10.1111/iej.13592

INTERNATIONAL ENDODONTIC JOURNAL | WILEY

ORIGINAL ARTICLE

Concentrated MTA Repair HP reduced biofilm and can cause reparative action at a distance

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Abstract

Aim: To evaluate *in vitro* whether MTA Repair HP can induce repair processes at a distance, including its effects on biofilm, cell viability, migration, production of TGF-β, phosphate and ALP, evaluated through MTA diluted extracts.

Methodology: Initially, antibacterial tests were performed with the bacterium *Streptococcus mutans* (ATCC 25175) in the presence of MTA extracts (dilutions of 1:1, 1:2 and 1:4). Growth inhibition assay by microdilution in broth, antibiofilm plate assay of young biofilm and antibiofilm assay in confocal microscopy of mature biofilm were carried out. Then, pulp cells were stimulated in the presence of several MTA dilutions, and cell viability (MTT assay), proliferation and migration capacity (scratch assay) were evaluated. To evaluate the capacity of 1:1, 1:2 and 1:4 dilutions of MTA Repair HP to promote the production of important agents of odontogenic differentiation and mineralization, ALP activity, TGF-β secretion and phosphate quantification were measured. Statistical differences were verified using one-way and two-way ANOVA and Tukey's post-tests.

Results: The test dilutions of MTA Repair HP did not inhibit planktonic *S. mutans* growth but were able to reduce young and mature *S. mutans* biofilm ($p < 0.001$). In addition, none of the MTA Repair HP dilutions was cytotoxic for pulp cells. The 1:2 and 1:4 dilutions of MTA Repair HP induced migration and proliferation of pulp cells ($p < 0.05$). ALP activity and TGF-β secretion were independent of the tested dilution

ARTIGO 3: Freire MS, Oliveira NG, Lima SMF, Porto WF, Martins DCM, Silva ON, Chaves SB, Sousa MV, Ricart CAO, Castro MS, Fontes W, Franco OL, Rezende TMB. IL-4 absence triggers distinct pathways in apical periodontitis development. J Proteomics. 2021 Feb 20;233:104080. doi: 10.1016/j.jprot.2020.104080. Epub 2020 Dec 15. PMID: 33338687.

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IL-4 absence triggers distinct pathways in apical periodontitis development

Mirna S. Freire [a,b,c], Nelson G. Oliveira [b], Stella M.F. Lima [b,d], William F. Porto [b,e], Danilo C. M. Martins [b,f], Osmar N. Silva [g], Sacha B. Chaves [h], Marcelo V. Sousa [i], Carlos A.O. Ricart [i], Mariana S. Castro [i], Wagner Fontes [i], Octavio L. Franco [a,b,f,j,**], Taia M.B. Rezende [b,d,f,*,1]

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[d] Curso de Odontologia, Universidade Católica de Brasília, UCB, Brasília, DF, Brazil
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ARTICLE INFO

Keywords:
IL-4
Apical periodontitis
Proteomics
Endodontics

ABSTRACT

Dental pulp is a specialized tissue able to respond to infectious processes. Nevertheless, infection progress and root canal colonization trigger an immune-inflammatory response in tooth-surrounding tissues, leading to apical periodontitis and bone tissue destruction, further contributing to tooth loss. In order to shed some light on the effects of IL-4 on periradicular pathology development modulation, microtomographic, histological and proteomic analyses were performed using 60 mice, 30 wild type and 30 IL-4$^{-/-}$. For that, 5 animals were used for microtomographic and histological analysis, and another 5 for proteomic analysis for 0, 7 and 21 days with/without pulp exposure. The periapical lesions were established in WT and IL-4$^{-/-}$ mice without statistical differences in their volume, and the value of $p < 0.05$ was adopted as significant in microtomographic and histological analyses. Regarding histological analysis, IL-4$^{-/-}$ mice show aggravation of pulp inflammation compared to WT. By using proteomic analysis, we have identified 32 proteins with increased abundance and 218 proteins with decreased abundance in WT animals after 21 days of pulp exposure, compared to IL-4$^{-/-}$ animals. However, IL-4$^{-/-}$ mice demonstrated faster development of apical periodontitis. These animals developed a compensatory mechanism to overcome IL-4 absence, putatively based on the identification of upregulated proteins related to immune system signaling pathways.

