Title: The upper respiratory tract microbiome of Australian Aboriginal and Torres Strait Islander children in ear and nose health and disease.

Running Title: Indigenous otitis media and the microbiome

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Abstract

Objective: To examine the nasal microbiota in relation to otitis status and nose health in Indigenous Australian children.

Methods: Children aged 2-7 years were recruited from two northern Australian (Queensland) communities. Clinical histories were obtained through parent interview and review of the medical record. Nasal cavity swabs were obtained, and the child's ears, nose and throat were examined. DNA was extracted and analysed by 16S rRNA amplicon next generation sequencing of the V3/V4 region in combination with previously generated culture data.

Results: 103 children were recruited (mean 4.7 years), 17 (16.8%) were 'healthy', i.e. normal examination and no history of otitis media (OM). Nasal microbiota differed significantly in relation to otitis status and nose health. Children with historical OM had higher relative abundance of *Moraxella* compared to healthy children, despite both having healthy ears at the time of swabbing. Children with healthy noses had higher relative abundance of *S. aureus* compared to those with rhinorrhoea. *Dolosigranulum* was correlated to *Corynebacterium* in healthy children. *Haemophilus* and *Streptococcus* correlated across phenotypes. *Ornithobacterium* was absent/low relative abundance in healthy children and clustered around otopathogens. It correlated with *Helcococcus* and *Dichelobacter*.

Conclusions: *Dolosigranulum* and *Corynebacterium* form a synergism that promotes URT/ear health in Indigenous Australian children. *Ornithobacterium* likely represents *Candidatus Ornithobacterium hominis* and in this population is correlated with a novel bacterium which appears to be related to poor upper respiratory tract/ear health.

Importance

Recurring and chronic infections of the ear (otitis media) are disproportionately prevalent in disadvantaged communities across the globe, and in particular, within Indigenous communities. Despite numerous intervention strategies, otitis media persists as a major health issue and is the leading cause of preventable hearing loss. In disadvantaged communities, this hearing loss is associated with negative educational and social development outcomes, and consequently, poorer employment prospects and increased contact with the justice system in adulthood. Thus, a better understanding of the microbial ecology is needed in order to identify new targets to treat, as well as prevent the infections. This study used a powerful combination of 16S rRNA sequencing and extended culturomics to show that *Dolosigranulum pigrum*, a bacterium previously identified as a candidate protective species, may require co-colonisation with *Corynebacterium pseudodiptheriticum* in order to prevent otitis media. Additionally, emerging and potentially novel pathogens and bacteria were identified.

Introduction

Otitis media (OM), an inflammation/infection of the middle ear, is a common paediatric condition[1]. However, in many indigenous populations globally there is a disproportionately high OM-associated burden, impacting negatively on schooling and employment outcomes[1, 2]. Previous microbiological studies relating to OM in indigenous populations have been largely limited to the main otopathogens (*Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*) using culture-dependent methods and seldom included healthy indigenous control children[3]. One study used 16S ribosomal RNA (rRNA) next

generation sequencing (NGS) to explore the middle ear effusion and nasopharyngeal/adenoid microbiota in relation to OM with effusion (OME) in 11 Aboriginal and/or Torres Strait Islander (referred to herein as Indigenous Australian) children[4], which confirmed the association of otopathogen-containing genera and OME.

We have previously used culturomics and species-specific quantitative PCR (qPCR) to explore the nasal microbiota in relation to ear health and OM in 103 Indigenous Australian children[5]. We found that children with historical or current OM/upper respiratory tract (URT) infection (URTI) had high otopathogen loads and higher detection of rhinovirus[5]. In contrast, *Corynebacterium pseudodiphtheriticum* and *Dolosigranulum pigrum* were associated with URT/ear health[5]. However, culture-based analyses can be insensitive to microbial population structure and fastidious or unculturable organisms, such as the recently described, *Candidatus Ornithobacterium hominis*[6, 7]. To address this limitation, 16S rRNA NGS, supplemented with the existing culturomics data, was used to investigate the broader bacterial microbiome and how it relates to ear and nose health and disease in Indigenous Australian children.

Materials and Methods

Additional detail of the materials and methods can be found in the supplementary material.

Population and sample collection

Indigenous Australian children aged 2–7 years old were recruited prospectively from one rural and one remote northern Queensland communities in Australia through October 2015–November 2017. Children whom received antibiotics within three weeks of sample collection

were excluded[5]. The study was approved by the Far North Queensland Human Research Ethics Committee (HREC/15/QCH/10-594).

A detailed description of the cohort, sampling and clinical data collection has been previously documented[5]. Briefly, demographic details and ear health history were collected for eligible children from parent interview and the child's medical record. Children underwent ear (otoscopy), nose and throat (ENT) examination. Ear status at time of swabbing was classified according to the most affected ear. Intra-nasal mucosal swabs (dry FLOQSwabs, Copan Diagnostics, USA) used for molecular analysis were collected in parallel with Rayon swab (Transystem^{TM} Minitip, Copan Diagnostics, USA) for culturomics[5]. All swabs were kept at 4°C from time of collection until arrival at the laboratory 24–48 hours later. Molecular swabs were then stored at -80°C.

