

**Table S1. Primers Used for Viral Screening**

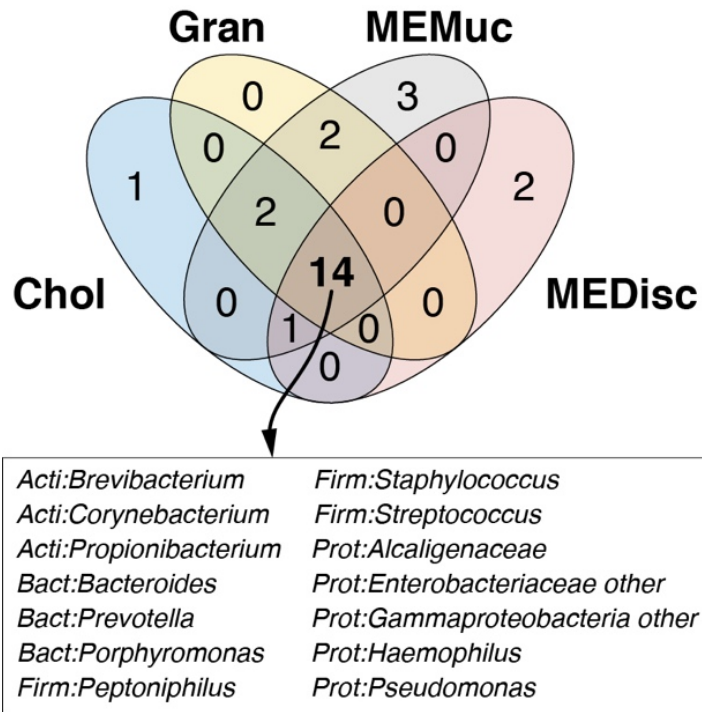
<i>Virus</i>	<i>Abbr.</i>	<i>Gene</i>	<i>Forward Primer</i>	<i>Reverse Primer</i>
HPV-E6	HPV	Oncoprotein E6	TCAGGACCCACAGGAGCG	CCTCACGTCGCAGTAACTGTTG
HPV-E7	HPV	Early protein E7	CCGGACAGAGCCCATTACAA	CGAATGTCTACGTGTGTGCTTTG
HPV-GP5/6	HPV	Late major capsid L1	GAAAAATAAACTGTAAATCATATT	TTTGTTACTGTGGTAGATACTAC
Adenovirus	HAdV	Hexon	GCCACGGTGGGGTTTCTAAACTT	GCCCCAGTGGTCTTACATGCACAT
Human Bocavirus NS-1	HBoV	Non-structural protein 1	TGCAGACAACGCYTAGTTGTTT	CTGTCCCGCCCAAGATACA
Rhinovirus	RV	Genome/5'UTR	TGGACAAGGTGCGAAGAG	CAAAGTAGTCGGTCCCATCC
Influenza B	InfB	Segment 4 hemagglutinin	AAATACGGTGGATTAATAAAAAGCAA	CCAGCAATAGCTCCGAAGAAA
Influenza A	InfA	Matrix proteins 1 & 2	GACCRATCCTGTACCTCTGAC	AGGGCATTYTGACAAAKCGTCTA
Human Metapneumovirus	hMPV	Nucleocapsid	CATATAAGCATGCTATATTAAGAGTCTCA	CCTATTTCTGCAGCATATTTGTAATCAG
Respiratory Syncytial Virus	RSV	cRNA/genome	GCTCTTAGCAAAGTCAAGTTRAATGATACA	GTTTTTGCACATCATAATTRGGAGT
Enterovirus	EV	Polyprotein	GGCCCCTGAATGCGGCTAATCC	GCGATTGTCACCATWAGCAGYCA
Human Coronavirus-229E	hCoV	Nucleoprotein	CAGTCAAATGGGCTGATGCA	AAAGGGCTATAAAGAGAATAAGGTATTCT
hCoV-OC43	hCoV	Nucleocapsid	CGATGAGGCTATTCCGACTAGGT	CCTTCCTGAGCCTTCAATATAGTAACC
