CLINICAL RESEARCH

Metatranscriptome and Resistome of the Endodontic Microbiome

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ABSTRACT

Introduction: In this study, we used metatranscriptomics for the first time to investigate microbial composition, functional signatures, and antimicrobial resistance gene expression in endodontic infections. **Methods:** Root canal samples were collected from ten teeth, including five primary and five persistent/secondary endodontic infections. RNA from endodontic samples was extracted, and RNA sequencing was performed on a NovaSeq6000 system (Illumina). Taxonomic analysis was performed using the Kraken2 bacterial database. Then, sequences with a taxonomic classification were annotated against the Universal Protein Knowledgebase for functional annotation and the Comprehensive Antibiotic Resistance Database for AR-like gene identification. **Results:** Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria represented the dominant phyla, whereas Fusobacteria, Spirochetes, and Synergistetes were among the nondominant phyla. The top ten species were mainly represented by obligate (or quasiobligate) anaerobes, including Gram-negative (eg, *Capnocytophaga* sp. oral taxon 323, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella oris*, *Tannerella forsythia,* and *Tannerella* sp. oral taxon HOT−286) and Gram-positive species (eg, *Olsenella uli* and *Parvimonas micra*). Transcripts encoding moonlighting proteins (eg, glycolytic proteins, translational elongation factors, chaperonin, and heat shock proteins) were highly expressed, potentially affecting bacterial adhesion, biofilm formation, host defense evasion, and inflammation induction. Endodontic bacteria expressed genes conferring resistance to antibiotic classes commonly used in dentistry, with a high prevalence and expression of tetracycline and lincosamide resistance genes. Antibiotic efflux and antibiotic target alteration/protection were the main resistance mechanisms. **Conclusions:** Metatranscriptomics revealed the activity of potential endodontic pathogens, which expressed putative virulence factors and a wide diversity of genes potentially involved in AR. *(J Endod 2024;50:1059–1072.)*

KEY WORDS

Antibiotic resistance; endodontic infections; human oral microbiome; Metatranscriptomics; moonlighting proteins

SIGNIFICANCE

Metatranscriptomic analysis of endodontic infections revealed virulence characteristics possibly related to the pathogenesis of apical periodontitis. The endodontic microbiome harbored several antimicrobial resistance genes, representing a reservoir of resistance, especially to tetracycline and lincosamide.

Over the last decades, high-throughput sequencing has enabled the screening of the 16S ribosomal RNA region in hundreds of thousands of microbial ecosystems, including the healthy and diseased oral microbiome[1], therefore also expanding the knowledge of the bacterial communities in infected root canals[2,3]. In particular, a recent study based on the combined analysis of 16S rRNA genes (DNA) and transcripts (RNA) of infected root canal microbiomes showed that only part of the community was transcriptionally active[4]. However, as this study was restricted to the analysis of 16S rRNA, no additional information about microbial interactions or virulence was revealed.

In recent years, metatranscriptomics (ie, the collective analysis of gene expression in a microbiome) has provided insights into how microbial communities behave in oral health and disease. Thus far, studies on the metatranscriptome of the oral microbiome have been restricted to caries and periodontal disease[5-13]. Such an approach expanded the knowledge of sugar metabolism in caries[10]. In the case of periodontal

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disease, it was found that microbial communities displayed conserved metabolic gene expression profiles despite their diverse composition[8,12]. Moreover, metatranscriptomics revealed "keystone" pathogens in periodontal disease[13]. To the best of our knowledge, metatranscriptomics has yet to be applied to the functional analysis of the endodontic microbiome; this could shed new light on the nature of endodontic pathogens and the core activities associated with apical periodontitis.

Metatranscriptomics also enables the profiling of the resistome, that is, the collective analysis of antimicrobial resistance (AR) genes[14-18]. As bacteria can express many AR genes in their natural environments, this technique provides information regarding the potential risk of AR in the community[14-18]. Metatranscriptomics has contributed to a deeper understanding of the AR gene activity in different environments, including the human upper respiratory tract[15], human/animal gut[16], wastewater[17], and hospital effluents[18]. Similarly, it could deepen the knowledge of the endodontic resistome. This information would be particularly important in cases requiring systemic antibiotic therapy (eg, spreading infections and systemic involvement) or intracanal antibiotic treatment (eg, regenerative endodontic treatment)[19,20]. Therefore, this preliminary study aimed to explore the endodontic microbiome's gene expression, including the search for virulence and antibiotic resistance profiles of endodontic pathogens.

MATERIALS AND METHODS

Patients and Samples

This preliminary analysis included ten patients (five with primary and five with persistent/secondary endodontic infections) participating in an ongoing study. This study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of the Institution. All participants signed an informed consent form before treatment.

The inclusion criteria were adult patients requiring endodontic treatment/retreatment of single-rooted teeth with radiographic evidence of apical periodontitis and no clinical symptoms. A negative response to sensitivity pulp tests confirmed pulp necrosis. Endodontic treatment failure was determined by the presence of apical periodontitis in teeth treated for more than four years. Teeth with significant loss of coronary structure that could not be adequately isolated with a rubber dam were excluded. In addition, patients who received antibiotic therapy during the previous three months or had periodontal pockets with depths greater than 4 mm were excluded from the study.

Sampling procedures were performed as previously described[4]. After removing the dental plaque and caries, the tooth and rubber dam were disinfected with 30% H_2O_2, 2.5% NaOCl, and 5% sodium thiosulfate. The access cavity was performed under irrigation with a sterile saline solution. The disinfection protocol was repeated before entering the pulp chamber, and the access cavity was completed. Then, a control sample from the disinfected cavity was performed with paper cones and stored at −80°C. The absence of bacterial DNA/complementary DNA in the control samples was verified by polymerase chain reaction using universal primers for bacteria (5′-GAG AGT TTG ATY MTG GCT CAG-3'; 5′- GAA GGA GGT GWT CCA RCC GCA-3′) as previously described[21].

In primary endodontic infections, the root canal was filled with sterile saline and the working length was established using an electronic apex locator (J. Morita Brasil, São Paulo, SP, Brazil). After applying a filing motion with a Hedström file, five sterile paper points were placed consecutively at working length (1 minute each) in cryotubes containing 300 μL of RNAlater solution (Life Technologies, Carlsbad, CA, USA) stored at −80°C. In persistent/secondary endodontic infections, the root filling was removed with reciprocating instruments (Wave One file, Dentsply Sirona, Ballaigues, Switzerland) and irrigation with sterile saline solution. Then, root canal sampling was performed as described above.

RNA Extraction, Library Preparation, and Sequencing

RNA from endodontic samples was extracted using the MasterPure Complete DNA and RNA Purification Kit (Epicentre Technologies, Madison, WI, USA) as previously described[4]. Briefly, cell lysis was performed using 450 μL of MasterPure tissue and cell lysis solution and 300 mg of glass beads (diameter, 0.1 mm; BioSpec Products Inc., Bartlesville, OK, USA) in a Mini-Beadbeater (BioSpec Products Inc.). Then, the protein was removed using Proteinase K and MasterPure protein precipitation solution. After total nucleic acid precipitation, DNA removal was performed using 200 μl of MasterPure DNase I solution following the manufacturer's instructions. An additional DNAse treatment was performed using DNase I (Invitrogen, São Paulo, Brazil) before the reverse transcription reaction. The absence of contaminating DNA in RNA samples was confirmed by polymerase chain reaction using universal primers for bacteria[21]. The complementary DNA synthesis was performed using total RNA as a template and random primers of the SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA). Then, the Illumina TruSeq Stranded Total RNA protocol was used to produce Illumina sequencing libraries. The libraries were then sequenced on a NovaSeq6000 system (Illumina, San Diego, CA, USA) at the Functional Genomics Center Zurich, ETH-University of Zurich.

