Impact of different oral treatments on the composition of the supragingival plaque microbiome

Alexander Rabe, Manuela Gesell Salazar, Stephan Michalik, Thomas Kocher, Harald Below; Uwe Völker and Alexander Welk

*Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, University Medicine Greifswald, Felix-Hausdorff-Str. 8, 17475 Greifswald, Germany; †Center for Dentistry, Oral and Maxillofacial Medicine, Department of Restorative Dentistry, Periodontology, Endodontology, and Preventive and Pediatric Dentistry, Dental School of University Medicine Greifswald, Fleischmannstraße 42-44, 17489; ‡Institute for Hygiene and Environmental Medicine, University Medicine Greifswald, Walter-Rathenau-Straße 49 A 17475 Greifswald, Germany

ABSTRACT

Background: Antiseptics are used to inhibit oral biofilm growth. However, they affect not only pathogenic but also commensal bacteria, which are a natural barrier against oral diseases.

Objective: Using a metaproteome approach combined with a standard plaque-regrowth study, this pilot study examined the impact of different concentrations of lactoperoxidase (LPO)-system containing lozenges on early plaque formation, and active biological processes.

Design: Sixteen orally healthy subjects received four local treatments as a randomized single-blind study based on a cross-over design. Two lozenges containing components of the LPO-system in different concentrations were compared to a placebo and Listerine®. The newly formed dental plaque was analyzed by mass spectrometry (nLC-MS/MS).

Results: On average, 1,916 metaproteins per sample were identified, which could be assigned to 116 genera and 1,316 protein functions. Listerine® reduced the number of metaprotein groups and their relative abundance, confirming the plaque inhibiting effect. The LPO-lozenges triggered mainly higher metaprotein abundances of early and secondary colonizers as well as bacteria associated with dental health but also periodontitis. Functional information indicated plaque biofilm growth.

Conclusion: The effects of Listerine® and LPO-system containing lozenges used for plaque inhibition are different. In contrast to Listerine®, the lozenges allowed maintenance of a higher bacterial diversity.

INTRODUCTION

Starting from birth, bacteria colonize the human supra-organism and have an enormous influence on the development of the immune system and thus on human health status [1]. Next to the gut, the second most complex bacterial ecosystem is the oral microbiome [2,3]. Besides the planktonically living bacteria in saliva, the bacteria in oral biofilms are of special interest [4,5]. Biofilms are defined as a community structure of microorganisms living in a matrix of synthesized exopolysaccharides [6,7]. The mechanical stability of the matrix and its high bacterial diversity lead to synergistic interactions, e.g. the extension of the genetic repertoire by horizontal gene transfer within the biofilm [4,8]. This organizational structure enables the biofilm to show a special resistance to external environmental influences such as nutrient limitation, the human immune system and antibiotics [6].

In principle, supragingival plaque is assumed to have a positive role, since it serves as a barrier against the colonization of pathogens [9]. However, a diet with a high carbohydrate content [10] combined with poor oral hygiene can lead to a bacterial shift [11,12] and cause diseases such as dental caries [13] or periodontitis [14]. Saliva is part of the 1st line defense against a dysbiotic biofilm, because it mechanically removes bacteria [12] but it also contains components of the innate immune system such as lactoperoxidase (LPO) [15,16].

The salivary glands produce among others the enzyme lactoperoxidase, which catalyzes an ionic substrate such as thiocyanate (SCN−) in the presence of H2O2 to form a highly reactive anti-microbial oxidation product, hypohiociyanite (OSCN−) [10,16,17]. Since the 1980s, an increased knowledge of the LPO system has been leading to toothpastes or mouthwashes, which contain components of the LPO system to support the
natural antimicrobial defense process [16,18]. The applicability of LPO products poses a challenge, as they cannot be used during the day between meals due to their volumes (mouth rinse) or additional materials like toothbrushes [18]. Furthermore, most of the antimicrobial substances used in oral health care products affect the whole microbiome. This means that all bacteria including the oral commensal flora will be reduced. However, it would be better if the commensal flora would not be reduced or even better, supported. Therefore, human own defense systems, such as the LPO system in saliva, are of interest.

Clinical studies provide insight into the effectiveness of the products, e.g. by performing plaque regrowth studies using traditional microbiological techniques [19]. Such a standard cross-over plaque-regrowth study [20] demonstrated that mouth rinse Listerine® Total Care™ (A – positive control) was statistically significantly more effective than the LPO-system-lozenges (B- 0.083% H2O2; accordingly a 1:2 H2O2/SCN- relation), (C- 0.04% H2O2 accordingly a 1:4 H2O2/SCN- relation), and the placebo lozenge (D) in inhibiting plaque. Listerine® rinse (A) as well as Lozenges (B) and (C) were statistically significantly more effective than the placebo lozenge (D), but no statistically significant differences could be observed between them. However, studies based on traditional microbiological evaluation techniques cannot address the effects of these treatments on the composition of the biofilm. Proteomics in combination with habitat-specific taxonomic and genomic databases allows studies of biofilms without the cultivation of bacteria and allows in-depth investigation of the behavior and composition of a biofilm directly in its natural habitat [21–25]. Thus, metaproteomic approaches that monitor changes at the protein level and their impact onto metabolic pathways within the bacterial community should be used in a complementary manner to improve the understanding of the microbiome by monitoring changes of gene expression [26] and to develop more personalized ways to positively support existing natural mechanisms of plaque control [27].

In this pilot study, we used an established metaproteomic approach [28,29] to evaluate the effect of the lozenges used in Welk et al. [19] on the microbiome composition and the changes at the protein level with respect to their functions in metabolic pathways in the bacterial community. To the best of our knowledge, this is the first study combining a well-recognized and established clinical model in dentistry [20] with a metaproteomic study [30].

The results of both studies are expected to support our long-term goal to develop a lozenge, which can be used as an easily applicable addition to daily oral hygiene, to positively influence the microbiome composition to ensure that commensal, non-pathogenic bacteria are the dominant species in the plaque biofilm.

Material and methods

This complementary study received a positive vote by the ethics committee of the University Medicine Greifswald and was conducted in accordance with the recommendations of the Declaration of Helsinki from 1996. The clinical trial was registered in the German Database for clinical trials (DRKS00022810, date of registry: 02.09.2020).

Clinical study design

The design of the 4-days standard randomized plaque-regrowth study [19,20] is displayed in Figure 1. All 16 study participants (six male and ten female) were oral healthy dental students of the Greifswald dental school, who gave their written informed consent for this study. The participants were between 20 and 30 years old with a mean age of 23.4 years.

Both test lozenges were based on sugar alcohols (xylitol, sorbitol, mannitol) and contained all components of the LPO system (10 mg LPO 350 U/mg (Sternewitz, Germany), 7.5 mg KSCN) and H2O2 either in high concentrations (Drug B: 0.083% H2O2 accordingly a 1:2 H2O2/SCN− relation) or low concentrations (Drug C: 0.04% H2O2 accordingly a 1:4 H2O2/SCN− relation). For Drug B and Drug C, carbamide peroxide (CPO) was used as the H2O2 donor, because it is very stable and releases H2O2 in a graduated way [31]. Drug D (placebo) was also a lozenge and had the same composition as Drug B and Drug C without the components of the LPO system. Drug A was Listerine® Total Care™ (Johnson & Johnson GmbH, Neuss, Germany) and is a commercially available mouth rinse containing essential oils. Using Drug A and Drug D as positive and negative control, allowed the results of Drug B and C to be attributed to the effect of two different hygiene measures and the LPO system.

In addition, each cycle started with a preparation phase followed by the treatment and a final recovery phase of at least 10 days. First, in the preparation phase, the participants suspended any kind of personal oral hygiene (timepoint: D0) for 3 days to support the recovery of the oral microbiome. On the fourth day (timepoint: D3) supragingival plaque was collected and served as the control sample. To ensure that plaque was totally removed, a professional dental cleaning was performed by the study dentist followed by the treatment phase (timepoints: D3 – D7), where the volunteers received one of the four drugs.

The mouth rinse solution (Drug A) had to be used twice daily in the morning and evening according to the manufacturer’s instructions. The Drugs B-D were sucked five times daily every 3 hours between every 8 o’clock am and 8 o’clock pm for a period of 10–15 minutes.
On the last day of the treatment phase (D7), plaque that had built up during treatment was collected and the teeth were professionally cleaned. The recovery period of at least 10 days began and the participants resumed their personal oral hygiene (Figure 1).

**Dental plaque collection procedure**

Using a sterile curette (Universal Curette, Hu-Friedy Mfg. Co. LLC, Frankfurt am Main, Germany), supra-gingival plaque was collected from at least 24 tooth surfaces of all four quadrants (Figure 2A). The curette with the collected plaque was transferred several times during sample collection to a sterile tube (SafeSeal micro tubes, Sarstedt AG & Co., Nümbrecht, Germany) containing 3 ml sterile 1x PBS (Life Technologies GmbH, Darmstadt, Germany) and shaken until the plaque was detached from the curette. Finally, the sample material was vortexed for 30 s to create a suspension. In the next step, 20 µl of a protease inhibitor (Sigma Aldrich, St. Louis, MO, USA; v/v 1:20) per 1 ml sample volume was added and samples were centrifuged for 3 min at 6,200 g and 4 °C. The remaining pellets were immediately frozen in liquid nitrogen and finally stored at −80°C.

