Chlorhexidine oral rinses for symptomatic COPD: a randomized, blinded, placebo-controlled preliminary study

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Online Data Supplement

Supplemental Methods

Study design

The chlorhexidine effect in the oral and lung microbiota study (CLIMB) is a randomized, blind, placebo-controlled, parallel-group preliminary study of the effects of chlorhexidine oral rinses on COPD. It was conducted at a single tertiary-care Veterans Affairs medical center (USA). Ethics approval was granted by the Minneapolis Veterans Affairs Medical Center (MVAMC) Institutional Review Board (#4526-A) and all procedures adhered to the study protocol (available in supplementary information).

Participants

Eligible CLIMB participants were those age 40-85 years receiving care at the MVAMC with a clinical diagnosis of COPD, a FEV_1/FVC ratio (post-bronchodilator) ≤ 70%, FEV_1 (post-bronchodilator) ≤ 65%, current or former smokers with lifetime cigarette consumption of ≥ 10 pack-years, presence of ≥ 4 natural teeth, and the presence of high likelihood of a chronic cough and sputum production defined as one of the following: 1) self-report of either cough or sputum production occurring “several days per week” or “almost every day”; or 2) a COPD exacerbation within the previous 12 months (defined as taking antibiotics and/or prednisone for respiratory symptoms, being hospitalized, or visiting the emergency department for respiratory illness). Participants were excluded if they were pregnant, not fully recovered for at least 30 days from a COPD exacerbation, treated with antibiotics (for any indication) in the last two months, had an active oral infection (e.g., dental abscess), currently used chlorhexidine oral rinses, had a known allergy or sensitivity to chlorhexidine, or used supplemental oxygen.

Presence of chronic cough, chronic sputum production, or COPD exacerbation in the last 12 months were used to enrich the study population with participants able to produce sputum and to report respiratory symptoms. The presence of at least four natural teeth was used to maintain consistency of the oral microbiota across participants, as the edentulous oral microbiota is different from the dentate oral microbiota. Likewise, participants who had not completely recovered from a COPD exacerbation, used antibiotics in the last two months, or had a dental infection were excluded to ensure that microbiota samples were collected from participants at their baseline.

Participants were recruited from those visiting the Emergency Department or admitted to the hospital for a COPD exacerbation, and among those participating in COPD case management due to frequent COPD exacerbations. All participants provided written informed consent.

Randomization and masking

Participants were recruited by the study coordinator and randomly assigned (1:1) via a random number generator to receive either 15 mL of twice-daily 0.12% oral chlorhexidine rinses (PerioGard®)[1] or matched placebo mouth rinses for eight weeks. Randomization was not stratified. Matched placebo was compounded by the research pharmacist and consisted of sterile water with blue dye (FD&C#2), polysorbate, and sodium saccharin for flavoring. The pharmacist conducted the allocation and assignment and was the only one unblinded to study assignment. Study medications were dispensed directly to participants in identical opaque

bottles. Participants as well as those interacting with participants (study coordinator and investigators) were blinded to group assignment during the conduct of the study. Investigators adjudicated antibiotic use and exacerbations after unblinding, but these data were used for post-hoc subgroup analyses and not in the primary or secondary outcome analyses.

Procedures

At visit 1, participants provided details of their medical history, performed spirometry, completed the St. George's Respiratory Questionnaire (SGRQ), were instructed on how to complete the Breathlessness, Cough, and Sputum Scale (BCSS)[2] daily diaries during the study, and then provided blood, oral, and induced sputum samples prior to randomization. Blood samples were used to determine white blood cell count and differential, fibrinogen, and C-reactive protein (CRP). Oral and sputum samples were obtained after at least a two-hour fast. Oral samples were obtained by swishing 15-mL sterile water in the mouth for 30 seconds and then spitting the water into a sterile cup. Sputum induction was performed with nebulized 3% saline (0.9% saline if FEV_1 <35%) for up to 20 minutes. Nebulization was terminated when participants either expectorated a 5 mL sputum sample into a sterile cup, 20 minutes of induction had elapsed, or the peak flow dropped to ≤ 80% of the baseline value. Unused sterile water was collected for use as control samples in microbiota analyses. Oral and sputum sample volumes were recorded. Using sterile technique, sputum samples were divided for cell count and gram stain performed by the clinical microbiology laboratory and microbiota analyses (including biomass quantification).

