

The effect of maxillary sinus antrostomy size on the sinus microbiome

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Background: The optimal maxillary antrostomy size to surgically treat sinusitis is not well known. In this study, we examined clinical metrics of disease severity and symptom scores, measured secreted inflammatory markers, and characterized the sinus microbiome to determine if there were significant differences in outcome between different maxillary ostial sizes.

Methods: Prospective randomized, single-blinded clinical trial enrolling 12 individuals diagnosed with recurrent acute or chronic rhinosinusitis. Each patient was blinded and randomized to receive minimal maxillary ostial dilation via balloon sinuplasty on 1 side vs a mega-antrostomy on the contralateral side. Data collected included symptom scores (20-item Sino-Nasal Outcome Test [SNOT-20]), endoscopy, and radiologic Lund-Mackay scores. During surgery and at their postoperative visit swabs were obtained from each maxillary sinus, and 16S DNA and inflammatory cytokine levels analyzed. The use of each patient as their own control allowed us to minimize confounding variables.

Results: There was statistically significant improvement in SNOT-20 symptom scores postoperatively in all patients.

There were no significant differences between maxillary ostial size in postoperative endoscopy scores, cytokine profile, or bacterial burden. There were statistically significant differences in relative postoperative abundance of *Staphylococcus*, *Lactococcus*, and *Cyanobacteria* between the mega-antrostomy and mini-antrostomy.

Conclusions: The method used in surgical maxillary antrostomies had no effect on endoscopy scores or cytokine profiles. Microbiome analysis determined significant differences between the different antrostomy sizes in postoperative *Staphylococcus*, *Lactococcus*, and *Cyanobacteria* abundance. The clinical significance of these changes in the sinus microbiome are not known but may be a result of increased access to postoperative sinonasal irrigations.
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Key Words:

maxillary; sinus; antrostomy; microbiome; cytokines

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Sinusitis affects approximately 10% of the U.S. adult population with a yearly economic impact of approximately \$15 billion.¹ Sinusitis symptoms may

include nasal blockage/obstruction/congestion, nasal discharge, facial pain/pressure, and the reduction or loss of smell.² The diagnosis of sinusitis is determined by assessing patient symptoms, nasal endoscopic findings, and/or radiologic characteristics of mucosal thickening or opacification on sinus computed tomography (CT). Our current knowledge of the pathophysiology of sinusitis focuses on sinonasal inflammation and the potential role of infection; however, our current diagnostic methods are not designed to detect inflammatory molecular markers or alterations in the sinonasal microbiome.

Acute sinusitis is self-limiting with complete symptom resolution within 4 weeks; surgical therapies are therefore not indicated. However, in circumstances of recurrent acute rhinosinusitis (RARS), defined as a minimum of 4 annual episodes of acute symptoms lasting greater than 10 days with distinct symptom free periods, or chronic rhinosinusitis (CRS), with symptoms greater than 12 weeks,² surgical therapy may play a role. A study comparing the

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outcomes of medical to surgical therapies in RARS concluded greater symptomatic improvement in those who underwent a targeted approach of maxillary antrostomies and anterior ethmoidectomies.³ Moreover, this limited surgical approach has been shown to be effective in persons with CRS who did not have additional risk factors of asthma, aspirin sensitivity, nasal polyposis, or a history of previous sinus surgery.⁴

The concept of minimally invasive sinus surgery has been a driving factor in the evolution from open procedures that stripped diseased mucosa to endoscopic procedures that focus on mucosal preservation and restoring normal mucociliary clearance.⁵ The principle of maxillary balloon sinuplasty is to cannulate the natural sinus ostia and then dilate the mucosa, thereby preserving the mucosa without removing the entire uncinate process.^{6,7} Several studies have shown that in select patients balloon sinuplasty results in comparable improvements in sinonasal symptoms, endoscopic findings, and radiographic Lund-Mackay scores compared to traditional endoscopic sinus surgery.^{8–11} However, these previous studies comparing optimal maxillary antrostomy size have been confounded by the heterogeneity of sinusitis between different patients. To our knowledge, there have been no studies that have utilized molecular inflammatory markers and alterations in the sinonasal microbiome to study the effects of maxillary antrostomy size. Our goal in this study was to determine if there were significant differences between inflammatory cytokine levels or postsurgical sinus microbiome in the same patient undergoing maxillary dilation on one side, and a maxillary mega-antrostomy and uncinectomy on the contralateral side.

Materials and methods

Patients were included if they were at least 16 years of age, diagnosed with RARS or CRS,² received prior maximal medical therapy (oral antibiotic and steroid treatment), and had findings of symmetric mucosal thickening involving the maxillary sinus and osteomeatal complex on sinus CT. Patients were excluded if they had clinical findings of obstructive nasal polyps, allergic fungal sinusitis, or had 1 of the following comorbidities: cystic fibrosis, primary ciliary dyskinesia, and/or other immunodeficiency disorders. All included patients gave informed consent under the University of Arizona Institutional Review Board (IRB) #1502660530. Prior to surgery, patients were given 20-item Sino-Nasal Outcome Test (SNOT-20) questionnaires,¹² and the sinuses were endoscopically and radiologically scored using the modified Lund-Kennedy¹³ and Lund-Mackay¹⁴ grading system. Maximal medical therapy was consistent with previously published guidelines for RARS to include antibiotics and oral steroids (Medrol dose pack; Pfizer Inc., New York City, NY). Our postoperative care was standardized to include 1 week of antibiotics and oral steroid taper with visits at 1 week, 6 weeks, and 3 months postoperation.

Laterality of the mega-antrostomy and mini-antrostomy was blinded to the patient and randomized prior to surgery. On the mini-antrostomy side, the lower half of the uncinate process was removed in order to visualize the natural maxillary ostium (os). Subsequent dilation was performed either with a balloon dilator (Relieva Scout 6 × 16 balloon sinuplasty system; Acclarent, Inc., Irvine, CA) via a maxillary sinus catheter which was dilated to a pressure of 12 atmosphere, or with a curved olive-tip suction. The diameter of the mini-antrostomy was approximately 1 cm. A mega-antrostomy was performed on the contralateral side which involved removing the entire uncinate process. The natural os of the maxillary sinus was enlarged anteriorly to the level of the lacrimal bone, superiorly to the height of the maxillary sinus, posteriorly to the depth of the posterior maxillary wall, and inferiorly to the level of the inferior turbinate. Two FLOQSwabs (Copan Flocked Technologies Srl, Brescia, Italy) were threaded through a sterile curved olive-tip suction and the maxillary sinus mucosa brushed. The swabs were immediately snap-frozen at –78°C using the CoolBox 30 system with dry ice (Corning Life Sciences, Corning, NY) and stored at –80°C until processing. A similar procedure was performed at subsequent postoperative visits. One to three postoperative samples were collected from each subject dependent on patient participation, and based on physician discretion following endoscopic evaluation.