**Dental Plaque sample preparation and nLC MS/MS Measurement**

The pellets were resuspended with 300 µl TE buffer (10 mM Tris; 1 mM EDTA; pH 8.0). Subsequently, the biofilm and its cells were disrupted by an ultrasound treatment (Labsonic U – B. Braun Melsungen AG, Melsungen Germany) on ice for 3 × 30 s and 50% power of the device to release the proteins. Cell debris and the cytosolic proteins were separated by centrifugation (30 min, 4°C, 16,200 g). The supernatant containing proteins was transferred to a new tube and stored on ice. The preparation of the protein mixture for the nLC-MS/MS measurement and the method for the mass spectrometric measurement were already described in detail [28,29]. Briefly, proteins were precipitated by TCA and washed several times with acetone. The vacuum dried pellet consisting of precipitated proteins was dissolved in 30 µl 8 M urea/thiourea solution. The protein concentration was determined with a Bradford Assay (Bio-Rad Laboratories GmbH, Munich, Germany). Cysteines were reduced with dithiothreitol (DTT) and alkylated with iodoacetamide IAA with subsequent digestion of 4 µg of the protein mixture using trypsin. The resulting peptide mixture was purified after a digestion period of 17 h by ZipTipC18 material (Merck KGaA, Darmstadt, Germany). Finally, 2 µg of peptides were separated with a reverse phase nano HPLC Ultimate® 3000 Nano HPLC (Thermo Scientific) and analyzed on a Q Exactive™ Plus (Thermo Scientific) in data-dependent mode.

**Metaproteome assembly, mapping, and annotation**

We designed a workflow based on open-source software applications to analyze our metaproteomic datasets, described in detail in one of our earlier metaproteome studies [28,29]. Figure 2B provides an overview of the most important steps and is...
centrifugation, performed MS/MS
UniProt microbial identification, sourceforge.net/
Comet analysis.

Figure 2. Workflow for supragingival plaque collection and preparation for nLC MS/MS Measurements (A). Collection of dental plaque in sterile 1x PBS from all four quadrants of the human mouth from at least 24 teeth using a sterile curette. After centrifugation, the pellet was resuspended in the TE buffer and treated with ultrasound. The protein mixture was precipitated with TCA and digested using trypsin to measure the peptide mixtures on a Q Exactive™ Plus in DDA mode.

Spectral processing and metaproteome annotation (B). The open-source software Trans-Proteomic Pipeline processed raw spectra of nano LC-MS/MS measurement. Peptides were identified based on the Comet algorithm and filtered regarding their FDR to increase the validity of peptides. A combined database consisting of human protein sequences from UniProt and bacterial protein sequences of the human oral microbial database provided the basis for protein identification. Taxonomic classification, functional prediction and relative quantification was performed by Prophane. Figure 2 is adapted from Rabe et al. [35,36].

briefly described subsequently. To evaluate our 128 MS/MS measurements, we used the Trans-Proteomic Pipeline (http://tools.proteomecenter.org/software.php) [32,33] and have chosen the Comet MS/MS search algorithm (http://comet-ms.sourceforge.net/) [34,35] for peptide and protein identification, based on a combined database with 1,079,744 bacterial sequences of the human oral microbial database (HOMD, www.homd.org) [36,37] and 20,154 human sequences from UniProt (UniProtKB/Swissprot, www.uniprot.org) [38]. Peptides and proteins were filtered according to their iProphet probability at 0.05 Protein FDR (iProphet iProb = 0.9015). Using an R script (version: 4.1.1) [39], only proteins identified with at least one unique peptide were used for further analysis. Finally, proteins were classified taxonomically using the Lowest-Common-Ancestor algorithm (LCA) [40] and regarding their functional TIGRFAM assignment (TIGRFAM library version 15.0; e-value: < 0.01) [41] by the bioinformatic pipeline Prophane (www.prophane.de) [42,43]. All proteins were relatively quantified using normalized spectral abundance factor (NSAF) values [44].

The measured MS/MS data of our study were uploaded to the publicly accessible MassIVE database (dataset name: MSV000089755; doi:10.25345/C57D2Q93).

Statistical analyses

The statistical calculations as well as the image creations were performed with R (version: 4.1.1) supported by the R Foundation for statistical computing [39].

The NSAF values for each treatment sample have been median normalized to their corresponding control. Values of treatments were divided by control values for each of the ratio calculations, whereby missing or infinite values were not considered.

We selected at minimum 50% valid values per sample for a paired two-sided Wilcoxon signed rank test, which was performed with a set confidence interval of 0.95. To detect significant changes, the cutoff was set to the fold-change = 1.5 and for the p-value = 0.05. Significant results are presented in Volcano and Violin plots created using different R packages (Supplemental Table 4).
The influence of treatments on the taxonomic composition of the plaque microbiome was visualized using the metacoder package (Supplemental Table 4) [45]. The natural logarithm of the ratios between treatments and controls before treatment (color scale) was plotted against the summed spectral counts (thickness of taxonomic clades).

**Results**

**Spectral processing results**

For our complementing 4-day plaque regrowth study, 4 μg protein of total plaque of 0.88–2.6 (median QHI; Oral hygiene index according to Quigley–Hein) was prepared from each of the 128 samples and analyzed by mass spectrometry using a Q Exactive Plus (Thermo Scientific). The MS/MS analyses of the whole sample set resulted in 5.4 million spectra, of which 2.5 million spectra (identification rate: 46.3%) could be assigned based on our database consisting of human and bacterial protein sequences. Across all samples, a total of 124,101 distinct peptides could be identified with a pepFDR ≤1.56%, thereof 106,980 were of bacterial and 17,121 of human origin.

At the protein level, we only considered proteins that had a protFDR ≤5.0% and contained at least one unique peptide to minimize the possibility of misclassification. Based on these quality criteria, an average of 1,916 (± 465) metaproteins of bacterial origin as well as 442 (± 171) human proteins were covered per sample.

Analyzing the same protein amount (4 μg) of plaque sample for the metaproteomic analysis, on average 23.5% less metaproteins, were observed after treatment (Ø 1057 metaproteins) with Listerine® compared to the negative control before the treatment (Ø 1382 metaproteins) at the genus level (Supplementary Figure 1). Accordingly, these proteins also covered a lower number of bacterial genera. This contrasts with slightly increased metaprotein numbers in treatment groups B (before treatment: Ø 1304 metaproteins; after treatment Ø 1425 metaproteins) and C (before treatment: Ø 1387 metaproteins; after treatment Ø 1486 metaproteins).

Relative quantification of the metaproteome data was performed using spectral counts, which were used to calculate the NSAF values for each protein. For Drug A (Listerine®, positive control), the relative metaprotein abundance of bacterial proteins per sample decreased from an average of 74.1% before the treatment to an average of 59.1% after the treatment, because Listerine® reduced the bacterial biofilm in general. Correspondingly, the relative abundance of human proteins increased. For both LPO component-based (10 mg LPO 350 U/mg (Sternenzym, Germany), 7.5 mg KSCN) lozenges Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) and Drug C (0.04% H₂O₂; 1:4 H₂O₂/SCN⁻ relation) as well as for D (placebo), the relative abundance of bacterial metaproteins remained constant with values averaging between 69.0% and 78.7% before and after treatment, indicating that the LPO-based lozenges had no decreasing effect on the bacterial biofilm in general.

**Taxonomic profile and changes at genus level**

To provide a general overview of the impact of treatments on the diversity of the plaque microbiome, we calculated the ratio of metaprotein abundances between the control and treatment time points by dividing the median normalized NSAF values for each treatment (D7) by its corresponding control (D3) and plotted them against the summed spectral counts in heat map trees (Figure 3).

Across all 128 samples, the metaproteins could be taxonomically assigned to a total of eight phyla, with the phyla Actinobacteria, Firmicutes, Fusobacteria, Proteobacteria, and Bacteroidetes dominating the composition of the plaque microbiome. Spirochaetes, Synergistetes, and Saccharibacteria played a minor role. At the genus level, the study covered metaproteins assigned to 116 genera across all samples and the high diversity remained constant after the different treatments in comparison to the control time points.

To evaluate whether the treatments caused changes in metaprotein abundances and thus altered plaque microbiome composition, we performed a paired two-sided Wilcoxon signed rank test with a confidence interval of 0.95 (cut-offs: fold-change = 1.5; p-value = 0.05) for genera that occurred in at least 50% of all samples. The Volcano (Figure 4) and violin plot (Supplemental Figure 2) show these significant metaprotein changes at the genus level. Figure 4 shows that Drug A (Listerine®, positive control) primarily led to a significant reduction of the relative abundance for metaproteins of the nine genera, such as Haemophilus, Leptotrichia or Tannerella, whereas higher metaprotein abundances could be identified for Rothia and Peptoniphilus. In contrast, the relative metaprotein abundances for the five genera Fusobacterium, Lachnospiraceae bacterium, Capnocytophaga and Johnsonella increased under Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation). However, lower metaprotein abundances could be identified for the genera Corynebacterium and Mobiluncus.