Significance: IL-4 might play a protective role in diseases involving bone destruction and its activity may contribute to host protection, mainly due to its antiosteoclastogenic action.

1. Introduction

The tooth is an organ formed by specialized structures including enamel, dentin, cement and pulp. This structure is surrounded by alveolar bone, periodontal ligament and gingiva [1]. Located inside the tooth, dental pulp is a specialized tissue able to respond to infectious processes, such as caries, by mineralized barrier formation, painful sensation and tissue repair [1]. However, the infection progress and root canal colonization trigger an immune-inflammatory response in tooth-surrounding tissues. This process can lead to apical periodontitis, which can promote bone tissue destruction and contribute to tooth loss if no treatment is performed [2,3]. In addition, this disease affects more

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Host defense peptides combined with MTA extract increase the repair in dental pulp cells: in vitro and ex vivo study

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Host Defense Peptides (HDPs) have, in previous studies, been demonstrating antimicrobial, anti-inflammatory, and immunomodulatory capacity, important factors in the repair process. Knowing these characteristics, this article aims to evaluate the potential of HDPs IDR1018 and DJK-6 associated with MTA extract in the repair process of human pulp cells. Antibacterial activity of HDPs, MTA and HDPs combined with MTA in Streptococcus mutans planktonic bacteria and antibiofilm activity was evaluated. Cell toxicity was assayed with MTT and cell morphology was observed by scanning electron microscopy (SEM). Proliferation and migration of pulp cells were evaluated by trypan blue and wound healing assay. Inflammatory and mineralization related genes were evaluated by qPCR (IL-6, TNFRSF, DSPP, TGF-β). Alkaline phosphatase, phosphate quantification and alizarin red staining were also verified. The assays were performed in technical and biological triplicate (n = 9). Results were submitted for the calculation of the mean and standard deviation. Then, normality verification by Kolmogorov Smirnov test, analyzing one-way ANOVA. Analyses were considered at a 95% significance level, with a p-value < 0.05. Our study demonstrated that HDPs combined with MTA were able to reduce biofilms performed in 24 h and biofilm performed over 7 days *S. mutans* biofilm ($p < 0.05$). IDR1018 and MTA, as well as their combination, down-regulated IL-6 expression ($p < 0.05$). Tested materials were not cytotoxic to pulp cells. IDR1018 induced high cell proliferation and combined with MTA induced high cellular migration rates in 48 h ($p < 0.05$). Furthermore, the combination of IDR1018 and MTA also induced high expression levels of DSPP, ALP activity, and the production of calcification nodules. So, IDR-1018 and its combination with MTA could assist in pulp-dentine complex repair process in vitro.

Maintenance of pulp vitality is essential for preserving dental structure and function[1]. Through maintaining pulp vitality, it is possible to induce closure of the root apex, an important characteristic for root thickness and length development. This fact will result in fracture resistance and greater longevity of the tooth that had its pulp tissue exposed. One method for preserving pulp tissue after its exposure is performing a direct pulp capping[2]. Direct pulp capping is a procedure in which the material is placed directly onto the exposed pulp to preserve its vitality and promote repair. It is usually indicated for cases where the pulp exposure is accidental and limited, without signs of irreversible pulp inflammation or necrosis[2]. This technique can use biomaterials, such as calcium hydroxide and calcium silicate-based materials, for example: Mineral trioxide aggregate (MTA),

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BASIC RESEARCH – BIOLOGY

Senescence on Dental Pulp Cells: Effects on Morphology, Migration, Proliferation, and Immune Response