hCoV-NL63	hCoV	Nucleocapsid	AGGACCTTAAATTCAGACAACGTTCT	GATTACGTTTTCGATTACCAAGACT
Beta-actin-2 (control)	β-actin	Exon 4	TCACCCACACTGTGCCCATCTACGA	CAGCGGAACCGCTCATTGCCAATG

**Table S2. Most Prevalent Cholesteatoma-associated Bacteria (showing all taxa found in >50% of Cholesteatomas)**

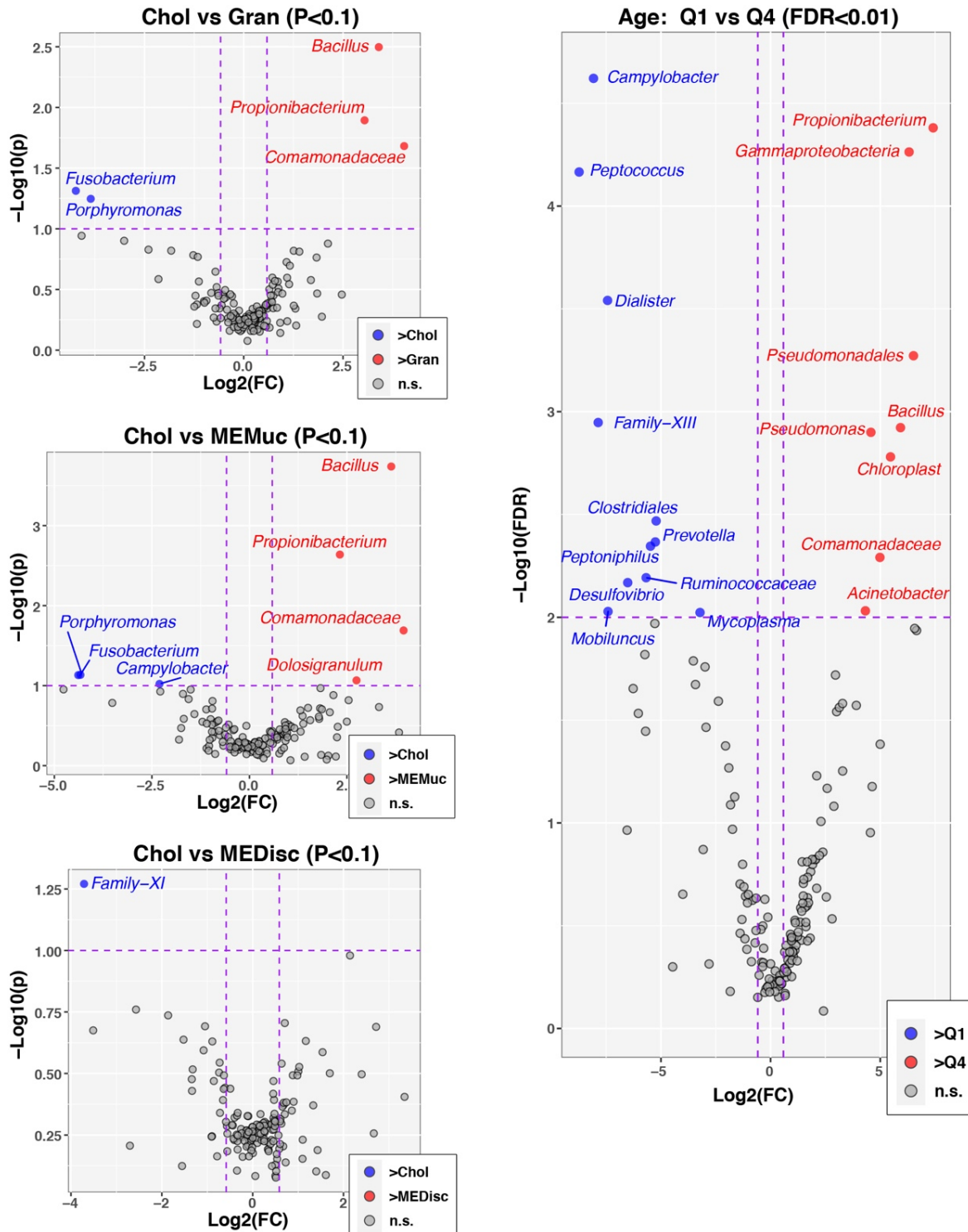
Taxon	Prevalence				Mean Abundance (s.d.)				Phylum
	CHOL N=25	GRAN N=34	MEMUC N=17	MEDISC N=27	CHOL	GRAN	MEMUC	MEDISC	
<i>Bact:Porphyromonas</i>	100	91	82	89	8.32 (12.05)	5.23 (10.00)	6.34 (10.36)	7.12 (13.67)	Bacteroidetes
<i>Firm:Streptococcus</i>	96	94	100	93	0.20 (0.67)	0.95 (1.61)	2.00 (4.18)	0.47 (1.00)	Firmicutes
<i>Fuso:Fusobacterium</i>	92	71	76	89	2.04 (3.97)	0.68 (1.35)	1.17 (2.45)	2.04 (5.09)	Fusobacteria
<i>Firm:Peptoniphilus</i>	92	91	88	96	7.27 (9.76)	5.84 (10.57)	5.54 (9.15)	3.05 (4.81)	Firmicutes
<i>Prot:Gammaproteobacteria</i>	88	91	88	96	0.60 (1.98)	6.96 (11.92)	5.69 (6.74)	4.02 (10.10)	Proteobacteria
<i>Prot:Pseudomonas</i>	88	94	100	81	2.30 (6.04)	2.72 (4.44)	0.84 (1.07)	1.11 (2.42)	Proteobacteria
<i>Prot:Haemophilus</i>	84	85	88	96	0.03 (0.08)	0.29 (0.61)	0.30 (0.67)	0.07 (0.20)	Proteobacteria