Treating the subjects with Drug C (0.04% H₂O₂; 1:4 H₂O₂/SCN⁻ relation) had a similar influence on the plaque metaproteome in comparison to the treatment with Drug D (placebo). Both treatments resulted in a significant decrease of metaprotein abundances for Granulicatella, as well as a higher abundance of Capnocytophaga. Additionally, an increased metaprotein abundance for the genus Neisseria occurred for Drug C.
showed statistical evidence that the species *Rothia dentocariosa* were present in higher abundance in samples, as compared to the control. Metaproteins of *Rothia* showed the greatest increase in abundance and *Aggregatibacter aphrophilus* showed the greatest decrease. *Leptotrichia* was the most represented genus with seven species, all of them displaying a reduction in abundance.

For Drug B (0.083% H$_2$O$_2$: 1:2 H$_2$O$_2$/SCN$^-$ relation), metaprotein abundances for 21 and 8 species were present in higher and lower abundance, respectively, in comparison to the control before treatment (Table 1). The metaprotein abundances with the greatest decrease were identified for *Cronobacter sakazakii* and with the greatest increase for *Lachnospiraceae bacterium ACC2*. All five different *Fusobacteria* showed an increase in metaprotein abundances.

For Drug C (0.04% H$_2$O$_2$: 1:4 H$_2$O$_2$/SCN$^-$ relation) and Drug D (placebo), metaprotein abundance changes were identified for 16 and 15 species, with metaproteins of 14 species showing higher abundance for Drug C (Table 1) and metaproteins of 11 species for Drug D (placebo) (Supplemental Table 2). Both treatments had the greatest similarities among the four treatments by sharing six species with a significant change of metaprotein abundances. Four of the six species originate from the genus *Capnocytophaga* and showed an increased metaprotein abundance, as well as *Neisseria flava* and *Leptotrichia sp. oral taxon 215*.

We observed significant metaprotein changes for *Capnocytophaga sp. oral taxon 329 F0087* and *Leptotrichia sp. oral taxon 215* during all four treatments.

In summary, our findings for the four treatments at the species level were consistent with the analysis results at the genus level. Drug A (Listerine®, positive control) showed a tendency to reduce the metaprotein abundances for most of the species, whereas drug B (0.083% H$_2$O$_2$: 1:2 H$_2$O$_2$/SCN$^-$ relation) tended to increase it. Drug C (0.04% H$_2$O$_2$: 1:4 H$_2$O$_2$/SCN$^-$ relation) and Drug D (placebo) showed a higher abundance for 11 species, with metaproteins of 11 species showing higher abundance for Drug C (Table 1) and metaproteins of 11 species for Drug D (placebo) (Supplemental Table 2). Both treatments had the greatest similarities among the four treatments by sharing six species with a significant change of metaprotein abundances.

Four of the six species originate from the genus *Capnocytophaga* and showed an increased metaprotein abundance, as well as *Neisseria flava* and *Leptotrichia sp. oral taxon 215*.

We observed significant metaprotein changes for *Capnocytophaga sp. oral taxon 329 F0087* and *Leptotrichia sp. oral taxon 215* during all four treatments.

In summary, our findings for the four treatments at the species level were consistent with the analysis results at the genus level. Drug A (Listerine®, positive control) showed a tendency to reduce the metaprotein abundances for most of the species, whereas drug B (0.083% H$_2$O$_2$: 1:2 H$_2$O$_2$/SCN$^-$ relation) tended to increase it. Drug C (0.04% H$_2$O$_2$: 1:4 H$_2$O$_2$/SCN$^-$ relation) and Drug D (placebo) showed a higher abundance for 11 species, with metaproteins of 11 species showing higher abundance for Drug C (Table 1) and metaproteins of 11 species for Drug D (placebo) (Supplemental Table 2). Both treatments had the greatest similarities among the four treatments by sharing six species with a significant change of metaprotein abundances.

Four of the six species originate from the genus *Capnocytophaga* and showed an increased metaprotein abundance, as well as *Neisseria flava* and *Leptotrichia sp. oral taxon 215*.

We observed significant metaprotein changes for *Capnocytophaga sp. oral taxon 329 F0087* and *Leptotrichia sp. oral taxon 215* during all four treatments.

In summary, our findings for the four treatments at the species level were consistent with the analysis results at the genus level. Drug A (Listerine®, positive control) showed a tendency to reduce the metaprotein abundances for most of the species, whereas drug B (0.083% H$_2$O$_2$: 1:2 H$_2$O$_2$/SCN$^-$ relation) tended to increase it. Drug C (0.04% H$_2$O$_2$: 1:4 H$_2$O$_2$/SCN$^-$ relation) and Drug D (placebo) showed a higher abundance for 11 species, with metaproteins of 11 species showing higher abundance for Drug C (Table 1) and metaproteins of 11 species for Drug D (placebo) (Supplemental Table 2). Both treatments had the greatest similarities among the four treatments by sharing six species with a significant change of metaprotein abundances. Four of the six species originate from the genus *Capnocytophaga* and showed an increased metaprotein abundance, as well as *Neisseria flava* and *Leptotrichia sp. oral taxon 215*.

We observed significant metaprotein changes for *Capnocytophaga sp. oral taxon 329 F0087* and *Leptotrichia sp. oral taxon 215* during all four treatments.

In summary, our findings for the four treatments at the species level were consistent with the analysis results at the genus level. Drug A (Listerine®, positive control) showed a tendency to reduce the metaprotein abundances for most of the species, whereas drug B (0.083% H$_2$O$_2$: 1:2 H$_2$O$_2$/SCN$^-$ relation) tended to increase it. Drug C (0.04% H$_2$O$_2$: 1:4 H$_2$O$_2$/SCN$^-$ relation) and Drug D (placebo) showed a higher abundance for 11 species, with metaproteins of 11 species showing higher abundance for Drug C (Table 1) and metaproteins of 11 species for Drug D (placebo) (Supplemental Table 2). Both treatments had the greatest similarities among the four treatments by sharing six species with a significant change of metaprotein abundances. Four of the six species originate from the genus *Capnocytophaga* and showed an increased metaprotein abundance, as well as *Neisseria flava* and *Leptotrichia sp. oral taxon 215*.
relation) as well as Drug D (placebo) also showed less pronounced effects on the metaprotein abundances on the species level.

**Bacterial functional profile of the plaque biofilm**

Metaproteomics enables the measurement and analysis of bacterial proteins, also allowing conclusions regarding interactions between microbes, functional properties of the community as well as to responses to changing environmental conditions [46]. Using a paired two-sided Wilcoxon signed rank test (confidence interval = 0.95; cut-offs: fold-change = 1.5; p-value = 0.05), we evaluated whether significant changes of abundance for metaprotein functions were detectable. Therefore, we analyzed all bacterial metaproteins with respect to their functional classification, which was based on the TIGRFAM system including three levels of classification, which differ in their granularity. One thousand three hundred and sixteen TIGRFAMs could be assigned to the bacterial metaproteins, which were distributed among 60 biological processes (Supplemental Table 3). At the lowest level of the TIGRFAM classification, no significant changes were observed. However, significant changes occurred in 19 biological processes, the second level of the TIGRFAM classification (Figure 6 and Table 2). Supplemental Figure 3 summarizes the treatment-related changes for those biological processes.

The most significant changes were observed for Drug A (Listerine®, positive control) under whose treatment metaproteins involved in 12 biological processes (Table 2) showed a reduced metaprotein abundance. Metaproteins involved in small-molecule interactions (PAS domain S-box protein [47]) mainly of the category ‘amino sugars’ [48,49] like glucosamine-6-phosphate deaminase, phospho-glucosamine mutase or N-acetyl-glucosamine-6-phosphate deacetylase showed the most significant differences between control and treatment.

For Drug B (0.083% H₂O₂: 1:2 H₂O₂/SCN⁻ relation), a significant increase in abundance was observed for metaproteins of the aromatic amino acid family, small-molecule interactions, iron metabolism, and for metabolism of unknown substrates (Table 2). Drug C (0.04%
Figure 5. Volcano plots showing significant changes of a paired two-sided Wilcoxon signed rank test (confidence interval = 0.95) for the metaprotein abundances assigned on the species level for all four treatments. Blue indicates a significant reduction in relative metaprotein abundance after treatment, red indicates a significant increase, and gray indicates no significant changes in relative metaprotein abundance after treatment.

\[ \text{species level} \]

![Volcano plots showing significant changes](image)

cutoffs: fold-change=1.5 / p-value=0.05

\[ \text{H}_2\text{O}_2; 1:4 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation} \] and Drug D (placebo) both showed an increase in abundance for proteins of the small-molecule interactions (PAS domain S-box protein [47]) and cations and iron carrying compounds like bacterioferritin, ubiquinone oxidoreductase or TonB-dependent siderophore receptor [50,51] (Table 2), with an additional increase in chemotaxis and motility, e.g. flagellar M-ring protein (FlIF) or flagellar motor protein switch protein (FlIM) [52] for Drug C (0.04% \text{H}_2\text{O}_2; 1:4 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}). Furthermore, for Drug C, we observed a reduced abundance for proteins of the histidine family (histidinol dehydrogenase, phosphoribosyl-ATP diphosphatase) and protein modification and repair (methionine aminopeptidase [53], L-isoaspartate O-methyltransferase [54]).

Overall, it can be concluded that for Drug A (Listerine®, positive control) most significant changes were observed for biological processes accompanied by a reduction in abundance. For Drugs B (0.083% \text{H}_2\text{O}_2; 1:2 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}), C (0.04% \text{H}_2\text{O}_2; 1:4 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}), and D (placebo), the number of significant changes was lower, but always showed an increase in abundance with two exceptions for Drug C. The small-molecule interactions were common to all treatments and were present in reduced abundance for Drug A (positive control) and with an increased abundance for Drugs B, C, and D (placebo).