DNA extraction and quality assurance

DNA was extracted via mechanical bead beating and tissue lysis, followed by automated MagNa Pure (Roche Diagnostics, Australia), as previously described[5]. Four clean negative control swabs were processed in parallel with the sample swabs. The quality of nasal sampling was assessed using a real-time PCR targeting the endogenous retrovirus-3 (ERV3) marker for human DNA[8]. Swabs that amplified with cycle thresholds ≤38 were considered to have adequate nasal epithelial cell content, and by extension, be of good collection quality. Swabs producing cycle thresholds >38 were excluded from further analysis.

16S Sequencing

All sample and negative control DNA extracts underwent 16S rRNA gene amplification using the 341F;806R primer set, followed by secondary indexing PCR. The equimolar library

pool was then sequenced on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 x 300 base pairs paired-end).

Sequence data processing

Primer sequences were removed from de-multiplexed reads using cutadapt (ver. 2.6). Using QIIME2 (ver. 2019.10.0), reads were filtered, dereplicated and chimeras removed by DADA2. Taxonomy was assigned to the resulting amplicon sequence variants (ASV) by aligning each (classify-consensus-blast) against the non-redundant SILVA database (release 138).

Data availability

Amplicon sequencing data has been deposited in NCBI's Short Read Archive under BioProject number PRJNA684919 (https://www.ncbi.nlm.nih.gov/sra/PRJNA684919) with accession numbers SRR13264782-SRR13264885.

Data analysis and statistics

Amplicon data analyses were performed in R (ver. 4.0.2). ASVs that were not bacterial, fungal or archaeal in origin, classified at below the phylum level, or that were classified as chloroplast or mitochondria, were discarded. Putative contaminants were identified using decontam (ver. 1.8.0) and microdecon (ver. 1.0.2) and removed. ASVs with a relative abundance <0.05% were also removed, with those samples with less than 4,000 reads remaining then discarded. Sample depth was limited to a maximum of 50,000 reads by using the rrarefy function in vegan (ver. 2.5-6).

Vegan was used to perform principal-component analysis (PCA), permutational multivariate analysis of variance (PERMANOVA) and analysis of multivariate homogeneity

(PERMDISP) on centred log-ratio (clr) transformed ASV counts collapsed to the genus level. Differentially abundant ASVs and genera were identified using DESeq2 (ver. 1.28.1). Alpha diversity metrics Chao1, Shannon, and Simpson were calculated using phyloseq (ver. 1.32.0) on samples rarefied to 10,000 reads. Significant differences in alpha diversity distributions were determined through either Mann-Whitney U tests or Kruskal-Wallis and Dunn's multiple comparisons tests, corrected for multiple testing using the Benjamini and Hochberg method. FastSpar (ver. 0.0.10) was used for correlation analysis of genera.

Culturomic analysis

Culture-based swabs were processed using an expanded agar protocol under aerobic and anaerobic conditions with Vitek MS MALDI-TOF (bioMérieux) isolate identification as previously described[5]. Agreement between culture and 16S sequencing was assessed using Cohen's Kappa.

Results

In total, 103 children were recruited; two children refused swabbing, resulting in 101 swabs for analysis. All swabs met quality assurance criteria on ERV3 testing. Raw sample 16S read depth ranged from 149–262,880 (median 119,693), with quality, contamination and non-specific filtering resulting in the remaining read depth ranging from 0–163,794 (median 66,929). Fourteen samples were subsequently excluded as they did not pass quality control. The agreement between culturomics and 16S sequencing was 59.2%, Cohen's kappa 0.08. The low level of agreement was predominately due to high sensitivity of detection by 16S sequencing, detecting on average of 14.3 (range 1–73) more genera per sample compared to culture.

Nasal Microbiota in relation to ear health

Only 17 children (16.8%) had no history of OM and normal ENT examinations at the time of swabbing (Never OM), 7 (6.9%) had a perforated TM, 18 (17.8%) had a middle ear effusion (Effusion), 4 (4.0%) had AOM, and 55 had a past history of OM, but normal TM at the time of swabbing (HxOM) (54.5%) (Table 1). Due to low numbers, AOM samples were excluded from further analyses. There was a significant difference in the nasal microbiota in relation to otitis status (PERMANOVA F = 2.101, $p = 0.0027$), although with dispersion differences (PERMDISP F = 3.341, $p = 0.0244$). Within children that had healthy TMs at the time of sampling, HxOM had higher mean abundance of *Moraxella* compared to Never OM (31.22% vs 20.22%, $p<0.05$) (Figure 1, Supplementary Table 1). The relative abundance of nine *Dolosigranulum* ASVs differed significantly in relation to otitis status; ASVs 588 and 2067 were more abundant in children with normal TMs, while ASVs including 1030, 1069 and 1528 were more abundant in children with OM (Supplementary Table 2) The relative abundance of *Dolosigranulum* was positively correlated with *Corynbacterium* in Never OM and both *Corynbacterium* and *Moraxella* in HxOM; there was no significant correlation between *Dolosigranulum* and the other main otopathogen-containing genera (Supplementary Figure 1). Children with Effusion had higher mean relative abundance of *Ornithobacterium* (34.1%) compared to Never OM (28.4%); although non-significant according to DESeq, was significant according to Dunn's test (adjusted $p = 0.018$, KrusW $p = 0.021$) (Supplementary Figure 2).

