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RESISTÊNCIA ANTIMICROBIANA E MICRORGANISMOS PRESENTES EM INFECÇÕES ENDODÔNTICAS E PERIODONTAIS: REVISÃO SISTEMÁTICA

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Tese apresentada à Banca Examinadora do Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba, como exigência parcial para obtenção do título de Doutor em Ciências Farmacêuticas.

Orientadora: Prof.ª Dr.ª Luciane Cruz Lopes

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Tese aprovada como requisito parcial para obtenção do grau de Doutor no Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba.

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DEDICATÓRIA

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Meu porto seguro.

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É sobre escalar e sentir que o caminho te fortaleceu…”

(Trem bala, Ana Vilela)

RESUMO

Introdução: A resistência aos antimicrobianos que os microrganismos vêm desenvolvendo é um problema de saúde pública que preocupa os órgãos de saúde do mundo. Embora o uso de antimicrobianos possa debelar as infecções odontológicas, os microrganismos ou se tornaram naturalmente resistentes aos antimicrobianos ou desenvolveram resistência por mutação devido à pressão seletiva ao longo dos anos. O conhecimento do perfil de resistência dos microrganismos presentes nas infecções endodônticas e periodontais deve orientar o uso adequado de antimicrobianos. **Objetivos:** Levantar e descrever o perfil de resistência antimicrobiana em infecções endodônticas e periodontais. **Método:** Por meio de revisão sistemática, selecionaram-se estudos observacionais realizados com dentição permanente, que avaliaram a resistência antimicrobiana em infecções endodônticas e/ou periodontais utilizando método de concentração inibitória mínima, zona de inibição e/ou detecção de genes de resistência por técnicas moleculares. Estudos que permitiram a ingestão de antimicrobianos até o momento da coleta foram excluídos. A busca foi realizada em dezembro de 2021 e incluiu estudos que foram publicados entre janeiro de 2011 e dezembro de 2021, nas seguintes bases de dados eletrônicas: PubMed, Ovid MEDLINE, Ovid Embase, BVS, CINAHL, Web of Science. Três duplas de revisores calibradas, de forma independente, selecionaram os títulos, resumos e textos completos e extraíram os dados de todos os estudos que atenderam aos critérios de elegibilidade. As seguintes medidas foram extraídas de cada estudo: características dos pacientes, diagnóstico da infecção, espécies microbianas avaliadas, antimicrobianos avaliados, identificação dos genes de resistência e fatores de virulência. A metodologia utilizada para avaliação da qualidade do estudo foi o *checklist* de avaliação crítica para estudos que reportam séries de casos do Instituto Joanna Briggs e adaptado a pergunta de pesquisa desta revisão sistemática de escopo. Os resultados foram apresentados segundo o diagnóstico da infecção (periodontal ou endodôntica) e sumarizados em análises descritivas. **Resultados:** Foram incluídos 46 estudos de série de casos (N= 3.050 pacientes) que avaliaram a resistência antimicrobiana de 50 espécies de microrganismos cultiváveis encontrados em infecções endodônticas e periodontais. Dos 22 estudos que avaliaram as infecções endodônticas, 12 reportaram *Enterococcus faecalis*, e dos 24 estudos que avaliaram as infecções periodontais, oito citaram espécies de *Prevotella* e *Porphyromonas* como as mais frequentes. Os antimicrobianos reportados com maior frequência de resistência foram eritromicina (57.0%) nas infecções endodônticas e ampicilina (39.5%) nas infecções periodontais. Sete estudos relataram a presença de genes de resistência para cada uma das infecções estudadas e seis

estudos verificaram a presença de fatores de virulência nas infecções endodônticas enquanto apenas dois estudos observaram esses fatores nas infecções periodontais. **Conclusão:** As cepas de microrganismos presentes nas infecções endodônticas e periodontais reportadas nos estudos incluídos foram bastante similares, no entanto, a resistência levantada variou de acordo com o diagnóstico da infecção, a técnica de avaliação de susceptibilidade/ resistência, a localização geográfica da população avaliada e o tipo de antimicrobiano utilizado. Não foram encontradas evidências com respeito a prevalência da resistência antimicrobiana nas infecções endodônticas e periodontais. Existe considerável incerteza com relação ao perfil de microrganismos e sua resistência nestas infecções, requerendo futuras pesquisas que deveriam focar em estudos populacionais regionais para dirimir este problema na era da crescente resistência aos antimicrobianos.

Palavras-chave: antimicrobianos; endodontia; odontologia; periodontia; resistência microbiana a medicamentos.

ABSTRACT

Introduction: The antimicrobial resistance that microorganisms have been developing is a public health problem that concerns health agencies worldwide. Although the use of antimicrobials can control dental infections, microorganisms have either become naturally resistant to antimicrobials or have developed resistance by mutation due to selective pressure, over the years. Knowledge of the resistance profile of microorganisms present in endodontic and periodontal infections should guide dentists to the proper use of antimicrobials. **Objectives:** Estimating and describing the antimicrobial resistance profile in endodontic and periodontal infections. **Methods**: Through a systematic review, observational studies were carried out with people over 16 years old, which evaluated the antimicrobial resistance in endodontic and/or periodontal infections using the method of minimum inhibitory concentration, zone of inhibition and/or detection of resistance genes by molecular techniques. Studies that allowed the ingestion of antimicrobials at the time of the collection were excluded. The bibliographic search was performed in December 2021 and included studies that were published between January 201 and December 2021 in the following electronic databases: PubMed, Ovid MEDLINE, Ovid Embase, BVS, CINAHL, Web of Science. Six reviewers, working in pairs and independently, selected titles, abstracts and full texts and extracted data from all studies that met the eligibility criteria. The following measures were extracted from each study: characteristics of patients, diagnosis of infection, microbial species assessed, antimicrobials assessed, identification of resistance genes and virulence factors. “The Joanna Briggs Institute” critical appraisal for case series was the tool selected to assess the risk of bias in the included studies and was adapted to the research question of this systematic scoping review. The results were presented according to the infection diagnosis (periodontal or endodontic) and summarized in descriptive analysis. **Results:** Forty-six case series studies (N= 3,050) that evaluated the antimicrobial resistance of 50 species of cultivable microorganisms found in endodontic and periodontal infections were included. Twelve out of the 22 studies that evaluated endodontic infections reported *Enterococcus faecalis*, and eight out of the 24 studies that evaluated periodontal infections cited *Prevotella* and *Porphyromonas* species as the most frequent. The antimicrobials reported with the highest frequency of resistance were erythromycin (57.0%) in endodontic infections and ampicillin (39.5%) in periodontal infections. Seven studies reported the presence of resistance genes for each of the infections studied and six studies verified the presence of virulence factors in endodontic infections, while only two studies observed these factors in periodontal infections. **Conclusions:** The strains of

microorganisms present in endodontic and periodontal infections reported in the included studies were quite similar, however, the antimicrobial resistance varied according to the diagnosis of the infection, the susceptibility/resistance assessment technique, the geographic location of the evaluated population, and the type of antimicrobial used. No evidence were found regarding the prevalence of antimicrobial resistance in endodontic and periodontal infections. There is considerable uncertainty regarding the profile of microorganisms and their resistance in these infections, requiring further research that should focus on regional population studies to address this issue in the era of increasing antimicrobial resistance.

Key words: anti-bacterial agents; dentistry; drug resistance, microbial; endodontics; periodontics.

SUMÁRIO

1 APRESENTAÇÃO

A resistência dos microrganismos frente aos antimicrobianos é um fenômeno global que, infelizmente, segue se ampliando e tem provocado discussões e até mesmo mudanças na política de saúde pública de vários países.

Países em desenvolvimento, como o Brasil, encontram um desafio ainda maior, pois, a incidência de doenças infecciosas é elevada e pacientes com uma infecção resistente podem não ter acesso a antimicrobianos específicos para o tratamento adequado.

Na Odontologia, um dos fatores que contribuem muito para o aumento da resistência dos microrganismos é o uso excessivo e a prescrição indiscriminada dos antimicrobianos frente às infecções endodônticas e periodontais.

Como o desenvolvimento de novos medicamentos não acontece na mesma velocidade em que a resistência aumenta, é importante conscientizar e orientar a comunidade odontológica quanto a correta indicação dos antimicrobianos, de acordo com o diagnóstico da infecção; o que envolve o conhecimento das características dos microrganismos presentes na cavidade bucal, e as características clínicas e demográficas de cada paciente.

Na contramão da tentativa de controle da prescrição antimicrobiana desenfreada, a pandemia do COVID-19 resultou num grande aumento do uso de antimicrobianos odontológicos para tratamento de infecções que seriam melhor gerenciadas por procedimentos clínicos locais, seguindo a rigor os protocolos de biossegurança.

Com formato inovador proposto pelo programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba, no qual os resultados são apresentados no formato de artigos científicos, esta versão conta com um protocolo publicado e dois artigos, a serem submetidos a revistas especializadas.

O referencial teórico foi construído considerando o racional das melhores evidências sobre o perfil de resistência aos antimicrobianos do microbioma presente nas infecções endodônticas e periodontais relacionando com as indicações do uso de antimicrobianos na prática odontológica.

2 INTRODUÇÃO

A resistência antimicrobiana (RAM) ameaça a prevenção e o tratamento eficaz de uma gama cada vez maior de infecções causadas por bactérias, parasitas, vírus e fungos. Isso ocorre quando esses microrganismos sofrem alterações metabólicas com o passar do tempo e não respondem mais aos medicamentos. Como resultado, os medicamentos tornam-se ineficazes e as infecções persistem no corpo, aumentando o risco de propagação de doenças, doenças graves e morte (WORLD HEALTH ORGANIZATION, 2020).

Os antimicrobianos trouxeram inquestionáveis benefícios para a medicina moderna: desde a sua descoberta em 1928 por Alexander Fleming até os dias atuais, a penicilina ainda é um dos antibióticos mais usado em Odontologia. Entretanto, o seu uso excessivo e indiscriminado, tanto por médicos e cirurgiões-dentistas quanto por pacientes, contribuiu, em muito, para o aparecimento de bactérias super-resistentes (RAMACHANDRAN; RACHURI; MARTHA; SHAKTHIVEL; GUNDALA; BATTU, 2019). O erro mais comum que as pessoas cometem é fazer uso de antibióticos para doenças não bacterianas (VENTOLA, 2015; GERMACK; SEDGLEY; SABBAH; WHITTEN, 2017).

As duas formas de resistência antimicrobiana mais frequentes são a intrínseca e a adquirida. A forma intrínseca pode ser considerada como "não-sensibilidade" do microrganismo ao antimicrobiano, ou seja, um medicamento específico não deve ser prescrito para tratar uma determinada infecção se o microrganismo não for sensível a ele. Com relação à resistência adquirida, ela pode ser entendida de duas maneiras: do ponto de vista biomecânico, a resistência antimicrobiana pode ser desenvolvida por quatro possíveis mecanismos de ação: 1) modificação do alvo, 2) o microrganismo produz enzima capaz de inativar ou modificar o medicamento, 3) o microrganismo desenvolve impermeabilidade ao medicamento ou 4) efluxo dos antimicrobianos para fora da célula. Do ponto de vista genético, a resistência pode ser adquirida por dois eventos totalmente distintos: ocorrência da mutação no genoma levando à herança vertical de resistência à descendência do microrganismo, ou a aquisição de informação genética exterior, de outros microrganismos, por transferência horizontal (PÉRICHON; COURVALIN, 2009; BELIBASAKIS *et al.*, 2020).

De maneira geral, a resistência surge como consequência de mutações em microrganismos e a pressão seletiva do uso de antimicrobianos fornece uma vantagem competitiva para cepas mutantes. Os clones resistentes resultantes são disseminados rapidamente em todo o mundo, e esta propagação é facilitada por transmissão genética entre espécies, saneamento básico e higiene em comunidades e em hospitais deficientes, e o aumento

da frequência de viagens, comércio e transmissão de doenças globais (LAXMINARAYAN *et al.*, 2013). Isto é consistente com o rápido surgimento de resistência antimicrobiana observada na clínica e prevê que novos antimicrobianos serão selecionados para determinantes de resistência pré-existentes que têm circulado dentro do pan-genoma microbiano por milênios (D'COSTA *et al.*, 2011).

Sendo os cirurgiões-dentistas responsáveis por cerca de 10% das prescrições antimicrobianas em países como os Estados Unidos, Portugal e Reino Unido, esse profissional apresenta uma responsabilidade vital, nacional e globalmente por contribuir para a redução da resistência antimicrobiana (FDI, 2021). E o primeiro passo é conhecer os patógenos mais comuns associados às infecções endodônticas e periodontais, e os seus perfis de susceptibilidade para uma prescrição antimicrobiana racional; pois, a composição microbiana e a resistência antimicrobiana de microrganismos associados ao abscesso periodontal variam entre as diferentes populações (IRSHAD *et al.*, 2020).

Assim, o papel do cirurgião-dentista não é mais apenas criar um ambiente biologicamente aceitável para preservar um dente, mas controlar de forma eficaz o biofilme microbiano para prevenir a transferência de genes resistentes a antimicrobianos (ARGs - *Antimicrobial Resistant Genes*) (DOMINGUEZ-PEREZ *et al.*, 2018).

A escassez de síntese de evidências qualificada sobre o perfil de resistência antimicrobiana nas infecções endodônticas e periodontais levou ao desenvolvimento dessa revisão sistemática, a fim de identificar os microrganismos circulantes isolados de infecções endodônticas e periodontais bem como seu perfil de resistência aos antimicrobianos.

3 REFERENCIAL TEÓRICO

3.1 Aumento global da taxa de resistência aos antimicrobianos

A crescente taxa de resistência dos microrganismos aos antimicrobianos tem gerado preocupação entre as autoridades de saúde da América Latina e do mundo (BAUMGARTNER; XIA, 2003; WORLD HEATLH ORGANIZATION, 2016; ELIAS *et al.*, 2017).

Pode-se entender que a maioria dos antimicrobianos é aplicada desnecessariamente, seja na agropecuária dirigida comercialmente, seja por médicos e cirurgiões-dentistas inseguros sobre o diagnóstico da doença (LAXMINARAYAN *et al.*, 2013). Essa exposição leva ao risco de desenvolver *Methicilin-Resistant Staphylococcus Aureus* (MRSA), *Vancomycin-Resistant Enterococci* (VRE) e *MultiDrug-Resistance* (MDR) de bactérias Gram-negativas (MARTÍN-LOECHES; DIAZ; VALLÉS, 2014). A multirresistência é resultado da co-resistência, na qual vários mecanismos estão associados no mesmo hospedeiro bacteriano e a utilização de uma parte de uma classe de medicamentos pode co-selecionar a resistência a outra classe de antimicrobianos com um modo de ação totalmente distinto (PÉRICHON; COURVALIN, 2009).

O alto consumo de antimicrobianos parece ter relação direta com o índice de desenvolvimento humano, a porcentagem da população urbana, densidade de estabelecimentos privados de saúde, expectativa de vida e percentual de mulheres, menores níveis de analfabetismo e menor porcentual da população de cinco a 15 anos de idade (KLIEMANN *et al.*, 2016). No caso dos países em desenvolvimento, o delicado equilíbrio está entre encorajar o uso para indicações apropriadas e a tendência avassaladora para o uso inadequado de antimicrobiano (LAXMINARAYAN; HEYMANN, 2012).

Como os cirurgiões-dentistas prescrevem aproximadamente 10% dos antimicrobianos dispensados na atenção primária e os dados obtidos na rede privada não são consistentes, é importante não subestimar a contribuição potencial da profissão odontológica para o desenvolvimento de microrganismos resistentes a esses medicamentos (SEGURA-EGEA *et al.*, 2016). Foi possível observar o aumento da resistência antimicrobiana de anaeróbios isolados de infecções endodônticas primárias de uma população brasileira específica numa avaliação de um período de nove anos (GOMES *et al.*, 2011).

Dessa forma, o gerenciamento de antimicrobianos parece representar uma das mais bem sucedidas estratégias para controlar o uso excessivo de antimicrobianos e diminuir a aquisição de organismos multirresistentes.

3.2 Infecções endodônticas e periodontais e a resistência antimicrobiana

As infecções endodônticas são doenças multimicrobianas associadas ao biofilme, que podem comunicar-se com o periodonto, principalmente, através de túbulos dentinários expostos, canais acessórios e laterais e até mesmo do forame apical (SIQUEIRA; RÔÇAS, 2014). A presença de uma resposta inflamatória perirradicular decorrente da necrose do tecido pulpar denomina-se periodontite apical primária (GOMES; HERRERA, 2018).

Periodontite é definida como doença inflamatória crônica multifatorial que afeta os tecidos de suporte dental, associada ao biofilme disbiótico e caracterizada pela destruição progressiva do aparato de inserção dental (ANSILIERO *et al.*, 2021).

A periodontite apical secundária (ou persistente) está associada a dentes que receberam tratamento endodôntico, mas que não obtiveram sucesso. Nesse caso, os microrganismos podem ter invadido o sistema de canais radiculares por meio da microinfiltração coronária da massa obturadora (infecção secundária) ou tolerado os procedimentos químico-mecânicos da limpeza e modelagem realizados anteriormente (infecção persistente) (GOMES; HERRERA, 2018).

A maioria das doenças pulpares e periodontais é melhor gerenciada por intervenção operatória e medidas de higiene bucal (SKUCAITE *et al.*, 2010). De acordo com a prática clínica, o tratamento da periodontite envolve a eliminação mecânica do biofilme microbiano causador da inflamação e/ou infecção (ARDILA; BEDOYA-GARCIA, 2020); bem como no caso das infecções endodônticas (sejam elas primárias, secundárias ou persistentes) que são solucionadas por meio da limpeza e (re)modelagem do sistema de canais radiculares. Fica claro que o sucesso do tratamento endodôntico visa erradicar a infecção, prevenir a reinfecção do canal e/ou da região perirradicular (HUSSEIN *et al.*, 2020).

Geralmente, os antimicrobianos não são indicados para tratamento de infecções crônicas, como no tratamento de periodontite apical secundária, pois, a ausência do tecido pulpar não permite que o suprimento sanguíneo chegue ao canal radicular. Nessa condição, a indicação de administração sistêmica de antimicrobiano é considerada negligente, pois, a concentração alcançada não é suficiente para inibir o crescimento microbiano (PINHEIRO *et al.*, 2004).

O tratamento apropriado por meio de administração de agentes antimicrobianos deve ficar restrito a apenas situações clínicas específicas, reservada para os casos em que haja sinais como celulite, linfadenite, limitação de abertura de boca, associados a sintomas como febre, perda de apetite e mal-estar geral (SIQUEIRA; RÔÇAS, 2013; MONTAGNER *et al.*, 2014).

Existem centenas de microrganismos associados a periodontite e a falta de conhecimento sobre a microbiota presente na infecção pode levar à seleção de um antimicrobiano para patógenos intrinsecamente resistentes, comprometendo a eficácia do tratamento e aumentando a probabilidade clínica de desenvolver resistência antimicrobiana (ALMEIDA et al., 2020).

Claramente, os benefícios do uso correto dos antimicrobianos incluem a resolução da infecção, a prevenção da disseminação e a minimização de sérias complicações da doença. Quando usados como adjuvante ao tratamento odontológico, o profissional deve optar pela administração no menor tempo possível, minimizar o uso de antimicrobianos de amplo espectro e monitorar o paciente diariamente (AHMADI; EBRAHIMI; AHMADI, 2021). O uso inapropriado de antimicrobianos pode levar não só ao aumento de eventos adversos e custo com cuidados em saúde como também contribui para a seleção de microrganismos resistentes.

3.3 Prescrição antimicrobiana na Odontologia

Os antimicrobianos estão entre os medicamentos mais comumente prescritos na clínica odontológica. No entanto, os resultados de alguns estudos indicaram que de 30% a 50% dos antimicrobianos prescritos ou não são necessários ou não são prescritos de forma ideal (FLUENT *et al.*, 2016; GERMACK *et al.*, 2017). O uso inapropriado ou irresponsável inclui: prescrição na ausência de infecção ou quando medidas locais são suficientes para o controle da infecção; prescrição profilática quando não há indicação; dose ou tempo de duração incorretos; espectro de ação antimicrobiana inadequado para infecção específica; tratamento não ajustado quando a cultura estiver disponível; uso de intravenosa quando a via oral pode ser usada; escolha incorreta de antimicrobiano para pacientes com histórico de alergia conhecido (SDCEP, 2016).

