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Aspirated bile: a major host trigger modulating respiratory pathogen colonisation in Cystic Fibrosis patients.

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Running Title: Bile aspiration modulates biodiversity.

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DNA Quality Control.

DNA quality was controlled through O.D. 260/280 ratio by nanodrop (Nanodrop Inc.) and the DNA was quantified using the Quant-iT PicoGreen dsDNA reagent and kit (Life Tech, Carlsbad, USA), following the manufacturer's instructions.

rRNA pyrosequencing.

16S rRNA genes were amplified at DNAVision using a primer set corresponding to primers ATTACCGCGGCTGCTGG and AGAGTTTGATCCTGGCTCAG. These PCR primers target the V1-V3 hypervariable 16S rRNA region. The forward primer contained the sequence of the Titanium A adaptor (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′) and a barcode sequence (pools of 8 samples). For each sample, a PCR mix of 100 µl was prepared containing 1 × PCR buffer, 2U of KAPA HiFi Hotstart polymerase blend and dNTPs (Kapabiosystems), 300 nM primers (Eurogentec, Liege, Belgium), and 60 ng gDNA. Thermal cycling consisted of initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 98°C for 20 s, annealing at 56°C for 40 s, and extension at 72°C for 20 s, with a final extension of 5 min at 72°C. Aliquots (3 µl) of PCR product were added to a new PCR mix (identical to the first round of PCR) for the nested PCR of 15 cycles. Amplicons were visualised on 1% agarose gels using GelGreen Nucleic Acid gel stain in 1x TAE (Biotium) and were cleaned using the Wizard SV Gel and PCR Clean-up System (Promega), according to the manufacturer's instructions.

Amplicon Quantitation, Pooling, and Pyrosequencing.

Amplicon DNA concentrations were determined using the Quant-iT PicoGreen dsDNA reagent and kit (Life Tech, Carlsbad, USA) following the manufacturer's instructions. Assays were carried out using 2 µl cleaned PCR product in a total reaction volume of 200 µl in black,

96-well microtiter plates. Following quantitation, cleaned amplicons were combined in equimolar ratios into a single tube. The final pool of DNA was eluted in 100 µl of nuclease free water, was purified using an Agencourt Ampure XP Purification system (Agencourt Biosciences Corporation-Beckman coulter, USA) and then resuspended in 100 µl of TE 1x. The concentration of the purified pooled DNA was determined using the Quant-iT PicoGreen dsDNA reagent and kit (Life Tech, Carlsbad, USA). Pyrosequencing was carried out using primer A on a 454 Life Sciences Genome Sequencer FLX instrument (Roche) following titanium chemistry.

***16S rRNA data analysis*.**

The sequences were assigned to samples according to sample-specific barcodes. This enabled collection of FASTA formatted files containing an average (± SD) of 10,597 ± 3625 sequences per sample. Sequences were then checked for the following criteria: (i) almost perfect match with barcode and primers; (ii) length of at least 240 nucleotides (barcodes and primers excluded); (iii) no more than two undetermined bases (denoted by N). The term "almost perfect match" signifies that one mismatch/deletion/insertion is allowed in the barcode, idem for the primer. Each sequence originating from pyrosequencing and passing QC was assigned to a family by the Ribosomal Database Project (RDP) classifier (v 2.1) with CE > 80%. The Shannon diversity and Chao richness estimates were calculated through the Mothur package.