Network analyses showed taxa correlations largely differed according to otitis status, with the notable exception of *Streptococcus* and *Haemophilus*, which correlated across all groups. Never OM children had a more complex network of correlated genera, compared to HxOM, despite both groups having normal TMs at the time of swabbing (Figure 2). *Dolosigranulum*

196 positively correlated with different genera across all otitis phenotypes, with exception of
197 Effusion; to *Corynbacterium* in the Never OM group and to *Moraxella* and *Neisseriaceae* in
198 the HxOM and TM perforation groups, respectively (Figure 2). Our culturomic data
199 suggested the species representing the associated genera were *D. pigrum* and *C.*
200 *pseudodiphtheriticum*[5]. *Ornithobacterium* correlated with *Helcococcus*, *Dichelobacter*
201 (Figure 2). There were no significant differences in alpha diversity in relation to otitis status
202 (Supplementary Table 3).
203

204 ## Nasal microbiota in relation to nose health

205 The nasal microbiota was significantly related to nose health (PERMANOVA $p < 0.001$, F =
206 2.98, PERMDISP F = 2.753, $p = 0.068$). Compared to purulent rhinorrhoea, children with
207 healthy noses had significantly higher mean relative abundance of *Staphylococcus* (6.68% vs
208 0.004%) and *Neisseriacea*e (0.868% vs 0.096%)(all $p < 0.001$) (Figure 1, Supplementary
209 Table 2). ASV analysis showed *Staphylococcus aureus* is likely accounting for the
210 *Staphylococcus* detections. Similar to ear health, multiple *Dolosigranulum* ASVs were
211 detected across nose phenotypes (Supplementary Table 2). Network complexity and
212 correlation patterns between *Dolosigranulum* and other bacteria in relation to nose status
213 were similar to those seen within otitis status (Figure 3). *Staphylococcus* correlated
214 negatively with *Moraxella* in healthy noses, however *Ornithobacterium* was present in all
215 phenotypes and correlated to *Helcococcus* and *Dichelobacter* and *Cardiobacteriaceae*
216 (Figure 3). There were no significant differences in alpha diversity in relation to nose health
217 (Supplementary Table 3).
218

Nasal microbiota in relation to season, household occupancy and community

No relationship was found between nasal microbiota and season (PERMANOVA $p = 0.456$, F = 1.00; PERMDISP, $p = 0.192$, F = 1.619) or household occupancy (PERMANOVA $p = 0.748$, F = 0.791; PERMDISP $p = 0.844$, F = 0.181). There were no significant differences in relative abundance or alpha diversity for these variables (Figure 1, Supplementary Table 3). The nasal microbiota differed significantly in relation to community of residence (PERMANOVA $p < 0.001$, F = 3.71), although with dispersion differences (PERMDISP, F = 7.87, $p = 0.005$). No separation was observed between the two communities on PCA (Figure 4).

Discussion

We demonstrated the nasal microbiota of Indigenous Australian children was related to ear and nose health. Healthy children with no history of OM showed a relationship between *Dolosigranulum* and *Corynebacterium*. We detected *Ornithobacterium* in children with OM, suggesting a potential role as a novel otopathogen in this population.

In relation to otitis status, *Moraxella* had a higher relative abundance in children with a history of OM, compared to children with no history of OM, despite both groups having healthy ears at the time of swabbing. In children with healthy noses, there was a negative correlation between *Moraxella* and *Staphylococcus.* Moraxella are common nasal colonisers whose abundance can increase during acute respiratory infections, leading to prolonged periods of enrichment within the nasal microbiota[9, 10]. Thus, the observed increase of Moraxella in HxOM children may be a downstream persistent effect of past respiratory infections (e.g. OM) leading to a remodelled microbiome distinct from children who did not contract OM. *In vitro* studies demonstrate some *Staphylococcus* species can inhibit the