Participants were instructed to swish 15 mL of the study medication (either 0.12% chlorhexidine or placebo) in their mouth for 30 seconds twice daily (morning and evening) followed by expectoration. The study mouth rinse was used twice daily for eight weeks. Participants were told to avoid routine dental clinic visits during the study period.

After four weeks of study mouth rinse use, the study coordinator conducted a mid-study phone call to assess for hospitalizations, Emergency Department visits, unplanned clinic visits, new medication use (including antibiotics), compliance with the study drug, BCSS diary completion, and to assess adverse events. Additional study drug was mailed to participants by the research pharmacist following this phone call.

Eight weeks after randomization participants returned for a second study visit. They were instructed not to use the study mouth rinse the morning of the visit. Participants returned completed BCSS diaries and used study medication bottles to the study coordinator, who noted any remaining volume of study drug. Participants again completed the SGRQ, completed questionnaires (assessing medication changes, hospitalizations, Emergency Department visits, unplanned clinic visits, new medication use), and provided samples (blood, oral rinses, and induced sputum) for biomarker and microbiota analyses.

The BCSS (a daily diary for tracking the severity of respiratory symptoms) was started on Day 1, the day of first treatment. Participants answered three symptom questions on a 0 to 4 scale and a total daily score was calculated from those answers. Baseline BCSS score was the average

of daily BCSS measurements from Days 1-7. Week 8 BCSS score was the average of daily BCSS measurements from Days 50-56.

The SGRQ is a self-administered questionnaire that measures pulmonary disease-related quality of life. It has been validated for use in many chronic lung diseases, including COPD.[3] The SGRQ is scored on a scale of 0 to 100, with 100 reflecting the most severe symptoms. The minimum clinically important difference in the SGRQ is widely accepted as being 4 units.[4]

The clinical laboratories at the MVAMC determined WBC and differential, fibrinogen, CRP levels, and sputum gram stain and culture results. All oral rinses, sputum samples, and unused sterile water (control samples) were frozen immediately and until DNA extraction. 16S rRNA quantification and 16S rRNA V4 MiSeq sequencing was performed at the University of Minnesota Genomics Center as previously described.[5]

Outcomes

The primary study outcome was change in oral and sputum microbiota biomass after 8 weeks of study medication use, compared to baseline values, in participants who used 0.12% chlorhexidine oral rinses vs. placebo as assessed by 16S rRNA quantification. To adjust biomass for the size of the sputum sample, raw counts were normalized by dividing by the sample volume or mass. Secondary outcomes (all compared to baseline values in participants receiving chlorhexidine vs. placebo) included: i) sputum and oral microbiota alpha diversity (as assessed by Shannon and Simpson diversity); ii) sputum and oral microbiota taxonomy; iii) inflammatory

markers (WBC, fibrinogen, and CRP); iv) BCSS scores (week 8 vs. week 1); v) SGRQ score; and vi) assessment of adverse events. Adverse events were assessed both during the mid-study phone call and at the second visit by assessing hospitalizations, new medication use, and death. Participants were asked specifically about known adverse events associated with chlorhexidine oral rinses (oral pain, decreased taste, and tooth discoloration) and open-ended questions about new symptoms.

Statistical analysis

The power analysis for the study examined the power to detect differences in lung microbiota diversity as measured by the Simpson (1-D) diversity measure, ranging from 0.22 to 0.32 between the treatment groups using a two-sample t-test with equal variances. Our data showed that age had a significant positive impact on Simpson diversity with a change in diversity of 0.34 and the averaged standard deviation in the Simpson measure among moderate and severely affected COPD patients of 0.281. If chlorhexidine were to have an effect size similar to the effect of age with 20 participants per group, there was 67%, 75%, 81%, 87%, 91%, and 94% power to detect a difference of 0.22, 0.24, 0.26, 0.28, 0.30, and 0.32 between treatment groups.

Baseline variables were compared using Fisher's Exact Test for categorical variables or the Wilcoxon Two-Sample Test for continuous variables. Means are presented with standard deviations (SD); mean differences and parameter estimates are presented with their associated standard error (SE).