Data Analysis

The raw reads (100 bp) were preprocessed using Trimmomatic v0.39[22] with the parameters "ILLUMINACLIP: adapters.fa:1:30:10 LEADING:0 TRAILING:0 SLIDINGWINDOW: 5:15 AVGQUAL:20 MINLEN:80". We then assembled the preprocessed reads using Trinity v2.11 with default settings[23]. We used Transdecoder v5.5.0[24] to reduce the redundancy of the resulting putative transcripts, and assembled transcripts, at least 150 bases long were retained. The mean length of all the transcripts analyzed was 295 bp. We mapped these assembled transcripts against the kraken2 bacterial database[25]. Kraken2 uses complete genomes from RefSeq (https://www.ncbi.nlm.nih.gov/refseq) as a reference database for bacterial classification. The sequences with a taxonomic classification were then annotated against UniProtKB (https://www.uniprot.org/) using DIAMOND v2.0.5[26] with parameters "-k 10 -e 0.001". Set-based gene analysis was performed using DAVID[27]. They were also annotated against the Comprehensive Antibiotic Resistance Database (CARD; card.mcmaster.ca) to identify the AR-like genes using an 80% amino acid identity. The AR gene families, antibiotic classes, and resistance mechanisms (RMs) were also classified using CARD. The CARD database provides a high-quality reference for searching molecular determinants of antibiotic resistance, containing 5010 reference sequences, 1933 mutations, and 3004 publications[28]. The comparison of the number of AR-like genes among phyla was performed using a two-way analysis of variance as implemented in the R function *stat_compare_means*.

RESULTS

All control samples of the access cavities were negative for bacteria. Clinical data and sequencing results of the cases included in this study are shown in Table 1. The amount of RNA was sufficient to analyze eight samples: five from primary (EP03, EP04, EP06, EP09, EP10) and three from persistent/secondary infections (EP05, EP07, EP08). Despite the limited number of root canal samples, the high sequencing depth allowed the identification of a large set of expressed genes, a total of 10,928 annotated transcripts in eight samples.

TABLE 1 - Patient Data, Clinical Features, and Sequencing Results of Selected Cases With Primary Endodontic Infections and Persistent/Secondary Endodontic Infections

Patient	Endodontic infections	Sequencing results	Gender	Age	Tooth	Periapical radiolucency	Pain	Swelling	Sinus tract	Apical diagnosis
EP01	Persistent/ secondary	N	Male	27	7	Y	N	N	Y	CAA
EP02	Persistent/ secondary	N	Female	35	7	Y	N	N	N	AAP
EP03	Primary	Y	Female	49	9	Y	N	N	N	AAP
EP04	Primary	Y	Male	37	9	Y	N	N	N	AAP
EP05	Persistent/ secondary	Y	Female	55	10	Y	N	N	N	AAP
EP06	Primary	Y	Female	32	9	Y	N	N	Y	CAA
EP07	Persistent/ secondary	Y	Male	37	9	Y	N	N	N	AAP
EP08	Persistent/ secondary	Y	Male	27	9	Y	N	N	N	AAP
EP09	Primary	Y	Female	33	10	Y	N	N	N	AAP
EP10	Primary	Y	Female	21	9	Y	N	N	N	AAP

AAP, asymptomatic apical periodontitis; CAA, chronic apical abscess.

Overview of the Microbial Community

A total of 166 species were identified in eight samples belonging to ten different phyla. The sample-wide variance in phyla abundance was high (Fig. 1*A*). Proteobacteria (28.06%), Bacteroidetes (26.70%), Firmicutes (19.68%), and Actinobacteria (19.58%) were detected in all samples and represented the most abundant phyla. Fusobacteria (2.68%), Synergistetes (1.88%), and Spirochetes (0.67%) were identified in at least five samples and represented more than 0.5% of the community. Of the 166 species, 53 species belonging to 31 genera represented the most abundant taxonomic groups (Supplemental Table S1). The ten most abundant genera and species are represented in Figure 1*B* and *C*, respectively.

Metatranscriptome and Functional Annotation

The assembly of the metatranscriptomes revealed 152 species transcriptionally active in at least two samples. The average number of annotated transcripts per species was 72, ranging from 0 (five species) to 2096 (*Cutibacterium acnes*). The percentages of annotated genes for each species, relative to the total number of transcripts in the same species, are shown in Supplemental Figure S1.

Across the metatranscriptomes, we identified 10,928 annotated transcripts, corresponding to 6,514 unique Uniprot IDs, which mapped to 3,084 unique gene IDs. In order to search for the genes with the most

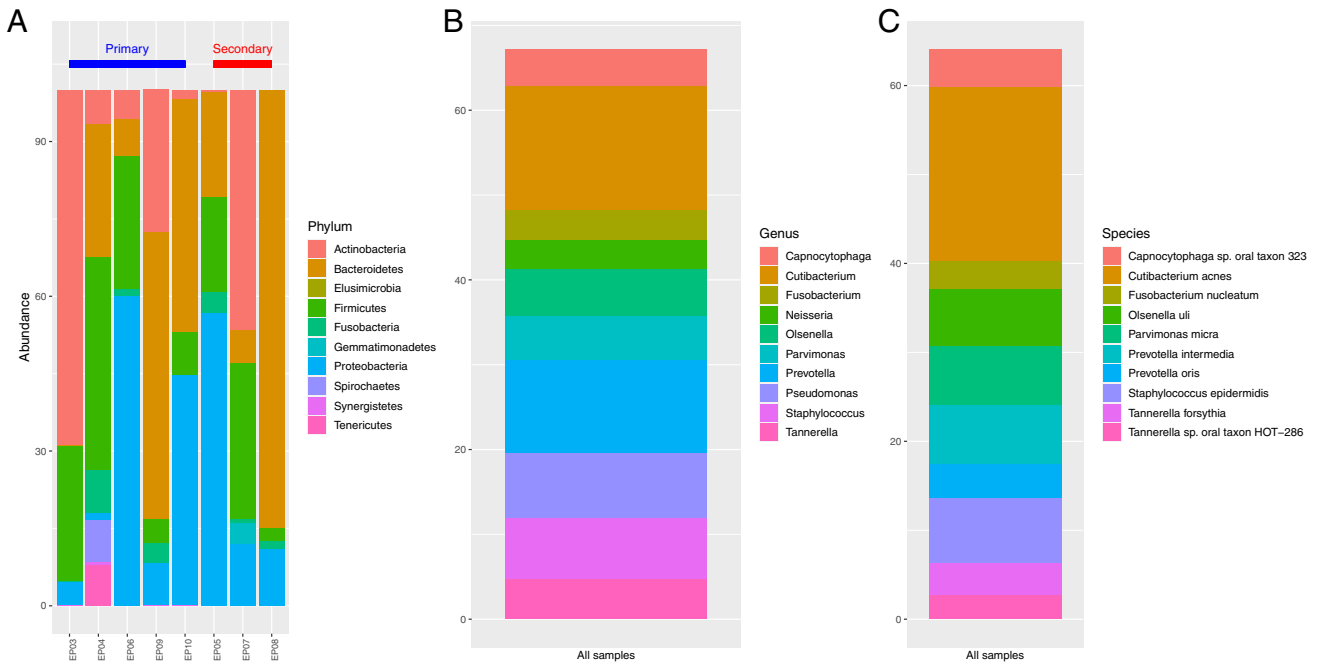

FIGURE 1 – (*A*) Relative abundance (%) of bacterial phyla in root canal samples of 5 teeth with primary endodontic infections and 3 with persistent/secondary endodontic infections; (*B*) Ten most abundant genera across all samples; (*C*) Ten most abundant species across all samples.

