Enhanced phylogenetic insights into the microbiome of chronic rhinosinusitis through the novel application of long read 16S rRNA gene amplicon sequencing*

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Abstract

Introduction: 16S rRNA next generation sequencing (NGS) has been the de facto standard of microbiome profiling. A limitation of this technology is the inability to accurately assign taxonomy to a species order. Long read 16S sequencing platforms, including Oxford Nanopore Technologies (ONT), have the potential to overcome this limitation. The paranasal sinuses are an ideal niche to apply this technology, being a low biomass environment where bacteria are implicated in disease propagation. Characterising the microbiome to a species order may offer new pathophysiological insights.

Methodology: Cohort series comparing ONT and NGS biological conclusions. Swabs obtained endoscopically from the middle meatus of 61 CRSwNP patients underwent DNA extraction, amplification and dual sequencing (Illumina Miseq (NGS) and ONT GridION). Agreement, relative abundance, prevalence, and culture correlations were compared.

Results: Mean microbiome agreement between sequencers was 61.4%. Mean abundance correlations were strongest at a familial/genus order and declined at a species order where NGS lacked resolution. The most significant discrepancies applied to Corynebacterium and Cutibacterium, which were estimated in lower abundance by ONT. ONT accurately identified 84.2% of cultured species, which was significantly higher than NGS.

Conclusions: ONT demonstrated superior resolution and culture correlations to NGS, but underestimated core sinonasal taxa. Future application and optimisation of this technology can advance our understanding of the sinonasal microenvironment.

Key words: nanopore, Illumina, NGS, 16S, microbiome

Introduction

The 16S rRNA gene has been a reliable target for microbiome characterisation since the inception of gene sequencing technology due to its ubiquity in all bacterial species. It contains constant genetic regions common to all bacteria that are optimal for primer binding, interlaced by hypervariable regions of DNA that are unique to each species allowing taxonomic discrimination. Next generation sequencers (NGS) superseded the first generation of sequencers some two decades ago, with an innovative and efficient parallel sequencing design⁽¹⁾. By sequencing short 2x300 base-pair(bp) segments of the 1500bp 16S rRNA gene, NGS could rapidly estimate the bacterial composition

of a sampled ecosystem, leading to widespread integration⁽²⁾. However, the design efficiency engendered certain limitations including incorrect taxonomic assignment, taxa bias and an inability to reliably assign sequences to a species order^(3–5). While NGS remains the widely accepted de facto standard of 16S microbiome sequencing, a new frontier of third generation sequencing is emerging.

Full length 'long read' sequencing is an evolution of NGS, with the capacity to sequence significantly longer nucleotide segments(10,000-50,000bp)⁽⁶⁾. In this manner, the entire 1500bp 16S rRNA gene can be sequenced, offering deeper phyloge-

netic resolution⁽⁷⁻¹⁰⁾. Oxford Nanopore Technologies (ONT) is a full length sequencing platform that uses an electric gradient to drive DNA/RNA through nanopores in an artificial biosensor membrane at a sequencing rate of 400 nucleotides per second, allowing long sequences to be detected in real time^(11,12). Validation of this technology has been performed in an array of biological niches, whereby ONT was compared with NGS sequencing^(13–17). Szoboszlay compared ONT and NGS in mock and fecal samples, concluding ONT provided a more accurate representation of mock communities and superior taxonomic assignment at a species resolution⁽¹³⁾. Matsuo reported comparable genus relative abundance and superior species resolution for ONT when applied to fecal samples⁽¹⁴⁾. Heikema and Rozas respectively came to similar conclusions regarding genus identification in the nose and skin, while simultaneously unearthing limitations of ONT in underestimating the relative abundance of certain taxa, advocating for further methodological optimisation(15,16).

The combination of a low bacterial biomass and a heavily implicated role of bacteria in chronic rhinosinusitis (CRS) has generated a unique demand for non-culture dependent research in the paranasal sinuses, resulting in extensive application of NGS. Early NGS studies offered conflicting abundance and diversity conclusions, largely driven by heterogeneous methodology and small sample sizes⁽¹⁸⁾, but progressively a consensus sinus microbiome has been established, with Staphylococcus and Corynebacterium predominating⁽¹⁹⁻²⁴⁾. The largest sinus microbiome study was a multicenter international series of 410 patients, which defined a core microbiome of Corynebacterium, Staphylococcus, Streptococcus, Haemophilus and Moraxella in health and disease⁽²⁵⁾. The limitation of existing studies is the inability to establish species level conclusions, which is fundamental to further advance our understanding of the role of bacteria in CRS. To date, ONT full length 16S rRNA gene amplicon sequencing has never previously been applied in the paranasal sinuses.

Herein, the objectives of this study are to compare the biological conclusions of ONT and NGS and to characterise the sinonasal microbiome to a species order. The hypothesis of this study is that ONT will provide superior taxonomic resolution and comparable taxonomic accuracy to NGS.

Materials and methods

Study design

Ethics approval was obtained from the Central Adelaide Local Health Network HREC(HREC/14/TQEHLMH/222 and LNR13604). This study was a paired design cohort series comparing ONT (GridION) and NGS (Illumina Miseq) sequencing platforms on extracted DNA from middle meatal swabs. We utilised swabs from 61 patients aged over 18 who had no documented history of immune suppression and had a clinical diagnosis of CRSwNP based on the EPOS diagnostic criteria⁽²⁶⁾. This included multiple CRS subtypes (idiopathic CRSwNP, eosinophilic CRS and allergic fungal rhinosinusitis). Clinical subgroup analyses are not presented in the current series, as this study forms part of a broader cohort series that will later examine unique clinical parameters. Written consent was obtained from participants at the time of swab collection.

Specimen collection and DNA extraction

Samples were obtained from the middle meatus under endoscopic guidance at the commencement of endoscopic sinus surgery, using sterile, guarded, flocked swabs. Swabs were stored in -80°Celsius freezers prior to DNA extraction. Extraction was performed by the Australian Genome Research Facility (AGRF, Melbourne, Australia) utilizing the Qiagen PowerSoil Pro DNA extraction kit (QIAGEN, Hilden, Germany). Quality control was performed with the Qubit Fluorometric Assays (Thermofisher, Waltham, MA, USA) Nanodrop Microdrop Spectrophotometers (Thermofisher), and gel electrophoresis. For NGS, the V3-V4(341F-806R) region was amplified utilizing a two-stage PCR protocol (Supplementary file 1) and sequenced using the Illumina MiSeq (Illumina Inc, San Diego, CA, USA) with 300bp chemistry. ONT sequencing was performed in-house at the Basil Hetzel Institute (University of Adelaide, Australia) applying an adaptation of the default ONT PCR protocol. In summary, initial denaturation 60 seconds (95°C); denaturation 25 x 20 seconds (95°C); annealing 25 x 30 seconds (62°C); extension 25 x 120 seconds (65°C); final extension 300 seconds (65°C), using the default ONT 16S Barcoding Kit (SQK16S024, ONT, Oxford, UK). Amplicons were primed using the ONT Flow Cell Priming Kit (EXP-FLP004, ONT) and added to the flow cell of the ONT GridION sequencer.

Bioinformatics pipeline

A quality threshold of >600 reads/sample was established, yielding 47 paired samples for downstream analysis. Demultiplexed fastq files were generated from Illumina MiSeq platform v2.6.2.1 (Illumina Inc) and Real Time Analysis (RTA) v1.18.54. QIIME2 V2020.2 were used for the NGS bioinformatics pipeline, applying SILVA database for taxonomic assignment. An in-house opensource pipeline, 'Coatofarms'⁽²⁷⁾ that implements EMU v3.4.5 was used for ONT bioinformatics, applying the default EMU database for taxonomic assignment⁽²⁸⁾. ONT taxonomic assignment was additionally performed against SILVA as a comparator.

Statistical analysis

Statistics were performed on GraphPad Prism 10(GraphPad Software, Boston, MA, USA) and 'R' (R Foundation for Statistical Computing, Vienna, Austria). Mean relative abundance (MRA) was calculated for all taxa. Wilcoxon paired signed-rank abundance

Table 1. Sequencing data - all samples.

	ONT	NGS
Samples Sequenced	61	61
Total Reads	843,203	1,764,669
Mean Reads / Sample	13,823	28,929
Number Samples <600 reads (Excluded)	13	2
Final Samples Analysed (Final Cohort)	47	47
Total Genera Identified	136	109
Mean Genera / Sample	8.0	8.3
Genera > 1% Relative Abundance	11	9
Total Species Identified	264	61
Mean Species / Sample	11.6	2.6
Species > 1% Relative Abundance	17	3

comparisons were applied to taxa with MRA>1%. Prevalence comparisons were performed with Chi-square and Fisher's exact test. Aldex2, an estimation of technical variation within each sample per taxon, was applied utilizing Dirichlet distribution and a closely related log-ratio (CLR) transformation⁽²⁹⁾. Wilcoxon tests were used for significance. Microbiome agreement was presented as descriptive data based on a calculation of the number of taxa in agreement for each sample; and the sum of the percentage of MRA in agreement per sample, presented as a mean, median and standard deviations⁽¹⁵⁾. An example of the agreement calculation agreement is provided in Supplementary file 2. Sequencing data was compared to bacterial culture results cultivated from the same site to assess the reliability of sequencing in identifying cultured organisms. The bacterial culture results presented were performed through hospital laboratories as part of the patients' standard of care. Alpha Diversity (Shannon's diversity, Simpson's diversity) and richness were calculated for each sample/group and Wilcoxon paired tests were performed to determine significance. Beta Diversity was calculated using a Bray Curtis dissimilarity model to calculate centroid ecological distances.

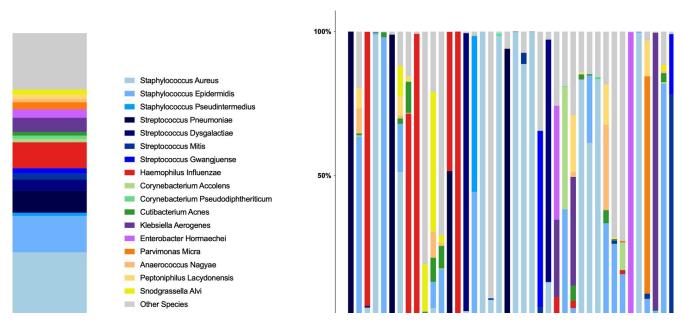
Results

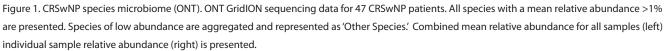
Demographics

Participant age ranged from 27-85 (mean=52.1; SD=13.6). Male to female ratio was 41:20. All patients had a diagnosis of CRSwNP (including eCRS, AFRS and idiopathic CRSwNP). Mean Lund-McKay score was 31.9 (SD=10.6) and mean SNOT22 was 25.9 (SD=14.5).