All analyses were performed using SAS version 9.4 (SAS Institute) and the intention-to-treat principle. A two-sided type I error of 0.05 was used. Correction of the Type I error rate for multiple testing was performed for the endpoints that report results from both oral wash and sputum samples using the Step-down Bonferroni method.[6]

For the primary analysis of both normalized oral wash and normalized sputum biomass count, values were transformed to the $\log_{10}$ scale and the mean difference between treatment groups was compared using the two-sample t-test. Additionally, for the analysis of sputum biomass count, a multiple imputation procedure was used to impute each unavailable sputum weight (PROC MI with seed=501213, MCMC method, and acceptable value range of 0.01 to 2.5). For each of the 25 datasets created by the procedure, the normalized biomass (count/mass) was calculated, the values were transformed to the $\log_{10}$ scale, and a t-test was performed. Lastly, PROC MIANALYZE was used to obtain an estimate from the t-test that accounted for the variability in the imputed values.

Linear regression was used to examine the effect of treatment group on the 8-week change in the Shannon and Simpson biodiversity indices, BCSS, SGRQ and inflammatory markers separately, with each model adjusted for the baseline value of the measure.

Subgroup analyses of participants who did not receive antibiotics during the study were also performed for the outcomes of biomass and biodiversity.

For taxa abundance analyses, the number of sequences assigned to each genus were determined for each sputum and oral wash sample. Treatment effects on the abundance of each genera were examined by modeling the 8-week change using linear regression, adjusted for baseline count. We restricted the analyses to the genera with <20% of values equal to zero. In addition, the proportion with organisms detected at Week 8 was compared between treatment groups for each genus using Fisher's Exact Test. Results were corrected for multiple comparisons.

A data monitoring committee did not oversee the study. The study was registered in ClinicalTrials.gov as NCT02252588.

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Table S1. Biomass (log_{10}) from oral wash and sputum samples by treatment group

	Oral Wash (log_{10} molecules/µL/mL)				**Sputum (log_{10} molecules/µL/gram)**			
	Chlorhexidine (N=24)		**Placebo (N=20)**		**Chlorhexidine (N=24)**		**Placebo (N=20)**	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Biomass at Baseline	24	5.22 (0.58)	20	5.38 (0.48)	21	6.07 (1.15)	13	6.66 (0.59)
Biomass at Week 8	20	4.94 (0.96)	20	5.24 (0.34)	17	6.35 (1.04)	15	6.31 (0.86)
Change in biomass	20	-0.24 (1.00)	20	-0.14 (0.32)	15	0.42 (1.24)	12	-0.38 (1.13)
Excluding antibiotic use	**Chlorhexidine (N=21)**		**Placebo (N=15)**		**Chlorhexidine (N=19)**		**Placebo (N=10)**	
Biomass at Baseline	21	5.23 (0.61)	15	5.48 (0.48)	19	6.14 (1.18)	10	6.77 (0.55)
Biomass at Week 8	17	4.93 (1.04)	15	5.29 (0.37)	16	6.39 (1.06)	11	6.19 (0.97)
Change in biomass	17	-0.26 (1.09)	15	-0.19 (0.31)	14	0.45 (1.28)	9	-0.62 (1.20)

Abbreviations: SD = Standard deviation.

Table S2. Linear regression results of the effect of treatment group on the change in biodiversity – subgroup of participants who did not use antibiotics during the study (N=32)

	Linear Regression			
Outcome	**Predictor**	**Estimate(SE)**	**Unadjusted P-value**	**Adjusted P-value[1]**
Shannon Diversity Index Change (Week 8- Baseline)				
Oral Wash (N=32)	Treatment Group[2]	-0.369 (0.097)	0.0007	0.0014
	Baseline Index	-0.168 (0.080)	0.0446	
Sputum (N=28)	Treatment Group	-0.675 (0.192)	0.0017	0.0017
	Baseline Index	-0.455 (0.132)	0.0020	
Simpson Diversity Index Change (Week 8- Baseline)				
Oral Wash (N=32)	Treatment Group	-0.026 (0.008)	0.0021	0.0042
	Baseline Index	0.052 (0.135)	0.7021	
Sputum (N=28)	Treatment Group	-0.087 (0.031)	0.0097	0.0097
	Baseline Index	-0.680 (0.188)	0.0013	

[1]A Step-down Bonferroni p-value adjustment is made for the two comparisons (oral wash and sputum) within each Diversity Index.

[2]Treatment group is coded as Chlorhexidine = 1, Placebo = 0.

