A metagenomic study of patients with alveolar osteitis after tooth extraction. A preliminary case-control study.

Aguilar-Durán, Laura[1]
Figueiredo, Rui[2]
Seminago, Ramón[3]
Roig, Francisco J[4]
Llorens, Carlos[5]
Valmaseda-Castellón, Eduard[6]

(1) DDS, Master of Oral Surgery and Implantology. Faculty of Dentistry – University of Barcelona. Spain.
(2) DDS, MS, PhD, Master of Oral Surgery and Implantology. Associate professor of Oral Surgery and Professor of the Master's degree program in Oral Surgery and Implantology. Faculty of Dentistry – University of Barcelona. Researcher at the IDIBELL institute. Spain.
(3) BSc Biology. Genomics Unit, CCiTUB – University of Barcelona. Spain.
(4) PhD. Biotechvana. Associate Bioinformatician of the Aquatic Pathogens with Public Health Impact Research Line at ERI BioTecMed – University of Valencia. Spain.
(5) PhD. Biotechvana. Associate Bioinformatician, CCiT – University of Barcelona. Spain.
(6) DDS, MS, PhD. Professor of Oral Surgery. Director of the Master's degree program in Oral Surgery and Implantology, Faculty of Dentistry – University of Barcelona. Researcher at the IDIBELL institute. Spain.

Short title: Microbiota of alveolar osteitis patients.

Key Words: Microbiota, molecular sequencing data, dry socket, tooth extraction, bacteria, microbiology.

Corresponding author:
Dr. Rui Figueiredo

Summary word count: 250
Total word count (Abstract to Acknowledgments): 2855
Total number of tables/figures: 3 tables, 2 figures
Number of references: 34

ABSTRACT

Objective: To identify the microbiome in sockets with alveolar osteitis and compare it with a control group using metagenomic techniques.

Materials and methods: A case-control study was conducted in subjects that had undergone a tooth extraction. Microbiological samples were taken from the sockets of 10 patients with dry socket after tooth extraction (AO group) and 10 patients in whom exodontia resulted in no postoperative complications (Control group). Bacterial DNA was isolated and the 16S rRNA gene was amplified and sequenced. Multiplexed tag-encoded sequencing of DNA from the samples was performed and the reads were processed by Metagenomic Rapid Annotation.

Results: A total of 151 different species were found: 55 bacteria were only found in the AO group, 51 were specific to the control group and 45 were common to both groups. The most frequently found genera in both groups was *Prevotella. Prevotella nanceiensis*, *Actinomyces odontolyticus*, *Treponema maltophilum*, *Veillonella dispar, Tannerella forsythia* and *Leuconostoc mesenteroides* were found in several patients with alveolar osteitis, with an abundance greater than 0.5%, and were absent in all the control group samples.

Conclusions: Patients who develop alveolar osteitis after dental extractions might have a different microbiota from that of patients without postoperative complications. Since this is a preliminary report, further research is needed to assess whether bacteria play an important role in the etiology of dry socket.

Clinical Relevance: This study seems to indicate that bacteria may play an important role in the alveolar osteitis etiology. Thus, new prevention and treatment strategies should be considered.

ACKNOWLEDGEMENTS

The authors would like to thank Mary Georgina Hardinge for English language editing of the manuscript and to Dr. Carlos Noguera for help with collecting the samples.

INTRODUCTION

Dry socket or alveolar osteitis is a common complication of dental extractions, with an estimated prevalence of around 0.5-5.0% [1]. However, some authors have found higher incidences after the surgical extraction of lower third molars [2-3]. This entity is characterized by an acute, severe pain, with no associated signs of infection or inflammation, starting 2 to 4 days after tooth extraction [2]. Several risk factors have been related to a higher incidence of this complication, including smoking, mandibular teeth, female gender, the use of oral contraceptives, and inadequate intraoperative irrigation, among others [1,2,4].

Although this postoperative complication has been extensively addressed in the literature, the etiology remains unclear. Most authors support the view that alveolar osteitis is associated with a fibrinolytic process that leaves the bony walls of the socket exposed to the oral cavity. This fibrinolytic activity has been linked to bacteria involved in periodontal diseases, such as *Treponema denticola* [5,6], and in pericoronitis, such as *Streptococcus spp.*, *Prevotella*, *Bacteroides* and *Peptostreptococcus* [7,8]. Peñarrocha et al.[9] found a significant increase in postoperative pain and alveolar osteitis after dental extractions in patients with poor oral hygiene and previous plaque accumulation. Several studies have also shown that both topical and systemic applications of antibiotics and antiseptics can significantly reduce the occurrence of this complication [2,3,10-13]. These findings suggest that microorganisms may play an active role in the occurrence of alveolar osteitis.

To date, more than 700 bacterial taxa have been detected in the oral cavity, many of which cannot be isolated by common culture methods [14]. Several methods have been used to analyse the composition of the oral microbiome, including microscopy, cultural analysis, enzymatic assays and immunoassays [15], but a substantial number of

microorganisms may be overlooked. Recent techniques, such as pyrosequencing, analyze the microbiome according to the community profile of the 16S ribosomal RNA gene (16S rRNA). It can be extracted from heterogeneous samples, amplified and sequenced [16]. This method can detect most species and identify bacteria that cannot be cultivated with standard techniques.