Bacterial DNA was extracted from 1 of the 2 maxillary mucosa swabs using the PowerSoil DNA isolation kits (MoBio Laboratories, Inc., Carlsbad, CA). The extracted samples were then purified and concentrated for 16S sequencing using the DNeasy PowerClean Pro cleanup kit (Qiagen NV, Venlo, the Netherlands). DNA concentrations were measured using a NanoDrop Lite spectrophotometer (ThermoFisher Scientific, Waltham, MA). The bacterial burden was measured using 16S real-time quantitative polymerase chain reaction (qPCR) and normalized against the amount of human DNA collected as a measure of surface area swabbed. Each reaction contained 20 ng of genomic DNA, 250 nM of forward and reverse primers, and 2X PerfeCTa SYBR Green FastMix (Quanta BioSciences, Inc., Gaithersburg, MD).¹⁵ Human IntraYd6-Alu primers were used: F 5'-GAGATCGAGACCACGGTGAAA-3', R 5'-TTTGAGACGGAGTCTCGTT-3'; PCR conditions: 95°C for 12 minutes, then 40 cycles alternating between 95°C for 15 seconds and then 61°C for 1 minute.¹⁶ Bacterial 16S rRNA primers were used as follows: F 5'-ACTCCTACGGGAGGCAGCAGT-3', R 5'-ATTACCGCGGCTGCTGGC-3'; PCR conditions: 95°C for 10 minutes, then 40 cycles alternating between 95°C for 30 seconds and then 68°C for 1 minute.¹⁷ The qPCR reaction was performed on a Rotor-Gene RG-3000 (Qiagen NV, Venlo, the Netherlands) in triplicate. *Escherichia coli* (Affymetrix, Inc., Santa Clara, CA) stock solution was diluted and used to create a standard curve (ranging from 1 pg to 10 ng) as a function of average cycle times. Concentrations of the bacterial DNA was calculated by

TABLE 1. Patient demographics. There was no statistical significance. Significance determined by two-tailed student's t-test

	(n = 12)	
Female, (n)	58% (7)	
Mean Age (Age Range)	48.8 (16–84)	
Ethnicity, (n)		
White	75% (9)	
Hispanic	17% (2)	
Native Americans	8% (1)	
Rhinosinusitis, (n)		
Chronic (CRS)	50% (6)	
Recurrent Acute (RARS)	50% (6)	
Asthma, (n)	42% (5)	
Allergic rhinitis (n)	58% (7)	
Predominant mega-antrostomy side (n, %)	Left (9, 75%)	
Clinical characteristics	Mega-antrostomy	Mini-antrostomy
Mean pre-OP modified Lund-Kennedy scores (score range)	1.08 (0–3)	0.92 (0–4)
Mean pre-OP maxillary Lund-Mackay score (score range)	0.50 (0–1)	0.50 (0–1)
Mean pre-OP total Lund-Mackay score (score range)	3.83 (0–12)	

comparing the calculated average cycle times against the standard curve generated.

To analyze the sinus microbiome, the hypervariable V4 region of the 16S rRNA gene was amplified from the same sample used for 16S qPCR, using barcoded 806R primers, 515 F primers,¹⁸ and MyFi Mix (Bioline USA Inc., Taunton, MA) in duplicate. Quality of the amplicons and the presence of potential contaminants was checked on an agarose gel. Amplicons were quantified using the PicoGreen assay kit (Invitrogen, Waltham, MA), according to the protocol provided by the manufacturer. From each sample, 240 ng of amplicon was pooled and cleaned using the UltraClean PCR clean-up Kit (MoBio Laboratories, Inc., Carlsbad, CA). A pooled library was quantified using the qPCR-based KAPA library quantification kit (Kapa Biosystems, Wilmington, MA). The library was diluted, denatured (0.2 N NaOH) and sequenced on the MiSeq platform (Illumina, Inc., San Diego, CA) using custom primers.¹⁸ Due to the limited sequence diversity among 16S rRNA amplicons, 5% of the PhiX control library (Illumina, Inc., San Diego, CA) made from PhiX174 was added to the run. Final concentration of 6.5 pM of the pooled 16S rRNA library was subjected to the paired-end sequencing using 2 × 151-bp MiSeq reagent kit V2 (Illumina, Inc., San Diego, CA). Sequencing was performed on the Illumina MiSeq (SN M02149, with the MiSeq Control Software v 2.5.0.5). After demultiplexing and quality filtering, 6,632,190 sequences remained with a median length of

251 bases. Out of 77 pooled samples, 54 samples from this study with a total number of 1,663,830 reads were taken for analysis presented in this study. Demultiplexing and filtering was done using the QIIME 1.9.1 software package.¹⁸ Sequences were assigned to operational taxonomic units (OTUs) with a 97% similarity threshold using QIIME's UCLUST-based open-reference OTU picking protocol against SILVA reference database (release 128, <http://www.arb-silva.de/download/archive/qiime/>).¹⁹ Sequences that did not match the reference database were clustered de novo and a phylogenetic tree was built.²⁰ The average number of sequences per sample was 30,811 ± 139,386 (mean ± SD, minimum 718, maximum 1,031,788). All samples were analyzed with the sampling depth of 718 sequences. The Core_diversity_analysis.py QIIME script (http://qiime.org/scripts/core_diversity_analyses.html) was run on the OTU tables including alpha and beta diversity as well as taxonomic summary as implemented in the QIIME software package.

The second maxillary mucosa swab was used to quantify inflammatory cytokines by eluting in 1 mL of 10 mM sodium-phosphate buffer containing 150 mM NaCl (pH 7.1) and 1% bovine serum albumin (Sigma-Aldrich Corporation, St. Louis, MO) for 15 minutes. The swabs were then centrifuged at 100 g for 1 minute to recover the remaining eluate.²¹ Cytokine levels were measured in triplicates using the Bio-Plex Pro human cytokine standard

