

# Evaluation of Pathological Microbes in Root Canals with Pulp Necrosis

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## ABSTRACT

**Aim:** The present study was conducted to evaluate the type of bacteria present in necrotic root canals of permanent teeth.

**Materials and methods:** All 60 participants with infected root canals were made to use 10 mL of mouthwash containing 0.12% chlorhexidine. Access to pulp chamber was established, and the sterile absorbent paper cones were inserted into the canal for 20 seconds. The contaminated paper cones were inoculated in a brain–heart infusion (BHI) agar culture medium and incubated in an oven for 48 hours at 37°C. Results were analyzed statistically with SPSS version 20.0 using Chi-square test and analysis of variance (ANOVA).

**Results:** In root canals with periapical lesions, gram-positive bacilli was present in 50 cases, gram-negative in 48 and yeast cells in 28; while in root canals without periapical lesions, gram-positive bacilli was present in 8. In 16 root canals of chronic apical periodontitis cases, gram-positive bacteria was present in 100%, gram-negative bacteria in 100%, and yeast cells in 20% cases. In 12 cases of periapical granuloma, gram-positive bacteria was present in 98%, gram-negative bacteria in 100%, and yeast cells in 40% cases. In 10 cases of chronic abscess with fistula, gram-positive bacteria was present in 86.2. In six cases of phoenix abscess, gram-positive bacteria was present in 100% and gram-negative bacteria in 100% cases.

**Conclusion:** There is predominantly gram-positive and -negative bacteria as well as the presence of yeast cells in periapical lesions.

**Clinical significance:** Knowledge about the bacterial species in root canal infection helps in its management and in selecting antibiotic.

**Keywords:** Bacteria, Gram positive, Periapical.

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## INTRODUCTION

The treatment of infected tooth with endodontic treatment ensures removal of pathogenic bacteria. However, sometimes failure of complete elimination from root canal leads to endodontic failure.<sup>1</sup> Chemically, the microorganisms can be eliminated using disinfectant. Various disinfectants have been introduced in the treatment of infected root canals such as NaOCl, H<sub>2</sub>O<sub>2</sub>, etc. The choice of irrigants depends upon cause and amount of infection. Thus, an endodontist should have a thorough knowledge of endodontic microbiota and infectious process and possible remedies to treat it.<sup>2</sup>

Bacteria exhibit their effects by releasing toxins and lipopolysaccharides which are constituent of the cell wall. The virulence is bacteria are of low intensity. The occurrence of bacteria in root canals causes pulpal as well as periapical infection. Endotoxins may trigger pain in infected root canal conditions by releasing vasoactive substances and neurotransmitters at nerve endings of periapical tissues. Endotoxins exerts effects by interacting with monocytes, macrophages, and other cells of the immune systems and ultimately with fibroblasts and resulting in the release of proinflammatory cytokines, e.g., tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$ .<sup>3</sup>

Studies have shown the presence of gram-positive, gram-negative, mixed aerobic and anaerobic bacteria that cause infection. Most common gram-negative anaerobic bacteria species involves *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Tanarella*, and *Treponema*.<sup>4,5</sup> Several techniques such as polymerase chain reaction may be useful in detection of microbes. This study was conducted to evaluate the type of bacteria present in the necrotic pulp canals of permanent teeth.

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**Conflict of interest:** None

## MATERIALS AND METHODS

The present study was conducted in the Department of Restorative Dentistry, College of Dentistry, Qassim University, Kingdom of Saudi Arabia. It comprised 40 patients of both genders with 60 infected root canals. The ethical approval was taken from institutional ethical committee of College of Dentistry, Qassim University. Informed consent was obtained from participants. Molar teeth with necrotic

root canals and asymptomatic teeth were included. All the canals for particular tooth were evaluated. Exclusion criteria were patients using antibiotics and who are under medical conditions. The study was done by a single-trained investigator.

Clinical examination and pulp vitality were performed. Intraoral radiographs were taken. All subjects were made to use 10 mL of mouthwash containing 0.12% chlorhexidine. After caries removal, the access to the pulp chamber was established.

Three to four sterile permeable paper points were inserted into each canal for 20 seconds. These polluted paper pointes were inserted in a BHI agar culture carrier and incubated in an oven for 48 hours at 37°C.

The Gram methodology was used. The prepared thin scrub was kept for air desiccation. Following this, it was passed by a burner flame for 3–4 times. Then for 4 minutes it was enclosed with ethanol. Later its surface was covered for 1 minute with crystal violet solution, cleaned with distilled water, and enclosed for 1 minute with Gram iodide solution. Then it was cleaned again using distilled water. Following this, acetone was added on the material till the purple color fade, in 10 seconds. The cleansed area was enclosed for 1 minute with safranin and cleaned with deionized water. The material was positioned in a vertical location on a holder and kept for air-dry.

