Prevalence of selected microorganisms in the pulp space of human deciduous teeth with irreversible pulpitis

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ABSTRACT
The aim of this study was to identify the presence of selected microorganisms from pulp space of human deciduous teeth with irreversible pulpitis. 40 children, 3 to 8 years old were involved in the study. The samples were collected from infected pulp spaces using sterile paper points were analyzed for selected pathogens (Enterococcus faecalis, Escherichia coli, Streptococcus mutans, Staphylococcus aureus, anaerobes and Candida albicans). S. mutans in 100% of samples, E. faecalis in 35%, E. coli in 15%, Staph. aureus in 5%, anaerobes in 20% and C. albicans in 15% samples were detected. It can be concluded that human deciduous teeth with initial stages of irreversible pulpitis have a considerable amount of organisms said to be resistant for routine treatment and also predominance of facultative anaerobes over strict anaerobes.

Introduction
Although oral health education and prevention are priorities in contemporary dentistry, pulp changes in deciduous teeth are very frequent and cannot be avoided at all times. Thus curative dentistry should simultaneously evolve and improve concepts and therapeutic procedures. The success of an endodontic treatment depends on many factors with the reduction or elimination of bacterial infection being the most important one. However for this to occur, precise identification of microorganisms participating in the pathogenesis of irreversible pulpitis is important in order to understand disease process and to provide effective antimicrobial treatment. Even with the standard antimicrobial regimens reinfections are not uncommon. Studies have shown that some species of microorganisms found in root canals are resistant to routine therapy and cause persistent infections.

Results of studies in which the microflora of teeth with persistence disease was studied showed a high prevalence of enterococci, streptococci, and Candida and are capable of invading dentinal tubules. Staphylococcus aureus are capable of surviving for extended periods because of resistance to drying and temperature changes. Data regarding such species in the pulp space of infected human deciduous teeth are limited in Indian scenario and is of importance since the complex root canal anatomy may limit the efficacy of chemomechanical preparation and root canal medication. Therefore the aim of this study was to identify the selected microorganisms from pulp space of human deciduous teeth with irreversible pulpitis.

Material and Methods
Fourty 3 to 8 year old children, of both sexes, visited department of Pedodontics and Preventive dentistry, A B Shetty memorial institute of dental
sciences, Mangalore were selected. The Ethical Committee NITTE University Mangalore approved the study protocol, and all of the patients gave informed consent before the sampling procedure was performed. The selected patients presented with the history of intermittent or continuous spontaneous pain or night pain not more than a week and the teeth were with intact root or less than two third of physiological root resorption, no clinical and radiographic evidence of periapical lesion, no periodontal pocket and no previous root canal intervention. Teeth with pulp chamber exposed to oral cavity were excluded from study. None of the patients received antibiotics 4 weeks prior to sampling.

Clinical procedure: The selected teeth were isolated with rubber dam and field of operation (exposed tooth, clamp & rubber dam) disinfected with 1% digluconate chlorhexidine. After caries removal sterile paper points were inserted into the pulp chamber for 60 seconds, and immediately placed in tubes containing reduced transport fluid. After sample collection teeth were treated with standard pulpectomy and obturation with zinc oxide eugenol cement.

Lab procedures: The samples were then streaked on to Mac Conkey agar, Muller Hinton agar, Sabroud’s Dextrose agar and Mutan- Sanguis agar (Himedia, Mumbai, India) for the specific isolation of Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, Candida albicans and Streptococcus mutans. These Petri plates were then incubated at 37° C aerobically for 18 to 24hrs. Samples streaked on Mutan sanguis agar were incubated microaerobically by the candle jar system for 2 to 3 days. Also the samples were streaked on sheep blood agar and incubated anaerobically using Gas-Pak system at 37° C for 2 to 6 days for specific isolation of anaerobes.

The colonies found on the agar plates were subjected to Gram staining for the preliminary identification by structural morphology and confirmed by specific biochemical tests. S. mutans by Esulin test and Catalase test, E. faecalis by Bile esculin test, E. coli by IMViC test, Staph. aureus by Catalase and Coagulase test, C. albicans by germ tube test and Anaerobes by growth in anaerobic environment. The anaerobes recovered found to be sensitive to metronidazole.

Lab procedures were conducted according to CLSI (Clinical and Laboratory Standard Institute) guidelines.

Results

The data obtained explained by descriptive statistics. (Table 1) S. mutans from 100% of samples, E. faecalis from 35% , E. coli from 15%, Staph. aureus from 5%, anaerobes from 20% and C. albicans from 15% were isolated.

Discussion

In previous studies as early as 1950s and 1960s researchers isolated mainly aerobic and facultative bacterial species from infected root canals due to the limitation of isolation technique and microbial culture. With evolution of technology, anaerobic techniques have been developed and the concept has been modified showing that pulpal and periapical infections are polymicrobial with predominance of strict anaerobes. Microflora of teeth with chronic periapical lesions and persistence disease has shown a high prevalence of enterococci, streptococci and Candida.
Primary endodontic infections are caused by oral microorganisms which are usually opportunistic pathogens. Theoretically any of these microbial species from microbiota may invade the pulp space and establish an infectious process. The most important route of pulpal invasion is through the tubules of carious dentin. This may take place even before the pulp is exposed directly to the oral environment by cavitation. The facultative anaerobes streptococci, staphylococci penetrate the dentin prior to the cavitation. The primary microorganism causing pulpitis is difficult to determine because of technical difficulties associated with obtaining samples for culturing and because of exact time of initial infection is difficult to ascertain.