Discussion

In this pilot study, metaproteomic techniques are used for the first time to evaluate the influence of a conventional antiseptic in comparison to an antimicrobial human defense system on supragingival plaque formation based on a standardized and widely accepted study model in dentistry [20]. For our study, we used Drug A (Listerine®, positive control) and Drug D (placebo) as positive and negative controls, to directly attribute the changes of the plaque-microbiome to the components of the LPO system, which was included in Drug B (0.083% \text{H}_2\text{O}_2; 1:2 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}) and Drug C (0.04% \text{H}_2\text{O}_2; 1:4 \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}) with different concentrations of hydrogen peroxide and in \text{H}_2\text{O}_2/\text{SCN}^- \text{relation}. 
Table 1. Summary of significant changed metaprotein abundances and their taxonomic assignment on the species level under Drug B (0.083% H₂O₂ accordingly a 1:2 H₂O₂/SCN⁻ relation) and Drug C (0.04% H₂O₂ accordingly a 1:4 H₂O₂/SCN⁻ relation). For each species, their association with healthy and/or diseased oral conditions is given and is color-coded for visual support.

| Species                        | Drug B          | Drug C          | Health/Disease Association                  |
|--------------------------------|-----------------|-----------------|---------------------------------------------|
|                                | Fold Change     | Fold Change     | p-values                                    | Fold Change     | Fold Change     | p-values| p-values| p-values| p-values|                        |
| Actinomyces johnsonii          | 2.418           | Down            | 0.011                                       | -               | -               | -       | -       | Commensal [102]         |
| Actinomyces oris               | 1.593           | Down            | 0.009                                       | -               | -               | -       | -       | Commensal [102]         |
| Mobiluncus mulleri             | 1.605           | Down            | 0.008                                       | -               | -               | -       | -       | Disease [102]           |
| Rothia aeria                   | 1.514           | Down            | 0.018                                       | -               | -               | -       | -       | Disease [104]           |
| Rothia dentocariosa            | 2.191           | Down            | 0.038                                       | -               | -               | -       | -       | Commensal and Disease [105–107] |
| Corynebacterium durum          | 2.661           | Down            | 0.045                                       | -               | -               | -       | -       | Commensal [104]         |
| Kingella oralis                | 1.580           | Down            | 0.044                                       | -               | -               | -       | -       | Unknown                |
| Cronobacter sakazakii          | 8.890           | Down            | 0.025                                       | -               | -               | -       | -       | Unknown                |
| Prevotella intermedia          | 2.092           | Up              | 0.012                                       | 0.029           | 1.548           | Down    | Disease [102]         |
| Capnocytophaga sp. oral taxon 326 | 1.963           | Up              | 0.021                                       | 0.000           | 2.066           | Up      | Commensal and Disease [102] |
| Capnocytophaga sp. oral taxon 329 F0087 | 2.048           | Up              | 0.002                                       | 0.000           | 2.447           | Up      | Commensal and Disease [102] |
| Capnocytophaga sp. oral taxon 332 | 2.266           | Up              | 0.002                                       | 0.000           | 3.490           | Up      | Commensal and Disease [102] |
| Gemella haemolysans            | 2.295           | Up              | 0.045                                       | 0.034           | 2.227           | Up      | Commensal and Disease [110] |
| Abiotrophia deflecta           | 4.377           | Up              | 0.024                                       | 0.0180          | 5.435           | Up      | Commensal and Health [111–113] |
| Streptococcus gordonii         | 1.518           | Up              | 0.029                                       | -               | -               | -       | -       | Commensal and Disease [114,115] |
| Streptococcus mutans           | 1.989           | Up              | 0.000                                       | 0.009           | 1.849           | Up      | Commensal and Disease [114,115] |
| Lachnospiraceae bacterium ACC2 | 4.538           | Up              | 0.016                                       | -               | -               | -       | -       | Unknown                |
| Johnella ignava                | 3.642           | Up              | 0.011                                       | -               | -               | -       | -       | Unknown                |
| Lachnospiraceae bacterium oral taxon 107 F0167 | 1.826           | Up              | 0.008                                       | -               | -               | -       | -       | Unknown                |
| Lachnospiraceae bacterium sp. oral taxon 082 F0431 | 1.893           | Up              | 0.025                                       | -               | -               | -       | -       | Unknown                |
| Fusobacterium necrophorum      | 1.978           | Up              | 0.012                                       | -               | -               | -       | -       | Commensal and Disease [118] |
| Fusobacterium nucleatum subsp. animalis | 1.520           | Up              | 0.002                                       | -               | -               | -       | -       | Commensal and Disease [118] |
| Fusobacterium nucleatum subsp. nucleatum | 1.646           | Up              | 0.004                                       | -               | -               | -       | -       | Commensal and Disease [118] |
| Fusobacterium nucleatum subsp. polymorphum | 1.701           | Up              | 0.003                                       | -               | -               | -       | -       | Commensal and Disease [118] |
| Fusobacterium periodonticum    | 1.550           | Up              | 0.002                                       | -               | -               | -       | -       | Commensal and Disease [118] |
| Leptotrichia goodfellowi       | 2.950           | Up              | 0.044                                       | 0.021           | 2.715           | Up      | Commensal and Disease [118] |
| Leptotrichia sp. oral taxon 215 | 2.390           | Up              | 0.001                                       | 0.011           | 1.979           | Up      | Commensal and Disease [120,121] |
| Agrobacterium tumefaciens      | 3.379           | Up              | 0.021                                       | -               | -               | -       | -       | Unknown                |
| Capnocytophaga granulosa       | -               | -               | -                                           | 0.018           | 1.614           | Up      | Commensal and Disease [120,122] |
| Capnocytophaga sputigena       | -               | -               | -                                           | 0.001           | 2.656           | Up      | Commensal and Disease [120,122] |
| Granulicatella adiacens        | -               | -               | -                                           | 0.039           | 1.619           | Down    | Commensal and Disease [111–113] |
| Neisseria elongata             | -               | -               | -                                           | 0.002           | 3.387           | Up      | Commensal and Disease [124,125] |
| Neisseria flava                | -               | -               | -                                           | 0.013           | 2.569           | Up      | Commensal and Disease [124–126] |
| Neisseria sicca                | -               | -               | -                                           | 0.006           | 2.328           | Up      | Commensal and Disease [124,125,127] |
| Propionibacterium propionicum  | -               | -               | -                                           | 0.050           | 2.475           | Up      | Unknown               |

Color legend: green – commensal or health associated; yellow – commensal and disease associated; red: disease associated; grey: no information available if the species is commensal, health or disease associated.
We benchmarked our results with the number of protein identifications and identified genera with the current literature. Compared to previous studies [55–58], we achieved higher protein identifications with 1,916 (± 465) bacterial metaproteins and 442 (± 171) human proteins per sample. One aspect to consider is that, with 16 subjects and 128 measured samples, we included more subjects and analyzed substantially more samples than comparable metaproteomic studies [55–58]. Further more, there are combined effects of a different sample preparation protocol as well as up-to-date mass spectrometers and data analysis strategies [59–61].

Bacterial metaproteins accounted for the largest proportion with on average three-quarters of the sample in comparison to human proteins. Since we scraped a biofilm from the supragingival area, the high level of bacterial proteins was to be expected, as a biofilm mainly consists of bacteria, extracellular polymeric substance (EPS) as well as other organic and inorganic components like Ca, Mg, SO₄, lipids or nucleic acids [4,62–64].

Upon exposure to Drug A (Listerine®, positive control) the relative abundance of bacterial metaproteins in total decreased, whereas the relative abundance of human proteins increased accordingly. Probably this is due to the inhibitory effect of Drug A (Listerine®, positive control) on plaque formation in general [65–67]. Drug A (Listerine®, positive control) was a commercially available antiseptic mouth rinse, whose bactericidal effect is based on essential oils and ethanol [68]. In our clinical part of the study, a reduced biofilm was also demonstrated by the observed median QHI value of 0.88 after treatment [19]. This was reflected in fewer identified bacterial metaproteins and their relative abundance, which was paralleled in changes in taxonomic and functional assignment. We identified the most significant reductions in small-molecule interactions, such as the PAS domain S-box protein [47], which plays a role in various signaling processes, such as histidine kinases or chemotaxis. Amino sugars, also with one of the highest reductions, are an important component of the peptidoglycan of the cell wall of bacteria and at the same time a source of energy, nitrogen, and

---

**Figure 6.** Volcano plot showing significant changes of a paired two-sided Wilcoxon signed rank test (confidence interval = 0.95) for the bacterial metaprotein functions for all four treatments based on the subrole level of the TIGRFAM classification. Blue indicates a significant reduction in relative metaprotein abundance after treatment, red indicates a significant increase, and gray indicates no significant changes in relative metaprotein abundance after treatment.
Table 2. Summary of significant changed metaprotein functions under treatment of Drug A (Listerine®, positive control), Drug B (0.083% H2O2 accordingly a 1:2 H2O2/SCN- relation), Drug C (0.04% H2O2 according to a 1:2 H2O2/SCN- relation) and Drug D (placebo) based on the subrole level of the TIGRFAM classification.