The hypothesis of the present study was that different microbiota are found in postextraction sockets with and without alveolar osteitis. Therefore, the aim of this study was to compare the microbiome in sockets with and without alveolar osteitis, using metagenomic techniques.

METHODS

Subject recruitment

A preliminary case-control study was conducted in subjects who underwent tooth extraction through the Oral Surgery and Implantology Master's degree program of the University of Barcelona. A total sample size of 20 patients was considered sufficient to assess if it would be feasible to perform a larger study. The patients were divided into 2 groups. The Alveolar Osteitis group (AO) comprised tooth extraction patients that presented moderate to severe postoperative pain (score of >40) measured on a 100-mm Visual Analogue Scale (VAS) with onset at least 48 hours after the surgical procedure. These cases had an empty socket and presented no apparent signs of infection (no suppuration). The Control Group (CG) was composed of patients that had undergone tooth removal with no postoperative complications (ratio 1:1). The time between tooth extraction and sample collection was similar in both groups since this variable was used as a criterion for matching cases to controls. The STROBE guidelines have been followed in reporting this study.

All the individuals included met the following criteria: (1) age between 18 and 90 years, (2) American Society of Anesthesiologists (ASA) health status score [17] not higher than 3, (3) patients who underwent tooth extractions. The protocol was approved by the institutional review board (Comitè Ètic d'Investigació Clínica, Hospital Odontològic de la Universitat de Barcelona; Protocol number 02/15) of the University of Barcelona Dental Hospital and complied with the Helsinki declaration guidelines for clinical research. All the patients gave signed informed consent to participate in the study.

Patients were excluded in the following situations: presence of purulent drainage or inflammation in the socket or use of an antibiotic or antiseptic mouthrinse (bisbiguanides, quarternary ammonium salts and essential oils) shortly before sample collection.

Data sampling

A single researcher (LAD) recruited the patients and examined all the clinical records. The data retrieved were age, gender, patient health status based on the ASA Physical Status Classification System [17], current medication, smoking habit (number of cigarettes/day), periodontal disease, history of infection or pericoronitis, surgical variables (date of extraction, local anesthetic, flap design, bone removal, tooth sectioning, suture and surgeon's experience), antibiotic and antiseptic administration and intraoperative complications.

Sample collection and DNA Isolation

The samples were collected by placing 3 sterile paper points inside the socket for 10 seconds. The samples were then placed in sterile snap-cap tubes and refrigerated at -40ºC until they were shipped for analysis.

In the laboratory, the samples were stored in a 1.5-mL microcentrifuge tube and frozen at -80ºC until further analysis. To release the bacteria, a phosphate-buffered saline (PBS) was used and the samples were vortexed for 5 minutes. The total DNA was purified with the QiAmp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for buccal swabs. The amount of DNA extracted was calculated using a Quibit system (Thermo Fisher Scientific, Waltham, MA USA).

PCR Amplification, Pyrosequencing and Bioinformatic Analyses

Variable regions V1 to V5 of the 16S rRNA genes were amplified using Fast Start High Fidelity PCR Systems (Roche, Mannheim, Germany) and sequenced with the GS Junior Titanium Sequencing kit (Roche, Mannheim, Germany).

Polymerase chain reaction (PCR) reactions for V1-V3 and V5-V3 primers were set up with annealing temperatures of 56ºC and 50ºC respectively. The PCRs were replicated and pooled for each sample. The amplicon library was cleaned with Agencourt AmPure Beads (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions, the concentration of amplicon was estimated using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA), and the size of the amplicon was analyzed using the Agilent 2100 Bioanalyzer with the Agilent DNA 7500 Kit (Agilent, Santa Clara, California, USA).

Multiplexed tag-encoded sequencing of DNA from the samples was performed on the GS Junior platform (Roche Applied Science, Indianapolis, IN, USA).

The primers used to amplify the 16SrRNA genes and to introduce Multiplex Identifiers (MIDs) to identify amplicons or samples are available on the NIH Human Microbiome Project website [18]. The resulting fast files were pre-processed with the Prinseq tool [19]

by size (over 50bp), quality (minimum quality 30), and N content (rejecting reads with over 5% of Ns and removing terminal Ns).

Taxonomic assignment

The processed reads of the 20 samples were uploaded to MG-RAST (Metagenomic Rapid Annotation using Subsystems Technology) [20] server annotations based on hierarchical classification with RDP (Ribosomal Database Project Release 11). The number of uploaded sequences ranged from 10842 to 17475 for AO and from 16740 to 36112 for CG, with a mean size of 200nt and 341nt respectively. MG-RAST default clustering parameters within the BLAT algorithm were used. Each read was taxonomically assigned down to the genus and species level with an 80% confidence threshold. Reads giving no bacterial hits were excluded. Artificial replicate sequences produced by sequencing artifacts were removed [21].

