ORIGINAL ARTICLE

Oral microbiota species in acute apical endodontic abscesses

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Background and objectives: Acute apical abscesses are serious endodontic diseases resulting from pulpal infection with opportunistic oral microorganisms. The objective of this study was to identify and compare the oral microbiota in patients ($N = 18$) exhibiting acute apical abscesses, originating from the demographic region in Portland, Oregon. The study hypothesis is that abscesses obtained from this demographic region may contain unique microorganisms not identified in specimens from other regions.
Design: Endodontic abscesses were sampled from patients at the Oregon Health & Science University (OHSU) School of Dentistry. DNA from abscess specimens was subjected to polymerase chain reaction amplification using 16S rRNA gene-specific primers and Cy3-dCTP labeling. Labeled DNA was then applied to microbial microarrays (280 species) generated by the Human Oral Microbial Identification Microarray Laboratory (Forsyth Institute, Cambridge, MA).
Results: The most prevalent microorganisms, found across multiple abscess specimens, include *Fusobacterium nucleatum*, *Parvimonas micra*, *Megasphaera* species clone CS025, *Prevotella multisaccharivorax*, *Atopobium rimae*, and *Porphyromonas endodontalis*. The most abundant microorganisms, found in highest numbers within individual abscesses, include *F. nucleatum*, *P. micra*, *Streptococcus* Cluster III, *Solobacterium moorei*, *Streptococcus constellatus*, and *Porphyromonas endodontalis*. Strong bacterial associations were identified between *Prevotella multisaccharivorax*, *Acidaminococcaceae* species clone DM071, *Megasphaera* species clone CS025, *Actinomyces* species clone EP053, and *Streptococcus cristatus* (all with Spearman coefficients > 0.9).
Conclusions: Cultivable and uncultivable bacterial species have been identified in endodontic abscesses obtained from the Portland, Oregon demographic region, and taxa identifications correlated well with other published studies, with the exception of *Treponema* and *Streptococcus cristae*, which were not commonly identified in endodontic abscesses between the demographic region in Portland, Oregon and other regions.

Keywords: *endodontic abscesses*; *oral microbiota*; *human oral microbial identification microarrays*; *anaerobic oral microorganisms*; Fusobacterium nucleatum; Streptococcus cristatus

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Endodontic disease is initiated by infection with multiple microorganisms and aggravated by an influx of inflammatory cells leading to pulpitis and periapical periodontitis (1–5). The formation of

acute apical abscess, an inflammatory response to pulpal infection, is further characterized by pain, sensitivity of the tooth to pressure, and swelling of surrounding tissues (1–5). The induction of an acute inflammatory response

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leads to bone resorption surrounding the tooth apex. The acute apical abscess is representative of an advanced stage of apical periodontitis and has the potential to expand from the root canal to form cellulitis, by disseminating an inflammatory response generating purulence (1–5).

Siqueira and Rôcas (5) have identified five levels of experimentation analyzing the microorganisms contained within acute apical abscesses: 1) culture methods targeting cultivable bacteria (open-ended or broad-range culture approach), 2) molecular identification tools, including polymerase chain reaction (PCR) and the checkerboard hybridization assay (using genomic probes to detect cultivable bacteria; closed-ended molecular detection approach), 3) broad-range PCR in addition to cloning and sequencing of targeted amplicons (open-ended molecular approach), 4) molecular analysis using PCR or DNA hybridization, including reverse checkerboard hybridization, and the use of human participants in large-scale clinical studies to assess prevalence and association of diverse bacterial strains (close-ended molecular approach), and 5) the use of next-generation sequencing (NGS) for extensive, open-ended analysis of abscess specimens.

On a broad taxonomic level, the most prevalent phyla identified in acute apical abscesses include: 1) the Firmicutes (including representative members within *Streptococcus, Parvimonas, Peptostreptococcus* and *Dialister*), 2) Bacteroidetes (including members within *Porphyromonas* and *Prevotella*), 3) Fusobacteria (including members within *Fusobacterium* and *Leptotrichia*), 4) Actinobacteria (including members within *Actinomyces, Atopobium,* and *Propionibacterium*), 5) Spirochaetes (including *Treponema*), 6) Synergistetes (including *Pyramidobacter*), and 7) Proteobacteria (including *Campylobacter* and *Eikenella*) (1–5).

