Assessment of the Endodontic Microbiota of Abscessed Primary Teeth using Microarray Technology

Abstract

**Context:** Knowledge of the microbial composition of abscessed primary tooth is limited. **Aim:** The aim was to investigate the presence of 10 oral bacterial species in samples from abscessed primary tooth root canals using microarray technology and to determine their association with clinical findings. **Subjects and Methods:** The samples were collected from root canals of 20 primary molars with acute primer infection. The bacterial composition of the samples was semi-quantitatively defined using a microarray system (ParoCheck®). Clinical parameters included the presence of spontaneous pain, mobility, percussion sensitivity and swelling. **Statistical Analysis:** Data were statistically analyzed by Student' t-test, Fisher’s exact Chi-square test, Freeman–Halton–Fisher’s exact test, and Spearman’s rho correlation analysis. **Results:** All the tested species were detected in the samples. F. nucleatum was the most frequent bacterium (100%), followed by P. micra (65%), P. intermedia (45%), and T. denticola (45%). According to paired bacterial combinations, F. nucleatum was significantly positively correlated with P. intermedia and P. micra (P < 0.05). T. denticola was significantly positively correlated with P. gingivalis, T. forsythia, C. rectus, and P. micra, while it was negatively correlated with Eikenella corrodens (P < 0.05). No statistically significant relationships were found between the presence of any bacteria and clinical findings. **Conclusion:** Microarray technology used in this study has demonstrated the presence of various bacteria with varying proportions in the root canals of abscessed primary teeth. The results regarding the high rate of certain bacterial combinations suggest the enhanced pathogenicity due to additive or synergistic effects of these microbial combinations.

Keywords: Abscessed primary teeth, microarray analysis, oral bacteria

Introduction

Although endodontic microbiota associated with acute or chronic dental infections have long been investigated by various researchers, knowledge about etiological factors involved in the establishment and progression of endodontic infections still remains limited.[1,2] Culture studies have suggested that a complex mixture of bacterial species, in approximately equal proportions of Gram-positive and -negative species dominated by obligate anaerobes, characterizes the etiology of endodontic infections.[3,4] In addition, ongoing research on the endodontic microbiome has shown that endodontic microbial diversity is greater than what culture-based methods have formerly been able to describe.[5] Culturing techniques used to identify species in the oral microbiome, however, remain challenging and time-consuming to apply given the anaerobic growth condition requirements of bacteria. In response, researchers have developed computerized diagnostic techniques such as polymerase chain reaction (PCR)-based DNA microarray systems to aid nucleotide sequence analysis and thereby detect complex endodontic microbiota with improved specificity and sensitivity.[4] For instance, the diagnostic ParoCheck® (Greiner Bio-One GmbH, Frickenhausen, Germany) microarray system kit, based on the detection of pathogen-specific 16S rRNA coding, comes highly recommended as a technique for identifying bacterial composition of endodontic microbiota.[4]

Because teeth with acute or chronic endodontic infections exhibit different clinical manifestations, researchers have attempted to identify bacterial species that

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correlate with various symptoms and signs.[7] Most previous studies have investigated the endodontic microbiota of permanent teeth,[3,8,9] whereas few have been performed on primary teeth.[4,10-12] Since most endodontic disorders derive from microbial infections, knowledge of the diversity of pathogens infecting the root canals of primary teeth can help develop more efficacious root canal therapies.[12]

The aim of the study reported here was to investigate the presence of 10 bacterial species in the root canals of abscessed primary teeth using microarray technology and to determine their association with clinical findings.

Subjects and Methods

Subject selection

Twenty children 4–8 years old (mean age: 6.35 ± 1.35 years), each of whom attended the clinics of the Department of Pediatric Dentistry at Istanbul University with an abscess related to primary molars, were enrolled for the study. Children who used any antibiotic for any reason in the 3 months before recruitment or who had a systemic disorder were excluded from the study. The Ethics Committee of the Istanbul Medical Faculty at Istanbul University approved the study protocol (file no. 2012/819-3101), and the researchers obtained written informed consent of each participant’s guardian before his or her enrolment in the study.

The same specialist assessed the abscessed tooth of each patient for clinical parameters (e.g. intraoral and extraoral), spontaneous pain, percussion sensitivity, mobility, and canal status (e.g. dry canal, presence of hemorrhage, and clear or purulent exudate). Only teeth without any previous root canal intervention were included as the sample of teeth, which ultimately consisted of 16 primary molars with intraoral abscesses and 4 primary molars with extraoral abscesses.

