REVIEW

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Factors influencing oral colonization of mutans streptococci in young children

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Abstract

This paper aims to critically review current knowledge about the key factors involved in oral colonization of the cariogenic group of bacteria, mutans streptococci (MS) in young children. MS, consisting mainly of the species *Streptococcus mutans* and *Streptococcus sobrinus*, are commonly cultured from the mouths of infants, with prevalence of infection ranging from around 30 per cent in 3 month old predentate children to over 80 per cent in 24 month old children with primary teeth. MS is usually transmitted to children through their mothers, and the risk of transmission increases with high maternal salivary levels of MS and frequent inoculation. Factors that affect the colonization of MS may be divided into bacterial virulence, host-related and environmental factors. Complex interaction among these factors determine the success and timing of MS colonization in the child. As clinical studies have shown that caries risk is correlated with age at which initial MS colonization occurred, strategies for the prevention of dental caries should include timely control of colonization of the cariogenic bacteria in the mouths of young children.

Key words: Mutans streptococci, cariogenic bacteria, dental caries, primary dentition.

Abbreviations and acronyms: ECC = early childhood caries; PCR = polymerase chain reaction.

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INTRODUCTION

Despite significant advances in preventive dentistry, dental caries continues to be one of the most common infectious oral diseases in mankind. An entity of dental caries, early childhood caries (ECC) may be defined as the presence of one or more decayed primary tooth surface in a child aged 71 months or younger.[1] The condition is responsible for the majority of dental abscesses and toothache in children who often require general anaesthesia for treatment.[2] The highest prevalence of ECC is found in the socially-disadvantaged groups, and recent surveys report rising caries rates in young children in Australia and other parts of the world.[3,4]

As in other types of dental caries, mutans streptococci (MS), consisting mainly of the species *Streptococci mutans* and *Streptococci sobrinus*, have been implicated as the principal oral bacteria responsible for the initiation and development of ECC.[5,6] Colonization with MS is thus a key event in the pathogenesis of EC that can be targeted for caries prevention in clinical practice.[2] The aim of the present paper therefore, is to critically review the current understanding of bacterial, host and environmental factors involved in MS colonization in children.

Oral microbes start to colonize an infant's mouth soon after birth. The numbers of oral bacteria increase gradually from exposure with microbial sources from the external environment.[7-9] *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus oralis* have been identified as the first and most dominant oral microbes to colonize the oral cavities of newborn infants. With the eruption of primary teeth, the number and complexity of the microflora in the oral environment increase.[7,9,10] The species colonizing the teeth after eruption include *Streptococcus sanguis*, *Staphylococcus spp.*, *Veillonella spp.*, *Neisseria spp.*, *Actinomyces spp.* and *Lactobacilli spp.* Oral streptococci including *S. oralis*, *S. anginosus* and *S. gordonii* are commonly reported to be present after first year of life.[7,9-11] In addition, anaerobes including *Fusobacterium* and *Prevotella* species can also be detected in young children.[8,10] In later childhood the bacterial diversity and numbers in the oral cavity increase as more teeth erupt and provide greater areas for the adherence and retention of microbes.[12]

Due to the paucity of longitudinal studies, relatively little is known regarding the timing of arrival of the key microbes found in the oral biofilm in children. Similarly, it is still unresolved as to the timing of acquisition of MS into the oral biofilm, and whether MS can be considered a component of the normal oral flora.[5,6,13]

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Mutans streptococci and caries in young children

The main MS species, *S. mutans* and *S. sobrinus*, are facultative anaerobes, non-motile and catalase-negative gram-positive cocci.[5,6] The central role of MS in the aetiology of ECC is now well established. In a recent review on the usefulness of bacterial testing in risk assessment for caries, Thenish *et al.*[3] examined cohort studies of 1–3 years follow-up of children aged 2 to 4.5 years of age residing in Scandinavia,[14-18] Cuba,[19] Japan[20] and USA.[21,22] Although numbers of subjects in the studies were small and the majority of investigators did not adjust for confounders in their respective investigations, the authors concluded that there was sufficient evidence that the presence of MS in plaque or saliva of caries-free preschool children is associated with significant increase caries risk in child.[3]

MS species that have been implicated in dental caries are *Streptococcus mutans* (serotypes *c, e, f*) and *Streptococcus sobrinus* (serotypes *d, g*).[5,6] MS have been isolated in 95 per cent of children with high caries rates and diverse ethnic and socio-economic backgrounds.[23-29] In children with ECC, MS comprises 30–50 per cent of plaque flora[30] and 10 per cent of salivary flora.[31] In contrast, less than 1 per cent of the oral flora in low caries risk children is MS.

