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WANESSA FERNANDES MATIAS REGIS

O PAPEL DA ASSOCIAÇÃO ENTRE *Streptococcus mutans* E *Candida albicans* NA CÁRIE DENTÁRIA

FORTALEZA
2023

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito parcial à obtenção do Título de Doutora em Odontologia.
Orientadora: Profª Drª Lidiany Karla Azevedo Rodrigues Gerage.

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"Quem a Deus tem,
mesmo que passe por momentos difíceis;
Sendo Deus o seu tesouro, nada lhe falta."

Santa Teresa D'Avila

RESUMO

Cárie dentária é uma doença mediada por biofilme que ocorre quando membros acidogênicos/acidúricos da microbiota oral residente obtêm uma vantagem ecológica sobre outras espécies, causando disbiose. O *Streptococcus mutans* (SM) é o microrganismo relacionado à doença mais estudado, no entanto, sozinho ele não é o agente etiológico desta condição, pois microrganismos como *Candida albicans* (CA) são comuns no biofilme cariogênico. Esse estudo revisou a literatura sobre características de virulência de SM e CA isolados ou em associação (Capítulo 1), além de investigar a ocorrência de CA e SM, dos seus sorotipos e de genes de ligação ao colágeno em amostras de dentina cariada de dentes permanentes em indivíduos brasileiros (Capítulo 2). No primeiro estudo, foram realizadas pesquisas em artigos originais e de revisão relacionados a características de virulência de SM e CA, tanto individualmente como em coagregação e como essa interação influencia o potencial cariogênico na desmineralização dentária, produção de polissacarídeos e expressão de genes de virulência. As glicosiltransferases de SM e moléculas de *quorum sensing* de CA podem estimular a produção de matriz de glucano e desempenham um papel significativo na interação entre SM e CA. No segundo estudo, 32 pacientes foram recrutados e 47 amostras de dentina cariada foram coletadas. As amostras foram classificadas segundo o sistema de pontuação radiográfica ICDAS em RB4 (⅓ médio da dentina; n=23) e RC5 (⅓ interno da dentina; n=24) analisadas por PCR para identificação de *Streptococcus* spp., sorotipos de SM (*c*, *e, f*, *k*), dos genes de ligação ao colágeno *cnm/cbm* e CA. Das 47 amostras, 95,7% (45/47) abrigavam *Streptococcus* spp., das quais em 36,1% (17/45) havia SM. Nas amostras SM+, a presença de um único sorotipo foi identificada em 4 para o *c* (23,53%), 10 para o *e* (58,82%), 2 para o *f* (11,76%) e 2 para o *k* (11,76%). Em duas amostras não foi possível tipificar o sorotipo (11,76%) e em outras duas os sorotipos estavam associados (*c*+*f*+*k* and *f*+*k*; 11,76%). Em RB4, foram encontrados *Streptococcus* spp. em 21 amostras (91,3%) onde SM estava presente em 9 (39,1%), dentre as quais, não foram identificados os sorotipos c, *f*, ou *k*, entretanto, o sorotipo *e* estava presente nas 9 (100%). Para RC5, *Streptococcus* spp. estava presente em todas as 24 amostras, sendo que 8 foram SM+ (33%), dentre elas, os sorotipos sozinhos ou combinados foram encontrados como se segue: *c* em 4 (50%), *e* em uma (12,5%) e os sorotipos *f* e *k* em duas (25%). O gene *cbm* foi encontrado em 12,5% (3/8) das amostras de RC5. O gene *cnm* e CA não foram encontrados nas amostras pesquisadas. A ocorrência do sorotipo *e,* considerado raro, em amostras RB4 evidencia a necessidade de uma investigação mais abrangente. Não foi possível mostrar nenhuma associação da presença de cárie dentinária moderada ou profunda com CA associada ou não ao SM, além disso, o gene *cnm* de SM não foi encontrado, independente da profundidade da lesão.

Palavras-chave: Virulência. Cárie Dentária. Reação em Cadeia da Polimerase. Polissacarídeos. Dentina. Serogrupo.

ABSTRACT

Dental caries is a disease caused by biofilm when certain acidogenic/aciduric members of the oral microbiota gain an ecological advantage over other species, leading to dysbiosis. While *Streptococcus mutans* (SM) is the most widely studied microorganism responsible for this disease, it is not the sole cause. Other microorganisms, such as *Candida albicans* (CA), are common in cariogenic biofilms. It is important to note that all evaluations presented in this text are objective and unbiased. This study conducted a literature review on the virulence characteristics of SM and CA, whether they occur independently or in conjunction (Chapter 1). Additionally, we examined the prevalence of CA and SM, along with their serotypes and collagen-binding genes, in samples of carious dentin taken from permanent teeth of Brazilian individuals (Chapter 2). The initial review analyzed primary and secondary articles on the virulence characteristics of SM and CA, examining the individual and conjoined impact on dental demineralization, polysaccharide production, and virulence gene expression. The SM's glucosyltransferase and CA's quorum sensing molecules can activate glucan matrix production, proving significant in SM and CA's interdependent association. In the second study, 32 patients were recruited and 47 samples of carious dentin were collected. The samples were classified according to the ICDAS radiographic scoring system as RB4 (⅓ middle dentin; n=23) and RC5 (⅓ inner dentin; n=24) and analysed by PCR for the identification of Streptococcus spp., SM serotypes (c, e, f, k), collagen-binding genes cnm/cbm and CA. Of the 47 samples, 95.7% (45/47) contained Streptococcus spp. and 36.1% (17/45) contained SM. In SM+ samples, the presence of a single serotype was identified in 4 for c (23.53%), 10 for e (58.82%), 2 for f (11.76%) and 2 for k (11.76%). Serotyping was not possible in two samples (11.76%) and serotypes were associated in two others (c+f+k and f+k; 11.76%). In RB4, Streptococcus spp. were found in 21 samples (91.3%), with SM present in 9 (39.1%), of which serotypes c, f or k were not identified; however, serotype e was present in all 9 (100%). For RC5, Streptococcus spp. were present in all 24 samples, 8 of which were SM+ (33%). Of these, serotypes alone or in combination were found as follows: c in 4 (50%), e in one (12.5%) and serotypes f and k in two (25%). The *cbm* gene was found in 12.5% (3/8) of the RC5 samples. The *cnm* gene and CA were not found in the samples examined. The occurrence of serotype *e*, considered to be rare, in RB4 samples highlights the need for comprehensive investigation. No association was found between the presence of moderate or deep dentinal caries lesions and CA, whether associated with SM or not. In addition, the SM *cnm* gene was not found, regardless of lesion depth.

Keywords: Virulence. Dental Caries. Polymerase Chain Reaction. Polysaccharides. Dentin. Serogroup.

SUMÁRIO

1. INTRODUÇÃO GERAL

Cárie dentária é uma condição complexa, caracterizada pela ausência de um único agente etiológico (PHILIP, SUNEJA & WALSH 2018). Essa condição é definida como uma doença biofilme-açúcar dependente, polimicrobiana, na qual microrganismos presentes na microbiota oral obtêm uma vantagem seletiva sobre outras espécies, resultando em um desequilíbrio disbiótico e no início do processo de doença (MARSH & MARTIN 1999).

Dentre os microrganismos residentes da cavidade oral, encontra-se o *Streptococcus mutans*, que embora não seja considerado o único agente etiológico da doença, desempenha um papel crucial na instalação e progressão dessa condição (BAKER *et al*., 2020) devido a seus mecanismos de virulência, dentre eles, três se destacam:

(1) Capacidade de sintetizar grandes quantidades de polímeros extracelulares de glucano, provenientes principalmente, mas não exclusivamente, da sacarose, que auxiliam na colonização permanente de superfícies dentárias duras e no desenvolvimento da matriz polimérica extracelular (LEMOS *et al*., 2019);

(2) Habilidade de transportar e metabolizar uma ampla gama de carboidratos em ácidos orgânicos (acidogenicidade) (LEMOS *et al*., 2019);

(3) Habilidade de prosperar em condições de estresse ambiental, especialmente em baixos níveis locais de pH (LEMOS *et al*., 2019).

Com base na composição de polímeros de ramnose-glicose da parede celular, o *S. mutans* é classificado em 4 sorotipos principais (*c, e, f* e *k*), os polissacarídeos nos sorotipos *c*, *e* e *f* consistem em polímeros de ramnose-glicose, enquanto, para o sorotipo *k*, as cadeias são compostas somente por polímeros de ramnose (NAKANO *et al*., 2004). O *S. mutans* também abriga proteínas de ligação ao colágeno (Cnm e Cbm) que estão relacionadas a sua ligação com o colágeno presente na dentina, podendo aumentar o risco ao desenvolvimento de lesões de cárie dentinária (SWITALSKI *et al*., 1993). Há relatos de que embora as duas proteínas apresentem alta homologia, a atividade de ligação ao colágeno das cepas positivas para Cbm é significativamente maior do que a atividade das cepas positivas para Cnm (OTSUGU *et al*., 2022).

Além do *S. mutans*, o fungo oportunista *C. albicans* também desempenha um papel ativo na patogênese da cárie e é frequentemente encontrado na cavidade oral de crianças com cárie da primeira infância e em cárie radicular (ZAREMBA *et al*., 2006; XIAO *et al.,* 2018). É um fungo que pode se apresentar no biofilme em forma de levedura, pseudo-hifas e hifas, a transição de levedura para hifa é amplamente reconhecida como uma característica de virulência de *C. albicans,* associada à formação de biofilme (JACOBSEN *et al*., 2012). *C. albicans* pode interagir com bactérias orais por meio de fixação física através das paredes celulares dos microrganismos, dos polissacarídeos extracelulares, da alimentação cruzada de metabólitos e alterações ambientais (BACHTIAR & BACHTIAR 2018). A capacidade de *C. albicans* de produzir enzimas proteolíticas, que facilita a adesão e promove a degradação colagenolítica da dentina, é outro fator que pode ser importante na progressão da cárie (XIANG *et al*., 2023), principalmente quando se considera lesões de cárie dentinária.

A habilidade da *C. albicans* em metabolizar glicose, frutose ou lactose para a produção de ácidos carboxílicos de cadeia curta, resultando na redução do pH do ambiente circundante e, consequentemente, na desmineralização dos tecidos dentários (KINKLE *et al*., 2009), juntamente com sua capacidade de tolerar condições extremas, como ambientes de pH reduzido (FALSETTA *et al*., 2014), bem como de regular os genes de virulência do *S. mutans* (TAO *et al*., 2017), têm suscitado um interesse crescente para investigar seu papel na cárie dentária.

Nesse contexto, o objetivo deste estudo foi investigar os papéis interativos de *S. mutans* e *C. albicans* na patogênese da cárie dentária, focando em seus distintos mecanismos de virulência e seu impacto combinado no potencial cariogênico na desmineralização dentária, produção de polissacarídeos e expressão de genes de virulência, além de investigar a ocorrência a presença de *S. mutans*, seus sorotipos *c*, *e*, *f* e *k*, dos seus genes de ligação ao colágeno *cnm* e *cbm*, além de *C. albicans* em amostras de dentina cariada humana provenientes de lesões dentinárias médias e profundas de dentes permanentes em indivíduos brasileiros.

2. PROPOSIÇÃO

Essa tese de doutorado está apresentada em capítulos, tendo como objetivos:

Capítulo 1: Descrever as características da bactéria *Streptococcus mutans* e do fungo *Candida albicans,* assim como revisar as literatura sobre os efeitos da associação desses microrganismos na cárie dentária. Outro objetivo desta revisão é discutir como essa interação influencia o potencial cariogênico na desmineralização dentária, produção de polissacarídeos e expressão de genes de virulência.

Capítulo 2: Investigar a ocorrência de *S. mutans,* dos seus sorotipos *c*, *e*, *f* e *k*, dos genes de ligação ao colágeno *cnm* e *cbm* bem como de *C. albicans* em amostras de dentina cariada humana coletadas de lesões dentinárias médias e profundas de dentes permanentes em indivíduos brasileiros.

3. CAPÍTULOS

REGIMENTO INTERNO

Esta tese está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Assim sendo, esta dissertação é composta de dois capítulos contendo artigos a serem submetidos para publicação em revistas científicas, conforme descrito abaixo:

CAPÍTULO 1: Interactions between *Streptococcus mutans* and *Candida albicans* in dental caries.

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CAPÍTULO 2: *Streptococcus mutans* serotyping, collagen-binding genes and *Candida albicans* in dentin carious lesions: a molecular approach.

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3.1 CAPÍTULO 1

European Journal of Oral Sciences

Interactions between *Streptococcus mutans* and *Candida albicans* in dental caries

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ABSTRACT

Dental caries is a prevalent chronic disease affecting a significant proportion of both adults and children worldwide. While *Streptococcus mutan*s (SM) is considered the microorganism most closely associated with dental caries, *Candida albicans* (CA) is often observed in a synergistic relationship with SM. However, the virulence mechanisms of these microorganisms individually and in association, and how this association influences cariogenic potential, are not fully understood. The aim of this review was to search for original and review articles related to the virulence characteristics of SM and CA, both individually and in coaggregation, and to verify how this influences the cariogenic potential in tooth demineralization, polysaccharide production, and virulence genes. Scientific research relevant to the key aspects of the review and those specifically addressing the analysis of virulence mechanisms of the microorganisms under investigation were included. The investigation indicates that the physiological interactions between SM and CA heighten acid production within their coexisting biofilm. Furthermore, the biofilm undergoes a surge in adhesion and anchorage sites, resulting in better cell survival and maintenance. The SM glucosyltransferases have a positive interaction with CA Farnesol and positively regulate genes that promote the two species' synergistic coexistence.