Microbiological sampling

Microbial samples were collected from root canals of primary molars with intra- or extraoral abscesses. Samples were collected from only one tooth per patient, and if the selected tooth was a multi-rooted tooth, then the sample was taken from the largest root canal.

Before collecting samples, a rubber dam was applied to each selected tooth for isolation. All processes, including instrumentation, were performed aseptically. The crown, the surrounding rubber dam, and the access cavity were cleaned with 3% H₂O₂, for 30 s, 2.5% NaOCl for an additional 30 s for disinfection, and 5% sodium thiosulfate to inactivate disinfectant agents.

Each access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile saline, and access to the root canal was achieved with sterile burs refrigerated by saline. If the root canals were dry, then a small amount of sterile saline solution was added into the canal, after which the root canal walls were scraped with a size 15 K-type hand file (Dentsply Maillefer, Ballaigues, Switzerland). Samples were collected immediately after crown access with two sterile paper points (ISO #, size 25). The main root canal depth was calculated from preoperative radiographs. Each sterile paper point was held in position for 30 s, placed in a sterile empty tube, and stored at −20°C until use.

Microbiological analysis

DNA extraction and purification of the samples were performed using GenElute Bacterial Genomic DNA Kit (Sigma–Aldrich, St. Louis, MO, USA) in accordance with the manufacturer’s instructions. PCR amplification was performed in a volume of 20 μL containing 18.8 μL of Master Mix delivered with ParoCheck kit (Greiner Bio-One GmbH) containing dNTPs and a forward as well as a 5′-end Cy5-labeled reversed primer universally targeting 16S rDNA, 1 μL of sample DNA, and 0.2 μL of Taq DNA polymerase (Fermentas UAB, Vilnius, Lithuania). Amplifications involved initial denaturation at 94°C for 1 min, followed by 45 cycles of denaturation at 95°C for 20 s, primer annealing at 60°C for 20 s, extension at 72°C for 30 s, and a final elongation after 45 cycles at 72°C for 1 min.

Hybridization was performed on a chip (ParoCheck; Greiner Bio-One GmbH) according to the manufacturer’s instructions. The slides were incubated at 60°C in a saturated humid environment for at least 5 min, after which 30 μL of hybridization buffer and 5 μL of the target PCR product were mixed at room temperature, and 25 μL of the solution was transferred onto the slide surface and overlaid with a coverslip (25 × 25 mm). Slide incubation was maintained for 10 min. Following the removal of the coverslip, the slides were treated with a buffer system provided with ParoCheck kit according to the manufacturer’s instructions. Centrifugation was used to dry the surface. The results were generated using a scanner (CheckScanner™; Greiner Bio-One GmbH) and ParoReport software provided with ParoCheck and based on Gene Pixt, Axon Instruments.

Ten species were identified by ParoCheck 10 microarray detection system: Aggregatibacter actinomycetemcomitans, Actinomyces viscosus, Tannerella forsythia, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Parvimonas micra, Porphyromonas gingivalis, Prevotella intermedia, and Treponema denticola. The results were estimated as absent, low, moderate, or high.

Statistical analysis

Data of the collected samples were assessed statistically with Statistical Package of the Social Sciences version 22.0 (IBM, Armonk, NY, USA). Student’s t-test
was used for descriptive statistics of mean, standard deviation, and frequency, as well as for quantitative data. Fisher’s exact Chi-square test and Freeman–Halton–Fisher’s exact test were used to evaluate clinical and radiographic findings according to the number of bacteria. Spearman’s rho correlation coefficient analysis was used to examine relationships between the bacterial groups. Significance was set at $P < 0.05$.

Results

Sampled teeth consisted of 12 primary second molars (60%) and eight primary first molars (40%). Of those teeth, five (25%) were located in the upper arch, and 15 (75%) were located in the lower arch. Clinical signs obtained in the primary molars are listed in Table 1. Hemorrhagic and purulent exudates were detected in 10 (50%) and 2 (10%) of the root canals, whereas 6 (30%) were dry.

All tested species were detected in the samples. The bacterial incidence and severity rates for 10 bacteria are shown in Table 2. *Fusobacterium nucleatum* was the most frequently isolated bacterium (100%), followed by *P. micra* (65%), *P. intermedia* (45%), and *T. denticola* (45%). On average, three species were detected per amplified sample.