O aumento da resistência antimicrobiana e os altos custos hospitalares tem requerido uma reavaliação do tempo de duração da prescrição (RAMACHANDRAN *et al.*, 2019). Levando-se em consideração que as infecções endodônticas apresentam evolução muito rápida, mas tem duração relativamente curta (dois a sete dias), em particular quando o foco da infecção

é eliminado (OBEROI *et al.*, 2015), a prescrição de antimicrobianos não deveria exceder cinco dias, acompanhada diariamente pelo cirurgião-dentista (GERMACK *et al.*, 2017).

Apesar de apresentar um espectro de ação que vai além das necessidades odontológicas, na maioria dos países, o antimicrobiano de eleição é a amoxicilina, que é rapidamente absorvida por via oral, não sofre interferência da alimentação e apresenta maior meia-vida plasmática e tecidual (ANDRADE; BENTES; BRITO, 2012).

Se a amoxicilina não for efetiva após dois ou três dias de administração, o metronidazol é recomendado como medicação suplementar. A combinação de amoxicilina com clavulanato de potássio apresenta um espectro de ação muito mais amplo pelo fato do clavulanato ser um inibidor competitivo da enzima ß-lactamase (BOLFONI *et al.*, 2018).

Ao mesmo tempo, a administração de antimicrobianos com um amplo espectro de ação pode eliminar muitas espécies microbianas que vivem em relação comensal com o corpo humano, o que acarreta o risco de desenvolvimento de resistência antimicrobiana (BAUMGARTNER; XIA, 2003).

O paciente que apresenta sensibilidade às penicilinas pode apresentar espécies não-usuais de microrganismos virulentos, multirresistentes, e ainda alguma deficiência no sistema imunológico. Nessas situações, a cultura e o teste de sensibilidade seriam ideais para a seleção do antimicrobiano adequado (AAE, 2017).

Apesar da clindamicina e a claritromicina não oferecerem vantagens sobre os antimicrobianos de primeira linha, podem ser consideradas uma opção de tratamento quando o paciente reporta alergia ou não responde à primeira opção. No entanto são medicamentos que oferecem risco ao surgimento de efeitos adversos severos além de contribuir para o desenvolvimento da resistência antimicrobiana (SDCEP, 2016; AAE, 2017).

Na Odontologia, a prescrição antimicrobiana pode ser considerada empírica porque os cirurgiões-dentistas, de maneira geral, não sabem quais microrganismos são responsáveis por determinadas infecções já que amostras do canal radicular ou da região periapical não são comumente coletadas e analisadas no exame clínico inicial (SEGURA-EGEA *et al.*, 2016).

A declaração do grupo escocês de prescrição de antimicrobianos (SAPG) reiterou que a antibioticoterapia só é apropriada se a drenagem imediata da infecção não for alcançada por meio de medidas locais ou quando houver evidência de disseminação de infecção ou envolvimento sistêmico, sugerindo que o sistema imune por si só não seja adequadamente capaz de gerenciar a infecção (SDCEP, 2016).

Muitos estudos avaliaram a tendência de prescrição de antimicrobianos odontológicos em todo o mundo. Por exemplo, um estudo retrospectivo na Croácia (BJELOVUCIC *et al.*, 2019) mostrou que antimicrobianos foram prescritos em quase metade das consultas de emergência odontológica para diagnósticos que não estavam relacionados a infecção; em Al Bahah, região da Arábia Saudita, observou-se que os antimicrobianos são administrados em várias condições para as quais não são recomendados (ALZAHRANI *et al.*, 2020). As evidências mostraram que há uma falta de conhecimento sobre os padrões de prescrição de antimicrobianos e a duração da terapia em dentistas na região de Tirana (Albânia) (ABAZI; MIHANI, 2018). Uma pesquisa realizada na Noruega (PREUS *et al.*, 2017) observou uma tendência de aumento das taxas de prescrição de antimicrobianos entre cirurgiões-dentistas, no período de 1990 a 2015, para a maioria das situações terapêuticas, chegando a conclusão de que muitos cirurgiões-dentistas não tem o conhecimento adequado sobre os antimicrobianos e os prescrevem em situações inapropriadas. Em Portugal a situação não é diferente, pois, uma parte consideravel dos cirurgiões-dentistas inquiridos prescreve antibióticos inadequadamente para condições endodônticas inflamatórias como a pulpite (SILVA *et al.*, 2017).

Os crescentes problemas de resistência nos últimos anos estão provavelmente relacionados ao uso excessivo ou incorreto de antimicrobianos, deixando claro a necessidade de desenvolvimento de diretrizes de prescrição e iniciativas educacionais para estimular o uso racional e adequado de antimicrobianos em Odontologia.

3.4 Formação de biofilme e o aumento da resistência antimicrobiana

Biofilmes são grupos de microrganismos sésseis que vivem dentro de uma matriz auto-produzida de substâncias poliméricas extracelulares que podem proteger os microrganismos contra desidratação e condições ambientais prejudiciais (MOHAMMADI *et al.*, 2013; DIESENDORF *et al.*, 2017). Por essa razão, microrganismos organizados em biofilmes são mais resistentes aos antimicrobianos do que as mesmas células desenvolvidas em um estado planctônico (HAQUE *et al.*, 2019).

Os biofilmes microbianos desempenham um papel essencial em várias doenças infecciosas como as infecções radiculares e perirradiculares e, a habilidade em formar biofilmes tem sido considerada como um fator de virulência (MOHAMMADI *et al.*, 2013). A matriz polissacarídica dos biofilmes retarda a difusão dos antimicrobianos quando enzimas extracelulares, como a β-lactamase, ficam presas e concentradas na matriz, inativando os antibióticos β-lactâmicos.

Além disso, ramificações apicais, canais laterais e istmos interligados aos canais radiculares principais são capazes de abrigar células microbianas organizadas em biofilme (RICUCCI; SIQUEIRA, 2010), pois, a região apical, de alta complexidade anatômica é usualmente inacessível aos instrumentos e agentes antimicrobianos durante o tratamento endodôntico. Essa estrutura de biofilme pode aderir-se à superfície radicular externa causando a periodontite apical persistente. Por essa razão, o tratamento endodôntico deve considerar as características de adesão e formação do biofilme por uma grande variedade de microrganismos (AL-AHMAD *et al.*, 2014).

Estima-se que o ecossistema que forma o biofilme é composto por, aproximadamente, 700 espécies, incluindo simbiontes inofensivos, comensais e patógenos oportunistas. Em pacientes saudáveis essa composição, geralmente, é estável. Porém, a diversidade microbiana pode variar de acordo com a pressão seletiva, que pode levar os microrganismos a expressarem genes de resistência antimicrobiana que garantem sua sobrevivência e sua persistência genética (ALMEIDA *et al.*, 2020).

Como outros biofilmes multiespécies, as espécies microbianas orais são organizadas em estreita proximidade, o que freqüentemente leva ao estabelecimento de interações, como sistemas de detecção de quorum, cadeias alimentares e troca de genes de virulência e genes de resistência antibiótica, incluindo aqueles que codificam resistência a antimicrobianos comumente usados, como β-lactâmicos, tetraciclina e macrolídeos (DOMINGUEZ-PEREZ *et al.*, 2018).

O mecanismo de tolerância aos antimicrobianos inclui a dificuldade de penetração na matriz do biofilme, que age como uma barreira; e os fatores determinantes da resistência comprometem o tratamento das doenças induzidas por biofilme. Por exemplo, vários determinantes de resistência que codificam a resistência à tetraciclina tet (Q), tet (M), eritromicina (ermF), aminoglicosídeos (aacA-aphD) e antibióticos β-lactâmicos (cfxA, blaSHV, blaTEM) foram coletados de pacientes saudáveis ou que apresentavam doença periodontal e observou-se maior prevalência dos genes de resistência à tetraciclina e a antibióticos β-lactâmicos em pacientes com periodontite, deixando claro a diversidade do resistoma antibiótico oral (OLSEN, 2015).

3.5 Genes de resistência e fatores de virulência

A resistência aos antimicrobianos é um fenômeno natural e de crucial importância no tratamento de doenças causadas por microrganismos patogênicos. As infecções endodônticas e

periodontais não são causadas por um único patógeno, porém, são desencadeadas quando ocorre a disbiose (quebra do equilíbrio entre microrganismos patogênicas e comensais) (RÔÇAS; SIQUEIRA, 2012).

A cavidade oral humana contém um ecossistema microbiano densamente povoado conhecido como microbioma (ALMEIDA *et al.,* 2020). Em face a pressão seletiva, os microrganismos podem expressar genes de resistência antimicrobiana (ARGs) e fatores de virulência.

A alta porcentagem de frequência de genes de resistência antimicrobiana indica que o perfil de resistência dos microrganismos vem sofrendo mudanças ao longo dos anos, pois esses genes podem espalhar-se pelas espécies microbianas da cavidade bucal, que vivem em biofilmes, e assim sustentar um reservatório de genes de resistência antimicrobiana (DOMINGUEZ-PEREZ *et al.,* 2018).

Determinantes de resistência em bactérias orais foram detectados por uma abordagem metagenômica envolvendo bactérias cultiváveis e ainda não cultiváveis e, ficou claro que o resistoma antimicrobiano oral é mais diversificado e abundante na periodontite do que em pacientes saudáveis (OLSEN, 2015). Essas associações tem sido reportadas entre as espécies microbianas endodônticas, incluindo *Prevotella* spp. dos abscessos dento-alveolares e *Enterococcus faecalis* em dentes com periodontite apical (RÔÇAS; SIQUEIRA, 2012).

O gene tetM tem sido muito prevalente em amostras orais, reduzem a eficácia das tetraciclinas e podem selecionar cepas resistentes. As beta-lactamases TEM são enzimas presentes em bactérias Gram-negativas e conhecidas por atacar vários antibióticos beta-lactâmicos. Essas enzimas conferem resistência às penicilinas e cefalosporinas precoces e estão amplamente distribuídas no biofilme periodontal, independentemente do estado da doença. Já os genes erm, que conferem resistência à eritromicina, foram amplamente demonstrados em isolados endodônticos (RÔÇAS; SIQUEIRA, 2013).

Diferentemente dos genes, os fatores de virulência (atividade da gelatinase, expressão de hemolisina, resposta a feromônios, produção de bacteriocina, expressão de fatores de aderência, substâncias de agregação e resistência a diversos antibióticos) são estratégias que os microrganismos utilizam para ″driblar" o sistema de defesa do hospedeiro e causar uma infecção para assegurar a sobrevivência e a persistência genética (LINS *et al.*, 2013). Fatores de aderência dos microrganismos influenciam na formação de biofilme e a capacidade de

formar biofilme é um fator de virulência importante que os microrganismos desenvolvem (ZOLETTI *et al.,* 2011).

3.6 Técnicas de identificação de microrganismos

Os métodos tradicionais de estudo da microbiologia das infecções do canal radicular variam desde os métodos dependentes de cultura até os métodos moleculares não dependentes de cultura. Recentemente, as técnicas de sequenciamento de última geração têm demonstrado que as infecções do canal radicular são muito mais diversas do que anteriormente considerado (PERSOON *et al.,* 2017).

As técnicas de cultura apresentam limitações como a necessidade de preservar a viabilidade bacteriana, as condições de transporte, a intensidade do trabalho, a necessidade de pessoal especializado, o período de tempo prolongado antes dos resultados e a amostragem rigorosa. Além disso, podem não identificar a presença de um microrganismo se a quantidade da amostra for baixa ou se estiver presente em uma área inacessível, e a falha em detectar a presença de um microrganismo não significa que ele estava ausente na região da coleta da amostra (PINHEIRO et al., 2003b).

O desenvolvimento dos métodos moleculares mostra que são técnicas adequadas e práticas para a detecção direta ou identificação de novos microrganismos, avaliando a existência de genes de resistência antimicrobiana, detecção de vários genes de fator de virulência e tipagem microbiana em investigações epidemiológicas (SHAHI *et al.,* 2018).

As técnicas de sequenciamento de DNA fornecem um grande número de leituras de sequência por execução, resultando em profundidade e cobertura de amostragem mais ampla e, permitindo a detecção não apenas da maioria da comunidade microbiana dominante, mas também os microrganismos menos abundantes (SIQUEIRA *et al.,* 2016).

3.7 Distribuição geográfica da resistência antimicrobiana

A composição da microbiota e o perfil de resistência antimicrobiana dos microrganismos associados ao abscesso periodontal variam entre as diferentes populações (IRSHAD et al., 2020). E, embora as diferenças dos achados laboratoriais, com relação a prevalência das espécies microbianas envolvidas nas infecções endodônticas em diferentes países, possam ser atribuídas as variações nas metodologias de identificação, também se tem suspeitado da influência geográfica na composição da microbiota do canal radicular (SIQUEIRA *et al.,* 2008).

A Organização Mundial da Saúde associa o alto consumo de antimicrobianos aos altos níveis de resistência antimicrobiana, o que poderia explicar a diferença encontrada nos valores de concentração inibitória mínima (CIM) para o mesmo antimicrobiano e espécies microbianas em diferentes países (ARREDONDO *et al.*, 2019).

A resistência aos antimicrobianos é considerada um fenômeno natural antigo e está presente em todas as regiões geográficas (D′COSTA; FROESE, 2011) e pode sofrer variações de acordo com as características demográficas da população, os hábitos culturais locais e a prescrição dos antimicrobianos.

4 OBJETIVOS

4.1 Geral

Levantar e descrever o perfil dos microrganismos e seus respectivos perfis de resistência antimicrobiana em infecções endodônticas e periodontais.

4.2 Específicos

1. Estimar a frequência dos microrganismos presentes nas infecções endodônticas e periodontais;
2. Estimar a frequência da resistência dos microrganismos frente aos agentes antimicrobianos;
3. Estimar a frequência dos genes de resistência e fatores de virulência presentes nas infecções avaliadas.

5 MÉTODO

O método proposto para o desenvolvimento desta tese foi registrado na base *Prospero* sob o nº CRD42018077810 e publicado na revista Medicine em 2018 (ABE *et al.*, 2018).

O protocolo publicado (ANEXO B) deu origem aos resultados, apresentados na forma de um artigo científico.

6 RESULTADOS

Os resultados estão apresentados no formato de artigo, conforme permitido nas normas do PPGCF – UNISO.

6.1 "Resistance profile to antimicrobial agents in the main circulating bacteria isolated from acute periodontal and endodontic infections in Latin America (MICROBE-DENT)"

Foi inserida nesta tese a versão original do artigo "Resistance profile to antimicrobial agents in the main circulating bacteria isolated from acute periodontal and endodontic infections in Latin America (MICROBE- DENT)". Protocolo da Revisão Sistemática publicado em outubro de 2018 (ANEXO B).

Revista: Medicine
Versão *online*: 1536-5964
Classificação Webqualis/Capes: A2
Área: Farmácia

6.2 "Antimicrobial resistance and microorganisms present in endodontic infections: a systematic review"

Foi inserida nesta tese a versão original do artigo "Antimicrobial resistance and microorganisms present in endodontic infections: a systematic review." formatado de acordo com as normas da revista *BMJ Open*, submetido em 18/02/2022 (ID-bmjopen-2022-061999).

Revista: *BMJ Open*
Versão *online*: 2044-6055
Fator de impacto (2022): 2.692
Classificação WebQualis/ Capes quadriênio 2013-2016: A1
Área: Farmácia

Title page

Title: "Antimicrobial resistance and microorganisms present in endodontic infections: a systematic review"

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Key words: Anti-bacterial agents. Dentistry. Endodontics. Drug resistance, microbial. Periodontics.

Word count - 3326

ABSTRACT

Objective To estimate and describe the prevalence of microbial resistance to antimicrobials in endodontic infections worldwide.

Design Through a systematic review, observational studies were carried out with permanent dentition, which evaluated the antimicrobial resistance in endodontic infections. Studies that allowed the ingestion of antimicrobials at the time of the collection were excluded.

Data sources MEDLINE (PubMed/ Ovid), EMBASE, BVS, CINAHL, and Web of Science from January 2011 until December 2021.

Data extraction and synthesis Six reviewers, working in pairs and independently, selected titles, abstracts and full texts extracting data from all studies that met the eligibility criteria: characteristics of patients, diagnosis of infection, microbial species assessed, antimicrobials assessed, identification of resistance genes and virulence factors. "The Joanna Briggs Institute" critical appraisal for case series was adapted to assess the risk of bias in the included studies.

Results Twenty-two studies (N= 1,263) met the inclusion criteria. *Enterococcus* species were the most cited microorganisms, and the virulence factors were related to enterococcal surface protein (esp) and gelatinase production (gelE). The antimicrobial resistance varied according to the diagnosis of the infection, the susceptibility/resistance assessment technique, and the type of antimicrobial assessed. The confidence on the studies was considered critically low.

Conclusion The most frequently cited microorganisms were present in both primary and secondary or persistent infection, and showed resistance to at least one of the antimicrobials tested.

Key words: Anti-bacterial agents. Dentistry. Endodontics. Microbial drug resistance. Periodontics. MICROBIOLOGY/ **BACTERIOLOGY/** ORAL MEDICINE

Registration: The protocol of this systematic review was registered in *Prospero* (CRD42018077810) and published in *Medicine* (http://dx.doi.org/10.1097/MD.0000000000013158)

Strengths and limitations of the study

- We undertook rigorous searches for the available literature and assessed the quality of the studies over the last ten years with the aim of avoiding interference in changing antimicrobial resistance characteristics.
- The information collected and related in this systematic review will guide future researches in order to evaluate the behavior of the microorganisms that make up the microbiome and their resistance profile to the most commonly prescribed antimicrobials in dentistry.
- One of the limitations of this review was the impossibility of estimating the prevalence of microorganisms found in endodontic infections, as the studies that met the eligibility criteria were only case series presenting a lack of information on the clinical and demographic characteristics of the patients.
- As the meta-analysis was not performed, it is not possible to infer hypotheses about the use of antibiotics and its implications, nor to suggest safer and more effective therapeutic protocols.

Introduction

Antimicrobial resistance has been increasing to dangerous high levels in all parts of the world. New mechanisms of resistance are emerging and spreading globally, threatening the ability to treat common infectious diseases[1].

During the treatment of endodontic infections, it is not routine to collect samples from the interior of the root canal or the periapical region to determine the causal agents. Because of that, in Dentistry, the antimicrobial prescription can be considered empirical[2 3]. A questionnaire applied to Brazilian endodontists revealed that many of them prescribe antimicrobials in situations when they would not be indicated[4].

Knowing the microbiological profile of endodontic infections, their geographic distribution and the resistance profile of the present microbiome, will allow the clinician to individually and assertively prescribe the antimicrobial, in order to control the increase in antimicrobial resistance[5].

The dearth of syntheses that estimate and describe the prevalence of microbial resistance to antimicrobials in endodontic infections worldwide led to the development of this systematic review. Therefore, this systematic review aimed to answer the focused question "What is the antimicrobial resistance profile of microorganisms in endodontic infections?", limiting the search for studies carried out from 2011 to 2021 to avoid a possible interference in the change in the resistance profile that may occur over the years.

Methods

This systematic review followed PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)[6] recommendations, was registered on the PROSPERO database under number CRD42018077810 and the protocol was published in *Medicine* (**Supplementary File 1**) .

Patient and public involvement

No patient involved.

Search strategy

The search was oriented by an experienced librarian in the following databases: MEDLINE (PubMed/ Ovid), EMBASE, BVS, CINAHL, and Web of Science. Additionally,

the website “bancodeteses.capes.gov.br” and Grey Literature Report were searched as grey literature.

The electronic search strategy was developed using the key words combining Medical Subject Heading (MeSH) terms. The Boolean operators ‘AND ’and ‘OR ’were applied to combine the terms and create a search strategy. The search strategies for each database and the following findings are summarized in **Supplementary File 2**. All articles selected were imported into the EndNote X9 (Clarivate, London, UK) reference manager to catalogue the references and facilitate the exclusion of duplicates.

Eligibility criteria

The studies were selected according to the following inclusion criteria:

• Population (P): patients with teeth diagnosed with endodontic infection (primary, secondary and/or persistent).

• Outcome (O): antimicrobial resistance reported through minimal inhibitory concentration, zone of inhibition and/or detection of resistance genes by culture-independent molecular techniques.

• Study design (S): observational studies.

• Timing: study published from January 2011 to December 2021. The resistance profile was considered to change every ten years, as many antimicrobials have entered in the market recently.

• Language: no restriction.