In acute endodontic infections, the most prevalent microorganisms include members from the phyla Firmicutes, Fusobacteria, and Bacteroidetes, or more specifically, the genera *Fusobacterium, Parvimonas, Peptostreptococcus, Dialister,* and *Atopobium*. In chronic endodontic infections, the most prevalent microorganisms include members from the phyla Firmicutes, Bacteroidetes, and Actinobacteria (1–5).

The primary objectives of this study were to identify the most prevalent oral microorganisms contained in endodontic abscesses and to identify important bacterial associations and potential synergistic relationships between species. The study hypothesis is that abscesses obtained from the demographic region in Portland, Oregon may contain unique microorganisms not identified in specimens from other regions. We sought to specifically compare microorganisms originating from the demographic region in Portland, Oregon with those contained in abscesses from Rio de Janeiro, Brazil (6), and found that taxa identifications correlated well, with the exception of *Treponema* and *Streptococcus cristae*.

The identification of bacterial species in endodontic abscesses, obtained from distinct sites in North and South America, may lead to the assessment of new candidate microorganisms and synergistic relationships associated with the progression of endodontic disease and the development of acute apical abscesses.

Materials and methods

Collection of abscess specimens

Abscess specimens were collected from patients at the OHSU School of Dentistry in 2007–09, following protocols approved by the OHSU Institutional Review Board (IRB #5362). Patients obtaining treatment (adult males and females; age range 16–60 years) had swelling associated with an acute apical abscess. Diagnostic terminology was based on guidelines obtained from the Pulpal & Periapical Diagnostic Terminology, formulated by the American Board of Endodontics (2007) and referenced in Ingle's Endodontics, 6th Edition (4). Specimen collections were obtained as previously described (7) using informed consent, and patient identifiers were anonymously coded. Prior to needle aspiration of the abscess, the oral cavity was rinsed with 0.12% chlorhexidine gluconate (Zila Pharmaceuticals, Phoenix, AZ) for approximately 15 s, and the overlying mucosal surface was disinfected using Povidone–Iodine swabs (Professional Disposables, Inc., Orangeburg, NY) for approximately 30 s. Paper point samples were collected from the site of aspiration and samples placed in growth medium for determination of bacterial contaminants. Clinical aspirates were obtained using a sterile 16-gauge needle and then immediately placed within vials (Port-A-Cult™, Becton Dickinson, Cockeysville, MD) for transport to the laboratory and subsequent freezing (−85°C).

DNA extraction and Human Oral Microbial Identification Microarray (HOMIM) analysis

Abscess specimens were clarified by low-speed centrifugation to remove human cells and debris, and then subjected to DNA extraction (using the PureLink Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA; now Life Technologies). Genomic DNA was electrophoresed in 0.75% agarose gels containing ethidium bromide (0.5 μg/ml) to verify nucleic acid integrity and then processed in the Human Oral Microbial Identification Microarray (HOMIM) Laboratory at the Forsyth Institute (Cambridge, MA). 16S rRNA-based oligonucleotide probes were generated and printed on aldehyde-coated glass slides (8–11). Abscess DNA fragments containing 16S rRNA gene sequences were generated by PCR, using the specimen DNA as template and 16S rRNA universal forward and reverse primers. Positive (16S universal primers) and negative buffer, and primer controls were used in the HOMIM analyses. The HOMD 16S rRNA

reference sequence files, including HOMD references and sequences, are available from the Human Oral Microbiome Database.

Data normalization and statistical analysis

Each probe was tested by z test to determine if the average of the signal intensity values was significantly higher than that of local background noise. After filtering, the median of the local background noise was subtracted from the corresponding mean signal intensity and then transformed into log base 2 scale. The background-corrected intensity values in log base 2 scale were subjected to global normalization in gene microarrays (12). In the log base 2 scale, the range of transformed intensity values was clearly quantitative between 0 and 16, and reflective of differences in microbial prevalence. After normalization, the duplicate spots for the same bacterial species were averaged. Prevalence and correlation values were computed when bacteria were detected in at least 50% of the patient specimens. We used R statistical language for all computations (13) and calculated Spearman's rank correlation coefficients to assess associations between species. In cases where two taxa were detected in 100% of the specimens, low correlation values (i.e. close to 0) are possible if their intensity values are not associated. Prevalence values for bacterial species identified in our microarrays were compared with prevalence values determined for corresponding species in the Rio de Janeiro study (6).