To estimate bacterial diversity, the number of operational taxonomic units (OTUs) in the samples was determined and a rarefaction analysis was performed. Rarefaction curves were obtained by plotting the number of observed OTUs against the number of sequences, using the MG-RAST platform [20] and the RDP database [22].

The total diversity was estimated, clustering sequences at 98% nucleotide identity over a 90% sequence alignment length, and rarefaction curves were obtained using the Gpro StaTool package [23] (Fig1a).

Venn analysis (Fig1b) and Principal Component Analysis (PCA) and heatmaps were generated using the GPRO StaTool [23]. The Venn analysis was run on the taxonomic diversity data. The PCA analysis was run on the taxonomic diversity and abundance of each individual sample and on the average of each group (AO and CG) (Fig1c).

RESULTS

Demographic, clinical and surgical characteristics of the subjects

Twenty patients were included in the study. Ten belonged to AO and ten to CG. The groups were similar with regard to age, gender and smoking habits (Table 1). No statistically significant differences were found in any of the other variables presented in Table 1, although patients with previous pericoronitis were more common in the AO group.

Microbial sequencing results

The quality of the reads from the sequencing process was evaluated by FastQC pipelines. All the samples presented an excellent average quality (PHRED values over 28). The non-assigned reads (non 16SrRNA or sequence artifacts) varied between samples, ranging from 1.68% to 16.38%. The samples used in this study were deposited in the SRA database of GenBank (https://www.ncbi.nlm.nih.gov/sra) under accession numbers SAMN09288233-SAMN09288252.

Oral microbial community

The rarefaction curves for sample size and total taxa identified show that all the taxonomic richness was accounted for in these metagenomes.

The taxonomic results showed 151 different species: 55 species were only found in the AO, 51 bacteria were specific to the CG and 44 were common to both groups (Fig1b, 1d, 1e and Tables 2 and 3).

The genus *Prevotella* was the most commonly identified in all the individuals studied.

Alveolar osteitis sites were colonized mainly by *Prevotella* (22%), *Fusobacterium* (7,6%), and *Porphyromonas* (5,8%), whereas the main bacterial groups in the control group were *Prevotella* (18%), *Capnocytophaga* (8%), *Streptococcus* (6%) and *Porphyromonas* (5%). All the bacterial species found can be observed in Tables 2 and 3.

The following bacterial species were identified exclusively in alveolar osteitis patient samples (AO group) and had a relative abundance of ≥0.5%: *P. nanceiensis* (2 out of 10 AO samples), *A. odontolyticus* (2 out of 10 AO samples), *T. maltophilum* (2 out of 10 AO samples), *V. dispar* (4 out of 10 AO samples), *T. forsythia* (2 out of 10 AO samples) and *L. mesenteroides* (3 out of 10 AO samples).

Although found in both groups (Table 3), the following pathogens were overrepresentated in the AO group: *Prevotella intermedia* (2% AO vs 0.04% CG), *Prevotella melaninogenica* (4% AO vs 0.09% CG), *Parviromas micra* (3% AO vs 0.4% CG) and *Fusobacterium nucleatum* (4% AO vs 0.5% CG). Also, *Porphyromonas gingivalis, T. forsythia* and a newly proposed periodontal pathogen, *T. maltophilum,* were only found in the AO group. Pathogens involved in pericoronitis, such as *Streptococcus* spp. and *Peptostreptococcus anaerobius,* were found in both groups but were more numerous in the AO group (0.8% vs 0.09% and 0.3% vs <0.09% respectively).

Of the 151 bacteria identified, 68 were not found in the Human Oral Microbiome Database [14] or the CORE Microbiome [24]. Most of these 68 belonged to the AO group.

DISCUSSION

The present study has shown the wide range and differing composition of the microbiota present in sockets with and without alveolar osteitis. A total of 151 different species were identified but only 45 were common to both groups. These findings might indicate that bacteria play an important role in the etiology of dry socket.

One of the main limitations of this study is the small sample size, which may jeopardize generalization of the outcomes. However, it must be taken into account that no data on studies of this topic using metagenomic techniques have been published. Thus, this paper adds new information to the literature on the microbiota of sockets with and without alveolar osteitis. Furthermore, the outcomes presented indicate that further large sample studies are needed to clarify the etiology of this complication. Next generation sequencing (NGS) of 16S rRNA has made it possible to identify a huge number of bacteria that may remain unnoticed if other common techniques are used. Standard culture media for bacteria were probably the first method that provided valuable data regarding the bacteria present in oral infections [25]. However, this technique is time-consuming and does not identify all the microorganisms present. Molecular methods such as polymerase chain reaction (PCR) using species specific oligonucleotides and DNA-DNA hybridization have also been widely employed in oral infections, but again do not provide an accurate view of the microbial community. PCR offers great sensitivity and only requires a small amount of DNA of the microbial sample [25], but its ability to discriminate and identify is limited to the oligonucleotides that have been selected, and therefore it neglects an important number of bacteria, especially those that have not been previously linked to the infection studied. NGS has significantly increased the diversity of oral human microbiome identification. Indeed, a recent report published by the authors' department identified 19 species in oral biofilm samples collected from implant abutments

[26] that were not currently in the Human Oral Microbiome Database (HOMD) [14] or the CORE Microbiome database [24].