Sequencing output

ONT generated 843,203 total reads (mean=13,823/sample) and NGS generated 1,764,669 reads (mean=28,929/sample). 14 samples with <600 reads were excluded (final cohort n=47). ONT assigned 136 genera(mean=8/sample) including 11 with >1% MRA (Table 1). NGS assigned 109 genera (mean=8.3/ sample) including 9 with >1% MRA. ONT assigned 264 species (mean=11.6/sample) including 17 with >1% MRA. NGS assigned 61 species (mean=2.6/sample) including 3 with >1% MRA. 88.13% of NGS reads could not be assigned to a species order.





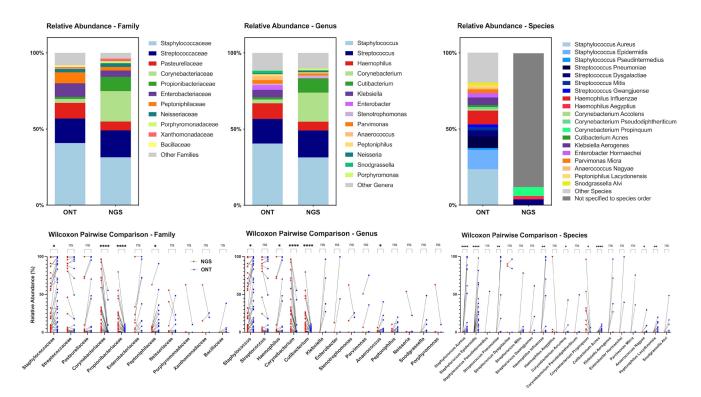


Figure 2. Paired Relative Abundance. Wilcoxon signed-rank tests.Relative Abundance Bar Graphs (Above): Mean Relative Abundance at a family (Left), Genus (Middle) and Species (Right) order, identifying all microbes with mean relative abundance >1%. The Species graph highlights the low resolution of NGS at this order with only 3 species identified in an abundance >1% and 88.13% of amplicons 'not specified to species order (dark grey). Wilcoxon Pairwise Comparison Graphs (Below): Wilcoxon paired signed-rank tests were performed for each organism >1% abundance at Family, Genus and species order, highlighting organisms with a significant difference in relative abundance based on the sequencing platform.

Species order microbiome

The sinonasal microbiome for the cohort of 47 patients presented to a species order using ONT sequencing is presented in Figure 1. 264 species were identified, including 16 discrete species of *Staphylococcus* (40.6% MRA, 85.1% prevalence). *Staphylococcus aureus* (23.7%) and *Staphylococcus epidermidis*(12.65%) had the highest MRA and prevalence, observed in 59.6%(28/47) and 61.7%(29/47) of samples respectively. *Streptococcus* (16 species, 16.1% MRA, 46.8% prevalence) and *Haemophilus* (5 species, 10.2% MRA, 40.4% prevalence) were also highly abundant, including *Streptococcus pneumoniae*, *Streptococcus dysgalactiae* and *Haemophilus influenzae*. *Corynebacterium* (9 species, 44.7% prevalence, 2.53% MRA) and *Cutibacterium* (3 different species, 42.6% prevalence, 1.34% MRA) were prevalent but low in abundance with *Corynebacterium accolens* (19.1% prevalence) and *Cutibacterium acnes* (40.4% prevalence) particularly prevalent.

Mean relative abundance comparison

ONT and NGS were strongly correlated at a familial order (Figure 2) with *Staphylococcaceae* the most abundant family for ONT and NGS. No significant difference in MRA was observed for 7 of 11 taxa with MRA >1% (Figure 2, Table 2). A statistically significant difference was observed for *Staphylococcaceae*

(ONT=40.81%, NGS=31.38%, p=0.013), Corynebacteriaceae (ONT=2.53%; NGS=19.96% NGS, p<0.001), Propionibacteriaceae (ONT=1.35%; NGS=9.35%, p<0.001) and Peptoniphilaceae (ONT=7.26%; NGS=2.38% NGS, p=0.013). Correlations remained strong at a genus order with no significant difference in MRA for 9 of 14 genera with MRA>1% (Figure 2, Table 2). Significant differences were observed for Haemophilus (ONT=10.21%; NGS=5.82%, p=0.048), as well as Staphylococcus, Corynebacterium, Cutibacterium and Anaerococcus (consistent with described familial discrepancies). 3.85% of NGS MRA was familial Enterobacteriaceae unable to be assigned to a genus order. This likely corresponded to Enterobacteriaceae genera observed with ONT, including Klebsiella (ONT=4.98%, NGS=0%, p=0.21) and Enterobacter (ONT=3.07%, NGS=0.27%, p=0.57).

Correlation between ONT and NGS declined significantly at a species order due to low resolution of NGS at this phylogeny, with only one species with MRA>1% (*Streptococcus dysgalactiae*) common to both datasets. 88.13% of NGS reads were unable to be assigned to a species. NGS assigned just 61 species and only 3 had >1% MRA (*Corynebacterium propinquum* 5.81%, *Streptococcus dysgalactiae* 3.78% and *Haemophilus aegyptius* 2.24%). The remaining 58/61 assigned species had a cumulative

Table 2. ONT and NGS relative abundance and prevalence comparisons.

	Mean Relative Abundance				Prevalence (cohort=47)			
Family	ONT (%)	NGS (%)	Wilcoxon	Corrected	ONT (n)	NGS (n)	Fisher's	Chi-Square
,			p-value	p-value			Exact	
Staphylococcaceae	40.81	31.38	0.001	0.01	41	41	>0.99	>0.99
Streptococcaceae	16.09	17.68	0.2	0.51	22	25	0.68	0.54
Pasteurellaceae	10.28	5.84	0.01	0.057	20	16	0.52	0.4
Corynebacteriaceae	2.53	19.96	<0.001	<0.001	21	36	0.003	0.002
Propionibacteriaceae	1.34	9.35	<0.001	<0.001	20	35	0.003	0.002
Enterobacteriaceae	8.94	4.19	0.07	0.32	13	16	0.66	0.50
Peptoniphilaceae	7.26	2.38	0.002	0.01	23	25	0.84	0.68
Neisseriaceae	2.25	2.39	0.9	0.94	13	16	0.66	0.5
Porphyromonadaceae	0.22	1.37	0.13	0.41	1	4	0.36	0.17
Xanthomonadaceae	0.96	1.66	0.44	0.68	2	5	0.43	0.24
Bacillaceae	1.07	0	0.05	0.21	6	2	0.27	0.14
Other	8.25	3.8						
Genus								
Staphylococcus	40.57	31.38	0.002	0.02	40	41	>0.99	0.77
Streptococcus	16.09	17.68	0.24	0.57	22	24	0.84	0.68
Haemophilus	10.21	5.82	0.005	0.048	19	16	0.67	0.52
Corynebacterium	2.53	19.04	<0.001	<0.001	21	36	0.003	0.002
Cutibacterium	1.34	9.33	<0.001	<0.001	20	35	0.003	0.002
Klebsiella	4.98	0	0.03	0.21	6	0	0.03	0.01
Enterobacter	3.07	0.27	0.25	0.57	3	1	0.62	0.31
Stenotrophomonas	0.96	1.66	0.75	0.75	2	3	>0.99	0.65
Parvimonas	2.4	1.22	0.16	0.57	5	4	>0.99	0.73
Anaerococcus	2.36	0.37	0.002	0.02	18	16	0.83	0.67
Peptoniphilus	1.57	0.49	0.03	0.21	14	14	>0.99	>0.99
Neisseria	0.52	1.2	0.16	0.57	6	9	0.57	0.4
Snodgrassella	1.71	0	0.02	0.13	7	0	0.01	0.01
Porphyromonas	0.22	1.37	0.13	0.55	1	4	0.36	0.17
Other	11.47	10.17						
Species	_	_	_	_	_	_	_	_
Staphylococcus aureus	23.7	0	<0.001	<0.001	28	0	<0.001	<0.001
Staphylococcus epidermidis	12.65	0	<0.001	<0.001	29	0	<0.001	<0.001
Staphylococcus pseudintermedius	1.15	0	>0.99	>0.99	1	0	>0.99	0.31
Streptococcus pneumoniae	7.73	0	<0.001	0.007	12	0	<0.001	<0.001
Streptococcus dysgalactiae	3.85	3.78	0.75	0.94	3	2	>0.99	0.65
Streptococcus mitis	2.08	0	0.008	0.08	8	0	0.006	0.003
Streptococcus gwangjuense	1.81	0	0.25	0.68	3	0	0.24	0.08
Haemophilus influenzae	9.05	0	<0.001	0.007	12	0	<0.001	<0.001
Haemophilus aegyptius	0	2.24	0.13	0.49	0	4	0.12	0.04
Corynebacterium accolens	1.19	0	0.004	0.04	9	0	0.003	0.002
Corynebacterium pseudodiphth.	1.13	0	0.008	0.08	8	0	0.006	0.003
Corynebacterium propinquum	0.13	5.81	0.002	0.02	2	12	0.007	0.004
Cutibacterium acnes	1.26	0	<0.001	<0.001	19	0	<0.001	<0.001
Klebsiella aerogenes	4.96	0	0.06	0.36	5	0	0.06	0.02

	Mean Relative Abundance				Prevalence (cohort=47)			
	ONT (%)	NGS (%)	Wilcoxon p-value	Corrected p-value	ONT (n)	NGS (n)	Fisher's Exact	Chi-Square
Enterobacter hormaechei	2.97	0	0.25	0.68	3	0	0.24	0.08
Parvimonas micra	2.4	0	0.06	0.36	5	0	0.06	0.02
Anaerococcus nagyae	1.14	0.01	0.001	0.01	11	0	<0.001	<0.001
Peptoniphilus lacydonensis	1.56	0	< 0.001	0.004	13	0	<0.001	<0.001
Snodgrassella alvi	1.71	0	0.02	0.12	7	0	0.01	0.01
Other species	19.53	0.03						
Not specified to species order	0	88.13						

relative abundance of 0.03%. Only one *Staphylococcus* species was identified by NGS: *Staphylococcus equorum*, an equine-host organism identified in a single sample (MRA=0.007%). In contrast, ONT demonstrated high resolution at a species order, identifying 264 different bacterial species including 16 different *Staphylococcus*, 16 *Streptococcus*, 12 *Neisseria* and 9 *Corynebacterium* species. 17 species had an abundance of >1% with *Staphylococcus aureus* (23.7%) and *Staphylococcus epidermidis* (12.65%) the most abundant (Table 2).

