Salivary biotypes of mutans Streptococci levels in schoolchildren aging 6-8 year old having a socioeconomic base

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Abstract
There is still little information on the mutans Streptococci biotypes in South America, specifically in Brazil, including regional and local information, associated to different socioeconomic categories. In this paper, the Streptococcus mutans levels and biotypes in Brazilian children aged 6-8 year old having a socioeconomic base was investigated. The sampling consisted of 200 selected children belonging to five different socioeconomic categories. Bacterial examination was made using children saliva diluted and cultivated in Bacitracin sucrose agar (SB-20). The plates were incubated by 48 hours/37°C at 10% CO₂ in anaerobiosis and after colonies growth samples identification was performed by biochemical tests. Results showed that 78% of the 491 tested samples harbored S. mutans, followed by S. sobrinus (11.6%), S. rattus (4.69%), S. mutans V (2.65%), S. cricetus (1.83%) and S. ferus (1.22%). The prevalence of Streptococcus mutans was found in every socioeconomic level. Among children harboring multiple species, the most frequent association detected was S. mutans/S. sobrinus (17%).

Key Words: mutans Streptococci, distribution, children, socioeconomic base.

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Introduction
Several epidemiological studies have been made about the prevalence, level and geographical distribution of the mutans Streptococci11. These microorganisms are subdivided in seven different species and eight serotypes12-14, having already been demonstrated that S. mutans, S. sobrinus, S. rattus, S. cricetus and S. ferus are cariogenic for experimental models14-22.

Many studies showed variations in the distribution of those microorganisms. In Europe and North America, serotypes c (80%) and with smaller frequency, d and a23 were found. In Egypt, the predominant were a and b serotypes24. Therefore, there is little information on the mutans Streptococci serotypes distribution in South America, specifically in Brazil, including regional and local information, associated to a different socioeconomic categories. The aim of this study was to isolate and identify salivary biotypes of mutans Streptococci levels in schoolchildren aged 6 to 8 year old having a socioeconomic base.

Material and Methods
1. Subjects
Two hundred children aging 6 to 8 year old without race or sex distinction, with different dental decay indexes without using antibiotics at least six months were used in this study. Children of five different socioeconomic categories (A - E), according to the ABA/ABIPEME criteria, from Piracicaba, SP, Brasil urban perimeter were selected.

2. Bacterial isolates
A gum-base piece of 1.5 grams was supplied to children chewed it for 30 seconds. The stimulated saliva was desposed and starting from this moment it was marked the time of 3 to 5 minutes and then collected it in tubes approximately of 3ml. The collected samples were conserved in ice during collection and transported to the Microbiology and Immunology laboratory. The time elapsed from the samples collection did not exceed 4 hours3. Samples were mixed for 30 seconds and the saliva samples were diluted (10⁻¹ to 10⁻⁴) in 0.05M Phosphate buffered/solution (PBS) pH7.3. Aliquots of 25mL of each dilution were inoculated in Bacitracin Sucrose Agar/SB-20° medium and then incubated at 37ºC for 48 hours in anaerobic water-jacketed CO₂ incubator (Cole-Parmer Instruments).

3. Biotyping
At least three typical colonies with morphological characteristics of S. mutans according to Van Palenstein-Helderman et al.26 and Torres et al.27 were transferred to 4ml of Brain Heart Infusion (BHI) medium, incubated at 37ºC for 24 hours and after growth stored at -70ºC for further biochemical identification tests28.

Results
Among the 491 mutans Streptococci isolated, S. mutans (78%) was predominant. S. sobrinus and S. rattus were 11.61% and 4.69% respectively, for all the socioeconomic categories as shown in Table 1.

Table 2 shows the distribution and frequency (%) of children harboring different species of Streptococcus group mutans according to the socioeconomic categories A, B, C, D and E. Most of the individuals (59%) were carriers of S. mutans followed by the association of S. mutans + S. sobrinus (17%) and S. mutans + S. rattus (7.5%). Other species showed less than 5.0% of association.

