Identification of the Predominant Oral Microbiome in Pericoronitis

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Abstract

Accumulation of mixed oral microflora appears to be one of the contributing factors to pericoronitis, an inflammation of the oral soft tissues surrounding the crown of a partially erupted, or impacted mandibular third molars. This study was aimed to identify the predominant infectious bacteria related to pericoronitis and their coexistence with other bacterial species at the infection site.

Plaque from pericoronal pockets of lower wisdom teeth of 25 patients that have been diagnosed with pericoronitis were sampled and subjected to a standard microbiological procedure for identification of bacterial species including cultivation on enriched agar plates, biochemical profiling and 16s rRNA PCR analysis. A total of 97 microorganisms were isolated and identified from the cultured samples and 94.73% were Gram-positive bacteria; with the highest incidence of Streptococcus gordonii, Streptococcus mitis, and Streptococcus anginosus. This study also revealed that facultative anaerobes were the predominant group causing pericoronitis (89%). The high occurence of multi-strain bacteria ranging from facultative anaerobic to aerobic bacteria display the importance of their infection networks in pericoronitis.

Knowledge gained from this study increases our understanding on the role of different pathogens in pericoronitis and provides new insight into the clinical management of patients and in the prevention of its recurrence.

Clinical article (J Int Dent Med Res 2019; 12(4): 1522-1526)

Keywords: Pericoronitis, Oral bacteria, Mandibular third molar.

Received date: 06 January 2019 **Accept date:** 28 May 2019

Introduction

Pericoronitis is an inflammation of the soft tissue surrounding the mandibular third molar tooth. It develops as the break of the partially erupted mandibular wisdom tooth through the gum, which has led to the invasion of multispecies bacteria around the tooth and cause infections.[1] The inflammation usually observed among young adults aged between 17 to 26 years old which is the time of the mandibular third molar tend to erupt and may occur bilaterally.[2] If it left untreated, it can cause significant morbidity as infection can spread to the nearby structures.[3]

Severity of pericoronitis varies, and can be classified into acute and chronic stages. The acute form of pericoronitis is of sudden onset and normally seen in patients having moderate or poor oral hygiene. Pericoronitis is considered as chronic when there are repeated episodes of acute pericoronitis, last for 2 to 4 days and they occur periodically. The common symptoms of pericoronitis include severe pain, limited mouth opening, extra oral swelling, halitosis, lymphadenitis, dysphagia and radiation of pain into adjacent muscle. The treatments for acute phase include debridement of plaques and food debris, drainage of pus, irrigation with sterile saline, evaluation and elimination of occlusal trauma.[4] Removal of tooth either partially or completely may overcome problems associated with the erupted third molars. However, tooth extraction should be avoided in the acute stage of pericoronitis because there are possibility of local tissue destruction and serious secondary infections. [5]

Accumulation of food debris in pericoronal pocket of the operculum and occlusal trauma of

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the pericoronal tissues by the opposing tooth may triggered pericoronitis.[1] Entrapment of plaque and food debris provides an ideal shelter for bacterial growth as the area is inaccessible with toothbrush and difficult to be cleaned. Study by Sixou et al.[6] demonstrated that the microflora on the infected pericoronal sites is mainly comprised of obligate anaerobes, microaerophilic and facultative anaerobes. Bacterial species which are predominant in pericoronitis of erupting mandibular third molars are *Streptococcus, Actinomyces* and *Propionibacterium sp*. There is also evidence of beta-lactamase producing bacteria such as *Prevotella, Bacteroides*, *Fusobacterium, Capnocytophaga and Staphylococcus sp*. identified at the erupted site.[7]

In spite of several studies have been carried out to identify the causative pathogens of pericoronitis, data from one study to another varied. Hence, the goal of this study was to identify the predominant infectious oral microbiome related to pericoronitis and to compare the distribution of oral microflora associated with pericoronitis and the healthy third molar flora among patients attending Universiti Sains Islam Malaysia (USIM) Dental Polyclinic, Malaysia.