Aldex2 abundance analysis

Aldex2 was utilised as an alternate differential abundance analysis, applying Wilcoxon pairwise comparisons for each genus. 196 genera were compared, with only 4 demonstrating a statistically significant difference (Supplementary file 3). These included *Corynebacterium* (p<0.001), *Laycella* (p<0.001), *Cutibacterium* (p<0.001) and *Lawsonella* (p=0.04).

Prevalence

Prevalence was compared applying Fisher's Exact and Chi-Square tests (Table 2). 10 of the 14 genera with MRA>1% showed no significant difference in prevalence. *Staphylococcus* (ONT=85.1%; NGS=87.2%; p>0.99), *Streptococcus* (ONT=46.8%; NGS=51.1%; p=0.84) and *Haemophilus* (ONT=40.4%; NGS=34.0%; p=0.67) were prevalent and comparable. *Corynebacterium* (ONT=44.7%; NGS=76.6%, p=0.003) and *Cutibacterium* (ONT=42.6%, NGS=74.5% p=0.003) were significantly more prevalent with NGS, while *Klebsiella* (6/47 ONT=12.8%, 0/47 NGS=0%, p=0.03) and *Snodgrassella* (7/47 ONT=14.9%, NGS=0%, p=0.01) were significantly more prevalent with ONT. Species order prevalence comparisons were limited due to the low resolution of NGS at this phylogeny. *Staphylococcus epidermidis* (61.7%), *Staphylococcus aureus* (59.6%) and *Cutibacterium acnes* (40.4%) had the highest prevalence by ONT (not detected with NGS).

Microbiome agreement

The sum of the percentage of MRA in agreement for each sample and the number of genera in agreement per sample

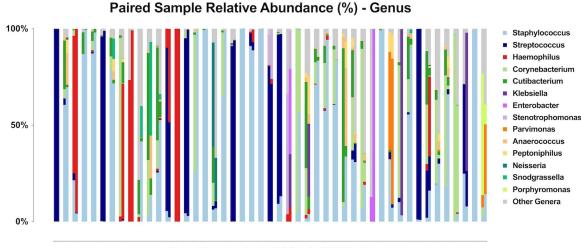
was calculated as described in Supplementary file 2. The mean percentage of relative abundance agreement per sample was 61.4% (SD=32.4; median=65.35%) (Figure 3). The mean number of genera in agreement 3.7 genera/sample (SD=2.2, median=4). 9 paired samples had MRA agreement >99%, while 6 samples had <20% MRA agreement.

Bacterial culture agreement

Sequencing results were compared with bacterial cultures obtained from the same middle meatal site at the time of surgery. A total of 38 species were cultivated from the patient cohort (Figure 4). ONT accurately identified the genus of the bacteria cultured in the corresponding patient in 34/38 (89.5%) samples, which was superior to NGS 30/38 (78.9%), without statistical significance (p=0.103). At a species order, ONT accurately identified the species cultured in 32/38 (84.2%) samples which was significantly more accurate than NGS, where just 2/38 (5.3%) species were correctly identified (p<0.001). ONT demonstrated a higher identification rate of cultured bacteria to a species depth (84.2%) relative to what NGS could identify at either a genus (78.9%) or species (5.3%) depth.

Alpha and Beta diversity

Alpha diversity and richness were calculated at a genus depth using Shannon's diversity index, Simpson's index and Chao1 (Figure 5). There was no significant difference in Shannon's diversity between ONT (mean=0.85) and NGS (mean=0.73, p=0.29). There was no significant difference in richness between ONT (mean=8.2) and NGS (mean=7.9, p=0.80). Suggesting ONT produces overall highly comparable conclusions to NGS using multiple commonly applied microbiome methods to assess alpha diversity. Bray-Curtis dissimilarity calculations were performed to establish beta diversity, calculating centroid ecological distance for each sample (Figure 6). On Wilcoxon testing, the mean centroid for ONT (0.55) and NGS (0.56) were highly comparable (p=0.66) suggesting inter-sample ecological dissimilarity in the cohort produced comparable conclusions in both sequencing modalities.



Paired Samples (n=47, NGS Left; ONT Right)

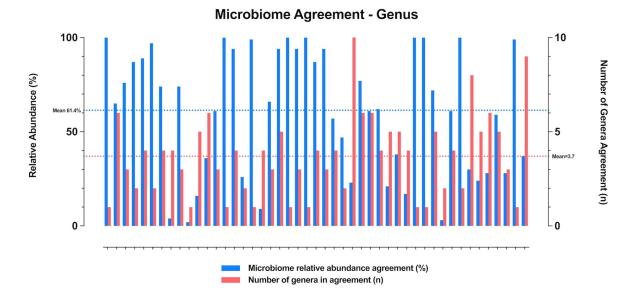


Table 2. ONT and NGS relative abundance and prevalence comparisons.

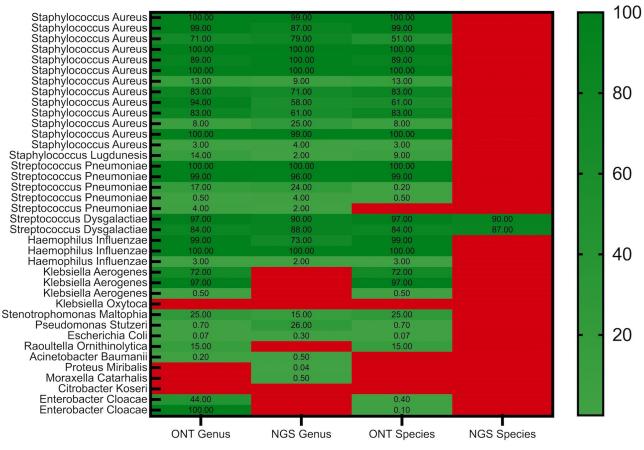
EMU versus SILVA database comparison

To confirm that the observed results related to differences between ONT and NGS and not differences in databases, we applied the SILVA database to the ONT dataset. The results were almost identical between ONT SILVA and ONT EMU dataset (Supplementary file 4). The mean difference in MRA for the 10 most abundant genera was 0.55% (SD=0.72%; range 0.01%-1.97%). The mean difference between the top 10 most abundant species was 0.85% (SD=0.66%; range 0.1%-1.68%). These results indicate that database had a negligible influence on the presented results.

Discussion

The paranasal sinuses possess a unique apposition of being both a low biomass environment and one where bacteria is heavily implicated in disease propagation. Accordingly, there is a substantial appetite for effective non-culture dependent techniques to enhance our understanding of the sinus microbiome. To our knowledge, this is the first time that ONT 16S rRNA sequencing has been applied in the paranasal sinuses, which has unearthed novel insights into the bacterial composition of CRS. By applying direct comparisons to NGS technology, we have elucidated strengths and weaknesses of each platform specific to sinonasal flora.

In this series comparable conclusions were established at genus order for abundance, alpha diversity and beta diversity. Correlations declined significantly at a species order. This mirrored the conclusions of other studies of a similar methodology, where ONT has consistently demonstrated superior phyloge-



Bacterial Culture Agreement

Figure 4. Bacterial culture agreement. 29/47 middle meatal swabs (from the same site that sequencing swabs were obtained) cultured 38 bacterial species, with *Staphylococcus aureus* (13) and *Streptococcus pneumoniae* (5) the most prevalent. Heatmaps assess the reliability of ONT and NGS to identify the cultured organism in the corresponding patient swab.

netic resolution^(9,10,13-16). Although select sinonasal NGS studies have reported taxa to a species order, this should be interpreted with trepidation, particularly in the absence of adjunct methods like amplicon sequence variance clustering or quantitative PCR. Yarza concluded that a minimum of 1300 nucleotides are necessary to accurately assign sequences to a species resolution⁽³⁰⁾, which significantly exceeds the limitations of NGS. Earl reported highly comparable genetic structures between *Staphylococcus* aureus and Staphylococcus epidermidis with 1.4% divergence and differing by as few as 23 nucleotides⁽³¹⁾. In our series, ONT could discriminate virulent *Staphylococcus aureus* and passive Staphylococcus epidermidis, two species with entirely divergent pathogenic potential. When contextualized with NGS studies that aggregate these species as a common genus, the potential to establishing clinically important inferences is limited. In this manner, ONT offers significant advantages in better understanding the microbiology of CRS.

It was critical to assess if the species conclusion established by ONT reflect the true bacteria in each sample. To assess this, we compared sequenced data to bacterial culture results obtained from the same site at the time of surgery. Correlations with the culture results were comparable at a genus order, with ONT reliably identifying 89.5% of cultured genera compared with 78.9% for NGS (p=0.103). NGS was most limited in identifying Enterobacteriaceae genera, (Klebsiella, Enterobacter, Citrobacter, Escherichia and Raoultella) which is likely due to NGS limitation of primer bias against this specific lineage. Critically, ONT correlated strongly at a species order reliably identifying 84.2% of cultured species in the patient matched sequences. This was significantly superior to NGS at a species order (5.3%; p<0.01) and notably exceeded the correlations of NGS at a genus order (78.9%; p=0.55). ONT accurately identified commonly cultured sinus species (S. aureus, S. lugdunesis, S. dysgalactiae, S. pneu*moniae*), difficult to culture species (*H. influenzae*) and species considered less endemic to the sinuses (Stenotrophomonas maltophia, Pseudomonas stutzeri, Escherichia coli, Raoultella ornithinolytica, Enterobacter cloacae). These results endorse the accuracy of ONT against a broad spectrum of common and atypical sinonasal organisms.

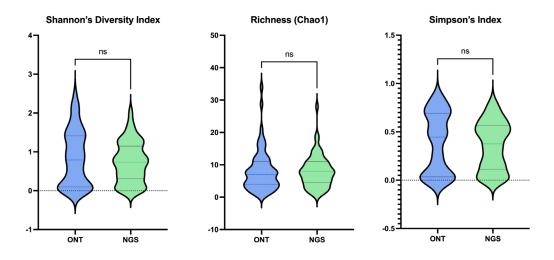


Figure 5. Alpha diversity calculations comparing ONT and NGS diversity at a genus level. No significant difference was observed on Wilcoxon paired analysis for Shannon's diversity index (mean ONT=0.85; NGS=0.74;p=0.29); Richness (Mean ONT=8.2;NGS=7.9;p=0.53) or Simpson's Index (mean ONT=0.39;NGS=0.36;p=0.52).