Keywords: Dental caries. Virulence. Polysaccharides. Chronic Disease. Microbiota.

Introduction

Dental caries is characterized by the emergence of dental signs and symptoms resulting from a chemical imbalance on dental surfaces, where the biofilm formed persists for a certain period of time (Heersema & Smyth 2019), often exposed to dietary carbohydrates (Fejerskov & Nyvad 2017). Untreated caries in permanent and deciduous teeth has been classified as one of the most prevalent conditions in the world (Marcenes *et al.*, 2013), therefore it is crucial to study microbiota related to dental caries in order to find out efficient ways of controlling it.

Of the greater than 600 species of bacteria, fungi, viruses, and archaea that have been identified in the human oral cavity, more than 30 microorganisms are predominantly found in cariogenic biofilms (Heersema & Smyth 2019). *Streptococcus mutans* is a Gram-positive bacterium that is notoriously cariogenic, due to its characteristics such as being a major producer of extracellular polymeric substances (EPS) and organic acids (acidogenicity) from metabolized carbohydrates, especially in the presence of sucrose Furthermore, it is able to survive in low pH environments (aciduricity) and overcome non-cariogenic species in the dental biofilm (Lemos *et al.*, 2013).

The EPS production is directly related to microbial adhesion to both surfaces and cell-to-cell, while the formation of the polymer matrix is related to the mechanical stability of the biofilm (Bowen *et al.*, 2018). To produce EPS, *S. mutans* secretes enzymes called glucosyltransferases (GTFs) that hydrolyze sucrose, a disaccharide formed by glucose and fructose, which presents a glycosidic linkage called atypical because it involves the functional groups of both monosaccharides (Bowen & Koo 2011).

Although *S. mutans* is the most studied microorganism involved in dental caries, not only other streptococci but also microorganisms from other kingdoms, such as *Candida albicans*, can contribute to the pathogenesis of the disease (Heersema & Smyth 2019; Hwang *et al*., 2015). The polymorphic commensal microbe, *C. albicans*, is capable of growing in distinct cell morphologies: yeast, pseudohyphae, and the most pathogenic form, hyphae (Lee *et al*., 2021). The hydrophobic cells of *C. albicans* are stickier than the hydrophilic ones and have the ability to adhere to soft and hard tissues (Masuoka & Hazen 2004). In addition, these fungi are capable of producing proteinases that can degrade human proteins (Mayer, Wilson & Hube 2013). In most of the cases, it coexists in equilibrium with the host; however, during periods of immune system suppression, it can become pathogenic (Gow *et al*., 2011).

Therefore, the virulence of *C. albicans* is associated with several characteristics, including changes in morphology, expression of adhesins, formation of biofilms, and release of hydrolytic enzymes, these factors allow fungal cells to colonize the host and induce both local and systemic disease, these infections can affect various areas such as the skin, gastrointestinal tract, reproductive system, and oral cavity (Mayer, Wilson & Hube 2013). The ability to form biofilms makes this fungus extremely tolerant to common antifungal and immune systems and able to associate, potentially increasing its resistance (Fernandes *et al*., 2016).

The inter-kingdoms associations, especially between bacteria and fungi, may be antagonistic or cooperative (Kim *et al*., 2017). Once together within biofilms, when under propitious conditions, these organisms may cooperate with each other for the provision of substrates, metabolites, and growth stimulators, as well as competition for nutrients may occur, leading to antagonistic interactions (Mayer, Wilson & Hube 2013).

In the oral cavity, co-adhesion between *C. albicans* and *S. mutans* has been linked to increased caries severity, particularly in cases of early childhood caries, root caries, and recalcitrant infections (Garcia *et al*., 2021; Lobo *et al*., 2019; Katrak *et al*., 2023). The pathogenicity of these organisms is influenced by host conditions and is enhanced by biofilm formation (Picco *et al*., 2019).

The coordination mechanism within biofilms, known as quorum sensing (QS), involves microorganisms that produce, release, detect, and respond to small hormone-like molecules called autoinducers for interspecies communication (Miller & Bassler, 2001). This mechanism depends on cell density and helps regulate behavior among microorganisms in a biofilm (Miller & Bassler, 2001). The autoinducers present in the microbial supernatant undergo concentration increase with the expansion of the microbial population and facilitate gene transcription and protein production (Rocha *et al.*, 2020).

In the case of *Candida albicans*, farnesol is identified as the primary QS molecule (Hornby *et al*., 2001). At high concentrations, farnesol inhibits the transition of yeast cells to their filamentous hyphal form. This hyphal form is associated with *C. albicans* infection and virulence processes (Fernandes *et al*., 2016). This inhibition allows farnesol to regulate *C. albicans*' ability to invade host tissues. Under extreme conditions, it can trigger cellular apoptosis and hinder germ tube formation, yet it cannot halt the elongation of pre-existing tubes (Fernandes *et al*., 2016).

Tyrosol, a compound derived from tyrosine, was later recognized as a second QS molecule in *C. albicans* (Chen *et al.*, 2004). Similar to farnesol, this substance is consistently released into the growth medium during the growth phase and has the ability to eliminate the usual lag phase observed when overnight cultures are transferred to fresh medium (Alem *et al*., 2006). Tyrosol accelerates the development of germ tubes by inducing the filament, so it seems that the morphogenesis process in *C. albicans* is regulated by the complex interplay of positive and negative influences exerted by tyrosol and farnesol, respectively. (Alem *et al*., 2006).

While *S. mutans* and *C. albicans* are commonly studied in scientific research related to dental caries, the specific virulence mechanisms influencing the development and progression of the disease remain unestablished (Bernard, Girardot & Imbert 2020). This review aims to examine how the virulence characteristics of these microorganisms affect demineralization of dental tissue, production of polysaccharides, and the expression of disease-related virulence genes.

Virulence mechanisms of *Streptococcus mutans* and *Candida albicans*: an individual analysis

The genus *Streptococci* is composed of spherical and oval cell microorganisms measuring approximately 0.5 to 0.75 μm in diameter, formed by a coccus arranged in chains (Clarke, 1924) These bacteria are considered a pandemic pathogen, since it is present in populations of diverse origins, ethnic, geographic and socioeconomic status (Spolidorio & Duque 2013). It is an opportunistic microorganism, belonging to the phylum Firmicutes; it is a facultative anaerobic and most isolated strains are α-hemolytic or non-hemolytic (Sztajer *et al*., 2014).

Dental caries is one of the most prevalent chronic diseases in the world, the difficulty in controlling the disease can be due to several factors related to its etiology, such as age, diet, oral hygiene (Koo & Bowen 2014). Within the dental biofilm, *S. mutans* contribute to the matrix composition, especially through the production of polysaccharides (Kim *et al*., 2017).

Its pathogenicity is influenced by the production of organic acids (lactic, formic, acetic and propionic acids), which, in turn, induce pH drop and biofilm virulence (Díaz-Garrido, Lozano & Giacaman 2016). The acid environment promoted by S. *mutans*

generates rapid pH modulations on the biofilm, which can drop the pH from neutral pH to acid values in less than 20 min (Matsui & Cvitkovitch 2010).

The microorganisms' ability to thrive in acidic environments results from the acid tolerance response, which is the capacity to adjust to acidic stress through previous exposure to low or sub-lethal pH levels (Matsui & Cvitkovitch 2010). Acidification of the polysaccharide-rich matrix favors the growth of microorganisms that are tolerant to acid stress and promotes demineralization of dental enamel; the role of matrix in the formation and structure of acidic environments in biofilms has been studied (Klein *et al*., 2015; Díaz-Garrido, Lozano & Giacaman 2016).

The synthesis of exopolysaccharides is an important virulence factor of *S. mutans*, since it promotes bacterial adhesion to the surface of the tooth, even in smooth surfaces, contributes to the structural integrity of the biofilms and alters its porosity, facilitating the diffusion of sugar in the matrix that leads to more pronounced drops in pH (Koo *et al.*, 2010). These polysaccharides, mainly glucans synthesized by microbial glucosyltransferase, are complex structures that change over time during the development of the extracellular matrix of the biofilm (Koo *et al.*, 2010).

If there is excess sugar available, in addition to producing organic acids and matrix, this microorganism is also able to synthesize intracellular polysaccharides, which are glycogen-like glucose polymers that act as a reserve of carbohydrates and decrease osmotic stress (Lemos *et al.,* 2019).

S. mutans is classified into four serotypes (*c*, *e*, *f* and *k*), in accordance with the chemical composition of their cell surface rhamnose-glucose polymers (Nakano *et al*., 2004). The distinguishing factors among different serotypes lie in their surface antigens, which play a pivotal role in interactions with dental surfaces and the formation of cariogenic biofilms. These antigens are responsible for enabling *S. mutans* to adhere to dental structures, such as enamel and dentin, thus facilitating its colonization (Matsumoto-Nakano, 2018). RgpG is an enzyme within the biosynthetic pathway of glucose polymers containing rhamnose, a significant surface antigen in oral *Streptococci* responsible for the differentiation of various serotypes (Yamashita *et al*., 1999).

Each serotype has unique traits and a geographic distribution that is not yet fully comprehended. Serotype *c* is widely found in various populations (Momeni *et al*., 2019), while serotypes *e*, *f*, and *k* exhibit a more restricted distribution (Elyassi, Babaeekhou &

Ghane 2022). Variation in *S. mutans* serotypes can influence its virulence, adherence to different substrates, and interactions with other microorganisms present in the oral biofilm (Matsumoto-Nakano 2018).

Like *S. mutans*, *C. albicans* is a typical native resident of the oral cavity (Salehi *et al*., 2020). However, in the presence of immunosuppression or changes in the host environment, this opportunistic microorganism can rapidly shift from a commensal colonization mode to a pathogenic state, causing various infections (Rapala-Kozik *et al.,* 2023).

Since its first microscopic detection in thrush swabs by Langenbeck, in 1839, *C. albicans* has been recognized as one of the most important pathogenic yeasts encountered as a cause of human infections (Sidrim & Rochal 2004). It has been characterized as a yeast capable of forming both pseudohyphae and hyphae, this morphological versatility significantly contributes to its pathogenesis, as hyphae are primarily responsible for tissue penetration, while yeast forms play a crucial role in early dissemination and less invasive infections (Mayer, Wilson & Hube 2013).

C. albicans biofilm formation occurs in four phases: adherence, initiation, maturation and dispersal. In the adherence phase, planktonic cells in yeast form adhere to the substrate (Mayer, Wilson & Hube 2013). Because of their high surface hydrophobicity, yeast cells can colonize both hard and mucosal surfaces (Sidrim & Rochal 2004). Furthermore, in the initiation phase, the cells form microcolonies and germ tubes to produce hyphae and there is an early production of extracellular matrix (Xu et al., 2020). In the maturation phase, the cells undergo a morphological transition to create the extracellular matrix (Fernandes *et al*., 2016). The accumulation of the matrix results in increased resistance of the biofilm to the antimicrobial agents and the host immune system (Xu et al., 2020).

Biofilm formation and adaptation of metabolic activity play an important role in the ability of *C. albicans* to colonize different regions of the human body and to survive interactions with the host immune system (Mayer, Wilson & Hube 2013). In the oral cavity, the co-adhesion of *C. albicans* with oral bacteria is crucial for its colonization and persistence in the biofilm (Falsetta *et al.*, 2014). *C. albicans* appears to provide sites of adhesion and growth stimulating factors for the bacteria, which in turn excrete lactate that can act as a carbon source for yeast growth (Metwalli *et al.,* 2013). Figure 1 shows a *S. mutans* cell inside a *C. albicans* blastopore.

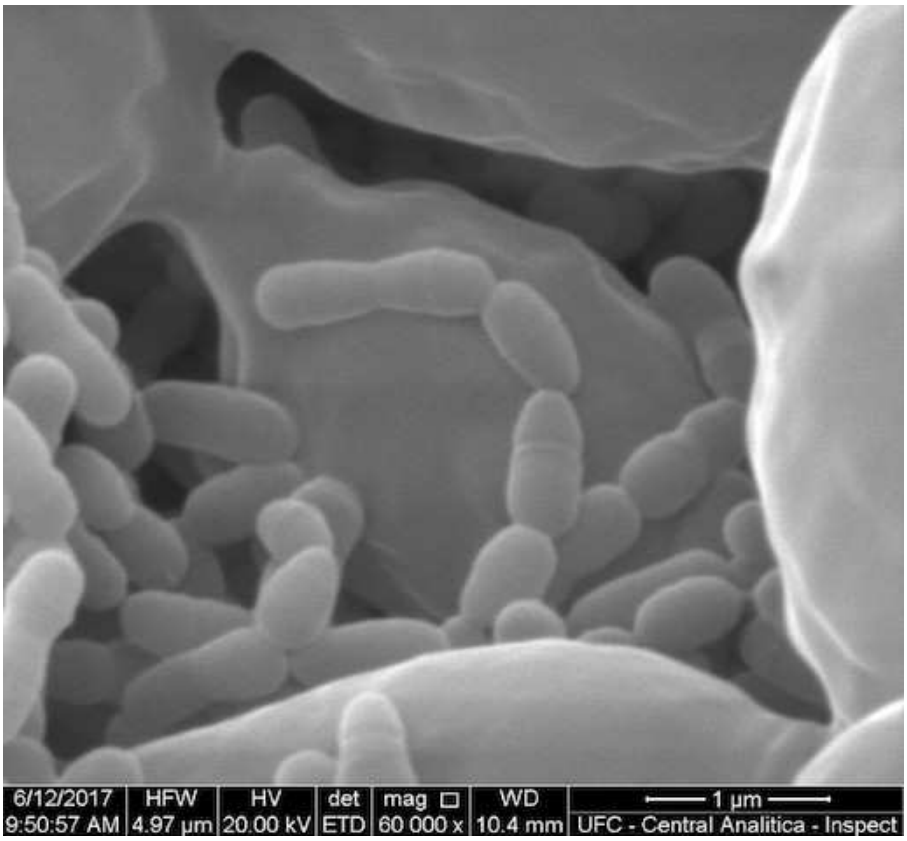

Figure 1. Scanning Electron Micrographs showing the *in vitro* biofilm of *S. mutans* and *C. albicans* on hydroxyapatite slabs after 48 h of cultivation. The yeast appears to provide binding sites for bacteria (source: author).