The distributions of the prevalence of the clinical symptoms according to species number (i.e. >3 or ≤3 different species) detected per amplified sample are shown in Table 3. There were no statistically significant differences between the clinical findings and the number of bacteria ($P > 0.05$).

Data for evaluating correlation between bacterial species are shown in Table 4. A statistically significant positive correlation was found between *A. actinomycetemcomitans* and *P. gingivalis* ($P = 0.003$; $P < 0.01$), *A. viscosus*, and *E. corrodens* ($P = 0.017$; $P < 0.05$), *T. forsythia*, and *C. rectus* ($P = 0.001$; $P < 0.01$), *T. forsythia* and *T. denticola* ($P = 0.004$; $P < 0.01$), *T. denticola* and *P. micra* ($P = 0.039$, $P < 0.05$), *P. intermedia* and *F. nucleatum* ($P = 0.011$, $P < 0.05$), *P. micra* and *F. nucleatum* ($P = 0.003$; $P < 0.01$), *T. denticola* and *P. gingivalis* ($P = 0.011$; $P < 0.05$), and *C. rectus* and *T. denticola* ($P = 0.004$; $P < 0.01$) with levels of 62.2%, 52.8%, 70.3%, 61.6% 46.4%, 56.1%, 62.1%, 56%, and 62.1%, respectively. *T. denticola* was significantly negatively correlated with *E. corrodens* ($P < 0.05$). There was no statistically significant correlation between other bacterial species ($P > 0.05$). According to paired bacterial combinations, *F. nucleatum* was significantly positively correlated with *P. intermedia* and *P. micra* ($P < 0.05$) and *T. denticola* was significantly positively correlated with *P. gingivalis*, *T. forsythia*, *C. rectus*, and *P. micra*, whereas it was negatively correlated with *E. corrodens* ($P < 0.05$).

Discussion

Although a complex mix of obligate anaerobes and facultative anaerobes is involved in the disease process of dental abscesses,[13,14] few studies have been performed to identify the bacteria in primary teeth with acute dental abscesses.[12,15,16] In response, the aim of the study was to detect bacterial species in the root canals of abscessed primary teeth with microarray technology based on the detection of pathogen-specific 16S rRNA coding DNA and to determine their association with clinical findings.

Using culture-based techniques is insufficient to assess bacterial diversity in any environment.[17,18] Numerous bacteria, including *Treponema* and *Prevotella* spp., have

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**Table 1: Distribution of clinical signs in abscessed primary molars**

| Clinical signs                  | $n$ | %  |
|--------------------------------|-----|----|
| Spontaneous pain               | 10  | 50 |
| Percussion sensitivity         | 9   | 45 |
| Mobility                       | 15  | 75 |
| Dry canal                      | 6   | 30 |
| Clear exudate                  | 1   | 5  |
| Canal content                  |     |    |
| Hemorrhagic exudate            | 10  | 50 |
| Purulent exudate               | 2   | 10 |
| Hemorrhagic/purulent exudate   | 1   | 5  |
| Type of abscess                |     |    |
| Intraoral                      | 16  | 80 |
| Extraoral                      | 4   | 20 |

**Table 2: Distribution of bacterial incidence rates**

|                          | Absent | Low level | Medium level | High level | Very high level |
|--------------------------|--------|-----------|--------------|------------|-----------------|
|                          | $n$ (%)|           |              |            |                 |
| *A. actinomycetemcomitans* | 18 (90)| 2 (10)    | -            | -          | -               |
| *A. viscosus*             | 13 (65)| 3 (15)    | 2 (10)       | 1 (5)      | 1 (5)           |
| *T. forsythia*            | 16 (80)| 2 (10)    | -            | 1 (5)      | 1 (5)           |
| *C. rectus*               | 16 (80)| 1 (5)     | -            | 3 (15)     | -               |
| *T. denticola*            | 11 (55)| 4 (20)    | -            | 2 (10)     | 3 (15)          |
| *E. corrodens*            | 14 (70)| 3 (15)    | 1 (5)        | 2 (10)     | -               |
| *P. intermedia*           | 11 (55)| 2 (10)    | 2 (10)       | 5 (25)     | -               |
| *P. micra*                | 7 (35) | 2 (10)    | 1 (5)        | 7 (35)     | 3 (15)          |
| *P. gingivalis*           | 16 (80)| -         | 3 (15)       | 1 (5)      | -               |
| *F. nucleatum*            | -     | 1 (5)     | 2 (10)       | 6 (30)     | 11 (55)         |
hardly been detected with conventional culture methods and have hardly been mentioned, if not overlooked, in previous studies. The molecular techniques developed have allowed other bacteria to be associated with periradicular lesions, severe periodontal diseases, and endodontic abscesses. Microarray technology has been used in microbiological studies with higher sensitivity than conventional culture methods, with cell detection limited to 100–500 cells. That method requires less time and labor than conventional PCR techniques by simultaneously detecting 10 bacterial species in several samples.

