CASE REPORT

Localized severe gingivitis caused by *Paenibacillus apiarius* in a 28-year-old male patient: A first case report

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Abstract

A 28-year-old male was admitted to the clinic with gingival complaints which on clinical examination revealed gingival disease and isolated periodontal pocket. There were signs of gingival inflammation, redness, sponginess of the gingival tissue, bleeding on provocation, changes in contour and presence of calculus and plaque. There was no radiographic evidence of crestal bone loss. A Gram-positive spore forming bacillus was isolated from subgingival sulcus area. The organism (strain G11) was initially identified as *Bacillus* sp. based on phenotypic properties then confirmed as *Paenibacillus apiarius* using Comparative 16S rDNA sequence analysis (accession no.: MF062074). The patient was treated with scaling, polishing, and root planning with curettage for isolated pocket. On follow-up, the patient progressively showed signs healing and complete recovered after 2 weeks with no further complication. We associate this uncommon bacterium with the infection given the fact that it was demonstrated in the lesions, isolated in a pure form, found facultative anaerobe and possessed a strong beta hemolysis virulence factor.

Keywords: 16S rDNA gene, *Bacillus apiarius*, gingival inflammation, periodontal disease

Introduction

Periodontal diseases are generally divided into two groups: Gingivitis, which causes lesions (wounds) that affect the gums and periodontitis, which damages the bone and connective tissue that supports the teeth. Gingivitis is caused by substances derived from microbial plaque accumulating at or near the gingival sulcus;¹² all other suspected local and systemic etiologic factors either enhance plaque accumulation or retention, or enhance the susceptibility of the gingival tissue to microbial attack.

Bacteria involved in the etiology of gingivitis include specific species of *Streptococcus, Fusobacterium, Actinomyces, Veillonella*, and *Treponema* and possibly *Bacteroides, Capnocytophaga*, and *Eikenella*.¹³ Microorganisms associated with both caries and periodontal diseases are metabolically highly specialized and organized as multispecies microbial biofilms. *Paenibacillus* species are not known to cause dental diseases, however, mutanase from *Paenibacillus* sp. was found active in the degradation of mutan and *Streptococcus mutans* biofilm. This bioactivity was observed as a potential agent for oral care and for controlling dental biofilm.⁴ Progression of these diseases involves multiple microbial interactions driven by different stressors. In gingivitis, plaque accumulation at the gingival margin leads to inflammation and increasing proportions of proteolytic and often obligatory anaerobic species.

The natural mucosal barriers and saliva are the main innate defense mechanisms against soft tissue bacterial invasion. Similarly, enamel and dentin are important hard tissue barriers to the caries process. This state of knowledge suggests that the etiologies of caries and periodontal diseases are mutually independent; the elements of innate immunity that appear to contribute to resistance to both are somewhat coincidental.³ The objective of this report was to describe a plaque-induced gingivitis in an adult male caused by a rare Gram-positive facultative anaerobic bacterium.

Case Report

A 28-year-old male was presented to the Clinic of The College of Dentistry, King Khalid University for general dental checkup. The patient was medically fit. On dental examination of intraoral soft tissue, the patient presented with the most common features of gingival disease include clinical signs of inflammation redness and sponginess of the gingival tissue, bleeding on provocation, changes in contour, and the presence of calculus or plaque with no radiographic evidence of crestal bone loss [Figure 1].
Specimen from the subgingival sulcus area was submitted to Microbiology Laboratory, College of Medicine, King Khalid University, for bacterial culture and identification. An aliquot was inoculated onto blood agar (BA) and Mueller-Hinton agar. Growth of the causative agent was obtained in BA after 2 days under an aerobic condition at 37°C. Subsequent subcultures were made on to BA medium for identification tests. Gram-stained smear shows the presence of spore-forming spindle-shaped Gram-positive cells. A dark green, beta hemolytic irregular, organism was isolated from dental specimens when cultured on BA (Difco, Becton, Dickinson and Company, Sparks, Maryland) for 24 h, incubated at 37°C [Figure 2].