Virulence mechanisms of *Streptococcus mutans* and *Candida albicans* in coaggregation in demineralization of dental tissues and production of exopolysaccharides (EPS)

The fermentation of carbohydrates by a specific group of microorganisms produces acids that lower the pH in the biofilms. (Matsui & Cvitkovitch 2010). The acidification is linked to the breakdown of dental tissues, which may happen when the equilibrium between the demineralization and remineralization processes is upset for an extended period (Eidt *et al.*, 2019). In dental caries, frequent consumption of sugar leads to dysbiosis in dental plaque whereby acid-tolerant microorganisms outcompete less acid-tolerant ones in response to biofilm acidification (Eidt *et al.*, 2019).

C. albicans is acidogenic, fermentative (capable to metabolize carbohydrates from the diet), cellular hydrophobic and can be considered a risk factor for the development of

dental caries, in the presence of *S. mutans*, *C. albicans* increases sucrose metabolism and acidogenicity of the biofilm, creating a highly acidic environment pH (<5,5) (Kim *et al*., 2017).

The fungus does not have the ability to metabolize sucrose due to the absence of gtfs. Nevertheless, it can catabolize fructose, glucose, or lactose, resulting in short-chain carboxylic acids that lower the pH of the environment (Xiang et al., 2023). This in turn facilitates the demineralization of hard tissues (Paes-Leme *et al*., 2009). *S. mutans*, on the other hand, can break down sucrose into fructose and glucose and promoting cross-feeding (Li *et al*., 2023).

Furthermore, *C. albicans* is capable of colonizing enamel, dentin, and cement surfaces and can penetrate deep into dentin tubules, thereby contributing to the development of caries on these surfaces in both children and adults (Salehi *et al.*, 2020). When *C. albicans* is present with *S. mutans*, the number and severity of smooth-surface lesions increases in comparison to the impact of infection caused by each microorganism separately (Metwalli et al., 2013).

One contributing factor is the lactate excretion of *S. mutans*, as it functions as a carbon source for yeast growth and lowers the oxygen tension levels preferred by *Streptococci*. Furthermore, it provides growth-stimulating factors for bacteria (Falsetta *et al*., 2014). The catalase produced by *C. albicans* enhances *S. mutans*' tolerance to oxidative stress when *S. mutans* and *C. albicans* interact, ultimately supporting the survival of both within the biofilm (Katrak *et al.*, 2023).

The demineralization capacity is greater when a dual-species biofilm of *S. mutans* and *C. albicans* (Figure 2) forms on tooth enamel than a monospecies biofilm of *S. mutans* alone (Kim et al., 2017). The intensified acid production resulting from the synergistic interactions between both microorganisms accelerates the progression of tooth decay (Eidt *et al.*, 2019).

The presence of *C. albicans* is necessary to promote biofilm maturation and create an acidic environment with *S. mutans* favorable to enamel demineralization (Kim *et al*., 2017). Different levels of cariogenicity amongst *C. albicans* biofilms could potentially be linked to the strain involved, and may also be a consequence of varying growth and experimental conditions from individual studies.

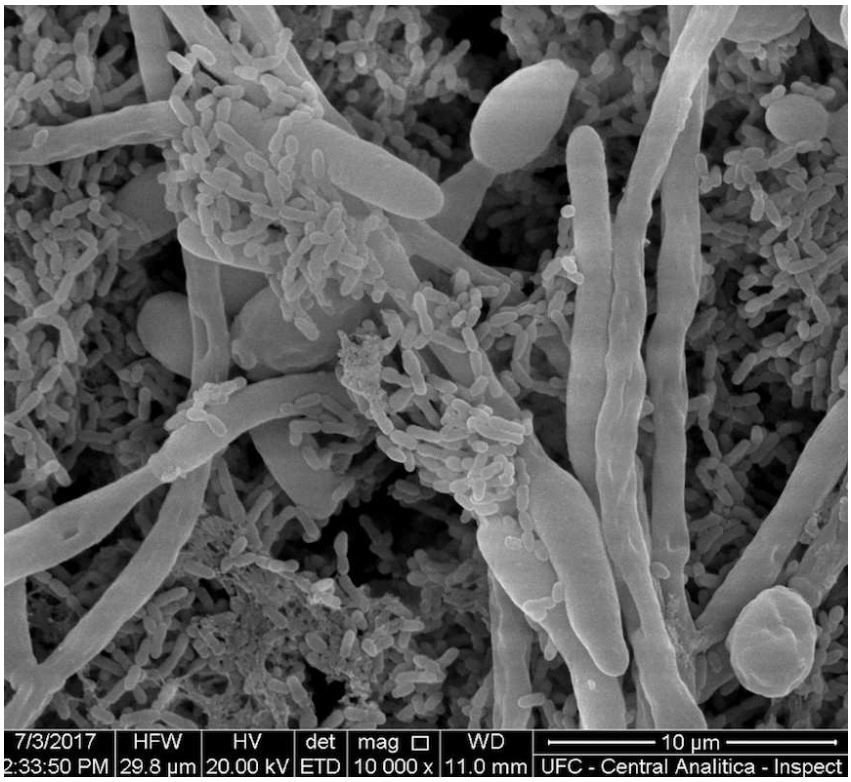

Figure 2. Scanning Electron Micrographs showing an *in vitro* dual-species biofilm of *S. mutans* and *C. albicans* formed on hydroxyapatite slabs after 48h of cultivation in RPMI-1640 medium (source: author).

The presence of *C. albicans* has been found to increase EPS production, resulting in duo-species biofilms accumulating a greater biomass and harboring more *S. mutans* cells than single-species biofilms. (Lee *et al*., 2021). *C. albicans* and *S. mutans* biofilms are more robust than *S. mutans* biofilms without *C. albicans* in enamel and dentin, the cariogenic potential of *S. mutans* and *C. albicans* biofilms increases by the interaction of the *C. albicans* cell wall with the EPS matrix formed by *S. mutans*, increasing tissue demineralization dental (Li *et al*., 2023).

The glucan matrix is a highly researched component of biofilm that offers a surface for microorganisms to adhere to the tooth surface, mechanical stability, and acidic microenvironments (Rainey *et al*., 2019). Glucans, which are polysaccharides that contribute to the formation of a sticky matrix within the biofilm, are produced by both microorganisms. They aid in the attachment of microbial cells to tooth surfaces and facilitate the accumulation and retention of sucrose and other dietary carbohydrates, ultimately leading to the development of dental plaque. (Khoury *et al.*, 2020).

To produce glucan, *S. mutans* captures sucrose and then utilizes enzymes to break it down into two smaller components, glucose and fructose, afterward, glucosyltransferase come into play, transferring glucose groups from one sugar molecule to another resulting in the formation of glucan (Lemos *et al*., 2019)

Furthermore, *C. albicans* produces extracellular matrix polysaccharides, such as α-mannan, one of the primary components of fungal cell walls, these polysaccharides are situated in the outermost layer of the *C. albicans* cell wall (Hwang *et al*., 2017). It is plausible that these biomolecules are involved in binding the Gtf exoenzyme to the fungal cell surface and in stimulating the production of the glucan matrix (Hwang *et al.*, 2017). These contributions play a vital role in the development and stability of biofilms, creating a protective matrix that shields microorganisms from environmental stressors, including antimicrobial agents and host immune responses (Hwang *et al*., 2015) (Figure 3).

The interplay between microorganisms within dental biofilms significantly impacts the development and progression of dental caries. The production of acids via carbohydrate fermentation drives pH reduction within the biofilm, ultimately leading to dental tissue demineralization (Lemos *et al*., 2019). The presence of *C. albicans* in combination with *S. mutans* has been identified as a potential risk factor, intensifying sucrose metabolism and biofilm acidogenicity, resulting in a highly acidic environment conducive to demineralization (Sampaio et al., 2019).

Although *C. albicans* does not efficiently metabolize sucrose due to a lack of gtfs, it can produce short-chain carboxylic acids by metabolizing fructose, glucose, and lactose, which lowers pH and causes demineralization of hard tissue (Klinke *et al*., 2009). The coexistence of these microorganisms in biofilms increases EPS production, resulting in greater biofilm maturation, biomass buildup, and demineralization of dental tissue (Klinke *et al*., 2009). The symbiotic interaction of *C. albicans* and *S. mutans* leads to the creation of a sticky matrix infused with glucans, essential for microbial adhesion and the development of plaque.

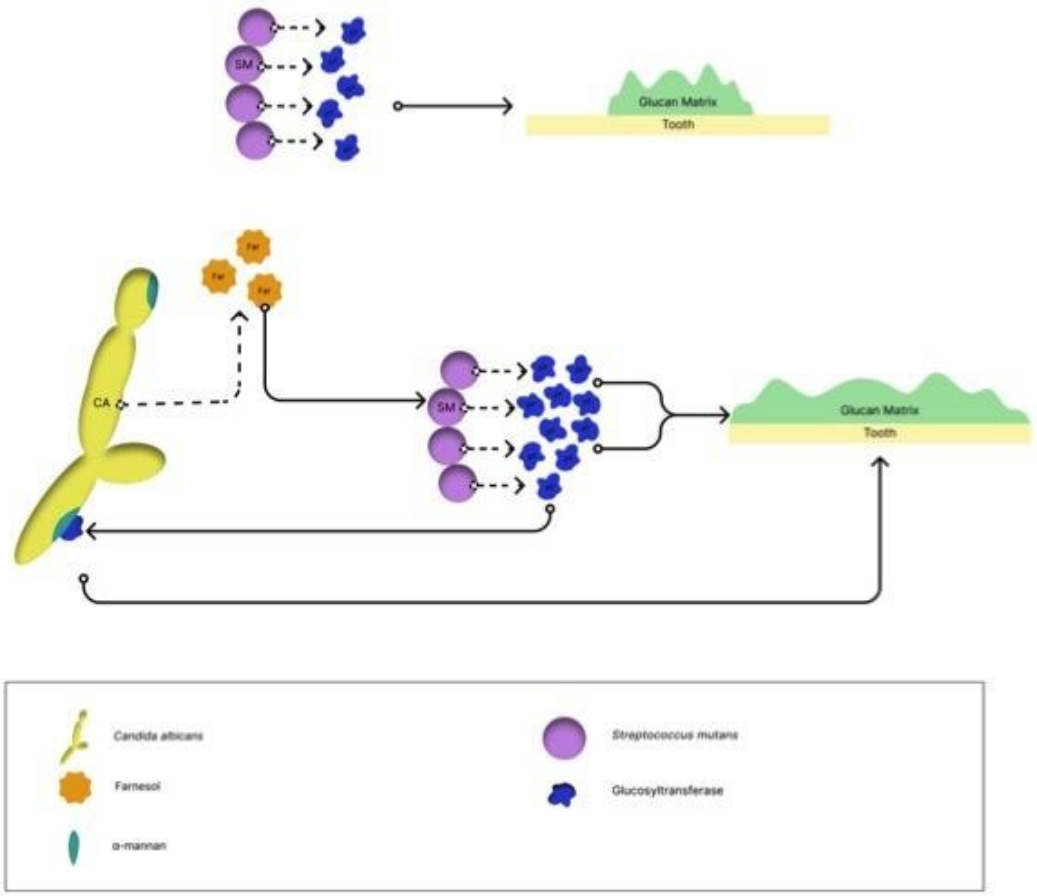

Figure 3. An overview of selected pathogenicity mechanisms in *S. mutans* and *C. albicans* to produce the glucan matrix. With the assistance of its enzymes (gtfs), *S. mutans* produces a glucan matrix, and when in the presence of *C. albicans*, this production is enhanced through the binding between α-mannan and gtfs (source: author).

Overall, these findings underscore the complex dynamics within dental biofilms and their profound impact on the onset and progression of dental caries. The cooperative relationship between *S. mutans* and *C. albicans* underscores their collective role in fostering an acidic environment within biofilms, thus accelerating the demineralization of dental tissues. As research continues to delve deeper into the mechanisms underlying these interactions, insights gleaned will be instrumental in formulating targeted strategies to mitigate the cariogenic potential of these biofilms. Table 1 shows a compilation of information regarding the virulence mechanisms: demineralization, extracellular polysaccharides and biofilm architecture of *S. mutans* and *C. albicans* in single- and dual-species.

Table 1. Compilation of information regarding the virulence mechanisms: demineralization, extracellular polysaccharides, and biofilm architecture of *S. mutans* and *C. albicans* in single and dual-species.