Alveolar osteitis is a painful complication that occurs frequently after dental extractions. Several reports have linked this disorder to risk factors like smoking [27], gender (women) [27], use of oral contraceptives [27], age [28], difficulty of the extraction [28] and previous history of infection [28]. Regarding prevention, the use of chlorhexidine and systemic antibiotics seems to be effective in reducing the incidence of alveolar osteitis [29-31].

Although many papers have been published on this topic, the etiology of dry socket remains unclear. Most authors agree, based on the clinical features of this complication (i.e. presence of an empty socket, pain onset between 48 and 96 hours after surgery and absence of inflammatory signs), that the blood clot either fails to form, or that it is subsequently lysed [6]. However, taking into consideration that antibiotics and antiseptics seem to reduce the incidence of alveolar osteitis and that most of the above-mentioned risk factors (smoking, age, previous history of infection, difficulty of the extraction) are also associated with increased rates of postoperative infections, it may be hypothesized that bacteria play an important role in the etiology of this disorder. Indeed, according to Serrati et al. [32], bacteria or debris might stimulate monocytes/macrophages to release cytokines which can provoke an up-regulation of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) that will lead to clot lysis. The results of the present study seem to support the view that this alteration might have an infectious background, since the microbiota found in the two groups were quite different. Due to the study design (small sample and metagenomic techniques), it is difficult to assess which bacteria are more likely to be linked to alveolar osteitis. However, the following species, which were found with an abundance of >0.5% in 2 or more AO group patients (and were

absent in the control group), are more likely to be associated with this complication: *P. nanceiensis*, *A. odontolyticus*, *T. maltophilum*, *V. dispar, T. forsythia* and *L. mesenteroides*. *P. intermedia, P. melaninogenica*, *P. micra* (3% AO vs 0.4% CG) and *F. nucleatum* might also be involved, since they were over-represented in the dry socket samples. *L. mesenteroides* may be an important microrganism in this complication, since it was found in 30% of the AO samples and this species has been used in the pharmaceutical and chemical industries as an anticoagulant to prevent the formation of blood clots [33-34]. Moreover, this bacteria was not present in the CG.

These outcomes indicate the need for additional research on the role of bacteria in such a clinically-relevant topic. Thus, future studies should consider using metagenomic techniques in larger samples of patients, which must also include a control group.

In conclusion, the microbiota of patients who develop alveolar osteitis after dental extractions might be different from that of patients without postoperative complications. Since this is a preliminary report, further research is needed to assess whether bacteria play an important part in the etiology of dry socket.

Compliance with Ethical Standards

Conflict of interest: Dr. Rui Figueiredo reports grants from the Faculty of Dentistry - University of Barcelona during the conduct of the study. He also reports grants, personal fees and non-financial support from Mozo-Grau (Valladolid, Spain), grants from Mundipharma Research (Cambridge, United Kingdom), personal fees from BioHorizons Ibérica (Madrid, Spain), Inibsa Dental (Lliça de Vall, Spain), Dentsply implants Iberia (Barcelona, Spain) and Araguaney Dental (Barcelona, Spain) outside the submitted work. Dr. Eduard Valmaseda-Castellón reports grants from the Faculty of Dentistry - University of Barcelona during the conduct of the study. He also reports grants, personal fees and non-financial support from MozoGrau, personal fees from BioHorizons Ibérica, personal fees from Inibsa Dental, and personal fees from Dentsply implants Iberia outside the submitted work. Dr. Laura Aguilar-Durán, Dr. Ramón Seminago, Dr. Carlos Llorens and Dr. Francisco J. Roig report grants from the Faculty of Dentistry - University of Barcelona to conduct the present study. The authors declare no other conflicts of interest regarding this study. The present research was conducted by the Dental and Maxillofacial Pathology and Therapeutics research group at the IDIBELL Institute and was funded by a postgraduate research grant from the Faculty of Dentistry of the University of Barcelona (4000€).

Funding: The present research was partially funded (4000€) with a postgraduate research grant from the Faculty of Dentistry of the University of Barcelona.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee

and with the 1964 Helsinki declaration and its latter amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Vezeau PJ (2000) Dental extraction wound management: medicating postextraction sockets. J Oral Maxillofac Surg 58:531-537
2. Blum IR (2002) Contemporary views on dry socket (alveolar osteitis): a clinical appraisal of standardization, aetiopathogenesis and management: a critical review. Int J Oral Maxillofac Surg 31:309-317
3. Noroozi AR, Philbert RF (2009) Modern concepts in understanding and management of the “dry socket” syndrome: comprehensive review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107:30-35
4. Alexander RE (2000) Dental extraction wound management: a case against medicating postextraction sockets. J Oral Maxillofac Surg 58:538-551
5. Nitzan D, Sperry JF, Wilkins TD (1978) Fibrinolytic activity of oral anaerobic bacteria. Arch Oral Biol 23:465-470
6. Nitzan DW (1983) On the genesis of “dry socket”. J Oral Maxillofac Surg 41:706-710.
7. Brescó-Salinas M, Costa-Riu N, Berini-Aytés L, Gay-Escoda C (2006) Antibiotic susceptibility of the bacteria causing odontogenic infections. Med Oral Patol Oral Cir Bucal 11:70-75
8. Poeschl PW, Spusta L, Russmueller G et al (2010) Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110:151-156
9. Peñarrocha M, Sanchis JM, Sáez U, Gay C, Bagán JV (2001) Oral higiene and postoperative pain after mandibular third molar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 92:260-264
10. Hedström L, Sjögren P (2007) Effect estimates and methodological quality of randomized controlled trials about prevention of alveolar osteitis following tooth extraction: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 103:8-15
11. Laird WR, Stenhouse D, MacFarlane TW (1972) Control of postoperative infection. A comparative evaluation of clindamycin and phenoxymethylpenicillin. Br Dent J 133:106-109

12. Larsen PE (1991) The effect of chlorhexidine rinse on the incidence of alveolar osteitis following the surgical removal of implacted third molars. J Oral Maxillofac Surg 49:932-937
13. Haraji A, Rakhshan V, Khamverdi N, Alishahi HK (2013) Effects of intra-alveolar placement of 0.2% chlorhexidine bioadhesive gel on dry socket incidence and postsurgical pain: a double-blind split-mouth randomized controlled clinical trial. J Orofac Pain 27:256-262
14. Human Oral Microbiome Database (HOMD) c2007-2018 Cambridge (MA): The Forsyth Institute; [accessed 2016 April]. http://www.homd.org/
15. Kilian M, Chapple IL, Hannig M et al (2016) The oral microbiome – an update for oral healthcare professionals. Br Dent J 221:657-666
16. Zarco MF, Vess TJ, Ginsburg GS (2012) The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis 18:109-120
17. ASA Physical Status Classification System (2014) Washington DC: American Society of Anesthesiologists; [updated October 15 2014; accessed April 20 2017] http://www.asahq.org/resources/clinical-information/asa-physical-status-classification-system
18. Group JCHMPDGWG. 16S 454 Sequencing Protocol HMP Consortium (2010) Available at: http://www.hmpdacc.org/doc/16S_Sequencing_SOP_4.2.2.pdf. Assessed January 25, 2016
19. Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864
20. Meyer F, Paarmann D, D'Souza M et al (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386-394
21. Gomez-Alvarez V, Teal TK, Schmidt TM (2009) Systematic artifacts in metagenomes from complex microbial communities. ISME J 3:1314-1317
22. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267
23. Futami R, Muñoz-Pomer L, Viu JM et al (2011) GPRO The professional tool for annotation, management and functional analysis of omic databases. Biotechvana Bioinformatics 2011-SOFT3

24. OSU CORE database, Oral Microbiome. Available at: http://microbiome.osu.edu/sequences. Assessed November 21, 2016
25. Charalampakis G, Belibasakis GN (2015) Microbiome of peri-implant infections: lessons from conventional, molecular and metagenomic analyses. Virulence 6:183-187
26. Cortés-Acha B, Figueiredo R, Seminago R, Roig FJ, Llorens C, Valmaseda-Castellón E (2017) Microbiota analysis of biofilms on experimental abutments mimicking denal implants: an in vivo model. J Periodontol 88:1090-1104
27. Bienek DR, Filliben JJ (2016) Risk assessment and sensitivity meta-analysis of alveolar osteitis occurrence in oral contraceptive users. J Am Dent Assoc 147:394-404
28. Taberner-Vallverdú M, Sánchez-Garcés MÁ, Gay-Escoda C (2017) Efficacy of different methods used for dry socket prevention and risk factor analysis: A systematic review. Med Oral Patol Oral Cir Bucal 22:e750-e758
29. Rodríguez Sánchez F, Rodríguez Andrés C, Arteagoitia Calvo I (2017) Does chlorhexidine prevent alveolar osteitis after third molar extractions? Systematic review and meta-analysis. J Oral Maxillofac Surg 75:901-914
30. Ramos E, Santamaría J, Santamaría G, Barbier L, Arteagoitia I (2016) Do systemic antibiotics prevent dry socket and infection after third molar extraction? A systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol 122:403-425
31. Lodi G, Figini L, Sardella A, Carrassi A, Del Fabbro M, Furness S (2012) Antibiotics to prevent complications following tooth extractions. Cochrane Database Syst Rev 11:CD003811
32. Serrati S, Margheri F, Bruschi S et al (2006) Plasminogen activators and inhibitor type-1 in alveolar osteitis. Eur J Oral Sci 114:500-503
33. Seo ES, Lee JH, Park JY, Kim D, Han HJ, Robyt JF (2005) Enzymatic synthesis and anti-coagulant effect of salicin analogs by using the Leuconostoc mesenteroides glucansucrase acceptor reaction. J Biotechnol 117:31-38
34. Ates O (2015) Systems biology of microbial exopolysaccharides production. Front Bioeng Biotechnol 3:200