Discussion
The identification of the microorganisms (491 samples) performed through the morphological and biochemical characteristics, according to the outline of Hardie28 allowed the identification of multiple types of the bacterium Streptococcus group mutans concerning to the different socioeconomic categories (A to E). Most of the isolates were identified as being S. mutans (78%) and S. sobrinus (11.6%).

These results are in agreement with the data of Marfort et al.2, Farghaly et al.29, Torres30 and Saarela et al.31, which found the serotype c (biotype I) as the prevalent specie in the population studied. In our study, the S. mutans serotype c (biotype I) was also the prevalent specie among all the population concerning to the five socioeconomic categories, followed by S. sobrinus serotype d (biotype IV), S. rattus serotype b (biotype II), S. mutans V serotype e (biotype V), S. cricetus serotype a (biotype III) and S. ferus (serotype c). These data suggest a possible predominance of the serotype c (biotype I) in our region.

The frequency of S. sobrinus (serotype d - biotype IV) was the second specie after the serotype c, being in agreement with the findings of Nyvad and Kilian32. Our results also showed that this specie was detected in association with the serotype c – biotypes I and V – (Table 2) being also found in association with other serotypes33-35. These authors still relate that the decay index was larger in the children with the combination of S. mutans and S. sobrinus species when compared to those harboring only S. mutans.

The species S. rattus (serotype b - biotype II), was detected and isolated in low proportion: at the socioeconomic category B and C or in association with other biotypes (Table 2). According to Farghaly et al.29, Nyvad and Kilian32 and Beighton et al.36 this serotype was not detected individually neither in association with other biotypes, as also found by Marfort3 and Brathal34. According to Torres et al.37, the detection of the serotype b (biotype II) may be associated to the socioeconomic status of the population, to the lifestyle (residents in the periphery) of the individuals and/or to the absence of provisioning water fluorotation. Our results showed this microorganism individually in the
socioeconomic categories B and C and in association with other serotypes (c and d) in all the socioeconomic categories, including the higher socioeconomic category A and B, residents in the central area of the city, provisioned by water fluoreration. The socioeconomic status of any population, may not necessarily be a decisive factor in the detection of this biotype. This kind of association is still less found in the literature, and being taken into account that the isolation of such serotype has been detected in low frequency in the population sampled, statements of such nature, need larger investigations.

Table 1. Distribution and prevalence (%) of mutans Streptococci biotypes between children having a socioeconomic base.

<table>
<thead>
<tr>
<th>Isolates species</th>
<th>Socioeconomic Categories</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td></td>
<td>60</td>
<td>67</td>
<td>81</td>
<td>93</td>
<td>82</td>
<td>383</td>
<td>78.00</td>
</tr>
<tr>
<td>Streptococcus sobrinus</td>
<td></td>
<td>11</td>
<td>5</td>
<td>8</td>
<td>14</td>
<td>19</td>
<td>57</td>
<td>11.61</td>
</tr>
<tr>
<td>Streptococcus rattus</td>
<td></td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>23</td>
<td>23</td>
<td>4.69</td>
</tr>
<tr>
<td>Streptococcus mutans V</td>
<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>13</td>
<td>2.65</td>
</tr>
<tr>
<td>Streptococcus cricetus</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>1.83</td>
</tr>
<tr>
<td>Streptococcus ferus</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>1.22</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>81</td>
<td>80</td>
<td>99</td>
<td>117</td>
<td>114</td>
<td>491</td>
<td>100.00</td>
</tr>
</tbody>
</table>

The detection of the S. mutans V (serotype e - biotype V), differs of the biotype I, by the melibiose use. In our sample, was present in most, in association with other serotypes (c and d), and only two individuals (socioeconomic categories A and D) showed this specie individually. These results are in agreement with the data obtained by Thomson et al.39 and Miranda et al.38, which also found such species in association with other serotypes. These authors also showed that this biotype is not related to high dental decay indexes even when associated with other biotype, not subject of our study.