Results

Out of 425 unique taxon-specific probes representing 280 species, 81 probes were reactive with the abscess specimen DNA. In positive control reactions, DNA from all abscess specimens generated amplicons using the 16S rRNA universal primers, and negative control reactions without DNA did not generate amplicons. The numbers of bacterial taxa identified within individual abscess specimens ranged from 2 to 30 (mean = 15.9; median = 17). The single abscess specimen with only two targeted species contained *Fusobacterium nucleatum* and *Parvimonas micra*. All other abscesses contained at least six targeted species.

Five taxa, including *F. nucleatum, P. micra, Megasphaera* species clone CS025, *Prevotella multisaccharivorax*, and *Atopobium rimae*, were highly prevalent across abscess specimens and were identified to be present in all specimens (18/18, 100%; Fig. 1). Several additional taxa, including *Porphyromonas endodontalis* (17/18, 94.4%), *Acidaminococcaceae* species clone DM071 (17/18, 94.4%), *Actinomyces* species clone EP053 (16/18, 88.9%), *Mycoplasma salivarium* (16/18, 88.9%), *Streptococcus cristatus* (15/18, 83%), and *Lactobacillus gasseri* (15/18, 83.3%) were identified in >83% of the abscess specimens examined in this study. Taxa with prevalence

values >0.39 (out of 81 taxa-specific probes detected) are shown in Fig. 1.

The five taxa with the highest average abundance, measured within individual abscess specimens and then numerically averaged across all abscess specimens, were *F. nucleatum, P. micra* (two distinct clones), *Streptococcus* Cluster III, *Solobacterium moorei*, and *Streptococcus constellatus* (Fig. 2).

F. nucleatum and *P. micra* were both highly prevalent across abscesses and highly abundant within individual abscess specimens. Interestingly, the other three taxa demonstrating high prevalence across all abscess specimens – *Megasphaera* species clone CS025, *P. multisaccharivorax*, and *A. rimae* – had moderate or lower abundance levels within individual abscesses (Fig. 2). Additional taxa with high prevalence (>83–94% range), including *P. endodontalis, Acidaminococcaceae* species clone DM071, *Actinomyces* species clone EP053, *M. salivarium, S. cristatus*, and *L. gasseri,* also had moderate or lower abundance levels within individual abscesses (Fig. 2).

The bacteria found most commonly associated with other bacteria, at levels above Spearman rank correlation coefficients of 0.9 were *P. multisaccharivorax/Acidaminococcaceae* species clone DM071 (Spearman = 0.961), *Acidaminococcaceae* species clone DM071/*Megasphaera* species clone CS025 (Spearman = 0.926), *Megasphaera* species clone CS025/*Actinomyces* species clone EP053 (Spearman = 0.924), *P. multisaccharivorax/Megasphaera* species clone CS025 (Spearman = 0.917), *Acidaminococcaceae* species clone DM071/*S. cristatus* (Spearman = 0.908), and *S. cristatus/Actinomyces* species clone EP053 (Spearman = 0.908). Interestingly, *Acidaminococcaceae* species clone DM071 is highly associated (Spearman coefficient >0.772) with seven other microorganisms (Table 1) and *Megasphaera* species clone CS025 is highly associated (Spearman coefficient >0.767) 0.767) with eight microorganisms (Table 1). *P. multisaccharivorax* and *S. cristatus* both have high associations (Spearman coefficients >0.828) with four and three other microorganisms, respectively (Table 1).

