

Bacteria That Cause Dentoalveolar Abscesses after Failed Endodontic Treatments: A Pilot Study

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Abstract

Failed endodontic treatments often lead to more severe conditions that need surgical procedures. The aim of this study was to identify which microorganisms caused dental abscesses.

50 pus samples from patients with dental abscesses were examined for bacterial growth and 50 samples from healthy gingiva of healthy individuals were swabbed for comparison of bacterial etiology. Isolated pathogenic bacterial were compared and bacteria were identified using MALDI-TOF.

Bacterial strains were positively identified in 42 out of 50 patients with dental abscesses. 16 different microorganisms from 100 subjects (patients with dentoalveolar abscesses and controls) were isolated. In 18 (36.0%) out of the 50 samples only aerobic flora was present, in 10 (20.0%) out of 50 only strictly anaerobic flora, and in 22 (44.0%) out of 50 abscesses mixed aerobic anaerobic flora was isolated.

Isolated oral microorganisms in our study did not vary with significance compared to healthy oral microbiota, thus commensal microbiota were the main cause of dental abscesses. Cultivating and culture testing take time providing results in few days, what is usually too late, and modern methods of microbial identification are expensive. Most of oral microbiota is uncultivable therefore modern methods of identification are necessary, especially at polymicrobial infections.

Experimental article (J Int Dent Med Res 2018; 11(3): 823-829)

Keywords: Bacterial identification, Dentoalveolar abscesses, Oral microbiota, Maxillofacial surgery, Novel microbiologic methods.

Received date: 29 January 2018

Accept date: 28 February 2018

Introduction

Dental abscess is a frequently occurring infectious process known to the health practice. The fate of the infection depends on the virulence of the bacteria, host resistance factors, and regional anatomy. Serious consequences arising from the spread of a dental abscess lead to significant morbidity and mortality.¹⁻³ Bacteriological agents implicated in causation of dental abscesses comprise of the complex mix of strict anaerobes and facultative anaerobes. Culture and molecular studies show that different bacteria have been identified in different types of endodontic infections.⁴ Depending upon the

recovery and cultural conditions, strict anaerobes outnumber facultative by a ratio which varies between 1.5 and 3:1 in mixed infections.^{5,6} Dental abscesses caused solely by strict anaerobes occur in approximately 20% of cases. Although there is a wide range depending upon recovery conditions (6-63%) it has been observed that pure cultures from acute dental abscesses are unusual⁷⁻¹¹ and mixed aerobic infections are also quite uncommon, accounting totally 6% of abscesses.¹¹ Polymicrobial nature of such infections and presence of cultivable and uncultivable microbes may pose a challenge toward diagnostic analysis in routine microbiological laboratories.

In the past, inappropriate methods of sampling hampered correct identification of the causative pathogens involved in the development of the dental abscess¹² choice of sample type and method of sampling are crucial to optimal diagnostic efficacy. Ideally, an aspirate through intact mucosa after disinfection by an appropriate antiseptic mouthwash, e.g., chlorhexidine should

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be collected. This will reduce contamination from the normal oral flora. Some researchers have also sampled purulent exudates from within infected canals.^{12,13}

Significant improvement in the routine diagnostic yield from acute dental abscesses has occurred with employment of meticulous specimen collection and processing on selective and nonselective agars under appropriate atmospheric conditions. However, despite the close attention to detail, it is apparent that many genera of bacteria have yet to be cultured. A major limitation of past cultural studies is that a large percentage of the oral microflora does not grow on conventional artificial culture media in the laboratory.¹⁴

Dental abscess and its complications position a substantial burden on individuals, communities, and the health-care system; hence, early diagnosis and appropriate intervention are extremely important. Determination of various host and environmental factors that put an individual at risk for development of 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. Increased reliance on novel molecular techniques has enriched our knowledge of the diverse polymicrobial collection that constitutes a dental abscess. Therefore, the aim of this study was with novel microbiologic methods to identify the bacterial strains that cause abscesses after failed endodontic treatment and compare the results with common oral microflora.

