

Supplemental File for

Title: Mupirocin-associated temporal changes in the nasal microbiota and host's antimicrobial responses: A pilot study in healthy staphylococcal carriers

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SECTION 1. Ecological metrics used in the current study

The current study used the following community richness, diversity, and evenness measures as described by Gotelli and Colwell (Chapter 4) and by Maurer and McGill (Chapter 5) in [1]; all calculations were cross-verified using separate R packages.[2, 3]

1-1. The observed number (S) is the total number of species (OTUs) actually observed.

1-2. Chao1 index for abundance data is defined as $S + \frac{f_1^2}{2f_2}$, where f_1 and f_2 is the relative abundance for singletons and doubletons, respectively.

1-3. Shannon diversity ($D_{Shannon}$) was estimated by $-\sum p_i \ln p_i$ where p_i was the relative abundance of the i th OTU.

1-4. Simpson diversity ($D_{Simpson}$) was calculated by $1-D$, where $D = \sum p_i^2$ was the estimated probability of two random OTUs from a community (sample) belonged to the same taxonomy unit.

1-5. Shannon evenness, $E_{Shannon} = D_{Shannon}/\ln(S)$.

1-6. Simpson evenness, $E_{Simpson} = D_{Simpson}/S$.

1-7. Morisita-Horn similarity (overlap) index is defined by $S_{MH} = 1 - \frac{\sum_{i=1}^S (p_{i1} - p_{i2})^2}{\sum_{i=1}^S p_{i1}^2 + \sum_{i=1}^S p_{i2}^2}$

whereas Morisita-Horn dissimilarity measure is the complementary form,

$$\frac{\sum_{i=1}^S (p_{i1} - p_{i2})^2}{\sum_{i=1}^S p_{i1}^2 + \sum_{i=1}^S p_{i2}^2} .$$

SECTION 2. Supplementary Tables

Table S1. Sample sequence metrics

Characteristics	No. subjects	No. samples	Sequence				Unique OTU ^a		
			Total N	Per subject Mean	Per subject SD	Per sample Mean	Per sample SD	Total N	Singletons 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

Table S2. Temporal changes in the relative abundance of most-changed OTUs ($\geq 2\%$) with relative abundance by pre-treatment nasal carriage status

Non-carrier (N=1)	T0	T1	T2	T3	T4	T5
Oxalobacteraceae family ^a	72.95%	49.84%	0.46%	2.08%	0.44%	1.60%
Propionibacterium acnes	6.87%	19.91%	12.17%	36.80%	13.79%	39.72%
Corynebacterium genus	13.81%	3.69%	77.60%	54.78%	72.98%	32.57%
Corynebacterium kroppenstedtii	0.03%	0.04%	0.06%	0.03%	10.65%	9.19%
Moraxellaceae family ^a	0.95%	0.55%	0.21%	0.06%	0.09%	7.80%
Neisseriaceae family ^a	0.12%	0.81%	0.28%	2.34%	0.08%	3.47%
Others	5.25%	25.15%	9.23%	3.91%	1.97%	5.66%
Carriers (N=3)						
Oxalobacteraceae family ^a	0.54%	81.20%	14.12%	44.05%	1.09%	25.75%
Staphylococcus aureus	14.02%	0.07%	0.06%	0.93%	0.31%	0.05%
Bacillaceae family	26.33%	0.90%	14.23%	7.66%	19.43%	17.12%
Corynebacterium genus	28.82%	3.14%	38.30%	21.28%	26.87%	23.14%
Streptococcus genus	3.45%	0.03%	0.92%	0.19%	1.68%	0.17%
Propionibacterium acnes	8.67%	3.04%	7.39%	9.57%	23.21%	5.41%
Staphylococcus epidermidis	8.46%	0.28%	3.11%	2.59%	5.26%	6.03%
Acinetobacter genus	0.08%	1.61%	1.68%	0.43%	0.17%	2.12%
Others	9.62%	9.72%	20.20%	13.31%	21.98%	20.21%

a. Gram-negative bacteria

Table S3. Community diversity estimates based on 10 rarefied data subsampled at the minimum sequence read of 41,698^a