244 growth of *M. catarrhalis*[11] and may account for their negative correlation within healthy
245 noses in our cohort.
246
247 A combination of 16S NGS and culturomic data strongly suggested there exist correlations
248 between *C. pseudodiphtheriticum* and *D. pigrum* in healthy children with no rhinorrhoea and
249 no historical OM. In non-Indigenous infants, *Corynebacterium* and *Dolosigranulum* are well-
250 recognised as being associated with a stable nasopharyngeal microbiota, conferring URT and
251 ear health[10, 12-15]. *In vitro* studies of *Corynebacterium–Dolosigranulum* relationships
252 demonstrated complex interactions that were species-specific, which may be dependent on
253 use host resources[16]. However, for the inhibition of *S. pneumoniae* both *C.*
254 *pseudodiphtheriticum* and *D. pigrum* were required; neither species could inhibit the growth
255 of *S. pneumoniae* alone[16]. These *in vitro* findings corroborate our *in vivo* data and warrant
256 further investigation, particularly with the view towards prevention/control of otopathogen
257 colonisation in the nose and consequent ear health benefits.
258
259 *Dolosigranulum* was ubiquitous in the nasal microbiota of our population, however
260 examination at the level of ASV suggests this may be a heterogeneous group. The method of
261 ASV analysis has greater precision than operational taxonomic units (OTU), used in prior
262 URT microbiota research, and therefore may be more sensitive in detecting strain-specific
263 differences[17]. There is one known species within the *Dolosigranulum* genus, however our
264 findings suggest the presence of more than one strain or species, particularly given the 16S
265 V3/V4 region as well as the wider *D. pigrum* genome has been reported to be highly
266 conserved[16]. We did not see an inverse relationship with any of the main otopathogens,
267 which has previously been described[18], which may be due to this population having a high
268 baseline level of otopathogen colonisation[5]. There is growing interest in *Dolosigranulum*

due to its association with URT and ear health, thus the use of whole genome sequencing and ASV analysis will provide further understanding of the nuances of this nasal commensal.

Ornithobacterium was absent or at low relative abundance in the nasal microbiota of children with no history of OM. This is likely to represent *O. hominis*, a newly described species of *Ornithobacterium*, which resides in the nasopharynx and is the only known human species in that genus[7]. The role of *O. hominis* in relation to respiratory/ear disease is still undetermined, however it was originally found in Australian children and Thai refugee camp infants with high rates of respiratory disease[19, 20]. Our findings suggest that *Ornithobacterium* may be associated with poor ear health. Furthermore, the network correlations supported relationships between *Ornithobacterium*, *Helcococcus* and *Dichelobacter* which may influence clinical outcomes. Intriguingly, the ASV and correlation network data suggests that there may be novel bacterial species within the nasal microbial ecosystem in genera which currently do not have human representatives (e.g. *Dichelobacter*, *Gracilibacteria*) or only have one species (*Dolosigranulum*). Along with *Ornithobacterium*, these genera warrant further investigations, particularly given their recurring relationships with genera associated with health and disease.

Although this is the largest NGS-based OM study in any indigenous population to date, limitations exist. Recruitment and sample collection in remote Australian communities is resource and time intensive, which impacted on sample size. Furthermore, recruitment of healthy children with no history of OM was challenging, despite our community-based sampling, reflecting the high burden of disease in remote Indigenous Australian communities[21]. We found that healthy children, with no history of OM, appeared to have differences in their nasal microbiota dependent on their community of residence, however the

small sample size limited further sub-group analysis. A well-recognised limitation of 16S rRNA sequencing is its poor resolution at the species-level. However, a combination of ASV and culturomics data partially overcame this limitation and provided novel species-level insights into the nasal microbial ecology in nose/ear health and disease. It is hoped that moving forward methods such as metagenomic shotgun sequencing can be optimised for the URT to provide a more comprehensive assessment of the URT microbiome in relation to health and disease.

In conclusion, our investigation of the nasal microbiota of Indigenous Australian children demonstrated that there is a potential synergism between *D. pigrum* and *C. pseudodiphtheriticum* that is associated with ear and nose health. Our ASV-level analysis suggested that *Dolosigranulum* is a heterogeneous genus. Finally, we have detected the likely presence of *O. hominis* and suggestions of other novel species within the nasal microbiota that associate with poor URT/ ear health.

Author's contributions:

ACol, AW, EGH, JA, MB, ACer, SB designed the study. ACol and AW collected samples. ACol, KC, SB conducted laboratory work. JZ and SB did the bioinformatics analysis. ACol, SB, JZ wrote the manuscript. All authors revised and approved the final manuscript.

Transparency declaration:

The authors declare that they have no conflicts of interest.

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411

412 *Table 1: Demographic and clinical details of participants*

Characteristic[a]	Remote Community (n = 59)	Rural Community (n = 44)	Difference between remote and rural (*p*-value)
Female gender	33 (47.7)	21 (47.7)	0.41
Age in months, mean (SD)	57.0 (13.4)	55.4 (18.6)	0.61
Educational attendance			<0.001
School	5 (8.5)	14 (31.8)	
Pre-school	43 (72.9)	11 (25.0)	
Daycare	5 (8.5)	18 (40.9)	
Home	6 (10.2)	1 (2.3)	
No. people in the home, mean (SD)	5.8 (2.2)	4.9 (1.6)	0.04
Pneumococcal vaccination[b]	56 (94.9)	37 (84.1)	0.04
No. children with a history of OM	51 (86.4)	25 (56.8)	<0.001
Never OM	5 (8.5)	12 (27.3)	0.01
Historical type of OM			0.07
AOM	25 (42.4)	18 (40.9)	
AOM with perforation	6 (10.2)	2 (8.0)	
OME	3 (5.1)	1 (4.0)	
CSOM	14 (23.7)	1 (4.0)	
Unknown	3 (5.1)	3 (12.0)	
Otoscopy at sampling			0.24
Bilateral normal TM	26 (44.1)	29 (65.9)	
Effusion	13 (22.3)	5 (11.4)	
AOM	2 (3.4)	2 (4.5)	
Perforation	5 (8.5)	2 (4.5)	