Materials and methods

Ethical Considerations

Ethical approval was obtained from the Faculty of Dentistry, Universiti Sains Islam Malaysia (USIM) Ethics Committee. A written informed consent was obtained from all subjects prior to sample collection.

Clinical sampling

Clinical sampling was conducted at USIM Dental Polyclinic between May 2017 and January 2018. Twenty-five adults aged 16 to 41 years, presented with partially impacted mandibular third molar and without systemic diseases were included in this study. The control group was the patients without any clinical signs and symptoms of pericoronitis. For both groups (pericoronitis and control), the subjects must not receive any antibiotic medication in the previous 6 months before the samples were taken. Plaque samples were taken aseptically from the pericoronitis pocket of the partially erupted mandibular third molar by a single clinician. A sterile Gracey curette was gently inserted to the base of the pocket and removed by a simple stroke. The subgingival plaques were transferred immediately into Thioglycolate broth, and subsequently send to the Microbiology Laboratory, USIM for further analysis.

Bacterial cultivation and identification

Plaque samples were homogenized by vigorous mixing on a vortex for 5 min. A total of 60 µl of each sample was plated on blood agar. Plates were incubated both aerobically and anaerobically (80% N_2; 10% H_2; 10 % CO_2) at 37ºC. The plates were observed after overnight incubation for aerobic and after 2-4 days for anaerobic bacteria. Single colonies were then subcultured on blood agar and brain heart infusion (BHI) broth, respectively for 24 hours at 37ºC. The colony morphology, Gram reactions and biochemical characteristics were then investigated according to the standards protocols.

DNA extraction

Bacterial genomic DNA was extracted using Promega Wizard Genomic DNA Purification Kit (Promega UK Ltd), according to the manufacturer's protocol. The concentration of the extracted DNA was determined using Nanodrop spectrophotometer (Implen P330, Germany) and quantified using gel electrophoresis.

Molecular identification and sequence analysis

PCR reaction was carried out using 16S rRNA universal primers (forward 5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GAC TAC GTG CCA GCA GCC-3'; reverse 5'-GGA CTA CCA GGG TAT CTA ATC C-3') (Sheffield et al., 1989). The PCR conditions were 95ºC, 3 min, 30 cycles of 94ºC, 1 min, 56ºC, 1 min, 72ºC, 2 min, and final extension at 72ºC for 5 min (Tian et al., 2010) . All PCR products were sequenced (ABI13730, Major Bio, China) and analysed using bioinformatics tools Basic Local Alignment Search Tool (BLASTn) and ClustalW.

Statistical analysis

Statistical evaluation was performed using the Statistical Packages for the Social Sciences (SPSS, USA) version 21. Descriptive statistics were presented as frequency and percentages. The values for each bacteria species were

compared between the pericoronitis group and control group.

Results

A total of 164 bacterial isolates have been identified from plaque samples of 50 subjects, which consist of 25 pericoronitis patients (study group) and 25 healthy adults (control group). There were 97 and 67 bacterial isolates detected among pericoronitis and healthy samples, respectively. Majority of the identified bacteria from both groups were Gram-positive which account for 95% of the total isolates. The identification results are summarised in Tables 1 and 2.

Gram-positive Cocci (n=82)	**No. of samples (%)**
Streptococcus gordonii	11(11)
Streptoccous mitis	10(10)
Streptococcus anginosus	8(8)
Streptococcus pneumoniae	8(8)
Streptococcus cristatus	7(7)
Streptococcus sanguinis	6(6)
Streptococcus himalayensis	5(5)
Streptococcus intermedius	4(4)
Streptococcus oralis	4(4)
Streptococcus constellatus	4(4)
Streptococcus sinensis	2(2)
Streptococcus sobrinus	2(2)
Streptococcus mutant	1(1)
Lactococcus lactis	1(1)
Staphylococcus epidermidis	1(1)
Streptococcus salivarius	1(1)
Streptococcus dentirousetti	1(1)
Streptococcus criceti	1(1)
Streptococcus downei	1(1)
Lactococcus salivarius	1(1)
Lactococcus delbrueckii	1(1)
Staphylococcus aureus	1(1)
Staphylococcus epidermidis	1(1)
Gram-Positive Rods (n=10)	**No. of samples (%)**
Rothia mucilaginosa	3(3)
Actinomyces naeslundii	2(2)
Actinomyces johnsonii	2(2)
Bacillus megaterium	1(1)
Bacillus cereus	1(1)
Bacillus licheniformis	1(1)
Gram-Negative Rods and Cocci (n=5)	**No. of samples (%)**
Neisseria sicca	2(2)
Enterobacter clocae	1(1)
Fusobacterium nucleatum	1(1)
Prevotella intermedia	1(1)