| Metaprotein Function (TIGRFAM subrole)                             | Drug A (Listerine®, positive control) | Fold Change | Fold Change Direction |
|-------------------------------------------------------------------|---------------------------------------|-------------|-----------------------|
| Glutamate family                                                  | 0.001                                 | 1.658       | Down                  |
| Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides | 0.003                                 | 1.683       | Down                  |
| Amino sugars                                                      | 0.0105                                | 2.104       | Down                  |
| Biosynthesis and degradation of polysaccharides                   | 0.0027                                | 1.511       | Down                  |
| Pentose phosphate pathway                                          | 0.030                                 | 1.602       | Down                  |
| Pyruvate dehydrogenase                                            | 0.001                                 | 1.555       | Down                  |
| Biosynthesis                                                      | 0.013                                 | 1.561       | Down                  |
| Protein and peptide secretion and trafficking                     | 0.043                                 | 1.610       | Down                  |
| Pyrimidine ribonucleotide biosynthesis                            | 0.039                                 | 1.595       | Down                  |
| Small molecule interactions                                       | 0.016                                 | 3.703       | Down                  |
| Carbohydrates, organic alcohols, and acids                       | 0.002                                 | 1.542       | Down                  |
| General (specific role is unknown)                                | 0.000                                 | 1.657       | Down                  |

| Drug B (0.083% H2O2 accordingly a 1:2 H2O2/SCN- relation)         |                                         |             |                       |
|-------------------------------------------------------------------|---------------------------------------|-------------|-----------------------|
| Aromatic amino acid family                                        | 0.017                                 | 1.770       | Up                    |
| Heine, porphyrin, and cobalamin                                   | 0.018                                 | 1.927       | Up                    |
| Small molecule interactions                                       | 0.003                                 | 1.615       | Up                    |
| Unknown substrate                                                 | 0.007                                 | 2.144       | Up                    |

| Drug C (0.04% H2O2 according to a 1:2 H2O2/SCN- relation)         |                                         |             |                       |
|-------------------------------------------------------------------|---------------------------------------|-------------|-----------------------|
| Histidine family                                                  | 0.047                                 | 1.632       | Down                  |
| Protein modification and repair                                    | 0.032                                 | 2.118       | Down                  |
| Chemotaxis and motility                                           | 0.007                                 | 2.253       | Up                    |
| Small molecule interactions                                       | 0.014                                 | 2.819       | Up                    |
| Cations and iron carrying compounds                               | 0.044                                 | 2.687       | Up                    |

| Drug D (placebo)                                                  |                                         |             |                       |
|-------------------------------------------------------------------|---------------------------------------|-------------|-----------------------|
| Small molecule interactions                                       | 0.002                                 | 2.071       | Up                    |
| Cations and iron carrying compounds                               | 0.005                                 | 1.674       | Up                    |

carbon via their degradation [48,49]. In summary, a significant reduction in several metabolic processes mostly affecting key metabolic pathways for growth and proliferation of bacterial cells occurred, which suggests a reduced growth of the bacterial populations after Listerine® treatment.

In contrast, we observed a slight increase in metaprotein abundance and identification with the other three treatments. The results indicate that there may be increased bacterial activity in the biofilm. The increased abundances of flagellar proteins (FlfF, FlfM) indicating the movement of, for example, still present planktonic initial colonizers moving chemotactically down the nutrient gradient (PAS domain S-box protein [47]) [52]. Another example is the TonB-dependent siderophore receptor relevant for iron supply to bacteria [51], which transports iron from the environment into the cell for deoxyribonucleotide synthesis or oxidative phosphorylation [50]. Another indication is the increased metaprotein abundances of proteins involved in the repair or degradation of damaged proteins (methionine aminopeptidase [53], L-isoaspartate O-methyltransferase [54]). Additionally, based on the median QHI for Drug B (QHI 1.6), Drug C (QHI 1.8), and Drug D (QHI 2.6) a less inhibitory effect on plaque formation could be determined [19].

Regarding the taxonomic diversity, phyla such as Actinobacteria, Firmicutes or Fusobacteria dominated the assignment of metaproteins and confirmed the results of previous studies [69–74]. The same applies to the genus level, where e.g. Actinomyces and Streptococcus are among the most represented genera [56,73,75,76]. However, the species level offers the greatest information content for dental practitioners, especially regarding the colonization of tooth surfaces by initial and secondary colonizers [77,78].

The metaprotein abundances and their assigned species showed only small changes after treatment with Drug D (placebo). There were a few significant changes, e.g. for increased metaprotein abundances of the secondary colonizers Capnocytophaga spp [77,79,80]. Functionally, the abundance of metaproteins in the categories of small-molecule interactions as well as the cations and iron carrying compounds increased. Drug D was designed as a placebo, and therefore we did not expect many significant changes in the metaproteome. We assume a slight influence by the sugar alcohols mannitol, sorbitol and xylitol contained in Drug D (placebo). Previous studies have already provided initial evidence that sugar alcohols can also have an influence on bacteria and their growth [81–86].
Drugs B and C contained all components of the LPO system with an equal level of LPO concentration whereas Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) contained the hydrogen peroxide donor CPO at a higher concentration than Drug C (0.04% H₂O₂; 1:4 H₂O₂/SCN⁻ relation).

Drug C had a minor effect on the plaque microbiome and the data generated are comparable to the results of Drug D (placebo). As an example, we also found higher metaprotein abundances for similar species, such as the secondary colonizer Capnocytophaga spp., Neisseria flava, or Leptotrichia sp [77–80]. Therefore, we suggest that the low concentration of CPO is not sufficient to make a decisive contribution to the growth of the plaque biofilm that goes beyond the effect of the placebo.

A decisive influence on the plaque metaproteome could be observed for Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) especially for metaproteins of beneficial species as well as first and second colonizers. As one example we detected an increased metaprotein abundance for Lachnospiraceae spp., and Abiotrophia defectiva, which are associated with dental health in caries-free children [75,87], whereas we could not find references in the literature for each identified species of Lachnospiraceae (see Table 1). Furthermore, metaprotein abundances of Streptococcus gordonii were only found significantly increased after treatment of our healthy subjects with Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation). It is one of the first colonizers of the oral cavity [88,89] and thus involved in the initial attachment to tooth surfaces and co-aggregates with a variety of bacteria. This bacterium has been further described to compete effectively with Streptococcus mutans due to the availability of oxygen and the production of hydrogen peroxide [90,91]. Additionally, the abundance of metaproteins of the secondary colonizers Capnocytophaga spp., which are described as commensals and associated with disease in the literature, was also elevated after treatment with Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) [92–94]. The bridging species Fusobacterium nucleatum subsp., reported to coaggregate with all early and late colonizer, or even the late colonizers Prevotella intermedia and Prevotella nigrescens showed also higher metaprotein abundances after treatment with Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) [77–80] both associated with periodontitis [95,96]. In summary, we identified positive changes regarding metaprotein abundances of health-associated bacteria for caries, but negative changes occurred in periodontitis-associated bacteria.

During the complex process of the development of dental caries, an increase in acidogenic bacteria like Streptococcus mutans is associated with an ecologic shift in the oral biofilm [88,90]. The treatment period extended over a duration of 4 days to allow a regrowth of the plaque biofilm but was too short to produce a shift of the biofilm towards a diseased status [97–100]. Therefore, no metaproteins from pathogenic species were expected. A more detailed analysis showed that we identified only 11 metaproteins for S. mutans, with only one metaprotein being statistically relevant because it was found in more than 50% of all samples. For this single identifier only, we found increased metaprotein abundances for Streptococcus mutans, not only after the treatment for Drug B (0.083% H₂O₂; 1:2 H₂O₂/SCN⁻ relation) but also for Drug C (0.04% H₂O₂; 1:4 H₂O₂/SCN⁻ relation) and Drug A (Listerine®, positive control). The metaprotein (identifier: smut_c_1_284) is a dehydrogenase in lipid metabolism that has not yet been further characterized. In comparison, we identified considerably more metaproteins for other species, such as for S. gordonii with 60 metaproteins or F. nucleatum with 235 metaproteins. In addition, other omics studies also identified pathogenic species in healthy subjects [101,102] and is consistent with the extended ecological plaque hypothesis [103,104]. Another point to consider is that the metaprotein abundances of the 10 days recovery phase including 3 days of absence of any oral hygiene procedure on day 3 (baseline oral biofilm) are already at a relatively high level. This baseline oral biofilm was just influenced by a test substance for the following 4 days without other oral hygiene procedures.

A unique challenge was to reconcile the results of the clinical part of the study with the results of the metaproteomic approach. A direct comparison of the observed QHI values of the clinical study [19] and the relative protein amounts (NSAF values) calculated in this metaproteomic study might be misleading because for all samples the same protein amounts were used for MS-based profiling even if treatments had different effects on total biofilm amount (QHI values). Nevertheless, we consider the combination of classical microbiological methods with metaproteomic data in addition with clinical parameters as a valuable approach. By integrating a multi-OMICs approach in the future, we expect to gain even deeper insights into the pathophysiology of dental disease.

**Conclusion**

Although the study of molecular mechanisms in complex biofilms using metaproteomic approaches is still in its infancy, we were able to elucidate the impact of four treatments on the plaque metaproteome and associate it with clinical parameters. It could be shown that the metaproteomic analyses not only contribute to the elucidation of the
taxonomic composition but also gather functional information for the plaque biofilm during treatment.

