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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) reveals the anaerobic *Slakia exigua* as unique etiology of a dental abscess

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ABSTRACT

Dental abscess is a frequently occurring infectious process known to the health practice. The rate of the infection depends on the virulence of the bacteria, host resistance factors, and regional anatomy. Serious consequences arising from the spread of dental abscess bacteria lead to significant morbidity and mortality. The microbial etiology of the acute dental abscess is polymicrobial, consisting of strict anaerobes, such as anaerobic cocci, *Prevotella* sp., *Fusobacterium* sp., and facultative anaerobes, such as *viridans* group streptococci and the *Streptococcus anginosus* group. However, numerous novel, uncultivable and fastidious organisms have been identified as potential pathogens by using culture-independent techniques. Improvements in sampling, culture and identification have led to a greater insight into the diversity of the microbiota in an acute dental abscess. This has resulted in the reporting of microorganisms which are probably more accurately described as 'unfamiliar' rather than 'new' implying their recent appearance. These include anaerobic members of the Gram-positive rods among which *Slakia exigua*. This paper describes for the first time in our country *S. exigua* identification as unique pathogen from dental abscesses using MALDI-TOF MS.

Keywords: *MALDI-TOF MS, Slakia exigua, dental abcess, anaerobic bacteria.*

1. INTRODUCTION

The oral cavity is recognized to be a source of systemic bacterial infections such as brain abscesses [1], endocarditis [2] and bacteremia [3]. Identifying the origin of systemic infection is important for making proper decisions and providing the appropriate treatment to patients.

Slakia exigua is a Gram-positive, non-spore-forming, non-motile, and strictly anaerobic bacillus that has been considered an oral asaccharolytic bacterial species included in the Coriobacteriaceae family. This bacterium was originally classified as *Eubacterium exiguum* in 1996 [4] and was reclassified as S. *exigua* in 1999 [5].

It grows poorly on culture media, being thus rarely isolated and it is associated with periodontal and periapical infections [6]. S. exigua has been identified in infected necrotic pulps and periradicular infections [7]. Booth et al. demonstrated that the presence in higher percentages of *S. exigua* has an important role in deep teeth infections and the supragingival plaque [8]. Also the presence of *S. exigua* in infections with intestinal origin has been shown [9]. Kim et al. [10] demonstated the isolation of this pathogen from oral infections but also from human wounds infections and abscesses. *S. exigua* was the most often identified using 16rRNA sequencing.

In 2015, Roingeard & Jaubert [11] (France), used matrix-assisted laser desorption/ionization time-of-fligt mass spectometry (MALDI-TOF MS) to identify for the first time *S. exigua* in a case of lung abscess in a man hospitalized for fever with a history of severe neurological deficit due to Ravine syndrome and which was ventilated by non-invasive tracheotomy.

In 2014, Matsumura et. al., [12] revealed the isolation of *S. exigua* along with two other anaerobes (*Dialister pneumosintes* and *Prevotella baroniae*) from an old patient with cavernous sinus thrombosis. In a patient with advanced rectal carcinoma, Bukki et al., [13] described for the first time *S. exigua* isolated from the cerebrospinal fluid. The culture was found polymicrobial, the cerebrospinal fluid being positive with aerobic and anaerobic bacteria. This was the first report which demonstrated the presence of *S. exigua* in the cerebrospinal fluid. However, the clinical significance of *S. exigua* in this case remained uncertain.

Deep neck and mediastinal abscesses are rare complication of the dental abscess but spread of oral infections to the neck is growing up [14].

2. EXPERIMENTAL SECTION

A young woman came as an emergency with signs and symptoms of acute dental abscess as pain, swelling, erythema, tenderness to palpitation and suppuration.

The X-ray evidenced the loss of bone around the tooth's root with the occurrence of a cavity leading into the soft pulp and a colored area denoting the infection.

After examination, the doctor observed pus collection in the alveolar bone at the root apex. The pus was drained and aspirated from within the infected canal. This harvesting of bacterial sample reduces contamination from oral microbiota.