The most significant limitation of ONT highlighted by this series was the discrepancies of MRA for two core sinonasal genera, Corynebacterium and Cutibacterium. Both are considered sinus commensals with theoretical probiotic, antibiosis and in some cases pathogenic potential⁽³²⁻³⁵⁾. Corynebacterium is highly abundant in numerous NGS studies and was the most abundant taxa in the 'International Sinus Microbiome' study⁽²⁵⁾, yet it was significantly underrepresented in our ONT MRA dataset (ONT=2.53%, NGS=19.04%, p<0.001). Despite its low abundance, it was the third most prevalent genus in the ONT dataset (44.7%), Similarly, Cutibacterium was the fourth most prevalent genus in the ONT sequences (42.6%) despite it also having underrepresented MRA (1.33% ONT, 9.33% NGS, p<0.001). The under estimation of these genera is not unique to our series. Heikema compared NGS and ONT in mock communities, identifying significant under estimation of Corynebacterium relative to the predicted mock community abundance⁽¹⁵⁾. Heikema deduced that the default ONT 16S rRNA primer (used in the current study) had a low affinity for the primer binding sites in multiple Corynebacterium species, resulting in impaired binding and low amplification. Rozas compared NGS and ONT on skin and mock cultures, identifying a similar PCR-bias with under representation of Corynebacterium, Cutibacterium and Micrococcus⁽¹⁶⁾. Rozas hypothesised that species with a high genetic GC-content were susceptible to under estimations of abundance which was partially validated when the PCR protocol was amended to mitigate this (changing the default polymerase from LongAmp Tag to KAPA). Like Heikema, Rozas also concluded that a lack of affinity to the ONT 16S reverse primer (1492R) was contributing to poor amplification, which was tested with the application of an alternate primer with a downstream primer target region (on the 23S gene), resulting in closer abundance approximations for the relevant species.

The current study applies the recommended ONT primers/PCR protocols, so was susceptible to the abundance discrepancies reported in other studies. Encouragingly, the described limitations appear to be amenable to protocol optimisation.

Cost and accessibility are critical additional considerations when comparing sequencing platforms. ONT has portable and benchtop products that can be readily integrated into independent laboratories. This promotes a streamlined workflow, optimised protocols, access to high-volume real-time sequencing and relinguishes the financial and time obligations of outsourcing to third parties⁽¹³⁾. ONT upfront costs are comparatively low, with the portable MinION and MinION Mk1C sequencers retailing at \$1000-5000, and the benchtop GridION (used in this study) retailing at \$50,000⁽³⁶⁾. Consumables constitute an ongoing expense including flow cells and library preparation kits. While Illumina does not currently have a portable device, it also offers the benefit of a benchtop model. The upfront cost for Illumina Miseg is approximately \$100,000⁽³⁷⁾ which may be a deterrent for independent laboratories, resulting in outsourcing of sequencing to third parties. Ongoing costs comparisons are variable, depending on the sequencing being performed, with some estimating higher per sample cost for ONT and others reporting them to be increasingly comparable⁽³⁸⁻⁴⁰⁾. Limitations of this study include the absence of a mock community to accurately interpret discrepancies in mean relative abundance. A commercially available mock community specific to sinonasal taxa does not exist, however our laboratory is working on a sinus-specific mock community to further optimise sequencing protocols in the future. Consistency at all stages of the wet and dry lab is critical, where feasible, for accurate methodological comparison. We have maximally strived to achieve this but

acknowledge unavoidable divergences. Sample collection and extraction were identical with DNA extracted from a common swab. Previous series have indicated that DNA from a common swab provide reproducible conclusions, even if primer targets or PCR protocols are amended^(4,41), offering some assurance for consistency in our methodology. Different amplification protocols optimised for ONT and NGS were used to maximize read yield. There was necessary divergence in bioinformatics, as ONT and NGS output data are compositionally different and require different clustering/denoising algorithms and different databases. EMU (ONT) uses a custom EMU database while QIIME2 (ONT) is compatible with SILVA or other NCBI derived databases. We used compatible databases for each pipeline, but acknowledge this has potential for database bias⁽⁴²⁾. To address this, we repeated ONT taxonomic assignment with SILVA and compared it to the original EMU results. Results were almost identical, affirming that database bias had a negligible impact. Interestingly, ONT SILVA had limitations assigning a small number of reads to a species, suggesting it may not be optimised for ONT. Therefore, EMU should be retained as the recommended ONT database. This study set out to accurately define the sinonasal microbiome to a species order. In this respect, ONT was superior to NGS and

to a species order. In this respect, ONT was superior to NGS and is endorsed as the preferred modality for future microbiome studies, which should include a concurrent focus on protocol optimisation. As we enter an era of full-length sequencing and metagenomics, embarking on new frontiers of microenvironmental discernment, we must simultaneously embrace the potential to reach new clinical and biological pinnacles whilst remaining humble in our understanding of the limitations and imperfections of this technology.

Conclusion

This is the first study to apply Oxford Nanopore full length 16S rRNA gene amplicon sequencing in the paranasal sinuses, which identified comparable biological conclusions to NGS at a genus order and significantly superior accuracy and resolution at a species order. Despite observed limitations in abundance estimations of sinonasal commensals, the species level characterization of sinus microbiome has provided novel insights into the microenvironment, not previously attained. Future application of full-length sequencing can advance our understanding of the role bacteria play in sinus health and disease.

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Authorship contribution

Study conception and design: AJP, SV, PJW, JC. Data collection: JC, KY, AJP, PW. Analysis and interpretation of results, draft manuscript preparation: All authors. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

AJP is a consultant for Medtronic and Neurent and receives speaking honorariums from Storz, GSK, Sanofi and Sequiris. He is a shareholder for Chitogel. PJW is a consultant for Neilmed, Stryker, Neurent, receives royalties from Integra, Fusetec and is a shareholder for Chitogel.

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Supplementary File 1. Australian Genome Research Facility (AGRF) PCR protocol applied to the NGS dataset in our cohort. Document provided by AGRF.

Materials and methods

PCR amplification and sequencing was performed by the Australian Genome Research Facility. PCR amplicons were generated using the primers and conditions outlined in the attached table. Thermocycling was completed with an Applied Biosystem 384 Veriti and using Platinum SuperFi II mastermix (Life Technologies, Australia) for the primary PCR. The first stage PCR was cleaned using magnetic beads, and samples were visualised on 2% Sybr Egel (Thermo-Fisher). A secondary PCR to index the amplicons was performed with Platinum SuperFi II mastermix (Life Technologies, Australia). The resulting amplicons were cleaned again using magnetic beads, quantified by fluorometry (Promega Quantifluor) and normalised. The eqimolar pool was cleaned a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation. The pool was diluted to 5nM and molarity was confirmed again using a Qubit High Sensitivity dsDNA assay (ThermoFisher). This was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 x 300 base pairs paired-end).

Target	Cycle	Initial	Initial Disassociate		Extension	Finish	
16S: V3 - V4	30	98°C for 30 sec	98°C for 30 sec 98°C for 10 sec		72°C for 30 sec	72°C for 5 min	
	165: V3 - V4 (341F-806R)						
Forward Primer (341F) CCTAYGGGRBGCASCAG							
Reverse Prim	ner (806R)	GGACTACNNGGGTATCTAAT					

Supplementary File 2. Microbiome agreement calculation.

Heikema et al. ⁽¹⁾ utilised this method to calculate the cumulate relative abundance (RA) agreement between NGS and ONT paired samples and the number of genera in agreement per sample. In this example (sample 7), *Streptococcus* was identified in 95.82% RA in NGS and 98.93%RA in ONT, with agreement of 95.82% for *Streptococcus*. *Moraxella* was identified in 3.48% RA in NGS and 1.07% RA in ONT with agreement of 1.07% for *Moraxella*. No other genera were identified in both samples. So the final agreement was 95.82% + 1.07%= 96.89% relative abundance agreement and a total of 2 genera in agreement.

Sample 7	Relative A	bundance (%)	Agreement		
GENUS	NGS	ONT	Relative Abundance (%)	Genera (n)	
16S: V3 - V4	30	98°C for 30 sec	98°C for 10 sec	60°C for 10 sec	

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All samples agreement table.

	Agreem	ent
Sample	Relative Abundance (%)	Genera (n)
1	100	1
2	65.35	6
3	75.93	3
4	87.26	2
6	89.19	4
7	96.89	2
8	73.99	4
9	4.45	4
10	74.27	3
12	1.9	1
14	16.33	5
15	35.55	6
16	61.43	3
17	99.98	1
18	93.81	4
19	26.29	2
20	99.33	1
21	8.53	4
22	65.83	3
23	94.33	5
24	99.9	1
25	93.83	3
26	99.89	1
27	87.33	4
28	93.58	3

Supplementary File 3.

Genus	significance (p_)
gCorynebacterium	1.03E-06
gLaceyella	1.57805612412796e- 06
gCutibacterium	0.000505205
gLawsonella	0.043206314
gKlebsiella	0.174471631
gStreptococcus	0.179348012
gBacillus	0.263256025
gSnodgrassella	0.275703742
gStaphylococcus	0.280137092
gNeobacillus	0.293867664

Genus	significance (p_)
gEscherichia-Shigella	0.355934586
gCrinalium	0.358337754
gMitochondria	0.36877446
gEscherichia	0.412804926
gRalstonia	0.432531345
gDelftia	0.450585337
gAnabaena	0.452618158
gAliterella	0.48875586
gOchrobactrum	0.491712684
gCyanothece	0.495889356
gGloeocapsa	0.497103958

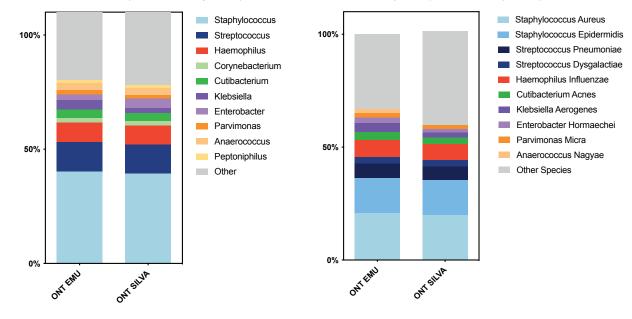
Short and long read sinus microbiome sequencing