<i>Firm:Staphylococcus</i>	84	88	76	93	7.95 (22.15)	6.20 (17.55)	7.22 (17.28)	5.11 (13.57)	Firmicutes
<i>Bact:Prevotella</i>	84	76	76	81	0.98 (2.22)	1.13 (2.74)	0.97 (1.52)	2.13 (5.69)	Bacteroidetes
<i>Acti:Propionibacterium</i>	84	91	100	89	0.34 (1.04)	6.19 (8.94)	5.10 (5.03)	4.68 (12.26)	Actinobacteria
<i>Acti:Corynebacterium</i>	80	94	88	100	11.53 (26.79)	5.63 (13.77)	2.08 (2.30)	7.87 (16.28)	Actinobacteria
<i>Prot:Alcaligenaceae</i>	80	85	88	78	4.67 (11.75)	1.56 (3.74)	2.00 (4.94)	2.71 (6.89)	Proteobacteria
<i>Acti:Brevibacterium</i>	80	76	76	78	3.68 (6.81)	1.67 (4.59)	2.23 (4.34)	5.62 (12.74)	Actinobacteria
<i>Firm:Parvimonas</i>	80	82	82	70	3.58 (7.88)	2.46 (6.93)	1.19 (2.82)	3.24 (6.42)	Firmicutes
<i>Bact:Bacteroides</i>	80	79	88	78	2.23 (3.77)	2.31 (3.81)	3.17 (4.09)	3.25 (7.23)	Bacteroidetes
<i>Prot:Enterobacteriaceae</i>	80	85	88	89	3.28 (8.87)	2.11 (7.29)	5.29 (15.19)	4.06 (10.86)	Proteobacteria
<i>Firm:Anaerococcus</i>	80	62	65	74	1.04 (3.00)	0.67 (1.65)	2.27 (6.20)	0.48 (1.42)	Firmicutes
<i>Prot:Pseudomonadales</i>	76	79	82	74	7.16 (21.60)	8.96 (19.36)	3.67 (12.16)	5.25 (17.19)	Proteobacteria
<i>Prot:Moraxella</i>	72	59	65	85	0.01 (0.02)	0.17 (0.78)	0.37 (1.01)	0.12 (0.53)	Proteobacteria