Methodological studies, studies that allowed the antimicrobial consumption until the time of sample collection and time series were excluded.

Selection of studies

Six reviewers working in pairs and independently (CCM, JPMVC, KK, SB-F, CCBM, CCG), screened titles and abstracts. The same reviewers were calibrated for each step of the process (assessed eligibility of each full-text article, data extraction and risk of bias assessment

of a determined number of studies with different quality levels). Disagreements were solved by consensus or a with the participation of a senior reviewer (LCL).

Data extraction

The information entered into in an Excel spreadsheet using a predefined data collection form and the same groups of independently reviewers extracted the data.

The following data were extracted from each study: author (year)/country, duration of the study, place of recruitment, characteristics of patients, diagnosis of infection, year of sample collection, microbial species evaluated, method of identification of microorganisms, evaluated antimicrobials, identification of resistance genes and virulence factors, and conflict of interest.

Data synthesis and statistical analysis

Due to the variety of resistances observed, the studies were grouped by antibiotic analyzed: anfotericin B, amifloxacin, amikacin, amoxicillin, amoxicillin + clavulanic acid, ampicillin, ampicillin + sulbactam, azithromycin, benzylpenicillin, cefachlor, cefixime, cefazolin, cefepime, cefotaxime, cefoxitin, cefuroxime, chloramphenicol, ciprofloxacin, clindamycin, cloxacillin, ceftazidime, cotrimoxazole, ceftriaxone, colistin/ polymyxin E, doxycycline, erythromycin, fosfomycin, fluconazole, gentamicin, imipenem, ketoconazole, levofloxacin, lincomycin, linezolid, meropenem, moxifloxacin, metronidazole, netilmicin, nitrofurantoin, nystatin, oxacillin, penicillin, piperacillin, piperacillin + tazobactam, polymyxin B, rifampicin, spiramycin, sulfamide, teicoplanin, tigecycline, tetracycline, tobramycin, vancomycin.

Thus, it was decided to group the reports of resistance, regardless of the strain detected in the included studies. In each identified study, it was identified the number of resistant strains out of the total investigated strains to calculate the percentage of resistance. As the resistance percentages are highly variable, a random effects model was chosen to group proportions in meta-analyses. To stabilize the variances, the Freeman-Tukey double arc sine transformation was used and to 95% confidence intervals considered Wilson scores. Heterogeneity (I^2) was calculated from the inverse variance model in a fixed-effect model.

The limits of I^2> 50% to consider heterogeneous was adopted. All analyzes were performed on Stata SE 14.2 (StataCorp, College Station, TX)[7].

Quality assessment and strength of evidence

The methodology used to assess the quality of the study was the checklist for case series from the Joanna Briggs Institute Critical Appraisal tools[8] and adapted to the research question of this systematic review. The risk of bias domains included were 1) selection bias (1.1 - inclusion criteria, 1.2 - diagnostic criteria, 1.3 - consecutive inclusion of patients, 1.4 - demographic characteristics of patients, 1.5 - clinical information of patients, 1.6 - location of the recruitment, 1.7 - complete inclusion of patients); 2) measurement bias (sample collection methodology); 3) selective reporting bias (outcome reporting).

Each domain or subdomain was assessed as follow:

1. Domain 1: Selection bias:

1.1. Inclusion criteria

"Were the inclusion criteria clearly defined?" The authors should provide the inclusion criteria (and exclusion if applicable) of the patients included in the studies. The inclusion criteria should present the disease's diagnose and the patient's medical history.

1.2. Diagnostic criteria

"Was the infection diagnostic established following a standardized criterion? The same criterion was used for all patients?" The authors should describe the measurement method of the condition (diagnostic) following a standard that should be replicated.

1.3. Consecutive inclusion of patients

"Did the study present the consecutive inclusion of patients?" Studies that present the consecutive inclusion of patients are more reliable than those which do not present it. The

authors should report if the inclusion of patients was consecutive or the period of time in which the samples were collected.

1.4. Demographic characteristics of patients

"Were the demographic characteristics of patients clearly reported?" The authors should describe the demographic characteristics of patients, as ethnicity, sex, age, oral hygiene habits and regional HDI (Human Development Index).

1.5. Clinical information of patients

"Was the clinical information of patients clearly reported?" The authors should clearly report the clinical information of patients, as the condition and stage of the disease, comorbidities, harmful habits (such as smoking and drinking alcohol), etc.

1.6. Location of the recruitment

"Was the location of the recruitment the same for all sample collection?" Some diseases or conditions may vary their prevalence according to the different geographical region and/or populations.

1.7. Complete inclusion of patients

"Did the authors report the complete inclusion of the patients?" The integrity of a case series contributes to its reliability. The authors should report if there was a loss of sample/participant and how it was solved.

2. Domain 2: Measurement bias (sample collection methodology)

"*Was the sample collection methodology adequate and standardized to all patients?"* The authors should determine if the measurement tools used were validated instruments as they have a significant impact on the validity of the outcome assessment.

3. Domain 3: Selective reporting bias (outcome reporting)

"Were the outcome or follow-up results (microbial resistance) clearly reported?" The results of any intervention or treatment should be clearly reported by the authors.

The GRADE approach framework (Grading of Recommendations, Assessment, Development and Evaluations) are used to assess the certainty of evidence when data are narratively summarized[9]. Under this approach, **high certainty** in the evidence means that researchers are very confident that the effect they found in the studies is close to the true effect; **low certainty** of evidence means that the result obtained will most likely be sufficiently different from what the research has found to affect a decision, and **very low certainty** of evidence means that the authors are certain they have little or no confidence in the effect.

Considering these aspects, an adaptation was performed, judging the quality based only on the risk of bias. In this way, each domain was evaluated and items 1.3, 1.4, 1.5, 1.7 and 2

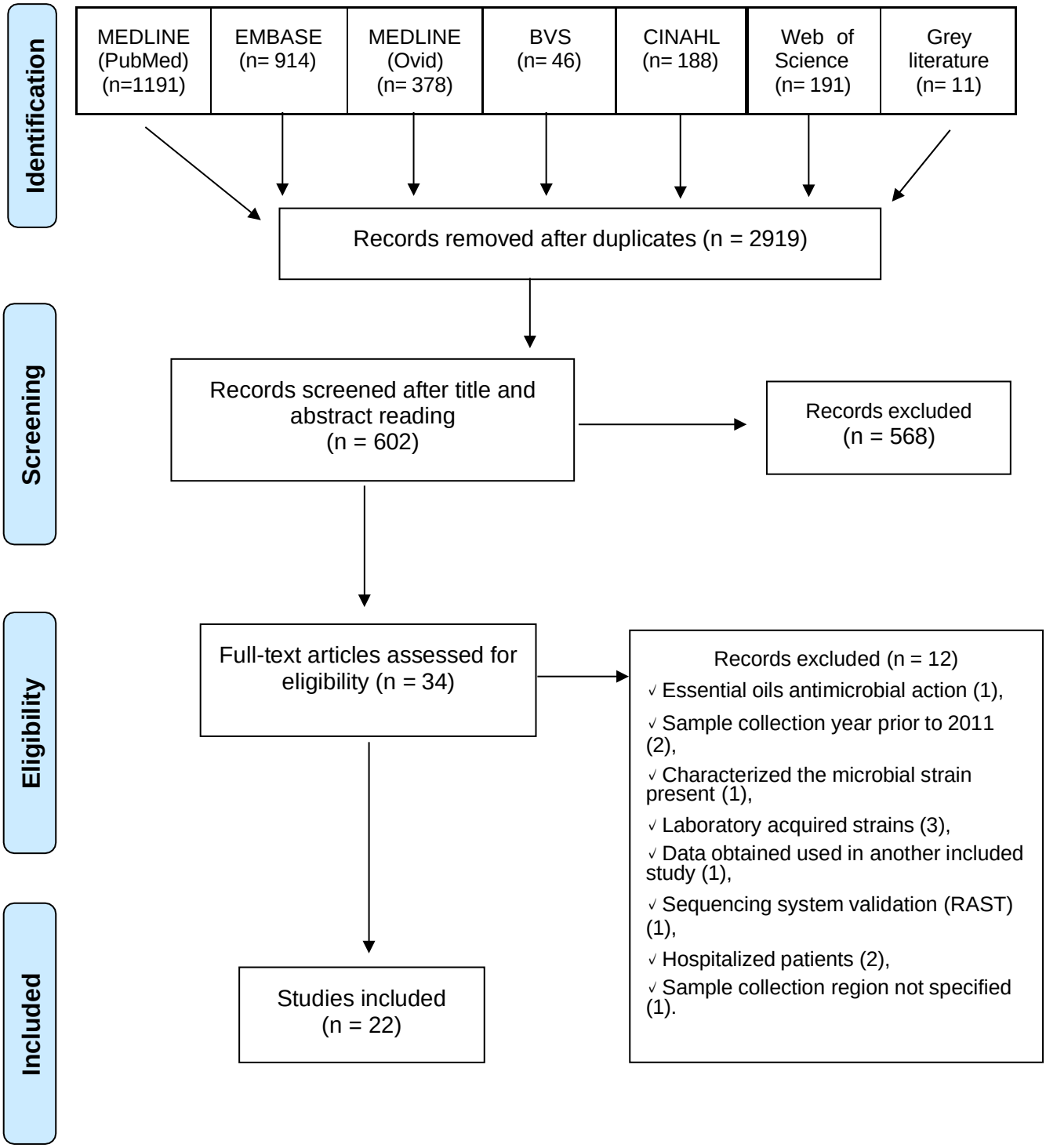
were used as critical domains. Observational studies, according to GRADE [9] initially present low quality, however, if the study does not present criteria that lower its quality, it can raise a level of confidence, being considered as “moderate " quality. That is, if the study presents “low risk of bias” in all items evaluated, the confidence in this study will be considered “moderate”. If the study presents “high risk of bias” in more than one domain considered critical, then confidence in the study will be “critically low”.

Results

Study selection

The search strategy identified 2.919 titles and abstracts from the cited databases and 602 studies were screened. From the evaluation of the full-text of each article, 66 studies were selected for data extraction and 22 studies met the eligibility criteria[10-31] and composed the final sample used in the qualitative and quantitative analyses (**Figure 1**). Forty-four studies were excluded for reasons listed in the table of characteristics of excluded studies (**Supplementary File 3**).

Figure 1 - Flow chart



Data extraction

Table 1 presents the summary of the included studies.

In all of the 1,263 patients who participated in the studies, only one tooth was evaluated with one of the possible diagnoses: healthy pulp, pulp necrosis associated with primary apical periodontitis (primary infection) or apical periodontitis after endodontic treatment (secondary or persistent infection).

- Geographic location: The majority of the studies was conducted in the Americas (13 studies) followed by Asia (6 studies), Europe (2 studies) and Africa (1 study). None of the studies was found neither in Antarctica nor in Oceania continents.
- Study design: all of the studies were case series. Other observational studies, as cohort or cross sectional studies, were not found.
- Duration of the study: only five studies reported the duration of the study[18 22 25 26].
- Place of recruitment: the sample collection was done in different places - 12 studies were conducted in a University/Dentistry College[10 12 13 15 18-22 25 28 29 31], one study was conducted in a public dental clinic[26], one study was conducted in a hospital dental clinic[24], three studies reported the association of more than one type of establishment[11 27 30], and four studies did not report the place of recruitment[14 16 17 23].
- Age: only 13 studies reported the participants' age, ranging from 11 to 94 years.
- Sex: only seven studies reported the sex of the patients[11 16 18 25 26 29 31].
- Diagnosis: nine studies reported primary infection[13-15 17 20 24 26 28 30], six studies reported secondary or persistent infection[11 19 21 22 27 31], five studies reported both infections[10 12 16 25 29], one study reported healthy pulp and both infections[18] and one study did not report the diagnosis of the infection[23].
- Microbial species assessed: 35 species were assessed (*Acinetobacter* spp., *a-Streptococcus* spp., *Aerococcus* spp., Anaerobes, *Anaerococcus prevotti, Bacteroides* spp., *Bifidobacterium* spp., *Campylobacter* spp., *Candida albicans, Citrobacter freundii, Clostridium* spp., *Dialister invisus, Eggerthella* spp., *Enterobacter* spp., *Enterococcus* spp., *Escherichia coli, Fusobacterium* spp., *Klebsiella* spp., *Lactobacillus* spp., *Lactococcus lactis, Moraxella* spp., *Parvimonas micra, Peptostreptococcus* spp., *Porphyromonas* spp., *Prevotella* spp., *Propionibacterium* spp., *Proteus* spp., *Pseudomonas* spp., *Pseudoramibacter alactolyticus, Roseomonas mucosa, Serratia* spp., *Staphylococcus* spp., *Streptococcus* ssp., *Treponema* spp., yeasts).

- Method of identification of the microorganisms: ten studies reported culture-dependent technique[12 16 18 20 23 24 26 29-31], six studies reported molecular culture-not dependent[10 11 13 15 25 28] and six studies reported both techniques[14 17 19 21 22 27].
- Antimicrobials assessed: 53 antimicrobials were assessed (anfotericin B, amifloxacin, amikacin, amoxicillin, amoxicillin + clavulanic acid, ampicillin, ampicillin + sulbactam, azithromycin, benzylpenicillin, cefachlor, cefixime, cefazolin, cefepime, cefotaxime, cefoxitin, cefuroxime, chloramphenicol, ciprofloxacin, clindamycin, cloxacillin, ceftazidime, cotrimoxazole, ceftriaxone, colistin/ polymyxin E, doxycycline, erythromycin, fosfomycin, fluconazole, gentamicin, imipenem, ketoconazole, levofloxacin, lincomycin, linezolid, meropenem, moxifloxacin, metronidazole, netilmicin, nitrofurantoin, nystatin, oxacillin, penicillin, piperacillin, piperacillin + tazobactam, polymyxin B, rifampicin, spiramycin, sulfamide, teicoplanin, tigecycline, tetracycline, tobramycin, vancomycin).
- Resistance genes and/or virulence factors identified: five studies assessed resistance genes[10 13 15 17 25], four studies assessed virulence factors[11 19 21 22] and three studies assessed both[14 27 28].
- Conflict of interest: none of the authors reported conflict of interest.

Table 1. Summary of the included studies.

Geographic location	Study reference	Population (N)	Participants' age (range)	Microbial species assessed (N)	Antimicrobials assessed (*resistance)	Resistance gene (Antimicrobial)	Virulence factors (Antimicrobial)
Africa (Burkina Faso)	Kaboré [26]	Patients (125)	19 - 40 years	*Lactococcus lactis* spp. *lactis* (5)	AMX*, AXL*, BZP*, CFX*, CRX*, CLR*, CIP*, CLI*, CTZ*, ERI*, ESP*, GEN*, LIN*, MTZ*, NTL*, OXA*, PIP*, PIT*, TBR*	NA	NA
Americas (Brazil)	Sousa [16]	Patients (60)	11 – 63 years	*Anaerococcus prevotii* (12) *Fusobacterium necrophorum* (11) *Prevotella intermedia/ nigrescens* (10) *Fusobacterium nucleatum* (10) *Parvimonas micra* (5)	AMX, AXL, AZI*, BZP, CFC, CLI, ERI*, MTZ*	NA	NA
Americas (Brazil)	Midena [20]	Patients (23)	NR	*Fusobacterium nucleatum* (5)	AMX, AXL, AMP, AZI, CLI, ERI*, MTZ	NA	NA
Americas (Mexico)	Medina-Palacios [31]	Patients (15)	22 – 72 years	*Enterococcus* spp. *Streptococcus* spp. *Bacteroides* spp. *Eggerthella* spp. *Aerococcus* spp. *Clostridium* spp. *Bifidobacterium* spp. yeasts	AMX*, AXL*, CLI*	NA	NA

Americas (Brazil)	Lins [14]	Patients (43)	NR	*Enterococcus faecalis* (20)	BZP*, ERI*, TET*, VAN*	*tetM, tetL* (TET)	*agg, esp gel*E
Americas (Brazil)	Montagner[17]	Patients (20)	NR	*Prevotella buccae* (8) *Prevotella disiens* (1) *Prevotella oralis* (3) *Prevotella intermedia/ nigrescens* (5) *Porphyromonas endodontalis* (1) *Porphyromonas gingivalis* (1) *Parvimonas micra* (9)	AMX*, AXL*, BZP*	*blaCfxA/ blaCfxA2* (ß-lactamase)	NA
Americas (Brazil)	Endo[19]	Patients (30)	19 – 65 years	*Enterococcus faecalis* (7)	AMX*, AXL, AZI*, BZP*, CIP*, CLR*, DOX*, ERI*, MOX*, RIF*, TET*, VAN*	NA	*ace, asa, cylA efaA, esp gelE*
Americas (Brazil)	Barbosa-Ribeiro [21]	Patients (20)	30 – 60 years	*Enterococcus faecalis* (20)	AMX*, AXL, AZI*, BZP*, CIP*, CLI*, CLR*, DOX*, ERI*, GEN*, MTZ*, MOX*, RIF*, TET*, VAN*	NA	*ace, asa, asa373, efaA, esp, gelE*
Americas (United States of America)	Jungermann [10]	Patients (50)	19 – 94 years	*Propionibacterium acnes* (2) α-*Streptococcus* spp. (3) *Moraxella* spp. (1) *Parvimonas micra* (1) *Prevotella corporis* (1) *Prevotella intermedia* (3) *Prevotella denticola* (1)	AFX, AMX*, AXL, CLI *, DOX *, MTZ*, TGC, TET*.	*bla-TEM1, bla-Z, blaCfxA* (ß-lactamase) *tetQ, tetM, tetW* (TET)	NA

				Prevotella buccae (2) *Fusobacterium* spp. (3) *Peptostreptococcus* spp. (5) *Lactobacillus* spp. (2)			
Americas (Brazil)	Zoletti [11]	Patients (85)	19 – 75 years	*Enterococcus faecalis* (20)	NA	NA	*ace, agg* *cpd, efa*A *ef*1841/ *fsrC* *esp, gelE*
Americas (Brazil)	Rôças & Siqueira [13]	Patients (26)	NR	*Campylobacter curvus* *Dialister invisus* *Fusobacterium nucleatum* *Parvimonas micra* *Prevotela* spp. *Propionibacterium propionicum* *Pseudoramibacter alactolyticus*	NA	*blaTEM, blaCfxA* (ß-lactamase) *ermC* (macrolides) *tetM, tetW* (TET)	NA
Americas (Brazil)	Rôças & Siqueira [15]	Patients (50)	19 – 64 years	NR	NA	*blaTEM blaCfxA* (ß-lactamase) *ermC* (macrolides) *tetM, tetQ, tetW* (TET)	NA
Americas (Mexico)	Dominguez-Perez [25]	Patients (64)	21 – 58 years	*Enterococcus* spp. (26) *Fusobacterium* spp. (31) *Porphyromonas endodontalis* (5) *Porphyromonas gingivalis* (0)	NA	*blaTEM-1* (ß-lactamase)	NA

				Prevotella spp. (27) *Streptococcus* spp. (29) *Treponema* spp. (1)		*ermC* (macrolides) *tetM, tetW* (TET)	
Americas (Brazil)	Lins [28]	Patients (20)	NR	*Enterococcus faecalis* (59)	NA	*tetM, tetL* (TET) *ermB* (macrolides) *vanA* (VAN)	*agg, asa373* *cylB, esp* *gelE*
Asia (Turkey)	Dumani [12]	Patients (231)	NR	*Enterococcus faecalis* (18) *Candida albicans* (18)	COL*, ESP*, PEN*, VAN* AFB, CET*, FLU*, NIS	NA	NA
Asia (Saudi Arabia)	Al-Badah [18]	Patients (82)	16 – 72 years	*Enterococcus faecalis* (21)	ERI*, LEV, LIZ*, MOX, NIT, TEI, TET*, TGC, VAN	NA	NA
Asia (Taiwan)	Lee [24]	Teeth (62)	NR	Anaerobes (83) *Campylobacter* spp. (2) *Streptococcus* spp. (3) *Staphylococcus* spp. (1) *Enterococcus faecalis* (8) *Pseudomonas* spp. (3) *Acinetobacter* spp. (2) *Citrobacter freundii* (1) *Escherichia coli* (2) *Klebsiella* spp. (2)	AXL*, AMP*, AMS*, CFZ*, CEF*, CFT*, CZD*, CLR*, CIP*, CLI*, LEV*, LIZ*, MTZ*, PEN*, SUL*	NA	NA

