Original Article

Study the Most Predominant Bacteria in Pericoronitis Patients

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Abstract

Background: Pericoronitis is a term used to describe inflammation of the soft tissues surrounding the crown of a tooth that is erupting or has an incomplete eruption. The development of mixed oral microflora appears to be one of the causes of pericoronitis, a bacteria-induced infection of the soft tissues of the mouth that encircle the crown of impacted or partly erupted mandibular third molars. **Objectives:** The purpose of this research was to discover the main pathogenic microbes linked to pericoronitis by molecular methods. **Materials and Methods:** A total of 80 study samples (50 cases; 30 control), 34 men and 46 women, aged 16–36 years. During the acute stage of pericoronitis, samples are collected by isolation of the lower third molar and dry area by air syringe and then a sterile 3-paper point is inserted in pericoronal pockets. After that, the paper point is put in an Eppendorf tube and stored at deep freeze until it was used for real-time polymerase chain reaction (PCR) technique. **Results:** The most common bacteria detected in real-time-PCR are *Streptococcus mutants*, *Prevotella intermedia*, and *Tanerella forthysia*. Our results in pericoronitis show that the percentage of gram-positive bacteria *S. mutans* is 64%, gram-negative *P. intermedia* 58%, and *T. forthysia* 36%. **Conclusion:** Bacteria detection was found to be remarkably high compared to that of controls. Distribution of infection according to gender is (66%) women and (34%) men, and the number of patients with pericoronitis at a young age (16–26) is significantly higher than in the older age groups.

Keywords: Pericoronitis, *Prevotella intermedia*, *Streptococcus mutants*, *Tannerella forthysia*

Introduction

Pericoronitis is a word used to describe inflammation of the soft tissues surrounding the crown of an erupted tooth or a tooth that has not fully emerged.[1] The condition is most frequently observed in late adolescence or early adulthood, and a number of variables are thought to have contributed to its development. Under the conditions of low immune resistance (stress, viral recovery), the oral microflora may acquire a pathologic potential and aid in the presentation of symptoms.[2] This area is covered by gingiva, and food debris lodges beneath the covering gingiva, which is then invaded by bacteria.[3,4] Dental plaque is thought to be one of the causes of oral cavity illnesses such as caries, gingivitis, periodontitis, and peri-implantitis.[5] Pericoronitis is a polymicrobial mixed infectious periodontal pathology.[6,7] The bacteria that develop in the distal pseudopocket are the primary source of pericoronal tissue inflammation and occlusal damage caused by the opposing tooth. The identification of the microbiota of the pericoronitis area is critical in the medical treatment plan.[8] Mostly anaerobic microorganisms make up the pseudopocket's microbial ecology.[8-12] Numerous potential pathogens have been mentioned in earlier reports, including various *Streptococcus* and *Staphylococcus* species, *Capnocytophaga* and *Fusobacterium* species, *Prevotella* and *Veillonella* species, *Porphyromonas gingivalis*, *Parvimonas micra* (previously *Peptostreptococcus micros* and *Micromonas micros*), and *Tannerella forsythia*.[13-15] Leung *et al.*[14] demonstrated a similarity between the species producing gingivitis or periodontitis and the multi-microbial, primarily anaerobic bacteria of pericoronitis. These microbes can survive in a setting with few bacterial commensals and little food. It progresses by forming a biofilm, which through an adaptive process, allows the bacterium to survive in difficult circumstances such as dental infections.[16] Cultivation studies can drastically

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underestimate microbial diversity for the reason numerous microbes cannot be cultured using conventional methods because the majority of the gram-negative bacteria that cause pericoronitis require anaerobic growth conditions.[17]

The isolation of DNA from the target environment is currently the best method for identifying microbial diversity without cultivation. When compared to healthy subgingival samples, quantitative polymerase chain reaction (qPCR) has been shown to be extremely sensitive and specific to recognize a pathogen in periodontitis samples.[18] In order to quickly, sensitively, and specifically identify bacterial pathogens in third molar pericoronitis, PCR has thus emerged as a key instrument.[19] Because of the advantages of PCR, it has been widely used for both diagnosing and identifying bacterial species.[20]

To our knowledge, real-time PCR will be used to detect periodontal bacteria in mandibular third molar pericoronitis. The purpose of this study was to discover the main pathogenic microbes linked to pericoronitis using molecular methods.