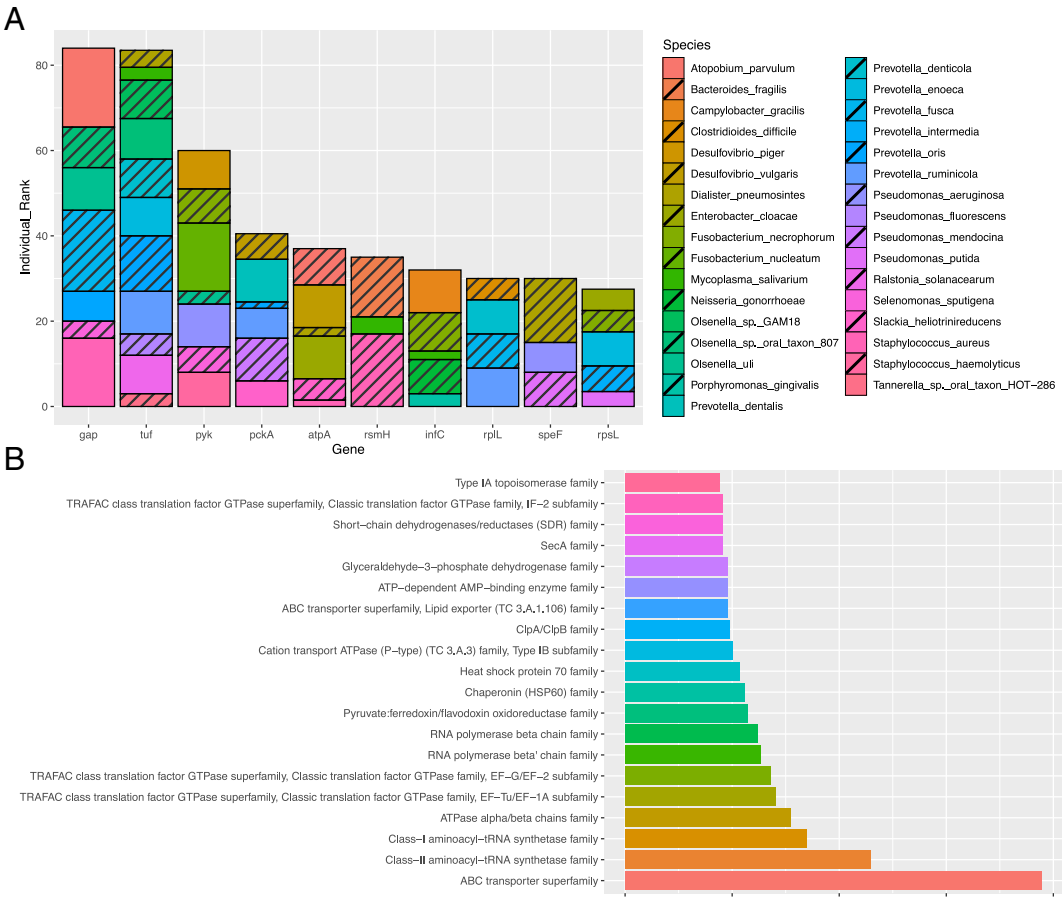

FIGURE 2 – (*A*) Top 10 most abundant annotated transcripts in the microbiome: glyceraldehyde-3-phosphate dehydrogenase (*gap*), elongation factor (*tuf*), pyruvate kinase (*pyk*), phosphoenolpyruvate carboxykinase (*pck*A), ATP synthase subunit alpha (*atp*A), ribosomal RNA small subunit methyltransferase H (*rsm*H), translation initiation factor IF-3 (*inf*C), large ribosomal subunit protein bL12 (rplL), inducible ornithine decarboxylase (*spe*F), and small ribosomal subunit protein uS12 (rpsL); (*B*) The 20 most prevalent protein families in the metatranscriptomic data from eight root canal samples.

prominent presence across the species, we ranked the top 10 most abundant annotated transcripts in each species and summed the rank across the species (Fig. 2*A*). Among the overall most expressed genes, the top 3 were glyceraldehyde-3-phosphate dehydrogenase, abbreviated GAPDH (*gap*), elongation factor (*tuf*), and pyruvate kinase, with an overall expression rank of 2 to 3 times (*gap* and *tuf*) and 1.5 to 2 times (pyruvate kinase) higher than the average of the genes ranked. In total, 1,612 protein families were associated with the annotated Uniprot IDs. The adenosine triphosphate-binding cassette (ABC) transporter superfamily was the most prevalent protein family. The 20 most prevalent protein families are listed in Figure 2*B*. As an additional functional annotation, we screened the three Gene Ontology (GO) databases: biological process (GO BP), molecular function (GO MF), and cellular component (GO CC). In total, 2,372 GO categories were associated with the 6,514 Uniprot IDs. Most of the identified GO terms belonged to GO MF (1,540), followed by GO BP (1,030) and GO CC (153). The 20 most prevalent GO terms are shown in Supplemental Figure S2 and included mainly adenosine triphosphate-binding functions in GO MF, translation and glycolytic processes in GO BP, and the major components of the cell (cytoplasm, membrane, and ribosome) in GO CC.

Resistome

A total of 423 AR-like genes were found in 85 species and classified into 141 different types of AR genes. AR-like genes conferring resistance to antibiotics commonly used in dentistry are shown in Table 2. Genes potentially associated with resistance to other antibiotics and multidrug resistance are shown in Tables 3 and 4, respectively.

The number of identified AR-like genes varied among the samples (Fig. 3*A*). The most prevalent AR gene family was the ABC antibiotic efflux pump, present in all samples (Fig. 3*B*). Antibiotic efflux was the most commonly associated RM, followed by antibiotic target alteration and protection (Fig. 3*D*). Several drug classes were associated with the AR-like genes, with a relatively high prevalence and expression of tetracycline and lincosamide resistance genes (Fig. 3*C*, Supplemental Tables S2). The species with the largest number of AR genes were *Cutibacterium acnes*, *Staphylococcus epidermidis*, *Olsenella uli*, *Parvimonas micra*, *Tannerella forsythia*, *Prevotella intermedia*, *Capnocytophaga* sp. oral taxon 323, and *Fusobacterium nucleatum* (Fig. 4*A*, Supplemental Tables S3). Given the variation of AR-like genes in different species, we tested whether specific phyla harbored a cumulative, more abundant resistome by comparing the distribution of AR-like genes in the species belonging to the same phylum. We did not find any significant difference (Supplemental Figure S3), indicating that the AR-like genes were similarly spread across the phyla of this microbiome. A pair-wise analysis of the abundances of microorganisms and AR-like

TABLE 2 - Antimicrobial Resistance Genes Conferring Resistance to Antibiotics Commonly Used in Dentistry

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Beta-lactam	*ACC-3 CAR-1*	ACC beta-lactamase CAR beta-lactamase	Antibiotic inactivation antibiotic inactivation	*Capnocytophaga, Cutibacterium, Parvimonas, Staphylococcus*
	GOB-15, GOB-25	GOB beta-lactamase	Antibiotic inactivation	
	mecB	Methicillin resistant PBP2	Antibiotic target replacement	
Fluoroquinolone	*Abau_AbaQ, norB*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Clostridium, Cutibacterium, Desulfobulbus, Mycoplasma, Neisseria, Parvimonas, Prevotella, Staphylococcus, Streptococcus*
	patA, patB	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	
Lincosamide		Miscellaneous ABC-F subfamily		
	lmrC	ATP-binding cassette ribosomal protection proteins	Antibiotic target protection	*Bacillus, Campylobacter, Cutibacterium, Staphylococcus, Streptococcus*
	lmrD	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	
Macrolide		Miscellaneous ABC-F subfamily		*Bacteroides, Campylobacter, Capnocytophaga, Cutibacterium, Desulfovibrio, Faecalibacterium, Hydrogenophilus, Neisseria, Olsenella, Parvimonas, Peptoniphilus, Prevotella, Propionibacterium, Pseudomonas, Ralstonia, Staphylococcus, Tannerella, Treponema, Xanthomonas*
	carA, oleB, srmB	ATP-binding cassette ribosomal protection proteins	Antibiotic target protection	
	macB, oleC	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	
Macrolide-	*tlrB*	Non-erm 23S ribosomal RNA methyltransferase (G748) Miscellaneous ABC-F subfamily	Antibiotic target alteration	*Cutibacterium, Olsenella, Treponema*
Lincosamide (ML)	*tlrC*	ATP-binding cassette ribosomal protection proteins	Antibiotic target protection	
Macrolide-Lincosamide-Streptogramin (MLS)	*erm(40), ErmE, ErmX*	Erm 23S ribosomal RNA methyltransferase	Antibiotic target alteration	*Cutibacterium, Neisseria, Rothia, Tannerella*
Nitroimidazole	*msbA*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Enterobacter, Faecalibacterium, Klebsiella, unclassified Lachnospiraceae, Olsenella, Parvimonas, Prevotella, Ralstonia, Tannerella, Treponema, Staphylococcus, Streptococcus*
Tetracycline	*otr(A), tet(W/N/W), tet32, tet36, tet44, tetB(P), tetO, tetQ, tetT, tetW*	Tetracycline-resistant ribosomal protection protein	Antibiotic target protection	*Alistipes, Cutibacterium, Desulfobulbus, Desulfovibrio, Enterobacter, Eubacterium, Faecalibacterium, Fusobacterium, Haemophilus, Hydrogenophilus, Mogibacterium, Mycoplasma, Olsenella, Orientia gen. nov., Parvimonas, Prevotella, Pseudomonas, Tannerella, Selenomonas, Staphylococcus, Streptococcus, Xanthomonas*
	otr(B), tap, tet(43), tetC, tetA(48)	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	
	tetA(46), tetA(60), tetB(46), tetB(60),Txr	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	

