Supplementary File

Title: Temporal changes in the nasal microbiota and host antimicrobial responses to intranasal mupirocin decolonisation: Observations in healthy staphylococcal carriers

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Content:

SECTION 1. QA procedures for RT-qPCR of selected antimicrobial peptide genes

Samples from the same subject were always measured in the same batch; any two out of four subjects were randomly paired in a batch regardless of pre-treatment Staphylococcal carriage status. For each target gene, we repeated the experiment for at least twice, each time using triplicate subsamples. We applied the $\Delta\Delta$Ct method [1] to estimate transcript levels comparing each post-treatment (T1 to T5) to the corresponding pre-treatment (T0) sample.

SECTION 2. Supplementary Tables

Table S1. Characteristics and nasal carriage status of participants

						Carriage status before and after mupirocin use[a]					
						T0	T1	T2	T3	T4	T5
Case no.	Age (years)	BMI (Kg/m^2)	Time at work (years)	Rhinitis history	Carriage status at FU	Before treatment	After treatment				
					(% FU visits)	<1week	<1week	3 weeks	5 weeks	9 weeks	13 weeks
A	42	18	18	Y	66.7%	N	N	N	N	N	N
B	39	23.4	1	N	100%	Y	N	Y	N	N	N
C	22	23.3	1	N	86.4%	Y	N	N	Y	Y	N
D	30	22.8	1.5	Y	71.7%	Y[b]	N	N	N	N	N

Abbreviations: BMI, body mass index; BL, baseline; FU, follow-up; IQR, interquartile range; MRSA, methicillin-resistant Staphylococcus aureus

a. Interval *before the beginning* and *after the end* of a five-day course of topical mupirocin use twice daily, expressed as median (IQR)

b. Methicillin-resistant strain (MRSA)

Table S2. Sample sequence metrics

Characteristics	No. subjects	No. samples	Sequence					Unique OTU[a]	
			Total	Per subject		Per sample		Total	Singletons
			N	Mean	SD	Mean	SD	N	n (%)
Total	4	24	1,958,527	489,631.8	40,213.6	81,605	19,454	341	27 (7.9%)
Timing									
pre-treatment	4	4	327,964	81,991.0	11,900.0	81,991	11,900	254	15 (5.9%)
Post-treatment	4	20	1,630,563	407,640.8	45,719.8	81,528	20,874	298	12 (4.0%)
Pre-treatment carriage									
Negative	1	6	497,381	497,381	-	82,897	19,231	239	8 (3.3%)
Positive	3	18	1,461,146	487,048.7	48,843.3	81,175	20,061	310	19 (6.1%)

Abbreviations: OTU, operational taxonomy unit; SL, similarity level; SD ,standard deviation

a. Including uncharacterised taxa with unknown or temporary taxonomic names

SESCTION 3. Supplementary Figures

Figure S1. Relative abundance of dominant genera and culture results for *S. aureus* (*) in individual participant at each sampling time (T0-T5). (UC=unclassified)

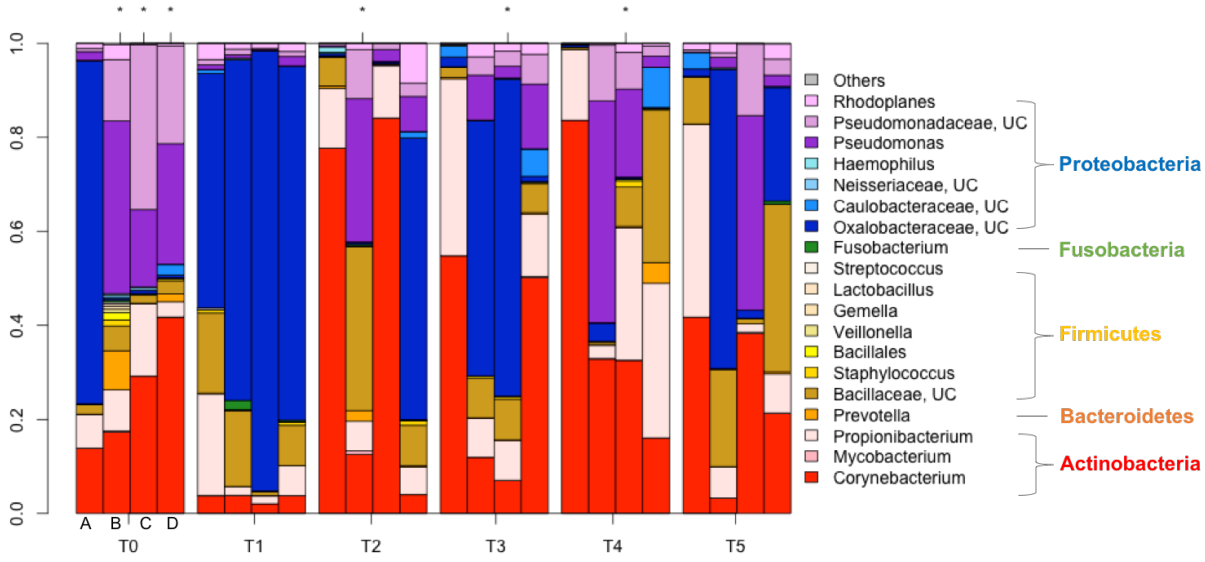


Figure S2. Alpha diversity measures at each sampling time. (pre_carrier=initial carriage status)

