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Original Article

Commensal mouth bacteria are the main cause of dentoalveolar abscesses in the maxillofacial region

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Abstract

Introduction: The aim of this study was to investigate the bacterial strains that most commonly cause abscesses after failed endodontic treatment.
Methodology: 102 pus samples from dentoalveolar abscesses were examined for bacterial growth. Additionally, 102 samples of healthy gingiva from the same patients were swabbed for comparison of etiology. The swabs were inoculated on blood, chocolate, and Schaedler agar plates; and incubated aerobically and anaerobically. Isolated pathogenic bacteria were compared to healthy oral flora from 50 healthy individuals. Bacterial strains were identified using the matrix assisted laser desorption ionization-time of flight (MALDI-TOF) method and susceptibility was tested using VITEK 2.
Results: The same microorganism was identified from the healthy and abscess side of the oral cavity in 50.0% of the cases. The most commonly identified healthy aerobic flora were coagulase-negative staphylococci, alpha-hemolytic *Streptococcus*, *Enterococcus*, and *Klebsiella* spp. The most identified anaerobes were *Actinomyces*, *Lactobacillus*, and *Bacteroides* spp. Identification of 6 vancomycin-resistant *Enterococcus*, 3 amoxiclav resistant *Actinomyces* spp., 1 extended-spectrum beta-lactamases (ESBL) *E. coli*, and 2 ESBL *Klebsiella* spp. were confirmed. A significant correlation was found between prescription of amoxiclav before surgery and isolation of amoxiclav-resistant *Actinomyces* spp. (p = 0.035).
Conclusions: Common oral flora caused dental abscesses. Not much antimicrobial resistance was detected among the bacterial isolates. However, the dentists used antibiotics irresponsibly because a few cases were identified where the bacteria were resistant to antibiotics used prior to removal of dentoalveolar abscesses.

Key words: mouth; microbiota; abscess, antibiotics; susceptibility.

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Introduction

Acute dentogenic infections are frequent, but underestimated diseases that often require surgical approach. They usually occur secondary to untreated caries, untreated pulp infection, trauma, or failed endodontic treatment. Antibiotic treatment of these infections is usually empirical; but it depends on the patient's comorbid conditions, inadequate choice of antibiotics, and their dosing; and can therefore often lead to a severe septic condition that may threaten the patient's life. Uncontrolled use of antibiotics can lead to antibiotic-resistant microorganisms, an ever-growing phenomenon [1–3].

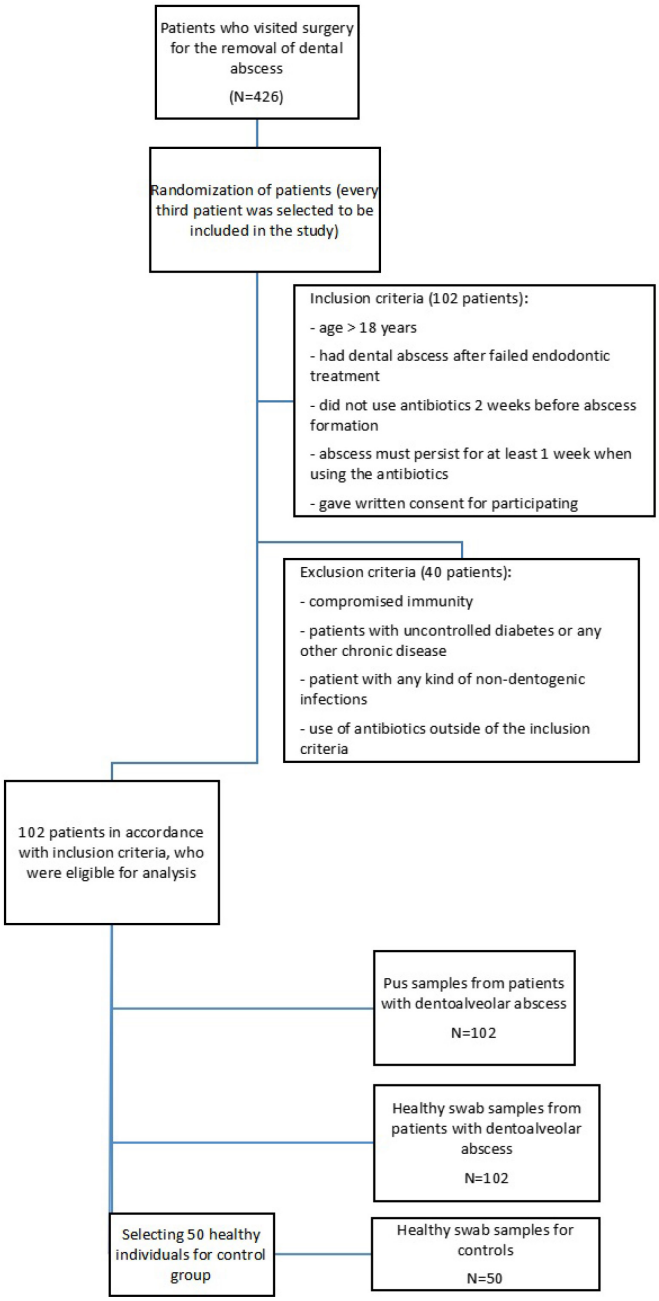
A vast number of microorganisms have been identified as potential pathogens; some of which are relatively new species, uncultivable, or fastidious [3]. Dentoalveolar abscesses are caused mainly by polymicrobial infections, encompassing different bacterial species including facultative anaerobes, strict anaerobes, anaerobic cocci, *Prevotella*, and *Fusobacterium* species [4]. When the infection is not treated early enough it can rapidly spread to areas near anatomic structures, resulting in serious complications such as septicemia, septic shock, brain abscess, cavernous sinus thrombosis, or even death [3].