TABLE 3 - Antimicrobial Resistance Genes Conferring Resistance to Other Antibiotics Used in Medicine

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Aminocoumarin	*mdtB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Bacteroides, Capnocytophaga, Cutibacterium, Desulfobulbus, Desulfovibrio, Eubacterium, Fusobacterium, Haemophilus, Lachnospiraceae, Mogibacterium, Neisseria, Olsenella, Parvimonas, Prevotella, Pseudomonas, Staphylococcus, Tannerella, Treponema*
	novA, RanA, RanB	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	
	kdpE	kdpDE	Antibiotic efflux	
Bicyclomycin-like antibiotic	*bcr-1*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Capnocytophaga, Staphylococcus*
Diaminopyrimidine	*dfrA26*	Trimethoprim resistant dihydrofolate reductase dfr	Antibiotic target replacement	*Capnocytophaga, Cutibacterium*
	dfrA31	Trimethoprim resistant dihydrofolate reductase dfr	Antibiotic target replacement	
Elfamycin	*facT*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium*
Glycopeptide	*vanC, vanD, vanL, vanN, vanO*	Glycopeptide resistance gene cluster, Van ligase	Antibiotic target alteration	*Campylobacter, Capnocytophaga, Cutibacterium, Desulfovibrio, Fusobacterium, Haemophilus, Olsenella, Prevotella, Pseudomonas, Staphylococcus, Tannerella, Xanthomonas*
	vanB, vanHA, vanHD, vanHF, vanHO	Glycopeptide resistance gene cluster, vanH	Antibiotic target alteration	
	vanKI	Glycopeptide resistance gene cluster, vanK	Antibiotic target alteration	
	vanRB, vanRE, vanRF, vanRM	Glycopeptide resistance gene cluster, vanR	Antibiotic target alteration	
	vanSB, vanSL, vanSN	Glycopeptide resistance gene cluster, vanS	Antibiotic target alteration	
	vanTC, vanTE, van TG	Glycopeptide resistance gene cluster, vanT	Antibiotic target alteration	
Mupirocin-like antibiotic	*Bbif_ileS_MUP, mupA, mupB*	Antibiotic-resistant isoleucyl-tRNA synthetase (ileS)	Antibiotic target alteration	*Bacillus, Capnocytophaga, Cutibacterium, Desulfovibrio, Eubacterium, Fusobacterium, Olsenella, Parvimonas, Prevotella, Selenomonas, Staphylococcus, Tannerella*
Peptide	*arnA, pmrF, ugd*	pmr phosphoethanolamine transferase	Antibiotic target alteration	*Bacteroides, Campylobacter, Capnocytophaga, Cutibacterium, Desulfovibrio, Fusobacterium, Neisseria, Olsenella, Parvimonas, Prevotella, Pseudomonas, Ralstonia, Staphylococcus, Tannerella, Treponema*
	bacA	Undecaprenyl pyrophosphate related proteins	Antibiotic target alteration	
	basS, cprR	pmr phosphoethanolamine transferase	Antibiotic target alteration, antibiotic efflux	
	bcrA, yojI	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	
	OmpA	General Bacterial Porin with reduced permeability to peptide antibiotics	Reduced permeability to antibiotic	
	pgpB	Lipid A phosphatase	Antibiotic target alteration	
	rosA	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	

(*continued on next page*)

TABLE 3 - Continued

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Phenicol	*catB8*	Chloramphenicol acetyltransferase (CAT)	Antibiotic inactivation	*Olsenella, Pseudomonas*
	pp-flo	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	
Phosphonic acid	*Abau_AbaF*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Eubacterium, Parvimonas, Staphylococcus*
	Ctra_murA_FOF	Antibiotic-resistant murA transferase	Antibiotic target alteration	
	FosD	fosfomycin thiol transferase	Antibiotic inactivation	
Pleuromutilin	*TaeA*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Barnesiella, Capnocytophaga, Cutibacterium, Enterobacter, Olsenella, Prevotella, Pseudomonas, Staphylococcus, Streptococcus, Tannerella*
Rifamycin	*RbpA*	RbpA bacterial RNA polymerase-binding protein	Antibiotic target protection	*Alistipes, Atopobium, Bacteroides, Campylobacter, Capnocytophaga, Cutibacterium, Desulfovibrio, Fusobacterium, Hungatella, Mogibacterium, Mycoplasma, Neisseria, Olsenella, Paeniclostridium, Parolsenella, Parvimonas, Pasteurella, Peptoniphilus, Porphyromonas, Prevotella, Pseudomonas, Selenomonas, Staphylococc, Streptococcus, Tannerella, Treponema*
	Bado_rpoB_RIF, rpoB2	Rifamycin-resistant beta-subunit of RNA polymerase (rpoB)	Antibiotic target alteration, antibiotic target replacement	
Streptogramin	*Vatl*	Streptogramin vat acetyltransferase	Antibiotic inactivation	*Cutibacterium*
Sulfonamide	*sul4*	Sulfonamide resistant sul	Antibiotic target replacement	*Cutibacterium, Prevotella*

genes in the samples indicated mid-to-high correlation levels between a dozen species and several AMR gene families (Fig. 4*B*). In particular, resistance-nodulation-cell division antibiotic efflux pump, pmr phosphoethanolamine transferase, and Cfr 23S ribosomal RNA methyltransferase were the AR gene families that showed the highest levels of correlations with the abundance of certain species. These species belonged to the phyla Proteobacteria, Firmicutes, Fusobacteria, and Spirochetes.

DISCUSSION

This preliminary study explored the root canal microbiome's composition, functional signatures, and resistome by performing next-generation, microbiome-wide RNA sequencing, generating the first dataset capturing the metatranscriptome of infected root canals. Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria represented the dominant phyla, whereas Fusobacteria, Spirochetes, and Synergistetes were among the nondominant phyla. The abundance of these phyla varied greatly between samples, reflecting a dynamic and competitive ecosystem certainly shaped by individual conditions of the host environment. These phyla have been identified in numerous other studies of the microbial populations in infected root canals[2,3]. In contrast to a previous RNA-based study[4], a higher abundance of Proteobacteria was found in the present study. The most likely reasons behind such differences are small sample size and high interindividual variation. At the genus and species levels, metatranscriptomics data confirmed the activity of potential endodontic pathogens, represented mainly by obligate anaerobes[29].

An interesting finding of the current study was the high expression levels of transcripts encoding moonlighting proteins. This term refers to proteins with two or more independent functions in the cell[30]. Many proteins participating in basic molecular mechanisms may also perform additional biological activities contributing to bacterial virulence, including glycolytic enzyme GAPDH, translational *tuf*, chaperonin, and heat shock proteins[30]. The glycolytic enzyme GAPDH (encoded by *gap,* the overall most expressed gene in the samples) can bind to various human proteins, facilitate host colonization, and evade host immune cells[30]. Likewise, translational *tuf* (the second most expressed gene in samples) can also favor bacterial adhesion[30]. Chaperonins/heat shock proteins that promote protein recovery from stress-induced unfolding may also be involved in bacterial adhesion and biofilm formation[30].

