Detection of different *Prevotella* species from deep dentinal caries of primary teeth - A culture and biochemical study

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**Abstract**

**Background:** Oral health is a significant component of general health, with dental caries affecting a person’s ability to eat, speak, or socialize. Cariogenic bacteria show transition from predominantly facultative Gram-positive bacteria in early caries to anaerobic Gram-positive rods and cocci and Gram-negative rods in deep carious lesions. Role of *Prevotella* spp. in deep dentinal caries in children has been elucidated in the present study.

**Materials and Methods:** A total of 31 patients with age range of 6–12 years were selected. Radiographic loss of at least 50% of the total dentinal thickness was considered as deep dentinal caries. The carious dentine of selected subjects was used for culture and biochemical analysis.

**Results:** Of 31 samples, 15 samples showed positivity for pigmented and non-pigmented organisms in culture study using blood agar and Kanamycin-Vancomycin BA. In these 15 samples, 13 showed positivity for *Prevotella* spp. in biochemical analysis. Pigmented *Prevotella* was 42% and non-pigmented *Prevotella* was 6.4% in these samples.

**Conclusion:** This study confirms the association of *Prevotella* spp. in causation of deep dentinal caries in mixed dentition age group.

**Introduction**

Oral health is significant component of general health, with dental caries affecting a person’s ability to eat, speak, or socialize.¹ Dental caries is one of the most prevalent health problems worldwide that causes demineralization and destruction of the hard tissues of teeth. It not only affects majority of adults but also children. A survey says about 6–9 children in every 10 are affected by dental caries.² Overall, the prevalence of dental caries in India is 80% in children and 60% in adults according to National Oral Health Survey.³ The prevalence of dental caries is said to be as high as 72% in the rural adolescents.⁴ Among various risk factors for dental caries, microbiological and dietary risk factors are chiefly involved in development and progression.⁵ The microbiological colonization of human teeth begins immediately on tooth eruption. Primary plaque colonizers can specifically adhere to acquired enamel pellicle formed by salivary proteins and glycoproteins.⁶ Among microorganisms, *Mutans Streptococci* are the most cariogenic pathogens for the initiation of dental caries as they are highly acidogenic and can dissolve hard tissues of teeth.⁶ As the lesion progresses, there is a transition from predominantly facultative Gram-positive bacteria in early caries to anaerobic Gram-positive rods and cocci and Gram-negative rods in deep carious lesions.⁷ Endodontic and/or periodontal infections are commonly found preceded by caries process and are associated with anaerobic bacteria, including that of the black-pigmented bacteria mainly *Porphyromonas* spp. and *Prevotella* spp. Advances in molecular approaches have revealed the existence of the proteolytic Gram-negative anaerobic genus *Prevotella* in deep dentinal caries sites which provide a unique environment for a complex array of novel and uncultured *Prevotella* and *Prevotella*-like bacteria.⁸ Culture of these bacteria is done by reproducing the conditions existing in the natural habitat from which the samples were taken. Although molecular techniques give accurate results, it also has its own limitations like detecting dead cells from samples producing false-negative results.⁹ Culture and biochemical testing revealed a discrepancy
among the number of *Prevotella* organisms and the scarcity of oral forms of the genus recognized. A study conducted by Martin et al. showed that dental caries in its advanced stages host’s unique environment for a complex array of novel and uncultured *Prevotella* and *Prevotella*-like bacteria which can dominate diverse polymicrobial community associated with the dental caries in early stages. Role of *Prevotella* spp. in periodontal and endodontic infections in adults is well known. However, the role of *Prevotella* spp. in deep dental caries in children without endodontic and periodontal infection is yet to be elucidated.

In this study, we report on the enumeration of bacteria both pigmented and non-pigmented *Prevotella* spp. isolated from deep carious dentine of children by both colony counting and biochemical analysis and define its association with deep dental caries.