Dental abscesses form when bacteria and/or their toxic products breach into the periapical tissues throughout the apical foramen and trigger inflammation processes, resulting in the formation of pus as inflammatory defense [2].

The role of antibiotic therapy is to reduce the microbial burden in established cases of infection

where the infection has overcome the local host defense mechanism; and to prevent infection in cases where either the host defense mechanisms are compromised, or infection can lead to greater morbidity. The guidelines for use of antibiotics in dentistry and maxillofacial surgery are less clear; and literature reports conflicting information [5–7]. The selected antibiotic should provide coverage against the microbes which are more frequently identified with surgical procedures and should at the same time cover the narrow spectrum. The ideal antibiotic that is used against oral cavity microorganisms should be effective against streptococci, anaerobic Gram-positive cocci, and anaerobic Gram-negative rods. Moreover, to ensure long-term efficacy the selected antibiotic should have a long half-life. Decision on the type of antibiotic should include cautious consideration of patient's conditions and co-morbidities, the surgical procedure the patient will undergo, and the likelihood of post-surgical infections [5,8]. However, it is important to remember that the long-term use of broad-spectrum antibiotics might result in higher risk of superinfection. Sometimes the microorganism develops antibiotic resistance to several groups of antimicrobial agents — a condition referred to as cross-resistance — and this is a serious problem [9–11].

Figure 1. Workflow of experimental design and patient selection.



General dental and medical practitioners do not always follow appropriate prescribing principles. Kuriyama *et al.* reported extremely successful rates of improvements with surgical drainages of the dentoalveolar infection, accompanied with rational and smart antibiotic prescriptions [12]. The process for guided treatment of a patient with acute dentoalveolar abscess involves surgical drainage, followed by elimination of the factor causing infection [12,13–15].

However, there is still a lack of knowledge on the use of a single or correct antibiotic regimen. Nonetheless, there are several suggested recommendations. Amoxicillin is established as the first choice of antimicrobial therapy. If there is resistance to amoxicillin, then either metronidazole [16] or amoxicillin combined with clavulanic acid [17] is considered as an alternative. Clindamycin is the choice of treatment in patients, who have developed allergy against the penicillin group of antibiotics [18].

The objective of the study was to identify bacterial strains that most commonly cause abscesses after failed endodontic treatment, to determine if common oral flora can also cause large dental abscesses. We isolated the pathogens and tested their antimicrobial susceptibility, to identify the antibiotics which should be chosen as a guideline for preventing postoperative infections and their complications in the oral and maxillofacial region.

Methodology

Study design

The current study was designed as a controlled, case-control observational trial. Selected patients and controls provided written and signed consent for participation in the study. The National Ethics Committee of Kosovo approved the study design. The research was conducted according to the standards of the World Medical Association Declaration of Helsinki.

Maxillofacial surgeries for removing infectious abscess (extraction of teeth and/or abscess incision) were performed at the Maxillofacial Surgery Department of University Clinical Centre of Kosovo (UCCK).

Unbiased randomization of the patients was obtained by randomly choosing the patients from the list of patients who visited the clinic. The inclusion criteria were: age over 18 years, with dentogenic abscess after failed endodontic treatment, patients who underwent maxillofacial surgery for removing infectious abscess, and did not use antibiotics two weeks prior to abscess formation (antibiotics could be used after abscess formation; however, the abscess must have persisted for at least one week when using the antibiotics). The exclusion criteria were patients with compromised immunity, patients with uncontrolled diabetes or any other chronic disease, patients with any kind of non-dentogenic infections, and use of antibiotics outside of the inclusion criteria (Figure 1). Every third patient in the list who fulfilled the inclusion criteria was included in the experiment.

Patients and controls

The study consisted of three groups. The case group consisted of samples from 102 patients with dental abscess; the control group contained the samples from the same patients taken from healthy side of the oral cavity; and the third group was the second control group and included 50 healthy individuals with no abscess or concomitant diseases. Healthy controls from the general population were selected such that basic characteristics of control patients matched with the patients with abscesses.

Samples

Swab samples of dentoalveolar abscesses were collected from 102 adult patients. In addition, 102 swab samples were collected from the healthy side of the different parts of the oral cavity from the same patients and were represented as healthy controls for investigating if healthy oral flora could cause abscesses. Moreover, 50 oral swabs from healthy individuals were taken. These samples represented control samples or the group for comparison between healthy oral flora and pathogens that could cause abscesses. A total of 254 swab samples were collected and sent for bacterial inoculation (Table 1).

During surgical procedure, a swab of pus was taken and stored in sterile transporting medium for bacterial cultivation (Transystem Stuart). Another swab from another part of the healthy oral cavity was simultaneously taken, while making sure that no influence or contamination from the abscess side could occur. The samples from healthy individuals were taken following the same protocol and stored in transporting media. All samples were transported within 48 h after sampling, at ambient temperature, in the transporting medium, in plastic bags, under anaerobic conditions that were ensured by the Anaerocult® system (Merck KGaA, Darmstadt, Germany). After arrival at the laboratory, the samples were inoculated on blood and chocolate agar for aerobes; and anaerobic blood agar plates (SNVS agar, SCS agar from Merck KGaA, Darmstadt, Germany) for anaerobes; and cultivated for bacterial growth.

Table 1. Distribution and anatomical region from which swabs were taken.

