Universidad Europea

TRABAJO DE FIN DE GRADO

Grado en Odontología

MICROFLORA ORAL EN EL NIÑO Y CARIES DENTAL

Madrid, curso 2020/2021

Número identificativo 63

Abreviaturas

A.naeslundii: Actinomyces naeslundii; CAC: cavidad activa con caries; CAS: saliva de niños con caries activa; CFS: saliva de niños sin caries; H. parainfluenzae: Haemophilus parainfluenzae; L.buccalis/shaii: Leptotrichia buccalis/shaii; S. mitis/mutans/sanguinis/sobrinus: Streptococcus mitis/mutans/sanguinis/sobrinus; N. flava/flavescens/pharynges: Neisseria flava/flavescens/pharynges; PA: película adquirida; V. atipyca/dispar/parvula: Veillonella atipyca/dispar/parvula.

Resumen

Introducción: La cavidad bucal en el niño está habitada por gérmenes comensales que forman la biopelícula bucal, mantienen la homeostasis y protegen contra microorganismos patógenos que pueden bajar los niveles de pH. Cuando se rompe el equilibrio entre estas bacterias se forma un proceso de desmineralización de las estructuras dentarias, iniciandose un proceso carioso. Hoy en día la caries dental es una de las enfermedades más frecuentes. En particular en los niños se debe a factores como los hábitos dietéticos, el nivel socioeconómico de los padres y un cepillado incorrecto.

Objetivo: El objetivo de este trabajo fue identificar las bacterias presentes en los diferentes estadios de la caries.

Metodología: El análisis se llevó a cabo a través de la búsqueda bibliográfica en buscadores científicos, utilizando palabras llaves como #MutansStreptococci, #ECC, #MicroflorDentalDecay, #OralBiofilm, #DentalPlaque.

Resultados: Entre los microorganismos principales que se encuentran, se asocian a salud las bacterias de las especies *Haemophilus parainfluenzae* y *Streptococcus sanguinis* y del género *Capnocytophaga*, *Corynbacterium*, *Neisseria* y

Porphyromonas; a enfermedad se asocian *S. mutans*, *S. sobrinus*, *Actinomyces*, *Leptotrichia*, *Scardovia wiggsae* y *Lactobacillus*.

Discusión: no todos los estudios observan las mismas bacterias, lo que puede explicarse por el diferente estadio de desmineralización de las caries estudiadas (ej. *Scardovia* y *Lactobacillus* se observan en los estudios que consideran las caries que afectan a la dentina), o por la forma en que se recogen las muestras, o porque los análisis no son lo suficientemente exhaustivos.

Conclusiones: Están presentes bacterias específicas para cada fase de desmineralización. Destacan las especies *S. mutans* y *S. sobrinus*, que tienen un papel predictor de la patología. En caries con afectación del esmalte se observa el género *Actinomyces* y las bacterias *Scardovia wiggsiae* y *Lactobacillus* cuando hay afectación de dentina. Además se observa una disminución en la variedad de la microflora a medida que avanza la caries.

Abstract

Introduction: The oral cavity in the child is inhabited by commensal germs that form the oral biofilm, maintain homeostasis and protect against pathogenic microorganisms that can lower pH levels. When the balance between these bacteria is broken, a process of demineralisation of the dental structures is formed, initiating a carious process. Nowadays, dental caries is one of the most frequent diseases. Particularly in children it is due to factors such as dietary habits, the socioeconomic level of the parents and incorrect brushing.

Objective: The aim of this study was to identify the bacteria present in the different stages of caries.

Methodology: The analysis was carried out through a bibliographic search in scientific search engines, using keywords such as #MutansStreptococci, #ECC, #MicroflorDentalDecay, #OralBiofilm, #DentalPlaque and reference books.

Results: Among the main microorganisms found, the bacteria that belong to the species *Haemophilus parainfluenzae* and *Streptococcus sanguinis* and the genus *Capnocytophaga*, *Corynbacterium*, *Neisseria* and *Porphyromonas* are not associated with caries; *S. mutans*, *S. sobrinus*, *Actinomyces*, *Leptotrichia*, *Scardovia wiggsae* and *Lactobacillus* are associated with the disease.

Discussion: not all studies observe the same bacteria, which may be explained by the different stage of demineralisation of the caries studied (e.g. *Scardovia* and *Lactobacillus* are observed in studies that consider caries affecting dentine), or by the way samples are collected, or because the analyses are not sufficiently exhaustive.

Conclusions: Bacteria specific to each stage of demineralisation are present.

S. mutans and *S. sobrinus* species stand out and play a predictive role in the pathology. In caries with enamel involvement, the genus *Actinomyces* and the bacteria *Scardovia wiggsiae* and *Lactobacillus* are observed when dentine is involved. In addition, a decrease in the variety of microflora is observed as the caries progresses.

Sommario

Introducción

La microflora oral es un conjunto de microorganismos que viven en la cavidad bucal y que tienen la capacidad de adherirse a las superficies dentales y a los tejidos blandos. Los microbios que la componen van cambiando a lo largo de la vida, dependiendo de la edad del huésped, presencia de enfermedad (caries y enfermedad periodontal) y serán diferentes según el lugar de la cavidad oral que colonizan (1).

En un individuo con salud dental la mayoría de los gérmenes que componen la biopelícula oral son comensales. El papel de estas bacterias es mantener la homeostasis y proteger frente a microorganismos patógenos y oportunistas que suelen disminuir el pH de la cavidad oral debido a sus capacidades acidogénicas (producir ácidos) y aciduricas (habilidad de vivir en un ambiente con pH ácido) (2).

Cuando se crea un desequilibrio entre el proceso de remineralización-desmineralización debido a bacterias que, a través sus capacidades de fermentar los hidratos de carbono que provienen de la dieta, producen ácidos, empieza la desmineralización de los tejidos duros y así el desarrollo de la caries dental. Esta última, consistente en la perdida de material dental, suele aparecer cuando el proceso de desmineralización es más frecuente del proceso de remineralización (3).

La caries es una infección polimicrobiana mediada por microorganismos orales comensales y se considera una enfermedad de origen multifactorial, debida a la interacción de varios factores. En su formación, influyen factores como la genética, la anatomía dentaria, la susceptibilidad del huésped y los microorganismos que componen la biopelícula oral (4)(5)(6)

Hoy en día la caries dental es considerada una de las enfermedades crónicas más frecuentes en las poblaciones de países desarrollados, tanto en los adultos como en niños. En estos últimos es debida sobre todo a factores como los hábitos dietéticos, el nivel socioeconómico de los padres y un cepillado incorrecto (4).

Se trata de un fenómeno que hay que detener en cuanto potencialmente destructivo para el aparato masticatorio y con importantes consecuencias bajo varios aspectos. Por ejemplo puede conducir a un estado de malnutrición, debido al dolor de esta enfermedad, y problemas psicológicos en las relaciones sociales (7) (vergüenza en el habla para el miedo de exhibir una dentición dañada).

1. Biopelícula oral

La cavidad oral es el único espacio del ser humano que presenta superficies descamativas y no descamativas, como los dientes. Estas últimas facilitan la colonización de microorganismos, con la consiguiente formación de la biopelícula oral que, si se forma sobre las superficies dentales, es conocida con el nombre de “placa dental”. Esta es el resultado de la interacción de varios microorganismos, que se adhieren a los dientes y que están embebidos en una matriz extracelular que ellos mismos producen (8).

La biopelícula presenta diferentes etapas de desarrollo, que empiezan desde la formación de una película adquirida hasta llegar, semana después, a la formación de una placa madura (8).

A continuación se desarrollan los estadios de formación de la biopelícula.

2. Película Adquirida y Colonización Primaria

Después del cepillado, los dientes entran en contacto inmediato con la saliva y se forma la capa de hidratación de Stern, que tiene la capacidad de absorber los complejos salivales como proteínas y glucoproteínas (mucinas, lisozima, a-amilasa) y compuestos bacterianos, entre ellos los glucanos, glucosiltransferasas, formando así la película adquirida (PA). Se trata de una capa delgada, amorfa acelular que tiene como fin principal lo de favorecer la adhesión de las bacterias a la superficie dental (8).

Los primeros microorganismos que colonizan los dientes, llamados colonizadores primarios, suelen ser bacterias Gram-positivos del género *Streptococcus*, sobre todo los que pertenecen a los grupos *S. sanguinis*, *S. mitis*, *S. oralis* y al género *Neisseria*, *Actinomyces* y *Veillonella* (8)(9).

Inicialmente, forman con la película adquirida enlaces no específicos y reversibles, a través fuerzas de Van der Walls, que sucesivamente se convierten en enlaces irreversibles y específicos. Esta última adhesión es debida a la interacción entre la PA y las adhesinas, moléculas que se encuentran sobre las superficies bacterianas. En esta primera fase de colonización, las bacterias son aerobios o aerobios facultativos (8)(10).

3. Colonización Secundaria y Terciaria

La placa dental sigue creciendo. Esto es debido a la multiplicación de los colonizadores primarios y a la coagregación y coadhesión de microorganismos secundarios y terciarios. Estos últimos dos son bacterias anaerobias y sus entradas

en la biopelícula es debida a la reducción de oxígeno producida por los colonizadores primarios (10).

Entre ellos se encuentran las especies *Fusobacterium nucleatum*, *Prevotella intermedia*, *Capnocytophaga*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* (10).

Es interesante el papel de los *Fusobacterium nucleatum* y *Porphyromonas gingivalis* porque actúan como puentes, para la unión física entre los primeros colonizadores y los tardíos. Esto es posible debido a sus capacidades de coagregación específicas con ambos colonizadores (11).

4. Placa Madura

La placa se considera madura cuando se encuentre en un estado de homeostasis con el huésped y actúe como una comunidad. Este nivel de equilibrio lo logra después varios procesos dinámicos, como los de antagonismo y sinergismo (10), y a través la comunicación y coordinación entre las bacterias, gracias al intercambio de pequeñas moléculas. Esta última interacción es conocida con el nombre de “transferencia horizontal de genes” (8).

5. Caries Dental

El equilibrio microbiótico de la placa se rompe cuando está sometido a cambios repentinos, y pasará da ser un biofilm sano a uno patógeno y empezará la enfermedad. Esta última es debida a la producción de ácidos provenientes de los

hidratos de carbono de la dieta, que son metabolizados por los microorganismos comensales. Los ácidos bajan el pH y causan la desmineralización de la estructura dentaria (8)(10).

Los estudios que se realizaron sobre ratas libres de gérmenes, demostraron que la presencia de placa dental y las bacterias que la componen no son los únicos factores que aumentan el riesgo de aparición de la caries, pero sí que su presencia es necesaria para el desarrollo de la enfermedad (12).

En definitiva, la placa constituye una condición que predispone a la formación de la caries.

Las caries dentales tienen diferentes manifestaciones clínicas, según su estadio de avance, y en cada una de estas etapas se observan varias bacterias entre las que se encuentran *Streptococcus mutans*, *Actinomyces*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, *Veillonella*, *Selenomonas* y *Atopobium* (6).

Las caries tienen varias etapas y entre las manifestaciones clínicas podemos encontrar: caries de esmalte activa o detenida, caries en dentina no cavitada, caries activa o detenida en dentina cavitada.

El color de la lesión cariosa cambia a medida que se acerca a la cámara pulpar, empezando desde un color blanquecino llegando a un color marrón oscuro en dentina (13).

5.a) Mancha Blanca

La caries en esmalte, conocida también con el nombre mancha blanca, es el primer signo de deterioro de la estructura dentaria (8)(14).

Dependiendo de si está activa o detenida, cambia la textura, pero no el color.

Es visible a simple vista y aparece como una lesión de color blanco tiza y opaca (8).

Se clasifica en:

- *Caries activa en esmalte*: al pasar la sonda se notará una superficie rugosa y sin brillo (8).
- *Caries detenida en esmalte*: al tacto la superficie dental será lisa, dura y aparecerá brillante (8).

Se considera como una etapa de transición entre el esmalte intacto y la lesión cavitada (14).

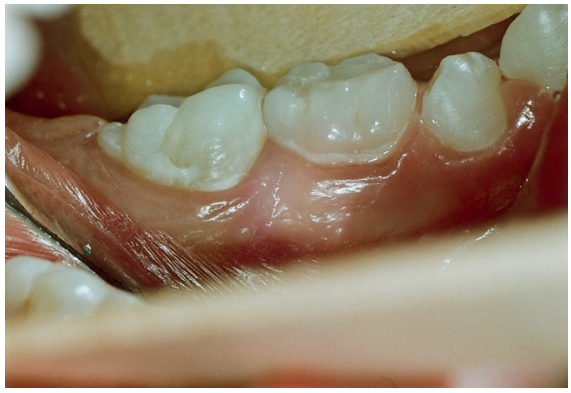

Figura 1: mancha blanca (15). *Página 10*.

5.b) Caries en dentina

A medida que la caries se extiende hacia la dentina la lesión adquiere un color más oscuro (16).

En esta tipología de caries encontramos:

- *Caries oculta*: se refiere a una lesión que no se ve a simple vista, a través de un examen visual; o mejor, según cuanto sea seca el área, se puede observar un esmalte opaco y de color más oscuro, hacía el color gris (8)(15); se puede detectar a través de un examen radiográfico, donde se observará una desmineralización de la dentina (8)(16);
- *Caries activa cavitada*: cuando se habla de cavidad se entiende la presencia física de un hueco en el diente (15); Cuando una lesión es activa, al pasar la sonda se nota un tejido de consistencia blanda, que se puede desprender, y de color marrón/amarillo (8); es una lesión que progresa con el tiempo (15);
- *Caries detenida cavitada*: es una lesión de color marrón oscuro, brillante y con la sonda se nota una superficie lisa y dura (8)(16); no suele progresar con el tiempo y puede ser presente desde años (15).

Sin embargo, a veces es difícil distinguir si una lesión es activa o detenida. Esto porque hay caries que pueden progresar rápidamente, muy lentamente o no progresar en ningún caso; el comportamiento dependerá del equilibrio biológico de la biopelícula (15).

6. Caries de Aparición Temprana

La caries de aparición temprana, conocida también con el nombre caries de biberón, de lactancia o rampante (8), es una de las enfermedades más frecuentes en los niños. Para que se considere como tal, tiene que estar presente en dientes temporales, en niños de 71 meses de edad o menores (15)(17); se define como la presencia de una o más superficies con caries, faltantes u obturadas (17)(7).

Es una forma muy agresiva, destructiva, de progresión rápida y extensa, que puede causar dolor y perdida de los dientes deciduos (7)(18).

Suele aparecer en superficies en las que normalmente no aparecen caries: los dientes más afectados son los incisivos centrales superiores, donde la lesión empieza como mancha blanca cerca del margen gingival (Figura 1) y, si no tratada, puede llevar a una completa destrucción de la corona (8)(15)(17).

Los dientes que presentan una menor probabilidad de padecer este tipo de caries, son los incisivos inferiores (19), porque protegidos por la lengua.

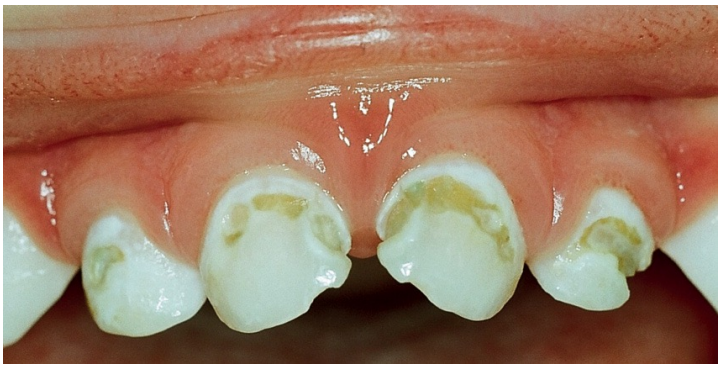

Figura 2: caries rampante o de aparición temprana (15). *Página 12*.

7. Bacterias principales

Entre las bacterias que constituyen un alto porcentaje del total de la microbiota oral se encuentran las que pertenecen al género *Streptococcus*, *Lactobacillus*, *Neisseria*, *Actinomyces*, *Veillonella* y *Leptotrichia* (14).

7.a) Streptococcus

Entre los primeros microorganismos colonizadores de la cavidad oral se encuentran las bacterias del género *Streptococcus*. Son cocos Grampositivos(20) y anaerobios

facultativos, es decir que no necesitan oxígeno para su desarrollo, pero crecen mejor en su presencia.

En estudios recientes, Richards y cols., subdividen los *Streptococcus* en ocho grupos y seis de ellos habitan la cavidad bucal: *mitis, sanguinis, anginosus, salivarius, downei* y *mutans* (2). Entre estos, algunos tienen propiedades patógenas y otras propiedades protectoras. Las más asociadas con el desarrollo de las caries dentales son las que pertenecen a la especie *Streptococcus mutans* (21).

Otra especie fuertemente relacionada a la futura aparición de caries, especialmente en las superficies lisas, es la de las bacterias del grupo *S. sobrinus* (22).

- ***Streptococcus mutans***

El *S. mutans* se considera como uno de los principales patógenos de las caries dentales, al ser microorganismos acidogénicos, acidúricos y acidófilos (23).

Las propiedades cariogénicas de estas bacterias son debidas a sus capacidades de metabolizar sacarosa, sintetizar glucanos extracelulares y de producir ácido láctico. Sobre todo la grande producción de esto último favorece la desmineralización de los tejidos duros, empezando el desarrollo de la lesión cariosa (24)(25). Además, el *S. mutans*, favorecen la adhesión a las estructuras dentarias de otros microorganismos (7).