Discussion

Oral microbiota contained within endodontic abscesses

For the current study, we examined endodontic abscesses for the presence of 280 oral bacterial species (425 taxa-specific probes) using microarray determinations conducted by the Human Oral Microbial Identification Laboratory at the Forsyth Institute (Cambridge, MA). Our findings confirmed and provided new information supporting the earlier research by Siqueira and Rôcas (6), describing the microbiota of endodontic abscesses obtained from the demographic region in Rio de Janeiro,
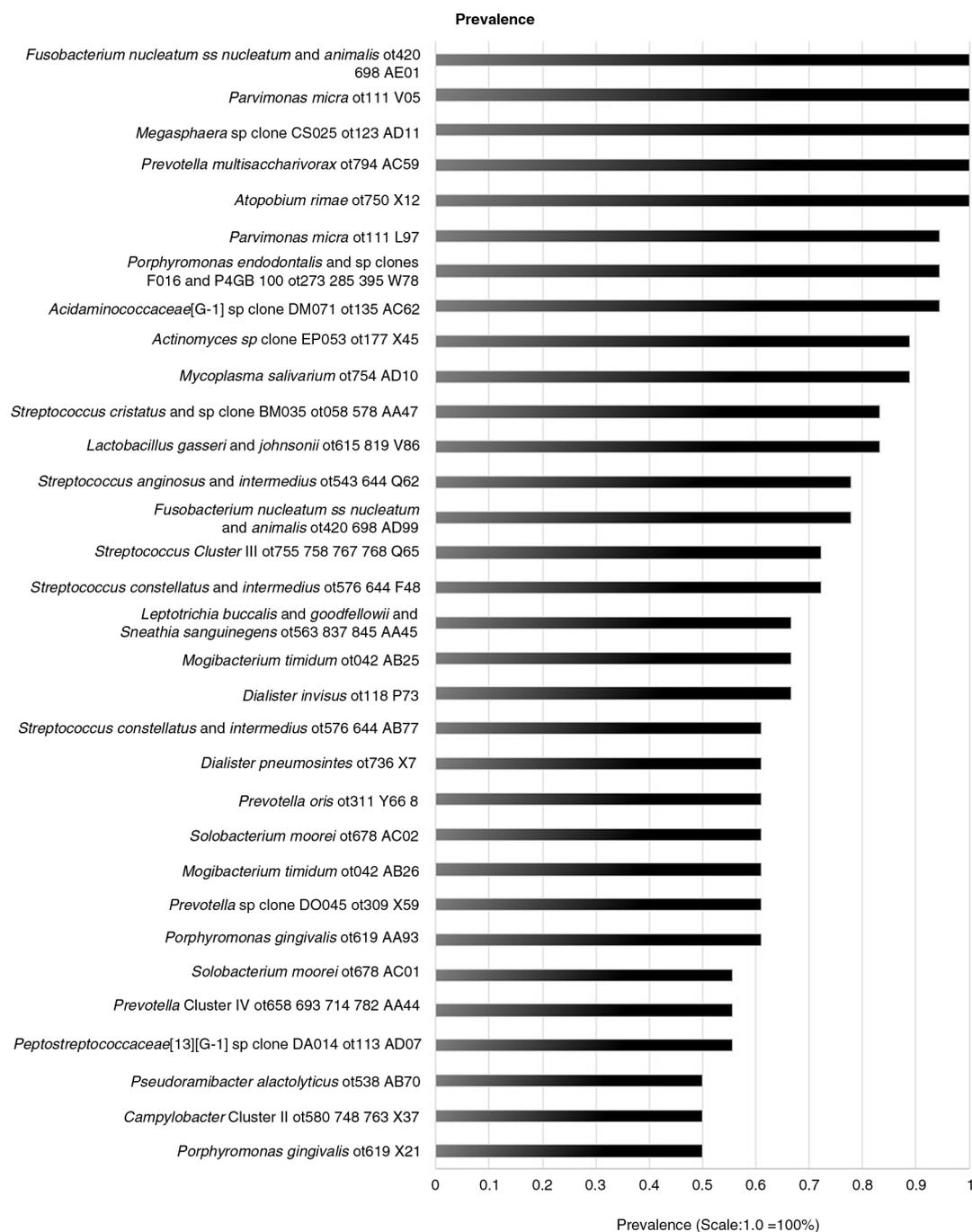
Prevalence

Fig. 1. Bar graph illustrating *prevalence* of bacterial species in endodontic abscess specimens ($N = 18$). *Prevalence* for each bacterial species is defined as the percentage across the entire number of abscess specimens (example: 100% prevalence is equivalent to bacterial species X found in 18 out of 18 abscess specimens). The horizontal axis is set to 0–1 scale; representing 0–100% prevalence. The range of prevalence values displayed in the bar graph is limited to those bacterial species >39%.