Reference	Strain		Substrate	Demineralization						Extracellular polysaccharides						Biofilm Architecture		
	SM	CA		SM		CA		SM+CA		SM		CA		SM+CA		SM	CA	SM+CA
				Surface	Inner	Surface	Inner	Surface	Inner	Sol	Ins	Sol	Ins	Sol	Ins			
Falsetta *et al.*, 2014	UA159	SC5314	Sprague Dawley Rats	++	NA	+	NA	+++	NA	++	++	+	+	+++	+++	++	+	+++
Barbosa *et al.*, 2016	UA159	ATCC 18804	*In vitro*	NA	NA	NA	NA	NA	NA	++	++	+	+	+++	+++	++	+	+++
Ellepola *et al.*, 2017	UA159	SC5314	96-well flat bottom plates	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	++	+++
Eidt *et al*., 2019	UA159	ATCC 90028	Bovine Enamel	+++	++	+	+	++	++	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sampaio *et al.,* 2019	UA159	ATCC 90028	Bovine Root Dentine	++	NA	+	NA	+++	NA	++	++	+	+	+++	+++	+	+	++
Icono *et al*., 2019	ATCC 25175	ATCC 10231	96-well flatbottom plates	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	++	+	+++
Dos Santos *et al.*,	UA159	ATCC18804	96-well flat	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	++	++	+

2020			bottom plates															
Khoury *et al.*, 2020	UA159	SC5314	Human Enamel	NA	NA	NA	NA	NA	NA	++	++	+	+	+++	+++	++	+	+++
Wu e*t al*., 2020	UA159*	SC5314	Culture plates	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	++
Kim *et al.*, 2021	UA159	SC5314	Hydroxyapatite discs	NA	NA	NA	NA	NA	NA	++	++	+	+	+++	+++	++	+	+++
Kim *et al*., 2021	UA159	SC5314	Human Enamel	++	NA	NA	NA	+++	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Wu *et al.*, 2022	UA159	SC5314	Bovine Coronal Dentine	NA	NA	+	NA	++	NA	NA	NA	NA	NA	NA	NA	NA	+	++
Yin *et al.*, 2022	UA159	SC5314	96-well flatbottom plates	NA	NA	NA	NA	NA	NA	NA	++	NA	NA	NA	+++	++	+	+++
Martorano-Fernandes *et al.*, 2023	UA159	SC5314	96-well flat bottom plates	NA	NA	NA	NA	NA	NA	++	++	+	+	+++	+++	+	+	+++

Virulence mechanisms of *S. mutans* and *C. albicans* in coaggregation that involve the expression of virulence genes

Complete genome sequencing has been carried out for four strains of *S. mutans*: UA159, NN2025, LJ23, and GS-5 (Nomura *et al*., 2014). Analysis of the UA159 complete sequence revealed that it contains proteins anchored with the LPXTG motif (**L**: leucine, **P**: proline, **X**: any amino acid, **T**: threonine, **G**: glycine) (Hu *et al*., 2021). This motif serves as a recognition signal for a specific enzymatic process known as sortase-mediated protein anchoring (Marraffini, Dedent & Schneewind 2006). These proteins play a significant role in the adhesion of *S. mutans* to various surfaces, including tooth enamel, which is crucial for the formation of dental plaque and biofilms, proteins anchored by the LPXTG motif are frequently associated with several processes, such as adhesion to host tissues, biofilm formation, and evasion of the host immune response (Marraffini, Dedent & Schneewind 2006).

Six genes encoding LPXTG-anchored proteins *pac*, *fruA*, *dexA*, *gbpC*, *wapA*, and *wapE* which have all been also identified in strains NN2025, LJ23, and GS-5 (Nomura *et al*., 2014). The major cell surface protein antigens of *S. mutans*, which include three types of glucosyltransferase, 190 kDa protein antigen (PA) and four types of glucan-binding proteins (Gbps), have been investigated in studies regarding its virulence in dental caries (Lemos *et al.*, 2019).

The *gtfB* gene of *S. mutans* encodes for glucosyltransferase B, an enzyme responsible for the synthesis of glucans known as α-glucans, which form the core of the extracellular matrix in cariogenic biofilms (Bowen *et al*., 2018). There are three main types of Gtfs produced by *S. mutans*: GtfB, GtfC, and GtfD. Each Gtf has a specific function in glucan synthesis. GtfB synthesizes water-insoluble glucans, while GtfC synthesizes both water-insoluble and water-soluble glucans. GtfD, on the other hand, produces water-soluble glucans (Koo *et al.*, 2010). These glucans provide a scaffold for bacterial attachment and help trap other bacterial species, contributing to the structural integrity of the biofilm (Koo *et al*., 2010).

The glucan matrix created by the activity of Gtfs facilitates bacterial accumulation and adhesion to the tooth enamel, enhancing the formation of biofilms (Zhang *et al*., 2021). Furthermore, the accumulation of dietary sucrose by the biofilm community promotes the synthesis of glucans through *gtfs*, leading to the further accumulation of bacteria and the production of acid as a byproduct (Klein *et al*., 2015). This acid production initiates the

demineralization of dental hard tissues, eventually resulting in the development of dental caries (Klein *et al*., 2015). This gene is capable of binding to the cell surface of *C. albicans* and producing large quantities of extracellular α-glucans on the fungal surface (Hwang *et al.*, 2010).

The GtfB protein of *S. mutans* has the capability to attach to the α-mannan located on the cellular surface of *C. albicans*, leading to the facilitation of coadhesion and the collaborative formation of symbiotic biofilms (Hwang *et al*., 2017). The interaction between *S. mutans* and *C. albicans* reveals that farnesol, a *C. albicans* QS molecule, can modulate the membrane permeability for protons and attenuate the glycolytic metabolism (ability to metabolize sugars, particularly glucose, to produce energy) in *S. mutans* resulting in decreased fitness of *S. mutans* in mixed biofilms (Černáková, Jordao & Bujdáková 2018). It is interesting to report that farnesol, induces *gtfB* gene expression from *S. mutans* that can interact with *C. albicans* and synthesize glucans *in situ*, contributing to biofilm EPS matrix development, which provide colonization sites for bacteria and growth factors for *C. albicans* (Xiang *et al.*, 2023).

GtfB increases the expression of the *hwp1, als1* and *als3* genes in *C. albicans,* these results suggest that GtfB derived from *S. mutans* may contribute to the increased accumulation of *C. albicans* in biofilms of species mixed with *S. mutans* (Ellepola *et al*., 2017). Although GtfB can bind to both *S. mutans* and *C. albicans*, its binding strength to *C. albicans* is notably greater (Ellepola *et al*., 2017).

The *als1* and *als3* genes in *Candida albicans* play a significant role in adhesion and biofilm formation (Martorano-Fernandes *et al*., 2023). These genes encode cell surface glycoproteins known as "agglutinin-like sequence" proteins. These proteins are involved in binding to host cells and extracellular matrix components, facilitating adhesion and colonization (Martorano-Fernandes *et al.*, 2023). The relationship between the *als1* and *als3* genes in *C. albicans* and *S. mutans* involves the potential modulation of als gene expression by *S. mutans*-derived factors, which could impact adhesion, biofilm formation, and the overall interactions between these two microorganisms (Martorano-Fernandes *et al.*, 2023).

Furthermore, at high concentrations, farnesol increases the sensitivity of *S. mutans* genes to oxygen and reduces the expression of genes related to bacterial virulence, including *luxS*, *brpA*, *ffh*, *recA*, *nth*, *smx*, *comC*, *comYB* (Lu *et al*., 2023). The *vicR* and *vicK* genes are part of the *VicRK* two-component regulatory system and play a crucial role

in stress responses and the regulation of biofilm formation in *S. mutans* (Lei *et al*., 2019). *C. albicans* appears to have the ability to upregulate the expression of these genes, leading to increased biosynthesis of EPS, enhanced biofilm formation, and subsequently, heightened cariogenicity (Liu *et al*., 2022).

The *hwp1* gene (Hyphal Wall Protein 1) represents a significant genetic element present in *C. albicans*, it encodes the Hwp1 protein, which assumes a pivotal role in virulence and pathogenicity. The functionality of the Hwp1 protein spans various phases of *C. albicans* life cycle, encompassing the attachment to host cells, the establishment of biofilms, and propagation within the host organism. It holds a critical position in the adhesion of the fungus to epithelial cells, thereby facilitating colonization and subsequent tissue invasion (Nobile *et al*., 2006).

The *hwp1* virulence gene in *C. albicans*, along with the *gtfB* virulence gene in *S. mutans*, contributes substantially to biofilm formation and the coaggregation between these two microorganisms (Martorano-Fernandes et al., 2023). The GtfB protein in *S. mutans* is accountable for glucan production, which is intricately associated with cell adhesion and aggregation. Research indicates that the interaction between *hwp1* and *gtfB* can potentiate the coaggregation of these microorganisms, thereby exerting an influence on the establishment of mixed biofilms (Li *et al*., 2023).

The interaction between bacteria and fungus present in biofilms can occur through extracellular signaling molecules by sending signaling molecules or through intercellular physical interactions that support the formation and development of cariogenic biofilms that contribute to the progression of caries lesions (Ellepola *et al*., 2017). During interactions between *C. albicans* and mitis-group streptococci, the bacteria adhere to hyphal-specific adhesins from *C. albicans* via polypeptides of the I/II antigen family SspA/B, however, the role of hyphal formation in interactions between kingdoms with cariogenic species remains unclear, it is supposed that the production of proteases such as SspA can possibly contribute to the severity of caries, as these enzymes are capable of degrading collagen (Xiang *et al*., 2023).

The interaction between these microorganisms is further complicated by the production of extracellular signaling molecules and intercellular physical interactions, which contribute to the formation and progression of cariogenic biofilms. Although many aspects of these interactions have been unraveled, further research is warranted to uncover the intricacies of their interplay and its wider impact on dental caries. Table 2 shows the

upregulation and downregulation of select virulence genes expressed by *S. mutans* and *C. albicans*, along with their behavior within the context of the dual-species.

Table 2. Illustrates the modulation of specific virulence genes upregulated or downregulated by *S. mutans* and *C. albicans* accompanied by their interactions.

Reference	Strain		Molecule	Gene of virulence			
	SM	CA		Upregulation		Downregulation	
				SM	SM	SM	SM
Feldman *et al*., 2016	UA159	SC5314	Thiazolidinedione-8	*gtfB , gtfC , gtfD, gbpB, brpA, luxS, nox, sodA*	NA	NA	*hwp1, csh1, als3, sod1, sod2, cat1,*
Tao *et al.*, 2017	UA159	SC5314	Mutanocyclin	NA	NA	NA	*hwp1, ece1, flo8 tec1; ihd1 , hyrt, rbt5, rhd3; csa1; rbe1; als; cht2, cht3, chs1.*
Ellepola *et al.*, 2017	UA159	SC5314	*gtfB*	NA	*hwp1, als1, als3*	NA	NA
Bachtiar & Bachtiar, 2018	UA159	SC5314	NA	*gtfB*	NA	NA	NA

Li *et al*., 2019	UA159	SC5314	Curcumin	NA	NA	*gtfB, gtfC, gbpB, comC, comD, comE*	*als1, als3*
Dos Santos *et al.*, 2020	UA159	ATCC18804	Supernatant of *S. mutans*	NA	*ywp1*	NA	*cph1, efg1 , hwp1*
Guo *et al*., 2021	UA159	SC5314	eDNA	*gtfC*	*spaP, eap1, hwp1, cdr2*	NA	*als3*
Yin *et al.,* 2022	UA159	SC5314	Caffeic acid phenethyl ester	NA	NA	*gtfB , gtfC, gtfD*	NA
Lu *et al.,* 2022	UA159	SC5314	NA	*dcr1*	*ndt80 , efg1, brg1*	NA	NA
Bao *et al*., 2023	UA159	SC5314	*Lactobacillus plantarum*	*atpD, eno*	*cht2*	*lacC, lacG*	*hwp1, ece*
Huang *et al.*, 2023	UA159	SC5314	*Galacto-oligosaccharide*	*atpD , eno , lacC, lacG*	NA	NA	*hwp1, ece*

Discussion

The relationship between bacteria and fungi existing in biofilms may happen via extracellular signaling molecules or intercellular physical interactions that promote the establishment and growth of cariogenic biofilms that advance the development of caries lesions (Ellepola *et al.*, 2017).

The co-aggregation of *S. mutans* and *C. albicans* can significantly augment the formation and stability of cariogenic biofilms, thereby leading to heightened acid production and caries progression. The frequent consumption of sugar further contributes to increased acid production by these microorganisms, furthermore, *C. albicans* secretes enzymes that degrade dentin collagen under acidic conditions, further increasing the virulence and cariogenicity of the biofilm (Li *et al.*, 2023).

The production of acids resulting from carbohydrate fermentation by specific microorganisms plays a critical role in the development of dental caries, as it leads to pH reduction within biofilms (Matsui & Cvitkovitch 2010). This acidification directly correlates with the dissolution of dental tissues when the balance between demineralization and remineralization processes is disrupted over an extended period (Eidt *et al*., 2019). Consequently, frequent sugar consumption leads to a dysbiotic shift in dental plaque, where acid-tolerant microorganisms outcompete less acid-tolerant ones, leading to biofilm acidification. Evidence in the literature suggests that biofilms formed by *S. mutans* and *C. albicans* possess a higher cariogenic potential compared to biofilms composed of a single species.

In the presence of *S. mutans, C. albicans* enhances biofilm acidogenicity, creating an environment with a significantly low pH (Kim *et al*., 2017). This fungus releases calcium ions (Ca2+) from hydroxyapatite under acidic conditions, causing a significant drop in pH from 7.0 to 3.0 during a 24-hour incubation period (Lemos *et al*., 2019). The presence of both microorganisms intensifies acid production through synergistic interactions, thereby accelerating tooth decay (Falsetta *et al.*, 2014; Kim *et al*., 2017; Eidt *et al*., 2019). *C. albicans* plays a crucial role in enhancing biofilm maturity and maintaining an acidic environment favorable to enamel demineralization in the presence of *S. mutans* (Kim *et al*., 2017).