The organism was labeled G11 and identified on the basis of colony morphology appeared on BA and on the basis of microscopic features following recommended guiding principles.[5]

In vitro susceptibility assay was performed using the well method on BA. The following drugs were tested: Doxycycline which revealed 35 mm inhibition zone; fusidic acid - 30 mm; meropenem - 30 mm; tobramycin - 0 mm; clindamycin - 10 mm; co-trimoxazole - 18 mm; vancomycin - 18 mm; nitrofurantoin - 30 mm; streptomycin - 28 mm; oxacillin - 38 mm; clarithromycin - 35 mm; amikacin - 20 mm; aztreonam - 35 mm; carbenicillin - 38 mm; chloramphenicol - 30 mm; fosfomycin - 32 mm; netilmicin - 23 mm; cefotaxime - 40 mm; rifampicin - 38 mm; gentamicin - 18 mm; cephalothin - 38 mm; erythromycin - 31 mm; cefuroxime - 40 mm.

The obtained 16S rDNA nucleotide sequences from strain G11 (920 bp; GenBank accession number: MF062074) was initially examined using the basic local alignment search tool (BLAST, USA) system to establish a rapid phylogenetic position. Following alignment of the isolate with Paenibacillus spp., the gene was analyzed using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6) software (Center for Evolutionary Medicine and Informatics, USA)[6] and compared with all known sequences of Paenibacillus spp. found in the GenBank database. The strain G11 was found close to Paenibacillus apiarius (99.55% similarity), and to Paenibacillus profundus (98.52%), Paenibacillus alvei (95.83%), and to Paenibacillus assamensis (95.33%) [Figure 3]. The strain G11 was thus confirmed as P. apiarius.

**Discussion**

The diversity of the periodontal ecological offer appropriate conditions for the colonization of species not usually considered members of the oral microbiota.[7] In this case, the patient was diagnosed with periodontal disease and a rare type of Gram-positive spore-forming *Bacillus* was isolated. This *Bacillus* is unique in its habitat, first described in association with bees, biochemical characteristics: Catalase negative, oxidase negative, motile, facultative anaerobe, and its spore morphology.[8]

To our understanding, this case denotes a first account of plaque-associated gingivitis caused by *Paenibacillus* species. Comparative analyses of the 16S rRNA gene DNA sequence showed that the proper phylogenetic position of the “*Bacillus apiarius*” Katznelson 1955 was in the genus *Paenibacillus*[8] as *Paenibacillus apiarius* for a Gram-positive, motile, spore-forming bacterium which was isolated from honeybees.[8] Many *Paenibacillus* species are known to be useful in promoting crop production and generating various useful environmental and agricultural compounds. Few species such as *Paenibacillus larvae* are causative agent of a lethal disease in honeybees or opportunistic pathogens in humans or causing spoilage of pasteurized dairy products.[8] Their association with oral biofilm is not known. We are describing this case in which severe gingivitis was encountered.

Biofilm is a complex bacterial community that is highly resistant to antibiotics and human immunity. Biofilm communities are the causative agents of biological developments such as dental caries, periodontitis, and peri-implantitis, and causing periodontal tissue breakdown dental caries, periodontal, and other oral diseases are linked to the ability of bacteria to form biofilm. Periodontal diseases have been associated to anaerobic Gram-negative bacteria forming a subgingival plaque. These include *Porphyromonas gingivalis*, *Actinobacillus*, *Prevotella*, and *Fusobacterium*.[10]
Conclusions

In conclusion, this report draws attention to the importance of careful clinical examinations to investigate gingival mucosa and to look for specific pathogen through laboratory diagnosis. To our knowledge, this report represents the first case description of plaque-associated gingivitis caused by *P. apiarius*.

References


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