	Case patients (N = 102)	Healthy controls (N = 50)
Total samples	204	50
Abscess	102	–
Teeth	26	13
Gingival sulcus	26	13
Tongue	25	12
Cheek mucosa	25	12

The samples were incubated aerobically at 37 °C for 2 days. Meanwhile, inoculated anaerobic plates were incubated under anaerobic conditions (5% CO_2, 10% H_2, and 85% N_2) for 2 days at 37 °C. The anaerobic modified atmosphere was generated by the Anoxomat System™ (MART Microbiology BV, Drachten, Netherlands). If there was no bacterial growth observed after 2 days, the incubation period was prolonged to 1 week.

After incubation, the growth of bacterial colonies on plates were evaluated. Different colonies of aerobic and anaerobic bacteria were first identified by eye, according to the morphological characteristics (shape, color, and thickness of colonies; smell; hemolysis on blood agar plate). After that, single colonies were taken for molecular identification.

Identification of bacterial suspension and growth

Bacterial strain identification was performed by the peptide mass spectrum for identification of bacterial proteins method on an Ultraflex Matrix Assessed Laser Desorption Ionization- Time of Flight/Time of Flight Mass Spectrometer (MALDI-ToF/ToF MS; Bruker Daltonic GmbH, Bremen, Germany). A single bacterial colony was administered by using a plastic loop on a special plate for MALDI/TOF identification. The colony was confluently and equally smeared in the marked circle on the plate. The sample was then overlaid by special matrix. The plate was inserted into the MALDI/TOF identifier and the bacteria or fungi

Table 2. Basic characteristics of subjects included in the analysis.

	Cases (N = 102)	Controls (N = 50)	*p* value
Age (years)	38.0 ± 17.5	36.9 ± 19.9	0.663
Gender			
M/F	56/46	25/25	0.658
Comorbidities	16 (15.7%)	0	0.096
History of gastric cancer	1	/	
History of breast cancer	1	/	
Acute bronchitis	4	/	
Controlled hypertension	8	/	
Controlled diabetes	2	/	
ATB before procedure	30 (29.4%)	/	/

M: male; F: female; ATB: antibiotic therapy.

was identified by the software according to the protein profile.

Antimicrobial susceptibility testing

All isolated bacteria were tested for antibiotic susceptibility using VITEK 2 compact analyzer (BioMerieux, Marcy-l'Étoile, France) with Gram-positive (GP) and Gram-negative (GN) cards. The antibiograms were performed using AST-533, AST-P 534, AST-P 536 cards for Gram-positive; and AST-N 019 and AST-N022 cards for Gram-negative bacteria.

Statistical analyses

Statistical analyses were performed with SPSS version 21 (IBM, Armonk, New York, USA) and Microsoft Excel (Microsoft, Redmond, Washington, USA). Analysis of variance (ANOVA) with series of post-hoc tests were used for the comparison of quantitative variables, and Pearson Chi square test for the comparison of qualitative variables. Statistical significance for all variables was set at $p < 0.05$.

Results

A total of 254 samples from 152 patients were collected (Table 2). The healthy control individuals and the cases had similar basic characteristics. Comorbidities were detected in 16 (15.7%) out of 102 case patients. Two patients had a history of cancer, which proved to be healed with no metastases. Four out of 16 patients had acute bronchitis; however bacterial infections were excluded as the cause of the disease and therefore could not influence the conclusive results of bacterial incubation.

Table 3 represents the list of antibiotics, which were administered as the first therapy by the endodontist after

Table 3. Antibiotics used in the 30 patients who developed dentoalveolar abscesses.

Antibiotics	Number of patients (N = 30)
Amoxicillin	12 (40.0%)
Amoxiclav	13 (43.3%)
Ampicillin	2 (6.7%)
Cefalexin	2 (6.7%)
Amoxiclav, Metronidazole	1 (3.3%)

Table 4. Aerobic and anaerobic bacterial species identified in the swabs taken from the oral cavity of the included patients and controls.

AERO	Cases (N = 102)		Controls (N = 50)
	Abscess samples	Healthy side	Healthy samples
Coagulase-Negative Staphylococci	2	17	20
Alpha-hemolytic *Streptococcus*	10	19	18
***Enterococcus* spp.**	20	20	18
***Klebsiella* spp.**	7	18	14
Streptococcus mitis	3	2	0
Streptococcus mutans	4	4	0
Streptococcus sanguinis	1	1	0
***Streptococcus* spp.**	6	2	2
Escherichia coli	6	2	2
Staphylococcus aureus	0	0	3
Yeast	4	2	3
Polymicrobial infection	13	0	0
Negative	19	4	0
ANR			
***Actinomyces* spp.**	29	18	8
***Bacteroides* spp.**	12	5	6
Fusobacterium nucleatum	3	3	0
***Lactobacillus* spp.**	2	8	10
Prevotella spp.	6	1	0
Negative	41	58	31

AERO: aerobic bacteria; ANR: anaerobic bacteria; yeast: *Candida albicans.* 0: no growth of bacteria was observed even after prolonged cultivation period (1 week); Polymicrobial infection: identification of microbes in polymicrobial sample was impossible, even with molecular methods such as the MALDI/TOF system.

unsuccessful cleaning of root canals. Amoxiclav and amoxicillin were the most frequently prescribed antibiotics after failed endodontic treatment, before the surgical procedure of removing dental abscess.