The level of cariogenicity exhibited by *C. albicans* biofilms may vary depending on the strain and the specific biofilm growth and experimental conditions employed in

different studies. While some studies report that the presence of *C. albicans* promotes EPS production and enamel demineralization (Falsetta *et al*., 2014; Kim *et al*., 2017), others suggest that the cariogenic potential of *C. albicans* on enamel is relatively low (Tao *et al*.,2022). This discrepancy can be attributed to the competition for fermentable sugars and antagonistic interactions between *C. albicans* and *S. mutans* within the mixed biofilm environment (Willems *et al*., 2016; Eidt *et al*., 2019).

Regarding EPS production, the interaction between *S. mutans* and *C. albicans* results in an increased accumulation of biomass and *S. mutans* cells within the biofilm. Glucans and α-mannans are among the EPS components produced by these microorganisms, α-mannan and β-1,3-Glucan from *C. albicans* stimulate *S. mutans* adherence and biofilm formation (Eidt *et al*., 2019; Khoury *et al*., 2020).

Mannans play a critical role in the adhesion of cells and the formation of biofilms, interestingly, GtfB demonstrates a pronounced binding affinity for purified α-mannan, showcasing significantly heightened binding strength in comparison to β-glucan. This interaction shows that the α-mannan constituents present on the surface of *C. albicans* potentially orchestrate a resilient connection between GtfB and the fungal cells. This mutual interaction provides a robust foundation, thereby enabling GtfB to yield EPS, thereby contributing substantively to the evolution of biofilms involving mixed species, namely *S. mutans* and *C. albicans* (Hwang *et al*., 2015).

Farnesol induces the expression of the *gtfB* gene in *S. mutans*, which synthesizes glucans and contributes to the development of the biofilm EPS matrix (Kim *et al.*, 2017). These interactions between *S. mutans* and *C. albicans* play a role in biofilm formation, providing colonization sites for bacteria and growth factors for *C. albicans* (Ellepola et al., 2017).

The extensive exploration of *S. mutans* and *C. albicans* interactions in the context of dental caries reveals their intricate relationship and its implications for biofilm development and caries progression. The presence of genes encoding LPXTG-anchored proteins in *S. mutans*, particularly GtfB, significantly influences biofilm formation, cohesion, and symbiotic interactions with *C. albicans*. This intricate interplay highlights the influence of various factors, including the upregulation of *als1* and *als3* genes in *C. albicans* driven by *S. mutans*-derived *gtfB* (Matsumoto-Nakano 2018). Moreover, the crucial role of the *hwp1* gene in *C. albicans* is evident, impacting the organism's virulence and pathogenicity through its multifaceted involvement in various phases of its lifecycle.

As a two-component system, VicRK is a common signaling mechanism found in many bacteria and fungi, allowing these microorganisms to detect and respond to various environmental changes and stresses (Lemos *et al*., 2013), *vicR* and *vicK* genes play a vital role in stress responses and regulation of biofilm formation in *S. mutans* (Lei *et al*., 2019).

The VicRK system in *C. albicans* senses the surrounding environment and triggers the expression of genes involved in adhesion, hyphal formation and extracellular matrix production (Liu *et al*., 2022). Hyphal formation is particularly important for biofilm development, as it allows *C. albicans* to form elongated filaments that intertwine with other cells, strengthening the biofilm structure and increasing its adherence to surfaces (Tao *et al.*, 2022). Overall, the VicRK system in *S. mutans* and *C. albicans* acts as a critical regulatory network that adjusts gene expression in response to environmental signals, ensuring proper coordination of biofilm formation.

In the case of *C. albicans* and mitis-group streptococci, bacteria adhere to *C. albicans*' hyphal-specific adhesins via polypeptides of the I/II antigen family SspA/B. Hyphal formation plays a crucial role in exacerbating the severity of mucosal infections. However, its role in interactions between different kingdoms, particularly with cariogenic species, remains uncertain. Nevertheless, it is known that the production of proteases, like SspA, can contribute to the severity of caries (Xiang *et al*., 2023).

The *als1* and *als3* genes in *C. albicans* are involved in adhesion and biofilm formation (Martorano-Fernandes *et al.*, 2023). These genes are potentially modulated by *S. mutans* factors, affecting interactions between the two. Farnesol sensitizes *S. mutans* genes to oxygen, reducing virulence-related gene expression. The *vicR* and *vicK* genes regulate biofilm formation in *S. mutans. C. albican*s upregulates these genes, increasing EPS biosynthesis, biofilm formation, and cariogenicity (Deng *et al*., 2021). The *hwp1* gene in *C. albicans* contributes to virulence and biofilm formation, and its interaction with *gtfB* amplifies coaggregation.

Interactions between *S. mutans* and *C. albicans* can occur via signaling molecules or physical interactions, influencing cariogenic biofilm progression. The roles of hyphal formation and proteases in these interactions are still unclear. The complexity of their interplay affects biofilm development and caries progression. Genes encoding LPXTG-anchored proteins, especially *gtfB*, play a significant role in this complex relationship. The upregulation of *als1 a*nd *als3* genes by *S. mutans*-derived *gtfB*, as well as the hwp1 gene's multifaceted impact in *C. albicans*, further complicate the interactions.

Additional investigation is required to comprehensively understand the specific mechanisms through which *C. albicans* contributes to caries and to explore potential therapeutic interventions aimed at addressing this fungal species.

Concluding remarks and Perspective

- The main targets for combating cross-kingdom biofilms would be Gtfs and farnesol, which play a significant role in the interaction between *S. mutans* and *C. albicans.*
- Disrupting their activity to inhibit EPS production could impair co-adhesion between the two microorganisms, presenting an attractive approach to preventing tooth decay.
- Targeting metabolic pathway regulators like farnesol could potentially interfere with cell-to-cell metabolic interactions and contribute to the treatment of dual-species biofilms.

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3.2 CAPÍTULO 2

Archives of Oral Biology

Title: *Streptococcus mutans* serotyping, collagen-binding genes and *Candida albicans* in dentin carious lesions: a molecular approach.

Short title: *S. mutans and C. albicans in dentinal caries lesions*

Highlights

- Dentin carious lesions contain different *S. mutans* serotypes.
- No *C. albicans* was found in carious dentin.
- Cbm positive strains were found in deep dentin carious lesions.

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ABSTRACT

Objectives: This study aimed to evaluate the occurrence of *Streptococcus mutans* and *Candida albicans* in medium and deep dentin caries lesions in Brazilian subjects. Serotyping of *S. mutans* (*c*, *e*, *f*, and *k*) and identification of the collagen-binding genes (*cnm/cbm*) were the secondary objectives.

Design: Dentin carious lesions were classified as RB4 (middle one-third; n=23) and RC5 (inner one-third; n=24) according to the ICDAS/ICCMS™ radiographic scoring system. Samples were collected and analyzed by PCR.

Results: *Streptococcus* spp. were present in 95.74% of samples, including 37.78% *S. mutans.* One serotype was identified in 3 samples for *c* (17.64%), 9 for *e* (52.94%) and 2 for *f* and 2 for *k* (11.7%). Undetermined serotypes were found in 2 samples. (11.76%) and in 2 others the serotypes were associated (*c*+*f*+*k* and *f*+*k*; 5.88%). In RB4, *Streptococcus* spp. was found in 21 samples (91.30%), of which *S. mutans* was present in 9 (39.13%), of which serotypes *c*, *f* or *k* were not identified, but serotype *e* was present in 9 (100%). In RC5, *Streptococcus* spp. was present in all samples, 8 were *S. mutans*-positive (33%), with the following serotypes alone or combined: *c* in 4 (50%), *e* in one (12.5%) and *f* and *k* in two (25%). The *cbm* gene was found in 37.5% (3) of RC5 samples. *Candida albicans* and *cnm* gene were not found. Serotype *c* was more frequent in RC5 ($p=0.029$) and serotype *e* in RB4 ($p<0.001$).

Conclusions: *S. mutans* serotype *e* may play an important role in medium dentinal lesions.

Keywords: Brazilian people, Dental Caries Susceptibility, *Streptococcus*, Dental Caries, Polymerase Chain Reaction

1. Introduction

Dental caries is a progressive pathology caused by a disturbed biofilm, which is linked to the recurrent intake of sugars and fermentable carbs, leading to the propagation of cariogenic bacteria (Selwitz, Ismail & Pitts 2007; Lamont, Koo & Hajishengallis 2018).

The production of acid by fermentable carbohydrates' metabolism by microorganisms in the dental biofilm and the consequent decrease in pH are the primary culprits for dental demineralization (Takahashi & Nyvad 2008). These continuous pH fluctuations are often balanced over time by compensatory remineralization mechanisms that prevent the onset of dental cavities (Takahashi & Nyvad 2008; Nyvad & Takahashi 2020). However, when high-frequency sugar consumption is established, acid production increases, disrupting the equilibrium between demineralization and remineralization mechanisms (Díaz-Garrido, Lozano & Giacaman 2016).

Streptococcus mutans is extensively studied as a cariogenic microorganism due to its ability to produce acid from carbohydrate fermentation, survive and metabolize under low pH conditions (Lemos *et al.*, 2019). Technical terms are consistently used throughout this objective and concise paragraph, and a logical connection between statements is established. Furthermore, *S. mutans* can produce extracellular polysaccharides from dietary sucrose, including proteins like glucan-binding protein that binds to the cell surface of the extracellular glucan matrix (Bowen & Koo 2011).

S. mutans are classified into four serotypes (*c*, *e*, *f*, and *k*), which differ in the chemical composition of their exopolysaccharides, known as rhamnose-glucose polymers (Lemos *et al*., 2019). The fundamental difference lies in the presence and arrangement of sugar side chains (glucose and rhamnose polymers) in the cell wall (outermost surface-exposed layer) (Kovacs *et al.,* 2019), which are composed of rhamnose polymers with α-1,2 and α-1,3 bonds and side chains of glycosidic residues connected by α-1,2 bonds (serotype *c*), β-1,2 bonds (serotype *e*), and α-1,3 bonds (serotype *f*). Serotype *k,* on the other hand, lacks these glucose side chains in the rhamnose backbone (Nakano *et al*., 2004).

The pan-genome refers to the complete set of genes within a certain species, consisting of both essential (core genome) and non-essential genes (non-core) that are shared by all strains (Tettelin *et al.*, 2008). The pan-genome of *S. mutans* comprises roughly 3,296 genes (Cornejo *et al.*, 2013). Among the non-core genes, notable examples include *cnm* and *cbm*, which encode the collagen-binding proteins Cnm and Cbm, respectively (Otsugu *et al*.,

2022). The Cnm (collagen-binding cell surface protein), initially recognized as a cell wall component and later acknowledged as a significant virulence factor (Sato *et al*., 2004; Momeni *et al.,* 2019; Lima *et al.*, 2021), harbors a conserved collagen-binding domain (Domain A) (Nomura *et al.*, 2020).

S. mutans cnm-positive strains have the ability to bind to the extracellular matrix and also present a sucrose-independent adhesion mechanism in the formation of biofilms, which affects the adhesion of *S. mutans* in oral and extraoral tissues (Naka *et al*., 2022; Misaki *et al*., 2023). Furthermore, Cnm-positive strains of *S. mutans* have been detected in 10-20% of healthy individuals (Nomura *et al.*, 2020). Another collagen-binding protein (CBP) found in *S. mutans i*s the Cbm protein. It is more abundant in *S. mutans* of serotype *k* (Nomura *et al*., 2012; Lapirattanakul *et al.*, 2013). The structure of Cbm is similar to collagen-binding domain A of Cnm (Sato *et al*., 2004). Additionally, the length of the *cbm* gene varies based on the number of repeats, similar to the cnm gene (Nomura *et al.*, 2012).

In addition to *S. mutans*, other microorganisms that break down collagen may also play a role in the development of carious lesions. One such organism is *Candida albicans,* a fungus that typically affects the whole body and is often detected in tandem with *S. mutans* in carious lesions in children (Portela *et al*., 2012; Metwalli *et al*., 2013; Xiang *et al*., 2023). Co-infection with both species in a rat caries model resulted in higher biofilm formation and tooth decay, as compared to the presence of only one species (Ghasempour *et al*., 2011). Moreover, it has been observed that *S. mutans* GTFs could adhere to *C. albicans* polysaccharides, thereby enhancing extracellular polymeric substances (EPS) production and the inclusion of the fungus into the biofilm (Patel, 2022).

This is supported by its capacity to generate carboxylic acids by means of carbohydrate metabolism, by adhering to dental surfaces and exposed dentin, and by demonstrating its aciduric and acidogenic capabilities (Falsetta *et al*., 2014). Additionally, *C. albicans* can secrete proteases, which are proteolytic enzymes that occupy a critical role in breaking down diverse host proteins, specifically type I collagen (Nakano & Kuramitsu 2006). Thus, under conditions conducive to the adhesion, colonization, and proliferation of this microorganism, caries development is accelerated (Oho *et al.*, 2000).

Co-adhesion between *C. albicans* and *S. mutans* is critical for their colonization and persistence in biofilms. *S. mutans* excretes lactate, which can serve as a carbon source for yeast growth, while *C. albicans* reduces oxygen tension and provides growth-stimulating factors for bacteria (Oho *et al*., 2000). In this context, it can be hypothesized that the

association between *S. mutans* and *C. albicans* is mainly prevalent in dentin caries, considering that the frequencies of *cnm* and *cbm* genes in oral strains are more frequently identified in the less common serotypes *e*, *f,* and *k*. The aim of the present study was to evaluate the occurrence of *C. albicans, Streptococcus* spp., serotypes (*c*, *e*, *f* and *k*) and *cnm/cbm* genes of *S. mutans* in carious dentin of permanent teeth using polymerase chain reaction (PCR) analysis.