<i>Acti:Actinomyces</i>	72	76	82	70	0.40 (1.40)	0.33 (0.55)	0.30 (0.47)	0.40 (0.84)	Actinobacteria
<i>Firm:Bacillus</i>	72	91	100	63	0.10 (0.33)	1.60 (2.56)	1.97 (3.01)	0.83 (2.15)	Firmicutes
<i>Prot:Proteus</i>	72	56	65	78	1.48 (3.42)	1.25 (3.23)	1.97 (4.40)	2.84 (7.27)	Proteobacteria
<i>Prot:Escherichia</i>	68	71	88	67	0.19 (0.48)	0.36 (0.69)	0.86 (1.17)	0.80 (1.90)	Proteobacteria
<i>Firm:Finegoldia</i>	68	68	71	70	1.64 (4.77)	1.44 (4.29)	1.90 (3.67)	1.17 (2.78)	Firmicutes
<i>Prot:Achromobacter</i>	64	62	59	67	3.55 (13.01)	2.75 (11.46)	0.29 (0.43)	1.45 (6.44)	Proteobacteria
<i>Prot:Campylobacter</i>	64	59	65	70	1.19 (2.16)	1.35 (4.23)	0.37 (0.92)	0.14 (0.34)	Proteobacteria
<i>Firm:Family-XI-Incertae-Sedis</i>	64	47	53	37	0.37 (0.73)	0.37 (0.92)	0.14 (0.21)	0.10 (0.28)	Firmicutes
<i>Prot:Acinetobacter</i>	60	65	76	63	0.28 (1.33)	0.59 (1.43)	2.46 (9.05)	0.23 (0.50)	Proteobacteria
<i>Firm:Family-XIII-Incertae-Sedis</i>	60	53	59	59	1.25 (3.25)	0.58 (1.40)	0.41 (0.80)	0.24 (0.41)	Firmicutes
<i>Proteobacteria</i>	60	62	59	63	0.01 (0.02)	0.06 (0.13)	0.15 (0.50)	0.03 (0.08)	Proteobacteria
<i>Spir:Treponema</i>	56	41	47	59	1.35 (2.81)	1.50 (4.04)	2.21 (4.25)	1.23 (3.24)	Spirochaetae
<i>Acti:Corynebacteriaceae</i>	52	68	71	59	0.19 (0.82)	0.38 (0.81)	0.22 (0.28)	0.18 (0.37)	Actinobacteria