The bacterial strains were identified by collecting pus material from dentoalveolar abscesses. A total of 254 swab samples were included in the analysis, of which 102 were pus samples from patients with dental abscesses. An additional 102 samples were swabs of healthy gingiva of the same patients, and 50 were swab samples from healthy individuals. Bacterial strains were identified in 92 out of 102 patients with dental abscesses. The list of aerobic and anaerobic bacteria is presented in Table 4. Sixteen different bacterial species were isolated from 102 patients with dentoalveolar abscesses. Only aerobic flora was present in 40 (39.2%) out of the 102 samples; only anaerobic flora was present in 16 (15.7%), and mixed aerobic and anaerobic flora was isolated in 46 (45.1%).

The same microorganism was identified from the healthy side of the oral cavity and from the sample of abscesses in 61 (50.0%) of 102 cases. Only polymicrobial infections could be identified in 13 samples of the cases, and these samples had the same infection agents as identified from the corresponding healthy swabs. Yeasts were identified as the causative microorganisms in 4 cases. Cultures of yeast were microscopically examined with native microscopic slides under an optical microscope, and *Candida albicans* was identified as the only causative pathogenic yeast.

The antibiotics prescribed after surgical removal of dentolaveolar abscess are listed in Table 5. Aerobic *Streptococcus* spp. were 94% sensitive to penicillin and amoxiclav, and 100% for clindamycin. Only 6% were resistant to penicillin. Hundred percent of streptococci were also sensitive to cefotaxime and erythromycin. Penicillin sensitivity was detected in all anaerobic bacteria. Three cases of *Bacteroides* spp. were resistant to penicillin. Most resistance was observed for amoxiclav. Hundred percent of the cases in the *Prevotella* group, were sensitive to penicillin, and 100% were also resistant to amoxicillin with clavulanic acid. Sensitivity to metronidazole and clindamycin was also present in 100% of isolates from this group. All anaerobic bacterial species isolated from odontogenic abscesses were susceptible to moxifloxacin. The identification of 6 vancomycin resistant *Enterococcus* spp. (VRE) and 3 amoxiclav resistant *Actinomyces* spp. should be emphasized. One ESBL *E. coli* and 2 ESBL *Klebsiella* spp were also identified. Another *E. coli* was resistant to ampicillin and amoxiclav. *Bacteriodes* spp. were resistant to penicillin, imipenem/cilastatin, and amoxiclav (Table 6).

Statistically significant correlation between prescribed amoxiclav and isolation of 3 amoxiclav-resistant *Actinomyces* spp. (p = 0.035) was observed. Similarly, there was correlation between prescription of amoxiclav and ampicillin and the resistance to these

Table 5. Antibiotics administered after surgery for the removal of dentoalveolar abscess.

Antibiotics	Number of patients (N = 102)
Amoxiclav, metronidazole	55 (53.9%)
Ampicillin, metronidazole	7 (6.9%)
Cefazoline, metronidazole	12 (11.8%)
Cefazoline, metronidazole, gentamicin	5 (4.9%)
Vancomycin, metronidazole	1 (1.0%)
Vancomycin, metronidazole, cefazoline	1 (1.0%)
Cefalexin, gentamicin	2 (2.0%)
Cefalexin, metronidazole	9 (8.8%)
Imipenem, metronidazole	2 (2.0%)
Ampicillin	5 (4.9%)
Amoxiclav	1 (1.0%)
Cefalexin	2 (2.0%)

Table 6. List of bacteria identified in this study and their resistance to standard antibiotic therapy.

AERO	Abscess samples		
	Resistance to ATB	ANR	Resistance to ATB
***Enterococcus* spp.**	VRE	*Bacteroides* spp.	Penicillin
***Enterococcus* spp.**	VRE		
E. coli	ESBL		
***Enterococcus* spp.**	VRE	*Actinomyces* spp.	Amoxiclav
Alpha-hemolytic *Streptococcus*	Penicillin		
***Streptococcus* spp.**	Penicillin	*Actinomyces* spp.	Amoxiclav
***Enterococcus* spp.**	VRE	*Actinomyces* spp.	Amoxiclav
Alpha-hemolytic *Streptococcus*	Penicillin		
***Enterococcus* spp.**	VRE		
E. coli	Ampicillin, Amoxiclav	*Bacteroides* spp.	Amoxiclav
Yeast; alpha-hemolytic *Streptococcus*	0	*Bacteroides* spp.	Penicillin, Imipenem/Cilastatin
Coagulase-negative staphylococci	0	*Bacteroides* spp.	Imipenem/Cilastatin
***Klebsiella* spp.**	ESBL	*Actinomyces* spp.	Amoxiclav
***Enterococcus* spp.**	VRE	*Actinomyces* spp.	Amoxiclav
***Klebsiella* spp.**	ESBL	*Fusobacterium* spp.	Ampicillin

AERO: aerobic bacteria: ANR: anaerobic bacteria: ATB: antibiotic; ESBL: extended-spectrum beta-lactamase; VRE: vancomycin resistant *Enterococcus* spp. 0: no antibiotic resistance could be established despite repetitive sampling.

antibiotics present in *Bacteroides* spp. and *E. coli*. Two patients were prophylactically unsuccessfully treated with amoxiclav.

Discussion

There are no guidelines for antibiotic use in dentistry, endodontics, and maxillofacial surgery. The current preventive measures followed in endodontics are not able to destroy the remaining bacteria after primary therapy; as a result, complications at the end can lead to the development of periapical lesions, cysts or abscesses, that need surgical removal. Antibiotics are a valuable tool for dentists or oral surgeons for the management of bacterial infections and are usually prescribed without the identification of pathogens. The current study was conducted to identify bacteria from dental abscesses and their antibiotic susceptibility.