2. Materials & methods

2.1. Ethics statement and population

This cross-sectional study used DNA extracted from previously collected human dentin carious samples. The research protocol was approved by the Ethics Committee of the Federal University of Ceará (No. 1.610.785). The use of stored and frozen samples was approved by the Research Ethics Committee of the Federal University of Ceará (No. 4.790.272).

The study population consisted of thirty-two patients from a convenience sample, aged 14-36 years, from the undergraduate dental program of the Faculty of Pharmacy, Dentistry and Nursing of the Federal University of Ceará in Fortaleza, residents of the city of Fortaleza, Ceará, Brazil. They were diagnosed with dentin carious lesions according to the ICDAS/ICCMS™ radiographic scoring system (Fakhruddin *et al*., 2021) and selected according to the inclusion criteria: teeth with pulpal health as assessed by clinical and radiographic examinations, asymptomatic or with signs and symptoms of reversible pulpitis. Sampling was performed on premolars and molars with ICDAS scores of moderate stages RB4 (radiolucency extending to the middle ⅓ of the dentin) and extensive stages RC5 (radiolucency extending to the inner ⅓ of the dentin, clinically cavitated or not).

2.2. Sample selection

Patients who met the inclusion criteria underwent the following procedures: periapical radiography, prophylaxis, local anesthesia, isolation of the surgical field with a rubber dam, and dentin carious tissues were collected using sterilized and DNAse-free spoon excavators (S.S. White Duflex, Rio de Janeiro, RJ, Brazil). The samples were immediately transferred to sterile DNAse-free microtubes (Axygen, Union City, California, USA) containing RNA stabilization solution (RNAlater® Sigma-Aldrich, Missouri, USA) and

stored at 4 °C for 18 h as recommended by the manufacturer. After this period, the tubes were stored at - 80 °C until genomic DNA extraction.

2.3 DNA extraction

Bacterial/fungal DNA was obtained by centrifugation of each sample in the microtube using InstaGene™ purification matrix (Bio-Rad 732-6030; Hercules, CA, USA). Briefly, dentin samples were transferred to a 1.5 mL microcentrifuge tube containing 1 mL of nuclease-free ultrapure water (Invitrogen™ Life Technologies, Carlsbad, CA, USA), vortexed, and centrifuged at 12,000 rpm for 1 min (Eppendorf Microcentrifuge, Model 5415R, Hamburg, Germany). After the centrifugation step, the supernatant was discarded, 200 µL of InstaGene matrix was added to the precipitate and the sample was incubated in a dry bath at 56 °C for 15-30 min (Loccus Biotechnology, São Paulo, SP, Brazil). The sample was then vortexed for 10 s and incubated again, this time at 100 °C for 8 min. Finally, the sample was vortexed at high speed for 10 s, centrifuged at 12,000 rpm (Eppendorf microcentrifuge, model 5415R, Hamburg, Germany) for 3 min (Eppendorf microcentrifuge, model 5415R, Hamburg, Germany) and the supernatant containing DNA was transferred to a new sterile microcentrifuge tube, resulting in a final elution volume of 160 µL. Total DNA concentration was determined by spectrophotometry at 260 nm using a UV-vis microvolume spectrophotometer (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA). DNA was spiked (20 ng/µL) and stored at -20°C for PCR analyses.

2.4 PCR analysis

A list of all primers used in this study can be found in Table 1. PCR reactions were performed in a thermocycler (CFX96™, Bio-Rad, Hercules, CA) using the Platinum ® PCR SuperMix kit with 2 µL of DNA and amplification of dentin samples was performed for each primer (Table 1).

Purpose	Primer	Sequence (5'-3')	Amplicon	Reference

			size (bp)	
Streptococcus spp.	*16S-F*	5'-TCCTACGGGAGGCAGCAGT-3'	466	Nadkarni *et al.*, 2002
	16S-R	5'-GGACTACCAGGGTATCTAATCCTGTT-3'		
S. mutans	*gtfB-F*	5'-ACTACACTTTCGGGTGGCTTGG-3'	517	Oho *et al.*, 2000
	gtfB-R	5′-CAGTATAAGCGCCAGTTTCATC-3		
Serotype *c*	*SC-F*	5'-CGGAGTGCTTTTTACAAGTGCTGG-3'	727	Shibata *et al.*, 2003
	SC-R	5'-CGGAGTGCTTTTTACAAGTGCTGG-3'		
Serotype *e*	*SE-F*	5'-CCCACAATTGGCTTCAAGAGGAGA-3'	517	Shibata *et al*., 2003
	SE-R	5'-TGCGAAACCATAAGCATAGCGAGG-3'		
Serotype *f*	*SF-F*	5'-CCC ACA ATT GGC TTC AAG AGG AGA -3'	316	Shibata *et al.,* 2003
	SF-R	5'-TGC GAA ACC ATA AGC ATA GCG AGG -3'		
Serotype *k*	*SK-F*	5'- ATTCCCGCCGTTGGACCATTCC-3'	294	Nomura et al., 2004
	SK-R	5'-CCAATGTGATTCATCCCATCAC-3'		
cnm	*cnm-F*	5’-TGGAGGTTCAGGGCAAGTATGTTGGTGATT-3'	579	Nomura *et al*., 2004
	cnm-R	5’-TGGAGGTTCAGGGCAAGTATGTTGGTGATT-3’		
cbm	*cbm-F*	5’-AGCTGAAGTTAGTGTTGTAAAACCTGCTTC-3'	393	Nomura *et al*., 2004
	cbm-R	5’-TAGGATCATCAACCTTAGTCAAGTACACGA3’		
Candida albicans	*calb-F*	5'-ATAAGGGAAGTCGG-CAAAATAGATCCGTAA-3́’	583	Fakhruddin *et al*., 2021
	calb-R	5’-CCTTGGCTGTGGTTTCGCTAG ATAGTAGAT-3'		
Candida albicans	*pac-F*	5’-GCTACCACTTCAGAATCATCATC-3'	950	Arastehfar *et al*., 2018
	pac-R	5’-AGATCAAGAATGCAGCAATACCAA-3'		

Table 1. Summary of primers used in this study.

For *Streptococcus* spp identification, thermal cycling conditions were 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min., with a final single extension of 72°C for 5 min, and then held at 4 °C (Nadkarni *et al*., 2002). For *S. mutans* identification, the following cycling parameters were used: 95 ºC for 4 min followed by 30 cycles of 95 ºC for 1 min, 55 ºC for 1 min and 72 ºC or 1 min with a final extension at 72 ºC for 10 min (Krzyściak *et al*., 2017). DNA samples from *S. mutans* UA159 (serotype *c*), NN2002 (serotype *e*), OMZ-175 (serotype *f*), or YT1 (serotype *k*) served as positive controls for the reaction. Nuclease-free water was employed as a substitution for DNA to serve as a negative contamination control. The samples underwent the following cycling parameters: 95°C for 4 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 7 min (NAKANO *et al*., 2004; MOMENI *et al*., 2019).

In addition, samples containing *S. mutans* DNA were analyzed for the detection of *cnm* and *cbm* by multiplex PCR using the same cycling parameters as for serotyping (Nomura *et al.*, 2012, Momeni *et al*., 2019). DNA samples of *S. mutan*s OMZ-175 (*cnm*+) and YT1 (*cbm*+) were used as positive controls.

For the identification of *C. albicans* (ATCC 10321) with the primer *calb*, the following reaction was used: initial denaturation at 93°C for 5 min, followed by 40 cycles of denaturation at 93°C for 30 sec; primer annealing at 55°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min (Fakhruddin *et al*., 2021). For primer *pac*, the reaction was performed by pre-denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and a final extension at 72°C for 8 min (Arastehfar *et al*., 2018).

At the end of the cycles, the samples were subjected to electrophoresis on a 1.5% agarose-tris-acetate-EDTA gel. A 100 bp DNA ladder was used as a molecular size standard. DNA bands were stained with SYBR Safe (Invitrogen™ Life Technologies, Carlsbad, CA, USA), added to the gel during the preparation phase and visualized under ultraviolet light (320 nm) on an L-PIX EX transilluminator (Loccus Biotechnology, São Paulo, SP, Brazil).

2.5 Statistical analysis

The data was tabulated in Microsoft Excel Microsoft Excel and exported to statistical software Statistical Package for Social Sciences (SPSS) version 27.0 (IBM Corp., Release 2016, IBM SPSS Statistics for Mac, Version 27.0 Armonk, NY: IBM Corp). The chi-squared test and, when this was not possible, Fisher's exact test was used for comparisons between the

presence and absence of the primer under investigation. In addition, for these comparisons, bivariate logistic regression was used to check the odds ratio and its 95% confidence interval.

3. Results

A total of 32 patients were initially enrolled in this study and 47 dentin carious samples were collected. Of these 47 samples analyzed, 95.7% (45/47) were positive for *Streptococcus* spp. and of these, 36.1% (17/45) were positive for *S. mutans*. Among the samples positive for *S. mutans*, single serotypes were present in 23.53% (4/17) for *c*, in 58.8% (10/17) for *e* and in 11.7% (2/17) for serotypes *f* and *k*. In addition, 11.7% of the samples (2/17) showed *S. mutans* with an undetermined serotype. Serotypes were also found in the same sample. Serotypes *c*, *e* and *k* were found in 5.8% (1/17) and *f* and *k* in 5.8% (1/17) of the samples analyzed (Figure 1).

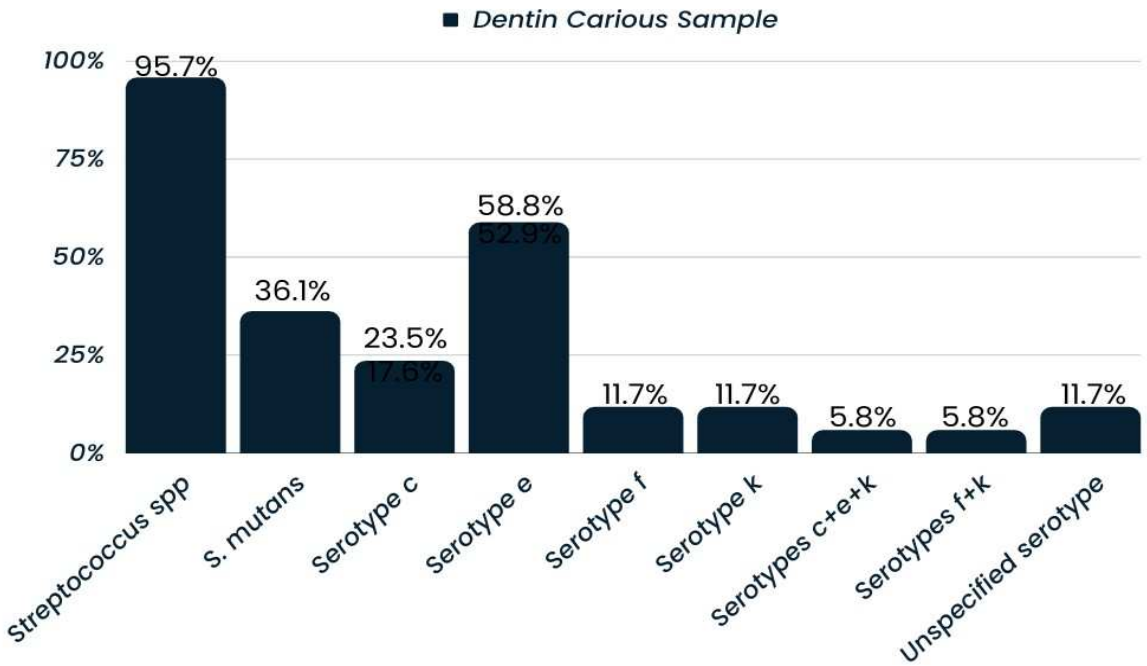

Figure 1. Occurrence of microorganisms found in samples of human carious dentin.

In RB4, *Streptococcus* spp. was present in 91.3% (21/23), while 39.1% (9/23) were *S. mutans*, all with serotype *e*. Serotypes *c*, *f*, *k* and the *cbm* gene were not identified in this type of lesion. In RC5 lesions, *Streptococcus* spp. was present in 100% (24/24), but *S. mutans* in only 33.3% (8/24), serotype *c* in 50% (4/8), serotype *e* in 12.5% (1/8) and serotypes *f* and *k* in 25% (2/8) (Figure 2).