According to the data of this metaproteomic analysis, we were able to show that the treatment based on the components of the LPO system induces a change in the plaque metaproteome that differs from that of a placebo and Listerine®. While the reduction of the Quigley-Hein index shown in the clinical study [19] for the antiseptics can be attributed to a reduction in the overall microbiome, our results suggest that the plaque reduction of the LPO-lozenges based more on an increase in bacterial diversity.

**Abbreviations**

CPO – carbamide peroxide; EPS – extracellular polymeric substance; DTT – dithiothreitol; FDR – false discovery rate; EDTA – ethylenediaminetetraacetic acid; HOMD – Human Oral Microbiome Database; HPLC – high performance liquid chromatography; H₂O₂ – Hydrogen peroxide; IAA – iso acid amide; LCA – Lowest-Common-Ancestor Algorithm; LPO – Lactoperoxidase; MS – mass spectrometry; MS/MS – tandem mass spectrometry; nLC – nano liquid chromatography; NSAF – normalized spectral abundance factor; OSCN⁻ – hypothyiocyante; pepFDR – peptide false discovery rate; protFDR – protein false discovery rate; SCN⁻ – thiocyanate; TCA – trichloroacetic acid; TE – Tris/EDTA; TIGRFAM - database of protein families

**Author contributions**

Study design: AR, MGS, HB, UV, and AW
Ethics application: HB and AW
Sample collection: AR and MGS
Sample preparation and nLC-MS/MS measurement: AR and MGS
Data analysis and analytical tools: AR, SM
Drafting of manuscript: AR
Editing of manuscript: MGS, SM, TK, HB, UV, and AW
Acquisition of funding HB, UV, and AW

**Acknowledgments**

We sincerely thank all the volunteers who participated in the study, which would not have been possible without them. We are also indebted to Ms Maral Zahedani, who provided dental care to the participants and collected the plaque samples. We would also like to thank Mr Patjek for performing the associated clinical study and its publication (19). We thank the research group of Stephan Fuchs and Katharina Riedel for developing Prophane.

In addition, we would like to express our gratitude to Les Laboratoires Servier and their service Servier Medical Art for their permission to use the image “Adult Teeth” (https://smart.servier.com/smart_image/adult-teeth-2/) based on the Creative Commons Attribution 3.0 Unported License. The image is used for Figure 2A.

We would like to thank Stephen Stacey for making the image “balance” freely available on FreImages.com (https://de.freimages.com/photo/balance-1172786). The image was used for Figure 2B.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This study was part of the Large Protection of Oral Health Project (LPO-Project) and was financed from the Ministry for Economics, Labor and Health in Mecklenburg-Western Pomerania and co-financed from the European Regional Development Fund (ERDF). Project numbers: AZ: V-630-VB243-2012/022, V-630-F-2012/023 and V-630-S137-2012/024.

**ORCID**

Alexander Rabe @ http://orcid.org/0000-0003-4045-4026

**References**

[1] Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. Science. 2012;336 (6086):1262–1267.
[2] Warinner C, Speller C, Collins MJ. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. Philos Trans R Soc Lond, B. Biol Sci. 2015;370(1660):20130376.
[3] The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–214.
[4] Costerton JW. Bacterial Biofilms: a common cause of persistent infections. Science. 1999;284:1318–1322.
[5] Zamboni C, Morvay AA, Sala C, et al. Antimicrobial effect of probiotics on bacterial species from dental plaque. J Infect Dev Ctries. 2016;10:214–221.
[6] Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microbiors. Clin Microbiol Rev. 2002;15:167–193.
[7] Jeon J-G, Rosalen PL, Falsetta ML, et al. Natural products in caries research: current (limited) knowledge, challenges and future perspective. Caries Res. 2011;45:243–263.
[8] Clegg MS, Vertucci FJ, Walker C, et al. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod. 2006;32:434–437.
[9] Finney M, Walker JT, Marsh PD, et al. Antimicrobial effects of a novel Triclosan/zinc citrate dentifrice against mixed culture oral biofilms. Int Dent J. 2003;53(6):371–378.
[10] Beighton D. Can the ecology of the dental biofilm be beneficially altered? Adv Dent Res. 2009;21(1):69–73.
[11] Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11):5721–5732.
[12] Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis. 2012;18(2):109–120.
[13] He J, Tu Q, Ge Y, et al. Taxonomic and functional analyses of the supragingival microbiome from caries-affected and caries-free hosts. Microb Ecol. 2017;75(2):543–554.
[14] Tanner ACR, Paster BJ, Lu SC, et al. Subgingival and tongue microbiota during early periodontitis. Journal of Dental Research. 2006;85(4):318–323.

[15] Faran Ali SM, Tanvir F. Oral microbial habitat a dynamic entity. J Oral Biol Craniofac Res. 2012;2(3):181–187.

[16] Tenovuo J. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: efficacy and safety. Oral Dis. 2002;8(1):23–39.

[17] Lenander-Lumikari M, Tenovuo J, Mikola H. Effects of a lactoperoxidase system-containing toothpaste on levels of hypophosphatase and bacteria in saliva. Caries Res. 1993;27:285–291.

[18] Jones SB, West NX, Nemesianov PP, et al. The antibacterial efficacy of a foam mouthwash and its ability to remove biofilms. BDI Open. 2018;4:17038.

[19] Welk A, Patjek S, Gärnter M, et al. Antibacterial and antiplaque efficacy of a lactoperoxidase-thiocyanate-hydrogen-peroxide-system-containing lozenge. BMC Microbiol. 2021;21(1):302.

[20] Addy M, Willis L, Moran J. Effect of toothpaste rinses compared with chlorhexidine on plaque formation during a 4-day period. J Clin Periodontol. 1982;10:89–99.

[21] Jagtap PD, Blakely A, Murray K, et al. Metaproteomic analysis using the galaxy framework. Proteomics. 2015;15:3553–3565.

[22] Bikel S, Valdez-Lara A, Cornejo-Granados F, et al. Combining metagenomics, metatranscriptomics and viromics to explore novel microbial interactions: towards a systems-level understanding of human microbiome. Comput Struct Biotechnol J. 2015;13:390–401.

[23] Abram F. Systems-based approaches to unravel multi-species microbial community functioning. Comput Struct Biotechnol J. 2015;13:24–32.

[24] Chistoserdov L. Functional metagenomics: recent advances and future challenges. Biotechnol Gen Eng Rev. 2010;26:335–352.

[25] Muth T, Renard BY, Martens L. Metaproteomic data analysis at a glance: advances in computational microbial community proteomics. Expert Rev Proteomics. 2016;13(8):757–769.

[26] Rodríguez-Valera F. Environmental genomics, the big picture? FEMS Microbiol Lett. 2004;231:153–158.

[27] Cross B, Faustoferri RC, Quivey RG. What are we learning and what can we learn from the human oral microbiome project? Curr Oral Health Rep. 2016;3(1):56–63.

[28] Rabe A, Gesell Salazar M, Michalik S, et al. Metaproteomics analysis of microbial diversity of human saliva and tongue dorsum in young healthy individuals. J Oral Microbiol. 2019;11(1):1654786.

[29] Rabe A, Gesell Salazar M, Völker U. Bottom-up community proteome analysis of saliva samples and tongue swabs by data-dependent acquisition nano LC-MS/MS mass spectrometry. Methods Mol Biol. 2021;2327:221–238.

[30] Wilmes P, Bond PL. Metaproteomics: studying functional gene expression in microbial ecosystems. Trends Microbiol. 2006;14(2):92–97.

[31] Dominguez Moreira S, Llina Puy C, Forner Navarro L, et al. Gingival bleeding reduction using a carbamide peroxide based tooth paste with lactoperoxidase. J Clin Exp Dent. 2011;e452–e455. DOI:10.4317/jced.3.e452

[32] Reiter L, Claassen M, Schrimpff SP, et al. Protein identification false discovery rates for very large proteomics data sets generated by tandem mass spectrometry. Mol Cell Proteomics. 2009;8(11):2405–2417.

[33] Shteynberg D, Deutsch EW, Lam H, et al. iProphet: multi-level integrative analysis of shotgun proteomic data improves peptide and protein identification rates and error estimates. Mol Cell Proteomics. 2011;10(12):M111.007690.

[34] Eng JK, Jahan TA, Hoopmann MR. Comet: an open-source MS/MS sequence database search tool. Proteomics. 2013;13(1):22–24.

[35] Eng JK, Hoopmann MR, Jahan TA, et al. A deeper look into Comet—implementation and features. J Am Soc Mass Spectrom. 2015;26:1865–1874.

[36] Chen T, Yu W-H, Izard J, et al. The human oral microbiome database: a web resource for investigating oral microbe taxonomic and genomic information. Database (Oxford). 2010;2010:baq013.

[37] Dewhurst FE, Chen T, Izard J, et al. The human oral microbiome. J Bacteriol. 2010;192:5002–5017.

[38] The UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017;45:D138–D169.

[39] R Development Core Team. R: a language and environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing; 2010. p. 1–3079.

[40] Huson DH, Auch AF, Qi J, et al. MEGAN analysis of metagenomic data. Genome Res. 2007;17(3):377–386.