Although the microarray technology used in this study was originally developed to detect periodontopathogens, Topcuoglu et al. have claimed that the system is convenient to use in primer endodontic infections of primary teeth. Their claim is based on the knowledge that some typical endodontic species, including Porphyromonas endodontalis, Enterococcus faecalis, Pseudoramibacter alactolyticus, Filifactor alocis, and Dialister pneumosintes, are rarely detected in root canals of primary teeth with primer endodontic infections. In contrast, some bacteria known as periodontopathogens, including P. intermedia, P. gingivalis, T. forsythia, E. corrodens, and F. nucleatum, have been closely associated with acute symptoms of endodontic infections, including abscesses.

A. actinomycetemcomitans, T. forsythia, C. rectus, T. denticola, E. corrodens, P. intermedia, P. micros, P. gingivalis, and F. nucleatum were the most frequently isolated species in endodontic infections of primary teeth. Although the microarray technology used in this study was originally developed to detect periodontopathogens, Topcuoglu et al. have claimed that the system is convenient to use in primer endodontic infections of primary teeth. Their claim is based on the knowledge that some typical endodontic species, including Porphyromonas endodontalis, Enterococcus faecalis, Pseudoramibacter alactolyticus, Filifactor alocis, and Dialister pneumosintes, are rarely detected in root canals of primary teeth with primer endodontic infections. In contrast, some bacteria known as periodontopathogens, including P. intermedia, P. gingivalis, T. forsythia, E. corrodens, and F. nucleatum, have been closely associated with acute symptoms of endodontic infections, including abscesses.

Few studies have evaluated root canal microbiota of primary teeth. The most commonly isolated species in endodontic infections of primary teeth are of Fusobacterium, Prevotella, and Porphyromonas genera. In a study by Gomes et al., P. gingivalis was detected in all root canals of necrotic primary teeth investigated, and P. nigrescens and F. nucleatum were also highly detected (93.3%). In contrast, Cogulu et al. found that T. denticola and P. gingivalis were the most prevalent species in their study. Meanwhile, Tavares et al. detected P. intermedia in 96.9% of the samples isolated from the root canal system of deciduous teeth exhibiting pulp necrosis with or without radiographically detectable radicular bone resorption. Neisseria mucosa (100%) and C. rectus (90%) have also been reported as bacteria species highly prevalent in necrotic primary root canals.

In this study, F. nucleatum (100%) was the most frequently isolated bacterium, followed by P. micros (65%), P. intermedia (45%), and T. denticola (45%). F. nucleatum has been one of the most commonly encountered micro-organisms in a majority of culture and molecular studies of acute apical abscesses. Yang et al. used PCR-based denaturing gradient gel electrophoresis and detected Prevotella spp. (24%) and Fusobacterium spp. (17.7%) as the most prevalent isolates in root canal samples of primary molars with acute periapical abscesses. Topcuoglu et al., who used the same method as that in this study, examined the root canals of primary teeth showing necrotic pulp and fistulae, in contrast to the examination of acute abscesses. Their results showed that F. nucleatum, P. intermedia, P. micros, T. denticola, and T. forsythia were detected in 96.7%, 86.7%, 83.3%, and 76.7%, respectively.

### Table 3: Evaluation of clinical signs according to the number of bacteria

| Clinical signs         | Number of bacteria | P     |
|------------------------|--------------------|-------|
|                        | n (%)              |       |
| Spontaneous pain†      | ≤3 (n=11)          | 5 (45.5) | 5 (55.6) | 1.000 |
|                        | >3 (n=9)           |        |        |       |
| Percussion sensitivity†| ≤3 (n=11)          | 4 (36.4) | 5 (55.6) | 0.653 |
|                        | >3 (n=9)           |        |        |       |
| Mobility‡              | ≤3 (n=11)          | 9 (81.8) | 6 (66.7) | 0.617 |
|                        | >3 (n=9)           |        |        |       |
| Canal content‡         | Dry canal          | 4 (36.4) | 2 (22.2) | 0.840 |
|                        | Clear exudates     | 1 (9.1)  | 0 (0)    |       |
|                        | Hemorrhagic exudate| 5 (45.5) | 5 (55.6) |       |
|                        | Purulent exudate   | 1 (9.1)  | 1 (11.1) |       |
|                        | Hemorrhagic and purulent | 0 (0)  | 1 (11.1) |       |
| Type of abscess†       | Intraoral          | 8 (72.7) | 8 (88.9) | 0.591 |
|                        | Extraoral          | 3 (27.3) | 1 (11.1) |       |