Los estudios demuestran que la adquisición de los *S. mutans*, en los niños, suele coincidir con la erupción de los dientes primarios (24) y también anterior, porque muchas veces la colonización por parte de estas bacterias cariogénicas es debida a la madre (transmisión vertical), que las transmite a través de los besos, cuando pone en boca el chupete del niño por “limpiarlo”, cuando sopla sobre la comida.

En general, la contaminación de la cavidad bucal de los niños, ocurre a través del paso de saliva por parte de personas vecinas a ellos (transmisión horizontal) (20).

- ***Streptococcus sanguinis***

Como se mencionó previamente, entre los grupos de *Streptococcus* que colonizan la boca no todos son asociados a la aparición de caries; hay grupos que se asocian a la salud dental. Sin embargo, esto no excluye su presencia en caso de caries.

Varios estudios coinciden en decir que los microorganismos que pertenecen a la especie *S. sanguinis*, debido a la catabolización de arginina, tienen la capacidad de elevar el pH (3), disminuyendo así la acidez de la cavidad bucal y prevenir la desmineralización del esmalte dental. Un otro estudio apoya la hipótesis que los *S. sanguinis* tienen un papel importante en el mantenimiento de la salud oral: cuando crecen en un entorno aeróbico inhiben la proliferación de los *S. mutans*, gracias a la producción de peróxido de hidrógeno (26).

7.b) *Lactobacillus*

Lactobacillus es una bacteria Gram-positiva, anaerobia facultativa, acidogénicas y aciduricas. No son exclusivos de la cavidad oral y pueden encontrarse también en el intestino y en la vagina (27)(20).

Los *Lactobacillus* se consideran un colonizador posterior y suelen estar presentes en mayor número cuando la caries progresa hacia la dentina, que en las primeras etapas de caries en esmalte (23). Esto porque estos microbios son tolerantes al ácido y pueden realizar glicolisis a niveles de pH muy bajos (pH 3).

Son grandes productores de ácido láctico, cuya presencia es fundamental para

generar y mantener un medio con pH bajo (28).

7.c) *Neisseria*

Es un coco gram-negativo que, según las condiciones de donde crece, metaboliza glucosa para producir ácidos orgánicos y utilizar ácido láctico (14).

Predomina en sujetos sin caries, debido a que contribuye en el metabolismo del ácido linoleico. Este último es un agente antimicrobiano que inhibe la proliferación de patógenos orales (6).

De igual manera se encuentra en estadios de caries avanzada, por su producción de ácido láctico (7).

7.d) *Actinomyces*

Son bacilos Gram-positivos, anaerobios obligados o anaerobios facultativos, que viven habitualmente en la cavidad oral de los niños, con propiedades acidogénicas y aciduricas, que contribuyen en el proceso de desmineralización del esmalte (29).

Intervienen en la formación de placa dental, favoreciendo la coagregación de bacterias más aciduricas, como *Veillonella* y *Streptococcus* (14)(30).

El género *Actinomyces* se asocia al comienzo de caries, porque parece que en medios muy ácidos (inferiores a pH 4), es decir en estadios avanzados de caries, algunas especies desaparecen, perdiendo sus actividades (14).

7.e) *Veillonella*

Las bacterias del género *Veillonella* son microorganismos Gram-negativos, anaerobios, que metabolizan el lactato (23).

Se suelen asociar a caries porque actúan como reservorio de ácido y favorece el crecimiento de bacterias acidogénicas (6).

7.f) *Leptotrichia*

La bacteria del género Leptotrichia es un microorganismo Gram-negativo, anaerobio que suele habitar en la cavidad oral.

Por su alto potencial cariogénico, debido a la capacidad de fermentar monosacáridos y disacáridos en ácido láctico, se suele asociar a caries (31).

Sin embargo, en cuanto su papel en la boca, existen opiniones contradictorias.

Un recién estudio asocia este microbio a pacientes sin caries, debido a su capacidad de sintetizar flavonoides. Estos últimos ayudarían a inhibir la agregación de *S. mutans* (6).

7.g) *Scardovia wiggsiae*

Scardovia wiggsiae es una bacteria acidógena y ácido-tolerante, con capacidad de tolerancia al ácido a niveles de pH muy bajos (26), que pertenece a la familia *Bifidobacteriaceae* (32).

Objetivos

Se plantea como objetivo general identificar los microorganismos que con más frecuencia se encuentran en cavidad oral de niños/as sanos/as con diferentes estadios de desarrollo de las caries.

Como objetivos secundarios:

- Determinar cuáles de estos microorganismos son cariogénicos y cuáles tienen un efecto protector;
- Identificar las correlaciones entre ciertos tipos de bacterias y la salud/no salud de los dientes

Metodología

El estudio se realizó mediante una búsqueda bibliográfica en buscadores científicos PubMed y Medline, utilizando palabras llaves como #MutansStreptococci, #ECC, #MicroflorDentalDecay, #OralBiofilm, #DentalPlaque y libros de consulta. El acceso ha sido a través la biblioteca Crai de la Universidad Europea de Madrid.

Para facilitar y organizar la selección de los artículos se han aplicado criterios de selección.

Criterios de inclusión:

- Artículos científicos de los últimos diez años.
- En idioma inglés y español.

- Artículos de revisión bibliográfica y ensayos clínicos.

Criterios de exclusión:

- Muestras de niños/as que han tomado antibióticos en el último mes.

A continuación, en la tabla 1 se muestra un resumen de las referencias seleccionadas.

Autor	Año	Tipo de estudio	Edad niños	Conclusión
Seneviratne CJ y cols.	2011	No reportado	No reportado	La placa dental es el agente etiológico por la mayoría de las enfermedades dentales
Tanner ACR y cols.	2011	Estudio longitudinal	2-6 años	Las bacterias más asociada a caries son S. mutans y S. wiggsiae
Teanpaisan R y cols.	2011	No reportado	2-5 años	Algunas especies de Lactobacillus pueden tener un efecto inhibidor contra S. mutans y S. sobrinus
Hughes CV y cols.	2012	Estudio longitudinal	2-6 años	S. mutans y S. sobrinus están asociado a caries de inicio temprano
Gross EL y cols.	2012	Estudio de casos y controles	1-3 años	Asocian a caries S. mutans, S. sobrinus, V. parvula, V. dispar y V. atypica
Cuenca E y Baca P	2013	Manual de teoría	/	/
Jiang W y cols.	2013	Estudio de casos y controles	3-6 años	Niños con caries tenían altos niveles de Streptococcus, Actinomyces y Granulicatella
Jiang W y cols.	2014	Estudio de casos y controles	3-7 años	Asocian a mancha blanca Actinomyces y Corynbacterium; a caries cavitada Streptococcus, Lactobacillus y Scardovia
Struzycha I	2014	Revisión bibliográfica	No reportado	
Rojas FS y Echevvería LS	2014	Revisión bibliográfica	/	La caries es una enfermedad multifactorial, que incluye factores genéticos, dietéticos y socioeconómicos
Krzyściak W y cols	2014	Revisión bibliográfica	No reportado	La virulencia de S. mutans depende de las condiciones ambientales
Kianoush N y cols.	2014	No reportado	No reportado	Colonización de bacterias diferentes según el gradiente del pH
Morou-Bermudez E y cols.	2015	Estudio longitudinal	3-6 años	La producción de álcalis por las enzima ureasa puedes ser un importante determinante ecológico en la placa dental de niños
Takahashi N	2015	Revisión bibliográfica	/	La actividad metabolica de los microbios está influenciada por el

				medio oral. Este ultimo puede ser modificado por las bacterias
Edwina Kidd OF	2016	Manual de teoría	/	/
Xuedong Z	2016	Manual de teoría	/	/
Corrêa-Faria P y cols.	2016	Estudio longitudinal	1-5 años	La presencia de caries en dientes primarios es un factor de riesgo para el desarollo de nuevas lesiones
Richards VP y cols.	2017	Estudio longitudinal	2-7 años	Niños sin caries: Streptococcus, Actinomyces, Haemophilus y Neisseria. Con caries: S. mutans, V. parvula, V. dispar, S. wiggsiae y L. salivarius
Pitts N y cols.	2017	Revisión bibliográfica	/	El equilibrio entre factores protectores y patológicos influye en el comienzo y progresión de la caries
Anil S y cols.	2017	Revisión bibliográfica	/	Los mayores factores de riesgos en caries de aparición temprana son microbiologicos, dietéticos y ambientales
Scalioni F y cols	2017	Estudio transversal	No reportado	La reducción de caries dentales no se atribuye a la técnica de hibridación in situ de S. mutans en la saliva inferior.
Kim BS y cols.	2018	Estudio longitudinal	No reportado	Microbiota salival es más variable en niños sin caries. En caries en dentina S. mutans y sin caries Neisseria, Lautropia y Leptotrichia.
Banas JA y cols.	2018	Revisión bibliográfica	/	Streptococcus mutans tiene un papel multifactorial en el proceso de la caries. Se sigue la hipótesis de la placa ecológica.
Hajishengallis E y cols.	2018	Revisión bibliográfica	/	Las caries de aparición temprana son una consecuencia de la interacción entre microorganismos, huésped y dieta.
Ghazal TS y cols.	2018	Analisis secundario de datos de un estudio Longitudinal	1 año	Relación entre la adquisición de S. mutans y caries dental
Velsko IM y cols.	2019		/	Existen diferencias microbianas sistemáticas entre la placa y el cálculo relacionadas con la fisiología de la biopelícula.

Hurley E y cols.	2019	Estudio de cohorte	≤ 5	CFS: Capnocytophaga, Porphyromonas, Leptotrichia, Neisseria, H. parainfluenzae y S. sanguinis. CAC: S. mutans, S. wiggsiae, BIfidobacterium y Neisseria.
Fakhruddin KS y cols.	2019	Revisión bibliográfica	1,5-11 años	Sin caries: S. sanguinis, N. flavescens y Corynbacterium. Con caries: Scardovia wiggsiae, Granulicatella y Firmicutes.
Dashper SG y cols.	2019	Estudio longitudinal	2 meses-4 años	Sujetos con altos niveles de S. mutans, S. sobrinus y V. parvula desarrollan caries
Valm AM	2020	Revisión bibliográfica	No reportado	La caries dental está mediada por interacciones sinérgicas dentro de la comunidad y influyen el comportamiento y el ambiente.
Kahharova D y cols.	2020	Estudio longitudinal	1-4 años	Ninos sanos: Streptococcus, Haemophilus, Neisseria y Actinomyces.

Tabla 1: Artículos seleccionados: autores, año de publicación, características metodológicas y conclusiones.

Resultados

Gross EL y cols., en 2012 (23), realizan un estudio di casos y controles sobre niños a partir de los 12 hasta los 36 meses de edad. El grupo de casos incluye 36 niños con caries y en el grupo de control los participantes (36) están libres de caries. Entre los requisitos de inclusión del primer grupo, los niños tienen que presentar al menos dos incisivos superiores que tienen una lesión de mancha blanca y/o una lesión cavitada no mayor de 1 mm. En todos los sujetos se recolecta una muestra de placa de un diente con esmalte sano. En los que presentan caries, las muestras se recogen desde las zonas de mancha blanca y lesión cavitada. Para individuar la composición de la

microbiota utilizan el análisis del gen 16S rRNA. Al término del estudio, asocian a niños libres de caries microorganismos del género *Streptococcus* y *Neisseria*, en particular las especies *S. sanguinis, S. mitis, N. flava, N. mucosa y N. pharyngis.* En los niños que presentan caries son observados bacterias de las especies *S. mutans, S. sobrinus, V. parvula, V. dispar* y *V. atypica*.

En 2013 Jiang W y cols. (30), realizan un estudio sobre 40 niños, 20 con caries (la patología tiene que afectar al menos diez dientes) y 20 sin lesiones, de edad entre 3 y 6 años. Se analizan muestras de placa supra gingival recogida en superficies intactas de al menos cuatro sitios diferentes. Los microorganismos se identifican a través el análisis del gen 16S rRNA. Al final del estudio observan que los niños con caries severa presentan un aumento de los niveles de los géneros *Streptococcus, Actinomyces* y *Granulicatella*.

Jiang W y cols., en 2014 (14) estudian 60 niños (edad entre 3 y 7 años), treinta sin caries y treinta con caries activa, en un mínimo de tres dientes, en esmalte y/o dentina. Se recolectan muestras de placa supra gingival. En los dientes sanos utilizan el mismo método del estudio previo y en los cariados la recogen en tres sitios diferentes (esmalte intacto, mancha blanca y dentina cavitada). En este último estudio los niños sin patología presentan niveles más altos, respecto a los otros grupos, de trece géneros, entre ellos se encuentran *Capnocytophaga*, *Fusobacterium*, *Porphyromonas*, *Treponema*, *Eikenella* y *Abiotrophia.* En caso de mancha blanca identifican las bacterias *Actinomyces* y *Corynbacterium* en mayor proporción respectos a los demás. Además, en lesiones de dentina cavitadas observan ocho géneros, como *Lactobacillus*, *Scardovia* y *Streptococcus*. Al final del estudio muestran

que la microbiota de sujetos sanos es más variable y que la de las lesiones iniciales es más compleja que la de las caries avanzadas.

En un estudio longitudinal llevado a cabo por Morou-Bermudez y cols. en 2015 (31), son examinadas 82 muestras de placa supra gingival recogida en 44 niños, de edad entre 3 y 6 años, en dos momentos a un año de distancia. Al inicio del estudio hay dos grupos de participantes: sin caries y con caries que, pero no afectan la dentina. La placa se recoge de cualquier superficie dental disponible y los microbios son identificados a través del análisis del gen 16S rRNA región V3-V5. En este estudio se explica que *Actinomyces naeslundii, Haemophilus parainfluenzae* y *Streptococcus salivarius* son productores de la enzima ureasa. Esto puede prevenir el desarrollo de caries, porque ayuda a neutralizar los ácidos producidos por otros microorganismos acidogénicos. En particular, cataliza la hidrólisis de la urea en ammonia y ácido carbónico, llevando a un aumento del pH local. Según este estudio, la bacteria *H. parainfluenzae* es relacionada con altos niveles de producción de ureasa. Entre los microorganismos que encuentran, se observó en caries activa el género *Leptotrichia;* entre las especies nominan *L. shaii* y *L. buccalis*. Con su estudio argumentan que los niveles de *Leptotrichia*, en particular la especie *L. buccalis*, aumentan al disminuir de la ureasa (produce álcalis). Otros que aumentan al disminuir de los niveles de actividad de la ureasa son las bacterias que pertenecen al género *Actinomyces, Corynbacterium, Prevotella* y *Streptococcus*. También hay bacterias que disminuyen al disminuir de la ureasa, como *Capnocytophaga* y *Porphyromonas.*

Richard y cols., en 2017 (3), realizan un estudio longitudinal de la duración de un año,

sobre 55 niños, de edad entre 2 y 7 años. Los participantes se agrupan según el estadio de caries y se seleccionan niños sin presencia de caries (ni en el momento del estudio ni en el pasado), niños con caries activa solo en esmalte y niños con al menos dos caries activas en dentina cavitada y no restauradas. Al final las muestras que se obtienen son 186, porque la placa se saca de al menos dos sitios dentales diferentes, pero con mismas condiciones de salud. Identifican como bacterias principales, en niños libres de caries, los *Streptococcus, Haemophilus, Actynomices* y *Neisseria*. Más específicatamente los del grupo *S. sanguinis, H. parainfluenzae, A. naeslundii* y *N. flavescens, N. pharynges, N. flava* y *N. mucosa*.

Además observan un alto número de *Lautropia mirabilis*, *Corynebacterium durum* y *Rothia aeria*. En presencia de caries activa en dentina, se detecta un número alto de *V. parvula* y *V. dispar* y *Leptotrichia sp.498*, aunque la especie dominante es la de *S. mutans*. Otros microorganismos presentes en altos niveles en caso de patología son *Scardovia wiggsiae* y *Lactobacillus salivarius*.

En un estudio longitudinal realizado en 2019 por Kahharova y cols (33), se seleccionan 503 niños de un año y sus principales cuidadores en función de determinadas características. A la edad de 1, 2 y medio y 4 años, se recolectan muestras de saliva y placa tanto del niño como de los cuidadores. Con el tiempo, se presentan cada vez menos personas en todos los controles y, al final de los tres controles, solo se presentan 266 parejas. De estos, 119 niños nunca han tenido caries en ningún momento, y son los que se toman en cuenta. También se excluyen aquellos que tienen una remineralización de la caries. Al final del estudio, se observa que los

microorganismos predominantes en la saliva de niños sanos en edad compresa entre 1 y 4 años eran *Streptococcus, Haemophilus* y *Neisseria*. En la placa dental no se encuentra el género *Haemophilus,* pero sí el género *Actynomices*, además de *Streptococcus* y *Neisseria*.