Unable to visualize TM	13 (22.0)	6 (13.6)	
Nasal discharge at sampling			0.01
Nil	30 (50.8)	35 (79.5)	
Serous	10 (16.9)	3 (6.8)	
Purulent	19 (32.2)	6 (13.6)	
Oropharynx at sampling			0.73
Tonsillitis	0	0	
Pharyngitis	2 (3.4)	1 (2.3)	
Season of collection			0.01
Winter	7 (11.9)	0	
Spring	29 (49.2)	16 (36.4)	
Summer	0	0	
Autumn	23 (38.9)	28 (63.6)	

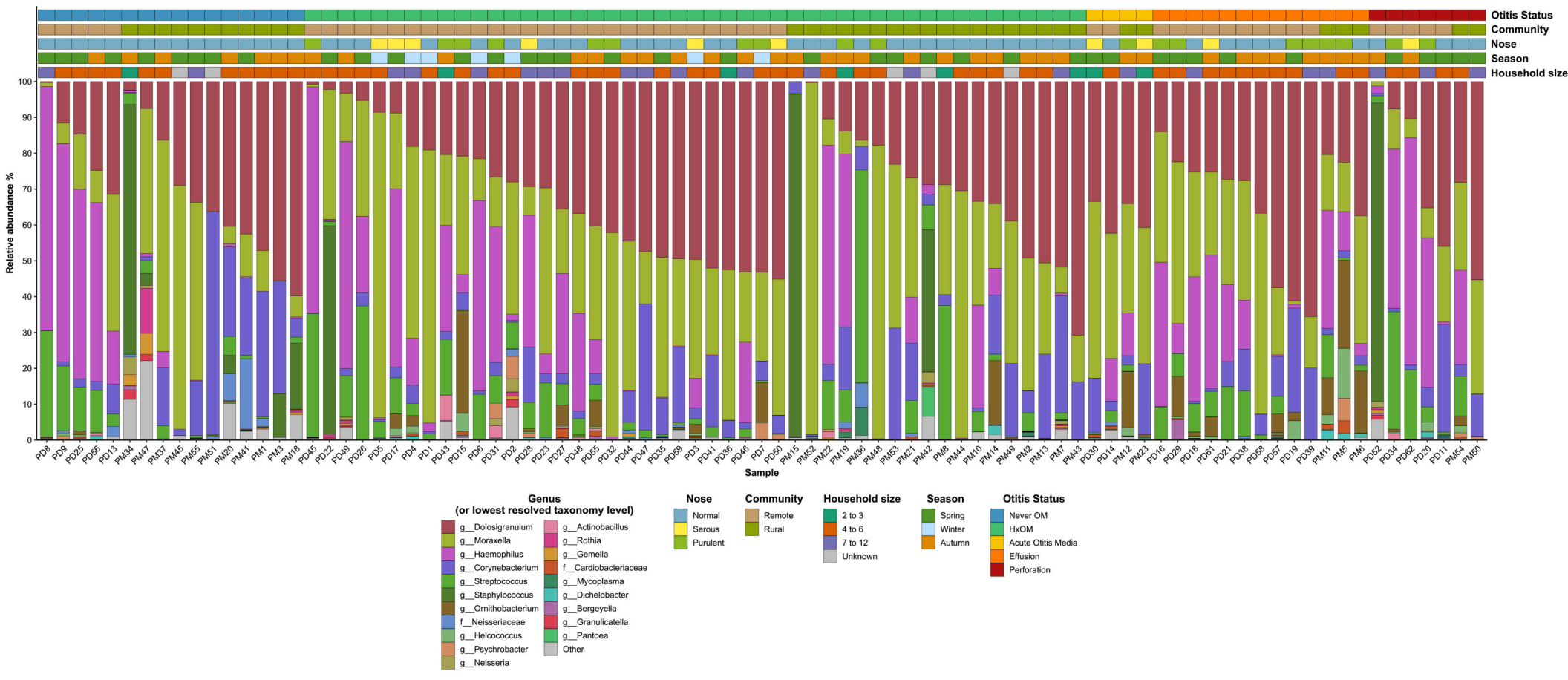
Note: [a] The data indicate number (percentage), aside from Age which represent mean (standard deviation (SD)); [b] as per the Australian Vaccination Schedule[22]; AOM, acute otitis media; CSOM, chronic suppurative otitis media; OM, otitis media; OME, otitis media with effusion; TM, tympanic membrane.