†Fisher’s exact test. ‡Freeman-Halton-Fisher’s exact test

### Table 4: Evaluation of correlation between bacterial species

| A. actinomycetemcomitans | A. viscosus | T. forsythia | C. rectus/showae | T. denticola | E. corrodens | P. intermedia | P. micros | P. gingivalis | F. nucleatum |
|--------------------------|-------------|--------------|------------------|--------------|--------------|--------------|-----------|--------------|-------------|
| A. actinomycetemcomitans | -           | -0.238       | -0.166           | 0.191        | -0.214       | 0.096        | -0.091    | 0.622**      | -0.113      |
| A. viscosus              | -0.204      | 0.271        | -0.019           | 0.528*       | -0.428       | 0.153        | 0.024     | 0.152        | 0.248       |
| T. forsythia             | 0.703**     | -0.616**     | -0.319           | 0.298        | 0.209        | 0.425        | 0.248     | 0.432        | 0.370       |
| C. rectus                | 0.621**     | -0.320       | 0.027            | 0.198        | 0.126        | 0.432        | 0.370     | 0.560*       | 0.360*      |
| T. denticola             | -0.553*     | 0.377        | 0.464*           | 0.560*       | 0.370        | 0.560*       | 0.360*    | 0.560*       | 0.370       |
| E. corrodens             | -0.345      | -0.223       | -0.320           | -0.198       | -0.198       | -0.223       | -0.320    | -0.198       | -0.198      |
| P. intermedia            | 0.344       | 0.115        | 0.561*           | -0.232       | 0.621**      | -0.047       |           |              |             |
| P. micros                | 0.621**     | -0.047       |                 |              |              |              |           |              |             |

Spearman’s rho correlation test: *P<0.05; **P<0.01
and 66.7% of the cases, respectively.\(^4\) In both that and this study, \textit{F. nucleatum} was the most prevalent bacteria and showed high percentages. However, the findings of Topcuoglu \textit{et al}.\(^4\) contrast the results of the study reported here in that \textit{P. intermedia}, \textit{P. micra}, \textit{T. denticola}, and \textit{T. forsythia} were detected in lower percentages. The difference between acute abscessed and chronic fistulised teeth might be interpreted to mean that bacteria such as \textit{P. intermedia}, \textit{P. micra}, \textit{T. denticola}, and \textit{T. forsythia} are more likely to be detected in chronic fistulised teeth.

In addition, this study demonstrated no statistically significant direct relationship between the presence of any bacteria and clinical findings, which stands in contrast to the data of Gomes \textit{et al}.,\(^6\) who reported a significant association between \textit{P. gingivalis} and swelling, mobility, and periapical radiolucency. The difference between the studies might derive from the fact that the study reported here used a smaller sample than that of Gomes \textit{et al}.,\(^6\)

Bacteria have to form complexes to survive under continuously changing environmental conditions.\(^26\) and the relationships between bacterial species and hosts can provide a better understanding of disease processes.\(^29\) Synergistic bacterial interactions allow different species to coexist in habitats in which interacting bacteria do not exist alone and increase the possibility of survival. Bacterial synergism may be another significant factor in the development of symptoms and signs of endodontic origin,\(^30,31\) and the presence of some pathogenic species in the root canal could provide an environment for the formation of acute periapical inflammation.\(^32\) In the study reported here, a statistically significant correlation emerged between \textit{T. forsythia} and \textit{T. denticola} in the same direction at the 61.6% level. The presence of the two bacteria has been associated with periodontal disease\(^33\) and clinical findings such as pain on palpation, swelling, and tenderness to percussion.\(^16\)

**Conclusion**

Our study has demonstrated that microbiota of abscessed primary teeth are highly diverse and commonly anaerobic. Findings regarding bacterial diversity have shown that most species are present in high percentages. The results regarding the high rate of certain bacterial combinations suggest the enhanced pathogenicity due to additive or synergistic effects of these microbial combinations. One should remember that microarray technology used for the rapid identification of potential pathogens in endodontic infections facilitates proper management of disease process.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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