Materials and methods

Study design

Study was designed as a controlled, case-control prospective trial. Selected patients and controls gave positive consent for participation in the study. National Ethics Committee of Kosovo approved the study design.

Patients were selected from the list of patients, who visited our clinic due to surgical procedure of removal of dentoalveolar abscess. The criteria for inclusion in the study were: >18 years of age, dental abscess after failed endodontic treatments, patients did not use antibiotics two weeks before abscess formation. Criteria for elimination from the study:

compromised immunity, patients with uncontrolled comorbidities, patient with infections, taking antibiotics.

Subjects

Study consisted of two groups of subjects; cases and controls. The case group consisted of 50 patients with dental abscess; the control group consisted of 50 healthy individuals with no abscess or concomitant diseases. Healthy controls were selected in terms that basic characteristics coincided with the data of case group.

Sampling

Pus from dentoalveolar abscesses were collected with a swab from 50 adult patients. In addition, 50 swab samples were collected from healthy controls for comparing the oral flora of healthy oral cavity and oral flora from abscesses. Totally 100 swab samples were collected for bacterial inoculation.

During surgical procedure of removing dentoalveolar abscess, a swab of pus was taken and stored in sterile transporting medium for bacterial cultivation-Transystem Stuart. Samples from healthy individuals were taken with the same principle. All samples were transported within 48 h after sampling at ambient temperature in transporting medium in plastic bags under anaerobic condition using the Anaerocult® system (Merck). Upon arrival at the laboratory, samples were inoculated and cultivated for bacterial growth on blood, chocolate agar for aerobes and anaerobic blood agar plates (SNVS agar, SCS agar) for anaerobes.

Samples were incubated aerobically in incubation chamber at 37 °C for 2 days. Meanwhile, inoculated anaerobic plates were incubated anaerobically (5% CO₂, 10% H₂ and 85% N₂) for 2 days at 37 °C using the Anoxomat System™ (MART Microbiology BV, Netherlands). If there was no bacterial growth after 2 days, the incubation period has been prolonged to one week.

After incubation the growth of bacterial colonies on plates was evaluated. Bacterial colonies were first identified by eye according to the morphological characteristics (shape, colour, thickness of colonies, smell, haemolysis on blood

agar plate). After that, single colony was administered for molecular identification.

Molecular identification of bacterial strains

Identification of bacterial strains was performed by acquisition of the peptide mass spectra for protein identification of bacteria on an Ultraflex Matrix Assessed Laser Desorption Ionization- Time of Flight/Time of Flight Mass Spectrometer (MALDI-ToF/ToF MS; BRUKER Daltonics GmbH, Germany). Bacterial colony was administered using plastic loop on a special plate for MALDI/TOF identification. Colony was confluent smeared on the marked part of the identification plate. The sample was overlaid by special matrix, and the plate was inserted into MALDI/TOF identifier. Identification of bacteria was given by computer software according to the protein profile.

Statistical analysis

Statistical analysis was performed by using Statistical Package for Social Sciences 21 (IBM, New York, USA). One-Eay ANOVA statistical test with post-hoc Tukey test were used with the comparison of quantitative variables and Pearson chi-square test for comparison of qualitative variables. Statistical significance was set at $P < 0.05$.

Results

Basic characteristics of subjects are presented in Table 1. Healthy controls did not statistically differ from the group of cases in any of the characteristics. Comorbidities were detected in 8 (16.0%) out of 50 case patients, two of them had simultaneously controlled diabetes.

In the analysis 100 swab samples were included, of which 50 were pus samples from dental abscesses, and additional 50 samples were swabs of healthy gingiva from healthy individuals. Bacterial strains were positively identified in 42 out of 50 patients with dental abscesses. Identified aerobic and anaerobic bacteria are presented in Table 2. 16 different microorganisms from 100 subjects (patients with dentoalveolar abscesses and controls) were isolated.

| | Cases (N=50) | Controls (N=50) | P-value |
|-------------------------|--------------|-----------------|---------|
| Age [years] | 39.2±14.5 | 36.9±19.9 | 0.663 |
| Gender | | | |
| M/F | 28/22 | 25/25 | 0.658 |
| Comorbidities | 8 (16.0%) | 0 | 0.096 |
| Controlled hypertension | 8 | / | |
| Controlled diabetes | 2 | / | |