[41] Haft DH, Selengut JD, Richter RA, et al. TIGRFAMs and Genome Properties in 2013. Nucleic Acids Res. 2013;41:D387–95.

[42] Schiebenhofer H, Schallert K, Renard BY, et al. A complete and flexible workflow for metaproteomics data analysis based on metaproteoanalyzer and prophane. Nat Protoc. 2020;15(10):3212–3239.

[43] Schneider T, Schmid E, de CJV, et al. Structure and function of the symbiosis partners of the lung lichen (Lobaria pulmonaria L. Hoffm.) analyzed by metaproteomics. Proteomics. 2011;11:2752–2756.

[44] Zybailov B, Mosley AL, Saridu ME, et al. Statistical analysis of membrane proteome expression changes in Saccharomyces cerevisiae. J Proteome Res. 2006;5:2339–2347.

[45] Foster ZSL, Sharpton TJ, Grünwald NJ. Metacoder: an R package for visualization and manipulation of community taxonomic diversity data. PLoS Comput Biol. 2017;13:e1005404.

[46] Zuñiga C, Zaramela L, Zengler K. Ecldication of complexity and prediction of interactions in microbial communities. Microbi Biotechnol. 2017;10(6):1500–1522.

[47] Henry JT, Crosson S. Ligand-binding PAS domains in a genomic, cellular, and structural context. Annu Rev Microbiol. 2011;65(1):261–286.

[48] Plumbridge J. Regulation of the utilization of amino sugars by Escherichia coli and Bacillus subtilis: same genes, different control. J Mol Microbiol Biotechnol. 2015;25:154–167.

[49] Kannan R, Harris CM, Harris TM, et al. Function of the amino sugar and terminal amino acid of the antibiotic vancomycin in its complexation with cell wall peptides. J Am Chem Soc. 1988;110:2946–2953.

[50] Briat J-F. Iron assimilation and storage in prokaryotes. J Gen Microbiol. 1992;138:2475–2483.
[51] Ferguson AD, Deisenhofer J, TonB-dependent receptors—structural perspectives. Biochim Biophys Acta - Biomembr. 2002;1565:318–332.

[52] Roman SJ, Frantz BB, Matsumura P. Gene sequence, overproduction, purification and determination of the wild-type level of the Escherichia coli flagellar switch protein FlfG. Gene. 1993;133:103–108.

[53] Ben-Bassat A, Bauer K, Chang S-Y, et al. Processing of the initiation methionine from proteins: properties of the Escherichia coli methionine aminopeptidase and its gene structure. J Bacteriol. 1987;169:751–757.

[54] Ryttersgaard C, Griffith SC, Sawaya MR, et al. Crystal structure of human L-isoaspartyl methyltransferase. J Biol Chem. 2002;277:10642–10646.

[55] Jersie-Christensen RR, Lanigan LT, Lyon D, et al. Quantitative metaproteomics of medieval dental calculus reveals individual oral health status. Nat Commun. 2018;9:4744.

[56] Belda-Ferre P, Williamson J, Simón-Soro A, et al. The human oral metaproteome reveals potential biomarkers for caries disease. Proteomics. 2015;15:3497–3507.

[57] Velsko IM, Fellows Yates JA, Aron F, et al. Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. Microbiome. 2019;7:102.

[58] Warinner C, Rodrigues JFM, Vyas R, et al. Pathogens and host immunity in the ancient human oral cavity. Nat Genet. 2014;46:336–344.

[59] Wilmes P, Heintz-Buschart A, Bond PL. A decade of metaproteomics: where we stand and what the future holds. Proteomics. 2015;15:3409–3417.

[60] Blackburn JM, Martens L. The challenge of metaproteomic analysis in human samples. Expert Rev Proteomics. 2016;13(2):135–138.

[61] Muth T, Benndorf D, Reichl U, et al. Searching for a needle in a stack of needles: challenges in metaproteomics data analysis. Mol Biosyst. 2013;9(4):578–585.

[62] Fleming H-C, Neu TR, Wozniak DJ. The EPS matrix: the “house of biofilm cells”. J Bacteriol. 2007;189(22):7945–7947.

[63] Costerton JW, Geczy GG, Cheng K-J. How bacteria stick. 1978;Sci. Amer. 238:86–95.

[64] Saxena P, Joshi Y, Rawat K, et al. Biofilms: architecture, resistance, quorum sensing and control mechanisms. Indian J Microbiol. 2019;59:3–12.

[65] Haffajee AD, Yaskell T, Socransky SS. Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. J Am Dent Assoc. 2008;139(5):606–611.

[66] Pilloni A, Pizzo G, Barlattani A, et al. Perceived and measurable performance of daily brushing and rinsing with an essential oil mouthrinse. Ann Stomatol (Roma). 2010;1(3–4):29–32.

[67] van Leeuwen MPC, Slot DE, van der Weijden GA. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: a systematic review and meta-analysis. Int J Dent Hyg. 2014;12:160–167.

[68] Ouhayoun J-P. Penetrating the plaque biofilm: impact of essential oil mouthwash. J Clin Periodontol. 2003;30(5):10–12.

[69] Xie G, Chain PSG, C-C L, et al. Community and gene composition of a human dental plaque microbiota obtained by metagenomic sequencing. Mol Oral Microbiol. 2010;25:391–405.

[70] Yeses V, González-Torres P, Carbonetto B, et al. Dental black plaque: metagenomic characterization and comparative analysis with white-plaque. Sci Rep. 2020;10:15962.

[71] Keijser BJF, Zaura E, Huse SM, et al. Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res. 2008;87:1016–1020.

[72] Zaura E, Keijser BJF, Huse SM, et al. Defining the healthy “core microbiome” of oral microbial communities. BMC Microbiol. 2009;9:259.

[73] Jiang W-X, Y-J H, Gao L, et al. The impact of various time intervals on the supragingival plaque dynamic core microbiome. PLoS ONE. 2015;10:e0124631.

[74] Adams SE, Arnold D, Murphy B, et al. A randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. Sci Rep. 2017;7:43344.

[75] Peterson SN, Snesrud E, Liu J, et al. The Dental Plaque Microbiome in Health and Disease. PLoS ONE. 2013;8(3):1–10.

[76] Utter MWJL, Borisy GG. Individuality, Stability, and Variability of the Plaque Microbiome. Front Microbiol. 2016;7:564.

[77] Kolonbrander PE, Andersen RN, Blehert DS, et al. Communication among oral bacteria. Microbiol Mol Biol Rev. 2002;66:486–505.

[78] Kolonbrander PE. Oral microbial communities: biofilms, interactions, and genetic systems. Annual Review of Microbiology. 2000;54(1):413–437.

[79] Aruni AW, Dou Y, Mishra A, et al. The biofilm community-rebels with a cause. Curr Oral Health Rep. 2015;2:48–56.

[80] Kolonbrander PE, Palmer RJ, Rickard AH, et al. Bacterial interactions and successes during plaque development. Periodontology. 2000;42:47–79.

[81] Maekawa M, Ushida K, Hoshi S, et al. Butyrate and propionate production from D-mannitol in the large intestine of pig and rat. Micro Ecol Health Dis. 2005;17:169–176.

[82] Sarmiento-Rubiano LA, Zúñiga M, Perea-Martinez G, et al. Dietary supplementation with sorbital results in selective enrichment of lactobacilli in rat intestine. Res Microbiol. 2007;158(8–9):694–701.

[83] Tamura M, Hoshi C, Horii S. Xylitol affects the intestinal microbiota and metabolism of daidzein in adult male mice. Int J Mol Sci. 2013;14:23993–24007.

[84] Söderling EM, Ekman TC, Taipale TJ. Growth inhibition of Streptococcus mutans with low xylitol concentrations. Curr Microbiol. 2008;56(4):382–385.

[85] Söderling EM, Hietala-Lenkkeri A-M. Xylitol and erythritol decrease adherence of polysaccharide-producing oral streptococci. Curr Microbiol. 2010;60(1):25–29.

[86] Sälli KM, Forssten SD, Lahtinen SJ, et al. Influence of sucrose and xylitol on an early Streptococcus mutans biofilm in a dental simulator. Arch Oral Biol. 2016;70:39–46.

[87] Kanasi E, Dwyerseh FE, Chalmers NI, et al. Clonal analysis of the microbiota of severe early childhood caries. Caries Res. 2010;44:485–497.

[88] Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. DNA Cell Biol. 2009;28:397–403.

[89] Luo CY, Corliss DA, Ganeshkumar N. Streptococcus gordonii biofilm formation: identification of genes that code for biofilm phenotypes. J Bacteriol. 2000;182:1374–1382.
[90] Kreth J, Zhang Y, Herzberg MC. Streptococcal antagonism in oral biofilms: streptococcus sanguinis and Streptococcus gordonii interference with Streptococcus mutans. J Bacteriol. 2008;190:4632–4640.

[91] Kaspar JR, Lee K, Richard B, et al. Direct interactions with commensal streptococci modify intercellular communication behaviors of Streptococcus mutans. ISME J. 2021;15:473–488.

[92] Idate U, Bhat K, Kotrashetti V, et al. Molecular identification of Capnocytophaga species from the oral cavity of patients with chronic periodontitis and healthy individuals. J Oral Maxillofac Pathol. 2020;24(2):397.