S. mutans has been identified as the principal cariogenic bacterium for caries initiation[5] while *S. sobrinus* is thought to enhance caries initiation progression and development.[5] Children harbouring both *S. mutans* and *S. sobrinus* species showed higher caries experience than children who carry only either *S. mutans* or *S. sobrinus*.[32-34] In addition, the presence of both *S. mutans* and *S. sobrinus* at localized sites has correlated strongly with the presence of early caries.[12,32,34]

Although clinical studies usually employed either pooled plaque or salivary samples, there are generally no significant differences in sensitivity and specificity of detection of MS from plaque or saliva samples.[3,35] Identification of MS has previously depended mainly on colony and biochemical characteristics based on the microbial growth on selective culture media.[36] Although conventional culture methods remain important, molecular techniques are fast replacing traditional methods for MS detection. Monoclonal antibodies for specific identification of all eight known species of MS have recently been reported.[37] Furthermore, polymerase chain reaction (PCR) techniques are now frequently used to detect small numbers of MS which are not usually detectable by culture techniques.[38] Importantly, molecular techniques are required for advanced microbial characterization such as genotyping of the bacteria.[39]

Virulence factors of mutans streptococci

Although MS organisms contain a wide range of traits such as acidogenicity and aciduranc that confer them with an ecological advantage over other oral bacteria for cariogenicity, not all strains are equally as virulent in causing dental caries.[40]

The major virulence factor of MS which is important in colonization is their ability to adhere to host surfaces.[8,19] This property involves a sucrose-independent initial adherence to the acquired salivary pellicle, followed by sucrose dependent cellular accumulation.[41,42] Adhesins from *S. mutans* (antigen I/II) and *S. sobrinus* (Spa A) interact with salivary proteins of the acquired pellicle on the tooth surface to promote bacterial adherence.[41,42] In addition, glucan binding proteins (GbpA, GbpB and GbpC) increase the binding of MS to glucans deposited on tooth surfaces, thereby contributing to the sucrose dependent adherence of *S. mutans* and *S. sobrinus* to teeth.[41,42] The glucans are products of MS enzymes, glucosyltransferase and fructosyltransferases which are found in both *S. mutans* and *S. sobrinus*.[43,44] These extracellular polysaccharides are central for the adhesion and colonization of MS. In addition, they increase the thickness of plaque on the tooth surface, resulting in enhanced rates of sugar diffusion and acid production at deeper plaque layers.[43-45]

Virulence properties of MS which are important for their proliferation and establishment are acidogenicity and acidurance which allows the organisms to survive at low pH levels and contributes significantly to their cariogenic potential.[46,47] This characteristic is derived from the ATPase-driven proton pumps (ATP hydrolysis) which actively remove protons from the cytoplasm.[48]

Another virulence property which is shown by half the MS species (*S. mutans*, but not *S. sobrinus*) is the ability to synthesize intracellular polysaccharides. The storage carbohydrates are metabolized by *S. mutans* to produce acid, which enhances cariogenicity by maintaining an acidic pH (<5.5) in the environment.[46,47] This property of *S. mutans* permits continual acid production after dietary carbohydrates have been depleted or during periods of low concentration of exogenous substrate. This activity maintains acidogenicity and fosters enamel demineralization during periods of low salivary secretion during sleep.[44]

Factors which affect mutans streptococci transmission

Clinical studies have provided microbiological, biochemical and molecular evidence that children generally acquire MS organisms through their mother.[49] These studies have shown that *S. mutans* strains isolated from mothers and their children exhibit similar or identical bacteriocin profiles[50,51] and identical plasmid or chromosomal DNA patterns.[52,53] Other studies have shown that there could also be transmission of MS from other family members and non-familial caretakers as children increase their social contacts outside the family.[53,54]