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g_Csciliatoria 0536983946 g_Allochromatium 0.634952576 g_Rathia 0.53745523 g_Stenidobacter 0.63555117 g_Sphingopysis 0.544526247 g_Chridobacter 0.636556117 g_Sphingopysis 0.554171208 g_Friedmanniella 0.63695707 g_Calothrix 0.56622497 g_Aloprevotella 0.64125074 g_Calothrix 0.568226052 g_Shingonela 0.642251745 g_Calothrix 0.5722265 g_Shonella 0.642251745 g_Advitella 0.57715528 g_Sporobecterium 0.642251745 g_Advitelabacter 0.5807681 g_Lactococcus 0.642251745 g_Advitelabacter 0.5807681 g_Lactococcus 0.642251745 g_Advitelabacter 0.5807681 g_Lactococcus 0.65487642 g_Aroindospermum 0.58735022 g_Actinobactilus 0.65487445 g_Prolynomoas 0.59809571 g_Actinobacter 0.65487445 g_Prolynomoas 0.5980786 g_Tichodesmium 0.65487445 g_Prolynomoas 0.59807927 g_Actinobacter	gBergeriella	0.531832504	gThermicanus	0.632189385
g. Rothia0.53745523g. Steroidobacter0.635243457g. Jehomophilus0.544526247g. Comamonas0.636556117g. Sphingomonas0.553864444g. Rahnella0.641259043g. Sphingopyis0.55171280g. Rahnella0.641259043g. Calothrix0.565623497g. Alkoprevotella0.641257072g. Calothrix0.568628097g. Phytisphaera0.642287128g. Calothrix0.56862632g. Dhytisphaera0.642287128g. Calothrix0.56876813g. Sporobacterium0.642287128g. Aggregatibacter0.5856768343g. Latcoccus0.64787428g. Acidiholdoster0.586768343g. Latcoccus0.644787468g. Perichonborus0.587785282g. Actihobacillus0.645870468g. Perichonborus0.586768343g. Latcoccus0.644787468g. Perichonborus0.586768343g. Actihobacillus0.655778615g. Perichonborus0.5887867g. Actinobacillus0.655778615g. Perichonborus0.59817867g. Calonocytophaga0.655078615g. Baekdua0.6925079817g. Calonocytophaga0.666372014g. Baekdua0.602560879g. Caronozaterium0.663231206g. Baekdua0.60397897g. Maighibuits0.663249759g. Microcystis0.60383868g. Nakamurella0.66314964g. Turkella0.601322454g. Chruella0.671719164g. Lactobactinu0.6163784368g. Nakamurella0.66314976g. Jurkerok0.61112691 <td< td=""><td>gParacoccus</td><td>0.534042442</td><td>gMethylocaldum</td><td>0.633762839</td></td<>	gParacoccus	0.534042442	gMethylocaldum	0.633762839
g_Haemophilus 0.544526247 g_Comamonas 0.636556117 g_Sphingomonas 0.553864444 g_Friedmanniella 0.638104241 g_Chrysobacterium 0.55652497 g_Alloprevotella 0.641259043 g_Chrysobacterium 0.55652497 g_Alloprevotella 0.64278772 g_Calothrix 0.55652497 g_Shinonella 0.64278772 g_Calothrix 0.57725286 g_Spatnonella 0.642787128 g_Neisseria 0.577745528 g_Sporobacterium 0.642787128 g_Adgregatibacter 0.586788343 g_Letococcus 0.64782221 g_Adjregatibacter 0.586789342 g_Actinobactlius 0.664879468 g_Porphyromonas 0.599090741 g_Actinobacterium 0.665878619 g_Methylomagnum 0.59678052 g_Algiphaera 0.6557861763 g_Methylomagnum 0.59647967 g_Algiphaera 0.65971062 g_Methylomagnum 0.59647962 g_Carnobacterium 0.6597141 g_Parvinonas 0.605730927 g_Carnobacterium 0.6597473 g_Matemobilimic oblum 0.60562386	gOscillatoria	0.536983946	gAllochromatium	0.634952576
g_sphingononas 0.553864444 g_Friedmanniella 0.638104241 g_sphingopyxis 0.5538717208 g_Rahnella 0.641259043 g_Calothrix 0.56602397 g_Alloprevotella 0.641259043 g_Calothrix 0.56602397 g_Bhyckphera 0.642275072 g_Calothrix 0.57222826 g_Salmonella 0.642278072 g_Asoultella 0.572715528 g_Sorobacterium 0.642780728 g_Adgregatibacter 0.586708343 g_Lectococcus 0.644128447 g_Adgregatibacter 0.5867058 g_Lectionciux 0.658876813 g_Cylindrospermum 0.58670814 g_Acrichabactur 0.658476425 g_Porphyromonas 0.5990724 g_Acrichabactur 0.658476425 g_Prosthecomicrobium 0.59417677 g_Algiphara 0.655778815 g_Prosthecomicrobium 0.59417677 g_Algiphara 0.658971412 g_Madellimicrobium 0.59417875 g_Carnostoctrium 0.658971412 g_Mabellimicrobium 0.607239427 g_Carnostoctrium 0.66331426 g_Friedola 0.607239432	gRothia	0.537453523	gSteroidobacter	0.635243457
g_Sphingopyxis 0.554171208 g_Rahnella 0.641259043 g_Chiryseobacterium 0.565622497 g_Alloprevotella 0.641234577 g_Galuthrix 0.565622497 g_Shinonella 0.642231745 g_Galuthrix 0.572119921 g_Zogloca 0.642231745 g_Raoutella 0.577145528 g_Sporobacterium 0.64473822214 g_Acidihalobacter 0.586763343 g_Letrococus 0.6447822214 g_Acidihalobacter 0.586763843 g_Letrobactilus 0.654876425 g_Dirlorborus 0.588182441 g_Acinetobacter 0.654876483 g_Prosincobrus 0.598176017 g_Algiphaera 0.655776815 g_Prosincobrus 0.598176017 g_Algiphaera 0.655776815 g_Prosincobrus 0.598176917 g_Algiphaera 0.655776815 g_Prosincomicrobium 0.596379052 g_Capnocytophaga 0.6580778815 g_Prosincomicrobium 0.596379052 g_Capnocytophaga 0.658071044 g_Bachdinacobina 0.600739474 g_Variovara 0.663231206 g_Farvimonas 0.600579847 <td>gHaemophilus</td> <td>0.544526247</td> <td>gComamonas</td> <td>0.636556117</td>	gHaemophilus	0.544526247	gComamonas	0.636556117
g_chrysobacterium 0.565623497 g_Alloprevotella 0.641948577 g_Calothrix 0.565623497 g_Alloprevotella 0.6412375072 g_Calothrix 0.5722826 g_Salmonella 0.642251745 g_Neisseria 0.577245528 g_Sorobacterium 0.642787128 g_Neisseria 0.58750758 g_Sorobacterium 0.644128447 g_Adgregatibacter 0.586768343 g_Lactococcus 0.64782221 g_Achinobacturium 0.586768343 g_Lactococcus 0.64782221 g_Adjindrospermum 0.586768343 g_Lactococcus 0.64782221 g_Arbinoborus 0.588162441 g_Actinobacillus 0.658876425 g_Porphyromonas 0.590909741 g_Trichodesmium 0.655876815 g_Borphyromonas 0.59047962 g_Calopocytophaga 0.6558778815 g_Borphyromonas 0.59947967 g_Rosevarius 0.65597472 g_Borphyromonas 0.606739947 g_Carnobacterium 0.663973947 g_Backduic 0.60739947 g_Carnobacterium 0.6639759472 g_Backduic 0.60739947	gSphingomonas	0.553864444	gFriedmanniella	0.638104241
g_Galothrix0.56892662g_Phycisphaera0.642375072g_Citrobacter0.57222826g_Salmonella0.6422371452g_Naoutella0.577314552g_Zoogleea0.642787128g_Agerapatibacter0.58500758g_Lactococcus0.644128447g_Addihalobacter0.58500758g_Lactococcus0.64782221g_Acidihalobacter0.586768343g_Lactococcus0.64782221g_Nohdrospermum0.58675082g_Actinobacillus0.65817863g_Porphyromonas0.59817871g_Actinobacter0.65847642g_Pseudanabaena0.599409741g_Trichodesmium0.65507881g_Pseudanabaena0.5943307g_Synechococcus0.65507815g_Backduia0.59847807g_Capnocytophaga0.65907104g_Backduia0.6097964g_Carnobacterium0.66947504g_Morea0.60573947g_Carnobacterium0.66349759g_Itricholospira0.60573947g_Carnobacterium0.66349759g_Tiricfala0.6073947g_Carnobacterium0.66349759g_Tiricfala0.6073947g_Mizrovarx0.66849759g_Tiricfala0.6073947g_Nabarurella0.66849759g_Lactobacillus0.611129691g_Natarurella0.66825874g_Natoroccus0.61112961g_Natarurella0.66825874g_Lactobacillus0.61112961g_Natarurella0.667173102g_Lactobacillus0.61472343g_Nertylobacterium0.67173102g_Lactobacillus0.614123436g_Nertylobacterium0.67218447	gSphingopyxis	0.554171208	gRahnella	0.641259043
g_Citrobacter 0.57222826 g_Salmonella 0.64221745 g_Raouhtella 0.573119921 g_Zoogloea 0.642787128 g_Akeidhabacter 0.585500758 g_Jerobacterium 0.64128447 g_Acidihabbacter 0.586768343 g_Jerbiconiux 0.647822221 g_Acidihabbacter 0.586768343 g_Jerbiconiux 0.654876425 g_Cylindrospermum 0.587350282 g_Actinobacillus 0.654879468 g_Prosincoborus 0.598182441 g_Acidihabacter 0.654879468 g_Prosincomicrobium 0.594176747 g_Meinpharen 0.655778815 g_Prosincomicrobium 0.594370379 g_Synechocccus 0.656875004 g_Methylomagnum 0.594370379 g_Synechocccus 0.656379047 g_Norea 0.602560079 g_Cannocytophaga 0.663719047 g_Parvimonas 0.600560386 g_Ialighilus 0.66375215 g_Parvimonas 0.600563386 g_Natamurella 0.66379275 g_Nicrocystis 0.61169348 g_Nelabaurella 0.671743088 g_Turicella 0.61169348	gChryseobacterium	0.565623497	gAlloprevotella	0.641948577
g_Racultella 0.573119921 g_Zoogloea 0.642787128 g_Neisseria 0.577745528 g_Sporobacterium 0.6447822221 g_Adgregatibacter 0.58500758 g_Lactococcus 0.647822221 g_Addihalobacter 0.586768433 g_Lactococcus 0.647822221 g_Addihalobacter 0.586768434 g_Leitoinux 0.654876425 g_Proinborus 0.588182441 g_Actinobacter 0.654876425 g_Proinborus 0.598176474 g_Algisphaera 0.655076815 g_Proinborus 0.594176747 g_Algisphaera 0.655071044 g_Morthylomagnum 0.596379052 g_Canpoxterium 0.655071042 g_Morea 0.6002560079 g_Canpoxterium 0.66331206 g_Flaubellimicrobium 0.600739947 g_Algiphilus 0.66331206 g_Flaubellimicrobium 0.600732942 g_Avarioorax 0.66331206 g_Flaubellimicrobium 0.600732942 g_Algiphilus 0.66331206 g_Flauboacterium 0.600732942 g_Araubacterium 0.66383166 g_Flavobacterium 0.61347255	gCalothrix	0.568926052	gPhycisphaera	0.642375072
g_Neisseria 0.577745528 g_Sporobacterium 0.644128447 g_Aggregatibacter 0.585500758 g_Lactococcus 0.644128447 g_Aggregatibacter 0.586768343 g_Lactococcus 0.647822221 g_Acidihalobacter 0.586768343 g_Lactococcus 0.654876425 g_Prolinoborus 0.588182441 g_Acinetobacter 0.654876425 g_Porphyromonas 0.59090741 g_Acinetobacter 0.655078815 g_Prolinoborus 0.594370379 g_Acinetobacter 0.6550778815 g_Proshecomicrobium 0.59437052 g_Capnocytophaga 0.65807101427 g_Morea 0.60256079 g_Carnobacterium 0.65931206 g_Arimetobaster 0.60256079 g_Carnobacterium 0.66231206 g_Tarvimonas 0.60256079 g_Carnobacterium 0.66231206 g_Tarvimonas 0.60256079 g_Carnobacterium 0.66331206 g_Tarvimonas 0.6025079472 g_Algiphilus 0.66231206 g_Tirichodespira 0.60733436 g_Nakamurella 0.66391496 g_Turicella 0.60733436	gCitrobacter	0.57222826	gSalmonella	0.642521745
g_Aggregatibacter 0.585500758 g_Lactococcus 0.647822221 g_Acidihalobacter 0.586768343 g_Lactococcus 0.647822221 g_Acidihalobacter 0.586768343 g_Lactinobacillus 0.651886865 g_Cylindrospermum 0.587350282 g_Actinobacillus 0.6564876425 g_Porphyromonas 0.590176747 g_Aleitobacter 0.656778815 g_Prosthecomicrobium 0.594350379 g_Synechococcus 0.6568770815 g_Prosthecomicrobium 0.596379052 g_Capnocytophaga 0.656871014 g_Baekduia 0.598417875 g_Algisphaera 0.6569710427 g_Moorea 0.600250079 g_Carnobacterium 0.6563752015 g_Parvimonas 0.600539947 g_Variovorax 0.6636497594 g_Ectothirchodospira 0.600739432 g_Variovorax 0.6636497591 g_Turcella 0.6007334368 g_Nehomeral 0.666811189 g_Lactobactilus 0.611129691 g_Nerkitoccus 0.671701864 g_Lactobactilus 0.611416348 g_Brevutnimonas 0.671873102 g_Lactobactilus	gRaoultella	0.573119921	gZoogloea	0.642787128
g_Acidialobacter 0.586768343 g_Herbiconiux 0.651886865 g_Cylindrospermum 0.586768343 g_Herbiconiux 0.651886865 g_Prolinoborus 0.588750282 g_Actinobacillus 0.651886865 g_Prolinoborus 0.588182441 g_Actinobacillus 0.654879468 g_Prophyromonas 0.590909741 g_Arichecbacter 0.655778815 g_Proshecomicrobium 0.594379052 g_Sapchococcus 0.656877004 g_Methylomagnum 0.595679052 g_Roseovarius 0.656971014 g_Baekduia 0.598417875 g_Roseovarius 0.659759472 g_Moorea 0.602560079 g_Carinobacterium 0.6637592472 g_Ntubellimicrobium 0.604775457 g_Algiphilus 0.66375215 g_Proshorenas 0.60563886 g_Flavobacterium 0.663872215 g_Microcytis 0.607329432 g_Aruella 0.666811189 g_Tychonema 0.610656622 g_Petpostreptococcus 0.6717543088 g_Latobacillus 0.61129691 g_Reguivicoccus 0.671873082 g_Microglationic-Paraburkhole	gNeisseria	0.577745528	gSporobacterium	0.644128447
g_Cylindrospermum 0.587350282 g_Actinobacillus 0.654876425 g_Prolinoborus 0.588182441 g_Actinobacillus 0.654876425 g_Porphyromonas 0.590909741 g_Actinobacillus 0.655778815 g_Prosthecomicrobium 0.59437052 g_Algisphera 0.655778815 g_Mostheomicrobium 0.59637052 g_Capnocytophaga 0.65687004 g_Morea 0.60250079 g_Caronocytophaga 0.659010427 g_Norea 0.60250079 g_Caronocytophaga 0.6563792472 g_Natorea 0.60250079 g_Caronocytophaga 0.663649759 g_Parvimonas 0.605739947 g_Algiphilus 0.663649759 g_Lictothiorhodospira 0.605739947 g_Arairovarax 0.663752215 g_Microcystis 0.60732432 g_Natamurella 0.6683866 g_Toricella 0.60732432 g_Nakamurella 0.668825874 g_Sutchobacillus 0.611129691 g_Nakamurella 0.668825874 g_Lactobacillus 0.6114720741 g_Natinipila 0.671701864 g_Niroletia-Caballeronia-Paraburkhol-	gAggregatibacter	0.585500758	g_Lactococcus	0.647822221
g_Prolinoborus 0.588182441 g_Acinetobacter 0.654879468 g_Porphyromonas 0.590909741 g_Trichodesmium 0.655778815 g_Prosthecomicrobium 0.594350379 g_Algisphaera 0.655778815 g_Mothylomagnum 0.596379052 g_Synechococcus 0.6558071014 g_Baekdula 0.598417875 g_Capnocytophaga 0.659759472 g_Moorea 0.602560079 g_Carnobacterium 0.662331206 g_Parvimonas 0.605683866 g_Algiphilus 0.66331206 g_Parvimonas 0.605683866 g_Aloyoorax 0.66331206 g_Microcystis 0.605739947 g_Varoorax 0.66311206 g_Tychonema 0.607329432 g_Cnuella 0.6631189 g_Tychonema 0.61056622 g_Petpotsreptococcus 0.671701864 g_Mareroccus 0.611129691 g_Acidicaldus 0.6712086 g_Burkholderia-Caballeronia-Paraburkhol 0.61482345 g_Metroylobacterium 0.67218981 g_Mareroccus 0.61416348 g_Inminial 0.6725089 g_Burkholderia-Caballeronia-Paraburkhol <t< td=""><td>gAcidihalobacter</td><td>0.586768343</td><td>gHerbiconiux</td><td>0.651886865</td></t<>	gAcidihalobacter	0.586768343	gHerbiconiux	0.651886865
g_Porphyromonas 0.590909741 g_Trichodesmium 0.655016365 g_Pseudanabaena 0.594176747 g_Algisphaera 0.655778815 g_Prosthecomicrobium 0.594350379 g_Synechococcus 0.656475004 g_Methylomagnum 0.596379052 g_Capnocytophaga 0.658071014 g_Baekduia 0.598417875 g_Capnocytophaga 0.658071014 g_Moorea 0.602560079 g_Carnobacterium 0.65631206 g_Parvimonas 0.60563886 g_Flavobacterium 0.666379202 g_Etothiorhodospira 0.605739947 g_Carnobacterium 0.6663752215 g_Flavobacterium 0.666372225 g_Cruella 0.6663752215 g_Floropoldia 0.60739947 g_Variovarax 0.663752215 g_Tricholesmira 0.605739947 g_Variovarax 0.666825874 g_Tricholesmira 0.60783486 g_Natamurella 0.668825874 g_Toricella 0.6017101664 g_Natamurella 0.671718464 g_Matoroccus 0.611129691 g_Natamurella 0.6721884 g_Latobacillus 0.61147255	gCylindrospermum	0.587350282	gActinobacillus	0.654876425
