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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 
an aerobiosis durante 2 días a 37 o 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 encontro 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

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 determinate 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 Turnequé (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; Detroi, 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 (H₂:CO₂:N₂ 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 μg/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 10⁵ UFC/ml of the test bacterium were applied in Wilkins-Chalgren agar. After 48 h of incubation at 35 °C in an anaerobic atmosphere (H₂:CO₂:N₂ 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 without *S. mutans*...
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 al., 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 μg/ml, respectively, for all antibiotics studied. The lowest mean value obtained was reported for penicillin. The MIC of all 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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REFERENCES


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