The tainted lamina was evaluated under 100× magnification using a drop of immersion oil. Dark blue used for gram-positive bacteria and pink red for gram-negative bacteria. Using objective lens, 100× optical microscope, morphological classification of microorganisms was achieved.

Results were analyzed statistically with SPSS version 20.0. Chi-square test and ANOVA were used for representation of the results. The *p* value was considered significant at lesser than 0.05.

## RESULTS

Table 1 shows that in root canals with periapical lesions, gram-positive bacilli was present in 50 cases, gram-negative in 48, and yeast cells in 28; while in root canals without periapical lesions, gram-positive bacilli was present in 8, gram-negative in 8, and yeast cells in 5 cases. The difference was highly significant ( $p < 0.05$ ).

**Table 1:** Comparison of the presence of microorganisms in root canals with or without periapical lesions

Microorganism	Root canal with periapical lesion (52)	Root canal without periapical lesion (8)	<i>p</i> value
Gram-positive	50	8	0.01
Gram-negative	48	8	
Yeast cells	28	5	

**Table 2:** Periapical lesions with the presence of microorganisms in the root canals

Periapical lesions (52)	Gram-positive bacteria (%)	Gram-negative bacteria (%)	Yeast cells (%)	<i>p</i> value
Chronic apical periodontitis (16)	100	100	20	0.05
Periapical granuloma (12)	98	100	40	
Chronic abscess with fistula (10)	86.2	99	80	
Periapical cysts (8)	100	100	42	
Phoenix abscess (6)	100	100	0	

Table 2 and Figure 1 show that in 16 pulp canals of persistent apical periodontitis cases, gram-positive microorganism was present in 100%, gram-negative bacteria in 100%, and yeast cells in 20% cases. In 12 cases of periapical granuloma, gram-positive bacteria was present in 98%, gram-negative bacteria in 100%, and yeast cells in 40% cases. In 10 cases of persistent abscess with fistula, gram-positive microorganism was present in 86.2%, gram-negative bacteria in 99%, and yeast cells in 80% cases. In eight cases of periapical cysts, gram-positive bacteria was present in 100%, gram-negative bacteria in 100%, and yeast cells in 42% cases. In six cases of phoenix abscess, gram-positive bacteria were present in 100% and gram-negative bacteria in 100% cases. The difference was significant ( $p < 0.05$ ).

## DISCUSSION

The study of periapical and pulpal diseases shows the existence of complex mixed infections. Various studies have been done so far which depicts the occurrence of gram-positive, gram-negative bacteria, and yeast cells. Microscopic and macroscopic studies have shown the pathological function in pathogenesis.<sup>6,7</sup> The polymicrobial behavior of endodontic infection can be confirmed and classified based on Gram color. The existence of aerobic microorganisms, strict anaerobes, yeasts, and facultative bacteria demands complete elimination for successful endodontic therapy.<sup>8</sup> This study was conducted to evaluate the type of bacteria present in necrotic pulp canals of permanent teeth.

We included 60 root canals with pulp necrosis in 40 patients. Only multirouted mandibular permanent molars were considered for the study. We found that in root canals with periapical lesions, gram-positive bacilli was present in 50 cases, gram-negative bacteria in 48, and yeast cells in 28; while in root canals without periapical lesions, gram-positive bacilli was present in 8, gram-negative bacteria in 8, and yeast cells in 5 cases.

Pourhajibagher et al.<sup>9</sup> in their study evaluated the prevalence of root canal pathogens from endodontic infections and found that of the 218 isolates, *Veillonella parvula* was present in initial endodontic infection, along with *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. It was observed that in secondary endodontic infections, *Enterococcus faecalis* was evident in 36.6% cases along with *Propionibacterium acnes*, *V. parvula*, and *Candida albicans*. They observed *V. parvula* and *E. faecalis* in initial and secondary root canal diseases.

In present study, common periapical lesions were chronic apical periodontitis, periapical granuloma, chronic abscess with fistula, periapical cysts, and phoenix abscess. We observed that of the 16 pulp canals of persistent apical periodontitis cases, gram-positive microorganism and gram-negative bacteria were present in 100% and yeast cells in 20% cases. In 12 cases of periapical granuloma, gram-positive bacteria was present in 98%, gram-negative bacteria in 100%, and yeast cells in 40% cases. Gram-positive bacteria was present in 10 cases of persistent abscess along with fistula, gram-negative bacteria in 99%, and yeast cells in 80% cases. In eight cases of periapical cysts, gram-positive bacteria was present in 100%, gram-negative bacteria in 100%, and yeast cells in 42% cases. In six cases of phoenix abscess, gram-positive bacteria was present in 100% and gram-negative bacteria in 100% cases.