<i>Prot:Alcaligenes</i>	52	32	29	59	0.41 (1.41)	1.98 (10.60)	0.08 (0.25)	3.24 (11.10)	Proteobacteria
<i>Acti:Mobiluncus</i>	52	44	71	48	2.12 (4.84)	2.27 (6.81)	0.88 (1.97)	0.31 (0.66)	Actinobacteria

**Table S3. PERMANOVA Results Stratified by Sample-type**

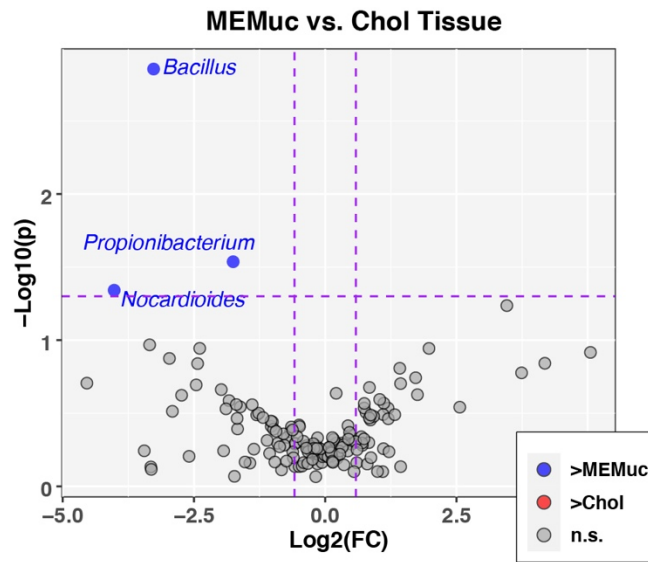
<b>A. Univariable</b>	<b>PERMANOVA P-Values</b>			
<b>Variable</b>	<b>Chol</b>	<b>Gran</b>	<b>MEDisc</b>	<b>MEMuc</b>
Age	0.19	<b>0.004</b>	0.34	0.20
Cholesteatoma Dx	na	<b>0.0005</b>	<b>0.002</b>	0.37
Quinolone	0.15	<b>0.04</b>	0.41	<b>0.008</b>
<b>B. Multivariable</b>	<b>PERMANOVA p-Values</b>			
<b>Variable</b>	<b>Chol</b>	<b>Gran</b>	<b>MEDisc</b>	<b>MEMuc</b>
Age	0.28	0.23	0.70	0.56
Cholesteatoma Dx	na	<b>0.03</b>	<b>0.002</b>	0.31
Quinolone	0.13	0.055	0.24	<b>0.02</b>



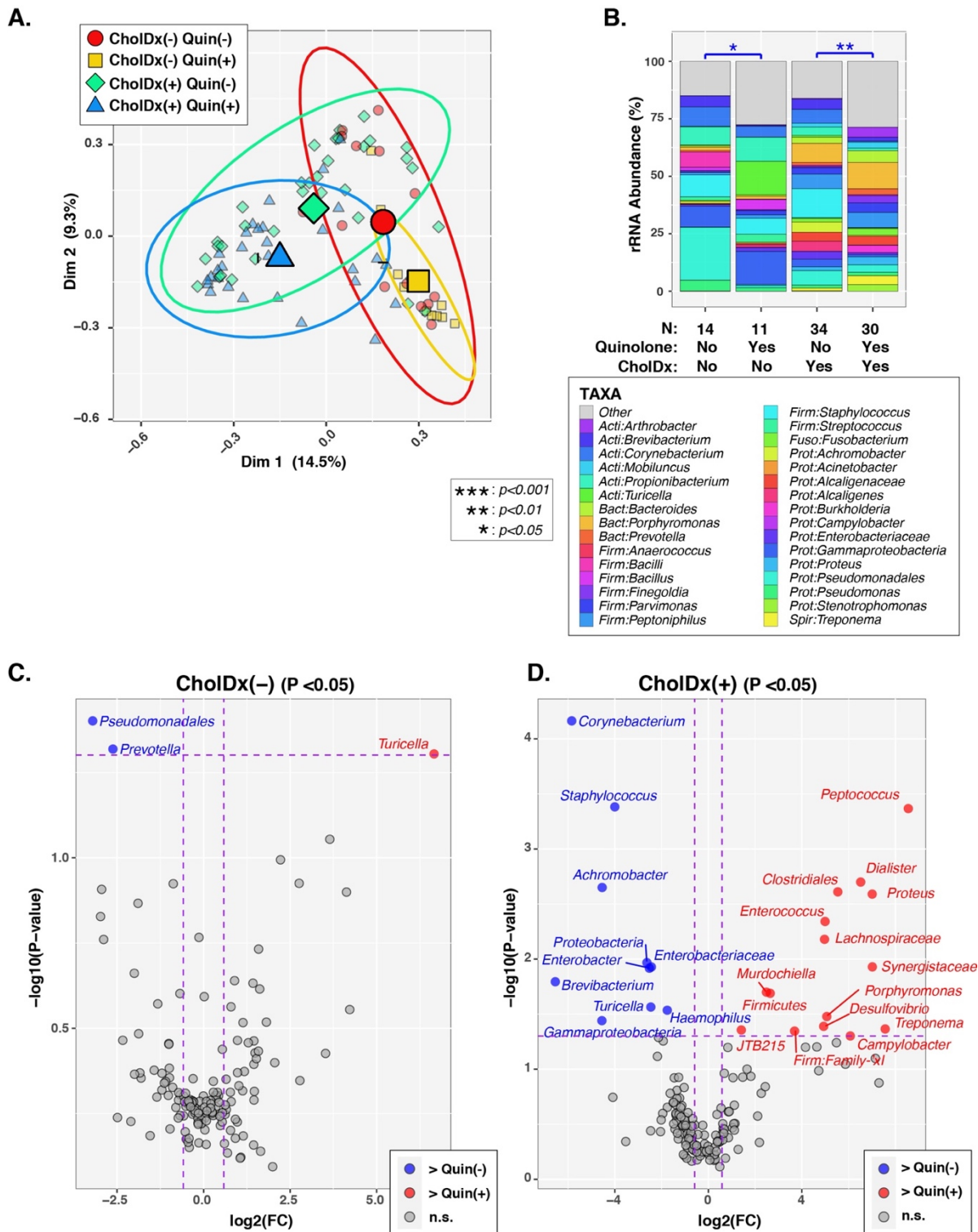
**Figure S1. Overlap in highly prevalent taxa.** Venn diagram shows numbers of taxa detected in  $\geq 75\%$  of patients for each sample-type. The 14 taxa identified in all four sample-types are listed below the diagram. Taxa names are pre-pended with phylum identifiers: Acti = Actinobacterium; Bact = Bacteroidetes; Firm = Firmicutes; Prot = Proteobacteria.