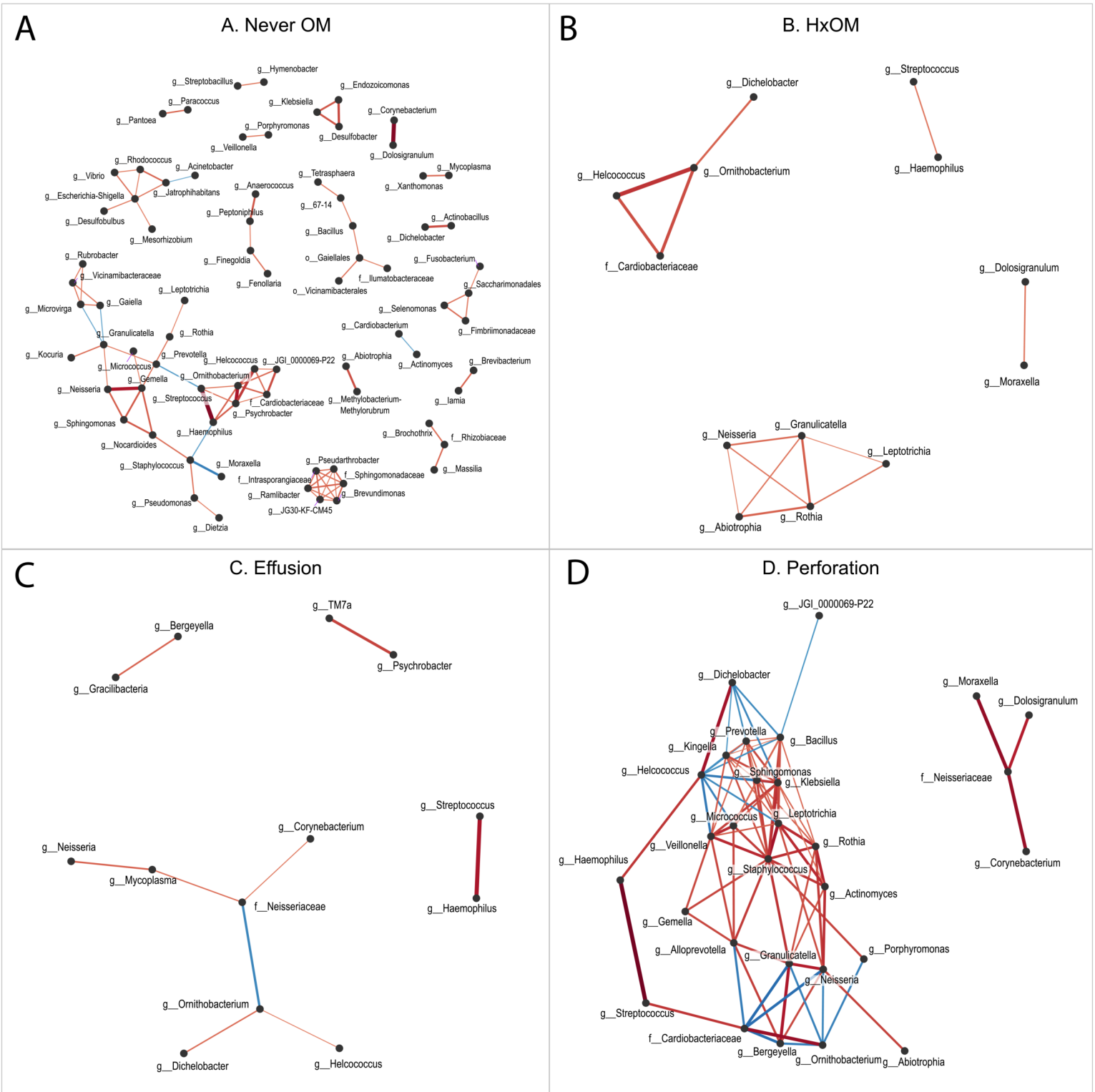
Figure 1: Mean relative microbial abundances of the 20 most abundant genera (or lowest resolved taxonomy level) across all samples illustrating differences between otitis Status, community of residence and other key variables. Microbes with lower abundances have been combined in the 'Other' (in grey). To improve interpretability, samples have been ordered by Otitis status, Community and Dolosigranulum abundance.
Note: OM = otitis media, HxOM = History of OM, but health tympanic membrane at time of collection.

Figure 2: Network correlation analysis showing differences in genera relationships in context of otitis status: A) Never OM; B): HxOM; C) Middle ear effusion; D) TM perforation.
Note: OM = otitis media, HxOM = History of OM, but health tympanic membrane at time of collection.