Hurley E y cols. (7) en 2019 realizan un estudio de cohorte sobre un total de 138 niños de edad igual o inferior a 5 años (68 con caries y 70 sin caries) (7). Recogen tres muestras: microbiota de lesión dentinaria profunda obtenida de un trozo de dentina, clasificada como cavidad activa con caries (CAC), saliva de niños con caries activa (CAS), saliva de niños sin caries (CFS). Las bacterias se identifican con el estudio del gen 16S rRNA región V4-V5. Hurley y cols. encuentran una similitud entre la microbiota de las muestras de saliva (CAS y CFS), donde encuentran altos niveles de *Haemophilus*, *Porphyromonas* y *Leptotrichia*. Este último es más abundante en la muestra de saliva de niños sin caries. De todas formas, en el grupo CFS predominan bacterias del género *Capnocytophaga*, *Leptotrichia*, *Neisseria* y *Porphyromonas* y las especies *S. sanguinis* y *H. parainfluenzae.* Como microorganismos asociados al grupo CAC identifican como más abundantes *S. mutans*, *Scardovia wiggsiae*, *Bifidobacterium*, *Prevotella* y *Neisseria*.

Otra investigación que se tiene en cuenta es la realizada por Dashper SG y cols. (29). Realizan un estudio longitudinal comparando 134 parejas de niños (edad entre 2 meses y 4 años) con sus madres seleccionadas por ellos, con las 259 parejas estudiadas por VicGin. Se recolectan muestras en diferentes estadios de edad: 1.9, 7.7, 13.2, 19.7, 39, 48.6 y 60 meses. Se observa que la diferenciación de la microbiota

empieza en un momento en que los niños todavía no han padecido caries; los sujetos donde eran presentes altos niveles de *S. mutans, S. sobrinus* y *V.parvula*, en los controles sucesivos, alrededor de los 5 años, desarrollan la patología. Entre otras bacterias, este estudio asocia a caries las bacterias *Scardovia wiggsiae* y *Leptotrichia shaii.*

Discusión

1. Streptococcus mutans y Streptococcus sobrinus

Comparando los estudios se observa que existe acuerdo unánime sobre el papel de la especie *Streptococcus mutans* en la desmineralización de las superficies dentales. Todos los estudios coinciden en asociar esta especie con caries (3)(14)(7)(23)(29)(30)(31)(33), tanto en lesiones iniciales (mancha blanca) como en estadios avanzados, en dentina. Sin embargo, su presencia no es exclusiva de la enfermedad; este microorganismo es considerado uno de los primeros colonizadores de la cavidad oral (20), y forma parte del biofilm dental del niño antes de que este desarrolle la enfermedad. Para que *S. mutans* pueda iniciar y favorecer la progresión de la caries, debe existir una intervención combinada con otros microorganismos, porque su presencia es sí necesaria, pero no suficiente (24).

Otra especie perteneciente al género *Streptococcus* que se asocia con caries es la de *Streptococcus sobrinus*. Gross EL y cols.(23) no la observan en ningún niño libre de patología; por el contrario solo está presente en los participantes con caries y en ocasiones sus niveles son más altos que los de *S. mutans* (siempre presentes).

Por tanto, se plantea la hipótesis de que *S. sobrinus* pueda ser un predictor de caries. A favor de la hipótesis antes mencionada, Dashper SG y cols.(29) observan que los niños que a los 3 años tienen altos niveles de *S. sobrinus*, junto a *S. mutans* y *V. parvula*, a los 4 y medio desarrollan la enfermedad.

2. Scardovia wiggsiae

Otra bacteria muy común en la caries es la de la especie *Scardovia wiggsiae*. Jiang (14), Richard (3) y Hurley (7) la observan en niños con lesiones profundas de dentina. También Dashper y cols. (29) la identifican en caso de patología, pero no especifican el estadio de la lesión.

Al contrario, Gross EL y cols. (23) en su estudio no diagnostican su presencia; es posible que su ausencia se deba a que los niños involucrados en el estudio no deben tener lesiones cavitadas mayores de 1 mm. Por lo tanto, se puede pensar que las caries afectan solo al esmalte o que, aunque estando en dentina, no son lo suficientemente profundas.

Incluso Morou-Bermudez y cols. (31) no observan la especie *Scardovia wiggsiae*. Entre los criterios de inclusión a cumplir, para poder participar en el estudio, está que en caso de caries esta no afecte la dentina.

Los estudios que han tomado en consideración caries que no afectan la dentina no consiguen observar esta bacteria, mientras la mayoría de los estudios que encuentran la especie la han visto en lesiones profundas.

Por lo tanto, se puede hipotetizar que la especie *Scardovia wiggsiae* intervenga en el proceso cariogénico solo en fase avanzada.

3. Leptotrichia

Con algunas opiniones en parte discordantes, una bacteria que suele aparecer en caso de patología es la perteneciente al género *Leptotrichia*.

Dashper SG y cols. (29) y Morou-Bermudez y cols. (31) asocian las especies *Leptotrichia shaii y Leptrotrichia buccalis* a caries.

Con respecto a la especie *L. shaii*, Richard y cols. la observan entre las bacterias en niños libres de caries (3). Describen una disminución de este microorganismo al comparar la muestra recolectada al inicio del estudio, en niños sin caries, con la obtenida a los 12 meses, en niños con caries activa en esmalte. Observan altos niveles sobre todo en ausencia de los microorganismos *Neisseria* y *S. sanguinis* (3).

A pesar de este desacuerdo con respecto a *L. shaii*, el estudio coincide con los estudios previamente citados, al asociar otras especies de *Leptotrichia* con caries; entre las más frecuentemente asociadas a patología se encuentran *L. wadei*, *L. sp.498* y *L. buccalis* (3).

4. Neisseria

Los estudios clínicos analizados evidencian niveles elevados del género *Neisseria* en niños sin caries.

El estudio de Gross EL y cols. (23) asocia a niños libres de caries las especies *N. flava*, *N. pharyngis* y *N. mucosa*. Además se nota que los niños que pertenecen al

grupo de control que no desarrollan caries tienen niveles más altos de *Neisseria* en comparación con los que padecen la enfermedad.

Estas tres especies se observan, en niños sin patología, también en el estudio de Richard y cols.(3). También describen y asocian a salud la bacteria *Neisseria flavescens*, una especie asacarolítica, entonces no productora de ácido. Debido a esta característica se puede asumir que no interviene en el proceso de desmineralización. El estudio de Hurley y cols. (7) está parcialmente de acuerdo. Los autores reflejan niveles de *Neisseria* más bajos en los sujetos en salud en comparación con los de los niños con patología en dentina. Sin embargo, informan que algunos estudios asocian la especie *N. flavescens* a la salud bucal.

5. Streptococcus sanguinis y Streptococcus mitis

En niños con salud bucal, considerando el género *Streptococcus*, destacan las especies *Streptococcus mitis* y *Streptococcus sanguinis*. Esta última se considera beneficioso por su capacidad de elevar el pH oral debido a la catabolización del aminoácido arginina (3). Su presencia destaca en los estudios de Gross EL y cols.(23), Jiang y cols. (14), Richard y cols. (3) y Hurley y cols(7).

En cuanto a la especie *S. mitis* no está descrita en muchas investigaciones. Los únicos autores que la describen son Gross EL y cols. (23) y Dashper y cols. (29).

En este último estudio (29) se aprecia que en las muestras de saliva de niños de 1.9 meses predominan siete microbios de colonización temprana, entre ellos *S. mitis*. En muestras posteriores se observa que las proporciones de esta bacteria disminuyen gradualmente con el aumento de la edad y por la colonización de bacterias acidogénicas.

Es necesario tener presente que la desmineralización ocurre cuando el pH de la cavidad oral disminuye, por lo tanto cuanto más bajo es, más ácido es el entorno oral. Da los estudios no emerge si *S. mitis* tiene un papel activo en la protección de la caries, pero se deduce que en el momento en que al medio oral se vuelve más ácido, entonces que se favorece la desmineralización, estas bacterias desaparecen.

Se puede deducir entonces que esta bacteria no está asociada a la formación de la caries.

6. Haemophilus parainfluenzae

Una bacteria que aparece con frecuencia en los estudios es la especie *Haemophilus parainfluenzae*. Los estudios realizados por Morou-Bermudez y cols. (31), Richard y cols.(3), Kahhrova y cols. (33) y Hurley y cols. (7) relacionan su presencia a ausencia de caries.

En particular Morou-Bermudez (31), con su estudio sobre la actividad de la ureasa, una enzima con la capacidad de neutralizar los ácidos producidos por otras bacterias y elevar los valores del pH, observa que *H. parainfluenzae* es productor de dicha enzima. Además nota que la bacteria es más abundante cuando la actividad de la ureasa es mayor. Entonces podemos hipotetizar su papel protector.

7. Actinomyces

Los estudios de Kahhrova y cols.(33) y Richard y cols. (3) asocian a salud el género *Actinomyces*. En particular, Richard y cols. (3), identifican la especie *A. naeslundii*, pero observan que entre las bacterias relacionadas con salud, es la única que no disminuye a medida que avanza la cavidad.

Jiang W y cols. (14) observan altos niveles del microbio en niños con lesión de mancha blanca si comparados con los valores de los participantes con caries cavitada o sin caries.

Morou-Bermudez y cols. (31) con su estudio afirman que la especie *A. naeslundii* es productora de la enzima ureasa. Por lo tanto, de acuerdo con las actividades beneficiosas de la ureasa, debería ser protector. En cambio, durante el estudio, observan que unos géneros de bacterias, dentro los cuales el género *Actinomyces*, aumentan a medida que disminuye la actividad de la ureasa. De todas formas, afirman que no es posible clasificar las especies que tenían la actividad opuesta a la ureasa.

Por tanto, según las características descritas en el apartado “Actinoymces”, previo a la descripción de los estudios, podemos pensar que esta bacteria esté presente en caries de esmalte y su presencia en dientes sanos puede explicarse a inicio de desmineralización, aún no visible.

8. Lactobacillus

Una bacteria de la que los estudios tienen opiniones contradictorias es *Lactobacillus*. Jiang W y cols. (14) y Richard y cols. (3), en caso de lesiones activas con dentina cavitada, la observan entre los microorganismos predominantes, en particular la especie *L. salivarius* (3).

Otros autores, como Hurley y cols. (7), afirman que el género *Lactobacillus*, a pesar de su papel en la progresión de caries, se encuentra en niveles muy bajos en pacientes con caries en dentina, en comparación con otros géneros. A sugerencia de un estudio de Rôça y cols., explican la baja presencia de *Lactobacillus* en caries muy avanzadas, a un cambio en la microbiota, que pasa de ser cariogénica a una microflora que estimula una mayor progresión hacia la pulpa.

Su ausencia en algunos estudios (Gross EL y cols. (23) y Morou-Bermudez y cols. (31)) puede explicarse por los criterios de inclusión de sus estudios, es decir, que los niños participantes deben tener una lesión solo en esmalte o una que no sea mayor de 1 mm (es probable que no llegue en dentina).

Sin embargo, a pesar de la cantidad, en los estudios el género *Lactobacillus* solo se observa en presencia de patología con afectación de dentina.

9. Veillonella

Un microorganismo que se observa con frecuencia en caso de caries en dentina es el género *Veillonella*. Entre las especies que destacan se pueden apreciar *V. parvula*, *V.dispar* y *V. atipyca*, como observan en los estudios de Gross EL y cols. (23), Jiang W y cols. (14) y Richard V y cols. (3).

En cuanto a *V. parvula*, Dashper y cols. (29) en sus estudios, sostienen que los niños que a los 3 años presentan altos niveles de este microbio, junto a *S. mutans* y *S. sobrinus*, a los 4 y medio desarrollan la enfermedad. Entonces lo consideran como una bacteria predictora de caries.

Sin embargo, todos los estudios coinciden en asociar el género *Veillonella* a caries.

10. Otras bacterias

Entre las otras bacterias que se pueden apreciar en algunos estudios se encuentran los géneros *Porphyromonas*, *Capnocytophaga* y *Corynbacterium*.

Porphyromonas y *Capnocitophaga* suelen ser asociado a salud. Morou-Bermudez y cols. (31) observan que sus valores disminuyen al disminuir de la ureasa.

Por lo tanto, se puede deducir que cuando los niveles de pH bajan (medio más ácido) se tendrán niveles menores.

En cuanto al género *Corynbacterium*, Jiang W y cols. (14) detectan niveles más altos en manchas blancas y Morou-Bermudez y cols. (31) observan que sus valores aumentan al disminuir de la ureasa (produce álcalis). Pero, estudios más recientes (Richard V y cols. (3), Kahharova y cols. (33) y Hurley y cols. (7)) lo detectan en grandes cantidades en niños sin caries.

11. Hipótesis de la placa ecológica

En general en los estudios analizados, en niños libres de caries se observa una gran variedad de microorganismos que disminuyen a medida que la desmineralización dental progresa.

Entre las especies principales de los sujetos en salud se identifican *Streptococcus sanguinis*, *Haemophilus parainfluenzae* y el género *Neisseria*. Entre otras, se observan bacterias del género *Capnocytophaga*, *Porphyromonas*, *Corynbacterium* y la especie *Streptococcus mitis*.

Esto podría sugerir la hipótesis de la placa ecológica, como lo argumentan Gross EL y cols. (23) y Jiang y cols. (14) en sus estudios.

Esta teoría define la caries como resultado de una alteración de la microbiota comensal que ocurre cuando hay una ingesta frecuente de carbohidratos y las especies productoras de ácido aprovechan sus presencias bajando el pH. Esto da como resultado una desaparición de bacterias sensibles a los ácidos y un predominio de microorganismos tolerantes a ellos (23).

También Hurley y cols. (7) en su estudio observan que las muestras de placa en caries de dentina muestran una menor diversidad de la microbiota en comparación con las muestras de saliva de los niños sanos.

Mientras los otros estudios no comentan sobre esto, Morou-Bermudez y cols.(31) afirman que no se nota mucha variación de las bacterias analizadas entre los diferentes estadios de la caries; pero declaran que los resultados pueden haber sido alterados por la técnica de análisis de las muestras. De hecho recolectan placa de todas las superficies dentales disponibles, con y sin caries sin diferenciarlas.

Entonces este estudio no da opiniones contrastantes.

Conclusiones

- En niños con un estado de salud bucal se encuentran las bacterias de la especie *Haemophilus parainfluenzae*, *Streptococcus sanguinis*, del género *Capnocytophaga*, *Corynbacterium* y *Porphyromonas*.
- El género *Neisseria* si está presente en proporciones elevadas ayuda a proteger de las caries. Por lo tanto, dicho microorganismo podría tener un carácter protector.

- Elevados niveles de *Streptococcus mutans* y *Streptoccous sobrinus* se suelen considerar como predictores de caries.
- Entre las bacterias en los diferentes estadios de la caries se encuentran *Actinomyces*, *S. mutans*, *S. sobrinus*, *Leptrotrichia*, *Lactobacillus* y *Scardovia wiggsiae*.
- De acuerdo con los resultados de los estudios, es posible asociar el género *Actinomyces* a caries con afectación del esmalte.
- Se puede deducir que el género *Lactobacillus* solo se asocia a caries, en particular a caries profundas con afectación de dentina.
- Se puede deducir que la especie *Scardovia wiggsiae* está presente en lesiones profundas que involucran la dentina.
- Se puede pensar que la patología sigua la hipótesis de la placa ecológica porque a medida que la caries avanza se puede observar una disminución en la variedad de los microorganismos y que permanecen microbios con tolerancia al ácido.

Responsabilidad social

La literatura científica afirma que existen bacterias responsables de producir caries dental. Entre estas bacterias caben destacar la especie *Streptococcus mutans*.

Sin embargo, cada bacteria es característica de una fase de desmineralización que sucesivamente conlleva a las caries.

La caries se puede prevenir mediante correctas técnicas de higiene, dieta con reducido aporte de carbohidratos y visitas periódicas con el objetivo de diagnosticar

de manera precoz la mancha blanca, que constituye el primer signo de la patología. Por lo tanto, se puede disminuir la incidencia mediante la prevención. En ámbito comunitario y social, el diagnóstico precoz constituye la base para el éxito del tratamiento.

Es posible que a través de análisis específicos, como la prueba de PCR para el análisis del gen 16S rRNA, muy utilizada en los estudios analizados, nos pueda ayudar en la detección temprana de bacterias cariogénicas como *S. mutans* y *S. sobrinus*.

Gracias a estas pruebas, es posible que la población infantil reciba el tratamiento más idóneo en el momento más adecuado y que además sea consciente sobre la probabilidad de riesgo de padecer caries.

Además de prevenir la aparición de la enfermedad en dientes deciduos, es importante tratarla lo antes posible para evitar que se afecte el diente permanente correspondiente.

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Anexos

Critical Reviews in Oral Biology & Medicine

Oral Microbiome Metabolism: From "Who Are They?" to "What Are They Doing?"

Journal of Dental Research
2015, Vol. 94(12) 1628–1637

DOI: 10.1177/0022034515606045
jdr.sagepub.com

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Abstract

Recent advances in molecular biology have facilitated analyses of the oral microbiome ("Who are they?"); however, its functions (e.g., metabolic activities) are poorly understood ("What are they doing?"). This review aims to summarize our current understanding of the metabolism of the oral microbiome. Saccharolytic bacteria—including *Streptococcus, Actinomyces*, and *Lactobacillus* species—degrade carbohydrates into organic acids via the Embden-Meyerhof-Parnas pathway and several of its branch pathways, resulting in dental caries, while alkalization and acid neutralization via the arginine deiminase system, urease, and so on, counteract acidification. Proteolytic/amino acid–degrading bacteria, including *Prevotella* and *Porphyromonas* species, break down proteins and peptides into amino acids and degrade them further via specific pathways to produce short-chain fatty acids, ammonia, sulfur compounds, and indole/skatole, which act as virulent and modifying factors in periodontitis and oral malodor. Furthermore, it is suggested that ethanol-derived acetaldehyde can cause oral cancer, while nitrate-derived nitrite can aid caries prevention and systemic health. Microbial metabolic activity is influenced by the oral environment; however, it can also modify the oral environment, enhance the pathogenicity of bacteria, and induce microbial selection to create more pathogenic microbiome. Taking a metabolomic approach to analyzing the oral microbiome is crucial to improving our understanding of the functions of the oral microbiome.