				Serratia spp. (2) *Enterobacter* spp. (2) *Proteus* spp. (2)			
Asia (Malaysia)	Abraham [29]	Patients (64)	18 – 60 years	*Candida albicans* (4)	AFB, CET, FLU, NIS	NA	NA
Asia (Iraq)	Hussein [30]	Teeth (80)	33 (mean) years	*Enterococcus faecalis* (55)	AMX, AZI, CFX*, CXA*, CLO*, CIP, LIN*, MTZ*, VAN	NA	NA
Asia (Iran)	Saffari [27]	Teeth (70)	NR	*Enterococcus faecalis* (22)	AMP* CIP*, ERI*, GEN, LIZ, RIF*, TEI, TET*, VAN	NA	*ace*, *chp* *cyl*, *ebp*, *esp*, *gel*E
Europe (Germany)	Diesendorf [23]	Patients (13)	NR	*Roseomonas mucosa* (7)	AMI, AMP*, AMS*, CFZ*, CZD*, CXA, CRX*, CIP, CTZ, FOS*, GEN, IMI, MER, PIP*, PIT*, POL, TET, TGC, TBR	NA	NA
Europe (Sweden)	Vidana [22]	Patients (30)	NR	*Enterococcus faecalis* (30)	AMP, CIP*, GEN*, IMI, LIZ, PIT, VAN	NA	*ace*, *asa*1 *cyl*A, *efa*A ef1841/ fsrC

esp, *gel*E

AFB - anfotericin B, AFX – amifloxacin, AMI – amikacin, AMX – amoxicillin, AXL – amoxicillin + clavulanic acid, AMP – ampicillin, AMS – ampicillin + sulbactam, AZI – azithromycin, BZP – benzylpenicillin, CFC – cefachlor, CFX – cefixime, CFZ – cefazolin, CEF – cefepime, CFM – cefotaxime, CFT – cefoxitin, CRX – cefuroxime, CLR – chloramphenicol, CIP – ciprofloxacin, CLI – clindamycin, CLO – cloxacillin, CZD – ceftazidime, CTZ – cotrimoxazole, CXE – ceftriaxone, COL – colistin/ polymyxin E, DOX – doxycycline, ERY – erythromycin, FOS – fosfomycin, FLU – fluconazole, GEN – gentamicin, IMI – imipenem, KET – ketoconazole, LEV – levofloxacin, LIN – lincomycin, LIZ – linezolid, MER – meropenem, MOX – moxifloxacin, MTZ – metronidazole, NTL – netilmicin, NIT – nitrofurantoin, NYS – nystatin, OXA – oxacillin, PEN – penicillin, PIP – piperacillin, PIT – piperacillin + tazobactam, POL – polymyxin B, RIF – rifampicin, SPI – spiramycin, SUL – sulfamide, TEI – teicoplanin, TGC – tigecycline, TET – tetracycline, TBR – tobramycin, VAN – vancomycin.

agg (aggregation substance), *esp* (*Enterococcal* surface protein), *gel*E (gelatinase), *ace* (angiotensin I- converting enzyme), *asa* (aggregation substance), *cyl* (citolisin), *efaA* (endocarditis specific antigen), *cpd* (protein code), *ef*1841/ *fsrC* (gelatinase-negative phenotype), *chp* (protein code gene), *ebp* (*elastin* binding protein)

*- resistance reported, NA – not assessed, NR – not reported.

Enterococcus species were the most frequent microorganisms, being reported in 12 studies, followed by *Fusobacterium* spp., *Prevotella* spp. (five studies each), *Parvimonas micra* (four studies) and *Streptococcus* spp. (three studies).

Among the most indicated antimicrobials for the treatment of endodontic infections, according to the American Association of Endodontists[32], amoxicillin, amoxicillin associated with clavulanic acid and ciprofloxacin were the most cited in this systematic review. And the antimicrobials that showed the highest resistance rates for different microorganisms were erythromycin (57.0%), metronidazole (53.9%), penicillin (47.2%) and clindamycin (40.6%) (**Table 2**).

Table 2. Frequency of antimicrobial resistance reported in endodontic infections according to the included studies.

Antimicrobial	**N (studies)**	**R % (95% CI)**	**I^2 (%, p)**	**% Weight**
amoxicillin	9	6.4 (0.0, 22.5)	70.2 (p= 0.0)	30.04
amoxicillin + clavulanic acid	8	7.9 (0.4, 20.1)	66.9 (p= 0.0)	57.71
ciprofloxacin	8	20.0 (2.0, 45.6)	83.3 (p= 0.0)	45.62
metronidazole	7	53.9 (24.7, 82,1)	90.3 (p= 0.0)	38.45
tetracycline	7	15.1 (0.0, 47.1)	86.7 (p= 0.0)	33.00
clindamycin	6	40.6 (17.4, 65.6)	72.1 (p= 0.0)	23.85
erythromycin	6	57.0 (20.6, 90.0)	90.2 (p= 0.0)	28.26
benzylpenicillin	5	2.4 (0.0, 16.6)	55.6 (p= 0.0)	100.00
azithromycin	4	7.2 (0.0, 41.7)	90.7 (p= 0.0)	11.53
doxycycline	3	0.6 (0.0, 14.3)	32.3 (p= 0.1)	23.62
penicillin	2	47.2 (19.2, 76.0)	67.4 (p= 0.0)	18.38

R- antimicrobial resistance, I^2- heterogeneity

The most cited genes in the included studies were related to tetracycline and erythromycin resistance, and ß-lactamase production.

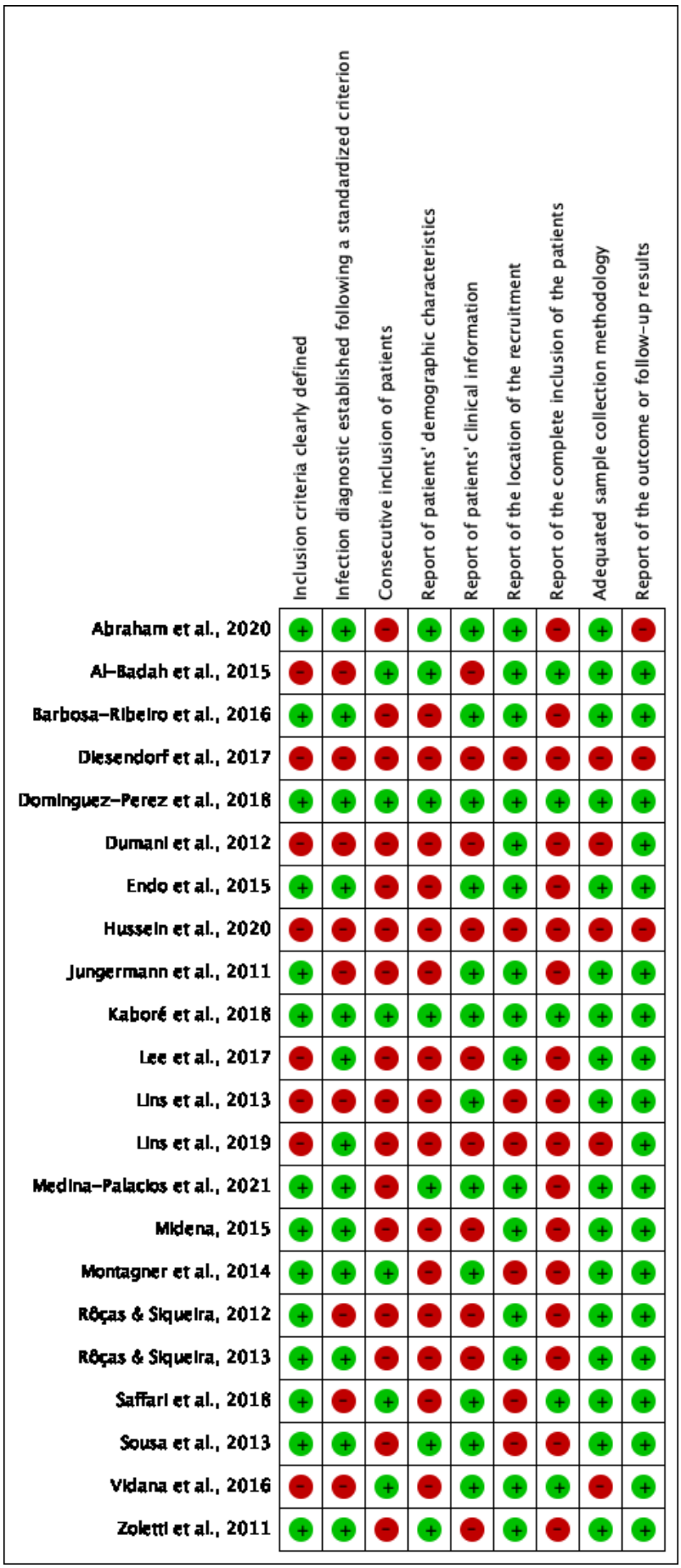
The most cited virulence factors were related to *Enterococcal* surface protein (esp), gelatinase production (gelE), angiotensin I - converting enzyme (ace), cytolysin (cylA), and endocarditis specific antigen (efaA).

The data obtained regarding the antibiotic resistance profile, the prevalence of microorganisms assessed in endodontic infections, and the antimicrobial resistance relationship did not allow the generation of statistical analysis for this systematic review.

Quality assessment

Figure 2 illustrates the risk of bias of the included studies for each domain evaluated and the analysis of the overall risk of bias. Only two studies[25] [26] presented all the criteria evaluated as low risk of bias. The remaining 20 studies presented at least one critical domain, therefore, they were graded as a critically low confidence.

Figure 2 - Risk of bias of the included studies.



It was not possible to analyze the risk of publication bias, as ten studies that evaluated the antimicrobial resistance of the same microorganisms against the same antimicrobials were not obtained.

The justifications for the modifications to the original registered protocol are presented in the **Supplementary File 4**.

Discussion

Enterococcus faecalis was the most assessed microbial species and showed the highest frequency of resistance to the following antimicrobials: ciprofloxacin, erythromycin and tetracycline, and the most cited virulence factor is associated to *Enterococcus* species. The other microorganisms that compounded the endodontic infection microbiome varied according to the geographic location of the included studies.

In this systematic review, only a survey of the frequency of resistance to some antimicrobials was performed, as the data from the included studies were insufficient to correlate with the microorganisms present in infections and generate statistical analysis. But it was possible to observe the correlation of the most frequently reported resistance genes (*tet*, *erm* and ß-lactamase production) with the most frequently reported antimicrobials of resistance, ß-lactams, tetracyclines, and macrolides.

A systematic review developed in 2016[33], in which seven studies were included, reported discrepant resistance data from this review: higher resistance rates for amoxicillin (7.7% against 6.4%), penicillin G (12.3% against 2.4%), tetracycline (40.0% against 15.1%), and lower resistance rates for amoxicillin-clavulanic acid (3.5% against 7.9%), clindamycin (13.1% against 40.6%), metronidazole (17.5% against 53.9%) and, erythromycin (26.0% against 57.0%).

Another systematic review[34] showed that the resistance genes that appeared more frequently in endodontic infections were those related to tetracycline, in nine studies. This systematic review, with 22 studies included, presented resistance genes capable of producing the ß-lactamase enzyme as the genes most frequently reported among the included studies.

This discrepancy could be explained by the presence of different microorganisms and sample size, by the geographic and temporal location of the studies and/or by the susceptibility analysis methodology (culture or non-culture dependent molecular technique), suggesting that the prescription of antimicrobials should be performed with caution by dentists.

Most of the included studies in this systematic review collected the sample using sterile paper tips to assess the minimum inhibitory concentration of antimicrobials in relation to the

microorganisms present (or identified) in the infections. However, persistent microorganisms are located in untouched root canal walls or in areas such as lateral canals, apical branches, dentinal tubules, and isthmuses. In most of these areas, microorganisms are inaccessible to instruments and substances used in endodontic treatment. Likewise, conventional microbiological sampling procedures with paper tips[17 19 20] cannot reach these areas and, consequently, the most important microbial species involved in the failure of endodontic treatment may not be collected and identified[35-37].

The use of the culture method to determine antimicrobial resistance was one of the main limiting factors of the studies, as the *in vitro* susceptibility assessment does not accurately reflect clinical efficacy. One study[19] identified seven samples of *E. faecalis* in the evaluation by culture, 13 samples using the conventional PCR gene sequencing technique and 23 samples when the nested PCR gene sequencing technique was used, confirming the limitation of the first technique.

The heterogeneous etiology of periodontitis, in which multiple microbial combinations may play a role in causing the disease, could justify the multi-resistance found in endodontic infections. The high presence of adhesion factors that influence biofilm formation and are able to increase antimicrobial resistance was reported in seven studies included in this review[11 14 19 21 22 27 28].

Several host and environmental factors related to individual geographic location can influence the microbial colonization of the oral cavity and are responsible for differences in the composition of these communities. These factors include genetic background, ethnicity, socioeconomic status, community water quality, supplies, eating habits, psychological stress, smoking, and the nature of the species colonizing other individuals in the same community[38], which emphasizes the importance of reporting demographic information from participants in studies that assess the behavior of infections.

As most of the studies included in this systematic review did not report neither important information about the clinical and demographic characteristics of the patients, nor the criteria for inclusion of these patients in the evaluated studies, it was not possible to determine the geographic distribution of the resistant microorganisms found.

The data reported in the included studies on the microorganisms assessed or the analysis of resistance to the antimicrobials tested were not sufficient to generate a meta-analysis, withal the high heterogeneity (I^2) between the antimicrobials assessed could engender a possible publication bias.

Strengths and limitations of the study

One of the limitations of this review was the impossibility of estimating the prevalence of microorganisms found in endodontic infections, as the studies that met the eligibility criteria were only of the case series type and there was a lack of information regarding the number of samples evaluated. The lack of information on the clinical and demographic characteristics of the patients included in the studies could also be considered an important limitation, as it did not allow the establishment of the geographic distribution of resistant microorganisms present in endodontic infections.

As the meta-analysis was not performed, it is not possible to infer hypotheses about the use of antibiotics and its implications, nor to suggest safer and more effective therapeutic protocols. However, the information collected and related in this systematic review will guide future researches in order to evaluate the behavior of the microorganisms that make up the microbiome and their resistance profile to the most commonly prescribed antimicrobials in dentistry.

Conclusion

The most frequently cited microorganisms were present in both primary and secondary or persistent infection, and showed resistance to at least one of the antimicrobials tested. It was not possible to estimate the prevalence of bacteria found in endodontic infections, as the studies included were case series presenting high risk of bias. Resistance genes were more frequent than virulence factors in both types of infections.

There is considerable uncertainty regarding the profile of microorganisms and their resistance to endodontic infections, therefore, further research is needed that should focus on regional population studies to resolve this problem in the era of increasing resistance to antimicrobials.

Titles of the figures:

Figure 1: Flowchart

Figure 2: Risk of bias of the included studies

Declaration:

Ethical statement: Not Applicable

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Contributors:

FCA is the principal investigator, wrote the protocol and the final version. KK performed extraction data and critique the literature and helped to write the final version. CCBM performed extraction data, critique the literature and helped to write the protocol. SBF performed extraction data and critique the literature and revised the manuscript. MTS provided insight on the epidemiological aspects of the review and helped to draft the manuscript. JPMVC performed extraction data, critique the literature and revised the manuscript. CCG performed extraction data and critique the literature. CCM performed extraction data and critique the literature. LCL is the review guarantor, advised on background, helped to write the protocol and revised the manuscript.

All authors approve the final version and take responsibility for its content.

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APÊNDICES

Reporting checklist for systematic review (with or without a meta-analysis).
Based on the PRISMA guidelines.

		Reporting Item	Page Number
Title			
Title	#1	Identify the report as a systematic review	1
Abstract			
Abstract	#2	Report an abstract addressing each item in the PRISMA 2020 for Abstracts checklist	3
Introduction			
Background/rationale	#3	Describe the rationale for the review in the context of existing knowledge	5
Objectives	#4	Provide an explicit statement of the objective(s) or question(s) the review addresses	5
Methods			
Eligibility criteria	#5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses	6
Information sources	#6	Specify all databases, registers, websites, organisations, reference lists, and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted	5-6
Search strategy	#7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used	6
Selection process	#8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and, if applicable, details of automation tools used in the process	6-7

Data collection process	#9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and, if applicable, details of automation tools used in the process	6-7
Data items	#10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (for example, for all measures, time points, analyses), and, if not, the methods used to decide which results to collect	7
Study risk of bias assessment	#11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and, if applicable, details of automation tools used in the process	8-10
Effect measures	#12	Specify for each outcome the effect measure(s) (such as risk ratio, mean difference) used in the synthesis or presentation of results	7
Synthesis methods	#13a	Describe the processes used to decide which studies were eligible for each synthesis (such as tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5))	7
Synthesis methods	#13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics or data conversions	7

Synthesis methods	#13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses	7
Synthesis methods	#13d	Describe any methods used to synthesise results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used	7
Synthesis methods	#13e	Describe any methods used to explore possible causes of heterogeneity among study results (such as subgroup analysis, meta-regression)	7-8
Synthesis methods	#13f	Describe any sensitivity analyses conducted to assess robustness of the synthesised results	7
Reporting bias assessment	#14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases)	9-10
Certainty assessment	#15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome	10
Data items	#10b	List and define all other variables for which data were sought (such as participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information	7
Results			
Study selection	#16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram (http://www.prisma-statement.org/PRISMAStatement/FlowDiagram)	10

Study selection	#16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded	10
Study characteristics	#17	Cite each included study and present its characteristics	10
Risk of bias in studies	#18	Present assessments of risk of bias for each included study	18
Results of individual studies	#19	For all outcomes, present for each study (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (such as confidence/credible interval), ideally using structured tables or plots	17
Results of syntheses	#20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies	17
Results of syntheses	#20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (such as confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect	17
Results of syntheses	#20c	Present results of all investigations of possible causes of heterogeneity among study results	n/a (insufficient data to generate statistical analysis)
Results of syntheses	#20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesised results	n/a (insufficient data to generate

			statistical analysis)
Risk of reporting biases in syntheses	#21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed	18
Certainty of evidence	#22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed	18
Discussion			
Results in context	#23a	Provide a general interpretation of the results in the context of other evidence	18-20
Limitations of included studies	#23b	Discuss any limitations of the evidence included in the review	20
Limitations of the review methods	#23c	Discuss any limitations of the review processes used	20
Implications	#23d	Discuss implications of the results for practice, policy, and future research	20
Other information			
Registration and protocol	#24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered	5
Registration and protocol	#24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared	5
Registration and protocol	#24c	Describe and explain any amendments to information provided at registration or in the protocol	18
Support	#25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review	21
Competing interests	#26	Declare any competing interests of review authors	21

Availability of data, code, and other materials	#27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review	21

Supplementary file 2. Search strategy.