The pus samples and the samples from the healthy side of the oral cavity were collected for identification of oral flora which could have caused dentoalveolar abscesses. Antibiotics were prescribed before removal of dentolaveolar abscesses in 30 (29.4%) out of 102 patients. The most commonly prescribed antibiotics were amoxiclav and amoxicillin. These antibiotics are effective against the entire spectra of bacteria and can inhibit the growth of most bacterial strains. Therefore, it is not surprising that the endodontists prescribed them.

Bacterial strains were identified in 92 out of 102 patients with dental abscesses. Polymicrobial infections were observed in some samples and it was not possible to precisely identify the causative pathogen. The MALDI/TOF method was used to identify the pathogens. However, when more than three different, but related, bacterial strains were present, it was almost impossible to identify them at the species level, despite the superiority of the methodology.

A number of different strains of anaerobic and aerobic bacteria were identified in all samples. The ratio between aerobes and anaerobes was nearly 1.5:1. In 61 out of 102 cases, the same microorganism was identified from the healthy side of the oral cavity and from the abscess. These results indicated that bacterial strains did not differ between the healthy side of the oral cavity and the abscesses; thus, confirming that normal oral microbiota can be pathogenic. The most commonly identified healthy aerobic flora were coagulase-negative staphylococci, alpha-hemolytic *Streptococcus*, *Enterococcus* spp., and *Klebsiella* spp. The most identified anaerobes were *Actinomyces* spp., *Lactobacillus* spp., and *Bacteriodes* spp. Thus, the same genera of microbes caused dental abscesses. Samples taken from healthy individuals also contained the same aerobic and anaerobic flora.

The dentoalveolar abscesses in our study were slightly more frequently caused by aerobic than anaerobic bacteria. Only aerobic bacteria were present in 39.2% of cases; only anaerobic bacteria were present in 15.7% of cases; and the majority (45.1%) of cases had mixed aerobic-anaerobic flora. Our results are not comparable to the study that described the proportion of 6% of aerobes, 50% of the anaerobes, and 44% mixed aerobic-anaerobic flora in isolates from 39 patients [19]. In another study that included 52 patients, the authors isolated 154 bacterial pathogens. Out of these, 6% were aerobic, 17% were anaerobic, and 75% were mixed aerobic-anaerobic [20]. In a similar study that included 17 patients, 127 bacterial pathogens were isolated, including 18% aerobic, and 82% anaerobic and mixed aerobic-anaerobic flora [21]. Our results are similar to the study, with regards to the percent of mixed aerobic-anerobic infections. Among aerobic agents, the predominant isolate in previous studies was *Streptococcus* [13]; and among anaerobic infectious agents, the most common isolates were *Fusobacterium* and *Bacteroides* [22,23]. In other reports the oral commensal flora included coagulase-negative staphylococci and Gram-negative cocci (Neisseriaceae, corynebacteria, spirochaetes, lactobacilli, Veillonellaceae, and mycoplasma). The pathogenic spectrum of bacteria in the oral cavity may include *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. pneumoniae*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Neisseria meningitidis*, Enterobacteriaceae, and Actinomycetes [24].

Our analysis also confirmed that there are more aerobic and facultative anaerobic bacteria than strict anaerobes in the healthy oral cavity, compared to the abscesses. Secondly, cultures of aerobic bacteria were more polymicrobial and diverse compared to the abscess samples. The 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. A similar study was performed by Ewringmann on rabbits, and he reported that the most frequently identified anaerobes belonged to the Gram-negative genera *Bacteroides* spp., *Fusobacterium* spp., and *Prevotella* spp.; and Gram-positive non-sporulating cocci *Peptostreptococcus* spp [25]. Among the aerobic bacterial strains, the majority (66.7%) were Gram-negative *Pseudomonas* spp., *Escherichia coli*, and *Pasteurella* spp.; and the remaining 33.3% were Gram-positive genera

Staphylococcus spp. and *Streptococcus* spp. These results are similar to our findings; although we identified additional genera — *E. coli*, *Bacteriodes*, *Prevotella*, and *Fusobacterium*; and we did not isolate *Pasteurella* spp. or *Pseudomonas* spp. The microflora of odontogenic infections typically consists of various strains of facultative and strict anaerobic bacteria, with increased resistance rates against various antibiotics [26]. Among these, strict anaerobes such as Gram-negative rods and Gram-positive cocci are dominant [27].

Most dentoalveolar abscesses develop as a consequence of increase in normal oral commensal bacteria which convert into opportunistic pathogens due to changes in the conditions of the oral cavity. As the number of bacteria grow, at some point it exceeds the minimum infective number, and thus causes a dentoalveolar infection [15]. Dentoalveolar infections of the periapical tissue are mostly caused by strict anaerobic Gram-positive cocci or Gram-negative rods, but are also often mixed with facultative anaerobes [13,15,28]. Most commonly identified bacterial strains in our study were *Enterococcus* spp. *Enterococcus faecalis* is a bacterial pathogen that has been found in various post-treatment (either endodontics or surgical) diseases [29,30]. The results of the current study are similar to the recent studies that concluded that *E. faecalis* is the main pathogen [29,30]. Although a single bacterial species can be a major pathogen in post-treatment diseases, tissue destruction is a consequence of not one but rather the synergistic activity of the group of bacteria that trigger host immune response [31,32]. *Enterococcus* spp. can form a bacterial biofilm — a complex of bacterial cells attached together in an extracellular matrix and glued to the surface. The microorganisms living in a biofilm interact with each other and represent a synergistic community where they organize, act, and respond as a whole [31–33]. Moreover, biofilms tend to consist of more potentially pathogenic bacterial groups, rather than just one species. Besides *Enterococcus* spp., we have also identified *Actinomyces* spp in many abscesses. We can conclude that dentolaveolar abscesses associated with failed endodontic treatment may have harbored various microorganisms, including *Actinomyces* species. However, the prevalence of infectious *Prevotella* spp. was low in our study.