Materials and Methods

A total of 80 study samples (50 cases and 30 controls), including 34 men and 46 women with the age range of 16–36 years who have pericoronitis during the acute stage participated. All samples were collected from the private orthodontic clinic and the specialized health center of dentistry in Babylon City, Iraq. During the same time span of study sample collection was performed from February 2022 to June 2022.

Specimen collection methods

The samples were taken from patients who had pericoronitis infection at the acute stage. A swab was taken by paper point (size 30) inserted at the lowest locations of the pericoronal pockets, just below the third molars' pericoronal soft tissues until resistance fell and wait for 30 s. Then the paper point was pulled away and immediately put in an Eppendorf tube containing (phosphate buffer solution) and labeled. Control group patients' samples were collected using sterile paper points placed at the distal site of the mandibular third molar and retained for 30 s. Then the tube was stored at –20ºC in a deep freeze until it was used for real-time PCR technique as shown in Figure 1.

Genomic DNA extraction

The DNA extraction approach presented in this study has improved the phenol/chloroform method of DNA extraction.[21] To lyse the cells, we used phenol rather than proteinase K, sodium dodecyl sulfate (SDS), or lysozyme. For 2 min at 8000 rpm, 1 mL of the bacteria was centrifuged to extract the DNA. After that, the cells underwent a 2-min, 8000 rpm centrifugation. The pellets were suspended in 200 µL of TE buffer (Tris EDTA buffer), which is composed of 10 mM Tris/HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and pH 8.0. Cells were lysed for 60 s by vortex mixing after being dissolved in 100 µL of saturated phenol (pH 8.0) in these tubes. Afterward, the materials were centrifuged to separate the aqueous phase and organic phase from them. The mixture was then centrifuged at 13,000 rpm for 5 min using 100 µL of chloroform and 40 µL of TE buffer. The lysate was purified with two or three rounds of extraction until there was no longer a white interface, then transferred 160 µL of the upper aqueous phase a brand-new. The RNA was digested using 40 µL of TE and 5 µL of RNase (10 mg/mL), which were combined and then incubated at 37°C for 10 min. Following thorough mixing, 100 µL of chloroform was added to the tube, and it was centrifuged. Then transferred 150 µL of the upper aqueous phase into a brand-new 1.5 mL tube. In the ensuing tests, the pure DNA in the aqueous phase was used right away or stored at -20°C.

Bacterial detection by real-time PCR

Real-time qPCR was carried out following the instructions accompanying the Master Mix kit from Rotorgene Q (Qi agene, Germany) employing the primer and the real-time PCR reaction ingredient as listed in Table 1. The tube,

Figure 1: Swab collection from patient

Table 1: Volumes of the real-time PCR protocol reaction

Component	Total volume 50 μL
Master mix Syper Green	20 µL
DNA	4 µL
Forward primer	1 µL
Revers primer	1 µL
$MgCl_2$	1 µL
Nuclease free water (NFW)	23 µL

PCR: polymerase chain reaction

where all the components were collected, was briefly centrifuged to spin down the contents and to eliminate any air bubbles. Then, the tube was set inside the qRT PCR well and the program was run.

Primers

The following primers were used in this study to identify the target genes in bacteria as listed in Table 2.

Detection of *Streptococcus mutans* by real-time PCR

- *Amplification curve*: By determining the cycle threshold (Ct) value that displayed the positive amplification (red line) and negative amplification (blue line) as shown in Figure 2.
- *Melting curve*: The selectivity and specificity for all analysis are checked by melting curve analysis.

Detection of *Prevotella intermedia* by real-time PCR

- *Amplification curve*: By determining the Ct value that displayed the positive amplification (red line) and negative amplification (blue line) as shown in Figure 3. The selectivity and specificity for all analysis are checked by melting curve analysis.

Detection of *Tannerella forsythia* by real-time PCR

- *Amplification curve*: By determining the Ct value that displayed the positive amplification (red line) and negative amplification (blue line) as shown in Figure 4.
- *Melting curve*: The selectivity and specificity for all analysis are checked by melting curve analysis.