Table S3. Linear regression analysis of the effect of treatment group on secondary outcomes

		Chlorhexidine (N=20)		**Placebo (N=20)**	**Treatment Group[1]**	**P-value[2]**
Outcome: 8-week Change[3]	N	Mean ± SD	N	Mean ± SD	Estimate (95% CI)	
C-reactive Protein (mg/L)	20	1.8 ± 7.5	20	0.4 ± 6.8	1.54 (-2.72, 5.80)	0.467
Fibrinogen (mg/dL)	19	22.5 ± 77.8	20	10.0 ± 77.0	20.19 (-28.52, 68.91)	0.406
Leukocytes (K/cmm)	20	0.2 ± 1.8	19	0.5 ± 1.8	-0.32 (-1.42, 0.78)	0.560

[1] Treatment group is coded as Chlorhexidine = 1, Placebo = 0.
[2] The p-value is for the comparison of chlorhexidine vs. placebo.
[3] Each model is adjusted for the baseline value of each outcome.
Abbreviations: SD = Standard deviation; CI = Confidence interval.

Table S4. Adverse events by treatment group

	Week 4 Phone Call		**Week 8 Visit**	
	Chlorhexidine[1]	**Placebo**	**Chlorhexidine**	**Placebo**
Number of Randomized Participants	**23**	**20**	**20**	**20**
Irritation and/or sores of the lining of the mouth, N(%)	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.3)
Allergic reaction (swelling, rash, or hives), N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Change in taste, N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Discoloration of teeth, N(%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
Other side effects, N(%)[2, 3]	2 (8.7)	3 (15.0)	2 (11.1)	0 (0.0)

[1] Due to study withdrawals, adverse effects were assessed for 23 of 24 chlorhexidine participants at Week 4 and 20 of 24 at Week 8.
[2] Other adverse effects in the chlorhexidine group - dry mouth (1 patient at Week 4), feeling of loose teeth + cough + green tinged sputum (1 patient at Week 4), widening gaps in teeth (1 patient at Week 8) and blue tongue for 15-20 minutes after using drug (1 patient at Week 4), widening gaps in teeth (1 patient at Week 8) and blue tongue for 15-20 minutes after using drug (1 patient at Week 8).
[3] Other adverse effects in the Placebo group - increased congestion (1 patient at Week 4), sinus/nasal infection (1 patient at Week 4), and dry mouth/dry cough (1 patient at Week 4).

Figure S1. Rarefaction curve.

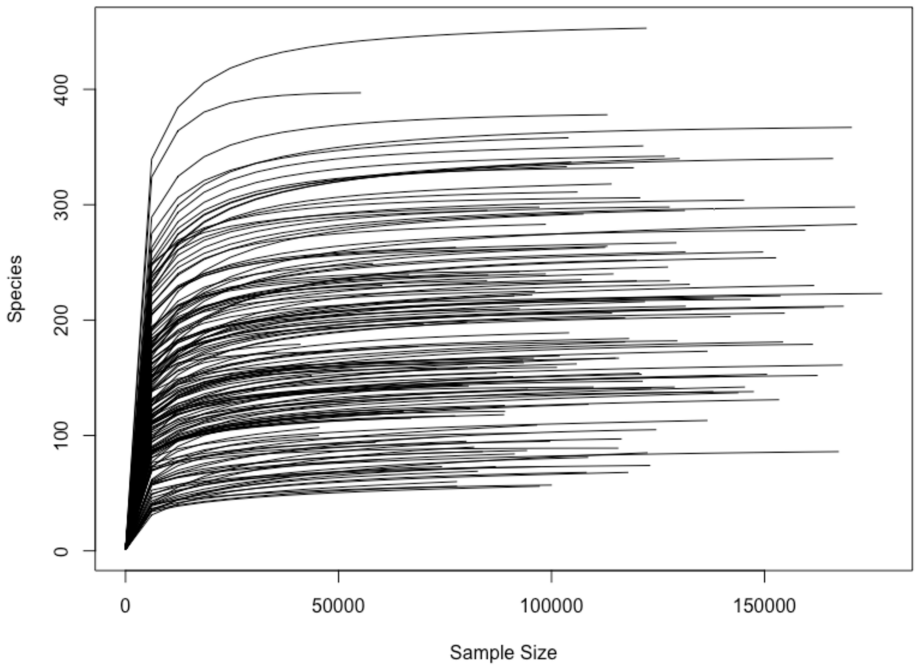

Figure S1. Rarefaction curve. Each sample is represented by a line which illustrates the number of species identified within a subset of sequences taken from that sample. Horizontal asymptotes indicate that additional sequences obtained from that sample are unlikely to identify additional species.

References

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