Table 1 Characteristics of the subjects			
Parameters	**Alveolar osteitis**	**Control group**	**p-value**
Patient age	30 (19 - 35)	29 (21 - 40)	
Gender (M/F)	3/7	4/6	0.639
Smoker (Y/N)	1/9	2/8	0.528
Oral contraceptives in women	2	0	
Oral hygiene (H/G/P)	4/5/1	7/3/0	0.257
History of infection or pericoronitis (Y/N)	6/4	2/8	0.063
Surgical extraction (Y/N)	6/4	5/5	0.653
Surgeon's experience (1st/ 2nd/ 3rd year resident)	5/3/2	2/4/4	0.341
M: male, F: female, H: healthy, G: gingivitis, P: periodontitis, Y: yes, N: no.			

Table 1: Main patient variables.

457

TABLE 2

Abundance and overrepresentation of identified species in Alveolar Osteitis group only

Species	Abundance	%	Gram	Species	Abundance	%	Gram
Acidithiobacillus ferrooxidans	117	0.09		*Halanaerobium saccharolyticum subsp. saccharolyticum*	930	0.8	-
Actinomyces odontolyticus	3250	3	+	*Halothiobacillus hydrothermalis*	1039	0.9	-
Aggregatibacter segnis	84	0.09	-	*Lactobacillus catenaformis*	4	<0.09	+
Anaplasma phagocytophilum	6	<0.09	-	*Lactococcus lactis subsp. lactis*	30	<0.09	+
Atopobium minutum	347	0.3	+	*Leptotrichia hofstadii F0254*	385	0.3	-
Bacteroides acidifaciens	313	0.3	-	*Leuconostoc mesenteroides*	819	0.7	+
Blautia producta	199	0.2	+	*Megasphaera micronuciformis*	16	<0.09	-
Butyrate-producing bacterium SM4/1	3	<0.09	+	*Mitsuokella multacida*	6	<0.09	-
Butyrate-producing bacterium SS3/4	328	0.3	+	*Mycoplasma salivarium*	25	<0.09	+
Butyricimonas synergistica	152	0.2	-	*Neisseria pharyngis*	342	0.3	-
Candidatus Desulforudis audaxviator MP104C	405	0.3		*Nymphaea alba*	1030	0.9	
Chryseobacterium sp. KM	563	0.5		*Parabacteroides goldsteinii*	109	0.09	-
Clostridium aminobutyricum	24	<0.09	+	*Porphyromonas gingivalis*	59	0.09	-
Clostridium longisporum	975	0.9	+	*Prevotella copri*	68	0.09	-
Clostridium sp. MK8	354	0.3	+	*Prevotella multiformis*	5	<0.09	-
Clostridium ultunense	1679	1	+	*Prevotella nanceiensis*	1521	1	-
Cytophaga sp. MBIC04667	3	<0.09	-	*Prevotella oulorum*	284	0.3	-
Dialister pneumosintes	1617	1	-	*Prevotella pleuritidis*	942	0.8	-
Embryophyte environmental sample	12	<0.09		*Prevotella veroralis*	836	0.7	-
Eubacterium cellulosolvens	5	<0.09	+	*Robinsoniella peoriensis*	4723	4	+
Eubacterium hallii	738	0.6	+	*Roseburia cecicola*	369	0.3	+
Eubacterium rectale	1159	1	+	*Streptococcus thermophilus*	171	0.2	+
Eubacterium saburreum	346	0.3	+	*Tannerella forsythia*	1398	1	-
Flavobacterium columnare	638	0.5	-	*Treponema lecithinolyticum*	185	0.2	-
Flavobacterium denitrificans	64	0.09	-	*Treponema maltophilum*	1146	1	-
Fusobacterium	7	<0.09	-	*Treponema*	79	0.09	-