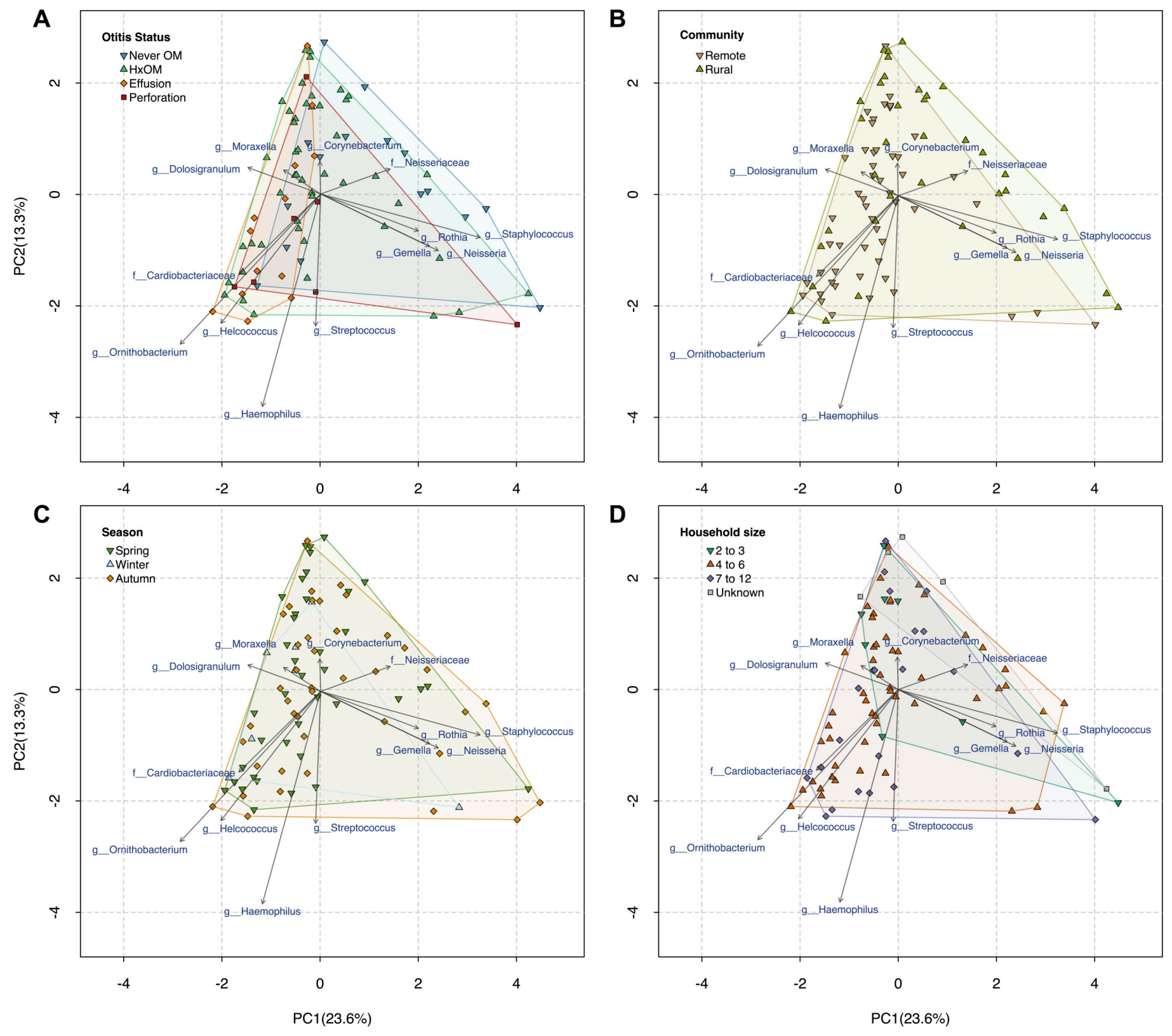
Figure 3: Correlation network analysis of genera in relation to nose health showing differential *Dolosigranulum* relationships.

Figure 4: Genus-level Principal Component Analysis showing no separation in relation to A) otitis status; B) community of residence; C) season of swab collection; D) number of people residing within the household.
Note: OM = otitis media, HxOM = History of OM, but healthy tympanic membrane at time of collection.


Otitis Status
Community
Nose
Season
Household size
Relative abundance %
100
90
80
70
60
50
40
30
20
10
0
PD8 PD9 PD25 PD56 PD13 PM34 PM47 PM37 PM45 PM55 PM51 PM20 PM41 PM1 PM3 PM18 PD45 PD22 PD49 PD26 PD5 PD17 PD4 PD1 PD43 PD15 PD6 PD31 PD2 PD28 PD23 PD27 PD48 PD55 PD32 PD44 PD47 PD35 PD59 PD3 PD41 PD36 PD46 PD7 PD50 PM15 PM52 PM22 PM19 PM36 PM48 PM53 PM21 PM42 PM8 PM44 PM10 PM14 PM49 PM2 PM13 PM7 PM43 PD30 PD14 PM12 PD23 PD16 PD29 PD18 PD61 PD21 PD38 PD58 PD57 PD19 PD39 PM11 PM5 PM6 PD52 PD34 PD62 PD20 PD11 PM54 PM50
Sample
Genus
(or lowest resolved taxonomy level)
g__Dolosigranulum
g__Moraxella
g__Haemophilus
g__Corynebacterium
g__Streptococcus
g__Staphylococcus
g__Ornithobacterium
f__Neisseriaceae
g__Helcococcus
g__Psychrobacter
g__Neisseria
g__Actinobacillus
g__Rothia
g__Gemella
f__Cardiobacteriaceae
g__Mycoplasma
g__Dichelobacter
g__Bergeyella
g__Granulicatella
g__Pantoea
Other
Nose
Normal
Serous
Purulent
Community
Remote
Rural
Household size
2 to 3
4 to 6
7 to 12
Unknown
Season
Spring
Winter
Autumn
Otitis Status
Never OM
HxOM
Acute Otitis Media
Effusion
Perforation