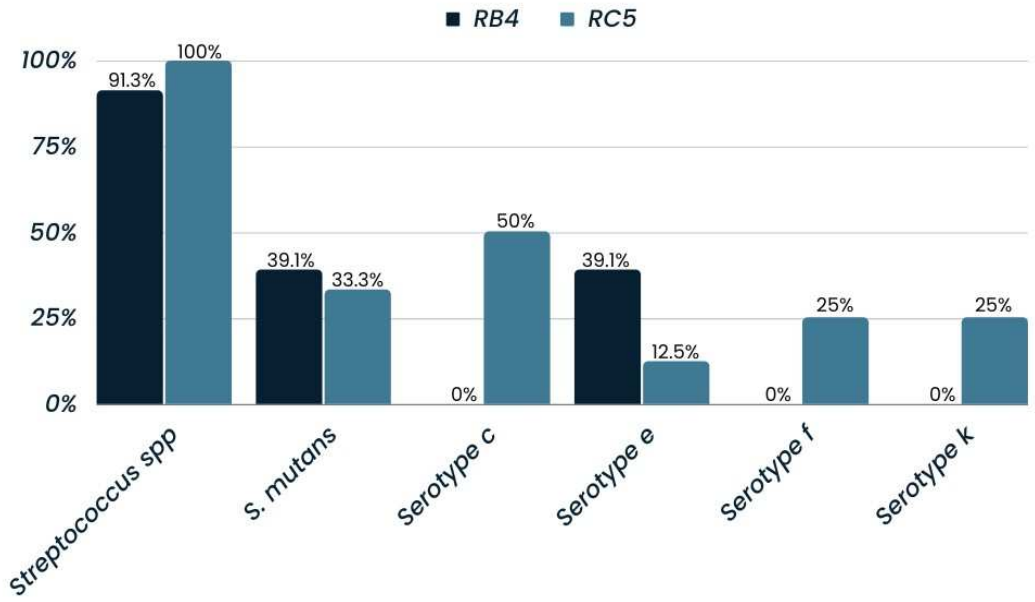

Figure 2. Occurrence of microorganisms found in samples of human carious dentin classified by depth (RB4: radiolucency extending to the middle ⅓ of the dentin and RC5: radiolucency extending to the inner ⅓ of the dentin, clinically cavitated or not)

The *cbm* gene was not identified in RB4 lesions. In RC5, *cbm* gene was found in 37.5% (3/8), with combined serotypes (*c*+*f*+*k* and *f*+*k*) or serotype *f*. The presence of serotype *c* was significantly more constant (p=0.029) in deeper lesions (RC5), whereas serotype e was more frequent in medium depth lesions (RB4) (p<0.001). The *cnm* and *C. albicans* (*calb* and *pac*) were not identified in any of the samples analyzed.

Lesion Depth	*Streptococcus* ssp.	P value	OD (CI95%)
RB4 (n=23); Positive n (%)	21 (91.3)	0.234#	Not estimated
RC5 (n=24); Positive n (%)	24 (100.0)		
	Streptococcus mutans (gftB)	P value	OD (CI95%)
RB4 (n=23); Positive n (%)	9 (39.1)	0.679*	1
RC5 (n=24); Positive n (%)	8 (33.3)		0.78 (0.24 – 2.56) p=0.680
	S. mutans (serotype *c*)	P value	OD (CI95%)
RB4 (n=9); Positive n (%)	0 (0.0)	0.029#	Not estimated
RC5 (n=8); Positive n (%)	4 (50.0)		
	S. mutans (serotype *e*)	P value	OD (CI95%)
RB4 (n=9); Positive n (%)	9 (100.0)	<0.001#	Not estimated
RC5 (n=8); Positive n (%)	1 (12.5)		
	S. mutans (serotype *f*)	P value	OD (CI95%)
RB4 (n=9); Positive n (%)	0 (0.0)	0.206#	Not estimated
RC5 (n=8); Positive n (%)	2 (25.0)		
	S. mutans (serotype *k*)	P value	OD (CI95%)
RB4 (n=9); Positive n (%)	0 (0.0)	0.206#	Not estimated
RC5 (n=8); Positive n (%)	2 (25.0)		
	cbm	P value	OD (CI95%)
RB4 (n=9); Positive n (%)	0 (0.0)	0.082#	Not estimated
RC5 (n=8); Positive n (%)	3 (37.5)		

*Chi-square test; #Fisher's exact test; OD: Odds ratio; 95% CI: 95% Confidence interval.

Tabela 2. Frequency of *Streptococcus* and *S. mutans* serotype in different lesion depths in the study population. RB4: ⅓ middle dentin and RC5:⅓ inner dentin.

The prevalence of *S. mutans* serotypes was initially determined by biochemical, immunodiffusion or immunofluorescence techniques and more recently by molecular

identification using PCR (Shibata *et al*., 2003). This technique is capable of detecting *S. mutans* and its serotypes from 1 to 10 pg of DNA (Nakano *et al.,* 2007). Researchers have found variants of *S. mutans* that have characteristics similar to specific serotypes, but that do not exactly match the markers of known serotypes (Rao & Austin 2014).

In study conducted in the Colombian population, the presence of different *S. mutans* serotypes in isolates with dental caries was investigated, 56.2% of *S. mutans* harboring serotypes *c,* 8.3% serotypes *e*, 9.9% serotypes *f* and 25.6% undetermined serotype (Rincón-Rodríguez *et al.*, 2019). In our study, 11.7% of the samples also contained *S. mutans* with undetermined serotypes, such discrepancies may arise from genetic variations within strains, the existence of new or unusual serotypes, or inherent restrictions in the testing techniques employed.

In this study, the occurrence of *S. mutans* serotypes in dentin carious lesions of different depths in Brazilian individuals was investigated and a higher occurrence of serotype *e* was found in RB4 samples of dentin. Our findings partially disagree previous results, since among the different *S. mutans* serotypes, serotype *c* is the most studied and the most prevalent, representing more than 70% of *S. mutans* isolates, while serotype *e* represents only about 20% (Nakano *et al,* 2008, Espinosa-Cristóbal *et al*., 2012, Momeni *et al*., 2019, Subramaniam and Suresh, 2019).

However, initially classified as a rare serotype, serotype *e* may be under-represented in studies due to the limited number of investigations on the occurrence of different serotypes and serotypes *e*, *f,* and *k* are increasingly being identified in different types of human samples. In a study investigating the distribution of *S. mutans* serotypes in an Iranian population using saliva and biofilm samples, a higher frequency of serotypes *e* and *k* was observed compared to previous reports in the literature. In addition, participants with serotype *e* had higher DMFT scores (Babaeekhou *et al*., 2021), which is partially consistent with the present data, as serotype *e* was the most dominant within the dentin carious samples, occurring alone in both RB4 and RC5 depths.

Conversely, in a previous study analyzing the presence of these serotypes in saliva samples from Indian patients, serotype *k* was found to be the most prevalent, followed by serotype *e*, while serotype *f* was not identified in any of the samples studied (Rao *et al*., 2014), which partially contrasts with our findings. It should be noted that the geographic distribution of these serotypes is still being determined worldwide, as several countries are conducting studies using different oral samples for data collection. . In addition, to the best of

our knowledge, none of the serotype prevalence studies conducted to date have used human permanent dentin samples obtained from carious lesions. Most studies have used biofilm and saliva samples, and the nature of the sample can significantly influence the distribution of predominant *S. mutans* serotypes.

Collagen-binding proteins have been implicated as an important factor in the ability of *S. mutans* to adhere to collagen-rich tissues such as dentin. In contemporary literature, the prevalence of *S. mutans cnm*+ is estimated to be around 10-20%, typically associated with serotype *f* or *k* (Nakano *et al*., 2007) and the prevalence of *cbm*+ strains is estimated at 2%, more commonly identified as serotype *k* (Nomura *et al.*, 2012). In our study, the *cnm* was not found in any of the samples analyzed, which partially contradicts the previously mentioned studies, as none of these previous investigations investigated the prevalence of the *cnm* gene in dentin caries samples. The *cbm*+ was predominantly found in serotype *f*, different from Nomura *et al*. (2012), where *cbm*+ was more prevalent in serotype *k*.

The *cnm* gene has mechanism of sucrose-independent adhesion in the biofilm formation involved in the colonization of oral tissues and adhesion in extraoral infections, that the way *cnm* is an important virulence factor in the initiation or exacerbation of systemic diseases (Nakano *et al.*, 2011, Nomura *et al*., 2013, Naka *et al.*, 2014). It is likely that the *cnm*+ is more prevalent in extraoral samples such as blood and heart valves and therefore was not identified in our study.

A study in the Brazilian population focused on the prevalence of serotypes in samples obtained from root canals of endodontic infections, only serotypes *c* and *k* were identified in these samples. Collagen-binding proteins were also studied and the *cbm* gene was detected in all samples analyzed, while none of the samples contained the *cnm* gene (Lima et al., 2020). These results are in partial agreement with our study, which showed a predominance of *cbm*+ in the analyzed samples. In addition, the *cnm* gene was not identified.

The fact that the *cbm* gene was more highly expressed than *cnm* gene was surprising, given the seemingly lower frequency of the *cbm* when compared to the occurrence of the *cnm.* In previous studies, which reported that the *cbm* gene is found in 5% of samples while it is approximately 20% (Nakano *et al*., 2010; Abranches *et al*., 2011; Nomura *et al*., 2013). It is important to emphasize that the *cnm* and *cbm* are particularly prevalent in serotypes *f* and *k*. In our study, in RB4 samples from dentin carious, the *cbm*+ was found in 17.6%, and it was predominantly present in serotype *f* and in samples with multiple serotypes, consistent with previous findings where CBP+ are more commonly found in serotypes *e*, *f*, and *k*. It is

intriguing to note that while they are associated with more severe cases of the disease, in our study, they were not found in samples from deep cavities; however, here, we cannot establish a direct relationship between severity and depth.

The biofilm-forming ability of *C. albicans* confers tolerance to antifungal agents and immune responses. Additionally, its association with bacteria such as *S. mutans* enhances its resistance (Garcia *et al*., 2021). The present study yielded negative results concerning a potential etiological relationship between *C. albicans* and the development of caries, as this microorganism was not detected in the PCR analysis. These findings contradict previous studies that reported a predominance of *C. albicans* in caries lesions (Ghasempour *et al*., 2011, Fakhruddin *et al*., 2020). It can be argued that the aforementioned study was conducted with children. Although the main causal factors of caries in adults and children are similar, there are unique factors in relation to each group, such as the ongoing development of the defense mechanism and the presence of distinct microbial flora.

The role of *C. albicans* in the aetiopathogenesis of adult caries remains uncertain, as there is currently no literature justifying its absence in coronal dentin and its presence in root dentin within carious lesions. Therefore, further studies investigating this topic are necessary, considering the limited research available in the current literature on the pathogenic microbiology of dentin carious in permanent teeth.

The current review sheds light on various aspects of *S. mutans* serotypes and their prevalence, particularly in the context of dental caries. A critical review of the available literature reveals both congruencies and disparities in the findings of different studies. In particular, geographical disparities have emerged as a significant factor influencing serotype distribution, as different populations exhibit different patterns of serotype prevalence. In addition, the choice of sample type, whether biofilm, saliva or dentin, has a significant impact on serotype distribution.

5. Conclusion

This study brings the following observations: (1) A novel prevalence hierarchy of the *S. mutans* serotypes (*e*, *c*, *f* and *k*) in dentin carious lesions from Brazil, (2) a positive association between caries and serotypes e (RB4) and c (RC5) and (3) the highest occurrence of the *cbm* gene in *S. mutans* serotype *f.*

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Wanessa Fernandes Matias Regis: Conceptualization, Methodology, Investigation, Validation, Supervision, original draft, writing - Review & Editing. **Ernanda Maria de Araújo:** Conceptualization, Methodology, Validation. **Francisco Ruliglésio Rocha**: Conceptualization, Methodology, Validation. **Myrna Maria Arcanjo Frota Barros:** Methodology, Validation. **Francisco Wilker Mustafa Gomes Muniz:** Methodology, Validation. **Beatriz Gonçalves Neves:** Methodology, Validation, Writing – Review & Editing. **Lidiany Karla Azevedo Rodrigues:** Conceptualization, Resources, Methodology, Review & Editing, Supervision, Funding acquisition, Project administration.

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CONCLUSÃO GERAL

Os microrganismos *S. mutans* e *C. albicans* apresentam mecanismos de virulência similares, porém existem muitas lacunas quanto a interação entre eles no desenvolvimento da doença cárie. Os principais mecanismos de interação são as glicosiltransferases do *S. mutans* e as moléculas de QS de *C. albicans*. No segundo estudo verificamos uma nova hierarquia de prevalência dos sorotipos de *S. mutans* (*e, c, f* e *k*) em amostras de cárie dentinária de dentes permanentes, também houve associação positiva entre cárie e os sorotipos *e* (RB4) e *c* (RC5), além da maior ocorrência do gene *cbm* em *S. mutans* sorotipo *f.*

ANEXOS

ANEXO A

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ENSAIO CLÍNICO CONTROLADO DA REMOÇÃO PARCIAL DE DENTINA CARIADA EM DENTES PERMANENTES COM LESÕES DE CÁRIE RASAS, MÉDIAS E

Pesquisador: Myrna Maria Arcanjo Frota

Área Temática:

Versão: 2

CAAE: 54451316.7.0000.5054

Instituição Proponente: Departamento de Clínica Odontológica

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.610.785

Apresentação do Projeto:

Pesquisa da doutoranda Myrna Maria Arcanjo Frota, orientada pela Profa. Lidiany Karla Azevedo, centrada no conceito de odontologia minimamente invasiva e na identificação do perfil bacteriano nas diferentes profundidades de lesão cariosa. É objetivo avaliar a eficácia do tratamento conservador com remoção parcial da dentina cariada em dentes permanentes com cáries rasas, médias e profundas, associando com o perfil bacteriano. Serão selecionados 48 pacientes atendidos nas Clínicas do Curso de Odontologia da FFOE (UFC), apresentando lesão ativa de cárie dentinária em molares ou pré-molares permanentes com indicação restauradora. O tratamento para lesões cariosas profundas consistirá da escavação incompleta da dentina cariada, aplicação de uma camada de cimento de hidróxido de cálcio, selamento provisório por um período de 6 meses no grupo controle e restauração definitiva já na primeira sessão no grupo da remoção parcial de dentina cariada, já para os grupos com lesões cariosas rasas e médias o tratamento será a realização da escavação incompleta da dentina cariada seguida de restauração definitiva. Radiografias interproximais e oximetria de pulso serão feitas logo após o selamento temporário ou restauração definitiva em intervalos de 6, 12, 18 e 24 meses. O estudo do perfil bacteriano da dentina será feito através de análise do q-PCR.Os resultados obtidos no estudo serão analisados através do teste ANOVA.