necrophorum				*medium*			
Fusobacterium nucleatum subsp. animalis	576	0.5	-	*uncultured alpha proteobacterium*	39	<0.09	
Granulicatella elegans	1182	1	+	*unidentified proteobacterium*	10	<0.09	
Haemophilus sp. CCUG 15949	69	0.09		*Veillonella dispar*	3368	3	-
Abundance and overrepresentation of identified species in Control group only							
Species	Abundance	%	Gram	Species	Abundance	%	Gram
Actinomyces israelii	263	0.1	+	Mesoplasma lactucae	932	0.4	+
Actinomyces viscosus	3035	1	+	Moraxella nonliquefaciens	5	<0.09	-
Arthrobacter agilis	9451	4	+	Mycoplasma faucium	31	<0.09	+
Bacteroides barnesiae	1	<0.09	-	Porphyromonas gingivicanis	1320	0.6	-
Blautia sp. Ser8	977	0.4		Prevotella aurantiaca	26	<0.09	-
Bradyrhizobium japonicum	207	0.09	-	Prevotella baroniae	757	0.3	-
Butyrivibrio fibrisolvens	4992	2	-	Prevotella bergensis	8	<0.09	-
Campylobacter gracilis	259	0.1	-	Prevotella bivia	2100	0.9	-
Campylobacter showae	360	0.2	-	Prevotella marshii	1128	0.5	-
Capnocytophaga canimorsus	4497	2	-	Prevotella oralis	161	0.09	-
Clostridium acetobutylicum	10	<0.09	+	Prevotella sp. RS2	1612	0.7	-
Clostridium aminovalericum	3921	2	+	Propionibacterium acidipropionici	223	0.09	+
Clostridium bifermentans	942	0.4	+	Pyramidobacter piscolens	6237	3	-
Clostridium hathewayi	1285	0.6	-	Rhodococcus sp. 28/19	339	0.1	+
Clostridium sphenoides	45	<0.09	+	Riemerella anatipestifer	24	<0.09	-
Corynebacterium kutscheri	1394	0.6	-	Streptococcus anginosus	6604	3	+
Desulfocaldus sp. Hobo	852	0.4		Streptococcus australis	4246	2	+
Elizabethkingia meningoseptica	57	<0.09	-	Streptococcus salivarius	2024	0.9	+
Ewingella americana	491	0.2	-	Synergistetes bacterium SGP1	9	<0.09	-
Fusobacterium canifelinum	74	<0.09	-	Treponema bryantii	1	<0.09	-
Gemella haemolysans	1372	0.6	+	Treponema denticola	4803	2	-
Geodermatophilus obscurus	3	<0.09	+	Treponema vincentii	63	0.04	-
Lachnospiraceae bacterium 14-2	993	0.4	-	Veillonella atypica	123	0.04	-
Leptotrichia wadei	43	<0.09	-	Veillonella parvula	828	0.3	-
Macrococcus carouselicus	2882	1	+				

Table 2. Abundance and overrepresentation of identified species identified in only one of the study groups (Alveolar Osteitis (AO) or Control group (CG)).

TABLE 3

Abundance and overrepresentation of identified species common to both groups

	Alveolar osteitis		Control Group			
Species	Abundance	%	Abundance	%	Over-represented	Gram
Abiotrophia defectiva	246	0.3	5132	2	Control	+
Abiotrophia para-adiacens	487	0.4	1925	0.8	Control	+
Actinomyces naeslundii	109	0.09	4378	2	Control	+
Atopobium vaginae	534	0.4	7712	3	Control	+
Butyrivibrio hungatei	4759	4	1650	0.7	Dry socket	-
Capnocytophaga gingivalis	509	0.4	9329	4	Control	-
Capnocytophaga ochracea	1717	1	2382	1	-	-
Capnocytophaga sputigena	201	0.2	6040	3	Control	-
Clostridium paradoxum	81	0.09	3703	2	Control	+
Desulfotomaculum thermosapovorans	84	0.09	303	0.1	-	+
Dialister pneumosintes	1617	1	189	0.09	Dry socket	-
Eikenella corrodens	386	0.3	1429	0.6	Control	-
Eubacterium sp. WAL 17363	1273	1	1253	0.6	-	+
Fusobacterium nucleatum	5109	4	1199	0.5	Dry socket	-
Fusobacterium nucleatum subsp. polymorphum	1676	1	3266	1	-	-
Fusobacterium periodonticum	1249	1	1333	0.6	-	-
Gemella morbillorum	300	0.3	390	0.2	-	+
Granulicatella adiacens	559	0.5	1599	0.7	Control	+
Haemophilus haemolyticus	2	<0.09	470	0.2	Control	-
Haemophilus parainfluenzae	1783	2	1327	0.6	-	-
Parvimonas micra	3149	3	922	0.4	Dry socket	+
Peptostreptococcus anaerobius	343	0.3	4	<0.09	Dry socket	+
Porphyromonas catoniae	4982	4	7425	3	-	-
Porphyromonas endodontalis	1500	1	2594	1	-	-
Prevotella buccae	7498	7	13532	6	-	-
Prevotella denticola	1765	2	2480	1	-	-
Prevotella enoeca	45	0.09	23	<0.09	-	-
Prevotella intermedia	1870	2	95	0.04	Dry socket	-
Prevotella loescheii	183	0.2	6599	3	Control	-
Prevotella maculosa	91	0.09	9	<0.09	Dry socket	-
Prevotella melaninogenica	4079	4	169	0.09	Dry socket	-
Prevotella nigrescens	1696	1	689	0.3	Dry socket	-
Prevotella oris	4060	4	4963	2	-	-
Prevotella pallens	277	0.3	4	<0.09	Dry socket	-
Prevotella ruminicola	45	0.09	75	0.04	-	-
Prevotella salivae	186	0.2	5588	2	Control	-
Selenomonas lacticifex	118	0.09	534	0.2	Control	-
Selenomonas ruminantium	736	0.6	940	0.4	-	-

Streptococcus	923	0.8	202	0.09	Dry socket	+
Streptococcus intermedius	922	0.8	24	<0.09	Dry socket	+
Streptococcus mitis	1516	1	827	0.3	Dry socket	+
uncultured bacterium	19265	17	39673	17	Control	
uncultured beta proteobacterium	113	0.09	580	0.3	Control	
uncultured Kingella sp.	750	0.7	4896	2	Control	
unidentified	78	0.09	7914	3	Control	
Veillonella rogosae	1460	1	89	0.04	Dry socket	-

Table 3. Abundance and overrepresentation of identified species common to both groups.