In our study we tried to know whether the species of microorganisms responsible for the persistence of endodontic infections are present at initial stages of pulpitis i.e.; when the infection is still contained within the pulp space. The microorganisms were isolated by regular culture methods, the advantage of this method over molecular analysis are broad range in nature, identification of unexcepted species, allow quantification of all major viable microorganisms in sample and wide availability.

In present study S. mutans was found in all the 40 specimens. Studies reported presence of streptococci in 96.7% and 85% respectively in infected root canals with periapical infections.

In previous studies the E. faecalis was consistently isolated from the teeth with chronic periapical infections or in cases of treatment failure due to re-infections. In our study E. faecalis was found in 35% of samples from inflamed pulp without periapical infection. E. faecalis has been reported in 10% to 30% of inflamed root canals. In untreated canals enterococci constitute around 5% of total microflora. Such results raise the question how and when they invade the root canal system. It can be hypothesized that E. faecalis could be present in untreated canals, but in such low numbers that it is not recovered. Due to changes in the root canal environment this microorganism may grow to higher and recoverable proportions. It may also enter the canal due to failure in isolation. There are some special properties of E. faecalis such as ability to grow in high salt concentration, tolerate a broad pH range, as well in the presence of intra canal medicaments. E. faecalis has some virulence factors such as secreted factor, adhesions, capsular polysaccharides and antibiotic resistance determinant could be the reason for their survival.

### Table 1. Prevalence of selected microorganisms in the pulp space of human deciduous teeth with irreversible pulpitis.

<table>
<thead>
<tr>
<th>Organisms isolated</th>
<th>Number of samples</th>
<th>Colony unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>40</td>
<td>$&gt; 10^5$</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>14</td>
<td>$&gt; 10^5$</td>
</tr>
<tr>
<td>E. coli</td>
<td>6</td>
<td>$&lt; 10^3$</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>2</td>
<td>$&lt; 10^3$</td>
</tr>
<tr>
<td>C. albicans</td>
<td>6</td>
<td>$&lt; 10^3$</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>8</td>
<td>$&lt; 10^4$</td>
</tr>
</tbody>
</table>
in harsh condition of root canal. As endopathogen they can invade the dentinal tubules and adhere to dentin surface\textsuperscript{16}.

\textit{E. coli} was also isolated from persistent periapical infections\textsuperscript{4}. Histological and radiographical evaluation of the periradicular tissues of cats after deposition of \textit{E. coli} endotoxin solutions in the root canals and concluded that endotoxin may have a role in the induction and perpetuation of periradicular inflammatory lesions\textsuperscript{17}. \textit{E. coli} were found in 15\% of samples in our study.

\textit{Staph. aureus} which are gram positive facultative anaerobes are able to remain viable for extended periods because of resistant to drying and temperature changes. A case of refractory endodontic lesion due to \textit{Staph. aureus} was reported by Chad M et al and the possible pathogenicity of this microorganism was discussed. The majority of \textit{Staph. aureus} also have the ability to develop resistance to antimicrobials rapidly thus of concern if found in inflamed pulp\textsuperscript{6}. \textit{Staph. aureus} was found in two samples in our study. Cohen et al reported the presence of \textit{Staph. aureus} in 2 out of 30 samples of infected deciduous teeth\textsuperscript{18}.

Sen et al showed the presence of yeasts in 4 of the 10 specimens of necrotic teeth\textsuperscript{19}. \textit{C. albicans} is the fungal species most often found in endodontic infections\textsuperscript{20}. In the present study \textit{C. albican} was isolated from 15\% of samples. Some characteristics such as invading the dentinal tubules and resistance to calcium hydroxide are common to yeasts and enterococci\textsuperscript{16,21}.

Studies of the bacteriology of infected root canals with periapical lesions have detected anaerobic bacteria in 96-100\% of samples\textsuperscript{1,12}. In the present study anaerobes found in 8 out of 40 specimens. A significantly smaller number of anaerobic species found in teeth with irreversible pulpitis without periapical infection and it may not be recovered if less in number in the initial stages of irreversible pulpitis\textsuperscript{22}.

Presence of these opportunistic microorganisms may vary person to person depending on individual’s oral hygiene and personal hygiene.

The primary objectives of pulpectomy are to eliminating intraradicular microorganisms and maintaining teeth and their supporting tissues in function and integrity. Root canal disinfection is accomplished through chemomechanical debridement and biomechanical preparation followed by quality root canal system obturation\textsuperscript{23}.

Because chemomechanical procedures are unlikely to eradicate the root canal infection completely in case of deciduous teeth, the use of antimicrobial intracanal medication may be a valuable adjunct in the treatment of teeth\textsuperscript{24}.

**Conclusion**

Within the limitations of this study it can be concluded that, initial stages of irreversible pulpitis have shown the presence of following organisms: \textit{Enterococcus faecalis}, \textit{Escherichia coli}, \textit{Streptococcus mutans}, \textit{Staphylococcus aureus}, anaerobes and \textit{Candida albicans} which were said to be found in persistent lesions. There was predominance of facultative anaerobes over strict anaerobes.

Microorganism eradication from an infected root canal system is the key to successful endodontic treatment. However the greater difficulties of instrumenting the root canal system in the primary dentition limit the ideal treatment. Thus attention...
must be paid for adequate chemomechanical preparation with effective irrigants and use of root filling materials with proven antibacterial and antifungal effectiveness.

References:


