SUPPLEMENTAL METHODS

Microbiome Complexity and *Staphylococcus aureus* in Chronic Rhinosinusitis

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**DNA extraction**

A phenol:chloroform bead-beating method was used to extract total genomic DNA from the swab heads. Swab heads were placed in sterile microcentrifuge tubes that contained 500 μl of Buffer B (142.9 mM NaCl, 142.9 mM Tris-Cl [pH 8.0], 14.3 mM EDTA, 5.7% SDS), and 500 μl of 1:1 phenol:chloroform. A total of ~0.25 g of 0.1-mm zirconia/silica beads was added to the suspension, and samples were mechanically disrupted in a Mini Beadbeater-16 (Biospec Products, Bartlesville, OK) at the highest setting for 2 minutes. The samples were centrifuged (>14,000 × g) for 5 minutes, and the aqueous layer was re-extracted two more times with an equal volume of phenol:chloroform and once with an equal volume of chloroform. DNAs were precipitated by the addition of 0.5 volume ammonium acetate (7.5 M) and 1 volume of 100% isopropanol, incubated at -80°C for 10 minutes, then centrifuged (>14,000 × g; 25 min). Nucleic acid pellets were washed with 250 μl of 70% ethanol and centrifugation (>14,000 × g; 5 min), dried in air, then re-suspended in 30 μl of sterile 1x Tris-EDTA (pH 8.0), and stored at –80°C until PCR processing. All DNA extraction and PCR steps were performed in a HEPA-filtered laminar flow hood that was decontaminated by UV light.

**PCR Assays**

*Quantitative.* A duplex quantitative PCR (QPCR) assay using previously published oligonucleotide primers for Total Bacteria (16S rRNA gene, FAM reporter)¹ and *S. aureus* (femA gene, TET reporter)² was conducted on an ABI 7300 thermocycler. PCR reactions
contained 10μl DyNAmo ColorFlash Probe qPCR Mastermix with ROX (Finnzymes Oy, Espoo, Finland), 3μl water, and 1μl each 20x PrimeTime™ primer/probe set (Integrated DNA Technologies, Inc., Coralville, Iowa), and 5μl of DNA template. Thermocycling was conducted as follows: 7 min initial denaturation at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60 °C. Standard curves were generated and absolute quantitation of DNA copy numbers per volume of template were used to compare the relative proportions of S. aureus to total bacteria.

NonQuantitative. A multiplex PCR assay for S. aureus specific methicillin-resistance genes (mecORF)³ was utilized to test for MRSA in specimens that were positive for S. aureus by QPCR and sequence. PCR reactions contained 1x NovaTaq PCR Mastermix (Merck Biosciences, Darmstadt, Germany) and 20nM each primer (Integrated DNA Technologies, Inc., Coralville, Iowa). Thermocycling conditions were as recommended by the manufacturer with an annealing temperature of 52° C. A Bioanalyzer2100 (Agilent Technologies, La Jolla, CA) was used to detect amplification.

Multiplexed Pyrosequencing

Amplicons of the 16S rRNA gene (~500 b.p.; primers 27FYM+3 and 515R)⁴ were generated via broad-range PCR (30-36 cycles) using 5’-barcoded reverse primers.⁵ PCR yields were normalized using a SequalPrep™ kit (Invitrogen, Carlsbad, CA), pooled, lyophilized, and gel purified, as previously described.⁷ Pooled amplicons were provided to the Center for Applied Genomics at the University of Toronto for pyrosequencing on a 454/Roche Life Sciences GS-FLX instrument using Titanium chemistry (Roche Life Sciences, Indianapolis, IN).

The software used for sequence analysis consisted of the function specific tools that were considered to be the standards in the academic community at the onset of this project. Pyrosequences were sorted into libraries by barcode using bartab. All pyrosequences were
screened for nucleotide quality (bases at 5’ and 3’ ends with mean Q <20 over a 10 n.t. window were discarded), ambiguous bases (sequences with >1 N were discarded), and minimum length (sequences <200 n.t. were discarded). The mean trimmed sequence length was ~340 b.p..

The *Infernal* RNA alignment tool\textsuperscript{8} was used to screen all sequences in terms of their fidelity to a Covariance Model (CM) derived from SSU rRNA secondary structure models provided by the lab of Dr. Robin Gutell.\textsuperscript{9,10} Sequences that did not adequately match a bacterial CM model were removed from all subsequent analyses. Chimera screening was performed by the tool *ChimeraSlayer*, which requires that sequences be previously aligned with *NAST-iEr*.\textsuperscript{11} Putative chimeras and other sequences that could not be aligned by *NAST-iEr* were removed from subsequent analyses.

Genus-level taxonomic calls were produced by the *RDP Classifier*, which performs naïve Bayesian taxonomic classification versus a training set.\textsuperscript{12} Species level taxonomy precision was obtained via BLAST\textsuperscript{13} against a database of sequences obtained from Silva 104\textsuperscript{14} tagged as isolates. Species-level results required that the candidate sequence overlap the database sequence by at least 95% with at least 99% sequence identity and that the Silva derived taxonomy of the database hit match the RDP classifier genus level taxonomy result. The principal output of the pipeline was a tab-separated file that enumerated the number of times each unique taxonomy line was observed in each library.

Bioinformatic analyses including diversity indices $S_{\text{obs}}$, $S_{\text{chao}}$ and Shannon’s Index ($H_o/H_{\text{max}}$), were conducted with software tool *biodiv* embedded within the sequence analysis pipeline.\textsuperscript{15} Diversity has two important features: 1) richness, which measures the number of different types of organisms or relatedness groups of pyrosequences in an environment and 2) evenness, which quantifies whether all groups are present in equal abundance or whether there is
a preponderance of a few dominant types.\textsuperscript{16} These microbial diversity measures can be estimated through biostatistical analysis of the distribution of pyrosequences obtained from a single specimen or patient cohort.
REFERENCES