Nobrega et al. evaluated the bacterial variety of root canals with acute apical abscess. They identified eight phyla and taxa belonging to the phylum Bacteroidetes, Firmicutes, and Proteobacteria. Most

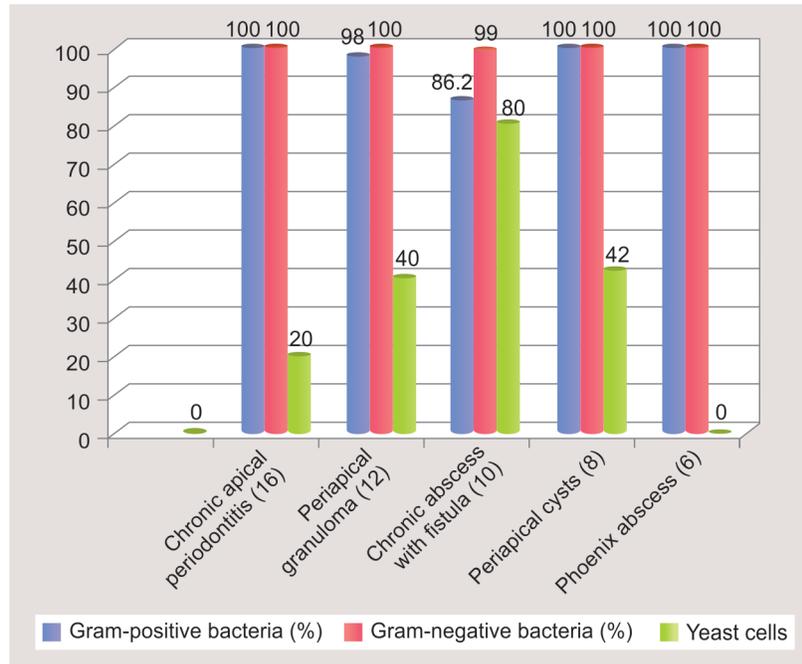


Fig. 1: Periapical lesions with the presence of microorganisms in the root canals

frequently detected microorganism were anaerobic gram-negative bacteria, i.e., Firmicutes and Bacteroidetes.<sup>10</sup>

Gomes in his review article stated that the development of apical periodontitis is mainly due to bacteria and their products. He further classified the various Gram-staining bacteria for endodontic infection.<sup>11</sup>

Vengerfeldt et al.<sup>12</sup> in their study assessed five cases of chronic periapical abscess, three cases of secondary chronic periapical abscess, and four cases of periapical abscess. Samples were collected under strict aseptic conditions. Majority of the microorganisms were Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Tenericutes, and Spirochaetes. Lana et al. performed the microbiological evaluation of the necrotic pulp and found polymicrobial infections such as microaerophilic bacteria, obligate, and facultative anaerobes.<sup>13</sup> Peciuliene et al. in their review stated that there is different root canal microflora for primary and retreatment cases due to the changes in ecological factor.<sup>14</sup> Jhajharia in her review article mentioned that the infected root canals have polymicrobial microbes, and *E. faecalis* is the most commonly found microorganism in infected root canals.<sup>15</sup>

Although some previous studies have evaluated the presence of gram-positive and -negative bacteria in root canals, but this study evaluates the presence of gram-positive and gram-negative bacteria in infected root canals along with the type of bacteria present in periapical abscess, cyst, fistula, and granuloma. Our results will help in the control of periapical infection with use of specific antibiotics and intracanal medicaments for successful endodontic treatment.

The limitation of the study was smaller sample size and the use of only multirrooted molar teeth. Further studies are needed with inclusion of a large sample size, deciduous teeth, even the involvement of maxillary and single-rooted teeth to provide better results.

## CONCLUSION

Authors concluded that there are predominantly gram-positive and -negative bacteria as well as yeast cells in periapical lesions.

## ETHICAL CLEARANCE

Obtained from institutional ethics committee.

## AUTHORS' CONTRIBUTIONS

Prashant Babaji contributed in manuscript preparation and drafting the work. Dr Ahmed Ali Alfawzan and Dr. Vardharajula Venkata Ramaiah revised it critically for important intellectual content. Dr. Mohammed Ali Habibullah contributed in conception or design of the work. Dr. Sabahat Ullah Tareen contributed in acquisition, analysis, or interpretation of data for the work.

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