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## PRESENCE OF *Streptococcus Mutans* IN SALIVA AND ITS RELATIONSHIP WITH DENTAL CARIES: ANTIMICROBIAL SUSCEPTIBILITY OF THE ISOLATES

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### RESUMEN

La caries dental es un proceso infeccioso, localizado y transmisible que conlleva a la destrucción del tejido dental duro. *Streptococcus mutans*, microorganismo normal de cavidad oral, tolerador y generador de ácidos, es considerado el principal agente causal de la caries. Diferentes estudios han demostrado la correlación y no correlación de *S. mutans* con la prevalencia de caries. El objetivo de este estudio fue determinar la relación entre la presencia de *S. mutans* y caries dental, y evaluar la susceptibilidad antimicrobiana de las cepas evaluadas. Con este fin se tomó saliva no estimulada de 53 niños con edades entre 3 y 5 años de la escuela Diego Torres (Turmequé-Boyacá). Las muestras de saliva se agitaron con vortex y se diluyeron en tampón fosfato 0.05 M. Se tomaron 100 ul de las diluciones y se cultivaron en agar Mitis Salivarius Bacitracina, para el aislamiento selectivo y recuento de *S. mutans*, y se incubaron en anaerobiosis durante 2 días a 37° C. Después de la identificación de las cepas aisladas, por medio de pruebas bioquímicas, se determinó la concentración mínima inhibitoria frente a la penicilina, amoxicilina, cefazolina, eritromicina, clindamicina, imipenem y vancomicina por el método de dilución en agar con concentraciones entre 0.003 y 32 ug/ml. La experiencia de caries en este grupo de niños fue de 66% (35/53) y *S. mutans* estuvo presente en 33 de los 53 niños (62%). Únicamente 21 de los 33 (64%) niños con *S. mutans* tuvieron caries. Catorce de los 20 niños (70%) en donde no se aisló *S. mutans* tuvieron caries. No hubo diferencias estadísticamente significativas en el número de unidades formadoras de colonias entre los grupos con o sin caries (p=0.21). Todos los 33 aislamientos de *S. mutans* fueron sensibles a penicilina, amoxicilina, cefazolina, eritromicina, clindamicina, imipenem y vancomicina; el 50 y 90% de las cepas fueron inhibidas, respectivamente, por concentraciones menores a 0.12 y 0.50 ug/ml, con todos los antibióticos evaluados. En conclusión, no siempre se encontró la relación de pareja *S. mutans*-caries dental, y las cepas de *S. mutans* aisladas fueron altamente sensibles a los antibióticos examinados.

**Palabras clave:** caries dental, saliva, *S. mutans*, susceptibilidad antimicrobiana

### ABSTRACT

Dental caries is a localized, transmissible, pathological infectious process that ends up in the destruction of hard dental tissue. *Streptococcus mutans* is considered to be the main cause of dental caries. Indeed, numerous reports have shown the close relationship between salivary levels of *S. mutans* and dental caries. The purpose of this study was to determine the relationship between the presence of *Streptococcus mutans* and dental caries, and to evaluate the antimicrobial susceptibility of the isolates. Unstimulated saliva was collected from 53 3 to 5-year-old children from the Diego Torres school in Turmequé (Boyacá, Colombia). Saliva samples were vortexed and serially diluted in 0.05 M phosphate buffer. Aliquots of 100 ul of the appropriate dilutions were cultured on Mitis Salivarius Bacitracin agar medium for the selective

isolation of *S. mutans*, and incubated anaerobically for two days at 37° C. The minimal inhibitory concentrations of the *S. mutans* isolates were evaluated against penicillin, amoxicillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin by an agar dilution method. The dental caries experience in these children was 66% (35/53) and *S. mutans* was found in the saliva of 33 children (62%); 21 of them had dental caries and 12 did not. In the 20 children from whom *S. mutans* was not isolated, 14 (70%) were found to have caries. There were no statistically significant differences in *S. mutans* counts between the group with dental caries and the caries-free group ( $p=0.21$ ). All isolates were highly sensitive to penicillin, amoxicillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin; 50 and 90% of the strains from *S. mutans* were inhibited by concentrations of less than 0.12 and 0.5 ug/ml, respectively, for all antibiotics studied. In conclusion, not all of the children hosting this microorganism had caries, and the *S. mutans* strains were highly sensitive to the antibiotics tested.