Keywords: amino acid, carbohydrate, dental caries, oral biofilm, oral malodor, periodontal diseases

Introduction

Oral biofilm is a thin microbial film that covers the surfaces of the human oral cavity. It consists of a tremendous number of microorganisms from a broad range of species. It is commonly considered that oral biofilm plays a role in oral health and disease. Recent advances in molecular biological techniques, such as next-generation DNA sequencing, have made it possible to analyze the oral microbiome more precisely and efficiently and to answer the question "Who are they?", which has resulted in the suggestion that an association exists between the microbial profile and oral health and disease. However, the functions of oral biofilm are poorly understood (i.e., "What are they doing?"), since they are based on its metabolic properties, which are complex and have not been fully elucidated.

Carlsson and his colleagues pioneered metabolic studies of caries-associated bacteria in the 1970s and 1980s (Carlsson 1986), while molecular biological analyses of microbial composition became common in biofilm research. In the 1990s, Marsh (1994) proposed the "ecological plaque hypothesis," in which the microbiota shifts from being healthy toward a pathogenic state through interactions between bacterial activity and the environment. He also stressed the importance of bacterial metabolic activity for oral diseases, such as dental caries and periodontal disease. During these periods, several researchers, including myself, continued to study the biochemical characteristics of bacterial metabolism—for example, metabolic pathways, mutual interactions between bacterial metabolism and environmental factors, and their involvement in oral health and disease (Takahashi 2005). In the 21st century, Kleinberg (2002) explored a mixed-bacteria ecologic approach to understand the role of bacterial metabolism in dental caries, and Nyvad and I (Takahashi and Nyvad 2008, 2011) extended Marsh's hypothesis and proposed the "extended ecological caries hypothesis," in which bacterial metabolic activity (acid production) regulates the development of caries through bacterial acid adaptation and selection in the oral microbial ecosystem. These findings suggest that bacteria-mediated oral diseases—such as dental caries, periodontal disease, and oral malodor, which occur at the interface between oral surfaces and indigenous microbiota—possess an etiology different from that of infectious diseases based on Koch's postulates.

In this review, I describe 1) the fundamental metabolic framework of oral bacteria and its relationship with oral diseases, such as caries, periodontal disease, and oral malodor;

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Published in final edited form as:

Microbiol Spectr. 2018 October ; 6(5): . doi:10.1128/microbiolspec.GPP3-0042-2018.

Biology of Oral Streptococci

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Summary

Bacteria belonging to the genus *Streptococcus* are the first inhabitants of the oral cavity which can be acquired right after birth and thus play an important role in the assembly of the oral microbiota. In this chapter, we will discuss the different oral environments inhabited by streptococci and the species that occupy each niche. Special attention is given to the taxonomy of *Streptococcus* as this genus is now divided into 8 distinct groups where oral species are found in 6 of them. Oral streptococci produce an arsenal of adhesive molecules that allow them to efficiently colonize different tissues in the mouth. Also, they have a remarkable ability to metabolize carbohydrates via fermentation thereby generating acids as byproducts. Excessive acidification of the oral environment by aciduric species such as *Streptococcus mutans* is directly associated with the development of dental caries. However, less acid-tolerant species such as *Streptococcus salivarius* and *Streptococcus gordonii* produce large amounts of alkali displaying and important role in the acid-base physiology of the oral cavity. Another important characteristic of certain oral streptococci is their ability to generate hydrogen peroxide that can inhibit the growth of *S. mutans*. Thus, oral streptococci can also be beneficial to the host by producing molecules that are inhibitory to pathogenic species. Lastly, commensal and pathogenic streptococci residing in the oral cavity can eventually gain access to the bloodstream and cause systemic infections such as infective endocarditis.

The oral environment

Warm, moist, and rich in nutrients, the oral cavity provides an ideal environment for colonization by a community of bacteria, fungi, protozoa, archaea, and viruses, often in the form of a complex structure called biofilm, or plaque (1). In addition, several microbial niches exist within the oral cavity (e.g. cheek, gingiva, teeth, tongue, palate) that vary in nutrient content, pH, oxygen tension, and shear force due to salivary flow and mastication. These physico-chemical characteristics select for suitable microorganisms for each oral niche such that the microbial compositions can differ greatly from one site to another. Saliva is the biological fluid of the oral cavity and its microbial composition is a collection of bacteria that have shed from various oral niches. As an easily accessible body fluid, saliva

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BACTERIAL INFECTIONS

Microbiomes of Site-Specific Dental Plaques from Children with Different Caries Status

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ABSTRACT The oral microbiota associated with the initiation and progression of dental caries has yet to be fully characterized. The Human Oral Microbe Identification Using Next-Generation Sequencing (HOMI*NGS*) approach was used to analyze the microbiomes of site-specific supragingival dental plaques from children with different caries status. Fifty-five children (2 to 7 years of age) were assessed at baseline and at 12 months and grouped as caries free (CF), caries active with enamel lesions (CAE), and caries active with dentin carious lesions (CA). Plaque samples from caries-free tooth surfaces (PF) and from enamel carious lesions (PE) and dentin carious lesions (PD) were collected. 16S community profiles were obtained by HOMI*NGS*, and 408 bacterial species and 84 genus probes were assigned. Plaque bacterial communities showed temporal stability, as there was no significant difference in beta diversity values between the baseline and 12-month samples. Irrespective of collection time points, the microbiomes of healthy tooth surfaces differed substantially from those found during caries activity. All pairwise comparisons of beta diversity values between groups were significantly different ($P < 0.05$), except for comparisons between the CA-PF, CAE-PE, and CA-PE groups. *Streptococcus* genus probe 4 and *Neisseria* genus probe 2 were the most frequently detected taxa across the plaque groups, followed by *Streptococcus sanguinis*, which was highly abundant in CF-PF. Well-known acidogenic/aciduric species such as *Streptococcus mutans*, *Scardovia wiggsiae*, *Parascardovia denticolens*, and *Lactobacillus salivarius* were found almost exclusively in CA-PD. The microbiomes of supragingival dental plaque differ substantially among tooth surfaces and children of different caries activities. In support of the ecological nature of caries etiology, a steady transition in community species composition was observed with disease progression.

KEYWORDS bacteria, caries, children, microbiome, supragingival, biofilms, dental plaque

Received 14 February 2017 **Returned for modification** 24 March 2017 **Accepted** 8 May 2017

Accepted manuscript posted online 15 May 2017

Citation Richards VP, Alvarez AJ, Luce AR, Bedenbaugh M, Mitchell ML, Burne RA, Nascimento MM. 2017. Microbiomes of site-specific dental plaques from children with different caries status. Infect Immun 85:e00106-17. https://doi.org/10.1128/IAI.00106-17.

Editor Vincent B. Young, University of Michigan-Ann Arbor

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Oral bacteria that colonize the teeth form dental plaque, a biofilm community that exists in equilibrium with host defenses and is generally compatible with the integrity of the tooth tissues (1). The development of carious lesions on tooth tissues involves a dynamic biological process wherein acids produced by bacterial fermentation of dietary carbohydrates effect the demineralization of dental tissues. Repeated acidification results in the emergence of acid-producing and highly acid-tolerant organisms, a selective process that upsets pH homeostasis and shifts the demineralization-remineralization balance toward the loss of tooth minerals (2–5). Oral environmental perturbations associated with changes in the availability of dietary carbohydrates and

ORIGINAL RESEARCH
Pediatric Dentistry

Incidence of dental caries in primary dentition and risk factors: a longitudinal study

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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DOI: 10.1590/1807-3107BOR-2016.vol30.0059

Submitted: Jul 03, 2015
Accepted for publication: Jan 20, 2016
Last revision: Mar 22, 2016

Abstract: The objectives of this prospective, longitudinal, population-based study were to estimate the incidence of dental caries in the primary dentition, identify risk factors and determine the proportion of children receiving dental treatment, through a two-year follow up. The first dental exam was conducted with 381 children aged one to five years, at health centers during immunization campaigns; 184 of them had dental caries and 197 had no caries experience. The second exam was carried out two years later at a nursery or at home with the same individuals who participated in the first exam. The diagnosis of dental caries was performed using the dmft criteria. Parents were interviewed regarding socioeconomic indicators. Descriptive, bivariate and adjusted Poisson regression analyses were performed. Among the 381 children, 234 were reexamined after two years (non-exposed: 139; exposed: 95). The overall incidence of dental caries was 46.6%. The greatest incidence of dental caries was found in the group of children with previous caries experience (61.1%). Among the children without dental caries in the first exam, 36.7% exhibited caries in the second exam. The majority of children (72.6%) received no treatment for carious lesions in the two-year interval between examinations. Children with previous dental caries (RR: 1.52, 95%CI: 1.12–2.05) had a greater risk of developing new lesions, compared with the children without previous dental caries. The incidence of dental caries was high and most of children's caries were untreated. Previous caries experience is a risk factor for developing new carious lesions in children.

Keywords: Dental Caries; Tooth, Deciduous; Oral Hygiene; Incidence.

Introduction

Dental caries is one of the most frequent chronic conditions in childhood. Approximately 50% of preschool children in different countries have caries experience.[1] This estimate is confirmed in studies conducted in Brazil, where prevalence rates range from 20.3%[2] to 53.6%.[3] Dental caries exerts a negative impact on the quality of life of both the child and the family,[2] and is considered a public health problem.

The etiology of dental caries in childhood is associated with eating habits,[4,5] irregular tooth brushing[6,7] and socioeconomic indicators.[8] Children from economically vulnerable families have a higher

Article:
Pitts, NB, Zero, DT, Marsh, PD et al. (7 more authors) (2017) Dental caries. Nature Reviews Disease Primers, 3. 17030. p. 17030.

https://doi.org/10.1038/nrdp.2017.30

J. Microbiol. Biotechnol. (2018), 28(3), 454–464
https://doi.org/10.4014/jmb.1710.10028

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Association of Salivary Microbiota with Dental Caries Incidence with Dentine Involvement after 4 Years

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Received: October 20, 2017
Revised: December 5, 2017
Accepted: December 21, 2017

First published online
January 11, 2018

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Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

Salivary microbiota alterations can correlate with dental caries development in children, and mechanisms mediating this association need to be studied in further detail. Our study explored salivary microbiota shifts in children and their association with the incidence of dental caries with dentine involvement. Salivary samples were collected from children with caries and their subsequently matched caries-free controls before and after caries development. The microbiota was analyzed by 16S rRNA gene-based high-throughput sequencing. The salivary microbiota was more diverse in caries-free subjects than in those with dental caries with dentine involvement (DC). Although both groups exhibited similar shifts in microbiota composition, an association with caries was found by function prediction. Analysis of potential microbiome functions revealed that *Granulicatella*, *Streptococcus*, *Bulleidia*, and *Staphylococcus* in the DC group could be associated with the bacterial invasion of epithelial cells, phosphotransferase system, and D-alanine metabolism, whereas *Neisseria*, *Lautropia*, and *Leptotrichia* in caries-free subjects could be associated with bacterial motility protein genes, linoleic acid metabolism, and flavonoid biosynthesis, suggesting that functional differences in the salivary microbiota may be associated with caries formation. These results expand the current understanding of the functional significance of the salivary microbiome in caries development, and may facilitate the identification of novel biomarkers and treatment targets.

Keywords: Microbiota, dental caries, children, function prediction, biomarkers

Introduction

Dental caries is one of the most common diseases of the oral cavity. In 2010, the prevalence of untreated caries in permanent teeth was 35.3% [1]. Severely decayed teeth may have an important impact on the general health, nutrition, growth, and body weight [2] of children by causing discomfort, pain, and sleeping problems [3].

Dental caries results from acids produced by commensal microbes within oral biofilms known as plaque [4]. Organic acids, including acetic, lactic, and propionic acids, are produced as fermentation by-products and dissolve the hydroxyapatite component of enamel and dentine [5], leading to tooth surface breakdown and subsequent cavity formation. In the cavitated lesion, exposed dentine collagen fibers are subject to enzymatic degradation by bacteria [6]. In contrast to most classical medical infections, caries is a polymicrobial infection mediated by commensal oral microbes. The oral environment is home to approximately 700 prokaryote species as determined by the Human Oral Microbiome Database [7]. Clonal analysis of the 16S rRNA gene revealed that diverse bacteria, including *Streptococcus mutans*, non-mutans streptococci, and members of the genera *Actinomyces*, *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*,

Hurley *et al. BMC Oral Health* (2019) 19:13
https://doi.org/10.1186/s12903-018-0693-1

BMC Oral Health

RESEARCH ARTICLE Open Access

Comparison of the salivary and dentinal microbiome of children with severe-early childhood caries to the salivary microbiome of caries-free children

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Abstract

Background: The main objectives of this study were to describe and compare the microbiota of 1) deep dentinal lesions of deciduous teeth of children affected with severe early childhood caries (S-ECC) and 2) the unstimulated saliva of these children and 3) the unstimulated saliva of caries-free children, and to compare microbiota compositional differences and diversity of taxa in these sampled sites.

Methods: Children with S-ECC and without S-ECC were recruited. The saliva of all children with and without S-ECC was sampled along with the deep dentinal microbiota from children affected by S-ECC. The salivary microbiota of children affected by S-ECC ($n = 68$) was compared to that of caries-free children ($n = 70$), by Illumina MiSeq sequencing of 16S rRNA amplicons. Finally, the caries microbiota of deep dentinal lesions of those children with S-ECC was investigated.

Results: Using two beta diversity metrics (Bray Curtis dissimilarity and UniFrac distance), the caries microbiota was found to be distinct from that of either of the saliva groups (caries-free & caries-active) when bacterial abundance was taken into account. However, when the comparison was made by measuring only presence and absence of bacterial taxa, all three microbiota types separated. While the alpha diversity of the caries microbiota was lowest, the diversity difference between the caries samples and saliva samples was statistically significant ($p < 0.001$). The major phyla of the caries active dentinal microbiota were Firmicutes (median abundance value 33.5%) and Bacteroidetes (23.2%), with *Neisseria* (10.3%) being the most abundant genus, followed by *Prevotella* (10%). The caries-active salivary microbiota was dominated by Proteobacteria (median abundance value 38.2%) and Bacteroidetes (27.8%) with the most abundant genus being *Neisseria* (16.3%), followed by *Porphyromonas* (9.5%). Caries microbiota samples were characterized by high relative abundance of *Streptococcus mutans*, *Prevotella* spp., *Bifidobacterium* and *Scardovia* spp.

Conclusions: Distinct differences between the caries microbiota and saliva microbiota were identified, with separation of both salivary groups (caries-active and caries-free) whereby rare taxa were highlighted. While the caries microbiota was less diverse than the salivary microbiota, the presence of these rare taxa could be the difference between health and disease in these children.

Keywords: Early childhood caries, Dentine, Saliva, Microbiota, Children

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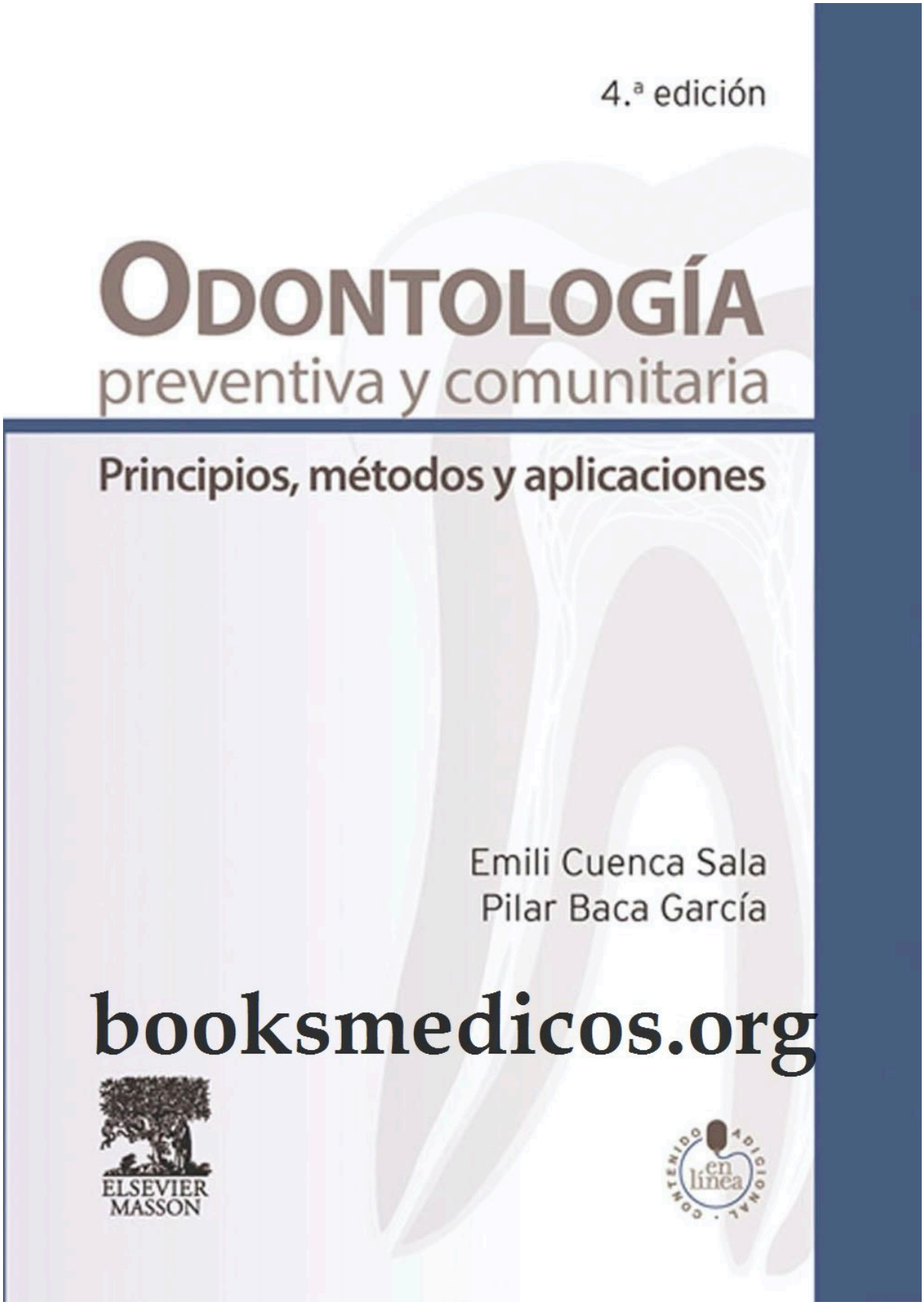
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Velsko et al. Microbiome (2019) 7:102
https://doi.org/10.1186/s40168-019-0717-3

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Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage

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Abstract

Background: Dental calculus, calcified oral plaque biofilm, contains microbial and host biomolecules that can be used to study historic microbiome communities and host responses. Dental calculus does not typically accumulate as much today as historically, and clinical oral microbiome research studies focus primarily on living dental plaque biofilm. However, plaque and calculus reflect different conditions of the oral biofilm, and the differences in microbial characteristics between the sample types have not yet been systematically explored. Here, we compare the microbial profiles of modern dental plaque, modern dental calculus, and historic dental calculus to establish expected differences between these substrates.

Results: Metagenomic data was generated from modern and historic calculus samples, and dental plaque metagenomic data was downloaded from the Human Microbiome Project. Microbial composition and functional profile were assessed. Metaproteomic data was obtained from a subset of historic calculus samples. Comparisons between microbial, protein, and metabolomic profiles revealed distinct taxonomic and metabolic functional profiles between plaque, modern calculus, and historic calculus, but not between calculus collected from healthy teeth and periodontal disease-affected teeth. Species co-exclusion was related to biofilm environment. Proteomic profiling revealed that healthy tooth samples contain low levels of bacterial virulence proteins and a robust innate immune response. Correlations between proteomic and metabolomic profiles suggest co-preservation of bacterial lipid membranes and membrane-associated proteins.

Conclusions: Overall, we find that there are systematic microbial differences between plaque and calculus related to biofilm physiology, and recognizing these differences is important for accurate data interpretation in studies comparing dental plaque and calculus.

Keywords: Ancient dental calculus, Oral microbiome, Metagenomics, Metaproteomics, Periodontal disease

Background

Dental calculus is a mineralized oral plaque biofilm that preserves biomolecules such as DNA and protein over long periods of time in the archeological record [1–7], and as such, it has the potential to offer insight into human microbiome evolution. Most clinical oral microbiome studies focus on dental plaque rather than calculus, in part because it is easier to study, it represents a living (and thus active) biofilm, and because dental plaque is directly responsible for oral pathology [8]. Comparatively less is known about the structure and formation of dental calculus, and studies of modern calculus are additionally hampered by the fact that deposits are smaller and less prevalent in living populations practicing tooth brushing and other forms of active oral hygiene [9, 10]. Although calculus forms from dental

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Dental Plaque Biofilm in Oral Health and Disease

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Dental plaque is an archetypical biofilm composed of a complex microbial community. It is the aetiological agent for major dental diseases such as dental caries and periodontal disease. The clinical picture of these dental diseases is a net result of the cross-talk between the pathogenic dental plaque biofilm and the host tissue response. In the healthy state, both plaque biofilm and adjacent tissues maintain a delicate balance, establishing a harmonious relationship between the two. However, changes occur during the disease process that transform this 'healthy' dental plaque into a 'pathogenic' biofilm. Recent advances in molecular microbiology have improved the understanding of dental plaque biofilm and produced numerous clinical benefits. Therefore, it is imperative that clinicians keep abreast with these new developments in the field of dentistry. Better understanding of the molecular mechanisms behind dental diseases will facilitate the development of novel therapeutic strategies to establish a 'healthy dental plaque biofilm' by modulating both host and microbial factors. In this review, the present authors aim to summarise the current knowledge on dental plaque as a microbial biofilm and its properties in oral health and disease.

Key words: *dental plaque biofilm, health and disease, properties*

Dental plaque biofilm – historical aspects

The relationship between microorganisms and dentistry dates back to the earliest observations of microorganisms. In a letter to the Royal Society in September 1683, Antoni van Leeuwenhoek described his observation of 'white little matter between his teeth' as 'an unbelievable great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time, the biggest sort bent … their body into curves in going forwards'[1,2]. Later studies revealed that the 'biggest sort' he referred in his letter could be *Selenomonas* species residing in the dental plaque[3,4]. Hence, dental plaque has been known to be a reservoir of microorganisms since the dawn of microbiology. However, until the 1980s it was assumed that microbes predominantly live in a suspended phase. Therefore, most studies on microbial diseases and drug-resistance mechanisms were based on this free-floating or 'planktonic' mode of growth. This concept would have influenced the genesis of Koch's postulates, which assumed that a specific pathogenic agent is accountable for a specific infectious disease. In its early days, dentistry embraced Koch's postulates and attempted to link specific pathogenic agents with particular dental diseases, such as *Streptococcus mutans* – which was discovered by Clarke as early as 1924[5] – with dental caries.

It is only as late as the 1970s that pioneering studies by Costerton and colleagues led to an understanding of the community lifestyle of microorganisms in nature[6]. Incidentally, dental plaque was one of the first few samples used in these ground-breaking studies on microbial biofilms. Costerton and colleagues showed that microbial cells adhere to the tooth surface and form

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HHS Public Access
Author manuscript
J Mol Biol. Author manuscript; available in PMC 2020 July 26.

Published in final edited form as:
J Mol Biol. 2019 July 26; 431(16): 2957–2969. doi:10.1016/j.jmb.2019.05.016.

The structure of dental plaque microbial communities in the transition from health to dental caries and periodontal disease

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Abstract

The human oral cavity harbors diverse communities of microbes that live as biofilms: highly ordered, surface-associated assemblages of microbes embedded in an extracellular matrix. Oral microbial communities contribute to human health by fine-tuning immune responses and reducing dietary nitrate. Dental caries and periodontal disease are together the most prevalent microbially-mediated human diseases, worldwide. Both of these oral diseases are known to be caused not by the introduction of exogenous pathogens to the oral environment, but rather by a homeostasis breakdown that leads to changes in the structure of the microbial communities present in states of health. Both dental caries and periodontal disease are mediated by synergistic interactions within communities and both diseases are further driven by specific host inputs: diet and behavior in the case of dental caries and immune system interactions in the case of periodontal disease. Changes in community structure (taxonomic identity and abundance) are well documented during the transition from health to disease. In this review, changes in biofilm physical structure during the transition from oral health to disease and the concomitant relationship between structure and community function will be emphasized.

Introduction

Numerous molecular based sequencing studies have resulted in a consensus among researchers that approximately 700 species or phylotypes comprise the bacterial component of the oral microbiome, while each individual human is estimated to carry a subset of between 50–200 species[1,2]. The human oral cavity includes different habitats for microbes including the epithelial mucosa; the papillary surface of the tongue dorsum; and the non-shedding, hard surfaces of the teeth, which themselves consist of two distinct compartments: the supragingival surface, i.e., above the gum line and the subgingival, i.e., that below the gum line[2]. Site specific, DNA sequencing studies of these different habitats have revealed that these different habitats support different microbial communities mediated by the characteristics of the surfaces available for attachment, oxygen availability, and exposure to

Banas and Drake *BMC Oral Health* (2018) 18:129
https://doi.org/10.1186/s12903-018-0595-2

BMC Oral Health

DEBATE Open Access

Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries?

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Abstract

The mutans streptococci were once the primary focus of research dedicated to understanding the etiology of dental caries. That focus has now shifted to an emphasis on the ecological balances and complexities within the entirety of the plaque microbiome. Within that framework there are considerable differences of opinion regarding the importance and relative contributions of the mutans streptococci. This article explores the basis for the various viewpoints, the limitations of current knowledge, and the confounders that make it difficult to arrive at a consensus.

Keywords: Dental caries, Mutans streptococci, Etiology

Background

Are the Mutans Streptococci MS; (Table 1) still considered relevant? The answer will likely depend upon whom is asked. If personal experience is a guide, the answers will range from yes, the MS remain the most prominent cariogenic species identified, to no, in the absence of MS other acidogenic species fulfill the same role. Debating the subject can be an interesting academic exercise but differences in opinion can also have serious implications related to research priorities, clinical strategies and educating the next generation of dental health professionals. This article discusses some of the challenges to acquiring a detailed understanding of the microbial contributions to caries, the basis for divergent opinions on the relative importance of the MS, and the potential that remains for improving caries prevention and treatment based on a prominent role for the MS.

The search for an understanding of the causes of tooth decay spans millennia. The ancient Greek philosopher and scientist Aristotle is credited with linking the consumption of figs and sweet foods with the development of decay. With the recognition of a microbial component as observed by Leeuwenhoek in the seventeenth century and put forth in the chemico-parasitic theory by W.D. Miller in the 1880s, many efforts focused on the possibility of linking dental caries with specific microbial pathogens. J. Kilian Clarke [1] first proposed a role for *Streptococcus mutans* in 1924. Not long afterward, Stephan and Miller [2] provided a compelling demonstration of plaque composition differences between sound and carious enamel sites by measuring changes in pH over time following a glucose rinse. Presented in what are now commonly referred to as 'Stephan curves' the patterns of pH decreases and recovery by plaque organisms fundamentally differ depending on the clinical status of the sites associated with the plaque. Plaque from carious lesions is more acidogenic than plaque from sound enamel sites. Thus, the concept of a caries--related plaque dysbiosis or imbalance of acidogens, later to form the central core of the Ecological-based Hypotheses [3–5], was suspected prior to widespread interest in the MS. The key uncertainty was whether there was a consistent basis or microbial specificity behind caries-related dysbioses. That uncertainty appeared to be resolved once the MS started to receive increased attention.

A wealth of studies employing a variety of subject groups and study designs supported correlations between levels of the MS and all types of caries [6, 7]. In addition, the MS had properties that could explain the epidemiological connection between sucrose consumption and caries, as well as its leading role in initiating the caries process in a manner consistent with the acid

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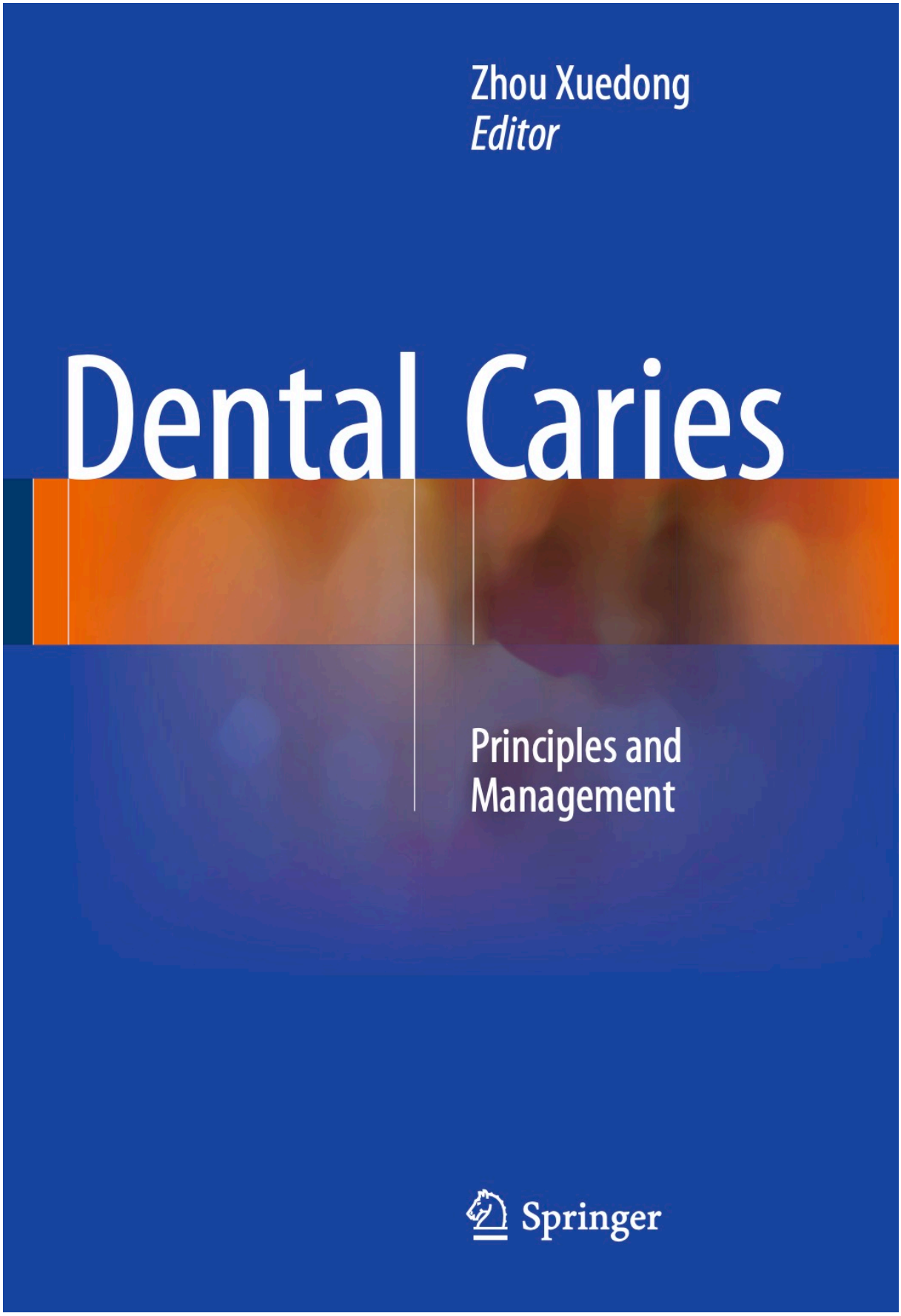
Zhou Xuedong
Editor
Dental Caries
Principles and
Management
Springer

Microb Ecol (2014) 67:962–969
DOI 10.1007/s00248-014-0372-y

HOST MICROBE INTERACTIONS

Pyrosequencing Analysis of Oral Microbiota Shifting in Various Caries States in Childhood

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Received: 30 June 2013 / Accepted: 20 January 2014 / Published online: 7 February 2014

Abstract Dental caries is one of the most prevalent childhood diseases worldwide, but little is known about the dynamic characteristics of oral microbiota in the development of dental caries. To investigate the shifting bacterial profiles in different caries states, 60 children (3–7-year-old) were enrolled in this study, including 30 caries-free subjects and 30 caries-active subjects. Supragingival plaques were collected from caries-active subjects on intact enamel, white spot lesions and carious dentin lesions. Plaques from caries-free subjects were used as a control. All samples were analyzed by 454 pyrosequencing based on 16S rRNA gene V1-V3 hypervariable regions. A total of 572,773 pyrosequencing reads passed the quality control and 25,444 unique phylotypes were identified, which represented 18 phyla and 145 genera. Reduced bacterial diversity in the cavitated dentin was observed as compared with the other groups. Thirteen genera (including *Capnocytophaga*, *Fusobacterium*, *Porphyromonas*, *Abiotrophia*, *Comamonas*, *Tannerella*, *Eikenella*, *Paludibacter*, *Treponema*, *Actinobaculum*, *Stenotrophomonas*, *Aestuariimicrobium*, and *Peptococcus*) were found to be associated with dental health, and the bacterial profiles differed considerably depending on caries status. Eight genera (including *Cryptobacterium*, *Lactobacillus*, *Megasphaera*, *Olsenella*, *Scardovia*, *Shuttleworthia*, *Cryptobacterium*, and *Streptococcus*) were increased significantly in cavitated dentin lesions, and *Actinomyces* and *Corynebacterium* were present at significant high levels in white spot lesions ($P<0.05$), while *Flavobacterium*, *Neisseria*, *Bergeyella*, and *Derxia* were enriched in the intact surfaces of caries individuals ($P<0.05$). Our results showed that oral bacteria were specific at different stages of caries progression, which contributes to informing the prevention and treatment of childhood dental caries.

Introduction

Early childhood caries (ECC) is one of the most prevalent chronic infectious diseases of childhood worldwide, affecting nearly two-thirds of children in China [1]. The oral cavity is a complex ecological habitat, as is reflected by its complex microbial community. Recent studies have shown that oral microbiota plays a vital role in maintaining the homeostasis of oral cavity [2]. Human dental plaque is a dynamic and complex biofilm where bacteria from saliva are adhered to tooth surfaces, embedded in a matrix of polymers. The development and maturation of dental plaque as a biofilm has profound implications in the etiology and progression of the most prevalent infections affecting humans, including dental caries [3]. To date, there are three major hypotheses for the etiology of dental caries: the non-specific plaque hypothesis, the specific plaque hypothesis, and the ecological plaque hypothesis that combines the specific and non-specific hypotheses to state that non-cariogenic bacteria have a role in the caries disease process through establishing and maintaining an optimum environment for caries-causing bacteria to thrive, resulting a

Wen Jiang and Zongxin Ling contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00248-014-0372-y) contains supplementary material, which is available to authorized users.

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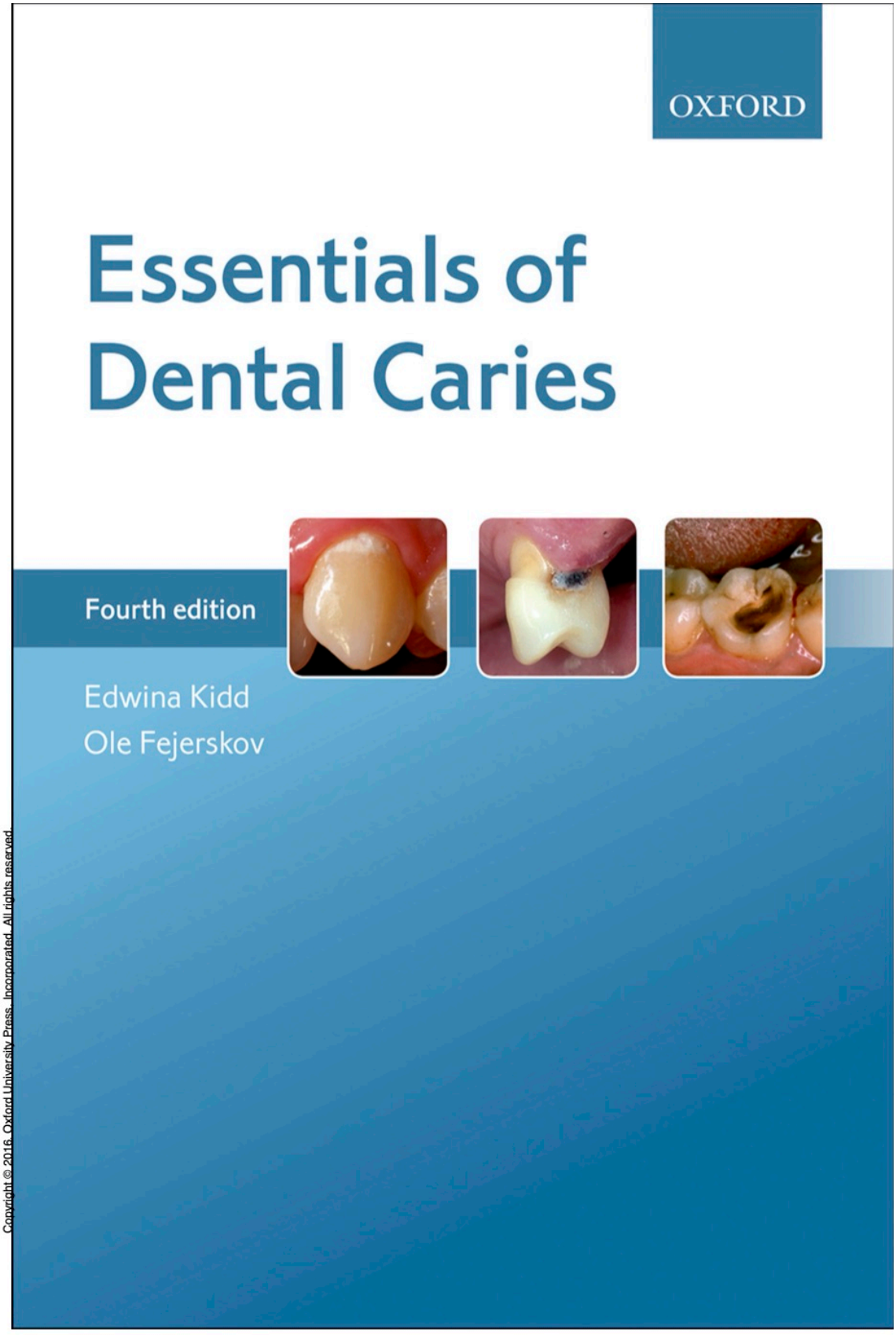

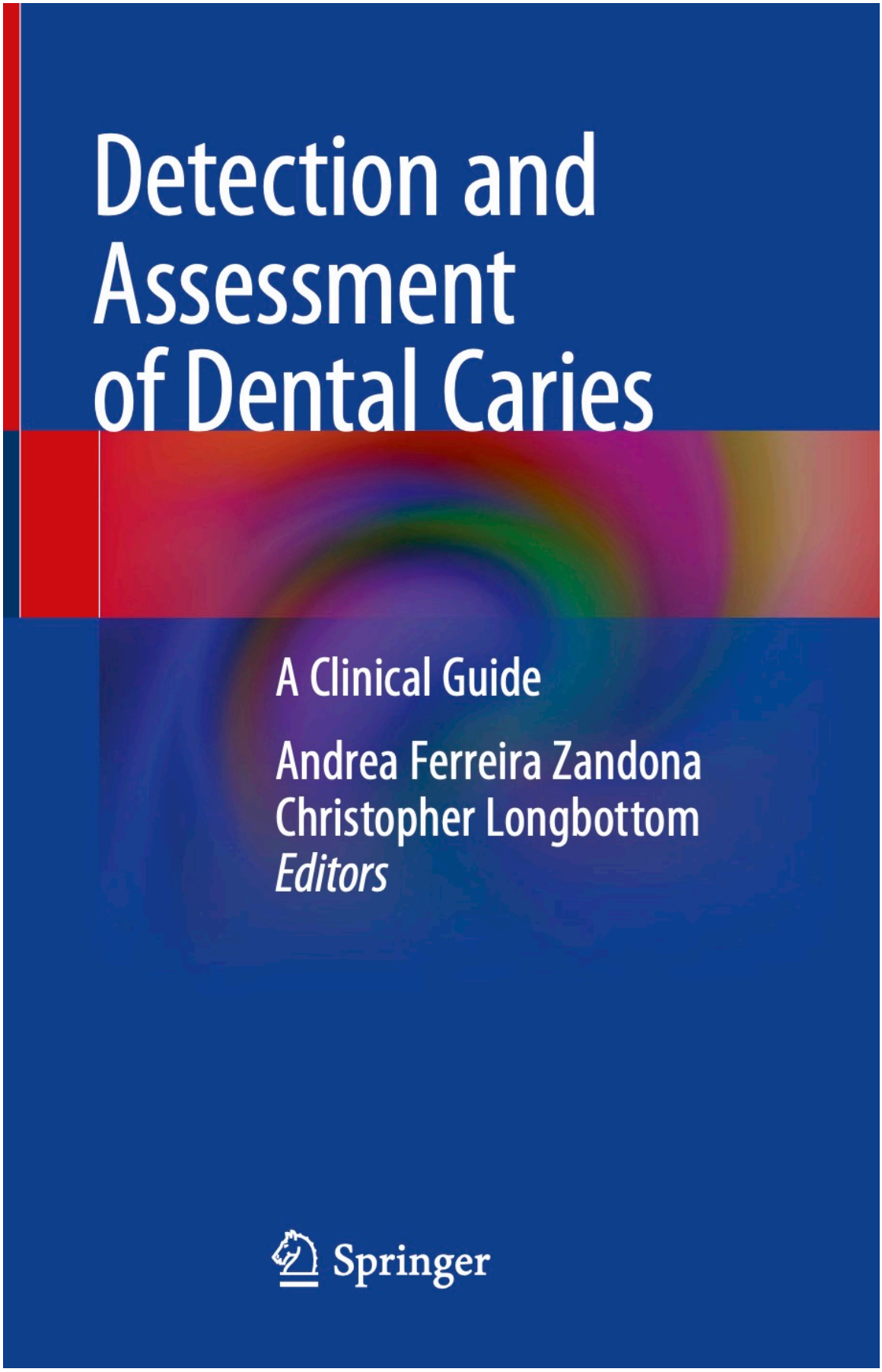
Detection and Assessment of Dental Caries
A Clinical Guide
Andrea Ferreira Zandona
Christopher Longbottom
Editors
Springer

frontiers in Pediatrics

REVIEW
published: 18 July 2017
doi: 10.3389/fped.2017.00157

Early Childhood Caries: Prevalence, Risk Factors, and Prevention

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Early childhood caries (ECC) is major oral health problem, mainly in socially disadvantaged populations. ECC affects infants and preschool children worldwide. The prevalence of ECC differs according to the group examined, and a prevalence of up to 85% has been reported for disadvantaged groups. ECC is the presence of one or more decayed, missing, or filled primary teeth in children aged 71 months (5 years) or younger. It begins with white-spot lesions in the upper primary incisors along the margin of the gingiva. If the disease continues, caries can progress, leading to complete destruction of the crown. The main risk factors in the development of ECC can be categorized as microbiological, dietary, and environmental risk factors. Even though it is largely a preventable condition, ECC remains one of the most common childhood diseases. The major contributing factors for the for the high prevalence of ECC are improper feeding practices, familial socioeconomic background, lack of parental education, and lack of access to dental care. Oral health plays an important role in children to maintain the oral functions and is required for eating, speech development, and a positive self-image. The review will focus on the prevalence, risk factors, and preventive strategies and the management of ECC.

Keywords: dental caries, early childhood caries, dietary habits, oral health, pediatric oral health, sociodemographic factors, infant feeding

OPEN ACCESS

Edited by:
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Reviewed by:
Alla B. Salmina,
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Agima Ljaljević,
Center of Health Promotion Institute of Public Health, Montenegro

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Specialty section:
This article was submitted to Child Health and Human Development, a section of the journal Frontiers in Pediatrics

Received: *28 March 2017*
Accepted: *27 June 2017*
Published: *18 July 2017*

Citation:
Anil S and Anand PS (2017) Early Childhood Caries: Prevalence, Risk Factors, and Prevention. Front. Pediatr. 5:157. doi: 10.3389/fped.2017.00157

INTRODUCTION

Early childhood caries (ECC) has been on the increase in many countries and has become a significant health problem especially in socially disadvantaged populations. ECC is defined as the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth in a child at 71 months of age or younger. It has several unique characteristics in clinical appearance such as rapid development of caries, which affects a number of teeth soon after they emerge in oral cavity. These lesions involve tooth surfaces that are less prone to caries development. Several terminologies were used to describe the condition such as, nursing bottle caries, nursing caries, rampant caries, baby bottle caries, baby bottle tooth decay, milk bottle syndrome, and prolonged nursing habit caries. ECC is a multifactorial disease that results from the interaction of factors that include cariogenic microorganisms, exposure to fermentable carbohydrates through inappropriate feeding practices, and a range of social variables. ECC is a severe health condition found among children living in socially disadvantaged communities in which malnutrition is a social and health disparity (1, 2). ECC is associated with other health problems, ranging from local pain, infections, abscesses, leading to difficulty in chewing, malnutrition, gastrointestinal disorders, and difficulty in sleeping (3).

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Author manuscript

Mol Oral Microbiol. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Mol Oral Microbiol. 2017 February ; 32(1): 24–34. doi:10.1111/omi.12152.

Advances in the microbial etiology and pathogenesis of early childhood caries

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Abstract

Early childhood caries (ECC) is one of the most prevalent infectious diseases affecting children worldwide. ECC is an aggressive form of dental caries, which left untreated, can result in rapid and extensive cavitation in teeth (rampant caries) that is painful and costly to treat. Furthermore, it affects mostly children from impoverished background, and thus constitutes a major challenge in public health. The disease is a prime example of the consequences arising from complex, dynamic interactions between microorganisms, host and diet, leading to the establishment of highly pathogenic (cariogenic) biofilms. To date, there are no effective methods to identify those at risk of developing ECC or control the disease in affected children. Recent advances in deep-sequencing technologies, novel imaging methods and (meta)proteomics-metabolomics approaches provide an unparalleled potential to reveal new insights to illuminate our current understanding about the etiology and pathogenesis of the disease. In this concise review, we provide a broader perspective about the etiology and pathogenesis of ECC based on previous and current knowledge on biofilm matrix, microbial diversity and host-microbe interactions which could have direct implications for developing new approaches for improved risk assessment and prevention of this devastating and costly childhood health condition.

Keywords

Dental caries; biofilm; matrix; diet; saliva; *Streptococcus*; *Candida*; microbiome

Introduction

Early childhood caries (ECC) is one of the most prevalent biofilm-dependent infectious diseases in childhood worldwide. It afflicts 23% of the preschoolers (< 6 years of age) in the US, and can be observed in toddlers as young as 12 months of age (Dye *et al.*, 2015). The disease most frequently targets children from poor socioeconomic families (>50%) and racial/ethnic minority backgrounds (Dye *et al.*, 2015). Left untreated, ECC can result in

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The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

Published in final edited form as:
Pediatr Dent. 2012 ; 34(2): 16–23.

Aciduric Microbiota and Mutans Streptococci in Severe and Recurrent Severe Early Childhood Caries

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Abstract

Purpose—Severe early childhood caries (ECC) results from bacterial acid production in an acidic environment. The current study determined *Streptococcus mutans, Streptococcus sobrinus* and acid-tolerant counts in severe-ECC.

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Polish Journal of Microbiology
2014, Vol. 63, No 2, 127–135

MINIREVIEW

The Oral Microbiome in Dental Caries

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Submitted 5 February 2013, revised 29 December 2013, accepted 1 February 2014

Abstract

Dental caries is one of the most common chronic and multifactorial diseases affecting the human population. The appearance of a caries lesion is determined by the coexistence of three main factors: acidogenic and acidophilic microorganisms, carbohydrates derived from the diet, and host factors. Socio-economic and behavioral factors also play an important role in the etiology of the disease. Caries develops as a result of an ecological imbalance in the stable oral microbiom. Oral microorganisms form dental plaque on the surfaces of teeth, which is the cause of the caries process, and shows features of the classic biofilm. Biofilm formation appears to be influenced by large scale changes in protein expression over time and under genetic control. Cariogenic microorganisms produce lactic, formic, acetic and propionic acids, which are a product of carbohydrate metabolism. Their presence causes a decrease in pH level below 5.5, resulting in demineralization of enamel hydroxyapatite crystals and proteolytic breakdown of the structure of tooth hard tissues. *Streptococcus mutans*, other streptococci of the so-called non-mutans streptococci group, *Actinomyces* and *Lactobacillus* play a key role in this process. Dental biofilm is a dynamic, constantly active metabolically structure. The alternating processes of decrease and increase of biofilm pH occur, which are followed by the respective processes of de- and remineralisation of the tooth surface. In healthy conditions, these processes are in balance and no permanent damage to the tooth enamel surface occurs.

Key words: cariogenic bacteria, dental caries, dental biofilm, oral microbiome

Introduction

Dental caries is one of the most common chronic infectious diseases worldwide and endangers humans throughout their life, not only during childhood or adolescence. It is the most common cause of tooth loss and pain in the oral cavity (Featherstone, 2004; Edelstein, 2006).

Epidemiological studies indicate a differentiated incidence of caries in various countries. In developed countries, a decreasing prevalence of the disease is observed. The reason for improvement of oral health conditions is attributed to diverse factors, including water fluoridation, use of fluoride toothpaste, a healthier diet containing sucrose substitutes and oral health education. (Konig, 2004; Marthaler, 2004). In developing countries, the incidence of dental caries still remains at a high level, and Poland unfortunately belongs to this group of nations (Robert and Sheiham, 2002; Wierzbicka *et al.*, 2012).

Dental caries, as a process determined by lifestyle, may be subject to activation in each period of human life if hygiene and diet are neglected even for a period as short as a few weeks (Nyvad and Fejerskov, 1997; ten Cate, 2001). In adverse conditions even the most resistant teeth will be affected by this disease (Kidd and Fejerscov, 2004). In the early stages the caries progress, can be stopped or reversed, but if left untreated, the disease may cause dysfunctions of the masticatory apparatus and systemic odontogenic infections.

The oral cavity as a complex ecosystem

The oral cavity is an extremely diverse, dynamic and unique ecosystem in the human body with a characteristic feature being the instability of its ecological conditions (Marsh, 2005). The oral cavity consists of a mucous membrane covered with a keratinized stratified squamous epithelium (*e.g.* the palate) and a non-keratinized epithelium, the papillary surface of the tongue and hard non-shedding structures of the teeth above and below the gingival margin, with different surfaces, grooves and hollows. These sites constitute separate ecological niches promoting the development of microorganisms, with each niche having a distinctive microbiome (Dewhirst *et al.*, 2010; Frandsen *et al.*, 1991; Paster *et al.*, 2006;).

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CARIES TEMPRANA DE INFANCIA: ¿ENFERMEDAD INFECCIOSA?

EARLY CHILDHOOD CARIES: INFECTION DISEASE?