Statistical analysis

The descriptive statistics, the statistical software for the social sciences, edition 22.0 (SPSS, IBM Company, Chicago, IL, USA) program was used. Continuous variables were shown using means and standard deviations. To compare the *P*-values of three groups, a chi-square calculator for independent samples was used. *P* values less than or equal to 0.05 were considered statistically significant, whereas

Table 2: Primer for bacterial detection

Bacteria	Primer	Reference	Product size
Streptococcus mutants	F AGCCATGCGCAATCAACAGGTT R CGCAACGCGAACATCTTGATCAG	[22]	415 bp
Prevotella intermedia	F CGTATCCAACCTTCCCTCC R ATTAGCCGGTCCTTCGAAG	[23]	389 bp
Tannerella forsythia	F GCGTATGTAACCTGCCCGCA R TGCTTCAGTGTCAGTTATACCT	[24]	360 bp

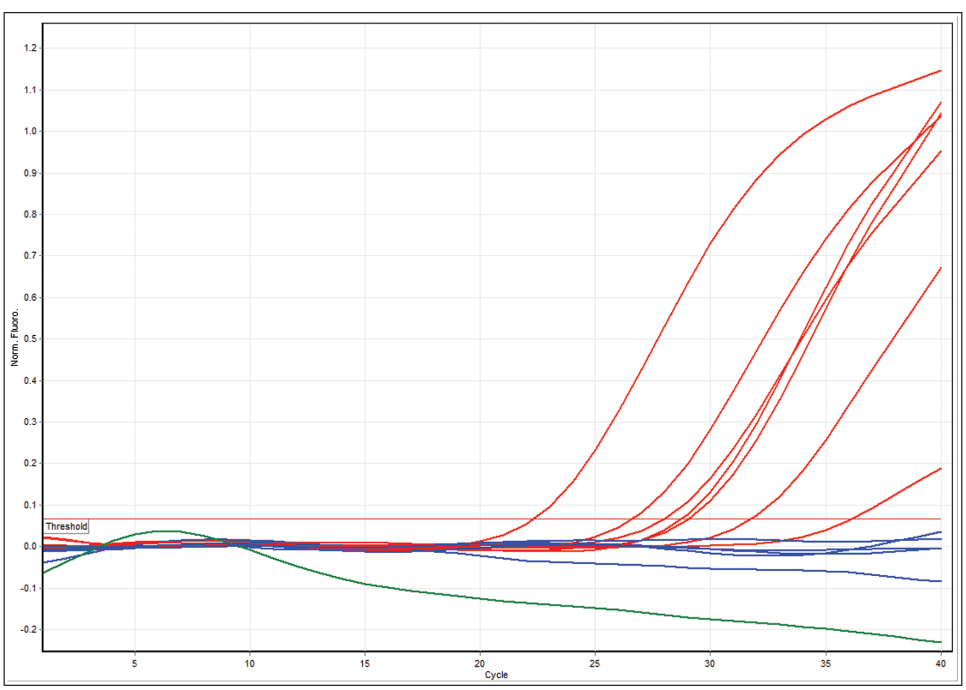

Figure 2: Amplification of *Streptococcus mutans*

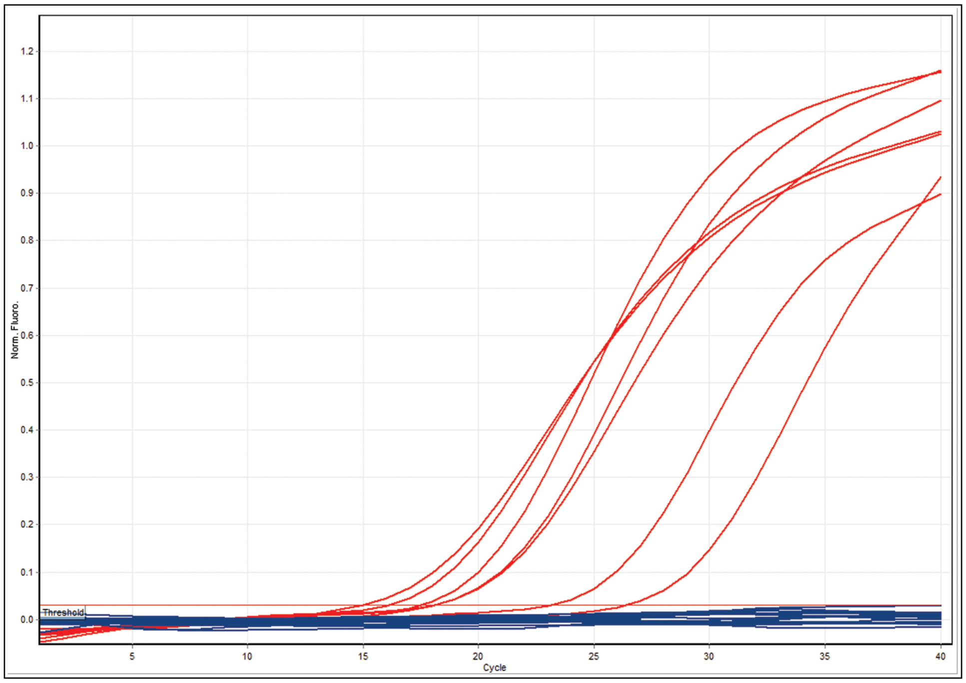

Figure 3: Amplification of *Prevotella intermedia*

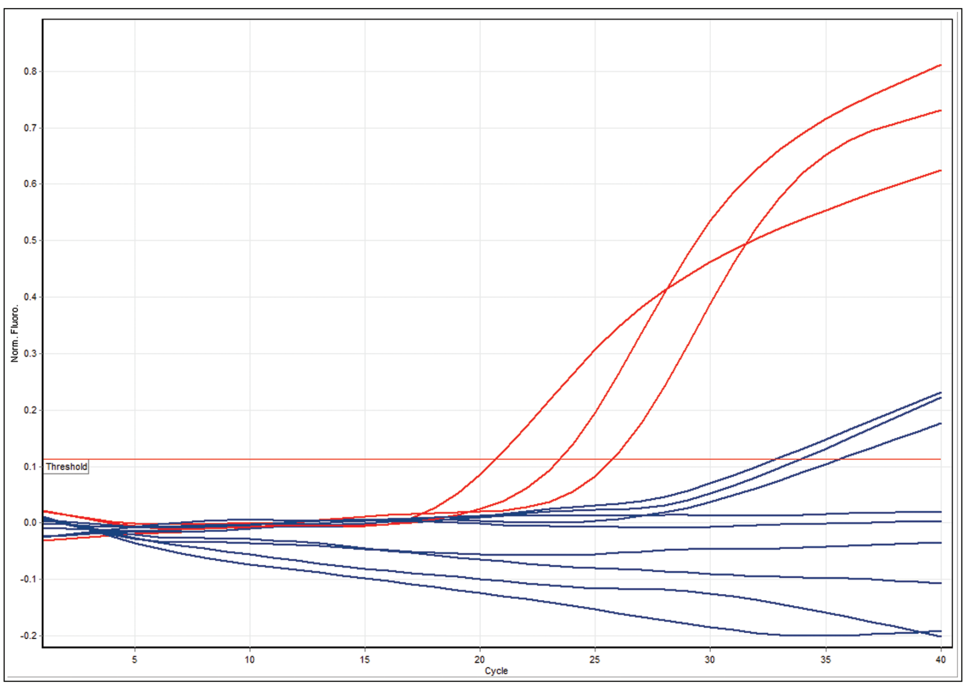

Figure 4: Amplification of *Tannerella forsythia*

P values higher than 0.05 were considered statistically insignificant.[25]

Ethical consideration

The study was carried out in conformity with the ethical standards outlined in the Helsinki Declaration. Before taking the sample, the patients verbal and analytical consent were approved by University of Babylon, Iraq, local ethics committee according to the document number 342 on February 7, 2022.

