Viridans Streptococci Associated with Periapical Dental Abscesses

Summary: Viridans streptococci isolated in apparently pure culture from periapical dental abscess have been examined. It has been found that each abscesses is associated with only one type of a given species of Streptococcus. The distribution of types of Strep. mitis found in abscesses differed from the distribution in healthy mouths, but no particular type occurred significantly more often in abscesses than in mouths.

The microbial flora of dental abscesses has been investigated by many workers (1-8) who isolated a variety of organisms including Staphylococcus epidermidis, Staph. aureus, Haemophilus spp., Bacteroides spp., coliforms and micrococci. However, viridans streptococci were isolated most frequently, both in pure and mixed culture. Akin and Agren (4), in their series of 27 specimens obtained, by direct sampling of the abscess either by aspiration or swabbing, isolated viridans streptococci in pure and mixed culture in 12 and 5 cases respectively; Feldman and Larje (5) examined 73 specimens obtained in a similar manner and found viridans streptococci in 17 and 29 specimens in pure and mixed culture respectively. Moore and Russell (6), in their comparative study of aspiration and swabbing of 50 abscesses, obtained viridans streptococci in 11 of the 25 aspirates which resulted in growth of a single organism.

Some methods of sampling dental abscesses are very prone to contamination by normal oral flora, which may in part account for the high percentage of isolates of viridans streptococci in early work. It is evident, however, that more sophisticated techniques, such as aspiration and sampling after flap retraction (5, 6) resulted in viridans streptococci being isolated in pure culture more commonly than any other organism.

"Viridans streptococci" are commensal organisms which may give rise to opportunistic infections (9); however, the workers listed above did not classify strains of viridans streptococci from periapical abscesses in detail. Miranda (10) differentiated his isolates into species but they were obtained from root canals with periapical reactions and not from the abscesses themselves. Carlsson (11) differentiated five species of viridans streptococci by their appearance on mitis-salivarius agar and production of an extracellular polysaccharide: Streptococcus sanguis, Strep. mutans, Strep. salivarius, Strep. mitis and Group IV. Employing 12 biochemical and physiological tests, he was able to classify Strep. sanguis and Strep. mitis into 9 types each, Strep. salivarius and Group IV into three and two types respectively. Colman and Williams (12) classified viridans streptococci into six taxonomic groups as follows: Strep. pneumoniae, Strep. salivarius, Strep. mutans, Strep. sanguis, Strep. mitior and Strep. milleri. This differentiation was based upon many tests but the taxa were not classified further into types. In the two systems, Strep. sanguis, Strep. mutans and Strep. salivarius have similar properties: Strep. mitior and Strep. mitis are equivalent species; Carlsson's Group IV is an acidogenic variant of Strep. mitis not defined in the other system; a new species Strep. milleri, is listed by Colman and Williams (12).

As stated earlier, viridans streptococci from dental abscesses have not previously been differentiated into species. In particular, the question whether any particular species or types within species predominate in such infected abscesses has not been answered. The present work attempts to provide some of the answers to these questions, using Carlsson's method.

Materials and Methods

Origin of cultures: Clinical material was collected at the Turner Dental Hospital, Manchester, either by aspiration or swabbing after flap retraction of the mucous membrane overlying the abscesses. Immediately before sampling, the area was thoroughly cleaned with 0.5% chlorhexidine in 70% alcohol (6). Each specimen was plated out onto two blood agar plates, one incubated aerobically, the other anaerobically, onto a chocolate incu- bat. Only when this procedure gave solely greenish streptococci, or a mixture of greenish streptococci and veillonellae, were the organisms examined further. In 20 such cases, one colony of streptococci from a plate was subcultured for further study; in each of a further 30 specimens, four well-separated colonies of viridans streptococci from a single plate were streaked onto blood agar, for subsequent characterization. Control samples were taken from the mouths of 50 dental students. A sterile cotton wool swab was rubbed...
over the dorsal and ventral aspects of the tongue, gums, soft palate and cheeks and was then streaked onto blood agar and incubated as before. Four greening colonies were picked from each plate. All cultures were maintained at 4°C and subcultured at monthly intervals on blood agar.

Colonial and cellular characteristics: These were examined on cultures grown aerobically and anaerobically on 5% horse blood agar, Mitis-Salivarius agar (Oxoid) and in Hartley's digest broth (Oxoid) containing 0.5% glucose.

Serological grouping: Precipitation tests were carried out on organisms grown in Hartley's digest broth (Oxoid) containing 0.5% glucose. The antigens were prepared by Lancefield's method (13) and the tests performed in capillary tubes using antisera A-S (Wellcome Laboratories). Further antisera were raised in rabbits to a representative of a Carlson type of each species that did not carry Lancefield's group antigens. Extracts of homologous and heterologous types were tested with each antisera. The strains employed were Strep. sanguis type b, Strep. mutans, Strep. mitis type c, and Strep. mitis type i.