Data from clinical studies show that children whose mothers harbour high concentrations of salivary MS acquire the bacteria at younger ages and in higher numbers compared with children of mothers who harbour low MS levels.[55,56] Berkowitz *et al.*[55] reported

that mothers with salivary levels of *S. mutans* greater than 10^6 organisms per millilitre of saliva have a greater than 50 per cent rate of transmission of the bacteria to their 10 to 16 month old children compared with a rate of only 30 per cent in the case of mothers with only 10^3 organisms per millilitre of saliva.[55] Other investigators have further reported that mothers who have greater caries experience, periodontal disease, poor oral hygiene, low socio-economic status and education as well as frequent snacking have higher risk of transmission of MS to the infant.[55-59]

Frequency of inoculation is likely to be an important factor in MS transmission from mother to infant.[49,57] Thus, mothers who share foods, drinks, utensils, toothbrushes and other items with their children have the highest risk of transmitting MS to their children.[58,60,61]

Some authors have hypothesized that there could be beneficial effects of early transmission of MS to the infant, suggesting that this may induce the development of anti-MS immunoglobulins as a result of natural inoculation.[62-65] However, although the putatively higher levels of immunity can theoretically reduce MS colonization and decrease dental caries experience in young children, this has not been observed in clinical practice.[62-65]

Similarly, it has been suggested that breast-feeding provides immunological protection against MS colonization from mothers to infants due to the presence of anti-*S mutans* IgA in breast milk.[66] However, protection against MS colonization by passively transferred IgA through breast-feeding has not been proven.

Successful colonization MS is likely to depend on the presence of a favourable environment of the dental microbial biofilm. Pioneer microbes entering the oral cavity can influence the colonization of later species and microbes arriving later will need to compete for colonization sites and nutrients.[67] As earlier-arriving species have an ecological advantage over those arriving later, it is likely that early arrival of MS will promote their persistance in the mouth compared to later arrival.[68,69]

Timing of initial mutans streptococci colonization in young children

As is the case regarding timing of development and maturity of the dental biofilm in children, the timing of initial MS acquisition in children is not yet established. Most studies suggest that the bacteria can be acquired at any time from under 6 months to over 3 years of age. Until recently, it was thought that MS require a non-shredding surface to colonize and multiply.[49,70] A small study by Caufield *et al.*[50] suggested that there is a "window of infectivity" for initial acquisition of MS at a mean age of 26 months (range 9–44 months). This observation has been supported by a few clinical studies which showed initial colonization of MS varied between 7–36 months which is a time period coinciding with eruption of the primary teeth.[10,14,71]

If colonization of MS occurs with tooth emergence, then several windows of infectivity may exist, such as time periods coinciding with eruption of primary incisors, primary molars and permanent first molars, respectively.[12,72] On the other hand, longitudinal studies by Wan and co-workers[60,61] and Law and Seow[73] showed that there was increasing MS colonization with increasing ages of the children, without any discrete windows of infectivity.[60,61,73]

There is now clinical evidence that MS can be detected in mouths of predentate children prior to the eruption of the first tooth.[61,74,75] Wan and co-workers showed more than 30 per cent of predentate children at the age of 3 months were infected with *S. mutans* and over 60 per cent showed presence of the bacteria by the age of 6 months.[61,75]

Karn *et al.* also reported a prevalence of 27 per cent of MS infection in children aged 12 months,[76] and Thorild *et al.* detected MS in 30 per cent of 18 month old children.[77] Higher infection rates were reported by Roeters *et al.*,[78] who found MS in 43 per cent of preschool children aged 2 to 5 years, and Milgrom *et al.*[74] who reported MS in 53 per cent of children aged 6 to 12 months and 72 per cent in those aged 13 to 24 months. Other investigators also reported similarly high levels in 24 month old children.[60,73]

The timing of MS colonization is of clinical significance as there is evidence that the earlier the MS colonization in a child, the higher is his/her caries risk.[14,74,79,80]

A few longitudinal studies have reported that children who had acquired MS before 2 years of age showed greater caries experience in both primary and permanent dentitions compared to children who were colonized at later ages.[14,74,79,80] The first report by Alaluusua and Renkonen[14] found that children who had MS by the age of 2 years showed higher caries experience compared to children who were colonized later in childhood. This observation, together with the fact that MS is usually transmitted by the mother, led to the concept that prevention of caries in children may be possible by reducing maternal MS counts and delaying MS colonization in the children.[14]

In later clinical studies, Kohler *et al.*[79] investigated children who were colonized at different times due to differing levels of maternal *S. mutans*, and correlated the colonization times with caries experience.[79] The authors reported that 4 year old children who were colonized at ages below 2 years had significantly lower caries experience compared with children who were colonized after 2 years of age. When the same cohort were followed-up at 7 years of age, the same group of children who were colonized after 2 years of age had the lowest levels of MS and significantly lower caries risk compared to those who were colonized earlier.[81] In other studies which attempted to reduce MS transmission by reducing maternal levels,