Database: **PubMed**

1. "dental pulp cavity"[MeSH Terms]
2. "dental care"[MeSH Terms]
3. “root canal”[MeSH Terms]
4. “endodontics”[MeSH Terms]
5. “periapical abscess”[MeSH Terms]
6. “periodontal disease”[MeSH Terms]
7. “periododntal abscesses”[MeSH Terms]
8. “drug resistance, microbial”[MeSH Terms]
9. “microbial sensitivity tests”[MeSH Terms]
10. “antifungal drug resistance”[MeSH Terms]
11. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7
12. 8 OR 9 OR 10
13. 11 AND 12

Search (((((((resistance, antifungal drug[MeSH Terms]) OR Antifungal Drug Resistance) OR Resistance, Antifungal Drug) OR Antibiotic Resistance, Fungal)) OR ((((((((((((((((((((((((((microbial sensitivity test[MeSH Terms]) OR Microbial Sensitivity Test) OR Sensitivity Test, Microbial) OR Sensitivity Tests, Microbial) OR Test, Microbial Sensitivity) OR Tests, Microbial Sensitivity) OR Drug Sensitivity Assay, Microbial) OR Antimicrobial Susceptibility Breakpoint Determination) OR Breakpoint Determination, Antimicrobial Susceptibility) OR Virus Drug Sensitivity Tests) OR Viral Drug Sensitivity Tests) OR Breakpoint Determination, Antibacterial Susceptibility) OR Fungus Drug Sensitivity Tests) OR Fungal Drug Sensitivity Tests) OR Minimum Inhibitory Concentration) OR Concentrations, Minimum Inhibitory) OR Concentration, Minimum Inhibitory) OR Inhibitory Concentration, Minimum) OR Inhibitory Concentrations, Minimum) OR Minimum Inhibitory Concentrations) OR Antibiogram) OR Antibiograms) OR Bacterial Sensitivity Tests) OR Tests, Bacterial Sensitivity) OR Sensitivity Tests, Bacterial) OR Test, Bacterial Sensitivity) OR Bacterial Sensitivity Test) OR Sensitivity Test, Bacterial)) OR (((((((antibiotic resistance[MeSH Terms]) OR Drug Resistances, Microbial) OR Antimicrobial Drug Resistance) OR Antimicrobial Drug Resistances) OR Antibiotic Resistance, Microbial) OR Antibiotic Resistance) OR Resistance, Antibiotic)))

AND ((((((((((((((((((((((((dental pulp cavity) OR cavity, dental pulp) OR pulp cavities, dental) OR pulp cavity, dental) OR cavities, dental pulp) OR dental pulp cavitites) OR dental pulp cavities) OR chamber, pulp) OR chambers, pulp) OR pulp chambers) OR pulp canal) OR canal, pulp) OR canals, pulp) OR pulp canals) OR root canal) OR canal, root) OR canals, root) OR root canals) OR pulp chamber)) OR ((dental care) OR care, dental)) OR Endodontics) OR ((((((((((((((Pulp Canals) OR Root Canals) OR Cavity, Dental Pulp) OR Pulp Cavities, Dental) OR Pulp Cavity, Dental) OR Cavities, Dental Pulp) OR Dental Pulp Cavities) OR Pulp Chamber) OR Chamber, Pulp) OR Chambers, Pulp) OR Pulp Chambers) OR Pulp Canal) OR Canal, Pulp) OR Canals, Pulp) OR Canals, Root) OR Canal, Root) OR Root Canal)) OR ((((((((((((((((((((Abscesses, Periapical) OR Periapical Abscesses) OR Dentoalveolar Abscess, Apical) OR Abscess, Apical Dentoalveolar) OR Abscesses, Apical Dentoalveolar) OR Apical Dentoalveolar Abscess) OR Apical Dentoalveolar Abscesses) OR Dentoalveolar Abscesses, Apical) OR Periodontitis, Apical, Suppurative) OR Periapical Periodontitis, Suppurative) OR Periapical Periodontitides, Suppurative) OR Periodontitides, Suppurative Periapical) OR Periodontitis, Suppurative Periapical) OR Suppurative Periapical Periodontitides) OR Suppurative Periapical Periodontitis) OR Alveolar Abscess, Apical) OR Abscess, Apical Alveolar) OR Abscesses, Apical Alveolar) OR Alveolar Abscesses, Apical) OR Apical Alveolar Abscess) OR Apical Alveolar Abscesses) OR Abscess, Periapical) OR periapical abscess)) OR (((((((periodontal disease[MeSH Terms]) OR Disease, Periodontal) OR Diseases, Periodontal) OR Periodontal Disease) OR Parodontosis) OR Parodontoses) OR Pyorrhea Alveolaris)) OR ((((periodontal abscess[MeSH Terms]) OR Abscess, Periodontal) OR Abscesses, Periodontal) OR Periodontal Abscesses))

Database: **Embase**

1. exp dental pulp cavity/ or dental pulp cavity.mp.
2.dental care.mp. or exp dental procedure/
3.endodontics.mp. or exp endodontics/
4.periapical abscess.mp. or exp tooth periapical disease/
5.periodontal disease.mp. or exp periodontal disease/
6.periodontal abscess.mp. or exp periodontal abscess/ or exp periodontal disease/
7.drug resistance, microbial.mp. or exp antibiotic resistance/
8.microbial sensitivity tests.mp. or exp microbial sensitivity test/
9.antifungal drug resistance.mp. or exp antifungal resistance/
10.1 OR 2 OR 3 OR 4 OR 5 OR 6
11.7 OR 8 OR 9
12.10 AND 11

Database: **CINAHL**

1.(MH "Dental Pulp Cavity") OR "dental pulp cavity"
2.(MH "Dental Care+") OR "dental care"
3.“root canal" OR (MH "Root Canal Therapy")
4.(MH "Endodontics+") OR "endodontics"

5.“periapical abscess" OR (MH "Periapical Diseases")
6.“periodontal disease" OR (MH "Periodontal Diseases+")
7.(MH "Periodontal Abscess") OR "periodontal abscess"
8.(MH "Drug Resistance, Microbial+") OR "drug resistance, microbial" OR (MH "Drug Resistance+")
9.(MH "Microbial Culture and Sensitivity Tests") OR "microbial sensitivity test"
10.1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7
11.8 OR 9
12.10 AND 11

Supplementary file 3. Reasons for exclusion.

Excluded studies (n = 12)

Author, year	**Reason**
Lysakowaska et al. 2016	Essential oils antimicrobial action
Divakar et al. 2017	Hospitalized patients
Rasteniene et al. 2015	Hospitalized patients
Delboni et al. 2017	Characterized the microbial strain present
Francisco et al. 2019	Laboratory acquired strains
Sabrah et al. 2013	Laboratory acquired strains
Seneviratne et al. 2017	Laboratory acquired strains
Durgesh et al. 2016	Sample collection region not specified
Narita et al. 2016	Sample collection year prior to 2011
Sun et al. 2012	Sample collection year prior to 2011
Slaton et al. 2017	Sequencing system validation (RAST)
Endo et al. 2014	The data obtained were used in another study (Endo et al. 2015)

Supplementary file 4. Modifications to the original registered protocol

1. Initially, it was planned to carry out a systematic review with meta-analysis. However, considering the study design found (case series), the extracted data did not allow the generation of meta-analysis, presenting the findings in detail as much as possible in a narrative and exploratory version of the data found.

2. Period of time: Changes were made in the survey period of the studies, extending to 10 years.
3. Exclusion criteria: Other exclusion criteria were added: studies in which patients used antimicrobials at the time of the sample collection, as the ingestion of the drug could interfere with the result of antimicrobial resistance.
4. The Kappa test in study selection was altered by a calibration of reviewers in all stages of the review (from study selection, data extraction to risk of bias analysis), and disagreements were resolved by a senior researcher.
5. Bacteria isolate with a resistance profile considered intermediate (I) should be considered sensitive (S) to the antimicrobial. As most authors considered the intermediate profile (I) as resistant (R) to the antimicrobial, we decided to reconsider what had been described in the protocol.
6. Considering the study design found, it was not possible to evaluate the initially proposed outcome (prevalence of antimicrobial resistance with 95% confidence interval). The profile of microorganisms present in infections was summarized according to their frequency in the study reports and the percentage of resistance to antimicrobials evaluated in each study.
7. Because only studies with case series design were included, The Joanna Briggs Institute critical appraisal tool for case series was used to assess the quality of the included studies rather than the tools originally proposed for cohort studies.
8. The protocol proposed the calculation of the 95% predictive distribution, in other words, the probabilistic interval for carrying out new studies in Latin America. The authors of the included studies were contacted to share the data obtained, however, the results obtained in data extraction were not sufficient to perform the statistical analysis.

6.3 Antimicrobial resistance and microorganisms present in periodontal infections: a systematic review"

Foi inserida nesta tese a versão original do artigo "Antimicrobial resistance and microorganisms present in periodontal infections: a systematic review." formatado de acordo com as normas da revista *Journal of Periodontology*.

Revista: *Journal of Periodontology*

Versão *online*: 1943-3670

Fator de impacto (2022): 6.693

Classificação WebQualis/ Capes quadriênio 2013-2016: A1

Área: Farmácia

ABSTRACT

Background: A variety of microbial species have been isolated from periodontal infections, however, there is still not enough clinical information regarding their ability to be resistant to antibiotics. The aim of this study was to estimate and to describe the antimicrobial resistance profile in periodontal infections. **Methods:** A systematic review was conducted according to PRISMA statement. The MEDLINE (PubMed/ Ovid), EMBASE, BVS, CINAHL, and Web of Science databases were searched from January 2011 to December 2021 of observational studies which evaluated the antimicrobial resistance in periodontal infections in permanent dentition. Studies that allowed the ingestion of antimicrobials at the time of the collection were excluded. Six reviewers, working in pairs and independently, selected titles, abstracts and full texts extracting data from all studies that met the eligibility criteria: characteristics of patients, diagnosis of infection, microbial species assessed, antimicrobials assessed, identification of resistance genes and virulence factors. "The Joanna Briggs Institute" critical appraisal for case series was adapted to assess the risk of bias in the included studies. **Results:** Twenty-four studies (N= 2.039) were included. *Prevotella* and *Porphyromonas* species were the most cited microorganisms, and the virulence factors were related to *Staphylococcus aureus.* The antimicrobial reported with the highest frequency of resistance was ampicillin (39.5%) and the most cited genes were related to macrolides. The quality of the included studies was considered critically low. **Conclusion:** No evidence was found regarding the profile of antimicrobial resistance in periodontal infections, requiring further research that should focus on regional population studies to address this issue in the era of increasing antimicrobial resistance.

Key words: Anti-bacterial agents. Dentistry. Microbial drug resistance. Endodontics. Periodontics.

Funding: This project was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—PROSUC—CAPES/UNISO.

Registration: The protocol of this systematic review was registered in *Prospero* (CRD42018077810) and published in *Medicine* (Abe et al., Medicine (2018) 97:48).

Background

Periodontal diseases are polymicrobial oral infections composed predominantly of species of gram-negative subgingival, capnophilic and anaerobic bacteria. In addition to mechanical debridement of infected periodontal pockets, clinical treatment protocols for severe forms of periodontitis often involve the adjunctive use of antibiotics[1].

The knowledge of the most common pathogens associated with periodontal abscess and apical periodontitis and their susceptibility profiles is necessary for a rational antimicrobial prescription[2]. The inappropriate use of antibiotics can lead not only to increased adverse events and health care costs but also to the risk of developing methicilin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multidrug-resistant (MDR) of gram-negative bacteria[3].

The indiscriminate prescribing of antimicrobials indicates a negative contribution of health professionals to antimicrobial resistance and shows the scarcity of knowledge between the public availability of antimicrobials without prescription, and the remaining use of antimicrobials; which is the hallmark of low- and middle-income countries[4].

Furthermore, resistance genes can easily spread under natural conditions.
This is consistent with the rapid emergence of resistance in the clinic and predicts that new antibiotics will be selected for pre-existing determinants of resistance which have been circulating within the microbial pan genome for millennia[5].

The dearth of syntheses that estimates and describes the profile of microbial resistance to antimicrobials in periodontal infections led to the development of this systematic review. Therefore, this systematic review aimed to answer the focused question "What is the antimicrobial resistance profile of microorganisms in periodontal infections?", limiting the search for studies carried out from January 2011 to December 2021 to avoid a possible interference in the change in the resistance profile that may have occurred over the years.

Materials and Methods

This systematic review followed PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) recommendations, was registered on the PROSPERO database under number CRD42018077810 and the protocol was published in *Medicine* (Abe et al., Medicine (2018) 97:48)[6].

Search strategy

The search was oriented by an experienced librarian in the following databases: Medline, Embase, BVS (bvsalud.org), CINAHL, and Web of Science. Additionally, the website "bancodeteses.capes.gov.br" and Grey Literature Report were searched as grey literature.

The electronic search strategy was developed using the key words combining Medical Subject Heading (MeSH) terms. The Boolean operators 'AND 'and 'OR 'were applied to combine the terms and create a search strategy. The search strategies for each database and the following findings are summarized in **Supplementary File 1**. All articles selected were imported into the EndNote X9 (Clarivate, London, UK) reference manager to catalogue the references and to facilitate the exclusion of duplicates.

Eligibility criteria

The studies were selected according to the following inclusion criteria:

• Population (P): patients diagnosed with periodontal infection (aggressive, chronic).

• Outcome (O): antimicrobial resistance reported through minimal inhibitory concentration, zone of inhibition and/or detection of resistance genes by culture-independent molecular techniques.

• Study design (S): observational studies.

• Timing: study published from January 2011 to December 2021.

• Language: no restriction.

Methodological studies, studies that allowed the antimicrobial consumption until the time of sample collection and time series were excluded.

Selection of studies

Six reviewers working in pairs and independently (CCM, JPMVC, KK, SB-F, CCBM, CCG), screened titles and abstracts. The same reviewers were calibrated for each step of the process (assessed eligibility of each full-text article, data extraction and risk of bias assessment of a determined number of studies with different quality levels). Disagreements were solved by consensus or a with the participation of a senior reviewer (LCL).

Data extraction

The information entered into in an Excel spreadsheet using a predefined data collection form and the same groups of independently reviewers extracted the data.

The following data were extracted from each study: author (year)/country, duration of the study, place of recruitment, characteristics of patients (age/ sex), diagnosis of infection, microbial species assessed, method of identification of microorganisms, antimicrobials assessed, identification of resistance genes and virulence factors, and conflict of interest.

Data synthesis and statistical analysis

Due to the variety of resistances observed, the studies were grouped by antibiotic analyzed: amikacin, amoxicillin, amoxicillin + clavulanic acid, amoxicillin + metronidazole, ampicillin, anfotericin B, azithromycin, cefazolin, cefepime, cefixime, cefotaxime, cefuroxime, cephalothin, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin,

clarithromycin, clindamycin, dicloxacillin, doxycycline, erythromycin, fluconazole, gentamicin, imipenem, itraconazole, kanamycin, levofloxacin, meropenem, metronidazole, miconazole, moxifloxacin, nystatin, ofloxacin, pefloxacin, penicillin, quinupristin/dalfopristin, rifampicin, roxithromycin, spiramycin, teicoplanin, tetracycline, tinidazole, trimethoprim-sulfamethoxazole (cotrimoxazole), voriconazole.

Thus, it was decided to group the reports of resistance, regardless of the strain detected in the included studies. In each study, it was identified the number of resistant strains out of the total investigated strains to calculate the percentage of resistance. As the resistance percentages are highly variable, a random effects model was chosen to group proportions in meta-analyses. To stabilize the variances, the Freeman-Tukey double arc sine transformation was used and to 95% confidence intervals considered Wilson scores. Heterogeneity (I^2) was calculated from the inverse variance model in a fixed-effect model. The limits of $I^2 > 50\%$ to consider heterogeneous was adopted. All analyzes were performed on Stata SE 14.2 (StataCorp, College Station, TX)[7].

Quality assessment and strength of evidence

The methodology used to assess the quality of the study was the checklist for case series from the Joanna Briggs Institute Critical Appraisal tools[8] and adapted to the research question of this systematic review. The risk of bias domains included were 1) selection bias (1.1 - inclusion criteria, 1.2 - diagnostic criteria, 1.3 - consecutive inclusion of patients, 1.4 - demographic characteristics of patients, 1.5 - clinical information of patients, 1.6 - location of the recruitment, 1.7 - complete inclusion of patients); 2) measurement bias (sample collection methodology); 3) selective reporting bias (outcome reporting).

Each domain or subdomain was assessed as follow:

- Domain 1: Selection bias:
  - Inclusion criteria

"Were the inclusion criteria clearly defined?" The authors should provide the inclusion criteria (and exclusion if applicable) of the patients included in the studies. The inclusion criteria should present the diagnosis of the infection and the patient's medical history.

- Diagnostic criteria

"Was the infection diagnostic established following a standardized criterion? The same criterion was used for all patients?" The authors should describe the measurement method of the condition (diagnostic) following a standard that should be replicated.

- Consecutive inclusion of patients

"Did the study present the consecutive inclusion of patients?" Studies that present the consecutive inclusion of patients are more reliable than those which do not present it. The authors should report if the inclusion of patients was consecutive or the period of time in which the samples were collected.

- Demographic characteristics of patients

"Were the demographic characteristics of patients clearly reported?" The authors should describe the demographic characteristics of patients, as ethnicity, sex, age, oral hygiene habits and regional HDI (Human Development Index).

- Clinical information of patients

"Was the clinical information of patients clearly reported?" The authors should clearly report the clinical information of patients, as the condition and stage of the disease, comorbidities, harmful habits (such as smoking and drinking alcohol), etc.

- Location of the recruitment

"Was the location of the recruitment the same for all sample collection?" Some diseases or conditions may vary their prevalence according to the different geographical region and/or populations.

- Complete inclusion of patients

"Did the authors report the complete inclusion of the patients?" The integrity of a case series contributes to its reliability. The authors should report if there was a loss of sample/participant and how it was solved.

2. Domain 2: Measurement bias (sample collection methodology)

"*Was the sample collection methodology adequate and standardized to all patients?"* The authors should determine if the measurement tools used were validated instruments as they have a significant impact on the validity of the outcome assessment.

3. Domain 3: Selective reporting bias (outcome reporting)

"Were the outcome or follow-up results (microbial resistance) clearly reported?" The results of any intervention or treatment should be clearly reported by the authors.

The GRADE approach framework (Grading of Recommendations, Assessment, Development and Evaluations) are used to assess the certainty of evidence when data are narratively summarized[9]. Under this approach, **high certainty** in the evidence means that researchers are very confident that the effect they found in the studies is close to the true effect; **low certainty** of evidence means that the result obtained will most likely be sufficiently different from what the research has found to affect a decision, and **very low certainty** of evidence means that the authors are certain they have little or no confidence in the effect.

Considering these aspects, an adaptation was performed, judging the quality based only on the risk of bias. In this way, each domain was evaluated and items 1.3, 1.4, 1.5, 1.7 and 2 were used as critical domains. Observational studies, according to GRADE[9] initially present low quality, however, if the study does not present criteria that lower its quality, it can raise a level of confidence, being considered as "moderate " quality. That is, if the study presents "low risk of bias" in all items evaluated, the confidence in this study will be considered "moderate". If the study presents "high risk of bias" in only one critical domain or in more than one non-critical domain, trust in that study will be considered "low". If the study presents "high risk of

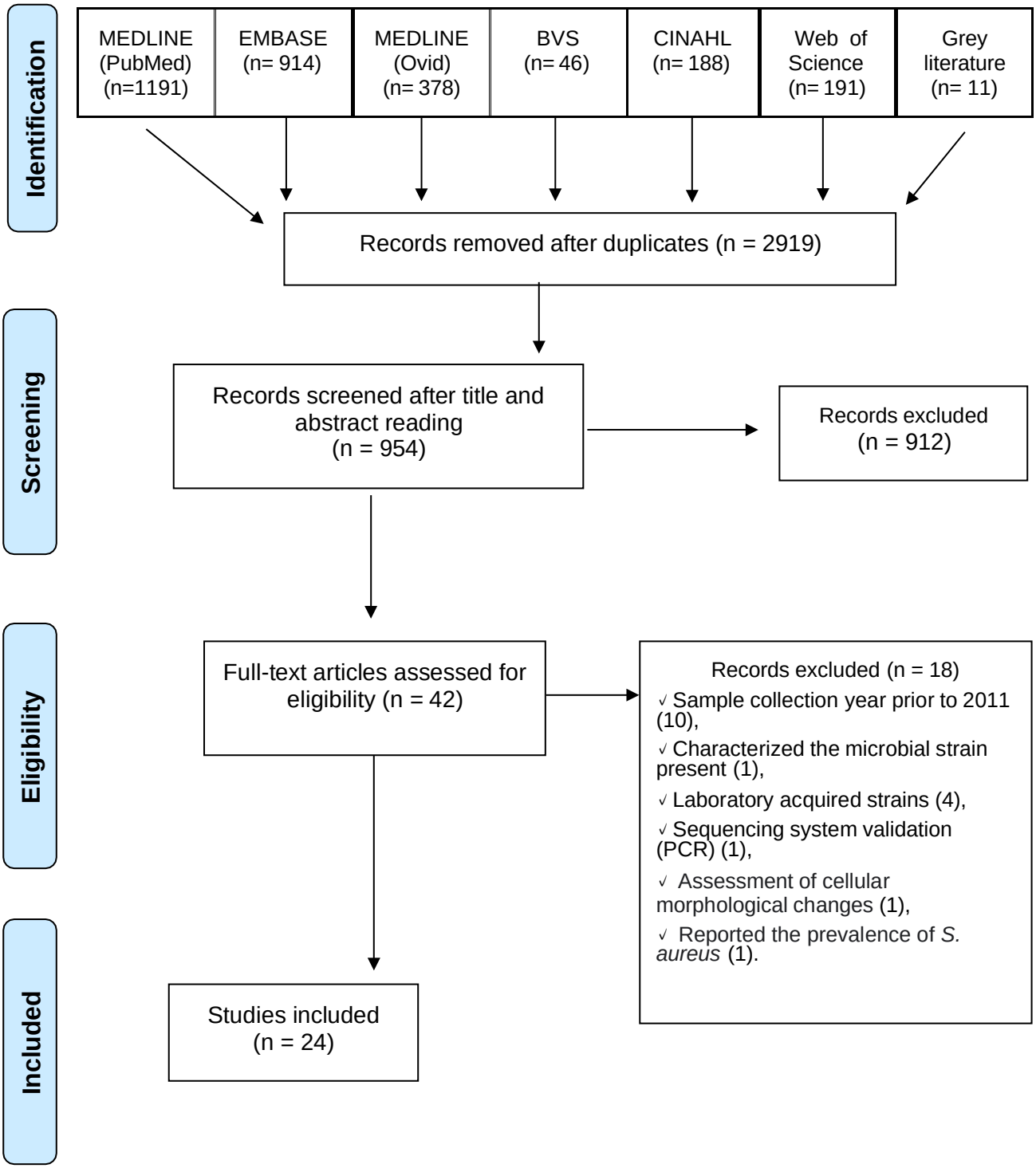
bias" in more than one domain considered critical, then confidence in the study will be "critically low".