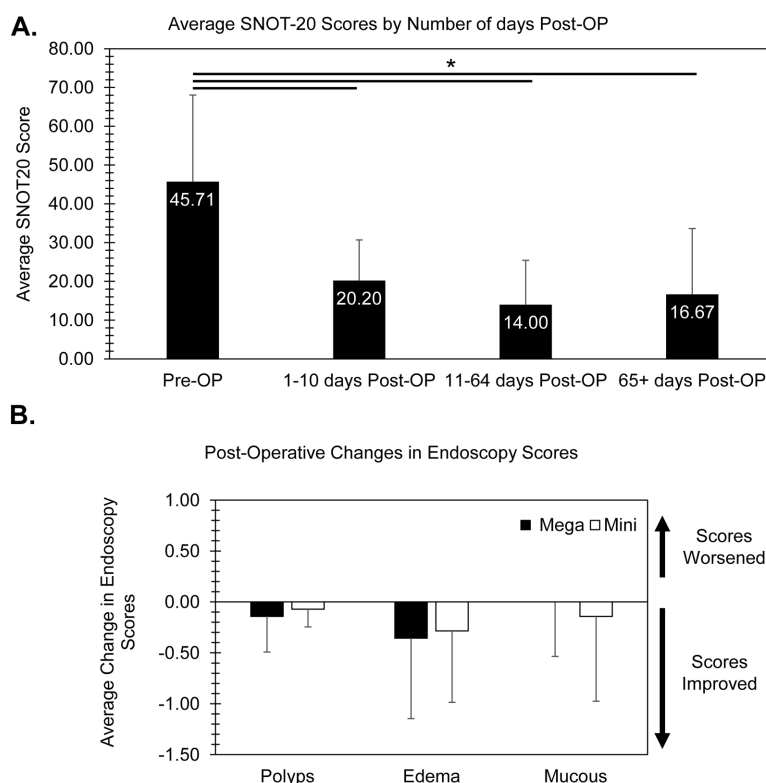


FIGURE 1. Patient symptom scores and endoscopic evaluation of the maxillary sinus pre-OP and post-OP. (A) SNOT-20 tests were given to study participants ($n = 7$) at pre-OP, and at different post-OP visits. The number of questionnaires averaged at each visit is as follows: pre-OP ($n = 7$); 1-10 days post-OP ($n = 5$); 21-64 days post-OP ($n = 5$); 65+ days post-OP ($n = 6$). *There was statistical significance between pre-OP and 1-10 days ($p = 0.038$); 11-64 days ($p = 0.016$); 65+ days post-OP ($p = 0.032$). (B) Modified Lund-Kennedy endoscopy scores ($n = 12$) were normalized between pre-OP and post-OP and averaged to show the change in scores between the mega-antrostomy and mini-antrostomy. Negative values indicate an improvement in scores between pre-OP and post-OP. There was no statistical significance. post-OP = postoperative; pre-OP = preoperative; SNOT-20 = 20-item Sino-Nasal Outcome Test.

27-plex, group 1 kit (Bio-Rad Laboratories, Inc., Hercules, CA). Specifically, only the following cytokines from the kit were measured: interleukin (IL)-1 α , IL-4, IL-6, IL-8, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)- γ , interferon-inducible protein 10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1). Some patient samples were excluded from the cytokine analysis because the swabs were contaminated with blood.

Results

There was an equal distribution of RARS and CRS in our study cohort with a greater than 40% prevalence of co-occurring asthma and allergic rhinitis. There were no preoperative differences between the sides that received a mega-antrostomy vs a mini-antrostomy based on endoscopic modified Lund-Kennedy and radiologic Lund-Mackay scores (Table 1). Postoperatively, there was a statistically significant improvement in SNOT-20 symptom scores for all patients (Fig. 1A). Further, there were no statistically significant differences between maxillary ostial size in postoperative endoscopy scores (Fig. 1B).

In total, 9 cytokines were measured in the maxillary sinuses after surgery. Concentrations of three

cytokines—IL-4, IFN- γ , and GM-CSF—were too low to be detected in all patients and were excluded from the analysis. The remaining 6 cytokines had concentrations that were within the detectable range. However, there was no statistically significant difference in postoperative cytokine profiles between the different ostial sizes (Fig. 2A, B).

There was no statistical difference in the total bacterial burden between the different ostial sizes, or in the change in average bacterial burden before and after surgery (Fig. 3A). In further characterizing the bacterial microbiome, there were no statistical differences in the postoperative sinus alpha or beta diversity between the mega-antrostomies and mini-antrostomies (Fig. 3B, C). The bacterial composition, however, was significantly different depending on the maxillary ostial size. Compositional analysis was conducted at a rarefaction measure of 718 sequences per sample (Fig. 4). Major taxa (relative abundance greater than 1%) that saw significant differences postoperatively between the mega-antrostomy and mini-antrostomy include (Table 2): *Streptococcaceae Lactococcus*, *Staphylococcaceae Staphylococcus*, and *Cyanobacteria Chloroplast*. In addition, there were significant changes in the following major bacterial taxa between preoperative and postoperative samples within the same antrostomy, but no significance differences when the mega-antrostomy and

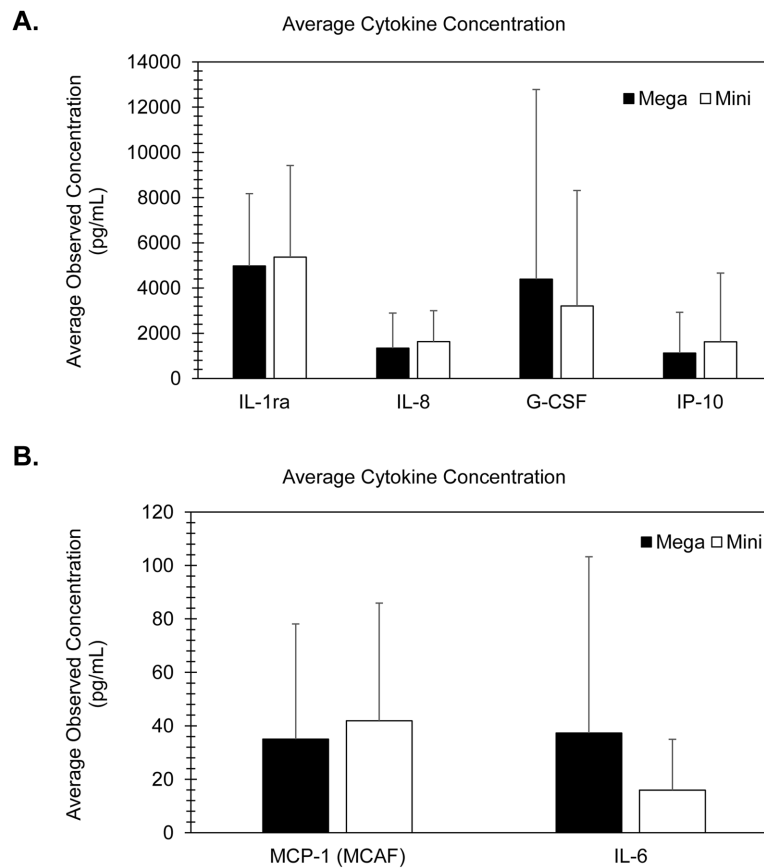


FIGURE 2. Sinonasal cytokine secretion in the mega-antroostomy vs mini-antroostomy postoperatively. (A, B) The average postoperative cytokine concentration was calculated by averaging the observed concentration of individual samples from the mega-antroostomy and mini-antroostomy from the patients' (n = 9) postoperative visits. The number of samples averaged for each cytokine is as follows: IL-1ra, IL-8, G-CSF, and IP-10 (n = 13 for mega-antroostomy and mini-antroostomy); MCP-1 (n = 13 and n = 12 for mega-antroostomy and mini-antroostomy, respectively); IL-6 (n = 9 and n = 10 for mega-antroostomy and mini-antroostomy, respectively). G-CSF = granulocyte-colony stimulating factor; IL = interleukin; IP-10 = interferon-inducible protein-10; MCP-1 = monocyte chemoattractant protein-1.

mini-antroostomy sinuses were compared: *Micrococcaceae* *Arthrobacter*, *Corynebacteriaceae* *Corynebacterium*, *Propionibacteriaceae* *Propionibacterium*, *Streptococcaceae* *Streptococcus*, *Comamonadaceae* *Delftia*, *Bradyrhizobiaceae* *Bosea*, *Lactobacillaceae* *Lactobacillus*, and *Pasteurellaceae* *Haemophilus* (Appendix Tables 1, 2 and 3). Statistical significance was determined using unpaired, 2-tail heteroskedastic Student *t* test, with a significance level of 0.05. Samples were grouped according to antrostomy size and the composition between each antrostomy was analyzed at every single time point. Changes within each antrostomy over time was also tested for significance.