All	Community richness		Community diversity		Community evenness	
	Observed OTU	Chao1	Shannon	Simpson	Shannon evenness	Simpson evenness
Overall	325 (323-327)	348.7±2.1	2.3679 ^b	0.821 ^c	0.409 ^d	0.00252 ^e
Per sample	87 (75.5-101.5)	111.5 (99.1-132.8)	1.69 (1.20-1.87)	0.66 (0.46-0.76)	0.37 (0.27-0.41)	0.007 (0.006-0.008)
Sampling time						
T0	105.5 (96.5-143.5)	127.6 (114.0-180.6)	1.78 (1.39-2.06)	0.76 (0.59-0.79)	0.38 (0.30-0.42)	0.006 (0.005-0.007)
Post-treatment	82 (74-95)	108.6 (94.2-128.0)	1.58 (1.20-1.87)	0.64 (0.45-0.71)	0.36 (0.27-0.41)	0.007 (0.006-0.009)
T1	75 (73-99)	102.3 (89.8-144.4)	1.33 (0.83-1.71)	0.45 (0.27-0.59)	0.31 (0.19-0.38)	0.006 (0.004-0.006)
T2	81 (62.5-100.5)	110.3 (88.8-141.4)	1.39 (0.82-2.32)	0.50 (0.33-0.75)	0.31 (0.19-0.53)	0.007 (0.005-0.009)
T3	82 (73.5-95.5)	113.9 (95.0-139.1)	1.64 (1.31-1.77)	0.62 (0.55-0.69)	0.38 (0.30-0.40)	0.008 (0.007-0.009)
T4	84.5 (77.5-94.0)	111.7 (104.3-120.8)	1.66 (1.14-1.95)	0.72 (0.55-0.79)	0.37 (0.26-0.43)	0.008 (0.007-0.009)
T5	90 (75-95)	104.6 (99-116.9)	1.69 (1.46-2.28)	0.69 (0.62-0.80)	0.37 (0.34-0.50)	0.008 (0.007-0.010)
Pre-treatment carriage						
Non-carrier	101 (92-119.5)	128.3 (114.0-149.7)	1.08 (1.02-1.64)	0.50 (0.44-0.71)	0.24 (0.21-0.35)	0.006 (0.005-0.006)
Carriers ^b	82 (74-92.5)	106.7 (93.1-125.0)	1.75 (1.38-1.94)	0.67 (0.53-0.78)	0.39 (0.32-0.42)	0.008 (0.007-0.009)

Abbreviations: IQR, inter-quartile range; OTU, operational taxonomic unit; S. aureus, Staphylococcus aureus; SE, standard error

a. All expressed as median (IQR)

b. SE=0.00015

c. SE=0.00025

d. SE=0.00025

e. SE=7.5E-06

Table S4. Diversity estimates for the community composition of the nasal microbiota at each post-decolonisation visit, median (IQR)

	No. Samples	Community richness		Community diversity		Community evenness	
		Observed OTU	Chao1	Shannon	Simpson	Shannon evenness	Simpson evenness
Sampling time							
T1	4	79.5 (75.8-92.5)	101.3 (96.3-116.7)	1.33 (1.06-1.54)	0.45 (0.35-0.53)	0.31 (0.19-0.37)	0.006 (0.004-0.006)
T2	4	90.0 (77.8-108.8)	125.0 (110.3-139.5)	1.39 (0.92-2.04)	0.50 (0.36-0.69)	0.30 (0.18-0.52)	0.006 (0.004-0.008)
T3	4	86.0 (77.0-100.0)	125.6 (110.8-140.0)	1.63 (1.41-1.76)	0.62 (0.56-0.68)	0.37 (0.29-0.40)	0.007 (0.006-0.008)
T4	4	98.5 (95.5-104.8)	119.8 (113.5-126.9)	1.66 (1.26-1.94)	0.72 (0.60-0.78)	0.36 (0.25-0.42)	0.007 (0.005-0.008)
T5	4	93.0 (85.3-100.8)	112.3 (103.5-125.1)	1.69 (1.55-2.01)	0.69 (0.64-0.76)	0.36 (0.33-0.50)	0.008 (0.006-0.009)

Abbreviations: OTU, operational taxnomony unit; IQR, interquartile range

Table S5. Results of differential expression analysis on OTUs by levels of HBD3 and HNP1, respectively, among healthy staphylococcal carriers following mupirocin decontamination