Concerning to the frequency of multiple serotypes of mutans Streptococci, the most frequent association founded was S. mutans/S. sobrinus (17%) approaching to the results obtained by Shklair and Keene39 and Fracalanzza40, which showed 27.6% and 21.4% respectively, followed by the association S. mutans/S. rattus (7.5%) and S. mutans/S. mutans V (3.5%) as shown in Table 2. Azevedo41, described the combination S. mutans/S. sobrinus in a smaller percentage (2.78%), followed by S. mutans/S. mutans V (1.85%), being the most frequent combination S. mutans/S. rattus (19.44%). Fracalanzza4 and Shklair and Keene38 showed, for the combination S. mutans/S. mutans V respectively, 28.6%, 1.85% and for the triple association this last author showed S. mutans/S. sobrinus/S. mutans V (71.0%), much higher than our results (1.0%).

This supposed disagreement concerning to the multiple types

Table 2. Children harboring multiple species and/or associations of mutans Streptococci according to the socioeconomic categories.

<table>
<thead>
<tr>
<th>Multiple Species and/or combinations</th>
<th>Socioeconomic Categories (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>S. mutans</td>
<td>25-62.5%</td>
</tr>
<tr>
<td>rattus</td>
<td>0</td>
</tr>
<tr>
<td>sobrinus</td>
<td>2 - 5.0%</td>
</tr>
<tr>
<td>cricetus</td>
<td>1 - 2.5%</td>
</tr>
<tr>
<td>mutans V</td>
<td>1 - 2.5%</td>
</tr>
<tr>
<td>mutans + S. sobrinus</td>
<td>5 - 12.5%</td>
</tr>
<tr>
<td>mutans + S. rattus</td>
<td>3 - 7.5%</td>
</tr>
<tr>
<td>mutans + S. mutans V</td>
<td>0</td>
</tr>
<tr>
<td>mutans + S. cricetus</td>
<td>0</td>
</tr>
<tr>
<td>mutans + S. ferus</td>
<td>0</td>
</tr>
<tr>
<td>sobrinus + S. ferus</td>
<td>0</td>
</tr>
<tr>
<td>sobrinus + S. mutans V</td>
<td>0</td>
</tr>
<tr>
<td>mutans + S. mutans V + S. sobrinus</td>
<td>0</td>
</tr>
<tr>
<td>mutans + S. sobrinus + S. rattus</td>
<td>1 - 2.5%</td>
</tr>
<tr>
<td>mutans + S. sobrinus + S. cricetus</td>
<td>1 - 2.5%</td>
</tr>
<tr>
<td>mutans + S. sobrinus + S. ferus</td>
<td>1 - 2.5%</td>
</tr>
</tbody>
</table>
combination, may be due to the saliva sampling performed in this research, instead of “pool of plaque”, as described and preconized by Masuda et al., showing also that 68% of the individuals harbored two or more serotypes of those microorganisms. These diversities in the multiple types of mutans Streptococci isolations, shows that geographical variations may be a limiting factor in the prevalence of certain species. The association of the mentioned biotypes may not be determined by mechanisms or well-known processes, happening therefore, by chance.

However, the statement of Beighton et al. and Thibodeau et al., in relation to the need of more researches to elucidate the mechanisms by which certain species of mutans Streptococci tend settling down, to prevail and to colonize the buccal cavity, individually or in association with another species must be take into account. Thus, new researches, seeking to elucidate such variability, should be done by oral researchers looking for its meaning in relation to dental decay.

Conclusion
- Seventy-eight percent of the 491 tested samples are colonies of S. mutans, followed by S. sobrinus, S. rattus, S. mutans V, S. cricetus, and S. ferus;
- The predominance of S. mutans is find in all socioeconomic categories;
- Among individuals harboring multiple species, the most frequent association is S. mutans/S. sobrinus.

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