Results

The study includes 80 subjects; 50 patients who had pericoronitis and 30 control subjects at different times. The study subjects consisted of 34 men and 46 women

Table 3: Distribution of *Streptococcus mutans*

	Streptococcus mutants		Total
	Positive	Negative	
Patient group	32 (64%)	18 (36%)	50
Control group	5 (16.67%)	25 (83.33%)	30

Table 4: Distribution of *Prevotella intermedia*

	Prevotella intermedia		Total
	Positive	Negative	
Patient group	29 (58%)	21 (42%)	50
Control group	1 (3.33%)	29 (96.67%)	30

Table 5: Distribution of *Tannerella forsythia*

	Tannerella forthysia		Total
	Positive	Negative	
Patient group	18 (36%)	32 (64%)	50
Control group		30 (100%)	30

with ages ranging between 16 and 36 years. Out of 80 subjects, 58 of them were under age 26 years old and 22 were above age 27 years. Female patients appeared more than male patients (66% vs. 34%).

Detection of *Streptococcus mutans* by RT-PCR technique

In this study, 80 samples were detected for the presence of *S. mutans* by RT-PCR technique. The results showed that there was a presence of *S. mutans* where positive in 32 (64%) cases of the patient group, while its presence was negative in 18 (36%) cases of the same group, while in control groups the presence of *S. mutans* was positive in 5 (16.67%) cases and negative in 25 (83.33%) cases of this group, as shown in Table 3.