Results

Study selection

The search strategy identified 2.919 titles and abstracts from the cited databases and 954 studies were screened. From the evaluation of the full-text of each article, 42 studies were selected for data extraction and 24 studies[2, 10-32] met the eligibility criteria and composed the final sample used in the qualitative and quantitative analyses (**Figure 1**). Eighteen studies were excluded for the reasons listed in the table of characteristics of excluded studies (**Supplementary File 2**).

Figure 1 - Flow chart



Data extraction

Table 1 presents the characteristics and main findings of the included studies.

Thirty-nine species of microorganisms evaluated were present in periodontal pockets and/or gingival sulcus of 2,039 patients diagnosed with periodontitis (aggressive, chronic, or non-specific).

- Country/continent: The majority of the studies was conducted in the Americas (12 studies), followed by Asia and Europe (five studies each), and Africa (two studies).
- Study design: all of the studies were case series. Other observational studies as cohort or cross sectional studies, were not found.
- Duration of the study: only nine studies reported the duration of the study[2, 11, 14, 19, 23, 25, 27-29].
- Place of recruitment: 15 studies[2, 11-14, 17, 21-25, 27-29, 31] were conducted in a University/Dentistry College. Three studies were conducted in a public dental clinic[19, 20, 32], other three studies were conducted in private clinics[10, 16, 30]. Two studies reported the association of more than one type of establishment[15, 26], and one study did not report the place of recruitment[18].
- Characteristics of the patients: 1) Age: only 18 studies reported the participants' age, ranging from 16 to 83 years. 2)Sex: the 19 studies that reported the patients' sex, observed the feminine majority.
- Diagnosis of the infection: three studies reported chronic periodontal infection[13, 21, 22], two studies reported a diagnosis of aggressive infection[20, 28], six studies reported a diagnosis of severe/moderate infection[10, 15, 16, 19, 24, 30], two studies reported more than one type of infection[17, 29] and 11 studies did not report the diagnosis of periodontal infection[2, 11, 12, 14, 18, 23, 25-27, 31, 32].
- Microbial species assessed: 39 species were assessed *(Actinomyces* spp., *Aggregatibacter actinomycetemcomitans, Alloprevotella* spp., *Anaerococcus* spp., Bacteroides, *Bifidobacterium* spp., *Campylobacter* spp., *Candida* spp., *Capnocytophaga* spp., *Citrobacter*

freundii, Clostridium spp., Dialister spp., *Eikenella* spp., Enteric rods/ pseudomonads, *Enterobacter* spp., *Enterococcus* spp., *Escherichia* spp., *Erwinia* spp., *Fusobacterium* spp., *Granulicatella* spp., *Hafnia alvei, Klebsiella* spp., *Leptotrichia* spp., *Morganella* spp., *Olsenella* spp., *Parvimonas micra, Peptostreptococcus* spp., *Porphyromonas spp., Prevotella* spp., *Propionobacterium* spp., *Pseudomonas* spp., *Raoutella* spp., *Rothia* spp., *Serratia* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Tannerella forsythia, Veillonella* spp.)

- Method of identification of the microorganisms: 16 studies reported culture-dependent technique[2, 10, 11, 13, 15, 16, 18, 20-22, 24, 25, 28, 30-32], two studies reported molecular culture-not dependent[17, 27] and six studies reported both techniques[12, 14, 19, 23, 26, 29].
- Antimicrobials assessed: 47 antimicrobials were assessed (amikacin, amifloxacin, amoxicillin, amoxicillin + clavulanic acid, ampicillin, anfotericin B, azithromycin, cefazolin, cefepime, cefixime, cefotaxime, cefuroxime, cephalothin, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, clarithromycin, clindamycin, dicloxacillin, doxycycline, erythromycin, fosfomycin, fluconazole, gentamicin, imipenem, itraconazole, kanamycin, levofloxacin, linezolid, meropenem, metronidazole, miconazole, moxifloxacin, nystatin, ofloxacin, pefloxacin, penicillin, quinupristin/dalfopristin, rifampicin, roxithromycin, spiramycin, teicoplanin, tetracycline, tinidazole, trimethoprim-sulfamethoxazole (cotrimoxazole), voriconazole).
- Resistance genes and/or virulence factors identified: *bla*CblA , *bla*CepA, *bla*CfxA, *bla*CSP-1, *bla*SHV, *bla*TEM, *bla*cfxA2/ *bla*cfxA3/ *bla*cfxA6, *tet*, *tet*B, *tet*L, *tet*M, *tet*Q, *tet*O, *tet*W, *tet*Q, *erm*(B), *erm*(C), *erm(*F), *nim*, aac, bbp, clfA, clfB, cna, coa, ebps, fnbA, fnbB, map/eap, mecA, pbp2b, sdrC, sdrD, sdrE, spa.
- Conflict of interest: none of the authors reported conflict of interest.

Table 1 - Characteristics and main findings of the included studies.

Continent (country)	Year (ref.)	Population (N)	Participants' age (range)	Microbial species assessed (N)	Technique assessment	Antimicrobials assessed (*resistance)	Resistance gene (Antimicrobial)	Virulence factors (Antimicrobial)
Africa (Morocco)	2019 [1]	Patients (45)	24,4 (mean)	*Aggregatibacter actinomycetemcomitans* (24) *Porphyromonas gingivalis* (30)	Culture	AMX, AXL, AZI*, MTZ*	NA	NA
Africa (South Africa)	2016 [2]	Patients (48)	52 (mean)	*Prevotella* spp. (49) *Bacteroides* (5) *Porphyromonas* spp. (6) *Fusobacterium* spp. (4) *Clostridium* spp. (3) *Propionobacterium* spp. (3)	Culture Molecular technique	AMP*, PEN*	$_{bla}$*cfxA* 2/ $_{bla}$*cfxA* 3/ $_{bla}$*cfxA* 6 (ß-lactamase)	NA
Americas (United States of America)	2011 [3]	Patients (37)	57,5 ± 12 (range)	*Porphyromonas gingivalis* (15) *Tannerella forsythia* (19) *Prevotella intermedia/ nigrescens* (36) *Fusobacterium nucleatum* (36) *Parvimonas micra* (37) *Campylobacter rectus* (20) *Streptococcus constellatus* (10)	Culture	AMX*, SPI*, MTZ* AMX + MTZ*, SPI + MTZ*	NA	NA

				Streptococcus intermedius (10)				
Americas (Colombia)	2013 [4]	Patients (86)	>20 years	*Klebsiella pneumoniae* (4) *Klebsiella oxytoca* (5) *Escherichia coli* (3) *Hafnia alvei* (2) *Erwinia* spp. (3) *Shigella* spp. (2) *Serratia liquefaciens* (5) *Serratia marcescens* (2) *Serratia odorifera* (1) *Enterobacter cloacae* (2)	Culture	AMI*, AXL*, AMP*, KAN*, CFM*, CZD*, CXE*, CIP*, CTZ*, GEN*	NA	NA
Americas (Colombia)	2014 [5]	Patients (87)	NR	*Porphyromonas gingivalis* (30)	Culture	MTZ, TET*	NA	NA
Americas (United States of America)	2014a [6]	Patients (400)	35 – 78 years	*Porphyromonas gingivalis* (312) *Prevotella intermedia/ nigrescens* (320) *Fusobacterium nucleatum* (122) *Parvimonas micra* (364) *Streptococcus constellatus* (147) *Aggregatibacter actinomycetemcomitans* (81) Enteric rods/ pseudomonads (9) *Enterococcus faecalis* (4)	Culture	AMX*, CLI*, DOX*, MTZ*, AMX+MTZ*	NA	NA

				Staphylococcus aureus (1)				
Americas (United States of America)	2014b [7]	Patients (50)	31 – 76 years	*Streptococcus constellatus* (33) *Streptococcus intermedius* (17)	Culture	AMX, AZI*, CIP*, CLI*, DOX*, MTZ*	NA	NA
Americas (Colombia)	2020 [8]	Patients (76)	26,1 (mean)	*Aggregatibacter actinomycetemcomitans* (37) *Porphyromonas gingivalis* (61) *Tanerella forsythia* (43)	Culture	AMX*, AZI*, MTZ*, MOX	NA	NA
Americas (United States of America)	2020 [9]	Patients (88)	35 – 83 years	*Porphyromonas gingivalis* (9) *Tannerella forsythia* (47) *Prevotella intermedia/ nigrescens* (80) *Fusobacterium nucleatum* (73) *Parvimonas micra* (88) *Streptococcus constellatus* (9) *Campylobacter rectus* (13)	Culture	AMX*, CLI*, DOX*, MTZ*, TIN*	NA	NA
Americas (Peru)	2021 [10]	Patients (8)	NR	*Rothia dentocariosa* *Eikenella corrodens* *Granulicatella adiacens* *Actinomyces naeslundii*	Culture	AMP*, AXL*, AZI*, CIP*, CLI*, CTZ*, DIC*,	NA	NA

						MTZ*, PEN*, TET*		
Americas (Brazil)	2021 (11)	Patients (18)	13 – 67 years	*Citrobacter freundii* *Enterobacter aerogenes* *Enterococcus avium* *Escherichia coli* *Klebsiella ozaenae* *Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Raoutella spp.* *Staphylococcus aureus* *Staphylococcus haemolyticus* *Streptococcus pneumoniae* *Streptococcus salivarius*	Culture	AMX*, AZI*, CEF*, CFM*, CLI*, CLR*, IMI*, TET*	NA	NA
Americas (Dominican Republic)	2015 (12)	Patients (77)	18 – 65 years	NA	Molecular technique	NA	*tet*, *tet*B, *tet*L, *tet*M, *tet*Q, *tet*O, *tet*W (TET)	NA
Americas (Brazil)	2020 (13)	Patients (110)	41,5 (mean)	NA	Molecular technique	NA	*aac* (fluroquinolones), *bla*$_{TEM,}$ *mecA* (ß-lactamase), *erm* (ERY), *pbp2b* (PEN)	NA
Americas (Mexico)	2019 (14)	Patients (268)	NR	*Staphylococcus aureus* (50)	Culture	AMP*, CEF*, CFM*, CRX*, CTZ*, DIC*,		bbp (bone sialoprotein binding protein)

					Molecular technique	ERY*, GEN*, LEV*, PEF*, PEN*, TET*		clfA, clfB (*S. aureus* binding to fibrinogen), cna (*S. aureus* binding to collagen) coa (coagulase enzyme), ebps (*elastin* binding protein) fnbA, fnbB (*S. aureus* binding to fibronectin), map/eap (extracellular adhesion protein), sdrC, sdrD, sdrE (sialoprotein and fibrinogen binding protein) spa (staphylococcal protein A)

Asia (India)	2015 (15)	Patients (80)	49 (mean)	*Staphylococcus aureus* (1) *Streptococcus mitis* (50) *Streptococcus oralis* (45) *Streptococcus sanguinis* (41) *Streptococcus parasanguinis* (12) *Streptococcus gordonii* (4) *Streptococcus anginosus* (6) *Streptococcus constellatus* (7) *Streptococcus mutans* (30) *Streptococcus hyointestinalis* (1) *Streptococcus sinensis* (2) *Streptococcus pluranimalium* (1) *Streptococcus thoraltensis* (1) *Streptococcus tigurinus* (1) *Granulicatella adiacens* (21) *Granulicatella elegans* (38)	Culture	AMP*, AZI*, CEM*, CFM*, CXE*, CLA*, CLI*, ERI*, LEV*, LIZ, OFX*, PEN*, QUI*, TET*, VAN	NA	NA
Asia (India)	2017 (16)	Patients (100)	18 – 75 years	Enterococci (46)	Culture	AMX*, CIP*, ERY*, GEN*, TEI*, VAN*	NA	NA
Asia (India)	2019 (17)	Patients (40)	NR	*Aggregatibacter actinomycetemcomitans* (40)	Culture	AMX*, AXL*, AZI*, CFZ*, CFM*, CXE*, CRX*, CLI*, DOX*, MTZ, MOX*, TET*	NA	NA

Asia (Pakistan)	2020 (18)	Patients (45)	45,4 ± 7,5 (range)	*Prevotella intermedia/ nigrescens* (10) *Porphyromonas gingivalis* (10) *Actinobacillus actinomycetemcomitans* (6)	Culture	AMX*, AZI, MTZ*, TET*	NA	NA
Asia (China)	2014 (19)	Patients (41)	47 (mean)	*Prevotella* genus (42) Não-*Prevotella* anaeróbios (18)	Culture Molecular technique	AMX*, CFX*, CED*, CLI*, DOX*, IMI, MTZ*, ROX*	bla_{CfxA} (ß-lactamase) *erm(*F) (ERY), *nim* (MTZ) *tet*Q (TET)	NA
Europe (Spain)	2017 (20)	Patients (61)	NR	*Candida* spp. (126)	Culture	AFB*, FLU*, ITR*, MIC*, NYS*, POS*, VOR*	NA	NA
Europe (United Kingdom)	2017 (21)	Patients (50)	NR	*Aggregatibacter actinomycetemcomitans* (56)	Culture	AMX*, AXL*, CZD*, CIP, CLI*, MTZ*, PEN*, TET*	NA	NA
Europe (France)	2014 (22)	Patients (42)	NR	*Capnocytophaga* spp. (48)	Culture Molecular technique	AMX*, AXL, CFM*, CZD*, CLI, ERY*	bla_{CfxA}, bla_{CSP-1}, (ß-lactamase) *erm*(C)/ *erm*(F) (ERY)	NA
Europe (Spain)	2019 (23)	Patients (52)	49,8 (range)	*Prevotella* spp. (100)	Culture	AZI*, ERY	*erm*B, *erm*F (ERY)	NA

					Molecular technique			
Europe (Spain)	2020 (24)	Patients (130)	24 – 82 years	*Actinomyces* spp.; *Alloprevotella* spp.; *Anaerococcus* spp.; *Bifidobacterium* spp.; *Campylobacter* spp.; *Capnocytophaga* spp.; *Dialister* spp.; *Eikenella* spp.; *Escherichia* spp.; *Fusobacterium* spp.; *Klebsiella* spp.; *Leptotrichia* spp.; *Morganella* spp.; *Olsenella* spp.; *Peptostreptococcus* spp.; *Prevotella* spp.; *Pseudomonas* spp.; *Rothia* spp.; *Serratia* spp.; *Staphylococcus* spp.; *Streptococcus* spp.; *Veillonella* spp.	Culture Molecular technique	AMX*, KAN*, CFM*, CLR*, ERY*, STR*, TET*	bla_{TEM}, bla_{SHV}, bla_{CfxA}, bla_{CepA}, bla_{CblA} (ß-lactamase)	NA

(ref) study reference: (1) Minguez *et al.,* (2) Binta & Patel, (3) Rams *et al.,* (4) Gamboa *et al*., (5) Gamboa *et al.,* (6) Rams *et al.,* (7) Rams *et al.,* (8) Ardila et al., (9) Rams *et al.,* (10) Aguilar-Luis *et al*., (11) Ansiliero *et al.,* (12) Collins *et al*., (13) Almeida *et al.,* (14) Uribe-Garcia *et al.,* (15) Dhotre *et al.,* (16) Bhardwaj *et al.,* (17) Bhat *et al.,* (18) Irshad *et al.,* (19) Xie *et al.,* (20) DeLaTorre *et al.,* (21) Akrivopoulou *et al*., (22) Ehrmann *et al.,* (23) Arredondo *et al.,* (24) Arredondo *et al.*

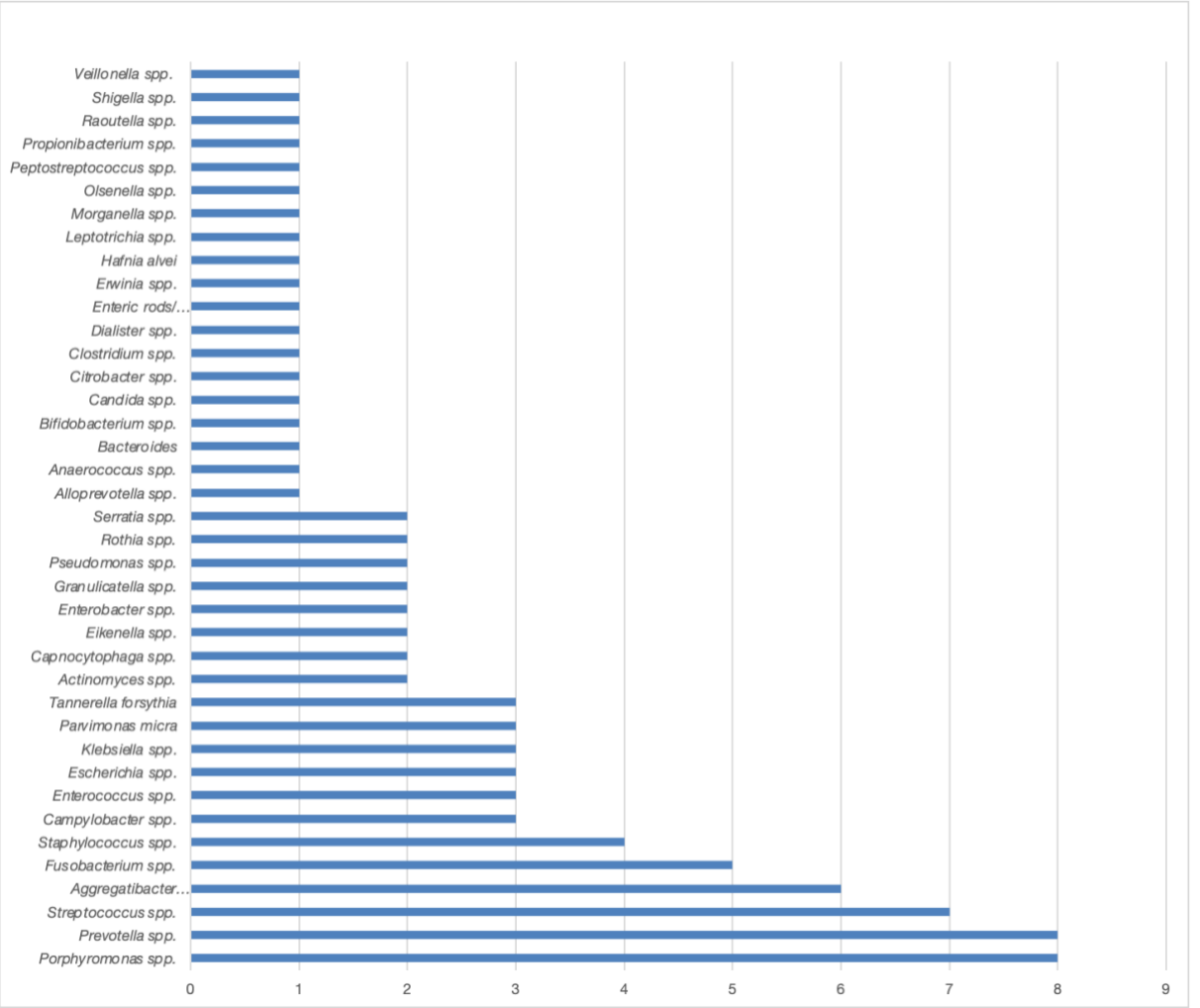
AFB - anfotericin B, AFX – amifloxacin, AMI – amikacin, AMX – amoxicillin, AXL – amoxicillin + clavulanic acid, AMP – ampicillin, AZI – azithromycin, CFZ – cefazolin, CEF – cefalotin, CEM – cefepime, CFX – cefixime, CFM – cefotaxime, CED – cefradine, CZD – ceftazidime, CXE – ceftriaxone, CRX – cefuroxime, CLR – chloramphenicol, CIP – ciprofloxacin, CLA – clarithromycin, CLI – clindamycin, CTZ – cotrimoxazole, DIC – dicloxacillin, DOX – doxycycline, ERY – erythromycin, FOS – fosfomycin, FLU – fluconazole, GEN – gentamicin, IMI – imipenem, ITR – itraconazole, KAN - kanamycin, LEV – levofloxacin, LIZ – linezolid, MOX – moxifloxacin, MTZ – metronidazole, MIC – miconazole, NIT – nitrofurantoin, NYS – nystatin, OFX – ofloxacin, PEF – pefloxacin, PEN – penicillin, POS – posaconazole, QUI – quinupristin/ dalfopristin, ROX – roxithromycin, SPI – spiramycin, STR – Streptomycin, TEI – teicoplanin, TET – tetracycline, TGC – tigecycline, TIN – tinidazole, VAN – vancomycin, VOR – voriconazole.