Antibiotic sensitivity: This was examined on DST agar (Oxoid) containing 5% lysed horse blood. Antibiotic discs (Mast Laboratories) containing the following were employed: penicillin G 1 unit, streptomycin 10 μg, tetracycline 25 μg, erythromycin 5 μg, sulphadiazine 200 μg, vancomycin 25 μg. A clear zone of 4 mm or more was taken as indicating sensitivity.

Production of extracellular polysaccharide: This was tested for by adding 1 ml and 3 ml ethanol to 1 ml supernatant of a culture in 5% sucrose broth.

Growth in 4% and 6.5% sodium chloride: These tests were performed using Phenol Red Broth Base (BBL) supplemented with 1% glucose, 0.3% yeast extract and 3.5% or 6% sodium chloride (w/v) respectively.

Growth on 10% and 40% bile blood agar: These tests were performed using Brain Heart Infusion agar (Oxoid) containing 5% horse blood, to which ox bile was added. The plates were examined after 48 hours.

Gelatinase production: This was tested in nutrient gelatine (Oxoid) incubated for 1 week.

Catalase: The presence of catalase was tested by adding one drop of 3% H₂O₂ onto a colony on Mitis-Salivarius agar.

Arginine hydrolysis: Ability to hydrolyse arginine was tested by the method of Cowan and Steel (14).

Aesculin hydrolysis: This was examined by the method of Cowan and Steel (14).

Optochin sensitivity: Growth in the presence of optochin was tested on DST agar by the method of Bowers and Jeffries (15).

Growth at 45°C: Ability to grow at 45°C was examined in Phenol Red Broth (BBL) containing 1% glucose after one week's incubation.

Acid production: Acid production from raffinose, inulin, mannoitol, sorbitol and trehalose was tested in Phenol Red Broth (BBL) to which 10% w/v filter-sterilised aqueous solution of carbohydrate was added to give a final concentration of 1%. The tests were performed in small bottles containing 3 ml broth. Terminal pH in 1% glucose, lactose and sucrose broths was examined after 1 week's incubation, using a pH meter.

Analysis of acid end products of glucose metabolism: A representative sample of each of the commonly occurring Carlsron types isolated from both abscesses and healthy mouths was selected for this study. Cultures were grown in 50 ml Brain Heart Infusion Broth (Oxoid). The supernatants were treated with 50% H₂SO₄ and the volatile acids extracted with diethyl ether; 0.5 N caproic acid was used as an internal standard. Methyl esters of the non-volatile acids were prepared by adding measured volumes of the methylaating agent, boron trifluoride in methanol, to the culture supernatants. The methyl esters were extracted with chloroform. Ether and chloroform solutions of known acids and methyl esters were examined, using a Perkin-Elmer gas chromatograph. The retention times of the known acids facilitated identification of acids extracted from the cultures; uninoculated broth was used as control. The height of the peak on the graph produced by each acid in a culture was divided by that due to added caproic acid. This ratio provided a semi-quantitative measure of each acid and thus enabled cultures to be compared with regard to their acid production.

Screening of cultures: The four streptococcal isolates obtained from each sample (see above) were initially examined as follows:

1. The colonial characteristics on two plates of Mitis-Salivarius agar, one incubated aerobically and the other anaerobically for 48 hours, were noted.
2. Terminal pH in 1% glucose broth was recorded. A difference of more than 0.5 units between cultures was considered to be significant.
3. Ability to grow at 45°C was tested.
4. Production of extracellular polysaccharide was examined.

If the four isolates from each sample gave identical results, they were taken as comprising one strain; if not, they were considered to be different strains and/or species.

Results

Screening of abscess isolates: Four isolates of viridans streptococci from each of 30 clinical samples were subjected to four screening tests as above. Of these 120 isolates, there were apparently 60 strains distributed among the different species as shown in Table 1. It was observed that in only one sample, did different species coexist, viz. Strep. sanguis and Strep. mitis. The 20 single isolates from the other series were classified as Strep. sanguis, 3; Strep. mutans, 1; Strep. mitis, 16. Thus, there were finally 80 different isolates to be comprehensively tested; four died out before this was done.

Table 1: Strains in 30 Abscesses

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of abscesses</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. sanguis</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Strep. mutans</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Strep. mitis</td>
<td>23</td>
<td>45</td>
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</tbody>
</table>

Screening of controls: 200 isolates of viridans streptococci from 50 normal mouths were similarly screened. There were apparently 157 strains as shown in Table 2. All samples contained at least two species. There were no Strep. mutans strains isolated.

Table 2: Strains in 50 Mouths

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of mouths</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. sanguis</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>Strep. mitis</td>
<td>47</td>
<td>122</td>
</tr>
<tr>
<td>Strep. salivarius</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Group IV</td>
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<td>3</td>
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</table>