[93] Ciantar M, Spratt DA, Newman HN, et al. Capnocytophaga granulosa and Capnocytophaga haemolytica: novel species in subgingival plaque. J Clin Periodontol. 2001;28(7):701–705.

[94] Gosses L, Amrane S, Mailhe M, et al. Capnocytophaga sputigena: an unusual cause of community-acquired pneumonia. IDCases. 2019;17:e00572.

[95] Lopes MP, Cruz AA, Xavier MT, et al. Prevotella intermedia and periodontitis are associated with severe asthma. J Periodontol. 2020;91:46–54.

[96] Lafaurie GI, Sabogal MA, Castillo DM, et al. Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: a Systematic Review. J Periodontol. 2017;88:1066–1089.

[97] Krzyszciak W, Jurczak C, Piątkowski J. The role of human oral microbiome in dental biofilm formation. In: Dhannasekaran D, Thajuuddin N, editors. Microbial Biofilms - Importance and Applications (London: IntechOpen). 2016:329–382.

[98] Chetruis V, Ion JR. Dental Plaque - Classification, Formation and Identification International Journal of Medical Dentistry. 2013;17(2):139–143. International Journal of Medical Dentistry.

[99] Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol. 1995;15:169–175.

[100] Wolff Mark S, Larson C. The cariogenic dental biofilm: good, bad or just something to control? Braz Oral Res. 2009;23(suppl 1):31–38.

[101] Jagtap P, McGowan T, Bandhakavi S, et al. Deep metaproteomic analysis of human salivary supernatant. Proteomics. 2012;12(7):992–1001.

[102] Rudney JD, Xie H, Rhodus NL, et al. A metaproteomic analysis of the human salivary microbiota by three-dimensional peptide fractionation and tandem mass spectrometry. Mol Oral Microbiol. 2010;25(1):38–49.

[103] Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology (Reading). 2003;149(2):279–294.

[104] Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. J Oral Microbiol. 2020;12(1):1701553.

[105] Henssge U, Do T, Radford DR, et al. Emended description of Actinomyces naeslundii and descriptions of Actinomyces oris sp. nov. and Actinomyces johnsonii sp. nov., previously identified as Actinomyces naeslundi genospecies 1, 2 and WVA 963. Int J Syst Evol Microbiol. 2009;59(3):509–516.

[106] Duke CM, Saylany A, Brown A, et al. Microbial supernatants from Mobiluncus mulieris, a bacteria strongly associated with spontaneous preterm birth, disrupts the cervical epithelial barrier through inflammatory and mRNA mediated mechanisms. Anaerobe. 2020;61:102127.

[107] Thiagarajan A, Balendra A, Hillier D, et al. The first report of survival post Rothia aeria endocarditis. BMJ Case Rep. 2013;2013(oct09 1):bcr2013200534–bcr2013200534.

[108] Brown JM, Georg LK, Waters LC. Laboratory identification of Rothia dentocariosa and its occurrence in human clinical materials. Appl Microbiol. 1969;17(1):150–156.

[109] Keng TC, Ng KP, Tan LP, et al. Rothia dentocariosa repeat and relapsing peritoneal dialysis-related peritonitis: a case report and literature review. Ren Fail. 2012;34:804–806.

[110] Willner S, Imam Z, Hader I. Rothia dentocariosa Endocarditis in an unsuspecting host: a case report and literature review. Case Rep Cardiol. 2019;2019:7464251.

[111] Treerat P, Redanz U, Redanz S, et al. Synergism between Corynebacterium and streptococcus sanguinis reveals new interactions between oral commensals. ISME J. 2020;14(5):1154–1169.

[112] Liu D, Bateman T, Carr E, et al. Endocarditis due to Gemella haemolysans in a newly diagnosed multiple myeloma patient. J Community Hosp Int Med Perspect. 2016;6:32357.

[113] Puzzolante C, Cuomo G, Meschiari M, et al. Granulicatella adiacens and abiotrophy defectiva native vertebral osteomyelitis: three cases and literature review of clinical characteristics and treatment approach. Case Rep Infect Dis. 2019;2019:5038563.

[114] Alberti MO, Hindler JA, Humphries RM. Antimicrobial Susceptibilities of Abiotrophia defectiva, Granulicatella adiacens, and Granulicatella elegans. Antimicrob Agents Chemother. 2015;60:1411–1420.

[115] Ruoff KL. Nutritionally Variant Steptococci. Amer Soc Mic bio. 1991;4:184–190.

[116] Abranches J, Zeng L, Kajfasz JK, et al. Biology of oral streptococci. Microbiol Spectr. 2018;6(5):1–18.

[117] Karpinski TM. Role of oral microbiota in cancer development. Microorganisms. 2019;7(1):20.

[118] Holm K, Bank S, Nielsen H, et al. The role of Fusobacterium necrophorum in pharyngotonsillitis - A review. Anaerobe. 2016;42:89–97.

[119] Brennan CA, Garrett WS. Fusobacterium nucleatum - symbiont, opportunist and oncobacterium. Nat Rev Microbiol. 2019;17:156–166.

[120] Henne K, Jing L, Stoneking M, et al. Global analysis of saliva as a source of bacterial genes for insights into human population structure and migration studies. BMC Evolutionary Biology. 2014;14(1). DOI:10.1186/s12862-014-0190-3.

[121] Henne K, Schilling H, Stoneking M, et al. Sex-specific differences in the occurrence of Fusobacterium nucleatum subspecies and Fusobacterium periodonticum in the oral cavity. Oncotarget. 2018;9(29):20631–20639.

[122] Eribe ERK, Paster BJ, Caugant DA, et al. Genetic diversity of Leptotrichia and description of Leptotrichia goodfellowii sp. nov., Leptotrichia hofstadii sp. nov., Leptotrichia shahii sp. nov. and Leptotrichia wadei sp. nov. Int J Syst Evol Microbiol. 2004;54:583–592.

[123] Eribe ERK, Olsen I. Leptotrichia species in human infections. Anaerobe. 2008;14:131–137.

[124] Kim WJ, Higashi D, Goytia M, et al. Commensal Neisseria kill Neisseria gonorrhoeae through a
DNA-dependent mechanism. Cell Host Microbe. 2019;26:228–239.e8.

[125] Liu G, Tang CM, Exley RM. Non-pathogenic Neisseria: members of an abundant, multi-habitat, diverse genus. Microbiology (Reading). 2015;161(7):1297–1312.

[126] Kageyama S, Nagao Y, Ma J, et al. Compositional shift of oral microbiota following surgical resection of tongue cancer. Front Cell Infect Microbiol. 2020;10:600884.

[127] Kozlova A, Palazzolo L, Michael A. Neisseria sicca: a Rare Cause of Bacterial Conjunctivitis. Am J Case Rep. 2020;21:e923135.

[128] Abusleme L, Dupuy AK, Dutzan N, et al. The sub-gingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J. 2013;7(5):1016–1025.

[129] Guilloux C-A, Lamoureux C, Beaurelle C, et al. Porphyromonas: a neglected potential key genus in human microbiomes. Anaerobe. 2021;68:102230.

[130] Romero-Velez G, Pereira X, Narula A, et al. Gemella morbillorum as a source bacteria for necrotising fascitis of the torso. BMJ Case Rep. 2020;13(1):1: e231727.

[131] Kilpper-Bälz R, Schleifer H. Transfer of Streptococcus morbillorum to the Genus Gemella as Gemella morbillorum comb. nov. 1988;Int J syst bacteriology. 38:442–443.

[132] Menon T, Naveen Kumar V. Catonella morbi as a cause of native valve endocarditis in Chennai, India. Infection. 2012;40(5):581–582.

[133] Lim YK, Kweon OJ, Kim HR, et al. Leptotrichia goodfellowii Infection: case Report and Literature Review. Ann Clin Lab Sci. 2016;46:83–86.

[134] Okumura E, Tsurukiri J, Yamanaka H, et al. Intracranial Hemorrhaging Following Cardiobacterium hominis Endocarditis. Intern Med. 2019;58(9):1361–1365.

[135] Nørskov-Lauritsen N. Classification, identification, and clinical significance of Haemophilus and Aggregatibacter species with host specificity for humans. Clin Microbiol Rev. 2014;27(2):214–240.

[136] Costescu Strachinaru DI, Gallez J-L, Paridaens M-S, et al. A case of Escherichia coli and Peptoniphilus species mixed osteomyelitis successfully identified by MALDI TOF-MS with a review of the literature. Acta Clin Belg. 2020;77(1):126–129.

[137] Downes J, Vartoukian SR, Dewhirst FE, et al. Pyramidobacter piscolens gen. nov., sp. nov., a member of the phylum ‘Synergistetes’ isolated from the human oral cavity. Int J Syst Evol Microbiol. 2009;59:972–980.

[138] Mack R, Slicker K, Ghamande S, et al. Actinomyces odontolyticus: rare etiology for purulent pericarditis. Case Rep Med. 2014;2014:734925.

[139] Yesilbas O, Yozgat CY, Nizam OG, et al. Life-threatening multiple brain abscesses secondary to Actinomyces odontolyticus. Pediatr Int. 2020;62:1307–1308.

[140] Ehrmann E, Jolivet-Gougeon A, Bonnaure-Mallet M, et al. Multidrug-resistant oral Capnocytophaga gingivalis responsible for an acute exacerbation of chronic obstructive pulmonary disease: case report and literature review. Anaerobe. 2016;42:50–54.