Brazil. In our Portland, Oregon abscess collection, we found similar microbial species as those defined in Siqueira and Rôcas (6) as well as several new strong bacterial associations, including those between *F. nucleatum* and *M. salivarium*, and between *F. nucleatum* and *S. cristatus*. Studies in Portland, Oregon, and Rio de Janeiro, Brazil, noted that *F. nucleatum* was the principal bacterial species

identified in endodontic abscesses and was used as a comparative baseline for positive bacterial associations. *F. nucleatum* was present in all abscesses ($N = 18$) in the current study and in 27 out of 44 abscesses (64%) in the study by Siqueira and Rôcas (6). *P. micra* and *Porphyromomas endodontalis* were also found in high prevalence in abscess specimens from the demographic
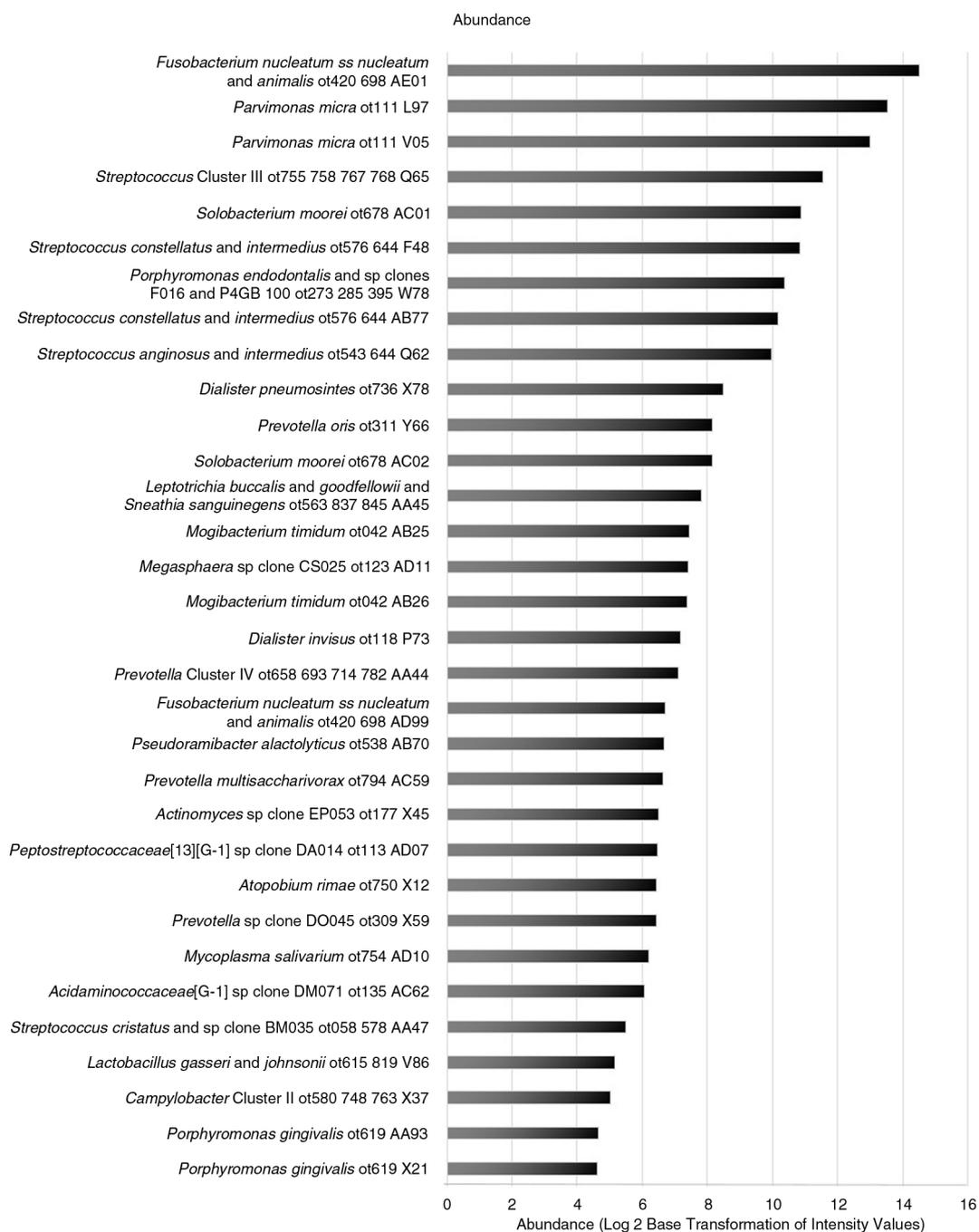
Abundance