Discussion

There are 3 major goals for a maxillary antrostomy: to improve sinus ventilation and drainage,^{6,22,23} to improve surgical access to the sinus for removal of tissue,⁵ and to allow access for saline irrigations and topical medication delivery in the postoperative period.^{23,24} There is currently debate in the field as to how large a maxillary sinus antrostomy should be to achieve optimal patient outcome.⁵ In

our study, maxillary antrostomy size did not have any significant effect on endoscopic scores, bacterial burden, or inflammation levels (as measured by cytokine secretion) postoperatively. However, certain bacterial taxa were significantly changed postsurgically.

Previous studies assessing sinus ventilation and drainage with respect to maxillary antrostomy size have reported conflicting results. Brumund et al.²⁵ found that a small antrostomy (2-4 mm) produced significant increases in maxillary sinus ventilation, and that no additional ventilation was achieved by creating a larger antrostomy (6-9 mm) in a sheep xenon-gas model. Other studies using computational flow dynamics to model human sinonasal air flow and ventilation patterns reported that larger antrostomies provide more sinus ventilation by increasing both airflow and flow velocity through the ostium.^{6,22} Hood et al.⁶ theorized that the natural ostium limited ventilation into the sinuses, which served to protect the sinuses from drying out the mucosal surfaces within the sinus cavity and maintain high nitric oxide concentrations limiting pathogen access. Based on this theory, the mini-antroostomy should theoretically have a lower bacterial burden, and in turn, less

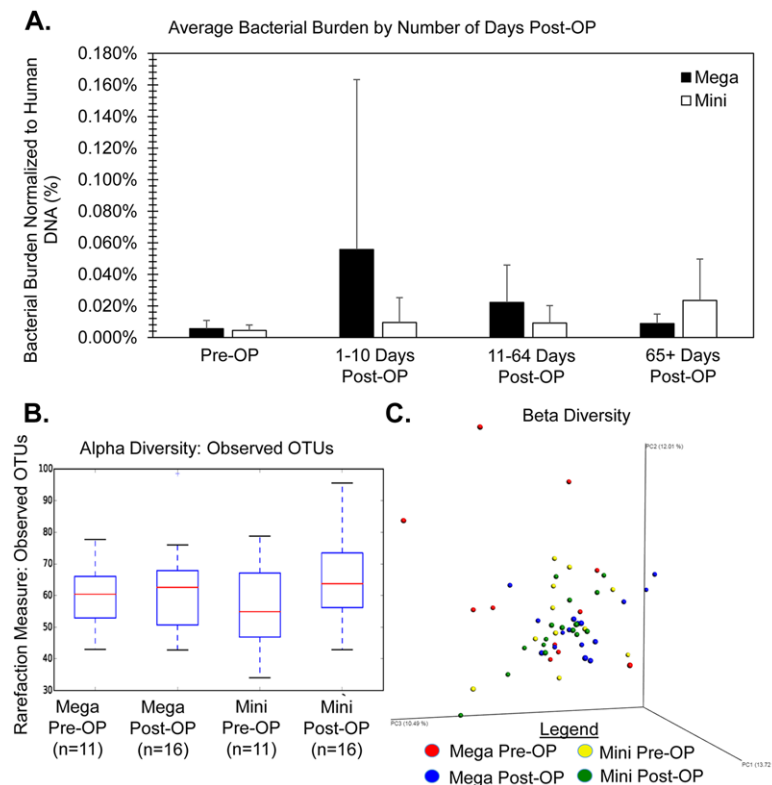


FIGURE 3. Bacterial burden and diversity of the maxillary sinus microbiome in mega-antroostomy vs mini-antroostomy. (A) Average bacterial burden normalized to human DNA was determined using 16S real-time qPCR. Pre-OP (n = 11), 1-10 days post-OP (n = 7), 21-64 days post-OP (n = 5), and 65+ days post-OP (n = 3). There was no statistical significance. (B) Alpha diversity was calculated at 718 sequences per sample using observed OTUs. There was no statistical significance in any of the indices calculated (including chao1, Shannon, PD whole tree, and margalef). (C) A PCoA of weighted UniFrac distances were calculated between all mega-antroostomy and mini-antroostomy pre-OP and post-OP visits. There was no statistical significance. OTU = operational taxonomic unit; PCoA = principal coordinates analysis; PD = Phylogenetic Diversity; post-OP = postoperation; pre-OP = preoperation; qPCR = quantitative polymerase chain reaction.

evidence of inflammation. The smaller ostial size should limit the air flow into the sinuses, thereby maintaining the high nitric oxide concentrations, controlling bacterial growth (as compared to the mega-antroostomy). However, in our clinical study, we did not find significant differences in inflammatory cytokine levels or overall bacterial burden levels postoperatively between either ostial sizes. The insignificant differences suggest that air flow patterns generated by the different maxillary antroostomy size may not contribute directly to sinonasal inflammation or bacterial burden.

One factor in enlarging the maxillary sinus antroostomy is to provide wider access for surgery and postoperative surveillance. In our study, we purposefully excluded sinonasal pathologies such as nasal polyposis and sinonasal tumors in which larger access could improve surgical outcomes. Postoperative visualization of the mini-antroostomy was more difficult than the mega-antroostomy; however, none of the patients in our cohort required revision surgery to correct restenosis or scarring of the mini-antroostomy. In previous studies, revision rates of balloon catheter dilations due to restenosis were similar to traditional endoscopic sinus surgery.^{10,26}

Bacterial 16S sequencing of both maxillary sinuses was conducted to determine the relative abundance of different

bacterial taxa present in the sinus microbiome before and after surgery. Postoperatively, there was a significant increase in the relative abundance of *Staphylococcaceae* *Staphylococcus* in the mini-antroostomy. There was no similar postoperative increase in *Staphylococcus* in the mega-antroostomy, and the difference in relative abundance of *Staphylococcus* between the mega-antroostomy and mini-antroostomy was statistically significant. The increase in *Staphylococcus* abundance seen in the mini-antroostomy is concerning because *Staphylococcus aureus* is one of the most common pathogens cultured in acute and CRS.²⁷⁻³¹ Previous studies have found that *S. aureus* biofilms are known to negatively affect endoscopic sinus surgery outcomes and are associated with ongoing mucosal inflammation and infections postoperatively.³² Our cohort of patients saw a statistically significant increase in *S. aureus* composition in the mini-antroostomy, but we did not see the expected association with inflammation. In our cytokine analysis, there was no statistically significant difference in inflammatory cytokines between both antroostomies postoperatively. Further, increased abundance of *S. aureus* can be associated with reduced bacterial diversity in the sinus microbiome,²⁹ although we did not see a significant decrease in diversity in our patients. *Streptococcaceae* *Lactococcus* was another bacterial taxa that showed significant