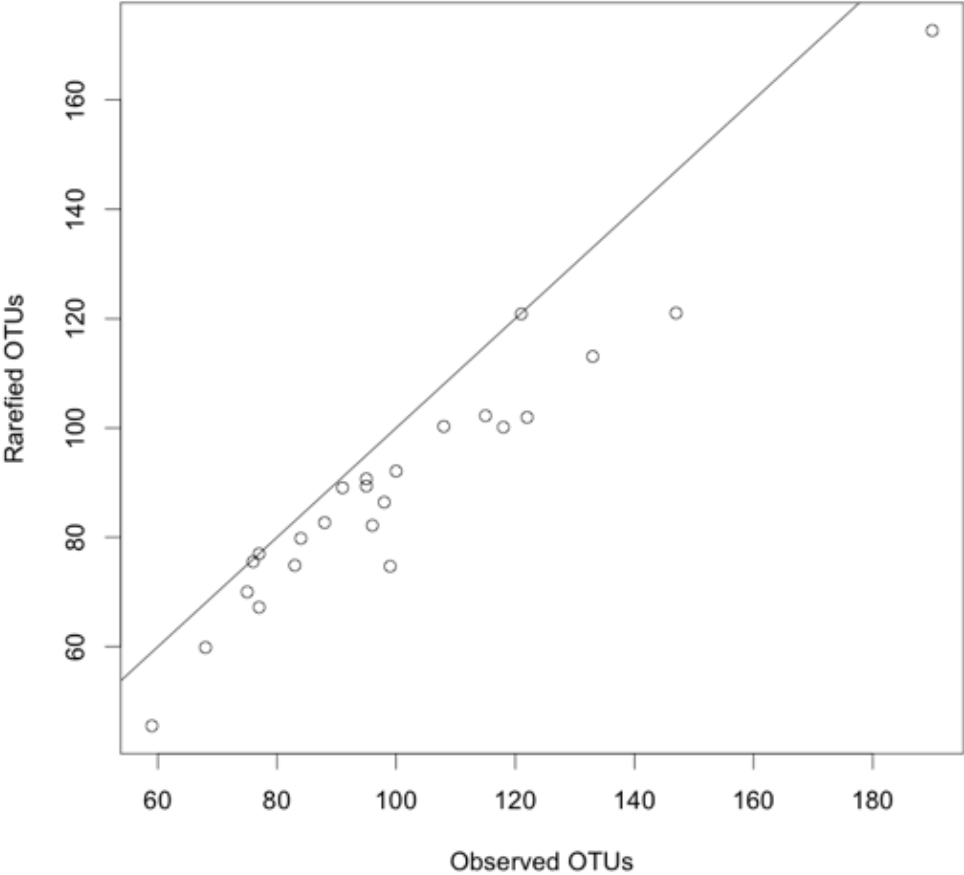
AMP	baseMean	log2FoldChange	lfcSE	padj	Family	Genus	Species
HBD3	835.648	-1.331	0.276	0.00015	Corynebacteriaceae	Corynebacterium	kroppenstedtii
	67.736	-0.881	0.220	0.00212	Gordoniaceae	Gordonia	NA
	14.776	-1.142	0.281	0.00212	Campylobacteraceae	Sulfurospirillum	NA
HNP1	835.6480	1.4004	0.2322	0.00000	Corynebacteriaceae	Corynebacterium	kroppenstedtii
	145.4689	-0.7110	0.1837	0.00427	Staphylococcaceae	Staphylococcus	NA
	84.0515	-0.7674	0.2002	0.00427	Caulobacteraceae	NA	NA
	364.3767	0.7949	0.2026	0.00427	Moraxellaceae	NA	NA

Abbreviations: HBD3, human beta-defensin 3; HNP1, human neutrophil peptide 1; lfc, log2-fold change; SE, standard error; padj, P-values adjusted for multiple testing using Benjamini-Hochberg correction; NA, unclassified

SESECTION 3. Supplementary Figures

Figure S1. Comparing the observed OTU number with that obtained by rarefying the sequence data with the minimum sequence read of each sample (a). Comparing the cumulative number of OTU observed in the current study with those obtained by using sequence read ("individual")-based and sample-based[4] rarefaction (b).

(a)



(b)