Table 1: Basic characteristics of subjects included in the study

| AERO | Abscess samples (N=50) | Healthy samples (N=50) |
|--------------------------------------|------------------------|------------------------|
| Coagulase-Negative Staphylococci | 2 | 20 |
| Alpha-hemolytic <i>Streptococcus</i> | 10 | 18 |
| <i>Enterococcus</i> spp. | 20 | 11 |
| <i>Klebsiella</i> spp. | 10 | 14 |
| <i>Streptococcus mitis</i> | 1 | 0 |
| <i>Streptococcus mutans</i> | 2 | 0 |
| <i>Streptococcus sanguinis</i> | 1 | 0 |
| <i>Streptococcus</i> spp. | 3 | 2 |
| <i>Escherichia coli</i> | 4 | 2 |
| <i>Staphylococcus aureus</i> | 0 | 3 |
| Yeast | 4 | 1 |
| Polymicrobial infection | 6 | 0 |
| ANR | | |
| <i>Actinomyces</i> spp. | 19 | 8 |
| <i>Bacteroides</i> spp. | 6 | 6 |
| <i>Fusobacterium nucleatum</i> | 3 | 0 |
| <i>Lactobacillus</i> spp. | 1 | 10 |
| <i>Prevotella</i> spp. | 6 | 0 |
| Negative | 21 | 31 |

Table 2: List of identified aerobic and anaerobic microorganisms from oral cavity swabs

* AERO - aerobic bacteria, ANR - anaerobic bacteria
 † 0 - no growth of bacterial was observed even after prolonged cultivation
 ‡ Polymicrobial infection - identification was due to polymicrobial sample impossible even with molecular methods

In 18 (36.0%) out of the 50 samples only aerobic flora was present, in 10 (20.0%) out of 50 only strictly anaerobic flora, and in 22 (44.0%) out of 50 abscesses mixed aerobic anaerobic flora was isolated.

Same microorganisms were identified from healthy oral cavity and from the abscesses. In 6 cases only polymicrobial infections could be evaluated and were impossible to precisely identify causative pathogen. In 4 cases yeasts

were identified, thus the colonies were microscopically examined with the native microscopic slides, and *Candida albicans* was identified as pathogenic yeast.

Discussion

Current preventive measures in endodontics could not be able to destroy the remaining bacteria after primary therapy so complications at the end lead to development of periapical lesions, cysts and abscesses that need surgical removal. The current study was conducted to identify bacteria from dental abscesses.

The samples of pus from dentoalveolar abscesses and samples from healthy oral cavity of controls were collected for identification of oral flora, which could have caused dental abscesses. Bacterial strains were identified in 42 out of 50 patients with dental abscesses. In some cases polymicrobial infections were observed, what unable to more precisely identify causative pathogen. Due to strict identification methods by MALDI/TOF, identification only on the level of genus was possible. Despite the superiority of methodology, when there are present more than three different but related bacterial strains, it is almost impossible to identify them on species level.

A wide range of aerobic and anaerobic bacteria were detected in all samples. The ratio between aerobes and anaerobes was nearly 2:1 for all 100 subjects. The results indicate that bacterial strains do not differ between healthy oral cavity and abscesses confirming that normal oral microbiota can be pathogenic and can cause dentoalveolar abscesses. Same microorganisms were identified from healthy oral cavity and from the pus samples. Most commonly identified healthy aerobic flora was coagulase-negative staphylococci, alpha-hemolytic *Streptococcus*, and *Enterococcus* spp. Most identified anaerobes were *Actinomyces* spp., *Lactobacillus* spp. and *Bacteriodes* spp.