TABLE 4 - Antimicrobial Resistance Genes Conferring Multidrug Resistance

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Peptide antibiotic, cephalosporin, penam	*abcA*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium*
Tetracycline antibiotic, penem, phenicol antibiotic, rifamycin antibiotic, carbapenem, diaminopyrimidine antibiotic, lincosamide antibiotic, fluoroquinolone antibiotic, cephalosporin, macrolide antibiotic	*adeN*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Parvimonas*
Tetracycline antibiotic, glycylcycline	*adeR*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Staphylococcus*
Disinfecting agents and antiseptics, fluoroquinolone antibiotic	*arlR*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Olsenella, Parvimonas, Prevotella, Staphylococcus*
Disinfecting agents and antiseptics, fluoroquinolone antibiotic	*arlS*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Capnocytophaga, Cutibacterium, Parvimonas, Staphylococcus*
Phenicol antibiotic, oxazolidinone antibiotic, streptogramin antibiotic, lincosamide antibiotic, glycopeptide antibiotic	*cfr(D)*	Cfr 23S ribosomal RNA methyltransferase	Antibiotic target alteration	*Prevotella*
Streptogramin antibiotic, lincosamide antibiotic, oxazolidinone antibiotic, phenicol antibiotic, pleuromutilin antibiotic, streptogramin A antibiotic	*clbC*	Cfr 23S ribosomal RNA methyltransferase	Antibiotic target alteration	*Cutibacterium*
Fusidane antibiotic, macrolide antibiotic, cephalosporin, fluoroquinolone antibiotic	*cmeB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Barnesiella, Pseudomonas, Tannerella*
Aminoglycoside antibiotic, aminocoumarin antibiotic	*cpxA*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium*
Penam, tetracycline antibiotic	*Cstr_tetA*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Prevotella*
Tetracycline antibiotic, rifamycin antibiotic, cephalosporin, disinfecting agents and antiseptics, penam, phenicol antibiotic, glycylcycline, fluoroquinolone antibiotic	*EcoI_acrA*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Efflux pump complex or subunit conferring antibiotic resistance	*Cutibacterium acnes*
Fluoroquinolone antibiotic, macrolide antibiotic, rifamycin antibiotic	*efrA*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Capnocytophaga, Neisseria*
Macrolide antibiotic, rifamycin antibiotic, fluoroquinolone antibiotic	*efrB*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Capnocytophaga, Hydrogenophilus, Olsenella, Propionibacterium, Pseudomonas, Staphylococcus, Xanthomonas*
Fluoroquinolone antibiotic, penam, tetracycline antibiotic, macrolide antibiotic	*evgA*	Major facilitator superfamily (MFS) antibiotic efflux pump, resistance- nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Haemophilus, Neisseria, Parvimonas, Prevotella, Pseudomonas*
Fluoroquinolone antibiotic, penam, tetracycline antibiotic, macrolide antibiotic	*evgS*	Major facilitator superfamily (MFS) antibiotic efflux pump, resistance- nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Bacteroides, Cutibacterium, Olsenella, Treponema*

(*continued on next page*)

TABLE 4 - Continued

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Nitroimidazole antibiotic, fluoroquinolone antibiotic, tetracycline antibiotic	*hp1181*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Moraxella, Xanthomonas*
Nucleoside antibiotic, lincosamide antibiotic	*lmrB*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Staphylococcus*
Peptide antibiotic, aminocoumarin antibiotic, carbapenem, rifamycin antibiotic	*LptD*	ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Enterobacter*
Lincosamide antibiotic, streptogramin antibiotic, pleuromutilin antibiotic	*lsaB*	lsa-type ABC-F protein	Antibiotic target protection	*Pseudomonas*
Pleuromutilin antibiotic, streptogramin antibiotic, lincosamide antibiotic	*lsaC*	lsa-type ABC-F protein	Antibiotic target protection	*Cutibacterium, Haemophilus, Orientia, Pseudomonas*
Tetracycline antibiotic, glycylcycline, carbapenem, penem, cephamycin, penam, phenicol antibiotic, disinfecting agents and antiseptics, monobactam, cephalosporin, rifamycin antibiotic, fluoroquinolone antibiotic	*marA*	Resistance-nodulation-cell division (RND) antibiotic efflux pump, General Bacterial Porin with reduced permeability to beta-lactams	Reduced permeability to antibiotic, antibiotic efflux	*Parvimonas*
Phenicol antibiotic, cephalosporin, carbapenem, penam, monobactam, penem, cephamycin	*mdsC*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Pseudomonas*
Peptide antibiotic, sulfonamide antibiotic, diaminopyrimidine antibiotic, aminocoumarin antibiotic, carbapenem, phenicol antibiotic, tetracycline antibiotic, penam, fluoroquinolone antibiotic, cephalosporin, macrolide antibiotic, monobactam, cephamycin, penem	*MexB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Pseudomonas*
Tetracycline antibiotic, fluoroquinolone antibiotic, disinfecting agents and antiseptics	*mexI*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Tannerella*
Carbapenem, macrolide antibiotic, disinfecting agents and antiseptics, tetracycline antibiotic, phenicol antibiotic, diaminopyrimidine antibiotic	*mexQ*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Pseudomonas*
Peptide antibiotic, cephalosporin, fluoroquinolone antibiotic, penam, tetracycline antibiotic, disinfecting agents and antiseptics	*mgrA*	Major facilitator superfamily (MFS) antibiotic efflux pump, ATP-binding cassette (ABC) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium*
Penam, macrolide antibiotic	*mtrA*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium*
Macrolide antibiotic, penam	*mtrD*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Neisseria, Fusobacterium, Olsenella, Staphylococcus*
Macrolide antibiotic, aminocoumarin antibiotic, tetracycline antibiotic, monobactam	*MuxB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Desulfovibrio, Enterobacter, Pseudomonas, Staphylococcus, Streptococcus, Tannerella*
Tetracycline antibiotic, macrolide antibiotic	*OprM*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Pseudomonas*

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TABLE 4 - Continued

Antibiotic classes	AR genes	AR gene families	Resistance mechanisms	Bacterial genera
Phenicol antibiotic, oxazolidinone antibiotic	*optrA*	Miscellaneous ABC-F subfamily ATP-binding cassette ribosomal protection proteins	Antibiotic target protection	*Bacteroidales, Pseudomonas*
Tetracycline antibiotic, glycylcycline, nitrofuran antibiotic, diaminopyrimidine antibiotic, fluoroquinolone antibiotic	*oqxB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Prevotella*
Peptide antibiotic, aminoglycoside antibiotic, diaminopyrimidine antibiotic, sulfonamide antibiotic, aminocoumarin antibiotic, penam, fluoroquinolone antibiotic, cephalosporin, carbapenem, macrolide antibiotic, monobactam, tetracycline antibiotic, phenicol antibiotic, cephamycin, penem	*Paer_CpxR*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Pseudomonas, Neisseria, Olsenella, Parvimonas, Prevotella, Pseudomonas, Staphylococcus, Streptococcus, Tannerella, Treponema*
Aminoglycoside antibiotic, fluoroquinolone antibiotic, tetracycline antibiotic, disinfecting agents and antiseptics, macrolide antibiotic, carbapenem, cephamycin, cephalosporin, penam, monobactam, phenicol antibiotic, penem	*ParS*	Outer Membrane Porin (Opr), resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux, reduced permeability to antibiotic	*Halomonas, Pseudomonas*
Disinfecting agents and antiseptics, aminoglycoside antibiotic, fluoroquinolone antibiotic	*PmpM*	Multidrug and toxic compound extrusion (MATE) transporter	Antibiotic efflux	*Pseudomonas, Tannerella*
Tetracycline antibiotic, phenicol antibiotic, oxazolidinone antibiotic	*poxtA*	Miscellaneous ABC-F subfamily ATP-binding cassette ribosomal protection proteins	Antibiotic target protection	*Cutibacterium, Pseudomonas*
Streptogramin antibiotic, pleuromutilin antibiotic, lincosamide antibiotic, streptogramin A antibiotic	*salA*	sal-type ABC-F protein	Antibiotic target protection	*Staphylococcus, Tannerella*
Aminoglycoside antibiotic, macrolide antibiotic, phenicol antibiotic, oxazolidinone antibiotic, diaminopyrimidine antibiotic	*Saur_LmrS*	Major facilitator superfamily (MFS) antibiotic efflux pump	Antibiotic efflux	*Staphylococcus, Olsenella, Prevotella, Tannerella*
Cephalosporin, cephamycin, aminoglycoside antibiotic, penam	*smeB*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Tannerella*
Cephalosporin, cephamycin, penam, aminoglycoside antibiotic	*smeR*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Campylobacter*
Cephalosporin, aminoglycoside antibiotic, cephamycin, penam	*smeS*	Resistance-nodulation-cell division (RND) antibiotic efflux pump	Antibiotic efflux	*Cutibacterium, Xanthomonas*
Streptogramin antibiotic, pleuromutilin antibiotic, streptogramin A antibiotic, lincosamide antibiotic	*vgaE*	vga-type ABC-F protein	Antibiotic target protection	*Staphylococcus epidermidis*
Aminoglycoside antibiotic, phenicol antibiotic, tetracycline antibiotic	*ykkD*	Small multidrug resistance (SMR) antibiotic efflux pump	Antibiotic efflux	*Staphylococcus, Parvimonas*