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ABSTRACT

Introduction: Evidence indicates that senescence can affect essential dental pulp functions, such as defense capacity and repair, consequently affecting the successes of conservative endodontic treatments. This study aims to evaluate the effects of senescence on the morphology, migration, proliferation, and immune response of human dental pulp cells. **Methods:** Cells were treated with doxorubicin to induce senescence, confirmed by β-galactosidase staining. Morphological changes, cellular proliferation, and migration were evaluated by scanning electron microscopy, trypan blue cells, and the scratch method, respectively. Modifications in the immune response were evaluated by measuring the genes for pro-inflammatory cytokines tumor necrosis factor alpha and interleukin (IL)-6 and anti-inflammatory cytokines transforming growth factor beta 1 and IL-10 using the real time polymerase chain reaction assay. **Results:** An increase in cell size and a decrease in the number of extensions were observed in senescent cells. A reduction in the proliferative and migratory capacity was also found in senescent cells. In addition, there was an increase in the gene expression of inflammatory cytokines tumor necrosis factor alpha and IL-6 and a decrease in the gene expression of IL-10 and transforming growth factor beta-1, suggesting an exacerbated inflammatory situation associated with immunosuppression. **Conclusions:** Cellular senescence is possibly a condition that affects prognoses of conservative endodontic treatments, as it affects primordial cellular functions related to this treatment. *(J Endod 2024;■:1–8.)*

KEY WORDS

Conservative endodontic treatments; pulp cells; senescence

SIGNIFICANCE

This study reveals that senescence in dental pulp cells causes morphological alterations, reduced cell function, and an exacerbated inflammatory and immunosuppressive response, potentially complicating conservative endodontic prognosis. Managing senescence is crucial for better treatment outcomes in endodontics.

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Senescence is a cellular state characterized by the irreversible interruption of the cell cycle halting replication in the G1 phase and remaining alive and metabolically active[1]. This state can be divided into replicative senescence, related to telomere shortening, and stress-induced senescence. Cellular senescence causes morphological alterations such as increased volume and irregular structure, and it is associated with changes in the cytoskeleton and vimentin filaments[1]. Senescent cells also exhibit increased activity of lysosomes and enzyme SA-β-gal, as well as upregulated expression of genes such as p21, p16, and p53[2]. This cellular state also results in metabolic reprogramming and the manifestation of secretory proteins, known as the senescence-associated secretory phenotype[3]. In general, this state contributes to aging, age-related diseases, and a decline in cellular functions[4].

Senescence can also affect the immune system[5]. Immunosenescence refers to changes in the immune system during aging, leading to a progressive decline in immune functions and increased susceptibility to diseases[5]. This stage results from two complementary processes: the effects of cellular senescence on immune cells and the weakening of the body's barriers indirectly caused by tissue cellular senescence, which promotes the release of signaling molecules to which immune cells respond[6]. Senescence-associated secretory phenotype proteins compromise immune system functions, including the elimination of senescent

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Antibiofilm and immunomodulatory resorbable nanofibrous filing for dental pulp regenerative procedures

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ABSTRACT

Multifunctional scaffolds with host defense peptides designed for regenerative endodontics are desirable nano-biotechnological tools for dentistry. Here, different scaffolds were tested for use during the pulp revascularization process, including poly(vinyl alcohol)-PVA hydrogels or resins, collagen hydrogels and poly(vinyl alcohol) PVA/Chitosan (PVA/CS) nanofibers. Based on time to degradation (21 days), nanofibers were chosen to be incorporated with ciprofloxacin and IDR-1002 (each at 50 mg/g). Nanofibers containing ciprofloxacin and IDR-1002 had anti-biofilm activity against *Enterococcus faecalis*, *Staphylococcus aureus* and a multispecies oral biofilm, besides anti-inflammatory activities. The *in vivo* subcutaneous tissue response to tooth fragments filled with nanofibers demonstrated a pulp-like tissue formation, when compared to empty teeth fragments. Thus, we designed a strong antimicrobial, immunomodulatory and regenerative candidate for pulp revascularization and regeneration procedures.

1. Introduction

Dental trauma can be due to acute percussive transmissions to the teeth and supporting structures, which can trigger fractures in the dental element and surrounding tissues [1]. Worldwide, about 20–30% of children under 12 have already suffered dental trauma to some degree [2]. The main etiological agents related to pulp necrosis in immature permanent teeth are trauma/injuries and dental caries [3]. Dental trauma can affect the pulp tissue, disrupting blood vessels and causing aseptic necrosis [4]. In addition, traumatized teeth can be more susceptible to the invasion of microorganisms into the pulp environment and, consequently, irreversible pulpitis and pulp necrosis [5].

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