Detection of *Prevotella intermedia* by RT-PCR technique

In this study, 80 samples were detected for the presence of *P. intermedia* by RT-PCR technique. The results showed that there was a presence of bacteria in pericoronitis patients in 29 (58%) positive samples with 21 (42%) negative samples while in control groups the presence of *P. intermedia* in 1 (3.33%) positive samples with 29 (96.67%) negative samples as shown in Table 4.

Detection of *Tannerella forsythia* by RT-PCR technique

In this study, 80 samples were detected for the presence of *T. forsythia* by RT-PCR technique. The results showed that there was a presence of bacteria in pericoronitis patients in 18 (36%) positive samples with 32 (64%) negative samples whereas in control groups *T. forsythia* is absent in all samples as shown in Table 5.

Discussion

The age group between 16 and 26 years is the more affected group with pericoronitis 36 (72%) than the age above 27 years which are 14 (28%). This result is in agreement with Yamalik and Bozkaya[26] result who found that the incidence peak of pericoronitis that occurred around lower third molar was seen between 19 and 23 years age group which is the period when mandibular third molars normally erupt. The present results were in disagreement with the results of Caymaz and Buhara[27] who explained that there is no significant correlation between age with pericoronitis.

The gender distribution of subject in this study revealed (66%) women and (34%) men. This result agreed with Bataineh who reported that pericoronitis cases were much seen in female patients (56.7%) than in male patients (43.3%)[28] and it agreed with Yamalik and Bozkaya[26] study. The present results disagree with several studies that have reported the distribution of pericoronitis between the genders to be insignificant with a slight female predominance[29] and disagree with result of Caymaz and Buhara[27] who found that there is no significant correlation of gender with pericoronitis.

Streptococcus mutans are considered one of the most important microorganisms in the etiology of dental diseases by acidic production.[30] This study set out with the aim of assessing the importance of RT-PCR in the detection of *S. mutans*. About 32 bacteria in pericoronitis case and five in the control group were detected by molecular method positive results for PCR by using a specific primer, sm 415, yet this primer uses for *S. mutans*.[22] The result recorded that the gram-positive bacteria *S. mutans* was predominant and highly significant (P = 000) in 32 (64%) cases of pericoronitis samples and 5 (16.67%) control samples. These bacteria are not present in 18 (36%) of cases and also does not occur in 25 (83.33%) of the control groups. This predominance may be due to its ability to cause dental caries which may progress to pulp infection, dental bad hygienic measurement with odontogenic infection, these microorganisms have several toxins that play crucial roles within host-pathogen interaction allowing the pathogen to colonize, proliferate, and disseminate tissues.[31] The present study was in agreement with Azemi *et al.*[32] study who found streptococcus mutans is most common bacteria in pericoronitis. The study also agreed with Rajasuo who recorded *S. mutants* as the most common bacteria that cause dental infections and in agreement with.[33,34]

The presence of *P. intermedia* in 29 (58%) cases of pericoronitis samples and 1 (3.33%) of control samples. These bacteria were not present in 21 (42%) of cases and also did not occur in 29 (96.67%) of the control groups. The present results agreed with Sixou *et al*. According to a study by Sixou *et al*.,[8] the most frequently detected microorganisms in pericoronitis microbiota were *P. intermedia* and disagree with Rajasuo *et al.*[34] who found that the presence of *P. intermedia* is (35%).

A recent study based on real-time PCR found statistically significant higher numbers of Tannerella forsythia detected in samples from pericoronitis patients.[15] The study disagrees with a present study which presents *T. forsythia* in 18 (36%) of pericoronitis samples and the absence of this bacteria in control samples. These bacteria were not present in 32 (64%) of cases and also did not occur in 30 (100%) of the control groups, whereas these results disagreed with Sencimen *et al.*[15] and Rajasuo *et al.*,[18] who found that the presence of *T. forsythia* increases in eight times the risk of developing pericoronitis when compared to individuals without it. Present results agree with Peltroche-Llacsahuanga *et al*.[12] and Jakovljevic *et al*.[35] results showed that *T. forthysia* was also associated with the development of pericoronitis and was present in 40% of their samples.

Conclusion

The present work investigates that the most common bacteria detected by real-time PCR were *S. mutans*. It is inferred that the percentage of infection with these bacteria was 64%, *P. intermedia* 58%, and *T. forthysia* 36%. There is a significant gender difference, with women getting more affected than men in infection. The number of pericoronitis patients under the 26 years (young age) group is significantly higher than in the older age groups.

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Conflicts of interest

There are no conflicts of interest.

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