Antibiotics may interfere with the balance of the normal oral flora and underlying resistant microorganisms. They may influence the cell adherence and decrease the amount of normal oral microflora, allowing resistant bacteria to outgrow the normal commensals. This is how the vancomycin resistant *Enterococci* (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), and multiple drug-resistant *Mycobacterium tuberculosis* have emerged [34]. These super-resistant strains can spread rapidly across the patient community, and there is no effective treatment available [24,35–38]. Frequent and irresponsible prescription of antibiotics, due to empirical and not rational reasons, has resulted in the creation of these super-resistant bacteria [15,35,36]. The spectrum of activity of clindamycin covers a range of microorganisms and most of those found in acute dentoalveolar infections, including those that have resistance to penicillin [28,37,39–41]. Clindamycin is one of the more appropriate antibiotic choices to manage acute dentoalveolar abscesses, as it has a wide spectrum of activity, is absorbed orally, and has good bone penetration [39–41].

The most prescribed antibiotic was amoxiclav (amoxicillin + clavulanic acid) in 55 (53.9%) cases. Despite testing a substantial number of samples, we observed a low percent of resistant bacteria. Among the antibiotic-resistant bacteria, there were 6 VRE, 3 amoxiclav-resistant *Actinomyces* spp., 1 ESBL *E. coli*, and 2 ESBL *Klebsiella* spp. Another *E. coli* was resistant to amoxiclav and ampicillin. The presence of resistance in aerobic bacteria is a cause of concern. According to guidelines from the College of General Dentistry and Faculty of Dental Surgery (FDS) of the Royal College of Surgeons of England, the first choice of treatment of dental abscess is still penicillin; and in patients who are allergic to penicillin, it is clindamycin [37]. The growing trend in bacterial resistance against penicillin, which is the most commonly used antibiotic, is mainly due to the production of beta-lactamase. In our study 3 cases of aerobic and 2 cases of anaerobic bacteria were resistant to penicillin. A study from the UK found that 9% of isolated *Streptococcus mitis* were resistant to penicillin [42].

Metronidazole is an antibiotic of choice in anaerobic infections caused by Gram-negative anaerobes; however, it is unreliable in the case of Gram-positive anaerobes and aerobic bacteria [43]. In our study, there was no resistance to metronidazole and moxifloxacin in any anaerobe. The antibiotic sensitivity of bacteria in our study does not match with the results from Germany [44], where sensitivity to penicillin in aerobic and anaerobic agents varied from 61–79%. We observed much higher sensitivity; however, our findings were consistent with the susceptibility to moxifloxacin reported in their study. The authors

reported that the sensitivity of aerobic and facultative anaerobic bacteria was 99%, and of anaerobic bacteria was 96%. Based on our results, moxifloxacin can be an appropriate choice of treatment because aerobic and anaerobic bacteria showed good sensitivity for moxifloxacin in vitro; but additional clinical research on its effectiveness in the treatment of odontogenic abscesses is necessary.

All amoxiclav-resistant *Actinomyces* spp. were found in patients treated with amoxiclav. Similar results were observed for *Bacteroides* spp. and *E. coli*, which were both resistant to amoxiclav and ampicillin, and the 2 patients were treated with amoxiclav before.

Based on the observations on antibiotic susceptibility, our study confirmed that identifying variations in bacterial sensitivity to antibiotics should be practiced for the rational use of antibiotics as prophylaxis of infections in the maxillofacial region. Substantial differences in susceptibility to antibiotics were found due to the diversity of the identified bacteria. Therefore, bacterial identification and antibiotic susceptibility tests are recommended to ensure effective post-treatment of odontogenic abscesses without complications.

Despite the diverse collection of subjects and samples in our study, the main limitation was that only 16 species were consistently isolated from the dentoalveolar abscesses. Conventional bacterial culture methods do not ensure the identification of approximately 50% of the oral microbiota, as these bacteria cannot be cultured [45]. Novel molecular methods for bacterial identification, such as 16S rRNA sequencing or mass spectrometry, may be used in the future for the identification of cultivable and uncultivable microorganisms [46,47].

Conclusions

There was no significant difference in the occurrence of 16 microorganisms in the case samples and in the healthy oral microbiota; thus, oral microbiota is the main cause of dental abscesses. Endodontic abscesses rarely result in life-threatening diseases and rapid microbial identification is not usually needed. Bacterial culture and test for antibiotic susceptibility take time and yield results in a few days. Therefore, antibiotics are prescribed empirically, without testing. Moreover, the indications of antibiotics have not been established. Oral surgeons prescribe amoxiclav because it theoretically works against a wide range of bacterial strains. However, due to limited knowledge about the pathogens and their roles in post-treatment infections, the usage of other antibiotics is lacking.

Although the prescribed antibiotic should be effective against the exact bacterial species responsible for the infection, dental abscesses are polymicrobial. In this aspect perhaps it would be more appropriate to administer an antibiotic that is effective against a wider spectrum of bacteria than to administer one that is only effective against one species. In such cases, even if the primary causative bacterial species is resistant to the selected antibiotic, the secondary species might be susceptible. In this study, in a few cases bacteria were resistant to antibiotics that were used prior to removal of dentoalveolar abscesses; indicating that the dentists used the antibiotics irresponsibly. However, further research is necessary to clarify the suitability of antibiotics in order to prevent oral cavity post-treatment infections.