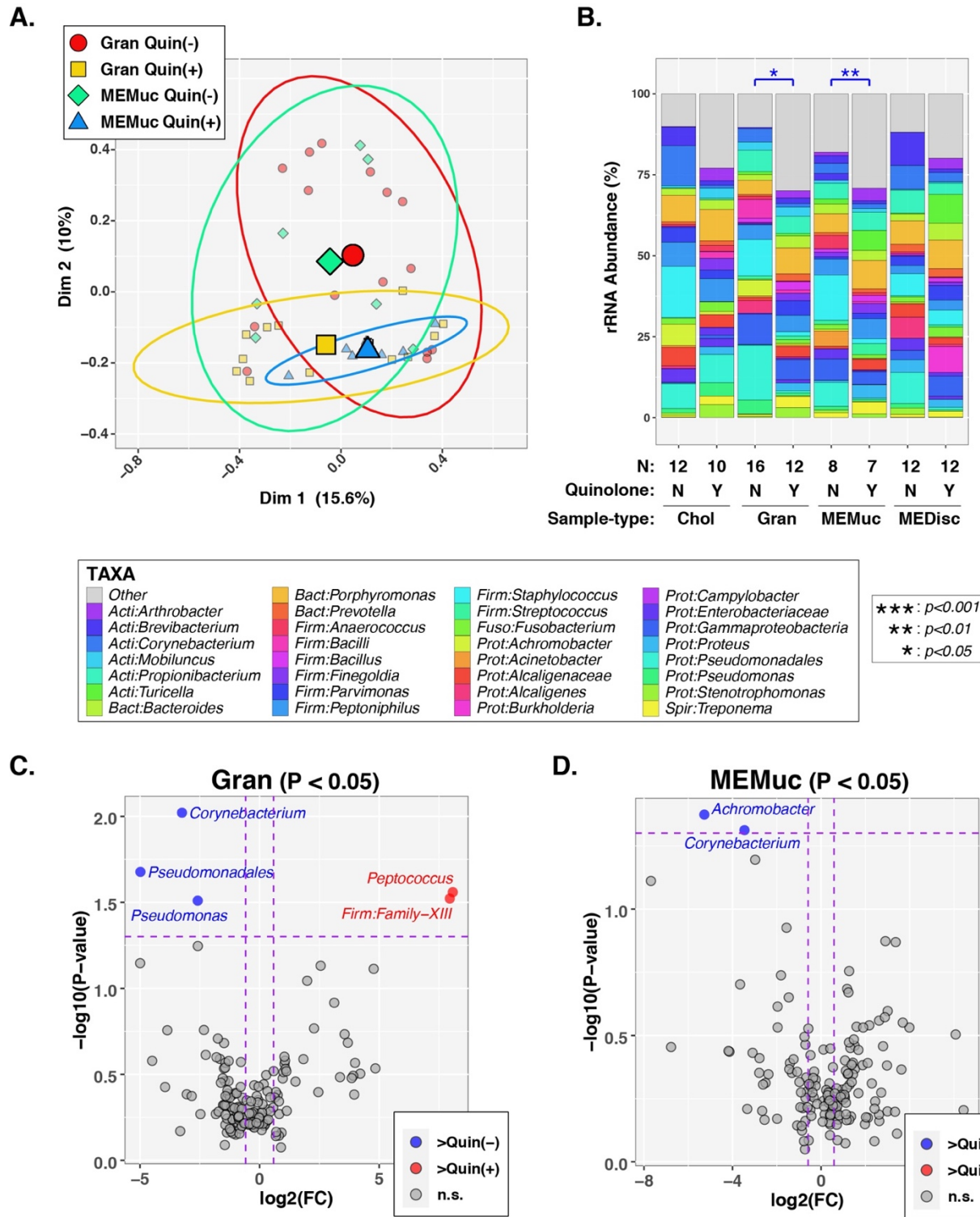
**Figure S2. Individual taxa differing by sample-type or age.** Between-group differences in the relative abundance of individual bacterial taxa were identified using the ANOVA-like differential expression (ALDEx2) test, which considers the compositional nature of microbiota datasets. *Vertical dashed lines* indicate fold-change cutoffs  $\geq 1.5$ . *Horizontal dashed lines* show p-value cutoffs for comparisons of sample-types (Left panels; nominal  $p \leq 0.1$ ) or age quartiles (Right panel; FDR-corrected  $p \leq 0.01$ ). **For sample-type comparisons**, blue circles in the upper left quadrants denote taxa enriched in Chol samples while red circles in the upper right quadrants denote taxa enriched in the other comparison group. **For comparison of age Q1 vs Q4**, blue circles in the upper left quadrants denote taxa enriched in Q1 samples while red circles in the upper right quadrants denote taxa enriched in Q4 samples. n.s.=not significant



**Figure S3. Individual taxa differing between Chol tissues and other ME sample-types among cholesteatoma patients.** Between-group differences in the relative abundance of individual bacterial taxa were identified using the ANOVA-like differential expression (ALDEx2) test, which considers the compositional nature of microbiota datasets. *Vertical dashed lines* indicate fold-change cutoffs  $\geq 1.5$ . *Horizontal dashed lines* show p-value cutoffs for comparisons (nominal  $p \leq 0.05$ ). *Blue circles in the upper left quadrant* denote taxa enriched in MEMuc relative to Chol while *red circles in the upper right quadrants* denote taxa enriched in Chol relative to MEMuc. No significant taxa were identified in comparing Chol to MEDisc or Gran.