FIGURE LEGENDS

Figure 1 A Rarefaction plot showing a curve of annotated species richness. This curve is a plot of the total number of distinct species annotations as a function of the number of sequences sampled. **B** Venn diagram demonstrating bacterial taxonomic distribution between diseased and healthy samples. **C** Principal Component Analysis (PCA) relationships among the groups of samples. The circles surround the samples belonging to the same group.

Figure 2 A and B Taxonomic spectrum visualized with Krona chart of metagenome read counts. The circles represent taxonomic classifications in ascending order up to the family level (outermost circle). Less abundant taxa are listed outside the charts together with their relative abundance. Obtained from raw data using the MG-RAST server (A: Alveolar Osteitis samples pool and B: Control Group).

Figure 1

Click here to access/download;Figure;Fig 1.tiff

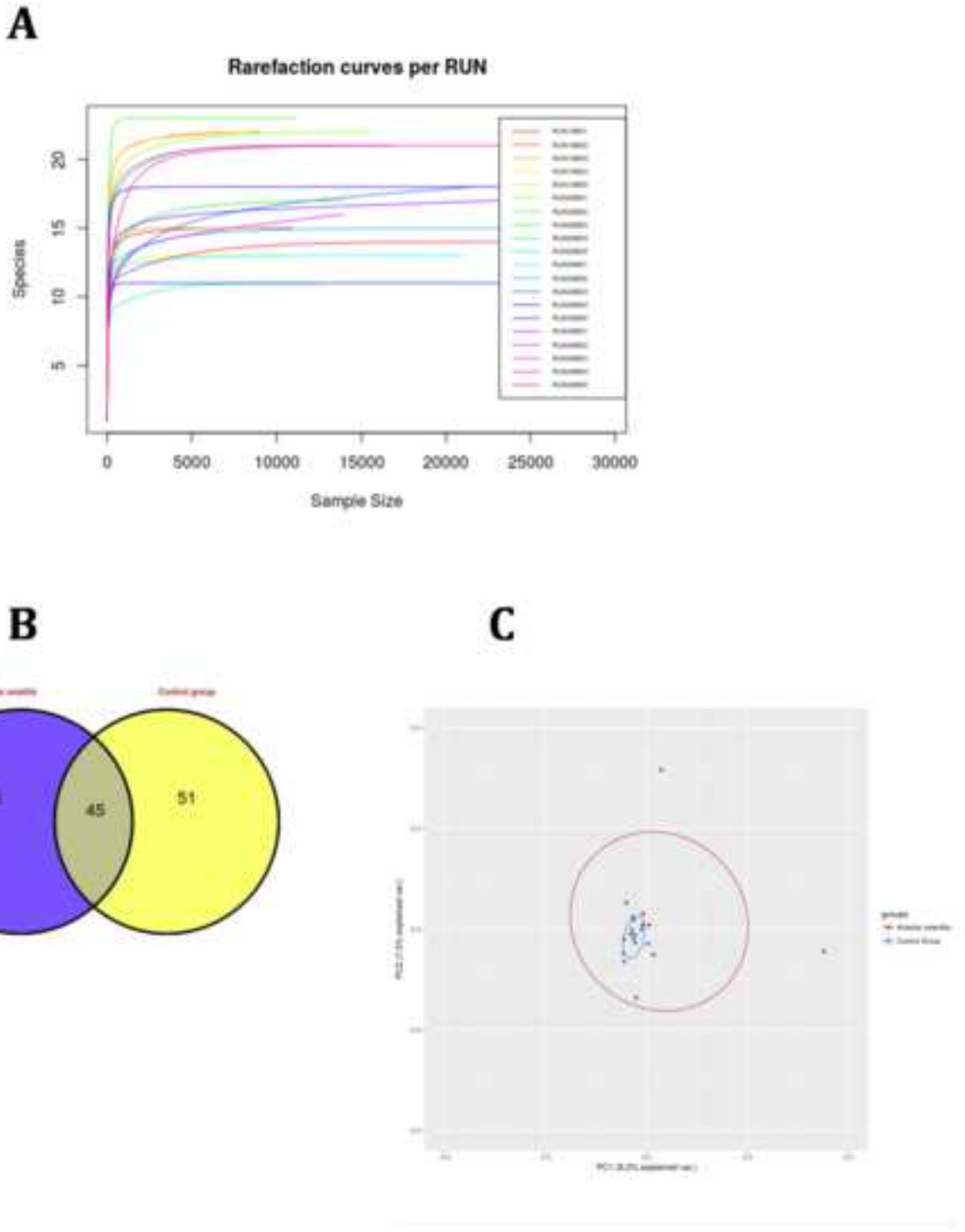

Figure 2

Click here to access/download;Figure;Fig 2.tiff

Figure 1