Fig. 2. Bar graph illustrating *abundance* of bacterial species in endodontic abscess specimens ($N = 18$). *Abundance* for each bacterial species is defined as the intensity signal (reflective of the numbers of bacterial species X) within each individual abscess specimen and then averaged for the entire abscess specimen set. Using the log base 2 scale, the range of transformed intensity values in the horizontal axis is 0–16.

regions in Portland, Oregon and Rio de Janeiro, Brazil. Interestingly, *Veillonella* species, which were detected in many culture-independent studies, but absent in Siqueira and Rôcas (6), were also found in low prevalence (2 out of 18 abscesses) in our study. In addition, *Treponema* species clones, numbering 25 taxa-specific probes in our study, were detected in very low abundance in many specimens;

only three abscess specimens generated moderate signals with the *Treponema* probes, *Treponema maltophilum* in two abscesses and *Treponema* cluster in the third abscess. This is in contrast to the findings of Siqueira and Rôcas (6) and to numerous culture-independent studies that have detected moderate-to-high numbers of the oral treponemes, *Treponema denticola* and *Treponema socranskii*, within

Table 1. Bacterial associations in endodontic abscesses (Spearman's rank correlation coefficient > 0.76)

Bacteria 1	Bacteria 2	Spearman
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Prevotella multisaccharivorax*_ot794_AC59	0.961
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Streptococcus cristatus* and sp clone BM035_ot058_578_AA47	0.911
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Lactobacillus gasseri* and *johnsonii*_ot615_819_V86	0.893
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Actinomyces* sp clone EP053_ot177_X45	0.882
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Parvimonas micra*_ot111_L97	0.856
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Porphyromonas endodontalis* and sp clones _F016 and P4GB_100_ot273_285_ 395_ W78	0.850
Acidaminococcaceae[G-1] sp clone DM071_ot135_AC62	*Parvimonas micra*_ot111_V05	0.772
Megasphaera sp clone CS025_ot123_AD11	*Acidaminococcaceae*[G-1] sp clone DM071_ot135_AC62	0.926
Megasphaera sp clone CS025_ot123_AD11	*Actinomyces* sp clone EP053_ot177_X45	0.924
Megasphaera sp clone CS025_ot123_AD11	*Prevotella multisaccharivorax*_ot794_AC59	0.917
Megasphaera sp clone CS025_ot123_AD11	*Streptococcus cristatus* and sp clone BM035_ot058_578_AA47	0.882
Megasphaera sp clone CS025_ot123_AD11	*Porphyromonas endodontalis* and sp clones _F016 and P4GB_100_ot273_285_ 395_ W78	0.816
Megasphaera sp clone CS025_ot123_AD11	*Parvimonas micra*_ot111_L97	0.804
Megasphaera sp clone CS025_ot123_AD11	*Lactobacillus gasseri* and *johnsonii*_ot615_819_V86	0.786
Megasphaera sp clone CS025_ot123_AD11	*Parvimonas micra*_ot111_V05	0.767
*Prevotella multisaccharivorax*_ot794_AC59	*Lactobacillus gasseri* and *johnsonii*_ot615_819_V86	0.886
*Prevotella multisaccharivorax*_ot794_AC59	*Streptococcus cristatus* and sp clone BM035_ot058_578_AA47	0.868
*Prevotella multisaccharivorax*_ot794_AC59	*Actinomyces* sp clone EP053_ot177_X45	0.865
*Prevotella multisaccharivorax*_ot794_AC59	*Porphyromonas endodontalis* and sp clones _F016 and P4GB_100_ot273_285_395_ W78	0.828
Streptococcus cristatus and sp clone BM035_ot058_578_AA47	*Actinomyces* sp clone EP053_ot177_X45	0.908
Streptococcus cristatus and sp clone BM035_ot058_578_AA47	*Lactobacillus gasseri* and *johnsonii*_ot615_819_V86	0.868
Streptococcus cristatus and sp clone BM035_ot058_578_AA47	*Parvimonas micra*_ot111_L97	0.829
Lactobacillus gasseri and *johnsonii*_ot615_819_V86	*Actinomyces* sp clone EP053_ot177_X45	0.793
*Mycoplasma salivarium*_ot754_AD10	*Fusobacterium nucleatum* ss nucleatum and animalis_ot420_698_AE01	0.829
Porphyromonas endodontalis and sp clones _F016 and P4GB_100_ot273_285_395_W78	*Lactobacillus gasseri* and *johnsonii*_ot615_819_V86	0.775
Streptococcus constellatus and *intermedius*_ot576_644_F48	*Streptococcus* Cluster III_ot755_758_767_768_Q65	0.873