**Key-words:** dental caries, saliva, *S. mutans*, antimicrobial susceptibility

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## INTRODUCTION

Dental caries is a localized and transmissible pathological infectious process that ends up in the destruction of hard dental tissue (Loesche 1986). *Streptococcus mutans*, an acidogenic and aciduric microorganism colonizing the oral cavity, is considered to be the main cause of dental caries (Loesche 1986). Different studies have shown a correlation between counts of *S. mutans* in the oral cavity and both the prevalence and incidence of caries (Loesche 1986, Lang et al., 1987, Beighton et al., 1989). However, in other studies, no correlation has been found between the quantity of *S. mutans* and the incidence of caries (Marsh et al., 1989, Macpherson et al., 1992). On the other hand, some researchers have suggested the importance of streptococci other than *S. mutans* in the generation of dental caries (van Houte et al., 1991).

In addition to dental caries and related pyogenic dental infections, *S. mutans* is also a very important endocarditis agent (Ullman et al., 1988). The participation of this microorganism in both oral and non-oral diseases has prompted interest in the knowledge of its susceptibility to antimicrobial agents. The aim of this study was to determine the relationship between the presence of *S. mutans* and dental caries in

children aged 3 to 5 years, and to evaluate the antimicrobial susceptibility of the isolates. This work is part of a research project on Microbial Oral Ecology in the Colombian population.

## MATERIALS AND METHODS

### Subjects

The present study was carried out between July and December 2001, and includes data on 53 3-to 5-year-old children from the Diego Torres school in Turmequé (Colombia). Written informed consent was obtained prior to enrolment from the parent(s) of all infants participating in the study. General information data were collected through interviews with the parent(s) during a visit to the school. The children who were microbiologically screened had not used antibiotics prior to the sampling.

### Microbiological procedures

Unstimulated saliva (Fure 1998) was collected by placing a plastic pipette in the buccal area and applying gentle suction. Samples were collected in the mid-morning, at least 1 hour after a meal, and were transported in a refrigerated recipient to the laboratory. Saliva samples were vortexed and serially diluted in 10-fold steps in 0.05 M phosphate buffer. Aliquots of 100 ul of the appropriate dilutions were cultured into

mitis salivarius bacitracin (MSB) agar for the selective isolation and enumeration of *S. mutans*. The MSB agar (Difco Laboratories; Detroit, MI) contained pancreatic digest of casein, proteose peptone no.3, proteose peptone, dextrose, saccharose 20%, dipotassium phosphate, trypan blue, crystal blue, agar, Chapman tellurite, and bacitracin 0.2 U/ml. The MSB agar plates were incubated anaerobically ( $H_2:CO_2:N_2$  10:10:80) for two days at 37° C (Fure 1998). Colony counts with a morphology typical of *S. mutans* were made on MSB agar (Emilson 1981). Microbial counts were expressed as colony-forming units (cfu) per ml of unstimulated saliva. Colonies on the MSB agar plates were visualized by Gram's stain and subjected to the following biochemical tests: raffinose, mannitol, melibiose, trehalose and inulin fermentation; esculin hydrolysis in the presence and absence of bile; urease; arginine hydrolysis; and the resistance to bacitracin. The biochemical profile of *S. mutans* is: raffinose, mannitol, melibiose, trehalose and inulin positive fermentation; esculin hydrolysis negative in the presence of bile and positive in the absence of bile ; negative urease; negative arginine hydrolysis; and resistance to 2U of bacitracin. Differences in the *S. mutans* counts between the population having caries and the caries-free population were tested with chi-square test.

### Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MIC) were evaluated against penicillin, amoxycillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin by using an agar dilution method with concentrations between 0.003 and 32 ug/ml (Liebana et al., 1989). The antimicrobial standard powders of penicillin, amoxycillin, cefazolin, erythromycin, clindamycin and vancomycin were purchased from Sigma Chemical (St Louis, MO, USA) and imipenem from Merck (Germany). By using a replicator device, standardized suspensions from  $10^5$  UFC/ml of the test bacterium were applied in Wilkins-Chalgren agar. After 48 h of incubation a 35 °C in an anaerobic atmosphere ( $H_2:CO_2:N_2$  10:10:80), the MIC was determined as the lowest concentration of antimicrobial agent that inhibited visible growth of an organism.

### RESULTS AND DISCUSSION

The dental caries experience in these children was 66% (35/53) and *S. mutans* was present in 33 of the 53 children involved in the study (Table 1). Therefore, the presence of *S. mutans* in this population was 62%. Only 21 of the 33 children with *S. mutans* (64%) had caries. In the 20 children group

TABLE 1. Relationship between *S. mutans* colonization and dental caries.

<i>S. mutans</i> presence	No. of infants with:		
	Caries	Caries-free	Total (%)
Positive	21	12	33 (62)
Negative	14	6	20 (38)
Total	35	18	53 (100)

from whom *S. mutans* was not isolated, 14 (70%) were found to have caries (Table 1). Not detecting *S. mutans* in these children was probably due to the fact that the numbers of this microorganism were below the detection limits of the current cultivation techniques. Another explanation could be the presence of other microorganisms (*Lactobacillus* and/or *Actinomyces*) that cause more advanced dental caries, for which isolation strategies have not yet been designed in this study. Bacterial counts of *S. mutans* ranged from  $10^3$  to  $> 10^7$  cfu / ml. There were no statistically significant differences in *S. mutans* counts between the group with dental caries and the caries-free group ( $p=0.21$ ). The next step would be to obtain information about the colonization and stability of these strains in the studied population. Identifying the time frame for the colonization and stable presence of *S. mutans* would be very useful for designing strategies for dental caries prevention. In order to accomplish this task, it may be necessary to resort to the techniques to amplify and genotype DNA.

Bacteremia originating in the oral cavity is rather common, and in some cases it can cause endocarditis. In addition to its participation in the etiology of dental caries, *S. mutans* also produces bacteremia, a systemic infection or subacute endocarditis resulting from dental treatments (Ullman et., 1988). Adequate treatment for these infections requires the determination of the antimicrobial susceptibilities of the *S. mutans* strains isolated from the oral cavity. *S. mutans* susceptibility to the antibiotics tested is illustrated in Table 2. All isolates were highly sensitive to penicillin, amoxicillin, cefazolin, erythromycin, clindamycin, imipenem and vancomycin; 50 and 90% of *S. mutans* strains were inhibited by using concentrations of less than 0.12 and 0.5 ug/ml, respectively, for all antibiotics studied. The lowest mean value obtained was reported for penicillin. The MIC of all

**TABLE 2. MIC range, MIC mean, MIC<sub>50</sub> and MIC<sub>90</sub> values (ug/ml) for selected antibiotics on 33 *S. mutans* strains**

Antimicrobial agent	
Penicillin	Range 0.007-0.06
	Mean 0.024
	MIC <sub>50</sub> 0.030
	MIC <sub>90</sub> 0.030
Amoxycillin	Range 0.03-0.50
	Mean 0.15
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.25
Cefazolin	Range 0.06-1
	Mean 0.20
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.25
Erythromycin	Range 0.015-0.50
	Mean 0.15
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.50
Clindamycin	Range 0.015-0.50
	Mean 0.14
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.25
Imipenem	Range 0.015-0.50
	Mean 0.12
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.25
Vancomycin	Range 0.03-1
	Mean 0.25
	MIC <sub>50</sub> 0.12
	MIC <sub>90</sub> 0.50

the strains from this study were found to be very similar to those observed by other authors (Liebana et al., 1991, De la Higuera et al., 1999).

The following conclusions can be drawn from the study: (1) the presence of *S. mu-*

tans in this population was 62%; (2) not all of the children hosting this microorganism had caries; and (3) *S. mutans* strains were highly sensitive to the antibiotics tested.

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