The sample has been cultivated on media containing reducing substances (Schadler, PAA – Phenylethyl Alchool Agar and Anaerobic Agar –Oxoid – UK) under anaerobic conditions using Gaspacks system (Becton Dickinson), at 37°C. Counts of the different colonies were made, and representative colonies were subcultured and subjected to standard procedures for bacterial identification, including Gram staining. After 8 days of culture media have been developed tiny, circular and translucent colonies.

S. exigua was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS-MALDI Biotyper, Bruker, Germania). MALDI-TOF MS represents a rapid and reliable technique for microbial identification as most results obtained using this method are similar to those of 16S rRNA gene sequence analysis but at a rapid rate and at a lower cost. This technique is based on fingerprinting analyses of primary ribosomal proteins, which are synthesized under all growth conditions and are the most abundant cellular proteins [15].This represents a fast and inexpensive technology for identification of anaerobic bacteria.

Antibiotic susceptibility testing was performed according to CLSI guidelines using Sensititre Anaerobe MIC Plate (TREK Diagnostic Systems, UK). Because of the slow growth colony, the interpretation was performed after 72 hours of incubation. The following antimicrobial agents were tested: ampicilin, amoxicillin/clavulanic acid, penicillin, imipenem, meropenem, clindamycin, metronidazol, and tetracycline. The isolates were categorized, by using interpretative criteria, as susceptible and resistant, if the minimal inhibitory concentration (MIC)s for each antimicrobial with the manufacturer's recommendations.

3. RESULTS SECTION

Using MALDI-TOF we obtained spectra with peaks between m/z 500 and up to about m/z 2000 (Figure 1) that specifically belonged to *S. exigua*.

The results of the *in vitro* antibiotic susceptibility testing of *S. exigua* isolate revealed the susceptibility to all tested antimicrobials: ampicilin, amoxicillin/clavulanic acid, penicillin, imipenem, meropenem, clindamycin, metronidazole and tetracycline. The production of β-lactamase was investigated by nitrocefin test (Becton Dickinson and Company, USA) and the strains ware negative for the β-lactamase production.

Further studies in which a greater number of strains of the species should be tested are needed.

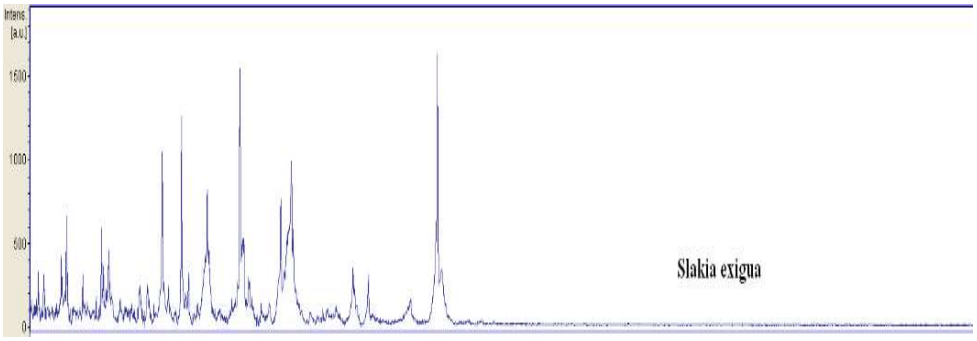

Figure 1. MALDI-TOF – spectrum of *Slakia exigua* with peaks between m/z 500 and m/z 2000.

4. CONCLUSIONS

Dental abscess and its complications pose a substantial burden on individuals, communities, and the health-care system; hence, early diagnosis and appropriate treatment are extremely important. Determination of various host and environmental factors that put an individual at risk for development of a dental abscess, influence the spread of infection from a localized collection at the apex of a tooth to a cellulitis and further life-threatening sepsis would aid treatment decisions.

Since *S. exigua* originally identified as an oral asaccharolytic species, was isolated in unique etiology from a dental abscess, this fact suggests that this species may play a pathogenic role in oral infectious diseases, including pulpal infections that could spread to the periradicular tissues and contribute to the pathology of periodontal disease.

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6. ACKNOWLEDGEMENTS

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