endodontic abscesses (14). In addition, *Enterococcus faecalis* was not detected in any of the abscess specimens in our study, consistent with the findings of others that *E. faecalis* was significantly more associated with asymptomatic, as opposed to symptomatic cases of endodontic disease (15).

In our study, five bacterial taxa were found in 100% of all surveyed abscess specimens ($N = 18$); these species included *F. nucleatum* and *P. micra*, both of which were found in high prevalence (64% and 52%, respectively) in the study by Siqueira and Rôcas (6). In

addition, *Megasphaera* species clone CS025, *P. multisaccharivorax,* and *A. rimae* were found at 100% prevalence in our study, albeit at moderate-to-lower abundance levels within individual abscesses, but varied from less than 5% (*Megasphaera* species clone CS025 and *P. multisaccharivorax*) to 10% prevalence (*A. rimae*) in the study by Siqueira and Rôcas (6). Strong associations were also found between bacterial taxa whose correlations had not previously been established. Several significant positive associations involving *F. nucleatum* as the reference strain included *F. nucleatum*/*M. salivarium*

($\rho = 0.829$), *F. nucleatum/Porphyromonas gingivalis* ($\rho = 0.691$), and *F. nucleatum/Megasphaera* species clone CS025 ($\rho = 0.684$) and were based on Spearman's rank correlation coefficients > 0.65.

We have minimized the risk of abscess sample contamination with the use of the chlorhexidine gluconate oral rinse and the disinfection of mucosal surfaces with Povidone–Iodine swabs. Paper point collections, conducted at the site of abscess aspiration, did not produce any microbial growth. Abscess specimens were collected and frozen at $-85°C$, prior to DNA extraction conducted as a batch in 2010. DNA was then sent to the Forsyth Institute for HOMIM analysis. The 5-year delay between HOMIM analysis and the preparation of this manuscript was due to the time required to obtain funding to recruit and pay biostatisticians qualified to analyze complex microarray data. We acknowledge that another potential negative control, which was not conducted during the experimental phase of this work in 2010, may have been the aspiration of tissue or blood from the mucosa of a healthy subject and the identification of any potential contaminants by microarray analysis. We also acknowledge that differences in the microbiota profiles identified in abscesses obtained from the demographic regions in Portland, Oregon, and Rio de Janeiro, Brazil, may be due to technical differences in the microarray methods, including the design of the 16S rRNA gene probes and the hybridization conditions used to target specific microbial sequences.

Coinfection of F. nucleatum *and other microorganisms may work synergistically within endodontic and periodontal disease*

Multiple microorganisms initiate endodontic infections, and genera such as *Fusobacterium, Treponema, Prevotella,* and *Streptococcus* dominate these lesions, although the range of bacteria present may vary (5, 16–18). Within specific genera, different species have been found to work synergistically, utilizing virulence factors from complementary microorganisms to their advantage (19).