**Materials and Methods**

A total of 31 patients attending the department of pedodontics of our institute, for regular dental checkup and treatment with age range of 6–12 years, were selected. Of these, subjects with deep dental caries without any symptoms such as pain, tenderness on percussion, swelling, sinus drainage, or pus discharge were excluded from the study. Radiographic loss of at least 50% of the total dentinal thickness was considered as deep dental caries. Subjects undergoing orthodontic treatment, preventive dentistry regimen, and antibiotic therapy were excluded from the study. Subjects with apparent systemic illness and carious permanent teeth were also excluded from the study. Informed consent was obtained from the respective parents or guardian of the subjects who were enrolled in the study has been obtained. Ethical clearance was obtained from the institute’s ethical review board.

**Methodology**

The unrestored teeth with coronal dentine caries were selected on the basis of clinical diagnostic tests indicating deep dental caries without obvious exposure of the pulp tissue and without periodontal pocket. The carious dentine of selected subjects was collected atraumatically using a sterile spoon excavator and transferred into a sterile 1.5 ml microcentrifuge tube containing reduced transport fluid (RTF) and transported to the laboratory.

**Determination of numbers of colony-forming unit (CFU)**

After collection of carious dentine, fragments were dispersed in RTF by first vortexing the fragments for 20 s. Samples were plated on blood agar (BA) and Kanamycin-Vancomycin BA (KV-BA) in duplicates and incubated in an anaerobic chamber for up to 5–6 days [Figure 1]. The total microbial load per milligram of dentine was determined by the measurement of the number of CFU and colony morphology on the agar plates. Load of *Prevotella* spp. was determined by counting the number of colonies of specified organism based on colony morphology which is pinpointed black/brown pigmented and non-pigmented moist colonies of *Prevotella* species. These colonies were confirmed by Gram staining followed by biochemical tests such as catalase, nitrate reductase, and sugar fermentation tests. Later, the speciation was made based on standard biochemical tests. The results are tabulated for statistical analysis.

**Results**

Of 31 samples, 15 samples (48.4%) showed positivity for pigmented and non-pigmented organisms in culture study using BA and KV-BA. 15 cases which were positive during culture showed maximum positivity for *Prevotella* spp., followed by *Porphyromonas* species [Tables 1 and 2]. Studies have been carried out with variable results regarding the isolation of pigmented *Porphyromonas* and *Prevotella* spp. through culture. The variations can be attributed to fastidious nature of these organisms and difficulty in culture of these organisms.

Of 15 samples showing pigmented and non-pigmented colonies in culture, 13 samples showed to contain *Prevotella* spp. in biochemical analysis. Remaining two samples turned out to be *Porphyromonas* spp. In 13 samples of *Prevotella* species, majority were pigmented and two samples contained both pigmented and non-pigmented microorganisms. In pigmented species, majority were *P. intermedia* and one sample was positive for *Prevotella melaninogenica*. The two samples with non-pigmented organisms were of *Prevotella buccae*.

Of total 31 samples, percentage of pigmented *Prevotella* was 42% and non-pigmented *Prevotella* was 64%. Fisher’s exact test to see the difference of proportions of *Prevotella* spp. in deep dental caries was non-significant (P = 0.226).

**Discussion**

The eruption of the permanent dentition begins at around age 6 years, with the mandibular central incisors and first mandibular and maxillary molars, and is almost complete by around age 12-14 years with the eruption of the second molars. This period (6–12 years) when the mouth contains both primary and permanent teeth is often described as the mixed dentition.

The periodontopathic bacteria are most commonly identified in subgingival area, supragingival area, saliva, dorsal, and lateral surfaces of tongue. Studies have also shown that the detection of these bacteria can vary according to various criteria such as phase of dentition, age, gender, and ethnicity.