* – resistance reported

NA – not assessed, NR – not reported.

Prevotella and *Porphyromonas* species were the most cited microorganisms, being reported in eight studies each, followed by *Streptococcus* spp. cited in seven studies, *Aggregatibacter actinomycetemcomitans* cited in six studies and *Fusobacterium* spp. cited in five studies (**Figure 2**).

Figure 2 – Microorganisms assessed



Among the most prescribed antimicrobials in dentistry, the most cited were amoxicillin (15 studies), metronidazole (12 studies) clindamycin (ten studies), azithromycin (nine studies) and tetracycline (eight studies). And the antimicrobials that showed the highest

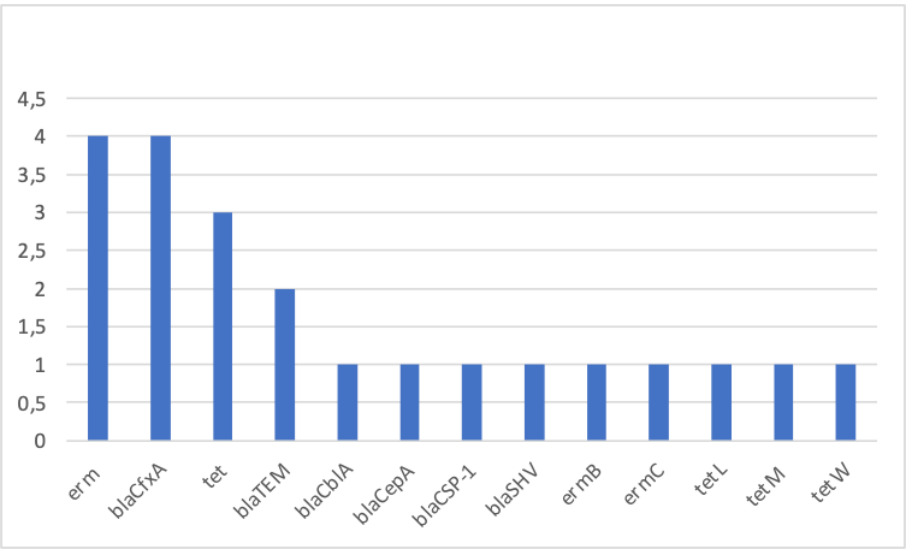
frequency of resistance were clindamycin (28.4%), azithromycin (28.1%) and metronidazole (21.6%) (**Table 2**).

Table 2. Antimicrobial resistance reported in periodontal infections according to studies.

Antimicrobial	**N (studies)**	**R % (95% CI)**	**I^2 (%, p)**	**% Weight**
amoxicillin	15	16.4 (8.7, 25.3)	94.1 (p= 0.0)	76.37
metronidazole	12	21.6 (10.4, 35.0)	97.5 (p= 0.0)	69.91
clindamycin	10	28.4 (15.0, 43.5)	95.8 (p= 0.0)	76.63
azithromycin	9	28.1 (17.0, 40.2)	78.6 (p=0.0)	89.96
tetracycline	8	6.0 (0.3, 15.8)	71.2 (p=0.0)	69.29
cefotaxime	7	23.0 (10.8, 37.1)	85.3 (p=0.0)	97.42
amoxicillin + clavulanic acid	6	38.4 (17.0, 61.8)	80.0 (p= 0.0)	48.77
ciprofloxacin	5	3.4 (0.0, 15.3)	67.2 (p= 0.141)	54.38
ampicillin	5	39.5 (17.5, 63.3)	92.3 (p=0.0)	63.47
doxycycline	5	21.0 (11.8, 31.6)	94.5 (p= 0.0)	76.38
penicillin	5	13.9 (0.0, 39.6)	95.4 (p=0.0)	81.62
R- antimicrobial resistance, I^2- heterogeneity				

The most cited genes in the included studies were related to erythromycin resistance (*erm*), *ß-lactamase* production (*$_{bla}$cfxA*) and tetracycline resistance (*tet*) (**Figure 3**).

Figure 3 – Resistance genes assessed in the included studies



Only one study[26] cited virulence factors and they were related to *Staphylococcus aureus* and extracellular adhesion.

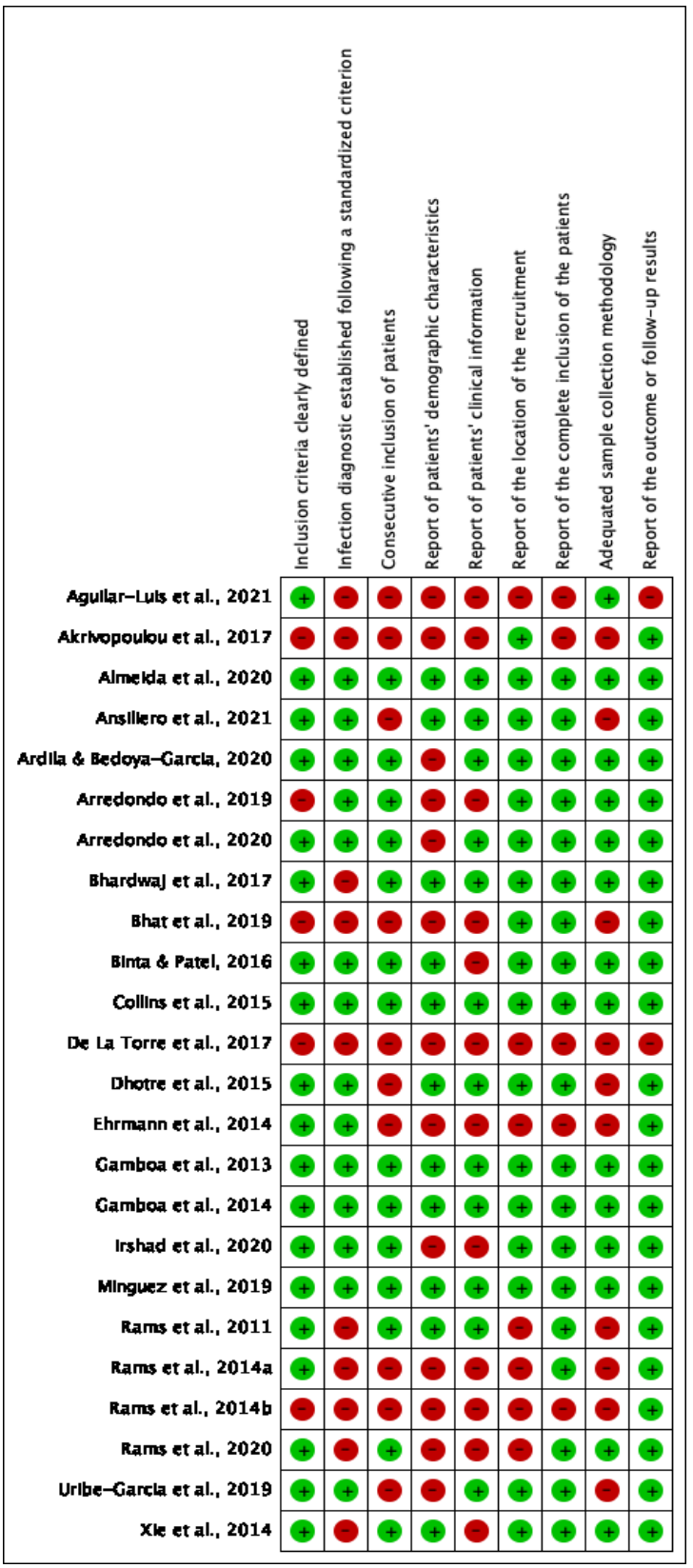
Five studies[18, 23, 26, 29, 32] reported multi-drug resistance.

The data obtained regarding the antibiotic resistance profile, the prevalence of microorganisms assessed in endodontic infections, and the antimicrobial resistance relationship did not allow the generation of statistical analysis for this systematic review.

Quality assessment

Figure 4 illustrates the risk of bias of the included studies for each domain evaluated and the analysis of the overall risk of bias. Only six studies[11, 13, 17, 21, 25, 27] presented all the criteria evaluated as low risk of bias. The remaining 18 studies presented at least one domain considered critical as a high risk of bias, therefore, they were graded as a critically low confidence.

Figure 4 – Risk of bias in the included studies



It was not possible to analyze the risk of publication bias, as ten studies evaluating the antimicrobial resistance of the same microorganisms against the same antimicrobials were not obtained.

Discussion

Despite the American Academy of Periodontology has published in 2017[33] a new classification of periodontal and peri-implant diseases and conditions, the studies included in this systematic review have still used the 1999 classification (aggressive, chronic periodontitis).

In the included studies of this systematic review, it was observed that the largest number of bacterial species was found in aggressive periodontitis and they were present in almost all studies.

Commensal bacteria present in sick and healthy patients may contribute to the development of MRSA when antimicrobials are used as adjuncts to periodontal treatment[34]. The heterogeneous etiology of periodontitis, in which multiple microbial combinations may play a role in the cause of the disease, could justify the multi-drug resistance found in periodontal infections reported in five studies included in this systematic review[18, 23, 26, 29, 32].

Another factor that contributes to the increase in antimicrobial resistance is the high presence of adhesion factors that influence biofilm formation[27, 35]. In the present study, the virulence factors were related to *Staphylococcus aureus* and extracellular adhesion. *Staphylococcus* spp. were cited in four studies because the oral cavity is considered a reservoir for these microorganisms and their presence may contribute to increased methicillin resistance though there is no evidence about the participation in the etiopathogenesis of periodontal disease[36].

The culture method used to determine antimicrobial resistance was one of the main limiting factors of the studies, because there is the possibility of losing the sample for evaluation. In addition, the *in vitro* susceptibility assessment does not accurately reflect the clinical efficacy[37].

One systematic review published in 2020[38] assessed the effects of systematic antimicrobials as an adjunct to non-surgical periodontal treatment and concluded that there is very low-certainty evidence (for long-term follow-up) to inform clinicians and patients if adjunctive systemic antimicrobials are of any help for the non-surgical treatment of periodontitis. In addition, none of the studies reported data on antimicrobial resistance and patient reported quality of life changes.

As most of the studies included in this systematic review did not report important information about the clinical and demographic characteristics of the patients, nor the inclusion criteria of these patients in the evaluated studies, it was not possible to determine the geographic distribution of the resistant microorganisms found at the same time that it was observed a high level of ß-lactamase, methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-antimicrobial resistant microorganisms (MDRO) in Latin American countries[28].

The data reported in the included studies on the microorganisms assessed or the analysis of resistance to the antimicrobials tested were not sufficient to generate a meta-analysis, withal the high heterogeneity (I^2) between the antimicrobials assessed could engender a possible publication bias.

Conclusion

It was not possible to estimate the prevalence of microorganisms present in periodontal infections, as the authors of the included studies evaluated only the bacteria most commonly

present in these infections. The most cited antimicrobials were not those that showed the highest rates of resistance, however, it was not possible to associate these rates with the microorganisms assessed.

There is considerable uncertainty regarding the profile of microorganisms and their resistance to periodontal infections, therefore, further research is needed focusing on regional population studies to resolve this problem in the era of increasing resistance to antimicrobials.

Conflict of interests

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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APÊNDICES:

Supplementary file 1. Search strategy.

Database: **PubMed**

1. "dental pulp cavity"[MeSH Terms]
2. "dental care"[MeSH Terms]
3. “root canal”[MeSH Terms]
4. “endodontics”[MeSH Terms]
5. “periapical abscess”[MeSH Terms]
6. “periodontal disease”[MeSH Terms]
7. “periododntal abscesses”[MeSH Terms]
8. “drug resistance, microbial”[MeSH Terms]
9. “microbial sensitivity tests”[MeSH Terms]
10. “antifungal drug resistance”[MeSH Terms]
11. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7
12. 8 OR 9 OR 10
13. 11 AND 12

Search (((((((resistance, antifungal drug[MeSH Terms]) OR Antifungal Drug Resistance) OR Resistance, Antifungal Drug) OR Antibiotic Resistance, Fungal)) OR ((((((((((((((((((((((((((microbial sensitivity test[MeSH Terms]) OR Microbial Sensitivity Test) OR Sensitivity Test, Microbial) OR Sensitivity Tests, Microbial) OR Test, Microbial Sensitivity) OR Tests, Microbial Sensitivity) OR Drug Sensitivity Assay, Microbial) OR Antimicrobial Susceptibility Breakpoint Determination) OR Breakpoint Determination, Antimicrobial Susceptibility) OR Virus Drug Sensitivity Tests) OR Viral Drug Sensitivity Tests) OR Breakpoint Determination, Antibacterial Susceptibility) OR Fungus Drug Sensitivity Tests) OR Fungal Drug Sensitivity Tests) OR Minimum Inhibitory Concentration) OR Concentrations, Minimum Inhibitory) OR Concentration, Minimum Inhibitory) OR Inhibitory Concentration, Minimum) OR Inhibitory Concentrations, Minimum) OR Minimum Inhibitory Concentrations) OR Antibiogram) OR Antibiograms) OR Bacterial Sensitivity Tests) OR Tests, Bacterial Sensitivity) OR Sensitivity Tests, Bacterial) OR Test,

Bacterial Sensitivity) OR Bacterial Sensitivity Test) OR Sensitivity Test, Bacterial)) OR (((((((antibiotic resistance[MeSH Terms]) OR Drug Resistances, Microbial) OR Antimicrobial Drug Resistance) OR Antimicrobial Drug Resistances) OR Antibiotic Resistance, Microbial) OR Antibiotic Resistance) OR Resistance, Antibiotic)))

AND ((((((((((((((((((((((((dental pulp cavity) OR cavity, dental pulp) OR pulp cavities, dental) OR pulp cavity, dental) OR cavities, dental pulp) OR dental pulp cavitites) OR dental pulp cavities) OR chamber, pulp) OR chambers, pulp) OR pulp chambers) OR pulp canal) OR canal, pulp) OR canals, pulp) OR pulp canals) OR root canal) OR canal, root) OR canals, root) OR root canals) OR pulp chamber)) OR ((dental care) OR care, dental)) OR Endodontics) OR ((((((((((((((((Pulp Canals) OR Root Canals) OR Cavity, Dental Pulp) OR Pulp Cavities, Dental) OR Pulp Cavity, Dental) OR Cavities, Dental Pulp) OR Dental Pulp Cavities) OR Pulp Chamber) OR Chamber, Pulp) OR Chambers, Pulp) OR Pulp Chambers) OR Pulp Canal) OR Canal, Pulp) OR Canals, Pulp) OR Canals, Root) OR Canal, Root) OR Root Canal)) OR ((((((((((((((((((((((((Abscesses, Periapical) OR Periapical Abscesses) OR Dentoalveolar Abscess, Apical) OR Abscess, Apical Dentoalveolar) OR Abscesses, Apical Dentoalveolar) OR Apical Dentoalveolar Abscess) OR Apical Dentoalveolar Abscesses) OR Dentoalveolar Abscesses, Apical) OR Periodontitis, Apical, Suppurative) OR Periapical Periodontitis, Suppurative) OR Periapical Periodontitides, Suppurative) OR Periodontitides, Suppurative Periapical) OR Periodontitis, Suppurative Periapical) OR Suppurative Periapical Periodontitides) OR Suppurative Periapical Periodontitis) OR Alveolar Abscess, Apical) OR Abscess, Apical Alveolar) OR Abscesses, Apical Alveolar) OR Alveolar Abscesses, Apical) OR Apical Alveolar Abscess) OR Apical Alveolar Abscesses) OR Abscess, Periapical) OR periapical abscess)) OR (((((((periodontal disease[MeSH Terms]) OR Disease, Periodontal) OR Diseases, Periodontal) OR Periodontal Disease) OR Parodontosis) OR Parodontoses) OR Pyorrhea Alveolaris)) OR ((((periodontal abscess[MeSH Terms]) OR Abscess, Periodontal) OR Abscesses, Periodontal) OR Periodontal Abscesses))

Database: **Embase**

1. exp dental pulp cavity/ or dental pulp cavity.mp.
2. dental care.mp. or exp dental procedure/
3. endodontics.mp. or exp endodontics/
4. periapical abscess.mp. or exp tooth periapical disease/
5. periodontal disease.mp. or exp periodontal disease/
6. periodontal abscess.mp. or exp periodontal abscess/ or exp periodontal disease/
7. drug resistance, microbial.mp. or exp antibiotic resistance/
8. microbial sensitivity tests.mp. or exp microbial sensitivity test/
9. antifungal drug resistance.mp. or exp antifungal resistance/

10. 1 OR 2 OR 3 OR 4 OR 5 OR 6
11. 7 OR 8 OR 9
12. 10 AND 11

Database: **CINAHL**

1. (MH "Dental Pulp Cavity") OR "dental pulp cavity"
2. (MH "Dental Care+") OR "dental care"
3. “root canal" OR (MH "Root Canal Therapy")
4. (MH "Endodontics+") OR "endodontics"
5. “periapical abscess" OR (MH "Periapical Diseases")
6. “periodontal disease" OR (MH "Periodontal Diseases+")
7. (MH "Periodontal Abscess") OR "periodontal abscess"
8. (MH "Drug Resistance, Microbial+") OR "drug resistance, microbial" OR (MH "Drug Resistance+")
9. (MH "Microbial Culture and Sensitivity Tests") OR "microbial sensitivity test"
10. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7
11. 8 OR 9
12. 10 AND 11

Supplementary file 2 – *Reasons for exclusion*

Excluded studies (n = 18)

Author, year	Motivo
Benachinmardi et al., 2013	Sample collection year prior to 2011
Benachinmardi et al., 2015	Sample collection year prior to 2011
Egwari et al., 2016	Sample collection year prior to 2011
Fernandez-Canigia et al., 2015	Sample collection year prior to 2011
He et al., 2013	Sample collection year prior to 2011
Koukos et al., 2013	Sample collection year prior to 2011
Lourenço et al., 2015	Sample collection year prior to 2011
Mombelli et al., 2015	Sample collection year prior to 2011
Oettinger-Barak et al., 2013	Sample collection year prior to 2011
Rasteniene et al., 2015	Sample collection year prior to 2011
Lee & Lee, 2019	Laboratory acquired strains
Okamoto-Shibayama et al., 2017	Laboratory acquired strains
Song et al., 2013	Laboratory acquired strains
Tantivitayakul et al., 2020	Laboratory acquired strains
Diaz et al., 2015	Assessment of cellular morphological changes
Stahli et al., 2020	Characterized the microbial strain present
Koukos et al., 2015	Reported the prevalence of *S. aureus*
Marin et al., 2019	Sequencing system validation (PCR)

7 CONSIDERAÇÕES FINAIS

O crescimento da resistência dos microrganismos frente a ação dos antimicrobianos é uma preocupação mundial e os profissionais de Odontologia exercem papel fundamental no controle desse crescimento por meio da prescrição consciente e coerente de antimicrobianos no tratamento das infecções orais.

O conhecimento das características dos patógenos que contaminam o sistema de canais radiculares e o periodonto, incluindo o seu perfil de resistência (por meio dos genes e fatores de virulência), e a indicação correta do antimicrobiano são fatores importantes para o controle da disseminação da resistência antimicrobiana

As cepas de microrganismos presentes nas infecções endodônticas e periodontais reportadas nos estudos incluídos foram bastante similares, no entanto, a resistência levantada variou de acordo com o diagnóstico da infecção, a técnica de avaliação de susceptibilidade/ resistência, a localização geográfica da população avaliada e o tipo de antimicrobiano utilizado.