Objetivo da Pesquisa:

Objetivo primário:

Avaliar a eficácia do tratamento conservativo com remoção parcial da dentina cariada e do tratamento convencional com remoção total através de parâmetros clínicos, radiográficos e oximetria de pulso.

Objetivo Secundário:

Avaliar clínica e radiograficamente a integridade da restauração e qualidade da dentina remanescente em diferentes profundidades de lesão cariosa.

Comparar o perfil bacteriano nas diferentes profundidades de lesão cariosa.

Avaliação dos Riscos e Benefícios:

A pesquisa é de risco moderado visto que os pacientes do grupo experimental que serão submetidos ao tratamento com remoção parcial de dentina cariada poderão ter índices de insucesso superiores quando comparados aos pacientes que serão submetidos ao tratamento com remoção total de dentina cariada. Além disso, os pacientes se submeterão à tomadas radiográficas periódicas.

Quanto aos benefícios destaca-se que todos os pacientes terão seus dentes restaurados. Existe a possibilidade de se demonstrar a não necessidade de remoção da cárie para estagnar a atividade bacteriana, havendo maior preservação de estrutura dental.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um ensaio longitudinal clínico controlado com mérito científico baseado no novo conceito de Odontologia minimamente invasiva.

Considerações sobre os Termos de apresentação obrigatória:

A pesquisadora apresentou ao comitê: projeto, folha de rosto, orçamento, cronograma, declaração de anuência dos pesquisadores envolvidos, autorização da clínica de dentística e do laboratório de microbiologia da FAMED-DPML, currículo lattes, carta de encaminhamento, TCLE Versão 2.

Conclusões ou Pendências e Lista de Inadequações:

Todas as solicitações foram atendidas. Não há pendências éticas nem documentais, s.m.j. deste colegiado.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_670312.pdf	06/06/2016 23:15:20		Aceito
Cronograma	CRONOGRAMA_DE_EXECUCAO.docx	06/06/2016 23:10:03	Myrna Maria Arcanjo Frota	Aceito
Orçamento	ORCAMENTO_DETALHADO.docx	06/06/2016 23:08:15	Myrna Maria Arcanjo Frota	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Doutorado_Myrna.docx	06/06/2016 23:05:44	Myrna Maria Arcanjo Frota	Aceito
Declaração de Instituição e Infraestrutura	DECLARACAO_DE_MUDANCA_DA_ANALISE_DE_DENTINA.doc	06/06/2016 22:58:00	Myrna Maria Arcanjo Frota	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_laboratorio_de_microbiologia_analise_de_dentina.pdf	06/06/2016 22:39:24	Myrna Maria Arcanjo Frota	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_DE_CONSENTIMENTO_LIVRE_E_ESCLARECIDO.docx	06/06/2016 22:36:19	Myrna Maria Arcanjo Frota	Aceito
Outros	CARTA_DE_ENCAMINHAMENTO_AO_CEP.docx	15/03/2016 11:22:18	Myrna Maria Arcanjo Frota	Aceito
Outros	Curriculos_Lattes_Myrna_Maria_Arcanjo_Frota.pdf	15/03/2016 11:20:34	Myrna Maria Arcanjo Frota	Aceito
Declaração de Pesquisadores	DECLARACAO_CONCORDANCIA_EM_PARTICIPAR_DA_PESQUISA.doc	15/03/2016 11:19:07	Myrna Maria Arcanjo Frota	Aceito
Declaração de Pesquisadores	TERMO_DE_RESPONSABILIDADE_DOS_PESQUISADORES.docx	15/03/2016 11:15:49	Myrna Maria Arcanjo Frota	Aceito
Folha de Rosto	Folha_de_Rosto_Final.pdf	15/03/2016 11:08:54	Myrna Maria Arcanjo Frota	Aceito
Outros	DECLARACAO_DE_AUTORIZACAO_PPGO.pdf	02/03/2016 14:33:33	Myrna Maria Arcanjo Frota	Aceito
Declaração de Instituição e Infraestrutura	CARTA_DE_ESPECIFICACAO_DO_LOCAL_DA_PESQUISA_AO_CEP.docx	02/03/2016 14:21:04	Myrna Maria Arcanjo Frota	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ANEXO B

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: PREVALÊNCIA DOS SOROTIPOS DE Streptococcus mutans, DOS GENES DE LIGAÇÃO AO COLÁGENO cnm/cbm E DE Candida albicans EM AMOSTRAS DE CÁRIE DENTINÁRIA

Pesquisador: Wanessa Fernandes Matias Regis Pinheiro

Área Temática:

Versão: 2

CAAE: 46268821.6.0000.5054

Instituição Proponente: Departamento de Clínica Odontológica

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.790.272

Apresentação do Projeto:

Streptococcus mutans é um dos principais microrganismos associados à cárie dentária. Proteínas de ligação ao colágeno (CBPs) (aproximadamente 120 kDa), denominadas Cnm e Cbm, são considerados como importante antígenos de superfície celular relacionados à aderência de S. mutans ao colágeno. Ademais, cepas de S. mutans CBP-positivas estão associadas a várias doenças sistêmicas envolvendo bacteremia, como endocardite

infecciosa. A infecção endodôntica em decorrência de lesões dentinárias profundas é considerada uma importante causa da bacteremia, mas pouco se sabe sobre a presença de S. mutans em lesões dentinárias. A associação de Streptococcus mutans e Candida albicans com cárie dentária é bem conhecida. Neste estudo transversal, a distribuição e de CBP + S. mutans e C. albicans em lesões de cárie dentinária de pacientes com cavidades de profundidade média e profundas serão investigadas com foco em CBPs. A avaliação da severidade de cárie foi realizada com o Sistema Internacional de Detecção e Avaliação de Cárie (ICDAS) e as amostras de dentina cariada foram coletadas antes da restauração dos dentes. O DNA das amostras de placa dental será extraído e analisado para identificação dos sorotipos de S. mutans (c, e, f e k) e C. albicans pela técnica da PCR. As amostras positivas para S. mutans serão analisadas para determinação dos sorotipos e identificação dos genes cnm e cbm. Estatística descritiva será

realizada e comparada com os dados da literatura científica.

Objetivo da Pesquisa:

Avaliar a prevalência do fungo C. albicans e dos sorotipos de S. mutans (c, e, f e k) e dos genes de ligação ao colágeno cnm/cbm, por uma abordagem de PCR, em amostras de cárie de dentina coletadas em pacientes adultos com lesões de cárie dentinária médias e profundas.

Avaliação dos Riscos e Benefícios:

Riscos:

Se trata de uma pesquisa de risco moderado, visto que as amostras estão armazenadas em microtubos de centrífuga e identificadas numericamente quanto ao tipo de amostra.

Benefícios:

O conhecimento da prevalência desses microrganismos beneficiará a população, pois ajudará na descoberta de novos métodos de prevenção da doença.

Comentários e Considerações sobre a Pesquisa:

O pesquisador deve atentar que o projeto de pesquisa aprovado por este CEP refere-se ao protocolo submetido para avaliação, ficando este isento de co-responsabilidade mediante pesquisas já realizadas. Portanto, conforme a Resolução CNS n. 466/12, o pesquisador é responsável por "desenvolver o projeto conforme delineado", e, se caso houver alteração nesse projeto, este CEP deverá ser comunicado em emenda via Plataforma Brasil, para nova avaliação.

Considerações sobre os Termos de apresentação obrigatória:

Os termos de presentação obrigatória foram devidamente apresentados.

Conclusões ou Pendências e Lista de Inadequações:

Diante da ausência de pendências ou inadequações, emito parecer favorável ao presente projeto.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1730737.pdf	19/05/2021 20:27:51		Aceito
TCLE / Termos de Assentimento / Justificativa de	TLCE.pdf	19/05/2021 20:26:52	Wanessa Fernandes Matias Regis Pinheiro	Aceito

Ausência	TLCE.pdf	19/05/2021 20:26:52	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Outros	Fiel_dep.pdf	18/05/2021 13:58:03	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Declaração de concordância	CONCORDANCIA.pdf	18/05/2021 13:57:12	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Outros	CARTA_SOLICITANDO_APRECIACAO_CEP.pdf	24/04/2021 10:56:51	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Orçamento	DECLARACAO_DE_ORCAMENTO_FINANCEIRO.pdf	24/04/2021 10:55:41	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Cronograma	CRONOGRAMA_CEP.pdf	24/04/2021 10:55:09	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Folha de Rosto	FOLHA_DE_ROSTO_CEP_WANESSA.pdf	07/04/2021 18:37:47	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DENTINA.docx	07/04/2021 17:18:29	Wanessa Fernandes Matias Regis Pinheiro	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_542042.pdf	07/04/2021 17:15:14	Wanessa Fernandes Matias Regis Pinheiro	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FORTALEZA, 18 de Junho de 2021

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador(a))

ANEXO C

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº AA2E736

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AA2E736**
Usuário:	**Wanessa Fernandes Matias Regis**
CPF/CNPJ:	**043.303.373-80**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Streptococcus mutans

Título da Atividade:	**PREVALÊNCIA DOS SORÓTIPOS DE Streptococcus mutans, DAS PROTEÍNAS DE LIGAÇÃO AO COLÁGENO cnm/cbm E DE Candida albicans EM SALIVA E BIOFILMES DE CRIANÇAS COM CARDIOPATIAS CONGÊNITAS E ADQUIRIDAS**

Equipe

Wanessa Fernandes Matias Regis	**Universidade Federal do Ceará**
Lidiany Karla Azevedo Rodrigues	**Universidade Federal do Ceará**

Data do Cadastro:	**06/05/2021 08:51:33**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético

Situação cadastral conforme consulta ao SisGen em **8:51** de **06/05/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

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Review

Effect of bioactive compounds on the regulation of quorum sensing network-associated genes and virulence in *Streptococcus mutans*—A systematic review

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ABSTRACT

Objective: The aim of this study was to systematically review the literature on the effect of bioactive compounds and their regulation of quorum sensing (QS)-related and/or -regulated-virulence genes expression in *Streptococcus mutans*.

Design: The search strategy was conducted through the electronic databases Pubmed, Scopus, and Web of Science for studies reporting the effects of natural and synthetic bioactive compounds on the regulation of QS-associated and/or -regulated genes of *S. mutans*.

Results: After full-text reading, 19 studies met the inclusion criteria, in most of them, QS-inhibitors from synthetic origin were evaluated, 16 articles described the effect of the compounds on biofilm formation cultivated in vitro and five studies described these effects on adhesion of biofilm-producing cells. Only 2 studies analyzed the potential target-component of the QS.

Conclusions: Mostly, the bioactive compounds affected the expression of QS-associated and/or -regulated genes and virulence traits (e.g. adhesion, biofilm formation, acid stress tolerance) of *S. mutans*. Further studies are necessary to elucidate the target-specific QS-system constituent used by bioactive compounds to achieve QS inhibition as well as validate the use of these compounds in controlling dental caries.

1. Introduction

Dental caries is a disease dependent on both biofilm aggregation over the tooth surface and its recurrent exposure to dietary carbohydrates (Fejerskov, 2004). *Streptococcus mutans* is a bacterium highly prevalent in dental biofilms and one of the most studied microorganisms related to dental caries (Krzyściak, Jurczak, Kościelniak, Bystrowska, & Skalniak, 2014; Loesche, 1986). *S. mutans* exhibit a high degree of virulence, mainly because of its capacity of producing great amounts of organic acids (acidogenicity) from metabolized sugars, surviving at low pH (aciduricity), and synthesizing extracellular polysaccharides substances (EPS) (Bowen & Koo, 2011; Marsh, 2003). These exopolysaccharides are mainly glucans produced by glucosyltransferases (Gtfs) that provide binding sites for microorganism agglomeration on the tooth surface and formation of pathogenic biofilms (Ren, Chen, Li, & Li, 2014; Scharnow, Solinski, & Wuest, 2019). *S. mutans* Gtfs are classified into three types (GtfB, GtfC, and GtfD), which are encoded by *gtfB*, *gtfC* and *gtfD* genes, respectively (Ren et al., 2016). The GtfB synthesizes mainly water-insoluble glucans (rich in α-1,3-glucosidic linkages), GtfC produces both water-insoluble and water-soluble glucans (rich in α-1, 6-glucosidic linkages), GtfD produces water-soluble glucans (Hanada & Kuramitsu, 1988; Aoki, Shiroza, Hayakawa, Sato, & Kuramitsu, 1986; Hanada & Kuramitsu, 1989). Furthermore, *S. mutans* produces multiple glucan-binding proteins (Gbps); GbpA (Russell, Coleman, & Douglas, 1985), GbpB (Smith, Akita, King, & Taubman, 1994), GbpC (Sato, Yamamoto, & Kizaki, 1997), and GbpD (Shah & Russell, 2004), which

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Title Page (with Author Details)

Antibiofilm activity of proteinase K and DNase I on dual-species biofilms of *Streptococcus mutans* and *Candida albicans*

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Title: Diversity of *Streptococcus mutans* serotypes isolated from dental plaque of Brazilian patients with a history of acute myocardial infarction

Short title: *S. mutans* serotypes in cardiac patient biofilm

Highlights

- Dental plaque from infarcted patients contains different *S. mutans* serotypes
- No occurrence of collagen-binding protein genes was found in *S. mutans* serotypes
- Non-*S. mutans* and normal cholesterol associated with lower heart attack risk

'Declarations of interest: none'.

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