The prevalence of dental morbidity is documented in terms of the number of teeth (T) or tooth surfaces (S) that have obvious decay (D), contain a dental restoration or filling (F), or are missing (M). Variability in microbial counts between samples

**Table 1: Number of positive cases positive for *Prevotella* sp. (pigmented and non-pigmented)**

<table>
<thead>
<tr>
<th>Total number of cases</th>
<th>Positive cases</th>
<th>Only pigmented</th>
<th>Pigmented and non-pigmented</th>
<th>Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 2: Number of samples positive for pigment and non-pigmented *Prevotella* sp. in deep carious lesions

<table>
<thead>
<tr>
<th>Total samples</th>
<th>Colonies positive for pigmented species</th>
<th>Average number of colonies</th>
<th>% of pigmented species</th>
<th>Colonies positive for non-pigmented species</th>
<th>Average number of colonies</th>
<th>% of non-pigmented species</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>15</td>
<td>4.0666×10⁶</td>
<td>48.38</td>
<td>3</td>
<td>3.1666×10⁶</td>
<td>9.67</td>
</tr>
</tbody>
</table>

Figure 1: (a and b) Growth seen in Blood agar and Kanamycin-Vancomycin blood agar

Table 3: Number of samples positive for *Prevotella* spp. in biochemical analysis

<table>
<thead>
<tr>
<th>Total number of cases</th>
<th>Samples taken for biochemical tests</th>
<th>Number of samples positive for <em>Prevotella</em> sp.</th>
<th>% of <em>Prevotella</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>15</td>
<td>7</td>
<td>22.5</td>
</tr>
</tbody>
</table>

Table 4: Number of samples positive for pigmented and non-pigmented colonies in biochemical tests

<table>
<thead>
<tr>
<th>S. No</th>
<th>Species</th>
<th>Number of cases positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>P. intermedia</em></td>
<td>6</td>
<td>19.35%</td>
</tr>
<tr>
<td>2.</td>
<td><em>P. melaninogenica</em></td>
<td>1</td>
<td>3.22%</td>
</tr>
<tr>
<td>3.</td>
<td><em>P. buccae</em></td>
<td>1</td>
<td>3.22%</td>
</tr>
</tbody>
</table>

*Prevotella intermedia: P. intermedia, Prevotella melaninogenica: P. melaninogenica, Prevotella buccae: P. buccae*

has been observed as seen in previous studies of Martin et al.\(^{[7]}\) and Massey et al. However, the results from the present study differed in that isolates positive for *Prevotella* species are less significant (22.5%) than previous studies (88%) \([\text{Table 3}].\) This difference can be attributed to samples collected from extracted teeth with symptoms of reversible or irreversible pulpitis in permanent dentition. However, in the present study, samples were collected from apparently asymptomatic teeth of primary dentition and also smaller sample size. Hence, there can be difference in the bacterial ecology in deep dentinal caries also as the lesion progress pulpally causing reversible or irreversible pulpitis. The study of Massey et al. gives a positive correlation between the presence of *Prevotella* spp. in the deep dentinal caries sample and thermal sensitivity as a relevant factor. Similarly, Massey et al.\(^{[17]}\) study also gives a positive correlation to the *Prevotella* spp. and extents of pulpal changes. In the present study, lesser isolates of *Prevotella* spp. can be attributed to asymptomatic sample isolates of deep carious dentin.

The present study showed 6 (19.4%) positive samples for *P. intermedia* \([\text{Table 4}].\) Among them, one sample is positive for both *P. intermedia* and *P. buccae*. A study conducted by Ooshima et al. in subgingival plaque samples, mixed dentition and permanent dentition showed 11% and 1.7% of samples positive for *P. intermedia* showing association of these bacteria from childhood to adulthood.\(^{[18]}\) The study also inferred *P. intermedia* as one of the transient microorganisms in periodontally healthy subjects and can raise other species with their presence. This study also showed a strict presence of *P. intermedia* in deep dentinal samples of mixed dentition pointed its role in the transition phase of tooth development from primary to mixed dentition. This gives a better insight into the association of these bacteria with dentinal caries. Further, its association with periodontitis has to be elucidated with a larger sample size.

In the earliest of these studies, errors may have been due to difficulties in the isolation of anaerobes or the use of an inadequate anaerobic environment. Better conformation can be done by advanced molecular tests such as viability nucleic acid assays using PCR, fluorescence microscopy, or flow cytometry.\(^{[19]}\)

This study confirms the association of *Prevotella* spp. in causation of deep dentinal caries in mixed dentition age group. Earliest detection of periodontopathic bacteria in childhood to prevent periodontal problems in the adulthood could become the future scope of the study.

References