Authors' contributions

Study conception and experiment design: SR, MS, SS, DS, NH; data acquisition: SR, DS, TJ; data analysis and interpretation: SR, MS, SS, NH, DPJ, AS, DS, TJ; statistical analysis: SR, DS, TJ; manuscript draft: SR, DS, TJ; manuscript critical revision for important intellectual content: MS, SS, NH, DPJ, AS; supervision: NH.

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Conflict of interests

No conflict of interests is declared.

References

1. Del Fabbro M, Corbella S, Sequeira-Byron P, Tsesis I, Rosen E, Lolato A, Taschieri S (2016) Endodontic procedures for retreatment of periapical lesions. Cochrane Database Syst Rev 10: CD005511. doi: 10.1002/14651858.CD005511.pub3.
2. Bertossi D, Barone A, Iurlaro A, Marconcini S, De Santis D, Finotti M, Procacci P (2017) Odontogenic orofacial infections. J Craniofac Surg 28: 197–202. doi: 10.1097/SCS.0000000000003250.
3. Shweta, Prakash SK (2013) Dental abscess: a microbiological review. Dent Res J (Isfahan): 10: 585–591.
4. Nair PN (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 15: 348–381. doi: 10.1177/154411130401500604.
5. Beltrami EM, Williams IT, Shapiro CN, Chamberland ME (2000) Risk and management of blood-borne infections in health care worker. Clin Microbiol Rev 13: 385–407. doi: 10.1128/CMR.13.3.385.
6. Lovato C, Wagner JD (2009) Infection rates following perioperative prophylactic antibiotics versus postoperative extended regimen prophylactic antibiotics in surgical

management of mandibular fractures. J Oral Maxillofac Surg 67: 827. doi: 10.1016/j.joms.2008.06.093.
7. Efron PA, Mazuski JE (2009) *Clostridium difficile* colitis. Surg Clin North Am 89: 483. doi: 10.1016/j.suc.2008.09.014.
8. Chaweewannakom U, Turajane T, Wongsarat T, Larbpaiboonpong V, Wongbunnak R, Sumetpimolchai W (2012) Cost analysis of peri-operative antibiotic administration in total knee arthroplasty. J Med Assoc Thai 95 Suppl 10: S42–47.
9. Kicinska AM, Lichodziejewska-Niemierko M, Bronk M, Sledzinska A, Samet A, Rutkowski B (2012) Sensitivity for antibiotics among the *E. coli* strains isolated from patients with different clinical stage of infection. Przegl Lek 69: 217–221. [Article in Polish].
10. Warnke PH, Lott AJS, Sherry E, Wiltfang J, Podschun R (2013) The ongoing battle against multi-resistant strains: in-vitro inhibition of hospital-acquired MRSA, VRE, *Pseudomonas*, ESBL *E. coli* and *Klebsiella* species in the presence of plant-derived antiseptic oils. J Craniomaxillofac Surg 41: 321–326. doi: 10.1016/j.jcms.2012.10.012.
11. Baumgartner JC, Xia T (2003) Antibiotic susceptibility of bacteria associated with endodontic abscesses. J Endod 29: 44–47. doi: 10.1097/00004770-200301000-00012.
12. Kuriyama T, Absi EG, Williams DW, Lewis MA (2005) An outcome audit of the treatment of acute dentoalveolar infection: impact of penicillin resistance. Br Dent J 198: 759–763. doi: 10.1038/sj.bdj.4812415.
13. Stefanopoulos PK, Kolokotronis AE (2004) The significance of anaerobic bacteria in acute orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 98: 398–408. doi: 10.1016/j.tripleo.2004.01.016.
14. Palmer NA (2003) Revisiting the role of dentists in prescribing antibiotics. Dent Update 30: 570–574. doi: 10.12968/denu.2003.30.10.570.
15. Dirks SJ, Terezhalmy GT (2004) The patient with an odontogenic infection. Quintessence Int 35: 482–502.
16. Roche Y, Yoshimori RN (1997) In-vitro activity of spiramycin and metronidazole alone or in combination against clinical isolates from odontogenic abscesses. J Antimicrob Chemother 40: 353–357. doi: 10.1093/jac/40.3.353.
17. Lewis MA, Carmichael F, MacFarlane TW, Milligan SG (1993) A randomized trial of co-amoxiclav (Augmentin) versus penicillin V in the treatment of acute dentoalveolar abscess. Br Dent J 175: 169–174. doi: 10.1038/sj.bdj.4808263.
18. Mangundjaja S, Hardjawinata K (1990) Clindamycin versus ampicillin in the treatment of odontogenic infections. Clin Ther 12: 242–249.
19. Brook I, Frazier EH, Gher ME (1991) Aerobic and anaerobic microbiology of periapical abscess. Oral Microbiol Immunol 6: 123–125. doi: 10.1111/j.1399-302X.1991.tb00464.x.
20. Goumas PD, Naxakis SS, Papavasiliou DA, Moschovakis ED, Tsintos SJ, Skoutelis A (1997) Periapical abscesses: causal bacteria and antibiotic sensitivity. J Chemother 9: 415–419. doi: 10.1179/joc.1997.9.6.415.
21. Khemaleelakul S, Baumgartner C, Pruksakorn S (2002) Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 94: 746–755. doi: 10.1067/moe.2002.129535.
22. Kuriyama T, Karasawa T, Nakagawa K, Yamamoto E, Nakamura S (2001) Incidence of beta-lactamase production and antimicrobial susceptability of anaerobic Gram-negative rods isolated from pus specimens of orofacial odontogenic infections. Oral Microbiol Immunol 16: 10–15. doi: 10.1034/j.1399-302x.2001.160102.x.
23. Facklam R (2002) What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev 15: 613–630. doi: 10.1128/CMR.15.4.613-630.2002.
24. Sweeney LC, Dave J, Chambers PA, Heritage J (2004) Antibiotic resistance in general dental practice - a cause for concern? J Antimicrob Chemother 53: 567–576. doi: 10.1093/jac/dkh137.
25. Ewringmann A (2017) Microbial flora and antibiotic susceptibility of purulent tooth diseases in rabbits. Tierarztl Prax Ausg K Kleintiere Heimtiere 45. [Article in German]. doi: 10.15654/TPK-170125.
26. Eckert AW, Just L, Wilhelms D, Schubert J (2012) Dentogenic infections - part I: the significance of bacterial isolation of dentogenic infections under routineous conditions. Wien Med Wochenschr 162: 316–320. [Article in German]. doi: 10.1007/s10354-012-0103-2.
27. Ogle OE (2017) Odontogenic infections. Dent Clin North Am 61: 235–252. doi: 10.1016/j.cden.2016.11.004.
28. Sandor GK, Low DE, Judd PL, Davidson RJ (1999) Antimicrobial treatment options in the management of odontogenic infections. J Can Dent Assoc 65: 508–514.
29. Kaufman B, Spangberg L, Barry J, Fouad AF (2005) *Enterococcus* spp.in endodontically treated teeth with and without periradicular lesions. J Endod 31: 851–856. doi: 10.1097/01.don.0000164133.04548.26.
30. Zoletti GO, Siqueira JF, Jr, Santos KR (2006) Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular lesions by culture-dependent and -independent approaches. J Endod 32: 722–726. doi: 10.1016/j.joen.2006.02.001.
31. Wang J, Chen W, Jiang Y, Liang J (2015) Imaging of extraradicular biofilm using combined scanning electron microscopy and stereomicroscopy. Microsc Res Tech 78: 947. doi: 10.1002/jemt.22555.
32. Ricucci D, Siqueira JF Jr (2010) Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 36: 1277–1288. doi: 10.1016/j.joen.2010.04.007.
33. Ricucci D, Candeiro GT, Bugea C, Siqueira JF Jr (2016) Complex apical intraradicular infection and extraradicular mineralized biofilms as the cause of wet canals and treatment failure: report of 2 cases. J Endod 42: 509–515. doi: 10.1016/j.joen.2015.12.014.
34. Pallasch TJ (2000) Global antibiotic resistance and its impact on the dental community. J Calif Dent Assoc 28: 215–233. doi: 10.1080/19424396.2000.12223071.
35. Flynn TR, Shanti RM, Hayes C (2006) Severe odontogenic infections, part 2: prospective outcomes study. J Oral Maxillofac Surg 64: 1104–1113. doi: 10.1016/j.joms.2006.03.031.
36. Lewis MAO (2008) Why we must reduce dental prescription of antibiotics: European Union Antibiotic Awareness Day. Br Dent J 205: 537–538. doi: 10.1038/sj.bdj.2008.984.
37. Addy LD, Martin MV (2005) Clindamycin and dentistry. Br Dent J 199: 23–26. doi: 10.1038/sj.bdj.4812535.
38. Stefanopoulos PK, Kolokotronis AE (2006) Controversies in antibiotic choices for odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: 697–698. doi: 10.1016/j.tripleo.2005.10.065.