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RESUMEN

La caries dental es una de las enfermedades más comunes en la infancia y las personas continúan siendo susceptible a través de la vida. Aunque actualmente puede ser detenida y potencialmente revertida en etapas tempranas, no es autolimitada, progresa en forma crónica si no existe un cuidado y control de los factores que la producen, llegando a la destrucción de dientes, dolor, alteraciones funcionales, sistémicas y consecuencias en la calidad de vida de las personas. La caries temprana de infancia, de inicio precoz en niños, es causada en forma frecuente por hábitos alimenticios inapropiados y la adquisición temprana de microorganismos como Streptococcus mutans. Se ha sugerido una transmisión vertical de madre a hijo como la vía principal de adquisición de Streptococcus mutans, y también se ha demostrado en la literatura, que existiría una transmisión horizontal entre niños y sus cuidadores, compañeros de jardín infantil y colegios. Por esta razón durante muchos años se ha definido la enfermedad caries como infecciosa y transmisible. Nuevos avances en técnicas moleculares han dado evidencia acerca de la microflora autóctona y cómo la placa dental o biofilm funciona como un sistema ecológico dinámico y complejo. Existe evidencia que la caries dental no es una enfermedad infecciosa clásica, como se creía hace unos años, por el contrario, esta enfermedad es el resultado de un cambio ecológico en la biopelícula adquirida en la superficie dental. Además la transmisión de Streptococcus mutans de la madre hacia el hijo no implica que la enfermedad se desarrolle, por el contrario, la caries dental hoy se describe como una enfermedad común, compleja y multifactorial, donde interactúan varios factores de riesgo, entre los más destacados conductuales, ambientales y genéticos.

Palabras clave: Caries temprana de infancia, Estreptococo mutans, transmisión vertical.

SUMMARY

The dental caries is one of the most common diseases in childhood and people remain susceptible through life. Even though this disease can be arrested and potencially reverse in early stages, it is not self-limitating, and progreses chronically if the causal factors are not controlled. It can even cause tooth destruction, pain, functional and systemic disorders and can affect the quality of life. The early childhood caries is frequently caused for unsuitable eating habits and the early acquisition of microorganisms such as Mutans streptococci. It has been suggested a vertical transmission from mother to child as the main way of acquisition of S.mutans, and has also been shown in the literature, that there would be an horizontal transmission between children and their caregivers, kindergarten and schools partners. For this reason for many years caries has been described as infectious and transmissible.

New advances in molecular techniques have provided evidence of the native microflora as dental plaque or biofilm functions as a complex and dynamic ecologic system. There is evidence that dental caries is not a classical infectious disease, as was thought a few years ago, however, this disease is the result of an ecological change in the acquired biofilm from dental surface. Vertical transmission does not mean that the disease develops, on the contrary, dental caries is described today as a common, complex and multifactorial disease, where multiple risks factors interact, where behavioral, environmental and genetic factors are the most predominant.

Key words: Early Childhood caries, Mutans streptococci, dental caries, vertical transmission.

Artículo recibido: 31-03-2014
Artículo aprobado para publicación: 29-04-2014

Original article
http://dx.doi.org/10.1590/1678-7757-2016-0241

JAOS 25 years
JOURNAL OF APPLIED ORAL SCIENCE

Salivary density of *Streptococcus mutans* and *Streptococcus sobrinus* and dental caries in children and adolescents with Down syndrome

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Abstract

Streptococcus mutans and *Streptococcus sobrinus* are strongly associated with dental caries. However, the relationship between oral streptococci and dental caries in children with Down syndrome is not well characterized. Objective: To assess and compare dental caries experience and salivary *S. mutans*, *S. sobrinus*, and *streptococci* counts between groups of Down syndrome and non-Down syndrome children and adolescents. Material and Methods: This study included a sample of 30 Down syndrome children and adolescents (G-DS) and 30 age- and sex-matched non-Down syndrome subjects (G-ND). Dental caries experience was estimated by the number of decayed, missing, and filled teeth in the primary dentition and the permanent dentition. Unstimulated whole saliva samples were collected from all participants. The fluorescence *in situ* hybridization technique was used to identify the presence and counts of the bacteria. The statistical analysis included chi-square, Student's t-test and Spearman's correlation. Results: The G-DS exhibited a significantly higher caries-free rate ($p<0.001$) and a lower *S. mutans* salivary density ($p<0.001$). No significant differences were found in the salivary densities of *S. sobrinus* or *streptococci* between the groups ($p=0.09$ and $p=0.21$, respectively). The salivary *S. mutans* or *S. sobrinus* densities were not associated with dental caries experience in neither group. Conclusion: The reduced dental caries experience observed in this group of Down syndrome children and adolescents cannot be attributed to lower salivary *S. mutans* densities, as determined with the fluorescence in situ hybridization technique.

Keywords: Down syndrome. Dental caries. Microbiology. Bacteria. Fluorescence *in situ* hybridization.

Submitted: May 24, 2016
Modification: August 22, 2016
Accepted: September 6, 2016

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OPEN ACCESS Freely available online

PLOS ONE

Beyond *Streptococcus mutans*: Dental Caries Onset Linked to Multiple Species by 16S rRNA Community Analysis

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Abstract

Dental caries in very young children may be severe, result in serious infection, and require general anesthesia for treatment. Dental caries results from a shift within the biofilm community specific to the tooth surface, and acidogenic species are responsible for caries. *Streptococcus mutans*, the most common acid producer in caries, is not always present and occurs as part of a complex microbial community. Understanding the degree to which multiple acidogenic species provide functional redundancy and resilience to caries-associated communities will be important for developing biologic interventions. In addition, microbial community interactions in health and caries pathogenesis are not well understood. The purpose of this study was to investigate bacterial community profiles associated with the onset of caries in the primary dentition. In a combination cross-sectional and longitudinal design, bacterial community profiles at progressive stages of caries and over time were examined and compared to those of health. 16S rRNA gene sequencing was used for bacterial community analysis. *Streptococcus mutans* was the dominant species in many, but not all, subjects with caries. Elevated levels of *S. salivarius, S. sobrinus*, and *S. parasanguinis* were also associated with caries, especially in subjects with no or low levels of *S. mutans*, suggesting these species are alternative pathogens, and that multiple species may need to be targeted for interventions. *Veillonella*, which metabolizes lactate, was associated with caries and was highly correlated with total acid producing species. Among children without previous history of caries, *Veillonella*, but not *S. mutans* or other acid-producing species, predicted future caries. Bacterial community diversity was reduced in caries as compared to health, as many species appeared to occur at lower levels or be lost as caries advanced, including the *Streptococcus mitis* group, *Neisseria*, and *Streptococcus sanguinis*. This may have implications for bacterial community resilience and the restoration of oral health.

Citation: Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, et al. (2012) Beyond *Streptococcus mutans*: Dental Caries Onset Linked to Multiple Species by 16S rRNA Community Analysis. PLoS ONE 7(10): e47722. doi:10.1371/journal.pone.0047722

Editor: Ping Xu, Virginia Commonwealth University, United States of America

Received June 29, 2012; **Accepted** September 14, 2012; **Published** October 16, 2012

Funding: This work was supported by grants R01DE016125, T32DE14320, and F30DE019339 from the National Institute of Dental and Craniofacial Research, National Institutes of Health (www.nidcr.nih.gov), and the OMNII Research Fellowship from the American Academy of Pediatric Dentistry (www.aapd.org). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Dental caries is the most common chronic disease of childhood [1]. It can occur in very young children, shortly after the eruption of teeth, and may be severe. For many children, early childhood caries is a source of pain and impaired quality of life, and for some it results in serious infection, hospitalization, and even fatality [2]. In this young age cohort treatment must often be completed under general anesthesia, accounting for a disproportionate fraction of total dental expenditures [3].

It is of particular importance to understand the microbial etiology of the onset of caries, since preventive interventions such as probiotics or vaccines will be most effective if they interrupt the process before irreversible damage is done to teeth. Once lesions advance beyond the white spot stage and the enamel surface is damaged, they cannot be biologically reversed. Moreover, the disease process may be refractory to ordinary preventive measures that involve biofilm removal such as tooth brushing, since the biofilm becomes more protected from mechanical disruption. Also, early caries experience appears to predispose to greater caries experience later in life, affecting the permanent dentition [4–7].

Recent advances, including data from the Human Microbiome Project, have lead to a new paradigm for understanding chronic, bacterially mediated diseases. Diseases of the oral cavity occur in a complex host-bacterial community interaction that often does not fit a single microbe pathogenesis model. Dental caries occurs as the result of a shift in the composition of a biofilm community specific to the human tooth surface. Frequent carbohydrate intake can disrupt the ecology of this community by the selection of acidogenic and acid tolerant species, and these acidogenic communities are responsible for caries development [8,9]. *Streptococcus mutans* appears to be the most common acid producer in caries initiation [10], but *S. mutans* is not present in all children with caries, and when found it is part of a complex microbial community [11–15]. Understanding the degree to which multiple acidogenic species provide functional redundancy and resilience to caries-associated communities is important for developing biologic

Original Paper

Caries Research

Caries Res 2018;52:246–252
DOI: 10.1159/000486103

Received: July 24, 2017
Accepted after revision: November 27, 2017
Published online: January 24, 2018

Mutans Streptococci and Dental Caries: A New Statistical Modeling Approach

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Keywords
Biostatistics · Dental caries · Microbiology · Oral epidemiology · Saliva · Survival analysis

Abstract
Survival analyses have been used to overcome some of the limitations encountered with other statistical analyses. Although extended Cox hazard modeling with time-dependent variables has been utilized in several medical studies, it has never been utilized in assessing the complex relationship between mutans streptococci (MS) acquisition (time-dependent covariate) and time to having dental caries (outcome). This study involved secondary analyses of data from a prospective study conducted at the University of Alabama at Birmingham. Low socioeconomic status, African-American preschool children from Perry County, AL, USA ($n = 95$) had dental examinations at age 1 year and annually thereafter until age 6 years by three calibrated dentists. Salivary MS tests were done at ages 1, 1.5, 2, 2.5, 3, and 4 years. The patterns of and relationship between initial MS detection (time-dependent covariate) and dental caries experience occurrence were assessed, using extended Cox hazard modeling. The median time without MS acquisition (50% of the children not having positive MS test) was 2 years. Approximately 79% of the children had positive salivary MS tests by the age of 4 years. The median caries experience survival (50% of the children not having dental caries) was 4 years. During the follow-up period, 65 of the children (68.4%) had their initial primary caries experience. Results of the extended Cox hazard modeling showed a significant overall/global relationship between initial caries experience event at any given time during the follow-up period and having a positive salivary MS test at any time during the follow-up period (hazard ratio = 2.25, 95% CI 1.06–4.75). In conclusion, the extended Cox modeling was used for the first time and its results showed a significant global/overall relationship between MS acquisition and dental caries. Further research using causal mediation analysis with survival data is necessary, where the mediator "presence of MS" is treated as a time-dependent variable.

It is now well established that mutans streptococci (MS) are the bacteria most commonly associated with the initiation of dental caries [Tanzer et al., 2001]. Of the MS found in the human oral cavity, *Streptococcus mutans* (*Sm*) usually predominates, while *Streptococcus sobrinus* (*Ss*) represents a minor fraction. Most children acquire MS soon after eruption of the primary teeth [Tanner et al., 2011; Gross et al., 2012]. Several studies have shown that the timing of the initial detection of MS varies be-

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Eur J Clin Microbiol Infect Dis (2014) 33:499–515
DOI 10.1007/s10096-013-1993-7

REVIEW

The virulence of *Streptococcus mutans* and the ability to form biofilms

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Received: 4 September 2013 / Accepted: 2 October 2013 / Published online: 24 October 2013

Abstract In some diseases, a very important role is played by the ability of bacteria to form multi-dimensional complex structure known as biofilm. The most common disease of the oral cavity, known as dental caries, is a top leader. *Streptococcus mutans*, one of the many etiological factors of dental caries, is a microorganism which is able to acquire new properties allowing for the expression of pathogenicity determinants determining its virulence in specific environmental conditions. Through the mechanism of adhesion to a solid surface, *S. mutans* is capable of colonizing the oral cavity and also of forming bacterial biofilm. Additional properties enabling *S. mutans* to colonize the oral cavity include the ability to survive in an acidic environment and specific interaction with other microorganisms colonizing this ecosystem. This review is an attempt to establish which characteristics associated with biofilm formation—virulence determinants of *S. mutans*—are responsible for the development of dental caries. In order to extend the knowledge of the nature of *Streptococcus* infections, an attempt to face the following problems will be made: Biofilm formation as a complex process of protein–bacterium interaction. To what extent do microorganisms of the cariogenic flora exemplified by *S. mutans* differ in virulence determinants "expression" from microorganisms of physiological flora? How does the environment of the oral cavity and its microorganisms affect the biofilm formation of dominant species? How do selected inhibitors affect the biofilm formation of cariogenic microorganisms?

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Introduction

In the 18th century, it was demonstrated that microorganisms live not only in a single-cell form, but are also capable of forming clusters suspended in a mucilaginous extracellular substance. The pathogenicity of certain microbial species such as *Streptococcus mutans*, *Staphylococcus epidermidis*, *Legionella pneumophila* or *Pseudomonas aeruginosa* is inseparably associated with their ability to form biofilms on solid surfaces, e.g., tissues, catheters or implants [1–4]. This feature allows microorganisms to form three-dimensional structures in which cells become more resistant to antibiotics and changing environmental conditions, among others, through changes occurring as a result of interbacterial interactions and the presence of an exopolysaccharide matrix protecting the entire structure [5, 6].

Microorganism pathogenicity

Interactions observed between pathogen and host have been the subject of research and discussion for many years. The historical approach to the problem of microorganism pathogenicity postulated by, amongst others, Koch, puts the pathogen or host in the main position by this featured affiliation to one of them. The term 'pathogenicity determinants' is related to the features which determine a microorganism's ability to cause disease, but which themselves are not required

Received: 22 March 2018 | Revised: 21 June 2018 | Accepted: 25 June 2018

DOI: 10.1111/odi.12932

REVIEW ARTICLE

WILEY ORAL DISEASES

Cariogenic microbiome and microbiota of the early primary dentition: A contemporary overview

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Abstract

Recent advances in the field of molecular microbiology provide an unprecedented opportunity to decipher the vast diversity of the oral microbiome in health and disease. Here, we provide a contemporary overview of the oral microbiome and the microbiota of early childhood caries (ECC) with particular reference to newer analytical techniques. A MEDLINE search revealed a total of 20 metagenomic studies describing cariogenic microbiomes of ECC, 10 of which also detailed the healthy microbiomes. In addition, seven studies on site-specific microbiomes, focusing on acidogenic and aciduric microbiota of deep-dentinal lesions, were also reviewed. These studies evaluated plaque and saliva of children aged 1.5–11 years, in cohorts of 12–485 individuals. These studies reveal a very rich and diverse microbial communities, with hundreds of different phylotypes and microbial species, including novel species and phyla such as *Scardovia wiggsiae*, *Slackia exigua*, *Granulicatella elegans*, *Firmicutes* in the plaque biofilms of children with ECC. On the contrary, bacteria such as *Streptococcus cristatus*, *S. gordonii*, *S. sanguinis*, *Corynebacterium matruchotii*, and *Neisseria flavescens* were common in plaque biofilm of noncarious, healthy, tooth surfaces in subjects with caries. The review illustrates the immense complexity and the diversity of the human oral microbiota of the cariogenic plaque microbiome in ECC, and the daunting prospect of its demystification.

KEYWORDS

early childhood caries, microbiome, microbiota, next-generation sequencing, primary dentition

1 | INTRODUCTION

The oral microbiome comprises a vast assortment of cohabiting plaque microbiota of more than 700 phylotypes of which approximately one half can be present in any individual at any one time (He & Shi, 2009; Kuramitsu, He, Lux, Anderson, & Shi, 2007; Palmer, 2014). The supragingival plaque biofilm, in particular, comprises a sub-micro-ecosystem of this polymicrobial community exhibiting a variety of patho-physiological characteristics (Takahashi & Nyvad, 2008, 2011).

In health, the oral microbiome has a symbiotic or a eubiotic relationship with the host but when a disease such as dental caries supervenes, this balance is perturbed and a dysbiotic relationship ensues. The dysbiosis is essentially due to the proliferation or overgrowth of cariogenic microbes such as *mutans*-group streptococci, which metabolizes dietary sugars, leading to a low pH eco-niche, demineralization of tooth structure, and dental caries (Marsh, Nyvad, & Baelum, 2008). In converse, some biofilm microbes through their arginine deiminase system can also produce alkali, resulting in a neutralizing micro-niche and a raised pH leading to remineralization

Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Inhibitory effect of oral *Lactobacillus* against oral pathogens

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Keywords
biofilm, inhibitory effect, oral diseases, oral *Lactobacillus*, oral pathogens.

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2011/0136: received 24 January 2011, revised and accepted 26 July 2011

doi:10.1111/j.1472-765X.2011.03132.x

Abstract

Aims: To determine the inhibitory effect of oral *Lactobacillus* against putative oral pathogens.

Methods and Results: Total 357 strains comprising 10 species of oral *Lactobacillus*, *Lactobacillus fermentum* (195), *Lactobacillus salivarius* (53), *Lactobacillus casei* (20), *Lactobacillus gasseri* (18), *Lactobacillus rhamnosus* (14), *Lactobacillus paracasei* (12), *Lactobacillus mucosae* (12), *Lactobacillus oris* (12), *Lactobacillus plantarum* (11) and *Lactobacillus vaginalis* (10) were used as producer strains. Inhibitory effect against a panel of indicators, periodontitis- and caries-related pathogens, was assessed. Most oral *Lactobacillus* was able to inhibit the growth of both periodontitis- and caries-related pathogens. The strongest inhibitory activity was associated with *Lact. paracasei*, *Lact. plantarum*, *Lact. rhamnosus*, *Lact. casei* and *Lact. salivarius*. *Lactobacillus* SD1–SD6, representing the six species with the strong inhibitory effect, inhibited growth of *Streptococcus mutans* ATCC 25175 in the biofilm model. Also, it was demonstrated that growth of *Strep. mutans* was inhibited in a mixture with *Lact. paracasei* SD1. The inhibition was enhanced in acidic condition and 5% glucose.