g_Pseudanabaena 0.594176747 g_Algisphaera 0.655778815 g_Prosthecomicrobium 0.594350379 g_Synechococcus 0.656475004 g_Methylomagnum 0.596379052 g_Capnocytophaga 0.658071014 g_Baekduia 0.598417875 g_Roseovarius 0.659759472 g_Morea 0.60075457 g_Algiphilus 0.662331206 g_Parvimonas 0.6057399472 g_Algiphilus 0.663475275 g_Ltothiorhodospira 0.60572255 g_Cruella 0.663752215 g_Tircella 0.607329432 g_Nakamurella 0.66825874 g_Tychonema 0.61129691 g_Negativicoccus 0.61714084 g_Microckus 0.611417255 g_Arcidicaldus 0.67184308 g_Lactobacillus 0.6114172567 g_Arcidicaldus 0.67184308 g_Microccus 0.6114169348 g_Methylobacterium 0.671873102 g_Lactobacillus 0.61720741 g_Arminipila 0.67265089 g_Burkholderia-Caballeronia-Paraburkhol 0.62263950 g_Inmirania 0.67347519 g_Arcibacterium 0.62263950	gProlinoborus	0.588182441	gAcinetobacter	0.654879468
g_Prosthecomicrobium 0.594350379 g_Synechococcus 0.656475004 g_Methylomagnum 0.596379052 g_Capnocytophaga 0.658071014 g_Baekduia 0.598417875 g_Roseovarius 0.659010427 g_Moorea 0.602560079 g_Carnobacterium 0.659759472 g_Rubellimicrobium 0.604775457 g_Algiphilus 0.662331206 g_Parvimonas 0.605739947 g_Tavobacterium 0.66349759 g_Etcthiorhodospira 0.60732942 g_Cnuella 0.66391496 g_Turicella 0.607329432 g_Nakamurella 0.666811189 g_Tavinonama 0.61056622 g_Negativiacccus 0.671743088 g_Geobacillus 0.611129691 g_Negativiaccus 0.671873102 g_Lactobacillus 0.61469348 g_Revundinonas 0.671873102 g_Burkholderia-Caballeronia-Paraburkhol 0.62280362 g_Imirania 0.67347519 g_Dietzia 0.62280379 g_Introscoccus 0.671403767 g_Methylococus 0.62638333 g_Yangia 0.67404767 g_Methyloacterium 0.67539927	gPorphyromonas	0.590909741	gTrichodesmium	0.655016365
g_Methylomagnum 0.596379052 g_Capnocytophaga 0.658071014 g_Baekduia 0.598417875 g_Roseovarius 0.659010427 g_Moorea 0.602560079 g_Carnobacterium 0.659759472 g_Nubellimicrobium 0.604775457 g_Carnobacterium 0.66563886 g_Parvimonas 0.605638866 g_Flavobacterium 0.663649759 g_Lectothiorhodospira 0.605739947 g_Variovorax 0.66361189 g_Ticrocystis 0.6072652 g_Cnuella 0.66391496 g_Tychonema 0.607329432 g_Nakamurella 0.66825874 g_Tychonema 0.61056622 g_Peptostreptococcus 0.671701864 g_Anaerococcus 0.611129691 g_Negativicoccus 0.671701864 g_Lactobacillus 0.614169348 g_Brevundimonas 0.671701864 g_Burkholderia-Caballeronia-Paraburkhol- G_Eburkholderia-Caballeronia-Paraburkhol- G_Eburkholderia-Caballeronia-Paraburkhol- G_Eburkholderia-Caballeronia-Paraburkhol- G_G2263959 g_Inmirania 0.67245089 g_Mathylococcus 0.624580333 g_Yangia 0.674404767 g_Mactibaterirum 0.625253409 g_Yangi	gPseudanabaena	0.594176747	gAlgisphaera	0.655778815
g_Baekduia 0.598417875 g_Roseovarius 0.659010427 g_Moorea 0.602560079 g_Carnobacterium 0.659759472 g_Rubellimicrobium 0.604775457 g_Algiphilus 0.662331206 g_Parvimonas 0.605683886 g_Flavobacterium 0.66349759 g_Ectothiorhodospira 0.60593947 g_Variovorax 0.663752215 g_Microcystis 0.60592725 g_Cnuella 0.666811189 g_Tychonema 0.607329432 g_Nakamurella 0.666825874 g_Tychonema 0.610656622 g_Peptostreptococcus 0.671701864 g_Anaerococcus 0.611129691 g_Acidicaldus 0.671795657 g_Lactobacillus 0.617720741 g_Araenococcus 0.671795657 g_Mixipalla 0.61720741 g_Aninipila 0.67231884 g_Methylococcus 0.622089362 g_Nitrosococcus 0.674110389 g_Methylococcus 0.622089362 g_Nitrosococcus 0.674110389 g_Methylococcus 0.622089362 g_Nitrosococcus 0.67410389 g_Methylococcus 0.62263959 g_Ni	gProsthecomicrobium	0.594350379	gSynechococcus	0.656475004
g_Moorea0.602560079g_Carnobacterium0.659759472g_Rubellimicrobium0.604775457g_Algiphilus0.66231206g_Parvimonas0.605633886g_Flavobacterium0.663649759g_Etothiorhodospira0.605739947g_Variovorax0.663752215g_Microcystis0.60592725g_Cnuella0.66381189g_Turicella0.607329432g_Maigakiibacter0.66681189g_Tychonema0.610656622g_Peptostreptococcus0.671701864g_Anaerococus0.611129691g_Negativicoccus0.671701864g_Lactobacillus0.614169348g_Aninipila0.6725089g_Mikpelleria-Caballeronia-Paraburkhor0.622089362g_Inmirania0.673218884g_Michylococcus0.6223395g_Nitrosoccus0.67110389g_Mathylococcus0.62263959g_Nitrosoccus0.674110389g_Michylococcus0.62263950g_Saccharimonadaceae0.67539927g_Actibacterium0.62263981g_Saccharimonadaceae0.67539927g_Mithylococcus0.62263981g_Cacdimonadaceae0.67539927g_Geitlerinema0.62263981g_Cacdimonadaceae0.67539927g_Geitlerinema0.625698819g_Cacdimonadaceae0.67523109	gMethylomagnum	0.596379052	gCapnocytophaga	0.658071014
g_Rubellimicrobium0.604775457g_Algiphilus0.662331206g_Parvimonas0.605568386g_Algiphilus0.66334206g_Etothiorhodospira0.605739947g_Variovorax0.663752215g_Microcystis0.60592725g_Cnuella0.66391496g_Turicella0.607329432g_Nakamurella0.66881189g_Tychonema0.61056622g_Peptostreptococcus0.671543088g_Gobacillus0.611129691g_Akdicaldus0.671701864g_Akaperococcus0.611469348g_Brevundimonas0.671873102g_Lactobacillus0.61720741g_Methylobacterium0.67321884g_Analomicrobium0.622089362g_Inimirania0.67347519g_Machbylococcus0.62235309g_Nitrosoccus0.674110389g_Machbylococcus0.62235409g_Varja0.674404767g_Machbylococcus0.62530891g_Saccharimonadaceae0.67539927g_Geitlerinema0.62569819g_Cacedimonas0.67533192g_Cacedimonas0.62569819g_Cacedimonas0.67533192	gBaekduia	0.598417875	gRoseovarius	0.659010427
g_Parvimonas0.60568386g_Flavobacterium0.663649759g_Etothiorhodospira0.605739947g_Variovorax0.663752215g_Microcystis0.60592725g_Cnuella0.66381496g_Finegoldia0.607329432g_Mizugakiibacter0.668811189g_Turicella0.607834368g_Nakamurella0.668825874g_Tychonema0.611129691g_Negativicoccus0.671701864g_Anaerococcus0.613417255g_Acidicaldus0.671701864g_Lactobacillus0.61720741g_Aninipila0.67265089g_Micholderia-Caballeronia-Paraburkhol0.618423435g_Inimirania0.673218884g_Methylobacterium0.62268959g_Vargia0.674110389g_Ancalomicrobium0.62263959g_Vargia0.67440767g_Actibacterium0.625235409g_Saccharimonadaceae0.675539927g_Geitlerinema0.62569819g_Kocuria0.675849598g_Revinipila0.62569819g_Caedimonas0.675839927g_Actibacterium0.62569819g_Caedimonas0.675839927g_Actibacterium0.62569819g_Caedimonas0.675839927g_Actibacterium0.62569819g_Caedimonas0.675839927g_Actibacterium0.62569819g_Caedimonas0.676523109g_Caedimonas0.676523109g_Caedimonas0.676523109	gMoorea	0.602560079	gCarnobacterium	0.659759472
g_Ectothiorhodospira 0.605739947 g_Variovorax 0.663752215 g_Microcystis 0.60592725 g_Cnuella 0.66391496 g_Finegoldia 0.607329432 g_Mizugakiibacter 0.668825874 g_Turicella 0.607834368 g_Nakamurella 0.668825874 g_Tychonema 0.61065622 g_Negativicoccus 0.671701864 g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.614169348 g_Negativicoccus 0.671701864 g_Kingella 0.61720741 g_Anninpila 0.67321884 g_Methylobacterium 0.62238962 g_Nitrosococcus 0.673410389 g_Dietzia 0.62235409 g_Nainipila 0.673410389 g_Methylococcus 0.624580333 g_Nainipila 0.673410389 g_Methylococcus 0.624580333 g_Saccharimonadaceae 0.675539927 g_Actibacterium 0.62589819 g_Saccharimonadaceae 0.675849588 g_Caedimonas 0.675849598 g_Acaedimonas 0.675849598	gRubellimicrobium	0.604775457	gAlgiphilus	0.662331206
g_Microcystis 0.60592725 g_Cnuella 0.66391496 g_Finegoldia 0.607329432 g_Mizugakiibacter 0.666811189 g_Turicella 0.607834368 g_Nakamurella 0.668825874 g_Tychonema 0.61055622 g_Peptostreptococcus 0.671543088 g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.613417255 g_Acidicaldus 0.671701864 g_Kingella 0.617702741 g_Aninipila 0.673218884 g_Nethyloderia-Caballeronia-Paraburkhol 0.622089362 g_Nitroscoccus 0.674110389 g_Dietzia 0.622089362 g_Nitroscoccus 0.674404767 g_Methylococcus 0.62263959 g_Nitroscoccus 0.67539927 g_Actibacterium 0.62253409 g_Saccharimonadaceae 0.675539927 g_Actibacterium 0.62568819 g_Saccharimonadaceae 0.675849598 g_Caedimonas 0.67553109 g_Caedimonas 0.67552109	gParvimonas	0.605683886	gFlavobacterium	0.663649759
g_Finegoldia 0.607329432 g_Mizugakiibacter 0.666811189 g_Turicella 0.607334368 g_Nakamurella 0.666825874 g_Tychonema 0.610656622 g_Peptostreptococcus 0.671543088 g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.614169348 g_Negativicoccus 0.671873102 g_Lactobacillus 0.617720741 g_Arminpila 0.67265089 g_Burkholderia-Caballeronia-Paraburkhol- 0.618423435 g_Alminpila 0.673218884 g_Mitugocccus 0.622089362 g_Nitrosococcus 0.674110389 g_Dietzia 0.62235409 g_Yangia 0.674404767 g_Actibacterium 0.62253409 g_Saccharimonadaceae 0.675539927 g_Actibacterium 0.62569819 g_Kocuria 0.675849598 g_Cedithorinas 0.6256087765 g_Cadimonas 0.675849598	gEctothiorhodospira	0.605739947	gVariovorax	0.663752215
g_Turicella 0.607834368 g_Nakamurella 0.668825874 g_Tychonema 0.610656622 g_Peptostreptococcus 0.671543088 g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.613417255 g_Acidicaldus 0.671873102 g_Lactobacillus 0.617720741 g_Aminipila 0.673218884 g_Burkholderia-Caballeronia-Paraburkhol 0.618423435 g_Athipila 0.67347519 g_Ancalomicrobium 0.622089362 g_Methylobacterium 0.67347519 g_Methylococcus 0.6220359 g_Yangia 0.675349927 g_Actibacterium 0.62235409 g_Nocuria 0.675849598 g_Geitlerinema 0.62598819 g_Caedimonas 0.675849598 g_Ruviolla 0.675231092 Gaedimonas 0.67523109	gMicrocystis	0.60592725	gCnuella	0.66391496
g_Tychonema 0.610656622 g_Peptostreptococcus 0.671543088 g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.613417255 g_Acidicaldus 0.671795657 g_Lactobacillus 0.614169348 g_Brevundimonas 0.671873102 g_Kingella 0.61720741 g_Aminipila 0.67265089 g_Burkholderia-Caballeronia-Paraburkhol- 0.618423435 g_Inmirania 0.673218884 g_Methylobacterium 0.622089362 g_Nitrosococcus 0.674110389 g_Dietzia 0.62263959 g_Yangia 0.67539927 g_Actibacterium 0.62535409 g_Saccharimonadaceae 0.675849598 g_Geitlerinema 0.62569819 g_Caedimonas 0.675849598 g_Rourialha 0.626007365 0.676523109 0.676523109	gFinegoldia	0.607329432	gMizugakiibacter	0.666811189
g_Geobacillus 0.611129691 g_Negativicoccus 0.671701864 g_Anaerococcus 0.613417255 g_Acidicaldus 0.671795657 g_Lactobacillus 0.614169348 g_Brevundimonas 0.671873102 g_Kingella 0.61720741 g_Aminipila 0.673218884 g_Burkholderia-Caballeronia-Paraburkhol 0.62089362 g_Inmirania 0.67347519 g_Ancalomicrobium 0.62263959 g_Nitrosococcus 0.674110389 g_Actibacterium 0.62253409 g_Saccharimonadaceae 0.675539927 g_Geitlerinema 0.62508819 g_Caedimonas 0.675849598 g_Caedimonas 0.675849598 g_Caedimonas 0.67583927	gTuricella	0.607834368	gNakamurella	0.668825874
g_Anaerococcus 0.613417255 g_Acidicaldus 0.671795657 g_Lactobacillus 0.614169348 g_Brevundimonas 0.671873102 g_Kingella 0.617720741 g_Aminipila 0.67265089 g_Burkholderia-Caballeronia-Paraburkhol- deria 0.618423435 g_Inmirania 0.673218884 g_Ancalomicrobium 0.622089362 g_Nethylobacterium 0.67347519 g_Dietzia 0.62263959 g_Yangia 0.674404767 g_Actibacterium 0.625235409 g_Saccharimonadaceae 0.675539927 g_Kocuria 0.675849598 g_Caedimonas 0.676523109	gTychonema	0.610656622	gPeptostreptococcus	0.671543088
g_Lactobacillus 0.614169348 g_Brevundimonas 0.671873102 g_Kingella 0.617720741 g_Aminipila 0.67265089 g_Burkholderia-Caballeronia-Paraburkhol- deria 0.618423435 g_Inmirania 0.673218884 g_Ancalomicrobium 0.622089362 g_Methylobacterium 0.67347519 g_Dietzia 0.62263959 g_Yangia 0.674404767 g_Actibacterium 0.625235409 g_Saccharimonadaceae 0.675539927 g_Caedimonas 0.675849598 g_Caedimonas 0.676523109	gGeobacillus	0.611129691	gNegativicoccus	0.671701864
g_Kingella0.617720741g_Aminipila0.67265089g_Burkholderia-Caballeronia-Paraburkhol- deria0.618423435g_Inmirania0.673218884g_Ancalomicrobium0.622089362g_Methylobacterium0.67347519g_Dietzia0.62263959g_Nitrosococcus0.674110389g_Methylococcus0.624580333g_Yangia0.674404767g_Actibacterium0.625235409g_Kocuria0.67539927g_Geitlerinema0.62569819g_Caedimonas0.675849598g_Rouvialla0.6260072650.676523109	gAnaerococcus	0.613417255	gAcidicaldus	0.671795657
g_Burkholderia-Caballeronia-Paraburkhol- deria0.618423435g_Inmirania0.673218884g_Ancalomicrobium0.62089362g_Methylobacterium0.67347519g_Dietzia0.62263959g_Nitrosococcus0.674110389g_Methylococcus0.624580333g_Yangia0.674404767g_Actibacterium0.625235409g_Saccharimonadaceae0.675539927g_Geitlerinema0.62569819g_Caedimonas0.67553109g_Caedimonas0.675531090.67553109	gLactobacillus	0.614169348	gBrevundimonas	0.671873102
deria g_Methylobacterium 0.67347519 g_Ancalomicrobium 0.62089362 g_Nitrosococcus 0.674110389 g_Dietzia 0.62263959 g_Yangia 0.674404767 g_Actibacterium 0.62535409 g_Kocuria 0.67539927 g_Geitlerinema 0.62569819 g_Caedimonadaceae 0.675849598 g_Caedimonas 0.676523109 0.676523109	gKingella	0.617720741	gAminipila	0.67265089
g_Ancalomicrobium 0.622089362 g_Nitrosococcus 0.67410389 g_Dietzia 0.62263959 g_Yangia 0.674404767 g_Methylococcus 0.624580333 g_Saccharimonadaceae 0.675539927 g_Geitlerinema 0.62569819 g_Caedimonas 0.676523109		0.618423435	gInmirania	0.673218884
g_Dietzia 0.62263959 g_Yangia 0.674404767 g_Methylococcus 0.624580333 g_Saccharimonadaceae 0.675539927 g_Actibacterium 0.625235409 g_Kocuria 0.675849598 g_Geitlerinema 0.626007365 g_Caedimonas 0.676523109			gMethylobacterium	0.67347519
g_Methylococcus 0.624580333 g_Saccharimonadaceae 0.675539927 g_Actibacterium 0.625235409 g_Kocuria 0.675849598 g_Geitlerinema 0.626007265 0.676523109	-		gNitrosococcus	0.674110389
g_Actibacterium 0.625235409 g_Saccharimonadaceae 0.675339927 g_Geitlerinema 0.625698819 g_Caedimonas 0.676523109 g_Rouvialla 0.626007265 0.676523109	5		g_Yangia	0.674404767
g_Geitlerinema 0.625698819 g_Caedimonas 0.676523109 g_Rouviella 0.626007265 0.676523109 0.676523109			gSaccharimonadaceae	0.675539927
g_Cdedimonas 0.676523109	5		gKocuria	0.675849598
g_Rouxiella 0.626007265 g_Hydrogenophaga 0.676571338			gCaedimonas	0.676523109
	gRouxiella	0.626007265	gHydrogenophaga	0.676571338