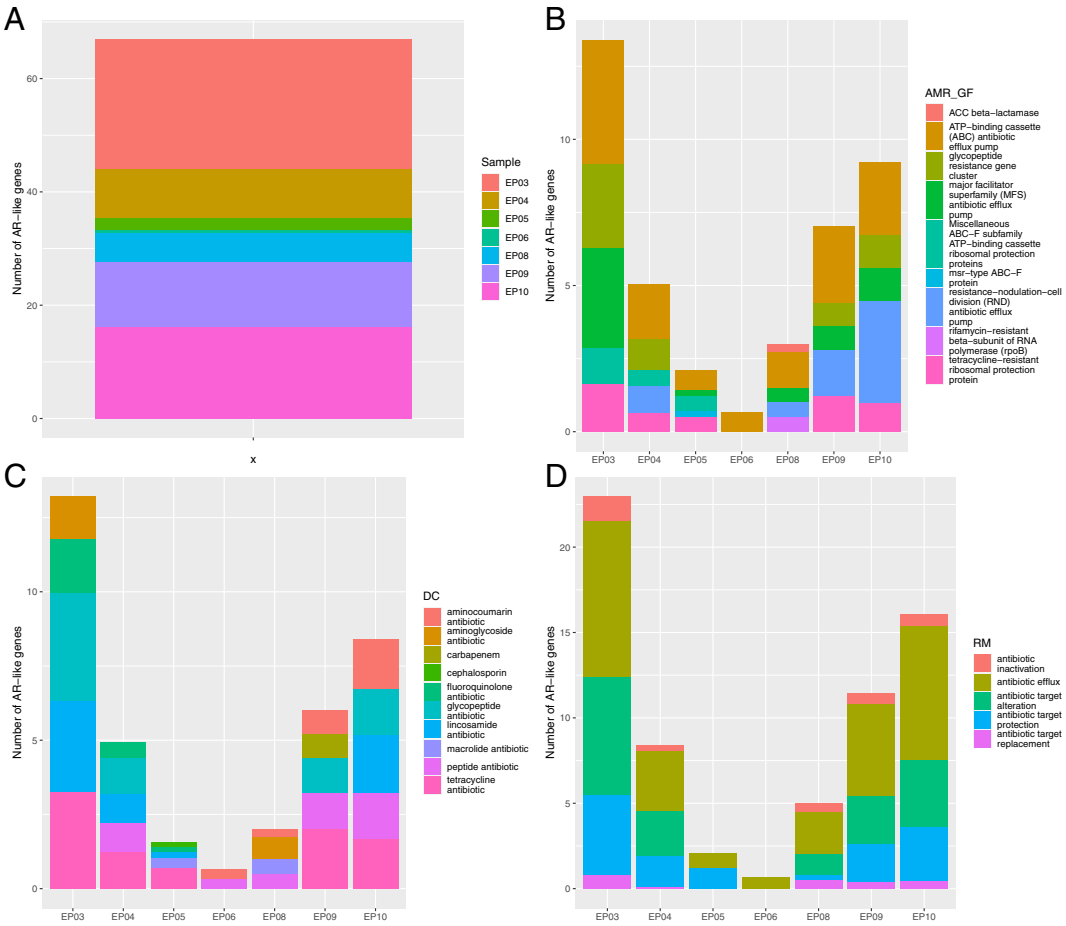

FIGURE 3 – (*A*) Number of antibiotic resistance (AR) genes, (*B*) AR gene families (AMR_GF); (*C*) drug classes (DCs); and (*D*) antimicrobial resistance mechanisms (RMs) in the metatranscriptomic data from eight root canal samples.

Furthermore, chaperonin can synergize with LPS from Gram-negative bacteria, activate cytokine synthesis, and stimulate bone resorption[30]. Considering the abundance of transcripts encoding moonlighting proteins in root canal samples and their potential effect on bacterial virulence, future studies should investigate the possible role of moonlighting proteins in the pathogenesis of apical periodontitis.

ABC transporters represented the most prevalent protein family. ABC transporters import essential nutrients for bacterial viability, including amino acids, peptides, and metals[31]. This importer system facilitates bacterial adaptation to changes in the host environment. They may also contribute to bacterial virulence by exporting virulent determinants such as toxins and proteins[31]. Likewise, this system can export toxic xenobiotic substances, contributing to the development of AR[31]. Indeed, the most relevant RM of endodontic bacteria was antibiotic efflux by membrane-located transporters.

The ability to pump drugs out of cells is bacteria's most widespread RM[32]. Endodontic bacteria expressed many AR-like genes related to efflux pumps, some of which were specific (eg, tetracycline pumps), whereas others could deliver a wide range of drugs conferring multidrug resistance. Some of these genes conferred additional resistance to disinfecting agents and antiseptics. Examples of bacterial genera expressing antibiotics and antiseptics resistance genes were *Capnocytophaga*, *Cutibacterium*, *Fusobacterium*, *Olsenella*, *Parvimonas*, *Prevotella*, *Staphylococcus*, and *Tannerella*. Considering the wide use of antiseptics in endodontics, future studies should investigate if the AR-like genes related to efflux pumps would affect the activity of endodontic antiseptics and contribute to bacterial selection.

Transcripts mapping to tetracycline resistance genes were the most abundant in the samples. In addition to antibiotic efflux, antibiotic target protection (eg, tetracycline-resistant ribosomal protection protein) was a common RM in the endodontic microbiome, conferring resistance to tetracycline and other drugs of this class (eg, minocycline). In this study, we identified transcripts mapping to 20 different AR-like genes conferring resistance to tetracyclines. These findings confirm the heterogeneity of the Tet family and its high prevalence in the oral microbiome, most likely resulting from many years of antibiotic selective pressure[33]. From a clinical point of view, our data confirmed that the endodontic microbiome could represent a reservoir of AR to tetracycline/minocycline, potentially impairing the clinical efficacy of these antibiotics in the local or systemic treatment of endodontic infections.

AR-like genes conferring resistance to lincosamide (eg, clindamycin) were also common in the resistome analysis. Antibiotic target protection and antibiotic efflux were the main RMs to this drug class. We also detected genes promoting antibiotic target alteration (eg, Erm 23S ribosomal RNA methyltransferase family) that confer resistance to macrolide-lincosamide-streptogramin,