39. Brook I, Lewis MA, Sandor GK, Jeffcoat M, Samaranayake LP, Vera Rojas J (2005) Clindamycin in dentistry: more than just effective prophylaxis for endocarditis? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100: 550–558. doi: 10.1016/j.tripleo.2005.02.086.
40. Zetner K, Schmidt H, Pfeiffer S (2003) Concentrations of clindamycin in the mandibular bone of companion animals. Int J Antimicrob Agents 4: 166–171.
41. Kirkwood KL (2003) Update on antibiotics used to treat orofacial infections. Alpha Omega 96: 28–34.
42. Warnke PH, Becker ST, Springer IN, Haerle F, Ullmann U, Russo PA, Wiltfang J, Fickenscher H, Schubertal S (2008) Penicillin compared with other advanced broad-spectrum antibiotics regarding antibacterial activity against oral pathogens isolated from odontogenic abscesses. Craniomaxillofac Surg 36: 462–467. doi: 10.1016/j.jcms.2008.07.001.
43. Paster BJ, Olsen I, Aas JA, Dewhirst FE (2006) The breadth of bacterial diversity in the human periodontal pocket and other oral sites. Periodontol 2000 42: 80–87. doi: 10.1111/j.1600-0757.2006.00174.x.
44. Tzanetakis GN, Azcarate-Peril MA, Zachaki S, Panopoulos P, Kontakiotis EG, Madianos PN, Divaris K (2015) Comparison of bacterial community composition of primary and persistent endodontic infections using pyrosequencing. J Endod 41: 1226–1233. doi: 10.1016/j.joen.2015.03.010.
45. Siqueira, JF, Roças IN, Oliveira JCM, Santos KRN (2001) Detection of putative oral pathogens in acute periradicular abscesses by 16S rDNA-direct polymerase chain reaction. J Endod 27: 164–167. doi: 10.1097/00004770-200103000-00004.
46. Clarridge JE 3rd (2004) Impact of 16S rRNA gene sequence analysis for identification of bacterial on clinical microbiology and infectious diseases. Clin Microbiol Rev 17: 840–862. doi: 10.1128/CMR.17.4.840-862.2004.
47. Munson MA, Pitt-Ford T, Chong B, Weightman A, Wade WG (2002) Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 81: 761–766. doi: 10.1177/0810761.