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Genus	significance (p_)	Genus	significance (p_)
gNitrosospira	0.677085058	gMicrolunatus	0.719727932
glphinoe	0.67743896	gAnoxybacillus	0.720296124
gThermus	0.678346618	g_Halomonas	0.720394138
gHafnia	0.680759403	gGloeothece	0.720513781
gPaenibacillus	0.680841011	gBifidobacterium	0.725640562
gChroococcidiopsis	0.683381247	gPelomonas	0.725642398
gAbiotrophia	0.683491274	gMethylocella	0.730478193
gLiberibacter	0.68459596	gTreponema	0.732576869
gPlasticicumulans	0.684712401	gEmpedobacter	0.734072829
gLeptotrichia	0.685641621	gCorynebacteriaceae	0.734427125
gGranulicatella	0.686660342	gMethylobacterium-Methylorubrum	0.737515941
gPseudonocardia	0.689586889	gAhniella	0.737568165
gLelliottia	0.689982784	gConexibacter	0.738460865
gPlanifilum	0.691704481	g_Proteus	0.738733637
gRoseomonas	0.692183055	gHowardella	0.738854769
gPeptoniphilus	0.694726346	gTannerella	0.739919427
gRhodococcus	0.695091889	g_Candidatus	0.740859172
gLeclercia	0.695215016	gDolosigranulum	0.740992597
gHalothiobacillus	0.696253018	gPhreatobacter	0.742203572
gBlastococcus	0.699096897	gBrevibacterium	0.743097574
gEikenella	0.700678119	gSelenomonas	0.744125318
gRathayibacter	0.701650756	gLautropia	0.744205659
gAureimonas	0.70232019	gMicrococcus	0.744276267
gRikenellaceae_RC9_gut_group	0.703549376	g_Salinicoccus	0.744328224
gStenotrophomonas	0.704460385	gSporosarcina	0.745352143
gThioalkalivibrio	0.704638575	g_Peptococcus	0.7457046
gMorganella	0.706651744	gVulcaniibacterium	0.74618523
gMycoplasma	0.706680415	gSkermanella	0.746201266
gReyranella	0.70937626	gNocardioides	0.746863125
gLentimicrobium	0.709751063	g_Yaniella	0.747222752
gDialister	0.711919409	gAchromobacter	0.747380391
gFacklamia	0.713662509	gAlloiococcus	0.750005059
gCatonella	0.713996587	gAmphibacillus	0.75069953
gCampylobacter	0.714434706	gLentimonas	0.750914816
gCraurococcus-Caldovatus	0.715618446	gNocardiopsis	0.751192207
gLongilinea	0.716472138	gFilifactor	0.752887712
gBradyrhizobium	0.71672831	gGeorgfuchsia	0.758495644
gClostridia	0.71766549	gBulleidia	0.759573958
gBrochothrix	0.718174767	gEnhydrobacter	0.759803094
gArthrobacter	0.718632703	gFusobacterium	0.769515369
gSerratia	0.719038696	gMoraxella	0.799196985