Table 1. Microorganisms detected from pericoronitis samples.

The most common bacterial isolates found in pericoronitis samples were facultative anaerobic (89%), followed by strictly anaerobic (6%) and aerobic bacteria (5.0%) as indicated in Table 3. A similar pattern was observed in healthy samples despite the different bacterial species distributions. The most frequently isolated facultative bacteria were identified as members of the *Streptococcus* followed by *Rothia, Staphylococcus*, *Lactococcus*, *Enterobacter* and *Lactobacillus* sp. Among the strictly anaerobic microorganisms, the genus *Actinomyces* (4/6) were commonly detected, whereas the genus *Prevotella* and *Fusobacterium* were detected in only 1/6 of the bacterial isolates. Aerobic bacteria detected were from the genus *Bacillus* and *Neisseria* with detection frequency of 3/5 and 2/5, respectively.

Gram-positive Cocci (n=49)	**No. of samples (%)**
Streptococcus mitis	8(12)
Streptoccous gordonii	7(10)
Streptococcus anginosus	6(9)
Streptococcus salivarius	6(9)
Streptococcus intermedius	4(6)
Streptococcus sanguinis	2(3)
Streptococcus mutant	2(3)
Staphylococcus aureus	2(3)
Staphylococcus thermophilus	2(3)
Lactobacillus salivarius	2(3)
Streptococcus oricebi	1(1)
Enterococcus hirae	1(1)
Staphylococcus hominis	1(1)
Streptococcus oralis	1(1)
Streptococcus cristatus	1(1)
Streptococcus sinensis	1(1)
Streptococcus dentirousetti	1(1)
Streptococcus sobrinus	1(1)
Gram-Positive Rods (n=15)	**No. of samples (%)**
Actinomyces naeslundii	7(10)
Actinomyces johnsonii	5(7)
Actinomyces odontolyticus	2(3)
Bacillus nealsoni	1(1)
Gram-Negative Rods and Cocci (n=3)	**No. of samples (%)**
Neisseria sicca	1(1)
Enterobacter clocae	1(1)
Klebsiella pneumoniae	1(1)

Table 2. Microorganisms detected from healthy samples.

Type of Bacteria Identified	Sample	
	Pericoronitis	Healthy
	n (%)	n (%)
Facultative anaerobic	86(89)	51(76)
Strictly anaerobic	6(6)	14(21)
Aerobic	5(5)	2(3)

Table 3. Comparison of bacterial distribution in pericoronitis and healthy samples.

The distribution of bacteria in pericoronitis and healthy samples is summarised in Figure 1. Our data indicates that there was no correlation between the distribution of microorganisms and the demographic variables of the subjects in both groups.

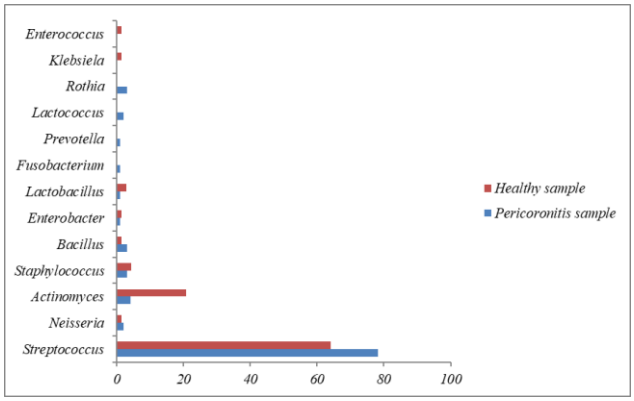

Figure 1. Distribution of oral bacteria in subgingival plaque of pericoronitis and healthy third molar (X-axis; frequency of detection, %; Y-axis: bacterial genera).