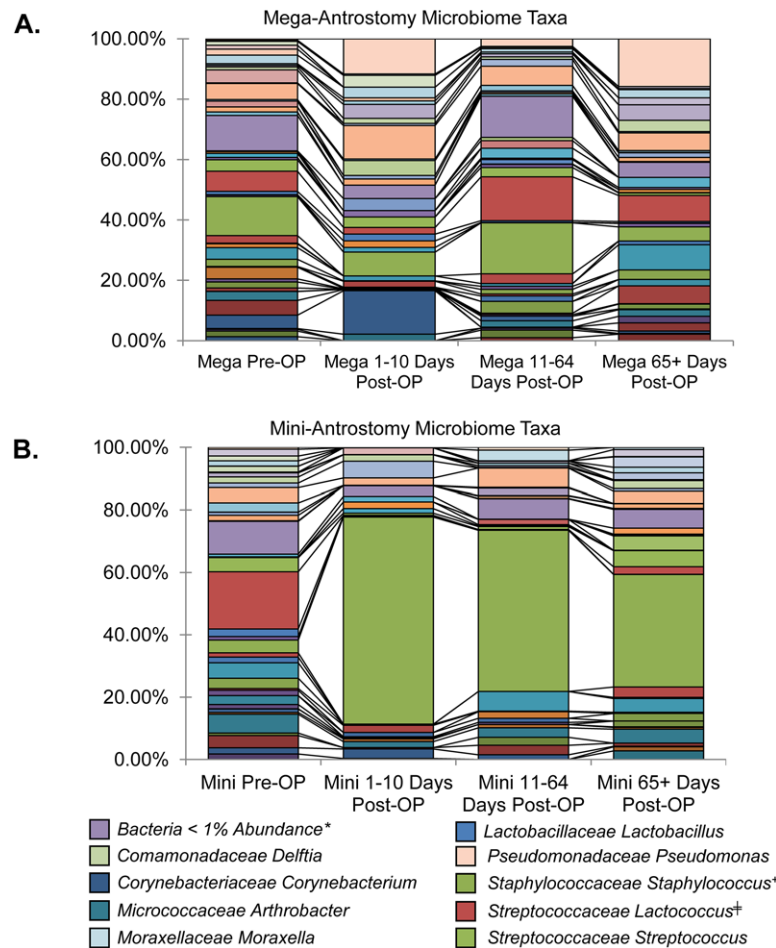


FIGURE 4. Taxonomic differences in the maxillary sinus. (A, B) The 2 bar graphs (n = 11) show the relative abundance of bacterial family present in the mega-antrostomy and mini-antrostomy (Family, Genus). Only bacteria greater than 1% in abundance was graphed. The # of samples averaged at each visit is as follows: pre-OP (n = 11); 1-10 days post-OP (n = 5); 11-64 days post-OP (n = 6); 65+ days post-OP (n = 5). There was statistical significance between the mega-antrostomy and mini-antrostomy in *Cyanobacteria Chloroplast* (Phylum, Class; abundance was <1%) at 11-64 days post-OP (*p = 0.02); *Staphylococcaceae Staphylococcus* (Family, Genus) at 1-10 days post-OP ([†]p = 0.04); *Streptococcaceae Lactococcus* (Family, Genus) at pre-OP and 11-64 days post-OP ([†]p = 0.002 and 0.03, respectively). Significance was determined by 2-tailed paired Student t test. post-OP = postoperation; pre-OP = preoperation.

post operative differences between the mega-antrostomy and mini-antrostomy: relative abundance increased in the mega-antrostomy while the mini-antrostomy saw postoperative decreases, with relative abundance falling as low as 0.03%. In a study conducted by Debarry et al.³³ *Lactococcus lactis* was 1 of 2 bacterial isolates which had allergy-protective properties that reduced allergic reactions in a rat model. In our cohort, there was no association between *Lactococcus* abundance and ostial size regardless of allergic rhinitis diagnosis. Finally, the relative abundance of *Cyanobacteria* chloroplasts was significantly greater in the mega-antrostomy than the mini-antrostomy at postoperative visits between 11 and 64 days. In a study by Aurora et al.³⁴ the most abundant species in CRS and in healthy patients was the *Cyanobacteria* phylum. In the mega-antrostomy, the levels of *Cyanobacterium* was increasing, while the mini-antrostomy saw significant decreases in *Cyanobacteria* abundance; however, neither antrostomy had levels as high as Aurora et al.³⁴ *Cyanobacteria* is

typically found in marine environments and terrestrial soil, indicating that the presence in our cohort may be due to environmental exposure.³⁵ The changes in *Staphylococcus*, *Lactococcus*, and *Cyanobacteria* abundance in the mega-antrostomy suggests that the larger ostium created during surgery may maximize the efficacy of postsurgical care, restoring the sinus microbiome back to a nondiseased state.

Multiple studies have investigated the effect of ostial size and the distribution of topical agents, efficacy of saline irrigations for sinus penetration, and efficacy of removing sinonasal secretions and biofilms.^{23,24,36} Grobler et al.²⁴ found that a mean maxillary sinus ostia of at least 9.2 mm allowed penetration of topical irrigations into the maxillary sinus; however, delivery increased as the ostia was enlarged. This may account for the microbial dysbiosis seen in those with minimal dilations of the maxillary os postsurgery, as postoperative sinonasal irrigations may not have adequately flushed the sinus.

TABLE 2. Percent change in bacterial abundance by antrostomy size*

Bacterial taxa (Family, Genus)	Mega pre-OP (%)	Mini pre-OP (%)	Mega 1–10 days (%)	Mini 1–10 days (%)	Mega 11–64 days (%)	Mini 11–64 days (%)	Mega 65+ days (%)	Mini 65+ days (%)
<i>Streptococcaceae Lactococcus</i>	1.05	2.41	2.27	0.09	0.60	0.00	0.48	0.07
<i>Staphylococcaceae Staphylococcus</i>	12.90	4.16	7.90	66.43	16.92	51.74	4.71	36.13
<i>Cyanobacteria Chloroplast (Phylum, Class)</i>	2.33	3.31	0.15	0.31	1.51	0.13	3.11	0.39
Percent change in bacterial abundance between antrostomy sizes								
Bacterial taxa (Family, Genus)	Pre-OP (%)	1–10 days post-OP (%)	11–64 days post-OP (%)	>65 days post-OP (%)				
<i>Streptococcaceae Lactococcus</i>	129.52	–96.04	–100.00	–85.42				
<i>Staphylococcaceae Staphylococcus</i>	–67.75	740.89	205.79	667.09				
<i>Cyanobacteria Chloroplast (Phylum, Class)</i>	42.06	106.67	–91.39	–87.46				

*Negative percent changes indicate that the bacterial taxa had smaller abundance in the mini-antrostomy, while positive percent changes indicate larger abundance. pre-OP = preoperation.

Limitations of this study include a small sample size, which may not overcome intrinsic patient-to-patient variations in cytokine profiles and microbiome composition. With a cohort of only 6 patients, the study by Liu et al.³⁷ had findings that strongly correlated with previous studies regarding the impact on antibiotics on the human microbiota. Even with the small sample sizes, Liu et al.³⁷ were able to see significant changes in endoscopic appearance and microbiota profile. Our study uses the patients as their own control, in addition to having a larger cohort than Liu et al.³⁷ Another limitation is that the SNOT-20 questionnaire is not validated to compare 2 sinuses within the same patient. However, the data gathered from these surveys showed statistically significant improvements in patient-reported outcomes postoperatively, consistent with other studies.^{9,11,38,39} Further studies evaluating the long-term effects of maxillary antrostomy size on the sinus microbiome and inflammatory profile are needed before generalizations on the entire CRS and RARS population can be made. However, our study makes significant contributions toward better understanding how antrostomy size may effect immune function and microbiome composition.