**Figure S4. Effects of quinolone use on ME microbiota in subjects with and without cholesteatoma diagnosis. Panel A.** Principal coordinates (PC) plots coded by cholesteatoma diagnosis [CholDx(+) vs. CholDx(-)] and quinolone use [Quin(+) vs. Quin(-)]. *Smaller symbols* designate individual subjects while *larger symbols* represent group means along both PC axes. Ellipses designate 90% confidence level for a multivariate t-distribution. **Panel B.** Barcharts summarizing the mean relative abundances of predominant taxa ( $>2\%$ RA) in each group; rarer taxa are grouped into the “Other” category. Results of PERMANOVA tests are indicated above barcharts; tests were conducted only for 1) CholDx(-)/Quin(-) vs. CholDx(-)/Quin(+) and 2) CholDx(+)/Quin(-) vs. CholDx(+)/Quin(+) subjects. *Blue lines/symbols* indicate p-values for pairwise comparisons. **Panel C.** Individual taxa differing between CholDx(-)/Quin(-) and CholDx(-)/Quin(+) subjects, assessed by Aldex2 and adjusting for age and sample-type. **Panel D.** Individual taxa differing between CholDx(-)/Quin(-) and CholDx(+)/Quin(+) subjects, assessed by Aldex2 and adjusting for age and sample-type. *Vertical dashed lines* indicate fold-change cutoffs  $\geq 1.5$ . *Horizontal dashed lines* show p-value cutoffs for comparisons (nominal  $p \leq 0.05$ ). *Blue circles in the upper left quadrants* denote taxa enriched in Quin(-) subjects while *red circles in the upper right quadrants* denote taxa enriched in Quin(+) subjects.



**Figure S5. Effects of quinolone use on middle ear microbiota in subjects, stratified by sample-type.** *Panel A.* Principal coordinates (PC) plots coded by sample-type (Gran vs. MEMuc) and quinolone use [Quin(+) vs. Quin(-)]. *Smaller symbols* designate individual subjects while *larger symbols* represent group means along both PC axes. *Ellipses* designate 90% confidence level for a multivariate t-distribution. *Panel B.* Bar charts summarizing the mean relative abundances of predominant taxa (>2%RA) in each group; rarer taxa are grouped into the “Other” category. Results of PERMANOVA tests are indicated above bar charts; tests were conducted only between Quin(+) and Quin(-) subjects within each sample-type category. *Blue lines/symbols* indicate p-values for pairwise comparisons. *Panel C.* Individual taxa differing between Gran/Quin(+) and Gran/Quin(-) subjects, assessed by Aldex2 and adjusting for age and cholesteatoma diagnosis. *Panel D.* Individual taxa differing between MEMuc/Quin(+) and MEMuc/Quin(-) subjects, assessed by Aldex2 and adjusting for age and cholesteatoma diagnosis. *Vertical dashed lines* indicate fold-change cutoffs  $\geq 1.5$ . *Horizontal dashed lines* show p-value cutoffs for comparisons (nominal  $p \leq 0.05$ ). *Blue circles in the upper left quadrants* denote taxa enriched in Quin(-) subjects while *red circles in the upper right quadrants* denote taxa enriched in Quin(+) subjects.