Discussion

It is well known that human oral cavity is colonised by more than 600 prevalent taxa of oral microbiome, with different subset predominating at distinctive microbial habitats such as teeth, tongue, cheeks and gingival sulcus. Many studies have shown that different oral structures and tissues are colonised by dissimilar microbial populations.[8] Previous study has reported that cultivation of 280 bacterial species from the oral cavity using anaerobic microbiological methods has led to an average of 600 common oral species being identified.[9] Bacteria can also be found colonising underneath the pseudo pocket overlying the crown of partially erupted third mandibular tooth in pericoronitis.

This study identified the predominant bacterial species in pericoronitis samples of USIM Dental Polyclinic, Malaysia. Our results demonstrated that the identified bacterial species were dominated by facultative anaerobic *Streptococcus* sp. that took account for 78% from the total isolates. These findings are in agreement with other studies including Peltroche-Llacsahuanga et al.[5] that detected majority facultative anaerobes bacteria such as *S. milleri, Stomacoccus mucilaginosus and Rothia dentocariosa* in pericoronitis samples. Another report by Sabra and Soliman[10] also detected facultative anaerobic and aerobic bacteria such as *S. viridans, Corynebacterium spp., Haemophilus spp. S. mutans, S. aureus, S. pneumonia, S. pyogenes and Pseudomonas spp* from pericoronitis samples.

In this study, we found that the most common species from pericoronitis samples were *S. gordonii*, *S. mitis*, *S. anginosus and S. pneumonia. S. gordonii* is long known as teeth primary colonisers and it normally initiates the formation of biofilms on tooth surfaces. It can also be found at the periapical lesions as it forms biofilms on the root canal surface.[11] The occurrence of *S. gordonii* in the pseudopocket of erupting mandibular third molar constantly linked to pericoronitis.[6,12] The elevated number of this species in pericoronitis- compared to the healthy samples as demonstrated in this study therefore implies its association with pericoronitis as described in the previous studies. Additionally, *S. gordonii* were also reported to cause bacterial endocarditis, which show its capability of being spread to extra oral site causing systemic infection.[13]

Interestingly, our data showed the presence of *S. pneumonia* in pericoronitis samples with a detection frequency of 8, but it was not detected in the healthy samples. *S. pneumonia* can cause a broad range of severe diseases including otitis media, sinusitis, septicaemia, pneumonia and meningitis. Its primary site for colonisation is the nasopharynx and based on our findings, it is likely that *S. pneumonia* has spread to the gum flap of erupting third molar due to its close proximity to the nasopharynx area. Limited studies have reported on the association of *S. pneumonia* and pericoronitis, therefore these findings warrant further investigation to decipher the pathogenesis and correlation of *S. pneumonia* in, and with pericoronitis.

S. anginosus has been described as one of the causative agents for pericoronitis[5] with frequency of detection reported ranges from 5% to 80%.[5,14-17] *S. anginosus* is a normal inhabitant of the oral cavity but it is also an opportunistic pathogen that can cause abscess-forming infections at various sites within the cavity. Likewise, *S. mitis* that is part of the healthy microflora is also commonly related to oral maxillofacial infections including dental alveolar abscess and sialadenitis. Our data indicated that high number of viridans group has outnumbered the *Actinomyces* despite the later has also been associated with numerous pericoronitis cases.[6]

Other species identified in this study are also inhabitants of healthy oral cavity but they may act as pathogens if predisposing factors or condition prevail, disturbance of the microbial

balance and disruption of mucosal barriers by spontaneous or induced trauma.[16] The competency of oral bacteria in causing infections including pericoronitis is associated with the production of quorum-sensing molecules[18] and thus highlights the importance of our findings.