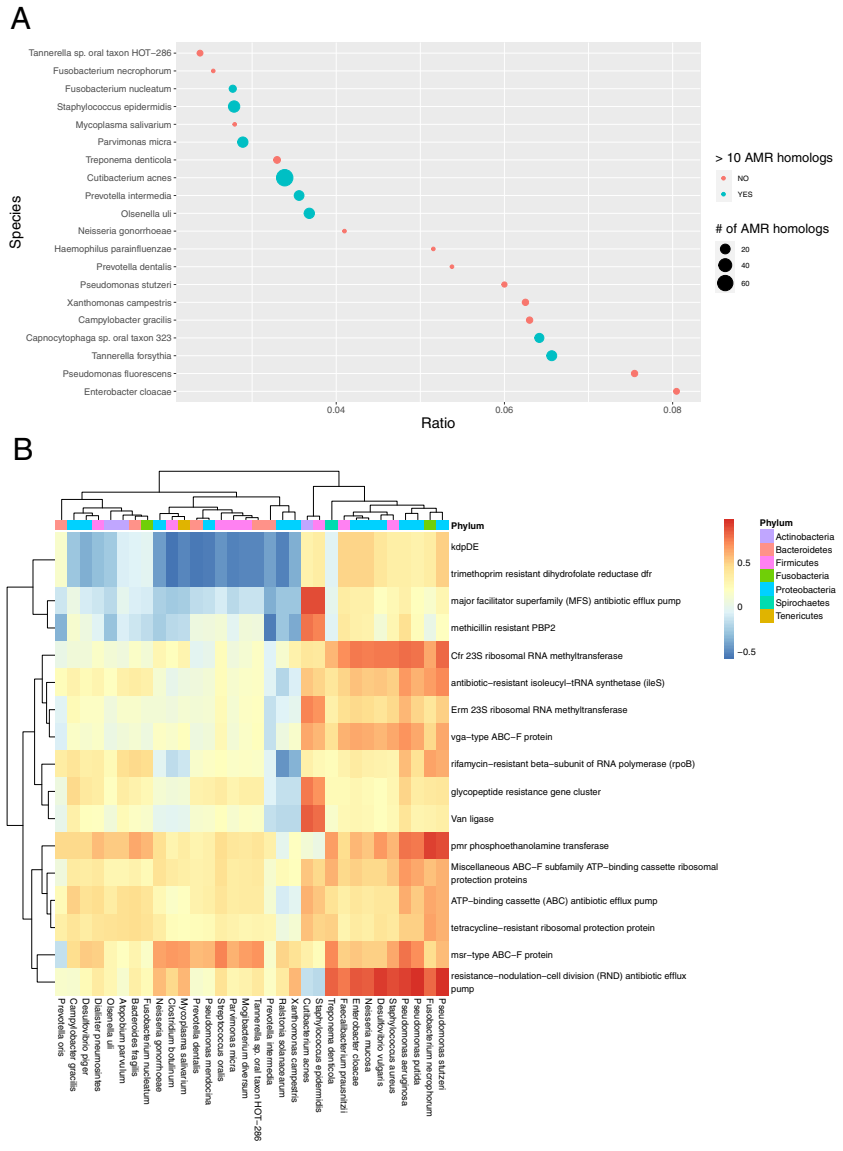

FIGURE 4 – (*A*) The 20 species with the largest number of antibiotic resistance (AR)-like genes; and (*B*) heatmap showing correlation levels between microorganisms and AR-like genes.

which are in agreement with previous metagenomic analysis of oral samples[33]. The resistome analysis also revealed AR genes conferring resistance to beta-lactams (eg, amoxicillin). Antibiotic target replacement (low-affinity penicillin-binding protein) and antibiotic inactivation by beta-lactamases were the main RMs to beta-lactams. Interestingly, endodontic bacteria expressed AR genes under natural conditions (ie, without antibiotic treatment), which agrees with recent reports showing that many AR genes are natural components of different microbial populations and may be constitutively expressed[14-18]. This reinforces the notion that indiscriminate use of antibiotics should be avoided due to the risk of imposing selective pressure and favoring the strains that naturally harbor those genetic elements.

Other antibiotic classes, including aminoglycoside (eg, gentamicin), fluoroquinolone (eg, ciprofloxacin), glycopeptide (eg, vancomycin), phenicol (eg, chloramphenicol), and nitroimidazole (eg, metronidazole) were also associated with genes expressed in the endodontic bacteria. It is important to note that *van* genes conferring resistance to glycopeptide share significant similarities with other housekeeping genes related to bacterial cell envelope maintenance[34]. Moreover, the complex RM to vancomycin involves the expression of more than 1 gene, promoting an enzymatic cascade. Therefore, additional methods to explore glycopeptide resistance, including phenotypic and proteomic analyses, are being explored[34].

This study showed preliminary metatranscriptome and resistome data from eight root canal samples. The metatranscriptomic analysis was restricted to bacteria as they are the main components of the endodontic microbiome[29]. However, future studies of the fungal fraction of the microbiome would provide valuable data on its possible role in endodontic infections. The advantages of the approach used in this study included the simultaneous analysis of bacterial presence and activity, providing insights into bacterial functions, virulence, and resistance profiles of the microbiome[12]. However, as post-transcriptional (translational) attenuation of genes may occur, metatranscriptomic data does not allow protein analysis (ie, the final gene product)[12]. For this purpose, additional approaches such as metaproteomics could provide complementary data on the protein profile of endodontic communities[35]. The metatranscriptomic data in this study reinforce previous findings on the endodontic metaproteome, which showed proteins related to the metabolic processes, adhesion, biofilm formation, stress response, and antibiotic resistance, among others[35]. Future studies integrating metatranscriptomic and metaproteomic analysis of representative samples from different clinical conditions and geographic locations could provide a better understanding of the functional endodontic microbiome.

In conclusion, metatranscriptomic analysis of infected root canals indicated the presence of a microbiome dominated by Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. Among the nondominant phyla, Fusobacteria, Spirochetes, and Synergistetes were observed in most samples. Transcripts encoding moonlighting proteins were abundant (eg, glycolytic proteins, translational *tuf*, chaperonin, and heat shock proteins), which may affect bacterial adhesion, biofilm formation, host defense evasion, and inflammation induction. The ABC transporter was the most prevalent protein family in root canals and may contribute to bacterial virulence and resistance. The resistome analysis highlighted the potential risk of tetracycline/minocycline resistance in the endodontic community. More importantly, endodontic bacteria expressed AR-like genes conferring resistance to different classes of antibiotics, which calls attention to the need for a more responsible use of antibiotics in Dentistry.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ericka T. Pinheiro: Conceptualization, Formal analysis, Investigation, Writing – original draft, Funding acquisition. **Lamprini Karygianni:** Conceptualization, Writing – review & editing, Funding acquisition. **George T.M. Candeiro:** Investigation. **Bruna G. Vilela:** Investigation. **Larissa O. Dantas:** Investigation. **Ana C.C. Pereira:** Investigation. **Brenda P.F.A. Gomes:** Writing – review & editing. **Thomas Attin:** Funding acquisition. **Thomas Thurnheer:** Conceptualization, Writing – review & editing, Funding acquisition. **Giancarlo Russo:** Formal analysis, Writing – original draft, Funding acquisition.

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The authors deny any conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version at www.jendodon.com (https://doi.org/10.1016/j.joen.2024.03.015).