Table 1. Factors which increase MS colonization

Bacterial factors	*Transmission* – Increased numbers of MS in mothers/close contacts – Increased frequency of contact with MS carriers *MS strains* – Virulent strains of MS *Biofilm* – Little competition with other species – Ecological sites available for colonization
Host factors	*Hereditary* – HLA genes with unfavourable immunological, salivary, – tooth, mucosal effects *Surfaces for microbial adherence* – Increase tooth surfaces – Altered mucosal surfaces *Saliva* – Reduced quantity and quality of saliva *Immunological* – Reduced oral immunity from congenital and acquired conditions *Diet* – Frequent ingestion of sweet snacks and drinks *Oral hygiene* – Lack of oral hygiene

Isokangas *et al.* also reported that colonization with MS before 2 years of age is associated with significantly earlier caries attack in the primary dentition compared to colonization after 2 years of age.[82]

Host factors which affect mutans streptococci colonization in young children

It is likely that MS colonization in children results from a complex interaction of the bacterial factors with host factors as listed in Table 1.

Hereditary factors

The influence of hereditary factors in dental caries has been well described,[83-85] and it is likely that many of these are associated with MS colonization in the child. It is reasonable to hypothesize that many genetic factors, such as the HLA genes, modulate the host's immunological responses which, in turn, influence MS colonization in the mouth. In addition, these genetic factors may also modulate bacterial colonization through changes in the saliva, tooth and mucosal surfaces.[83-85]

Twin studies are useful to determine the relative contribution of hereditary and environmental factors in a variety of traits, including the factors affecting oral colonization. Comparing results of identical or monozygotic twin pairs who share all their genes, and non-identical or dizygotic twin pairs, who share approximately half of the segregating genes, allows computation of the relative amount of genetic contribution to trait variation. In this regard, Goodman *et al.* first proposed that streptococci salivary levels are modulated by genetic factors whereas lactobacilli levels were correlated mainly with environmental factors.[86] This hypothesis was extended by molecular studies of Acton *et al.* which suggest that MHC genes may modulate susceptibility for the colonization of MS levels, although conflicting results were reported by Ozawa *et al.*[83,85] In a recent study of preschool twins, genetic influence was reported to contribute to approximately 52 per cent in the variation in salivary MS levels, while the remainder of the variation was thought to result from environmental factors.[84]

Tooth and mucosal surface factors

Although non-shedding hard tissue surfaces provide the main adherence surfaces for MS colonization, there is increasing evidence that oral mucosa may have a role in initial colonization of the bacteria. In recent studies, oral mucosal surface changes manifested as developmental nodules (Bohn's nodules),[75] and oral clefts[70] have been reported to provide increased retention sites to facilitate MS colonization in predentate children.

Abnormalities in hard tissue surfaces and the presence of cleft palate obturators can also modulate the adherence and colonization of MS.[70] Enamel hypoplasia leads to loss of enamel integrity and tooth surface irregularity which enhance the colonization of MS on the tooth surfaces due to increased bacterial adherence, plaque retention and decrease in carbohydrate clearance.[87,88] Enamel defects can result from a variety of inherited and acquired systemic aetiologic factors ranging from genetic abnormalities such as *Amelogenesis imperfecta* to congenital abnormalities of mineralization such as hypo phosphatasia, to metabolic defects such as rickets and prematurity of birth.[87,89-91] It is thus reasonable to expect that children who have these conditions and are predisposed to enamel defects are also at higher risk for early MS colonization.[87,92]

Saliva

The protective role of saliva against oral bacteria is well established.[93] Saliva contains several antimicrobial components that mediate selective adhesion and colonization of mutans streptococci on the tooth surfaces. Agglutinins include mucins, glycoproteins, fibronectin, lysozyme and salivary immunoglobulin A (sIgA) promote agglutination of MS and enhance bacteria removal.[93,94] Other proteases and galactosides in saliva destroy the surface protein antigens of *S. mutans* to prevent adherence of *S. mutans*.[95]