Supplementary file 4. ONT data comparisons utilising the default recommended EMU database compared with SILVA on the same raw dataset. Relative abundance conclusions are highly comparable between the two database datasets.



Genus	ONT EMU (%)	ONT SILVA (%)	Difference (%)	Species	ONT EMU (%)	ONT SILVA (%)	Difference (%)
Staphylococcus	40.21	39.29	0.92	Staphylococcus Aureus	20.68	19.74	0.94
Streptococcus	13	12.85	0.15	Staphylococcus Epidermidis	15.53	15.53	0
Haemophilus	8.48	8.12	0.36	Streptococcus Pneumoniae	6.45	6.06	0.39
Corynebacterium	1.95	1.97	0.02	Streptococcus Dysgalactiae	2.96	2.86	0.1
Cutibacterium	3.76	3.61	0.15	Haemophilus Influenzae	7.51	7.1	0.41
Klebsiella	4.03	2.05	1.98	Cutibacterium Acnes	3.52	3.03	0.49
Enterobacter	2.52	4.18	1.66	Klebsiella Aerogenes	4.02	2.02	2
Parvimonas	1.96	1.74	0.22	Enterobacter Hormaechei	2.44	1.64	0.8
Anaerococcus	3.04	3.03	0.01	Parvimonas Micra	1.96	1.74	0.22
Peptoniphilus	1.3	1.27	0.03	Anaerococcus Nagyae	1.68	0	1.68
Other	80.24	78.12	2.12	Other Species	33.25	41.8	8.55

Relative Abundance - Genus (Database Comparison)

Relative Abundance - Species (Database Comparison)