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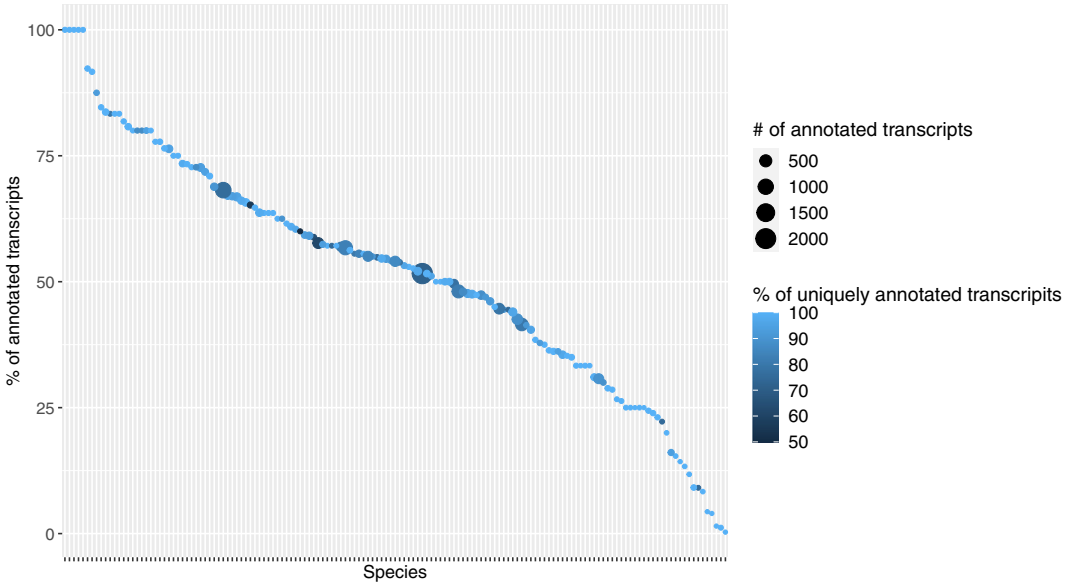
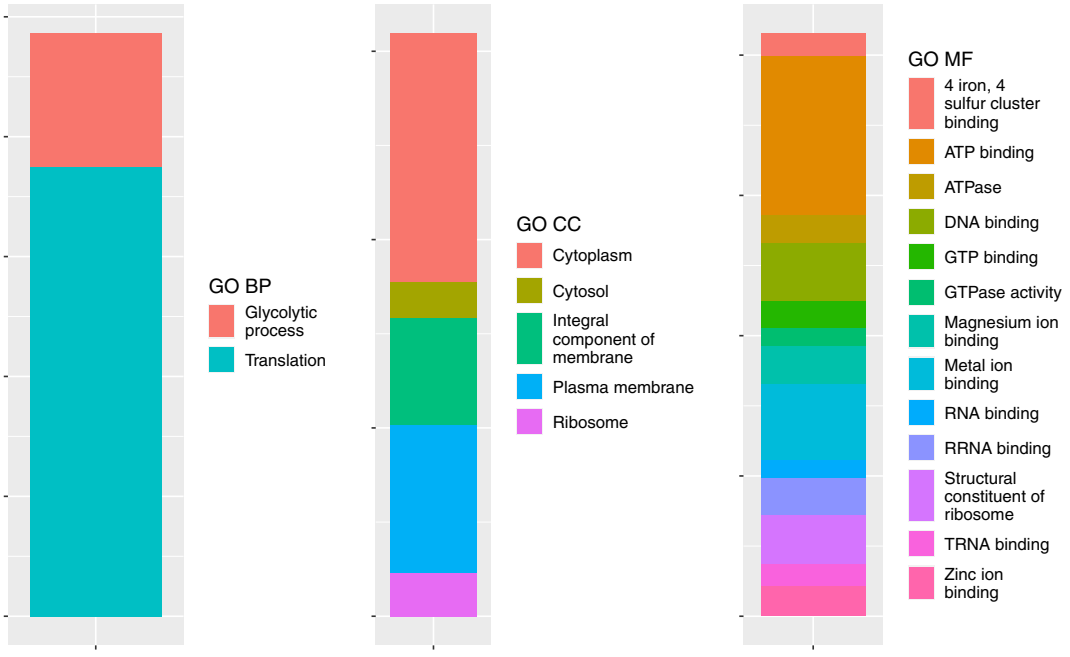
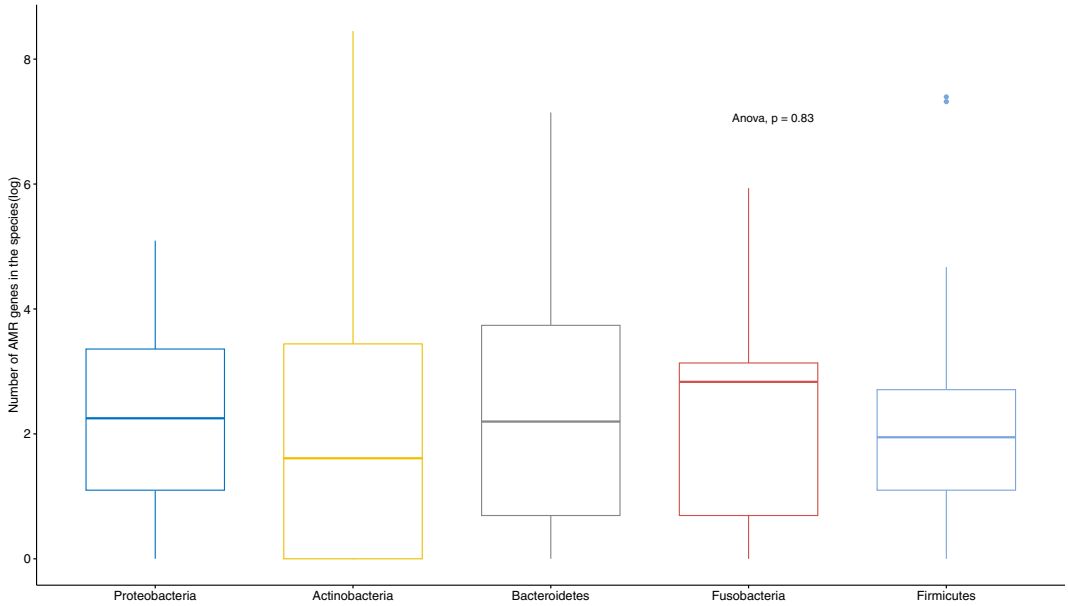
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE 1 – Number of annotated genes for each species and relative percentages of annotated and uniquely annotated genes.



SUPPLEMENTARY FIGURE 2 – The 20 most prevalent gene ontology (GO) categories across all infected root canals: biological process (GO BP), molecular function (GO MF), and cellular component (GO CC).



SUPPLEMENTARY FIGURE 3 – Distribution of AR-like genes in the species belonging to the same phylum. The comparison of the number of AR-like genes among phyla showed no difference using a 2-way ANOVA test ($P > .05$).

SUPPLEMENTARY TABLE 1 - The Most Abundant Phyla and Genera in Root Canal Samples

Actinobacteria	Bacteroidetes	Elusimicrobia	Firmicutes	Fusobacteria	Gemmatimonadetes	Proteobacteria	Spirochaetes	Synergistetes	Tenericutes
Actinomyces *Atopobium* *Cutibacterium* *Olsenella* *Propionibacterium* *Rothia*	*Bacteroides* *Capnocytophaga* *Prevotella* *Tannerella*	*Eubacterium*	*Bacillus* *Clostridium* *Filifactor* *Mogibacterium* *Parvimonas* *Selenomonas* *Staphylococcus* *Streptococcus*	*Fusobacterium*	*Gemmatirosa*	*Desulfovibrio* *Enterobacter* *Haemophilus* *Klebsiella* *Neisseria* *Pasteurella* *Pseudomonas* *Ralstonia*	*Treponema*	*Aminomonas* *Jonquetella*	*Mycoplasma*

SUPPLEMENTARY TABLE 2 - The 20 Antimicrobial Resistance Genes With the Highest Expression Levels in Root Canal Samples

CARD_ID	Mean expression
gb|AAK37618.1|ARO:3005008|Txr	1611.22
gb|AYV52072.1|ARO:3002882|lmrD	57.38
gb|CAD55718.1|ARO:3000197|tet36	21.67
gb|AAB05622.1|ARO:3002921|vanRB	20.50
gb|CAA10975.1|ARO:3000194|tetW	19.46
gb|BAI83385.1|ARO:3003440|mecB	19.19
gb|AEA37904.1|ARO:3003112|lsaC	18.50
gb|AAS76623.1|ARO:3004606|erm(40)	17.50
gb|CAA03986.1|ARO:3000343|tap	17.38
gb|BAE85481.1|ARO:3003727|vanKI	14.88
gb|AAG06465.1|ARO:3005063|cprR	14.63
gb|AAC75136.1|ARO:3000793|mdtB	12.25
gb|ANZ79240.1|ARO:3004035|tetA(60)	12.00
gb|AHA41500.1|ARO:3002913|vanO	11.50
gb|ACJ41547.2|ARO:3004574|Acinetobacter	10.88
gb|ACS83748.1|ARO:3000573|tet(43)	10.63
gb|AMP42147.1|ARO:3004442|tet(W/N/W)	10.50
gb|CDO61516.1|ARO:3003949|efrB	9.25
gb|AAD04032.1|ARO:3002892|otr(B)	9.13
gb|CAA79727.1|ARO:3000191|tetQ	8.88

SUPPLEMENTARY TABLE 3 - Number of Antimicrobial Resistance (AR) Genes in Relation to the Total Number of Annotated Genes. The Species With At Least 10 AR-Like Genes Are Listed.

No. of AR-like genes	No. of annotated genes	Ratio	Species
71	2096	0.03	*Cutibacterium acnes*
29	1041	0.03	*Staphylococcus epidermidis*
23	625	0.04	*Olsenella uli*
23	796	0.03	*Parvimonas micra*
21	320	0.06	*Tannerella forsythia*
20	562	0.03	*Prevotella intermedia*
17	265	0.06	*Capnocytophaga sp. oral taxon 323*
10	361	0.03	*Fusobacterium nucleatum*