Conclusions: The results have shown that oral *Lactobacillus* SD1–SD6 showed a strong inhibitory effect against *Strep. mutans* and *Streptococcus sobrinus*, as well as, Gram-negative periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

Significance and Impact of the Study: The results indicated that *Lactobacillus* may be of benefit as probiotics for the prevention of oral diseases.

Introduction

Lactobacillus is Gram-positive, rod-shaped, nonspore forming and catalase negative bacteria, which are found in diverse environments such as food, vegetables and sewage (Prescott *et al.* 2002). Several species of *Lactobacillus* are also found in humans including the oral cavity, intestine and vagina. *Lactobacillus* species present in the resident flora of humans are usually pathogenically innocent, and some of them have been used as probiotics (Ahola *et al.* 2002; Annuk *et al.* 2003). However, the association between dental caries and the prevalence of lactobacilli has been recognized for several decades. Oral lactobacilli were previously believed to be the prime micro-organism in caries development because of their high numbers in carious lesions and their acidogenic and aciduric properties. Subsequent research has shown that they are associated with the caries progression in dentine rather than with the caries initiation in the enamel (van Houte 1980; Loesche 1986).

In spite of the cariogenic potential of lactobacilli, some of them are generally associated with health. Certain species of *Lactobacillus* may specifically play an important role in the maintenance of health by stimulating natural immunity and contributing to the balance of microflora in various habitats. The latter could occur by interaction

Published in final edited form as:
Clin Oral Investig. 2014 March ; 18(2): 659–669. doi:10.1007/s00784-013-1009-0.

pH gradient and distribution of streptococci, lactobacilli, prevotellae, and fusobacteria in carious dentine

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Abstract

Objectives—Caries process comprises acidogenic and aciduric bacteria that are responsible for lowering the pH and subsequent destruction of hydroxyapatite matrix in enamel and dentine. The aim of this study was to identify the correlation between the pH gradient of a carious lesion and proportion and distribution of four bacterial genera; lactobacilli, streptococci, prevotellae, and fusobacteria with regard to total load of bacteria.

Materials and methods—A total of 25 teeth with extensive dentinal caries were sampled in sequential layers. Using quantitative real-time PCR of 16S rRNA gene, we quantified the total load of bacteria as well as the proportion of the abovementioned genera following pH measurement of each sample with a fine microelectrode.

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Electronic supplementary material The online version of this article (doi:10.1007/s00784-013-1009-0) contains supplementary material, which is available to authorized users.

Conflict of interest The authors declare no potential conflicts of interest in regard to authorship and/or publication of this article.

SCIENTIFIC REPORTS
nature research

OPEN

Temporal development of the oral microbiome and prediction of early childhood caries

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Human microbiomes are predicted to assemble in a reproducible and ordered manner yet there is limited knowledge on the development of the complex bacterial communities that constitute the oral microbiome. The oral microbiome plays major roles in many oral diseases including early childhood caries (ECC), which afflicts up to 70% of children in some countries. Saliva contains oral bacteria that are indicative of the whole oral microbiome and may have the ability to reflect the dysbiosis in supragingival plaque communities that initiates the clinical manifestations of ECC. The aim of this study was to determine the assembly of the oral microbiome during the first four years of life and compare it with the clinical development of ECC. The oral microbiomes of 134 children enrolled in a birth cohort study were determined at six ages between two months and four years-of-age and their mother's oral microbiome was determined at a single time point. We identified and quantified 356 operational taxonomic units (OTUs) of bacteria in saliva by sequencing the V4 region of the bacterial 16S RNA genes. Bacterial alpha diversity increased from a mean of 31 OTUs in the saliva of infants at 1.9 months-of-age to 84 OTUs at 39 months-of-age. The oral microbiome showed a distinct shift in composition as the children matured. The microbiome data were compared with the clinical development of ECC in the cohort at 39, 48, and 60 months-of-age as determined by ICDAS-II assessment. *Streptococcus mutans* was the most discriminatory oral bacterial species between health and current disease, with an increased abundance in disease. Overall our study demonstrates an ordered temporal development of the oral microbiome, describes a limited core oral microbiome and indicates that saliva testing of infants may help predict ECC risk.

Early Childhood Caries (ECC) is a complex, multifaceted disease involving interactions between the oral microbiota, host susceptibility at the tooth, mouth and person level, and environmental factors, especially behaviours relating to early feeding practices, ingestion of free sugars and oral hygiene[1–3]. ECC is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces (dmfs) in any primary tooth in a child under the age of six years[4]. Australian children at four years of age have an average decayed, missing, and filled teeth (dmft) score of 1.94[5]. However, this score masks the true pattern and burden of disease as 60% of those examined were caries free. Additionally, the burden of disease associated with ECC is disproportionately borne by children from vulnerable and disadvantaged families[6].

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Curr Microbiol (2013) 67:537–542
DOI 10.1007/s00284-013-0393-7

Pyrosequencing Analysis of Oral Microbiota in Children with Severe Early Childhood Dental Caries

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Received: 18 March 2013 / Accepted: 15 May 2013 / Published online: 7 June 2013

Abstract Severe early childhood caries are a prevalent public health problem among preschool children throughout the world. However, little is known about the microbiota found in association with severe early childhood caries. Our study aimed to explore the bacterial microbiota of dental plaques to study the etiology of severe early childhood caries through pyrosequencing analysis based on 16S rRNA gene V1–V3 hypervariable regions. Forty participants were enrolled in the study, and we obtained twenty samples of supragingival plaque from caries-free subjects and twenty samples from subjects with severe early childhood caries. A total of 175,918 reads met the quality control standards, and the bacteria found belonged to fourteen phyla and sixty-three genera. Our results show the overall structure and microbial composition of oral bacterial communities, and they suggest that these bacteria may present a core microbiome in the dental plaque microbiota. Three genera, *Streptococcus*, *Granulicatella*, and *Actinomyces*, were increased significantly in children with severe dental cavities. These data may facilitate improvements in the prevention and treatment of severe early childhood caries.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-013-0393-7) contains supplementary material, which is available to authorized users.

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Introduction

Early childhood caries (ECC) are one of the most common chronic, infectious diseases during childhood, affecting over 40 % of 5-year-old children in the US and China [3, 11]. Advanced forms of this disease are a major reason that young children visit the hospital and are of high clinical significance because they increase the risk that the caries will spread to the permanent dentition [19]. Accumulating evidence suggests that dental plaque microbiota are closely correlated with severe ECC [18]. *Streptococcus*, *Veillonella*, *Actinomyces*, and *Granulicatella* have been found to be significantly increased in subjects with dental caries, indicating a positive role in the progression of caries [1, 17]. In addition, culture-independent methods further expanded the range of bacteria, such as *Gemella* and *Granulicatella*, associated with dental caries [13]. However, there are few studies that actually explored the overall extent of diversity within the microbiota of children with severe ECC.

Recent advances in sequencing technology, such as high-throughput pyrosequencing, have revealed an unexpectedly high diversity within the human oral microbiota: dental plaques pooled from adults have been shown to contain approximately 10,000 microbial phylotypes, and the ultimate diversity of oral microbiome has been estimated to contain approximately 25,000 phylotypes [16]. These results suggest that this technology could gain a better understanding of the complexities of the oral microbiome [9].

The purpose of the present study was to explore the dental plaque microbiota of severe ECC and caries-free children through the utilization of massively 16S RNA V1–V3 region-tagged parallel pyrosequencing. Our data will define the microbial difference between caries-free and severe ECC groups, which may facilitate better prevention and treatment of dental caries.

PLOS | ONE

RESEARCH ARTICLE

Urease and Dental Plaque Microbial Profiles in Children

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OPEN ACCESS

Citation: Morou-Bermudez E, Rodriguez S, Bello AS, Dominguez-Bello MG (2015) Urease and Dental Plaque Microbial Profiles in Children. PLoS ONE 10(9): e0139315. doi:10.1371/journal.pone.0139315

Editor: José A Lemos, University of Florida, UNITED STATES

Received: April 17, 2015

Accepted: September 11, 2015

Published: September 29, 2015

Data Availability Statement: All sequence and related metadata files are available from the Harvard Dataverse Network (doi:10.7910/DVN/RBYNUF).

Funding: This study was supported by K23 DE015285 (PI: Morou-Bermudez, Evangelia) from the National Institute for Dental and Craniofacial Research, by 2U54MD007587 from the National Institute On Minority Health And Health Disparities of the National Institutes of Health (PI: Cruz, M and Luciano C), and by RCMI grant #G12 MD007600 (PI: Emma Fernandez-Repollet). Data generation for this publication was performed at the Sequencing and Genomics Facility (SGF) of the University of Puerto Rico. Data was generated from equipment financially

Abstract

Objective

Urease enzymes produced by oral bacteria generate ammonia, which can have a significant impact on the oral ecology and, consequently, on oral health. To evaluate the relationship of urease with dental plaque microbial profiles in children as it relates to dental caries, and to identify the main contributors to this activity.

Methods

82 supragingival plaque samples were collected from 44 children at baseline and one year later, as part of a longitudinal study on urease and caries in children. DNA was extracted; the V3–V5 region of the 16S rRNA gene was amplified and sequenced using 454 pyrosequencing. Urease activity was measured using a spectrophotometric assay. Data were analyzed with Qiime.

Results

Plaque urease activity was significantly associated with the composition of the microbial communities of the dental plaque (Baseline P = 0.027, One Year P = 0.012). The bacterial taxa whose proportion in dental plaque exhibited significant variation by plaque urease levels in both visits were the family Pasteurellaceae (Baseline P<0.001; One Year P = 0.0148), especially *Haemophilus parainfluenzae*. No association was observed between these bacteria and dental caries. Bacteria in the genus Leptotrichia were negatively associated with urease and positively associated with dental caries (Bonferroni P<0.001).

Conclusions

Alkali production by urease enzymes primarily from species in the family Pasteurellaceae can be an important ecological determinant in children's dental plaque. Further studies are

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1464–1474 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.02427-10

Cultivable Anaerobic Microbiota of Severe Early Childhood Caries▽¶

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Received 29 November 2010/Returned for modification 10 January 2011/Accepted 19 January 2011

Severe early childhood caries (ECC), while strongly associated with *Streptococcus mutans* using selective detection (culture, PCR), has also been associated with a widely diverse microbiota using molecular cloning approaches. The aim of this study was to evaluate the microbiota of severe ECC using anaerobic culture. The microbial composition of dental plaque from 42 severe ECC children was compared with that of 40 caries-free children. Bacterial samples were cultured anaerobically on blood and acid (pH 5) agars. Isolates were purified, and partial sequences for the 16S rRNA gene were obtained from 5,608 isolates. Sequence-based analysis of the 16S rRNA isolate libraries from blood and acid agars of severe ECC and caries-free children had >90% population coverage, with greater diversity occurring in the blood isolate library. Isolate sequences were compared with taxon sequences in the Human Oral Microbiome Database (HOMD), and 198 HOMD taxa were identified, including 45 previously uncultivated taxa, 29 extended HOMD taxa, and 45 potential novel groups. The major species associated with severe ECC included *Streptococcus mutans*, *Scardovia wiggsiae*, *Veillonella parvula*, *Streptococcus cristatus*, and *Actinomyces gerencseriae*. *S. wiggsiae* was significantly associated with severe ECC children in the presence and absence of *S. mutans* detection. We conclude that anaerobic culture detected as wide a diversity of species in ECC as that observed using cloning approaches. Culture coupled with 16S rRNA identification identified over 74 isolates for human oral taxa without previously cultivated representatives. The major caries-associated species were *S. mutans* and *S. wiggsiae*, the latter of which is a candidate as a newly recognized caries pathogen.

Early childhood caries (ECC), dental caries of the primary dentition, also known as nursing (bottle) caries, is the most common chronic infectious disease of childhood in the United States, affecting 28% of the population (6). Advanced forms of this disease, severe ECC, can destroy the primary dentition and is the major reason for hospital visits for young children (42). Severe ECC disproportionately affects disadvantaged ethnic and socioeconomic groups and can affect over 50% of the children in these groups (2, 19, 23, 38, 55).

Dental caries is caused by an interaction between acidogenic bacteria, a carbohydrate substrate which is frequently sucrose, and host susceptibility (51). The acidogenic and acid-tolerant bacterial species *Streptococcus mutans* is recognized to be the primary pathogen in early childhood caries (4, 8, 31, 50). *S. mutans* is detected in caries-free populations but is not detected in all cases of childhood caries (1, 27), suggesting that other species may be cariogenic pathogens.

Studies of severe ECC using culture have historically focused on selected bacterial groups, particularly *S. mutans* and other *Streptococcus* species and *Lactobacillus*, *Actinomyces*, and *Veillonella* species (27, 31, 33, 52). Isolates were generally identified phenotypically, sometimes only to the genus level, and thus, their relationships to currently defined human oral taxa on the basis of a 16S rRNA-defined taxonomy (11) are unclear. Culture studies demonstrated a strong association of *S. mutans* with ECC and severe ECC and also reported significant associations with selected *Actinomyces* and *Lactobacillus* species. Primary isolation on acid media has been used to select for acid-tolerant species that would be present in active carious lesions. Total counts were higher on acid agar from children with initial caries (45) and severe ECC (21) than from caries-free children. Acid broth enrichment was found to select for *Streptococcus oralis*, *S. mutans*, *Actinomyces israelii*, and *Actinomyces naeslundii* in severe ECC (31).

PCR of the 16S rRNA gene with cloning and subsequent sequencing has been used to evaluate the diversity of the microbiota of early childhood caries, and combined with use of species/taxon-specific probes to the 16S rRNA gene to evalu-

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▽ Published ahead of print on 2 February 2011.
¶ The authors have paid a fee to allow immediate free access to this article.

Research Reports: Clinical

Maturation of the Oral Microbiome in Caries-Free Toddlers: A Longitudinal Study

Journal of Dental Research
2020, Vol. 99(2) 159–167

DOI: 10.1177/0022034519889015
journals.sagepub.com/home/jdr

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Abstract

Understanding the development of the oral microbiota in healthy children is of great importance to oral and general health. However, limited data exist on a healthy maturation of the oral microbial ecosystem in children. Moreover, the data are biased by mislabeling "caries-free" populations. Therefore, we aimed to characterize the healthy salivary and dental plaque microbiome in young children. Caries-free (ICDAS [International Caries Detection and Assessment System] score 0) children (n = 119) and their primary caregivers were followed from 1 until 4 y of child age. Salivary and dental plaque samples were collected from the children at 3 time points (T1, ~1 y old; T2, ~2.5 y old; and T3, ~4 y old). Only saliva samples were collected from the caregivers. Bacterial V4 16S ribosomal DNA amplicons were sequenced using Illumina MiSeq. The reads were denoised and mapped to the zero-radius operational taxonomic units (zOTUs). Taxonomy was assigned using HOMD. The microbial profiles of children showed significant differences (P = 0.0001) over time. Various taxa increased, including *Fusobacterium, Actinomyces*, and *Corynebacterium*, while others showed significant decreases (e.g., *Alloprevotella* and *Capnocytophaga*) in their relative abundances over time. Microbial diversity and child-caregiver similarity increased most between 1 and 2.5 y of age while still not reaching the complexity of the caregivers at 4 y of age. The microbiome at 1 y of age differed the most from those at later time points. A single zOTU (*Streptococcus*) was present in all samples (n = 925) of the study. A large variation in the proportion of shared zOTUs was observed within an individual child over time (2% to 42% of zOTUs in saliva; 2.5% to 38% in dental plaque). These findings indicate that the oral ecosystem of caries-free toddlers is highly heterogeneous and dynamic with substantial changes in microbial composition over time and only few taxa persisting across the 3 y of the study. The salivary microbiome of 4-y-old children is still distinct from that of their caregivers.

Keywords: saliva, plaque, caries-free children, caregiver, 16S rRNA gene amplicon sequencing, fungal qPCR

Introduction

The oral microbiome is unique to each individual and comprises a diverse community of microorganisms, including bacteria, archaea, fungi, protozoa, and viruses (Wade 2013). Even among healthy individuals, there are substantial differences in the composition of the resident oral microbiome (Aas et al. 2005). Furthermore, the microbial composition differs across microniches within a healthy oral cavity (Zhou et al. 2013). Equilibrium among the commensal microbiota of the oral ecosystem, interacting with each other and the host, is considered one of the most important factors for maintaining a healthy microbiota (Zaura et al. 2014). In addition, various internal and external factors, such as diet, oral hygiene, use of antibiotics, and others, affect the composition and the stability of the oral microbiome (Dagli et al. 2016).

To date, emphasis has been on describing the differences of caries-affected versus caries-free children (Luo et al. 2012; Jiang et al. 2016), while there is limited knowledge on the "normal" (healthy) microbiome, especially in children. Development and maintenance of the normal microbiome throughout childhood are not well studied due to various limitations, particularly cross-sectional study designs, targeted microbial and clinical diagnostic methods, or small sample sizes (Xin et al. 2013; Lee et al. 2016; Li et al. 2018).

Development of enamel lesions is preceded by microbial ecological shifts toward aciduric and acidogenic microbiota (Marsh 1994). Defining dental caries as a "cavity" leads to a mislabeling of "caries-free" participants in clinical studies where diagnostic thresholds determine what is recorded as "diseased" or "sound" (Pitts 2004). Most studies that focus on

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A supplemental appendix to this article is available online.

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