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Conclusion

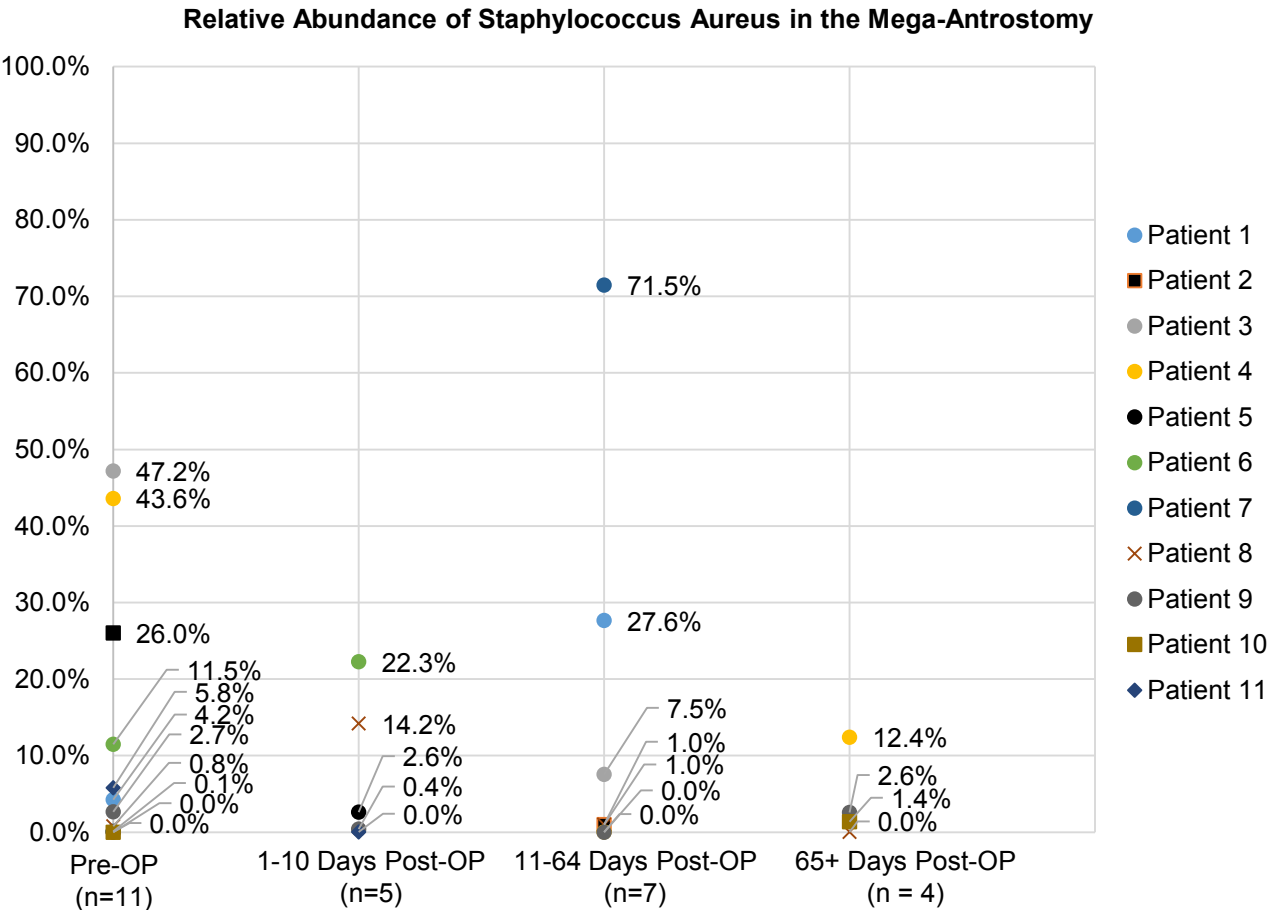
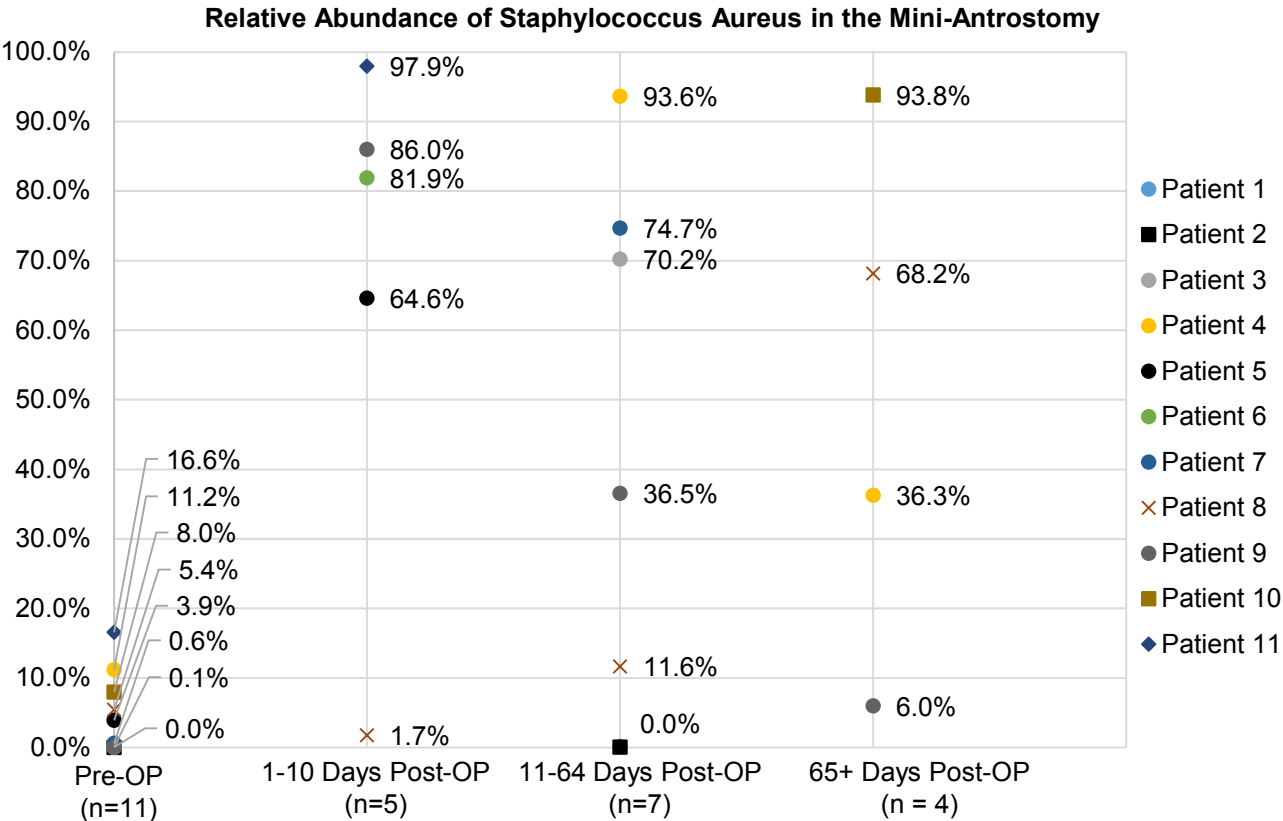
The size of the maxillary antrostomy had no effect on postoperative patient outcome questionnaires, endoscopy scores, cytokine profiles, or total bacterial burden. Even though patients had 2 different antrostomy sizes, the patients in this cohort reported improved symptom scores consistent with those seen in patients with traditional bilateral sinus surgery. The microbiome analysis did show differences between the 2 antrostomy sizes postoperatively, and these differences may be related to postsurgical care. ④