A
A. Never OM
g__Hymenobacter
g__Streptobacillus
g__Endozoicomonas
g__Paracoccus
g__Klebsiella
g__Corynebacterium
g__Pantoea
g__Porphyromonas
g__Desulfobacter
g__Veillonella
g__Dolosigranulum
g__Rhodococcus
g__Acinetobacter
g__Vibrio
g__Tetrasphaera
g__Mycoplasma
g__Escherichia-Shigella
g__Jatrophihabitans
g__Anaerococcus
g__Xanthomonas
g__67-14
g__Desulfobulbus
g__Peptoniphilus
g__Actinobacillus
g__Bacillus
g__Mesorhizobium
g__Dichelobacter
g__Rubrobacter
g__Finegoldia
o__Gaiellales
g__Fusobacterium
g__Vicinamibacteraceae
g__Fenollaria
f__Ilumatobacteraceae
o__Vicinamibacterales
g__Leptotrichia
g__Saccharimonadales
g__Microvirga
g__Gaiella
g__Selenomonas
g__Granulicatella
g__Rothia
g__Fimbriimonadaceae
g__Cardiobacterium
g__Kocuria
g__Prevotella
g__Abiotrophia
g__Actinomyces
g__Brevibacterium
g__Micrococcus
g__Helcococcus
g__JGI_0000069-P22
g__Gemella
g__Ornithobacterium
g__Neisseria
g__Streptococcus
f__Cardiobacteriaceae
g__Methylobacterium-Methylorubrum
g__Iamia
g__Psychrobacter
g__Sphingomonas
g__Haemophilus
g__Brochothrix
f__Rhizobiaceae
g__Nocardioides
g__Staphylococcus
g__Moraxella
g__Pseudarthrobacter
g__Massilia
f__Intrasporangiaceae
f__Sphingomonadaceae
g__Ramlibacter
g__Brevundimonas
g__Pseudomonas
g__JG30-KF-CM45
g__Dietzia
B
B. HxOM
g__Dichelobacter
g__Streptococcus
g__Helcococcus
g__Ornithobacterium
g__Haemophilus
f__Cardiobacteriaceae
g__Dolosigranulum
g__Moraxella
g__Granulicatella
g__Neisseria
g__Leptotrichia
g__Abiotrophia
g__Rothia
C
C. Effusion
g__TM7a
g__Bergeyella
g__Psychrobacter
g__Gracilibacteria
g__Streptococcus
g__Corynebacterium
g__Neisseria
g__Mycoplasma
f__Neisseriaceae
g__Haemophilus
g__Ornithobacterium
g__Dichelobacter
g__Helcococcus
D
D. Perforation
g__JGI_0000069-P22
g__Dichelobacter
g__Moraxella
g__Dolosigranulum
g__Prevotella
g__Bacillus
g__Kingella
g__Helcococcus
g__Sphingomonas
g__Klebsiella
f__Neisseriaceae
g__Micrococcus
g__Leptotrichia
g__Veillonella
g__Rothia
g__Haemophilus
g__Staphylococcus
g__Actinomyces
g__Corynebacterium
g__Gemella
g__Alloprevotella
g__Granulicatella
g__Porphyromonas
g__Neisseria
g__Streptococcus
f__Cardiobacteriaceae
g__Bergeyella
g__Ornithobacterium
g__Abiotrophia
Correlation
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0


A. Normal nose
g__Helcococcus
g__Dichelobacter
g__Ornithobacterium
g__Streptococcus
g__Haemophilus
g__Corynebacterium
g__Dolosigranulum
g__Staphylococcus
g__Moraxella
g__Leptotrichia
g__Actinomyces
g__Rothia
g__Gemella
g__Neisseria
g__Abiotrophia
g__Granulicatella
B. Serrous rhinorrhea
g__Mycoplasma
g__Corynebacterium
g__Streptococcus
g__Haemophilus
g__Helcococcus
g__Ornithobacterium
C. Purulent rhinorrhea
g__Haemophilus
g__Streptococcus
g__Helicobacter
g__Alloprevotella
g__Streptobacillus
g__Dolosigranulum
g__Moraxella
g__Dichelobacter
g__Helcococcus
f__Cardiobacteriaceae
g__Ornithobacterium
g__Mycoplasma
g__Klebsiella
g__Rothia
g__Abiotrophia
Correlation
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0


A
Otitis Status
Never OM
HxOM
Effusion
Perforation
B
Community
Remote
Rural
C
Season
Spring
Winter
Autumn
D
Household size
2 to 3
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g__Staphylococcus
g__Gemella
g__Neisseria
f__Cardiobacteriaceae
g__Helcococcus
g__Streptococcus
g__Ornithobacterium
g__Haemophilus
PC1(23.6%)
PC2(13.3%)