F. nucleatum has been found to enhance the adhesion and invasion of other oral microorganisms (20). Furthermore, coinfection of *F. nucleatum* with other oral bacteria may inhibit host immune response, allowing for their persistent growth (20). This cooperative growth may explain the presence of *M. salivarium, P. gingivalis, or Megasphaera* clone with *F. nucleatum* in endodontic and periodontal disease. In addition, *F. nucleatum* was found to be associated with *S. cristatus*, which has been specifically shown to inhibit the pro-inflammatory actions of the former microorganism (21). The synergistic interactions of enhancing virulence and inhibiting host immune response are believed to be contributory determinants of endodontic and periodontal disease.

Microbiota in endodontic abscesses may differ based on geographic considerations

The inter-study variability of microbiota species identified in periodontal and endodontic diseases may be based on the geographic location of the patient pool and specimen acquisition. Several studies have shown that body sites typically colonize different microbes depending on geographic locations. For example, Kemppainem et al. (22) demonstrated that gut flora colonization and diversity in infants at risk for Type I diabetes may be based on country of birth. Furthermore, different single-nucleotide polymorphisms (SNPs) of *Mycobacterium tuberculosis* were found to occur in different geographic locations (23). Machado de Oliveira et al. (24) and Siqueira and Rôcas (5) also previously noted differences in bacterial community profiles and certain microorganisms from endodontic abscesses in patients from Portland, Oregon, and Rio de Janeiro, Brazil. Although common microbiota profiles were found between these two geographic locations, the prevalence of microbes varied between studies. Interestingly, consistent with a nested PCR analysis conducted using abscess specimens jointly obtained from Portland, Oregon, and Rio d Janeiro, Brazil (25, 26), *T. denticola* and *Tannerella forsythia* were not present, or present in extremely low levels in our microarray analyses, and were present at high prevalence in specimens described in the Rio de Janeiro study (6). In addition, it is widely accepted that ethnic and geographic differences exist in periodontal microbiota profiles (27), and we conclude, in part by extrapolation, that geographic location may play a role in the pathogenic microbial causes of endodontic and periodontal infections.

Potential role of streptococci species and S. cristatus *in dental disease*

S. cristatus has been demonstrated to inhibit host immune response by attenuating interleukin-8 in the inflammatory pathway (21) and has been implicated as opportunistically pathogenic by its role in severe early childhood caries (S-ECC) and by its association with periodontal bacteria (28, 29). *F. nucleatum* uses a nuclear factor-kappa B (nuclear translocation) inflammatory pathway to induce a pro-inflammatory cascade, which is blocked by *S. cristatus* (21). *S. cristatus* is also negatively correlated with *P. gingivalis* (29), described as a potential causative determinant of adult periodontitis. The pathogenicity of *P. gingivalis* lies in part by its ability to adhere to oral surfaces with its long fimbriae; *S. cristatus* appears to inhibit the expression of the fimbrillin (*FimA*) gene, which is involved in the adhesion and subsequent colonization of *P. gingivalis* (30). *S. cristatus* also produces arginine deiminase (*ArcA*), which represses *FimA* production and inhibits the colonization of *P. gingivalis* (31). Through these mechanisms, *S. cristatus*

has been implicated to attenuate the pathogenic activity of *P. gingivalis* and significantly contributes to decreased inflammatory destruction in periodontal disease, including decreased alveolar bone loss (32).

Furthermore, the ability of *S. cristatus* to undergo transformation at an increased frequency has been proposed to contribute to the virulence of this microorganism (33).

Implications of mixed microbial infections and concluding remarks

Bacterial interactions within endodontic infections may synergistically or antagonistically affect growth and underscore the complexity of the ecological determinants that support the metabolic efficiency and pathogenic potency of the composite bacterial community. Our study supports the findings of previous research in defining the most prevalent bacteria within endodontic abscesses and identifies strong bacterial associations, some of which may be unique for the demographic region in Portland, Oregon, or patient population. Furthermore, the presence of *S. cristatus* within endodontic abscesses, which has not been identified in previous endodontic disease studies, may indicate a new role as a member of a pathogenic microbial ecology.

Acknowledgements

Conflict of interest and funding

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