The microorganisms that have cause dentoalveolar abscesses in our study were more aerobic than anaerobic bacteria. In 36.0% of cases they were strictly aerobic, in lower 20.0% of cases the flora was strict anaerobes, and most 44.0% of cases the oral flora were mixed aerobic-anaerobic. Most identified healthy aerobic flora were coagulase-negative

staphylococci, alpha-hemolytic *Streptococcus*, *Enterococcus* spp. and *Klebsiella* spp. *Streptococcus haemolyticus* represents a standard bacteria against which antibacterial actions do not work properly. *E. faecalis* is the most resistant species in the oral cavity and possible cause of failure of root canal treatment.¹⁵ Most identified anaerobes were *Actinomyces* spp., *Lactobacillus* spp. and *Bacteriodes* spp. Our results do not coincide with the findings that describe the proportion of 6% of aerobes, 50% of the anaerobes and 44% share of mixed aerobic anaerobic flora in isolates of 39 patients.⁹ Much more aerobes were find from our analysis. In a study by Goumas et al¹¹ on 52 patients, the authors isolated 154 bacterial agents, of which the aerobic share was 6%, 17% were anaerobes, and 75% were mixed aerobic-anaerobic flora. In a similar study, authors isolated 127 bacterial agents with 18% aerobic and 82% anaerobic and mixed aerobic anaerobic flora.⁵ Our results are in concordance with the percent of mixed aerobic-anerobic infections. Among aerobic agents, the predominant isolate was genus *Streptococcus*¹⁶ and from anaerobics mostly *Fusobacterium* and *Bacteriodes* were found.^{17,18} Other bacteria found in the oral commensal flora can include coagulase-negative staphylococci, gram-negative cocci, what was also proved in our analysis. Bacteria that are potentially pathogenic and are sometimes found in the oral cavity include *Staphylococcus aureus*, *Enterococcus faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, members of the *Enterobacteriaceae* family and *Actinomyces*.¹⁹

Our analysis and results confirmed that in healthy oral cavity there are more aerobic and facultative anaerobic bacteria than strict anaerobes, compared to the abscesses. Secondly, cultures of aerobic bacteria were more polymicrobial and diverse compared to the abscess samples. Commonly present oral bacteria can therefore cause infections and enable the development of dentoalveolar abscesses in patients, who have failed endodontic treatment, especially if the patient is host for anaerobes. This oral microflora can therefore cause dentolaveolar abscesses in parts of oral cavity that is damaged or contains open wound. Our patients with dental abscesses were not immuno-compromised patients and still common oral flora caused the development of

dental abscesses. Similar study was performed by Ewringmann on rabbits.²⁰ The most commonly isolated anaerobes were gram-negative *Prevotella* spp., *Fusobacterium* spp., *Bacteroides* spp. and gram-positive non-sporulating cocci mostly *Peptostreptococcus* spp. Of the aerobes, the author confirmed that 66.7% were gram-negative mostly *Pasteurella* spp., *Escherichia coli*, *Pseudomonas* spp., while 33.3% were gram-positive mostly *Streptococcus* spp., *Staphylococcus* spp. The results of bacteria are similar to our findings, while we have also found some sample of *E. coli*, *Bacteriodes*, *Prevotella* and *Fusobacterium*. The microflora of dental infections is typically polymicrobial consisting of various facultative and strict anaerobes.²¹ The dominant isolates are strictly anaerobic gram-negative rods and gram-positive cocci.²²

As was proved in all mentioned studies most dentoalveolar infections arise from overgrowth of normal commensal microflora within the oral cavity, as a result of changes in conditions of local environment, leading to opportunistic infections. Once microbial growth exceeds the minimum infective dose, a dentoalveolar infection and abscess development may arise.²³ It is widely accepted that dentoalveolar infections affecting the periapical tissues are predominantly consisted of strictly anaerobic gram-positive cocci and gram-negative rods mixed with facultative anaerobic flora.^{16,23,24} Mostly identified bacterial strain in our study was *Enterococcus* spp. as a presented of facultative anaerobic gram-positive cocci. The role of *Enterococcus faecalis* is a key-stone pathogen in posttreatment disease.^{25,26} In many studies it has been identified as one of the most frequent microorganisms that cause post-treatment diseases.²⁵⁻²⁷ *E. faecalis* as facultative anaerobe is able to survive long period without nutrients.²⁸ Thus, it invades oral cavity developing bacterial biofilm, which provides protection against irrigating and other disinfection agents and it makes it difficult to eliminate.²⁸⁻³⁰ Our findings are in line with studies that have shown the status of *E. faecalis* as the main pathogen in post-treatment oral diseases.^{31,32} Moreover, although certain bacteria can be recognized as a key pathogen, it is the synergistic activity of the whole bacterial community that interferes with host immune system and causes tissue destruction.^{33,34} *Enterococcus* spp. with the formation of bacterial biofilm, attaches to a solid