Acknowledgments

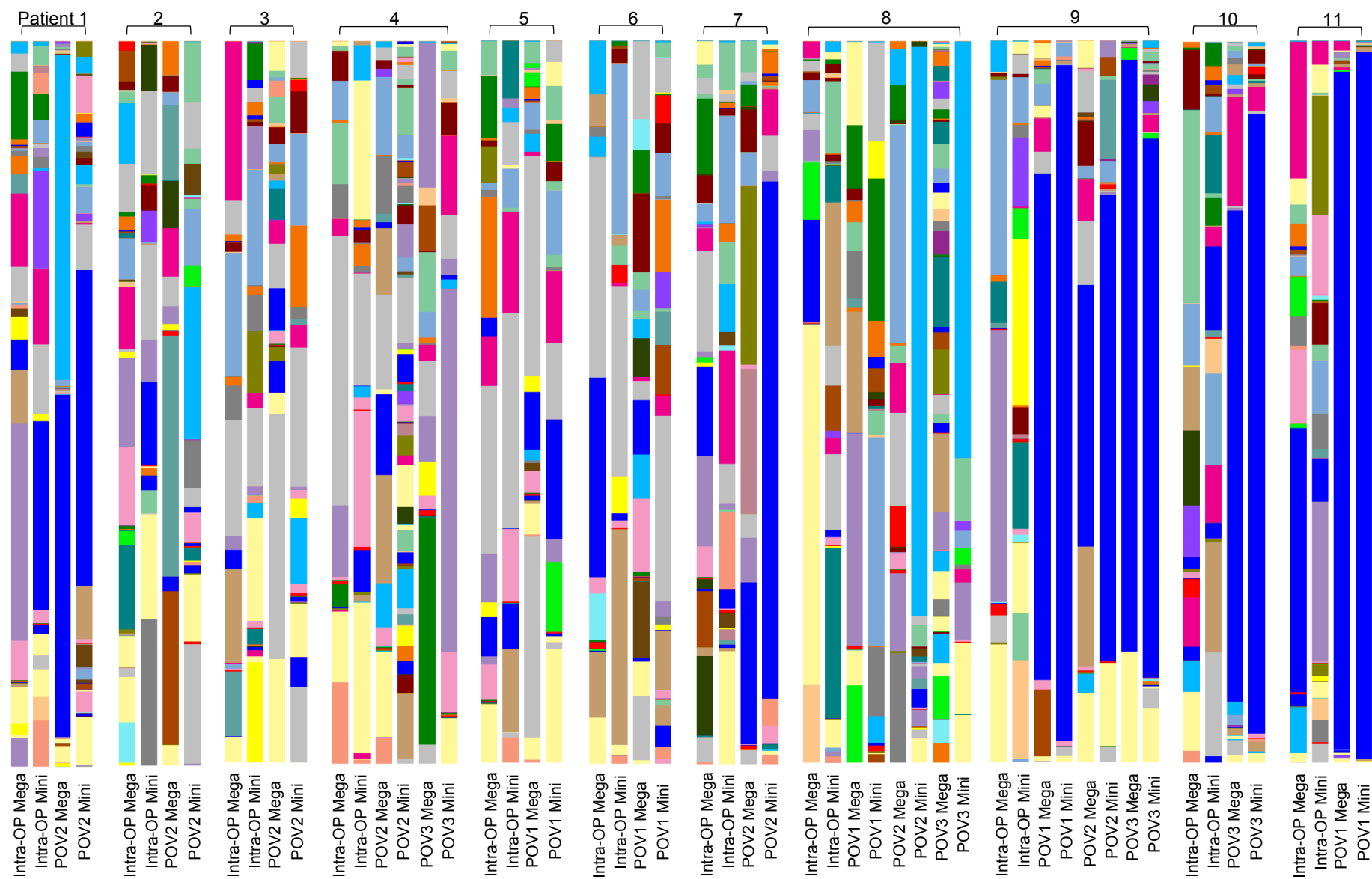
We thank Dr. Joe G.N. Garcia and his laboratory (Department of Medicine, University of Arizona College of Medicine, Tucson, AZ) for allowing us to use their Luminex MagPix instrument and Dr. Heddwen Brooks and her laboratory (Department of Physiological Sciences, University of Arizona, Tucson, AZ) for allowing us to use their Rotor-Gene instrument. We also thank Erin Romero (Department of Otolaryngology–Head & Neck Surgery, University of Arizona, Tucson, AZ) for her assistance in consenting patients and sample collection.

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Appendix Figure 1



Appendix Figure 2



NOTE: Refer to Appendix Table 3 for the legend to this figure.

D_0_Archaea;D_1_Euryarchaeota;D_2_Thermoplasmata;D_3_Thermoplasmatales;D_4_Marine Group II;Other

D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteria;D_3_Acidobacteriales;D_4_Acidobacteriaceae (Subgroup 1);D_5_Terriglobus

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 2;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6/D_3_uncultured Acidobacteria bacterium;D_4_uncultured Acidobacteria bacterium;D_5_uncultured Acidobacteria bacterium

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6/D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;Other;Other

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinobacteriales;D_4_Actinobacteriaceae;D_5_Ferrihrix

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinobacteriales;D_4_Actinobacteriaceae;D_5_Illumatobacter

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinobacteriales;D_4_Actinobacteriaceae;D_5_uncultured

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinobacteriales;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinobacteriales;D_4_uncultured;Other

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinomycetales;D_4_Actinomycetaceae;D_5_Actinomycetes

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Actinomycetales;D_4_Actinomycetaceae;D_5_Mobiluncus

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Bifidobacteriales;D_4_Bifidobacteriaceae;D_5_Bifidobacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Bifidobacteriales;D_4_Bifidobacteriaceae;D_5_Gardnerella

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Corynebacteriales;D_4_Corynebacteriaceae;D_5_Corynebacterium

D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Corynebacteriales;D_4_Corynebacteriaceae;D_5_Corynebacterium 1

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D_0_Bacteria;D_1_Bacteroidetes;D_2_Sphingobacteria;D_3_Sphingobacteriales;D_4_Chitinophagaceae;D_5_uncultured

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D_0_Bacteria;D_1_Bacteroidetes;D_2_Sphingobacteria;D_3_Sphingobacteriales;D_4_Saprospiraceae;D_5_uncultured

D_0_Bacteria;D_1_Bacteroidetes;D_2_Sphingobacteria;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;D_5_Pedobacter