Moreover, salivary flow and buffering capacity (carbonic acid-bicarbonate system, phosphate and protein systems) facilitate oral clearance, which influence MS colonization by neutralizing acid resulting from MS fermentation of substrate.[96] Oral clearance is thought to be slowest at night-time when the salivary flow rate is lowest. Such conditions enhance colonization of MS and plaque growth.[96]

Children with compromised salivary flow tend to be at highest risk for early MS colonization. They may have congenital abnormalities and undergone surgical

resection of salivary glands, or have compromised salivary gland function after radiotherapy or intake of medications such as salbutamol.[97,98]

Immunological factors

Although immunological factors are likely to play an important role in infections in general, the significance of immunological factors in MS colonization and dental caries is still unclear.[99] There is evidence that most children are orally immunocompetent with specific immune factors at birth or soon after,[100] and antibodies levels against *S. mutans* tend to increase with age.[94,101] Specific immune factors derived from saliva (sIgA) or serum and gingival crevicular fluid (IgG, IgA and IgM) influence the colonization and pathogenic activity of MS. Both sIgA and IgG inhibit *S. mutans* adherence to saliva-coated hydroxapatite and epithelial surfaces and neutralize *S. mutans* enzymes and virulence factors.[62,64,102] Moreover, IgA can enhance lactoferrin, peroxidases and lysozyme activities in saliva that may reduce *S. mutans* colonization. IgG antibodies found in gingival crevicular fluid enhance phagocytosis and killing of *S. mutans* and other oral micro-organisms through complement activation or opsonisation.[101] IgM antibodies that can agglutinate *S. mutans* and fix complement are important in the first few days of the primary immune response.[101]

However, the role of immunoglobulins in colonization of MS and dental caries is unclear.[103-105] High anti-MS immunoglobulins have been shown to be associated both with and without caries experience in separate studies.[103-105]

Feeding habits and sugar exposure

Prolonged bottle feeding with sugar-containing drinks, and night-feeding of sweetened fluids, as well as frequent snacking are well-known factors which are associated with increased MS levels and development of caries in young children.[74,106] Sucrose has been identified as the main cariogenic sugar which can be metabolized by MS to produce the plaque dextrans essential for bacterial adherence and colonization in the oral cavity.[6,107] Possible sources of sucrose in young children include fruit juices, sweet solids and drinks that are readily metabolized by MS to acids which demineralize tooth structure.[108] In addition, high frequency of sugar in both solids and fluids increases the acidity of plaque and enhances the establishment and dominance of the aciduric MS.

The longitudinal studies of Wan *et al.*[60,61] and Law and Seow[73] provide strong evidence that frequent consumption of sugar-containing snacks are associated with earlier colonization of MS in infants. Furthermore, babies who are started on solids earlier are at higher risk of MS colonization, probably due to the fact that many children are given sweet foods as their first solids to encourage their acceptance of first solids.[60]

Although the cariogenicity of human breast milk and bovine milk have not been investigated extensively under *in vivo* conditions, both are generally thought to be minimally cariogenic from *in vitro* studies.[109] On the other hand, a recent animal study by Bowen and Lawrence[110] suggests that at high frequency exposures, human breast milk had greater cariogenicity compared to bovine milk, probably due to the fact that human breast milk contains higher lactose concentration (7 per cent) than bovine milk (4 per cent).[110,111] Human breast milk has antibacterial activity due to the presence of immunoglobulins (sIgA), enzymes and specific antibacterial agents such as lactoferrin, interferon and lysozymes which can theoretically prevent and reduce *S. mutans* colonization.[66] However, the clinical effects of these antimicrobial factors on MS colonization are still unknown.

Systemic antibiotics

It has been suggested that long-term antibiotic therapy in early childhood may eliminate or reduce MS colonization,[72,112] although the extent of protection from single courses of antibiotics is unknown. On the other hand, the effects of antibiotics are often confounded by the caries-promoting effects of sucrose found in some medications, as well as the effects of enamel hypoplasia which can result from systemic infections necessitating the antibiotics.[88] These factors may explain the findings in the report of Dasanayake *et al.*[113] who showed higher MS prevalence and increased caries risks in children who were frequently exposed to sucrose-containing medications.

CONCLUSIONS

The colonization of MS in a child's mouth has significant implications for caries risk. Factors which affect colonization include bacterial virulence factors interacting with host genetic and environmental factors. Future strategies aimed at controlling MS colonization are likely to be most effective if they draw on our growing understanding of the nature of the interplay between bacterial and host factors in this critical process.

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