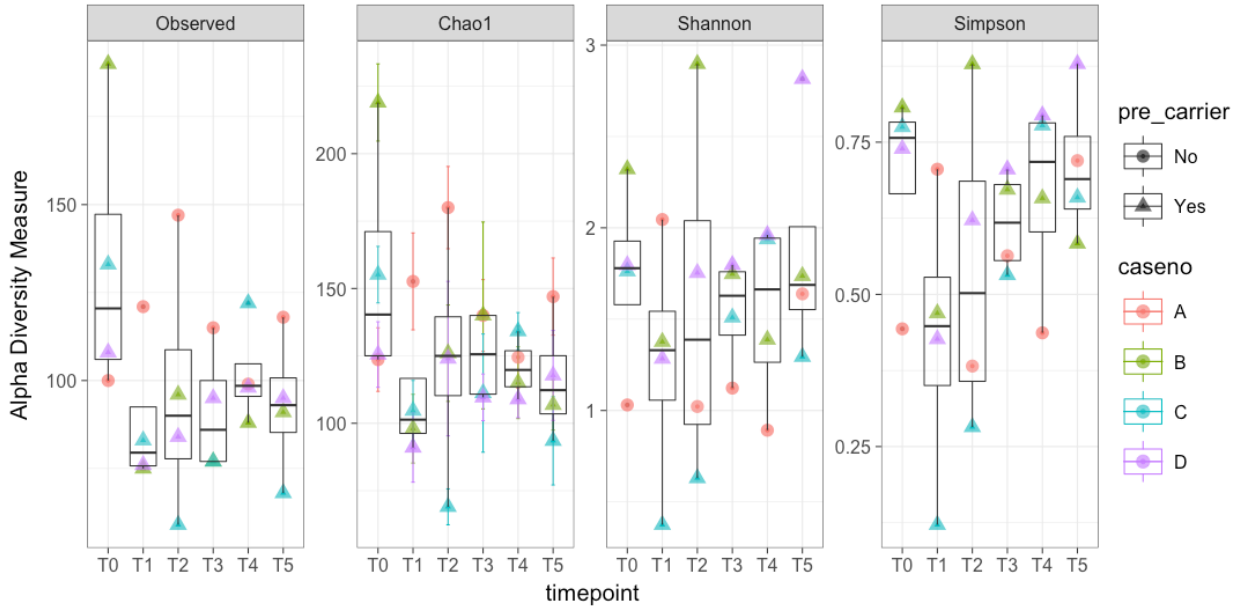
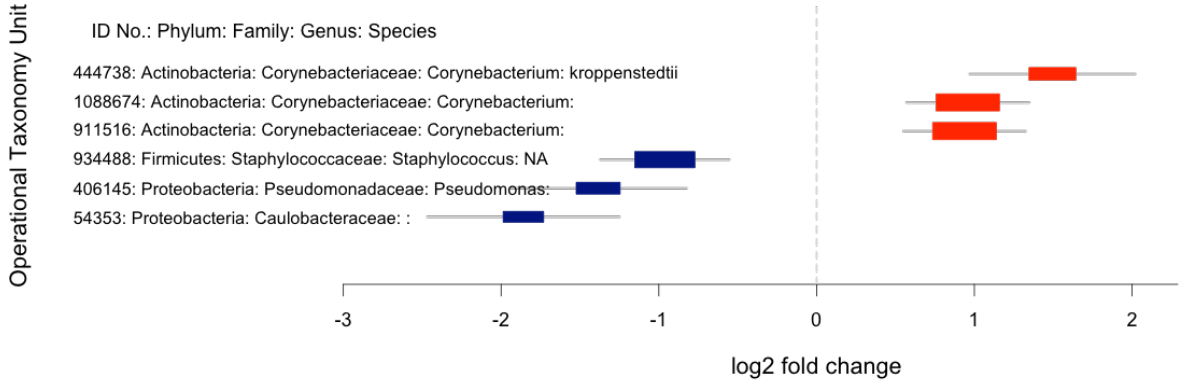
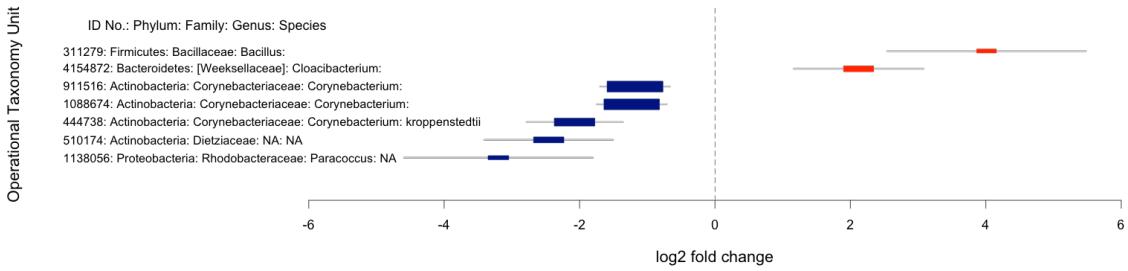


Figure S3. Results of univariate differential abundance analysis showing specific OTUs that were substantially enriched (red) or depleted (blue) in samples with higher expression levels of HNP1 (a) or of HBD3 (b) than those with lower levels (NA=unclassified).

(a) High versus low expression levels of HNP1



(b) High versus low expression levels of HBD3



REFERENCES

1. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL *et al*: **The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments**. *Clin Chem* 2009, **55**(4):611-622.