Não existe atualmente evidência com respeito a prevalência da resistência antimicrobiana nas infecções endodônticas e periodontais. Portanto, existe considerável incerteza com relação ao perfil de microrganismos e sua resistência nestas infecções, requerendo futuras pesquisas que deveriam focar em estudos populacionais regionais para dirimir este problema na era da crescente resistência aos antimicrobianos.

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ANEXO A – ORIENTAÇÕES PARA APRESENTAÇÃO DE DISSERTAÇÕES/TESES DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS DA UNIVERSIDADE DE SOROCABA

UNISO
Programa de Pós-Graduação em Ciências Farmacêuticas

Orientações para apresentação de dissertações/teses do Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba

As dissertações/teses do Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade de Sorocaba (PPGCF-Uniso) poderão ser apresentadas em dois formatos: o tradicional ou em formato de artigo(s) científico(s).

Os trabalhos de investigação que possam resultar em patentes poderão ser apresentados na forma convencional, a critério do grupo de pesquisadores envolvidos, reservadas as particularidades exigidas em relação ao sigilo.

O formato tradicional segue o padrão descrito nas normas do "Manual para normalização de trabalhos acadêmicos" da Universidade de Sorocaba.

As dissertações entregues no formato de artigo científico têm como exigência a publicação ou, no mínimo, a submissão prévia de **pelo menos um** artigo em revista científica com classificação mínima Qualis/Capes B2 (de acordo com a categorização da WebQualis mais recente, na data do envio/publicação) e podem ser inseridos no idioma e na formatação estabelecida pelo(s) respectivo(s) periódico(s). Os demais artigos podem não ter sido submetidos ainda.

As teses entregues no formato de artigo científico têm como exigência a publicação ou, no mínimo, a submissão prévia de **pelo menos dois artigos** em revista científica com classificação mínima Qualis/Capes B2 (de acordo com a categorização da WebQualis mais recente, na data do envio/publicação) e podem ser inseridos no idioma e na formatação estabelecida pelo(s) respectivo(s) periódico(s). Os demais artigos podem não ter sido submetidos ainda.

Para aclarar membros da banca que desconhecem esta versão alternativa da dissertação/tese recomenda-se anexar este documento no final das versões encaminhadas aos membros da banca.

A dissertação/tese no formato de artigo(s) científico(s) deverá possuir os elementos apresentados no Quadro 1.

Quadro 1 - Elementos para a construção da dissertação no formato de artigo(s) científico(s).

Elementos pré-textuais	*1. Folha de rosto*
	2. Errata (Opcional)
	3. Folha de aprovação
	4. Dedicatória (Opcional)
	5. Agradecimentos (Opcional)
	6. Epígrafe (Opcional)
	7. Resumo na língua vernácula

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	8. Resumo em inglês (Abstract)
	9. Lista de abreviaturas e siglas; lista de tabelas e lista de símbolos (opcionais). Estas listas não devem conter as informações apresentadas nos artigos científicos.
	10. Sumário
Elementos textuais	*11. Introdução ou apresentação:* trata-se da parte inicial do texto com formulação clara e simples do tema investigado, constando a delimitação do assunto tratado, sua relevância e justificativa.
	12. Revisão de literatura: quando a revisão de literatura for concebida como artigo de revisão, este item deverá ser incluído no item resultado(s).
	13. Objetivos: geral e específico
	14. Material e Métodos (opcional). Quando parte dos resultados não for apresentada no formato de artigo, este item deverá ser incluído após os objetivos específicos. Quando o autor quiser apresentar o(s) método(s) de forma mais detalhada do que no artigo, este item pode também ser apresentado em separado.
	15. Resultados (pode ser apresentado no formato de artigos): deve(m) ser inserida(s) a(s) cópia(s) de artigo(s) derivado(s) da dissertação, previamente publicados, submetidos ou não para publicação em revistas científicas. Sugere-se que cada artigo seja antecedido de uma breve apresentação seguida dos elementos de identificação do artigo (autores, título, revista de publicação, volume, páginas). Os artigos anexados poderão ser apresentados nos formatos exigidos pelas revistas, as quais os artigos foram publicados e/ou submetidos. Parte dos resultados pode ser apresentada em separado dos artigos, quando conveniente.
	16. Discussão (opcional): O autor pode ampliar a discussão dos resultados, quando conveniente.
	17. Conclusão ou Considerações finais: esta parte deverá conter a conclusão do trabalho ou as considerações do autor sobre os resultados alcançados frente aos objetivos propostos.

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Elementos pós-textuais	*18. Referências:* Devem seguir as normas do "Manual para normalização de trabalhos acadêmicos" da Universidade de Sorocaba. Não devem ser inseridas as referências apresentadas nos artigos.
	19. Apêndices (Opcional)
	20. Anexos (Opcional)

ANEXO B - PUBLICAÇÃO DO PROTOCOLO DA REVISÃO SISTEMÁTICA

Resistance profile to antimicrobial agents in themain circulating bacteria isolated from acute periodontal and endodontic infections in Latin America (MICROBE- DENT) A systematic review protocol

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Abstract

Background: Antimicrobial resistance is currently considered the main risk to global health. A variety of microbial species have been isolated from endodontic and periodontal infections. However, clinical endodontic and periodontics bacterial isolates have notbeen sufficiently characterized with regard to their capacity for antibiotic resistance. We aim to assess the existing evidence toestimate the prevalence of the main antimicrobial resistance and multidrug resistant organisms in endodontics and periodontics and
to describe their geographic distribution in Latin America.

Methods: All types of designs and will be restricted to Latin American studies will be included in this systematic review. MEDLINE, Embase, CINAHL, BVS (LILACS, BBO - bvsalud.org), IBECS (bases.bireme.br), Google Scholar, Cochrane Central Register ofControlled Trials, and Web of Science databases will be searched from 2013 to December 31, 2018 for all types of study designs that
report microbial infection in endodontics and periodontics and their resistance and that define the microbiological methods used to identify microorganisms. The selection of articles for inclusion will be performed by 2 reviewers using predefined eligibility criteria. The Cochrane and ROBINS-I risk of bias assessment tools will be used to assess the methodological quality of randomized control trials. TheNewcastle–Ottawa scale will be used to assess the quality of methodology in observational studies. The overall quality of evidence willbe assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE) using the same principles anddomains applied in the quality assessment of prognostic studies. The heterogeneity of the findings will be assessed using both the x^2 test and the I^2 statistic. Sensitivity analysis will be performed by subgroup analyses and meta-regression to investigate the effect of study-
level characteristics, such as age, gender, and methodological quality score, whenever possible. Publication bias across studies will beevaluated by visual inspection of the funnel plots and Begg's test for the results covered in 10 or more studies.

Results: The evidence derived by this study will inform best practices for patients with endodontic and periodontal problemsreceiving antimicrobial agents.

Conclusion: Successful completion will significantly impact clinical practice and contribute to improved prescribing competency.

Protocol registration: PROSPERO—CRD42018077810.

Abbreviations: BBO = Bibliografia Brasileira de Odontologia (Brazilian Bibliography of Dentistry), BVS = Biblioteca Virtual emSau´de (Virtual Health Library), CENTRAL = Cochrane Central Register of Controlled Trials, CI = confidence interval, CINAHL = Cumulative Index to Nursing and Allied Health Literature, Development and Evaluation, embase = Excerpta Medica Database, GRADE = Grading of Recommendations Assessment, IBECS = Índice Bibliográfico Español en Ciencias de la Salud (Spanish Bibliographic Index in Health Sciences), LILACS = Literatura Latino-Americana e do Caribe em Ciências da Saúde (Latin American

and Caribbean Health Sciences Literature), MDRO = multidrug-resistant organisms, MEDLINE = Medical Literature Analysis and Retrieval System Online, MeSH = Medical Subject Headings, MIC = minimal inhibitory concentration, PRISMA-P = Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols, PROSPERO = International Prospective Register of Systematic Reviews, ReBEC = Registro Brasileiro de Ensaios Clínicos (Brazilian Registry of Clinical Trials), ROBINS-I = Risk of Bias inNonrandomized Studies of Interventions, WHO = World Health Organization.

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of interest to disclose.

Supplemental Digital Content

is available for this article.

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1. Introduction

Antimicrobial resistance is a natural phenomenon for the sake of the survival and maintenance of the species and is present in all geographic regions.[1] It is a worldwide public health problem with few therapeutic options and a negative impact on patients infected by multidrug resistant organisms (MDROs).[2] MDROs, according to the most widely used criteria in the literature,[3] are labeled as such because of their in vitro resistance to more than one antimicrobial agent.

The antimicrobial resistance of anaerobes isolated from primary endodontic infections has increased in the last decade in the Brazilian population.[4] Considering that the bacteria from root canals could potentially develop antimicrobial resistance, their capacity to form a biofilm may facilitate the dissemination of antimicrobial resistance by horizontal gene transfer.[5]

Infections caused by drug-resistant bacteria are associated with increased morbidity and mortality and increased costs. Clinically, patients with acute apical abscess experience mild-to-severe pain, swelling and even trismus. Systemic manifestations could occur, including fever, lymphadenopathy, malaise, headache, and nausea.[6] Acute dental abscesses have caused serious complications and even death.[6,7]

The increasing rate of resistance of microorganisms to penicillins or other antibiotics has generated concern among health authorities in Latin America.[8–10] It is more threatening when considering the very limited number of new antimicrobial agents that are in development.[3]

In 2004, a study intended to detect bacterial species from abscess samples collected in Oregon and Rio de Janeiro suggested that the differences found in the bacteria detected or cultured in the studies could be associated with the geographic location.[11] It is estimated that, due to its size and alarming magnitude, the epidemiology of resistance may show remarkable geographical variability and rapid temporal evolution.

A systematic review including 7 studies that evaluated 374 patients from different countries worldwide revealed that antimicrobial resistance rates varied according to the previous use of antibiotics.[12] However, the authors did not evaluate the risk of bias and disregarded the findings' chronology.

The data generated by this search could also help managers of public health systems to make better decisions, in addition to serving as an educational tool for prescribers to acquire a greater understanding and awareness of the importance of the rational use of antimicrobials, in line with the recommendations of the World Health Organization.[10]

Therefore, the objectives of this systematic review are to estimate the prevalence of the main microbially resistance and multidrug resistance organisms, to analyze the time course tendencies of resistance and multidrug resistance, and to describe the geographic distribution of resistance and multidrug resistance organisms.

2. Systematic review question

What is the resistance profile to antimicrobial resistance in main circulating bacteria isolated from acute periodontal and endodontics infections in Latin America?

3. Methods

3.1. Standards

The systematic review will be performed according to the recommendations specified in the Cochrane Handbook for Interventional Reviews and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) statement (Additional file-1, http://links.lww.com/MD/C637).

3.2. Protocol and Registration

We registered our review protocol with the International Prospective Register of Systematic Reviews https://www.crd.york.ac.uk/prospero/ (PROSPERO-CRD42018077810). Ethical approval is not required because this is a literature-based study.

3.3. Eligibility criteria

3.3.1. Inclusion criteria. This systematic review will include all types of designs and will be restricted to Latin American studies meeting the following criteria: investigate microbial infection in endodontics and periodontics, report resistance to microbial infection in endodontics and periodontics, and discuss the microbiological methods used for the identification of microorganisms. Antimicrobial resistance is understood as the ability of a microorganism to resist the effects of an antimicrobial agent that previously could successfully treat the disease. Antimicrobial resistance will be defined as the resistance of an isolated pathogen to the antibiotic in question using a standardized antimicrobial susceptibility test model such as the agar diffusion test (Kirby–Bauer method) or other standard methods for determining the zone of inhibition or minimal inhibitory concentration (MIC) of the isolate. Additionally, we will include studies that detected the bacterial resistance genes of antibiotics by molecular techniques and those with samples collected from the buccal cavity (saliva, supragingival biofilm, or root canals with primary endodontic infections). MDROs, according to the most widely used criteria in the literature,[3] are labeled as such because of their in vitro resistance to more than one antimicrobial agent.

3.3.1.1. Participants. The studies should include patients with permanent dentition and endodontic and/ or periodontal microbial infection.

3.3.1.2. Timing. The last 5 years (from 2013 to 31 December 2018)

3.3.1.3. Language. There will be no restrictions based on language.

3.3.2. Exclusion criteria. We will exclude *crossover* studies and those with incomplete data or information, studies in which dataon microbial agents could not be isolated, primary studies or systematic reviews with the qualitative synthesis of information, therapeutic guides, guidelines, abstracts, conferences, books, book chapters, and methodological studies.

3.4. Measure outcomes

The main outcomes will be to find the prevalence of antibiotic resistance (with 95% confidence intervals), proportion of drug resistance transmitted and acquired, rate of failure during antibiotic treatment, percentage and the overall percentage of resistance for each antimicrobial agent.

3.5. Search methods for primary studies

We will not impose any language restrictions or publication status.

3.5.1. Electronic searches. We will search in the following electronic databases, with no publication status restrictions: MEDLINE, Embase, CINAHL, Google Scholar, Cochrane Central Register of Controlled Trials, and Web of Science. The BVS (bvsalud.org) will be used to search for studies in different databases, such LILACS (lilacs.bvsalud.org), BBO (bases.bireme. br), and IBECS (bases.bireme.br). In *Portal de Periodicos* (periodicos.capes.gov.br), we will search for dentistry and oral sciences sources. For primary studies, we will search in ReBEC (http://www.ensaiosclinicos.gov.br), Clinicaltrials.gov and the WHO Register (who.int).

3.5.2. Searching other resources. Additionally, we will use the website "bancodeteses.capes.gov.br" to identify dissertations inthe field, and websites such as the Grey Literature Report (http://www.greylit.org) will be searched as grey literature. If necessary, the lead authors of the studies will be contacted for further information.

3.6. Search strategy

For the profile of the circulating agents of antimicrobial resistance in Latin America, the search strategy will be conducted individually with MeSH terms such as: resistance to antimicrobial drugs and endodontic and/or periodontal infections. The search strategy to be used is described in Additional file-2, http://links.lww.com/MD/C637. This same strategy will be tailored for each database or library listed. This search strategy will be performed in cooperation with a research librarian.

3.7. Eligibility determination

Following a calibration exercise, peer reviewers will evaluate titles and abstracts, independently and in duplicate, according to the eligibility criteria. Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org) will be used to manage the screening among reviewers.

The full-text publications of articles selected as potentially eligible will be acquired. After a second calibration exercise, the same pairs of reviewers will independently apply the eligibility criteria to the potentially eligible full texts using standard forms. Differences will be resolved by consensus among all reviewers. To exclude studies that published their results in more than one article (data replication), a reviewer will review all eligible articles and identify those with one or more in common authors. In case of publication of data from the same cohort, the article with the most complete data will be used.

To evaluate the concordance of the selection for the full text, the Kappa test will be used. Kappa values between 0.40 and 0.59 will be considered to represent weak agreement, between 0.60 and 0.74 to represent intermediate agreement, and 0.75 or more to represent excellent agreement. Reviewers will use a standardized, pretested data extraction form with instructions on how to extract them. For articles published only in summary or articles that have important information missing, complete information on the methods and results will be obtained by contacting the authors.

3.8. Data extraction

Two reviewers, in pairs and independently, will be calibrated by extracting at least 3 articles and then coming to a consensus. This procedure should occur until the reviewers are able to extract the data.

Data on the patient's nosological status (type of infection, diagnosis), including the number of subjects included in the study, the description of recruitment, city, location, date of the research, exposure to antibiotics, sample size, type (saliva, supragingival biofilm, root canal with primary endodontic infection, etc.), methods of determining sample size, conflicts of interest, biological material used, methods used to measure results (type of medium of culture, type of collection, etc.), antimicrobial agents tested, number of bacterial lines and number of resistant species, will be collected from all studies.

The total percentage of an antimicrobial agent will be calculated for each study, regardless of the bacterial species tested.

The overall percentage resistance for each antimicrobial agent tested will be the average between the total number of resistant isolates and the total number of isolates evaluated. Microbial isolates with an intermediate profile will be considered susceptible to the antimicrobial agent.[13]

3.9. Risk of bias in individual studies

The risk assessment of bias will be independently assessed by at least 2 reviewers in duplicate using the instrument for nonrandomized studies by Cochrane (Collaboration tool for assessing the risk of bias—ROBINS-I)[14] and considering specific tools for prevalence studies.[15] For observational studies, the Newcastle–Ottawa quality scale for cohort studies will be used. If differences are observed, they will be resolved by consensus among all reviewers. Incomplete results will be stipulated as having a low risk of bias, with a loss of follow-up of $<10\%$.

Two reviewers will independently evaluate the quality of each study included, and any disagreement will be resolved by consensus or by one arbitrator to judge unresolved disagreements.

3.10. Confidence in pooled estimates of effect

The overall quality of evidence will be assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE) using the same principles and domains applied in the quality assessment of prognostic studies.[16]

We will perform subgroup analysis for geographic area (country) and by dental specialty (endodontics and periodontics). If possible, we will also perform subgroup analyses for age,gender, and antimicrobial class. Analysis will be performed using R, V.3.2.3, and the meta V.4.3-2 and metaphorpackages.

3.11. Data synthesis

The results will be analyzed separately according to the study design, and when possible, will be described qualitatively in tables of evidence. A descriptive summary will be created to determine the amount of evidence found and the variation between studies. The data will be grouped by microbial agents and antibiotic agents.

Statistical analysis of data (meta-analysis) will only be performed if appropriate data is found. The random-effects model will be used to calculate the pooled prevalence and corresponding 95% CI. If possible, the prevalence of the main circulating agents of antimicrobial resistance in periodontal and endodontic infections in Latin America will be adjusted, considering the population of each Latin American country.

The 95% predictive distribution, that is, the probabilistic interval of the realization of new studies to be carried out in Latin America, will be calculated.

The heterogeneity of the findings will be assessed using both the x^2 test and the I^2 statistic. Sensitivity analysis will be performed by subgroup analyses and meta-regression to investigate the effect of study-level characteristics, such as age, gender and methodological quality score, whenever possible. Publication bias across studies will be evaluated by visual inspection of the funnel plots and Begg's test for the results covered in 10 or more studies.

3.12. Ethics and dissemination

No ethical approval is required as no primary, personal or confidential data are being collected in this study. Successful completion will significantly impact clinical practice and contribute to improved prescribing competency. This will inform best practices for patients with endodontic and periodontal problems receiving antimicrobial agents. The results of this study will be published in a peer-reviewed journal and presented at conferences.

4. Discussion

Our review will assess the existing evidence to estimate the prevalence of the main antimicrobial resistance and MDROs in endodontics and periodontics and to describe their geographic distribution in Latin America.

The data generated by this search could also help managers of public health systems to make better decisions, in addition to serving as an educational tool for prescribers to acquire a greater understanding and awareness of the importance of the rational use of antimicrobials, in line with the recommendations of the World Health Organization.[10]

The findings will be disseminated to national and international scientific sessions and published in a peer-reviewed journal. Successful completion will significantly impact on clinical practice and contribute to improve prescribing competency. This will inform best practice of patients with endodontic and periodontal problem receiving antimicrobial agents, and help facilitate evidence-based shared care decision-making. This study will also identify key areas for future research.

4.1. Strengths and limitations of this study

- To our knowledge, this is the first systematic review protocol that has attempted to evaluate the prevalence of antimicrobial resistance in the areas of endodontics and periodontics in Latin America.
- This protocol was written according to the PRISMA-P guidelines, and the review will be written using a standardized methodology with a full bibliographic search, study selection, data extraction, and bias risk assessment performed by 2 independent researchers.
- The chosen time period appears short (5 years) but represents the time necessary to verify changes in the profile of resistance.
- Using all types of designs and limiting the studies to Latin American research in the area of dentistry, where there is a clear lack of high-quality trials, will increase the internal validity.

Author contributions

FCA is the principal investigator and wrote the protocol. MFF wrote the search strategy. The guarantor of the review is LCL. FCA and LCL will individually perform the abstract extraction and critique the literature, and CCB will be the third reviewer. CCB provided insight on the epidemiological aspects of the review and helped draft the manuscript. FCA, CCB, MFF and LCL advised on background and revised the manuscript. All authors approve the final version and take responsibility for its content.

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