Conclusions

Current work in our laboratory is now aim at understanding the virulence factors of the predominant species causing pericoronitis. Thorough investigation on the virulence factors of these species is crucial that it may provide new insight into the clinical management of patients and in the prevention of its recurrence.

Declaration of Interest

The authors report no conflict of interest and would like to thank the Ministry of Higher Education Malaysia for the financial support provided under the Fundamental Research Grant Scheme (FRGS/1/2016/SKK14/USIM/03/1).

References

1. Moloney J, Stassen LFA. Pericoronitis: treatment and a clinical dilemma. *J Ir Dent Assoc.* 2009;*55*:190–192.
2. Niedzielska IA, Drugacz J, Kus N, Kreska J. Panoramic radiographic predictors of mandibular third molar eruption. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;102:154–9.
3. Renton T, Wilson NHF. Problem with erupting with wisdom tooth: signs, symptoms, and management. *Br J Gen Pract.* 2016;66:236-237.
4. Dhonge RP, Zade RM, Gopinath V, Amirisetty R. An Insight into pericoronitis. *Int J Dent Med Res*. 2015;1:172-175.
5. Peltroche-Llacsahuanga H, Reichhart E, Schmitt W, Lutticken R, Haase G. Investigation of infectious organisms causing pericoronitis of the mandibular third molar. *J Maxillofac Surg.* 2000;58:611-616.
6. Sixou JL, Magaud C, Jolivet-Gougeon A, Cormier M, Bonnaure-Mallet, M. Evaluation of the mandibular third molar pericoronitis flora and its susceptibility to different antibiotics prescribed in France. *J Clin Microbiol*. 2003;41:5794–5797.
7. Gutiérrez-Pérez JL. Third molar infections. *Med Oral Patol Oral Cir Bucal.* 2004; 9:120-125.
8. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J. Clin. Microbiol*. 2005; 43:5721-5732.
9. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe AFE, Dewhirst FE. Bacterial diversity in human subgingival plaque. *J Bacteriol*. 2001;183:3770-3783.
10. Sabra SM, Soliman MM. The prevalence of impacted mandibular wisdom with associated physical signs and microbial infections among under graduate girls at Taif University, KSA. *World Appl Sci J.* 2013;21:21-29.
11. Chávez de Paz L, Svensäter G, Dahlén G, Bergenholtz G. Streptococci from root canals in teeth with apical periodontitis receiving endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;100:232-41.
12. Vickerman M, Campbell J. Phylogenetic Analysis of Microbial Species in Pericoronitis. *Int J. Oral Maxillofac. Surg.* 2011;69:10.
13. Durack DT. Prevention of infective endocarditis. *N. Engl. J. Med.* 1995;332:38-44.
14. Rajasuo A, Jousimies-Somer H, Savolainen H, Leppanen J, Murtomaa H, Meurman JH. Bacteriologic findings in tonsillitis and pericoronitis. *J Clin Infect Dis*.1996;23:51-60.
15. Leung WK, Theilade E, Comfort MB, Lim PL. Microbiology of the pericoronal pouch in mandibular third molar pericoronitis. *J Oral Microbiol Immun.*1993;8:306-312.
16. Wade WG, Gray AR, Absi EG, Barker, GR. Predominant cultivable flora in pericoronitis. *Oral Microbiol Immun*.1991;6:310–312.
17. Mombelli A, Buser D, Lang NP. Suspected periodonto pathogens in erupting third molar sites of periodontally healthy individuals. *J Clin Periodontol*.1990;17:48-54.
18. Hannan S, Ready D, Jasni AS, Rogers M, Pratten J, Roberts AP. Transfer of antibiotic resistance by transformation with eDNA within oral biofilms. *FEMS Immunol Med Microbiol*. 2010;59:345-9.