D_0_Bacteria;D_1_Bacteroidetes;D_2_Sphingobacteria;D_3_Sphingobacteriales;D_4_Sphingobacteriaceae;Other

D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Anaerolineales;D_4_Anaerolineaceae;D_5_uncultured

D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Kallitenuales;D_4_AKIW781;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Chloroflexi;D_2_KD4-96;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_C0119;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_G30-KF-AS9;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_P2-11E;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Chloroflexi;D_2_TK10;Other;Other;Other

D_0_Bacteria;D_1_Chloroflexi;D_2_Thermomicrobia;D_3_G30-KF-CM45;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Bromus tectorum;D_4_Bromus tectorum;D_5_Bromus tectorum

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Cercis gigantea;D_4_Cercis gigantea;D_5_Cercis gigantea

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Gossypium arboreum;D_4_Gossypium arboreum;D_5_Gossypium arboreum

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Lolium perenne;D_4_Lolium perenne;D_5_Lolium perenne

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Musa itinerans;D_4_Musa itinerans;D_5_Musa itinerans

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Nicotiana otophora;D_4_Nicotiana otophora;D_5_Nicotiana otophora

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Solanum melongena (eggplant);D_4_Solanum melongena (eggplant);D_5_Solanum melongena (eggplant)

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Solanum torvum;D_4_Solanum torvum;D_5_Solanum torvum

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_Ziziphus jujuba;D_4_Ziziphus jujuba;D_5_Ziziphus jujuba

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Chloroplast;Other;Other;Other

D_0_Bacteria;D_1_Cyanobacteria;D_2_Cyanobacteria;D_3_SubsectionIII;D_4_FamilyI;Other

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;Other;Other

D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Vampirovibrionales;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Deinococcus-Thermus;D_2_Deinococci;D_3_Deinococcales;D_4_Trueperaceae;D_5_Truepera

D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Lineage IV;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;D_5_Anaerobacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;D_5_Anoxybacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;D_5_Bacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;D_5_Oceanobacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Bacillaceae;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Family X;D_5_Thermicanus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Family XI;D_5_Gemella

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae;D_5_Ammophilus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae;D_5_Aneurinibacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Paenibacillaceae;D_5_Paenibacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;Ambiguous_taxa

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;D_5_Lysinibacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;D_5_Planococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;D_5_Planomicrobium

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;D_5_Sporosarcina

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Planococcaceae;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;D_4_Staphylococcaceae;D_5_Staphylococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales;Other;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;Ambiguous_taxa;Ambiguous_taxa

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_16d63.751;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Aerococcaceae;D_5_Abiotrophia

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Aerococcaceae;D_5_Aerococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Aerococcaceae;D_5_Eremococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Aerococcaceae;D_5_Facklamia

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Aerococcaceae;D_5_Facklamia

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Attopococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Carnobacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Dolosigranulum

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Granulicatella

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;D_5_Trichococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Carnobacteriaceae;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Enterococcaceae;D_5_Enterococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Lactobacillaceae;D_5_Lactobacillus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Leuconostocaceae;D_5_Leuconostoc

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Streptococcaceae;D_5_Lactococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Streptococcaceae;D_5_Streptococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;Other;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;Other;Other;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiaceae 1;D_5_Clostridium sensu stricto 1

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XI;D_5_Anaerococcus

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XI;D_5_Ezakiella

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XI;D_5_Pingmodia

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XI;D_5_Parvimonas

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XI;D_5_Pentophilus

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XVII;D_5_uncultured

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136 group

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-001

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Moryella

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Oribacterium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Rosebacteria

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Shuttleworthia

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Stomatobaculum

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Eubacterium oxidoreducens group

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptostreptococcaceae;D_5_Intestinibacter

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptostreptococcaceae;D_5_Peptoclostridium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Fastidiosipila

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Eubacterium coprostanoligenes group

D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;Other;Other

D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Faecalibaculum

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Dialister

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Megasphaera

D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Veillonellaceae;D_5_Veillonella

D_0_Bacteria;D_1_Fusobacteria;D_2_Fusobacteria;D_3_Fusobacteriales;D_4_Fusobacteriaceae;D_5_Fusobacterium

D_0_Bacteria;D_1_Fusobacteria;D_2_Fusobacteria;D_3_Fusobacteriales;D_4_Leptotrichiaceae;D_5_Leptotrichia

D_0_Bacteria;D_1_Fusobacteria;D_2_Fusobacteria;D_3_Fusobacteriales;D_5_Sneathia

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_BD2-11 terrestrial group;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatimonas

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_Gemmatirosa

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Gemmatimonadetes;D_3_Gemmatimonadales;D_4_Gemmatimonadaceae;D_5_uncultured

D_0_Bacteria;D_1_Gemmatimonadetes;D_2_Longimicrobia;D_3_Longimicrobiales;D_4_Longimicrobiaceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Omnitrophica;D_2_uncultured bacterium;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Parcubacteria;D_2_Candidatus Azambacteria;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium

D_0_Bacteria;D_1_Parcubacteria;D_2_Candidatus Azambacteria;Other;Other

D_0_Bacteria;D_1_Plancymycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_Tepidisphaeraceae;D_5_uncultured bacterium

D_0_Bacteria;D_1_Plancymycetes;D_2_Phycisphaerae;D_3_Tepidisphaerales;D_4_Tepidisphaeraceae;Other

D_0_Bacteria;D_1_Plancymycetes;D_2_Plancymycetacia;D_3_Plancymycetales;D_4_Plancymycetaceae;D_5_Pirellula

D_0_Bacteria;D_1_Plancymycetes;D_2_Plancymycetacia;D_3_Plancymycetales;D_4_Plancymycetaceae;Other

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Alphaproteobacteria Incertae Sedis;D_4_uncultured;D_5_uncultured bacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteriaceae;D_5_Brevundimonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacteriales;D_4_Caulobacteriaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Bradyrhizobiaceae;D_5_Bosea

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Bradyrhizobiaceae;D_5_Bradyrhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Devesia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Hyphomicrobiaceae;D_5_Hyphomicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methylobacteriaceae;D_5_Methylobacterium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methylobacteriaceae;D_5_Microvira

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methylobacteriaceae;D_5_uncultured

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methylobacteriaceae;Other

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Methylocystaceae;D_5_Hartmannibacter

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Phyllobacteriaceae;D_5_Mesorhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;D_5_Rhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales Incertae Sedis;D_5_Bauldia

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Xanthobacteriaceae;D_5_Azorhizobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;Other;Other

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacteriales;D_4_Rhodobacteriaceae;D_5_Paracoccus

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodobacteriales;D_4_Rhodobacteriaceae;D_5_Rubellimicrobium

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Acetobacteraceae;D_5_Roseomonas

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Rhodospirillaceae;D_5_Deulfivococcus

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_Rhodospirillales Incertae Sedis;D_5_Reyranella

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;Ambiguous_taxa

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Betula phytophilla

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Coulboulia dactyloides

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Coulboulia raticulata

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Oryza meyeriana

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rickettsiales;D_4_Mitochondria;D_5_Solanum melongena (eggplant)

D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_