surface in a nutrient-containing fluid, the microbial cells are embedded in an extracellular matrix, and interact with each other. The microorganisms living in a biofilm can self-organize, resist environmental changes, act synergistically and respond to the changes in the environment as a community.^{34,35} Bearing in mind the tendency of microorganisms to form intraradicular and extraradicular biofilms, the future metagenomic studies should be oriented on the pathologic potential of bacterial biofilms rather than on a single microorganism, despite relatively few bacterial species are involved in persistent abscesses. *E. faecalis* has the ability to survive in unfavorable conditions. The study by Dewa Ayu et al.³⁶ found the the average amount of *E. faecalis* is almost twice as much in the cases of endodontic infections. Microorganisms after primary endodontic treatment penetrate into the root canal and dental tubulus, what explains average yield relative amount of *E. faecalis* in endodontic retreatments, which are almost seven times higher than in the group of primary endodontic treatment.³⁶ Beside *Enterococcus* spp., in many cases of abscesses we have also identified *Actinomyces* spp., which in terms is also frequently associated with failed endodontic treatment even with the help of resistance to antibiotics.

Our study had some limitations. Despite quite high collection of subjects and samples, main limitation of our study was that only 16 species have been consistently isolated from dentoalveolar abscesses due to the limitations of culture-dependent methods. Approximately 50% of the oral microbiota is still uncultivable, therefore modern methods of identification are necessary, especially at polymicrobial infections.³⁷ Thus, the role of more fastidious or still uncultivable microorganisms in the pathogenesis of primary endodontic infections or persistent dentoalveolar abscesses may have been underestimated. Molecular methods for bacterial identification based on 16S rRNA sequencing represent a valuable tool for both identification of cultivable and uncultivable pathogens and determination of their taxonomic position.³⁸ Direct amplification of 16S rDNA genes from extracted DNA from microorganisms in clinical samples, followed by sequencing of the genes, could allow identification of bacterial communities in their entirety, without the biases of culturing.³⁹ MALDI/TOF, which was used in the

current analysis, is one of the modern technologies in microbiology, but identification still requires the first step of cultivating the clinical samples. Therefore, uncultivable bacteria could still not be identified. According to Unlu et al.⁴⁰ the most common abscess formation sites are mandibular posterior (74.8% of cases), maxillar posterior (9.6%); maxillar anterior (8.9%) and mandibular anterior (6.5%) regions. In our study we have not analyzed the anatomical regions, where abscesses were presented and an evaluation of diagnostic methods was also not included which could identify the anatomic locations of infections. The accuracy of abscess detection in head and neck infections is improved by the combination of clinical examination and contrastenhanced CT.⁴⁰ Another limitation was that our study was designed as a pilot study so we did not test the antibiotic resistance of isolated bacteria. Further study could also cultivate the samples from healthy side of oral cavity from the individuals with dental abscesses and compare the isolated microorganism between the swabs.

Conclusions

The isolated oral microorganisms in our study did not vary with significance compared to healthy oral microbiota, thus commensal microbiota is the main cause of dental abscesses.

Endodontic abscesses rarely cause life-threatening diseases consequently rapid microbiologic identification is not usually necessary. Cultivating and culture testing take time providing results in few days, what is usually too late, and modern methods of microbial identification are expensive.

Most of oral microbiota is uncultivable therefore modern methods of identification are necessary, especially at polymicrobial infections. Further investigations are necessary to estimated more bacterial strains and perhaps to include analysis of bacterial resistance to antibiotics.

Acknowledgements

All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission. No funding was received.

Declaration of Interest

Authors declare no conflict of interest.

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