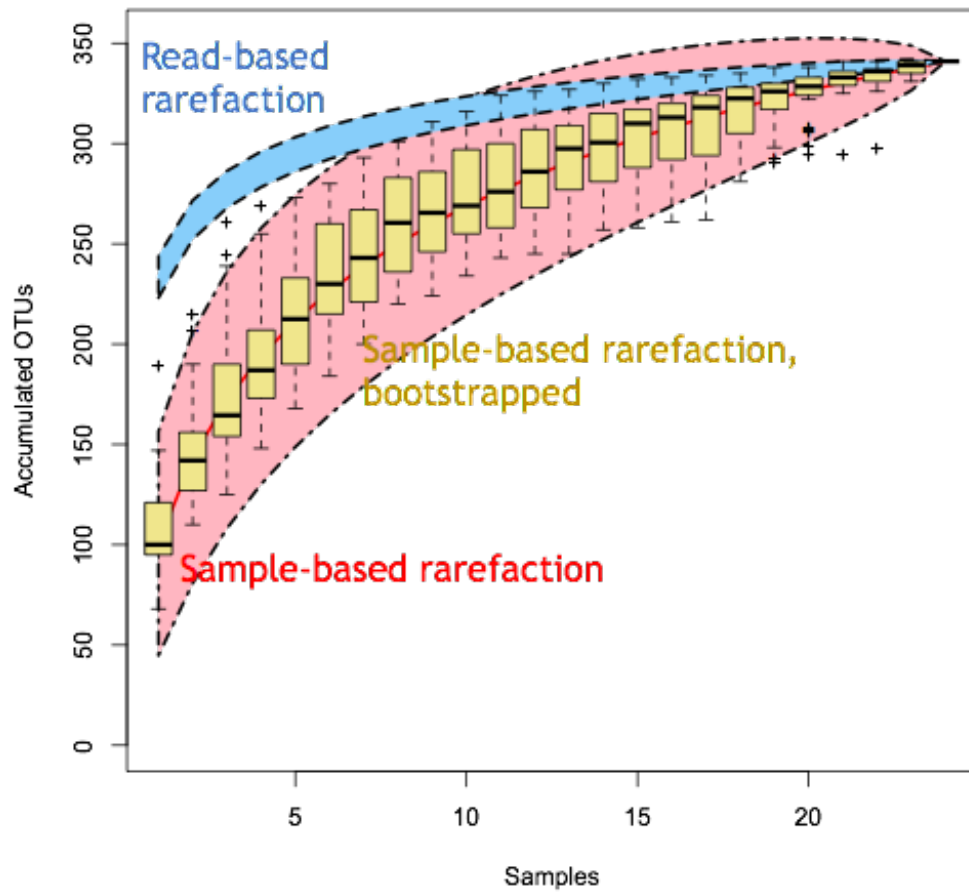


Figure S2. Linear correlation between the original dissimilarity metric (Morisita-Horn) and MDS-transformed distance.

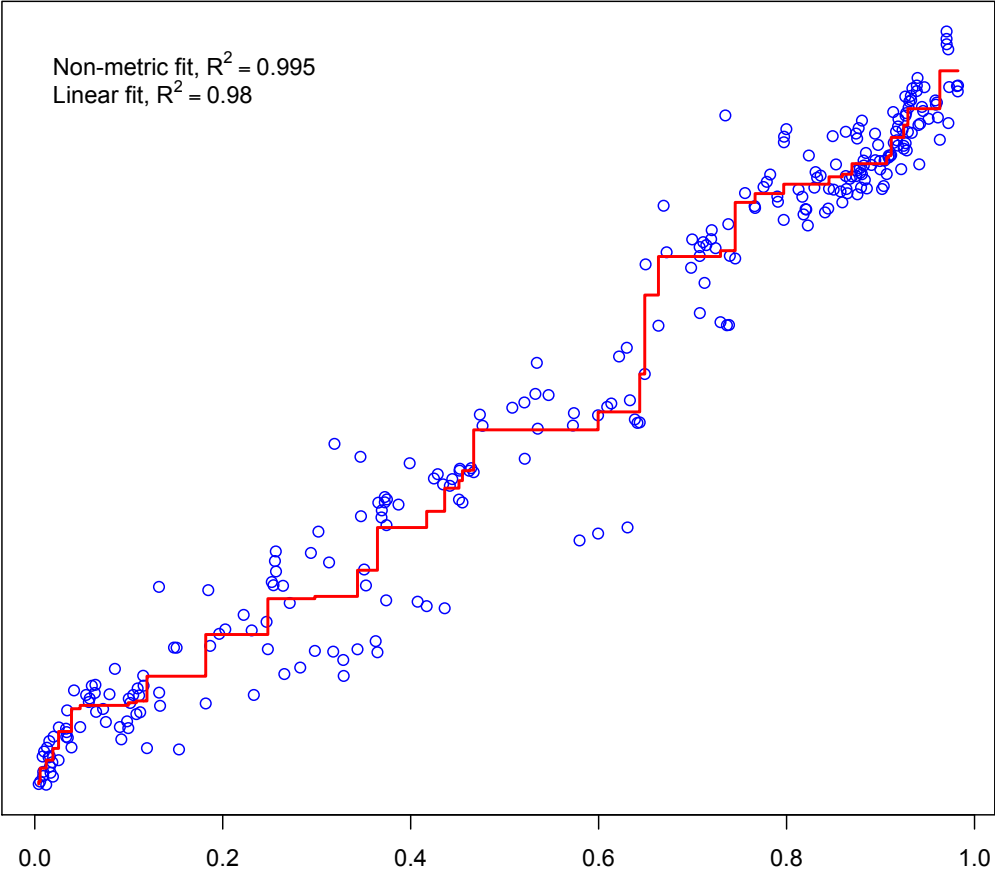


Figure S3. Samples clustered by two major axes using constrained correspondence analysis (CCA) without specifying "environmental" variables in the model yet (the "null" model). (pre_carrier: the initial carriage status- yes versus no)

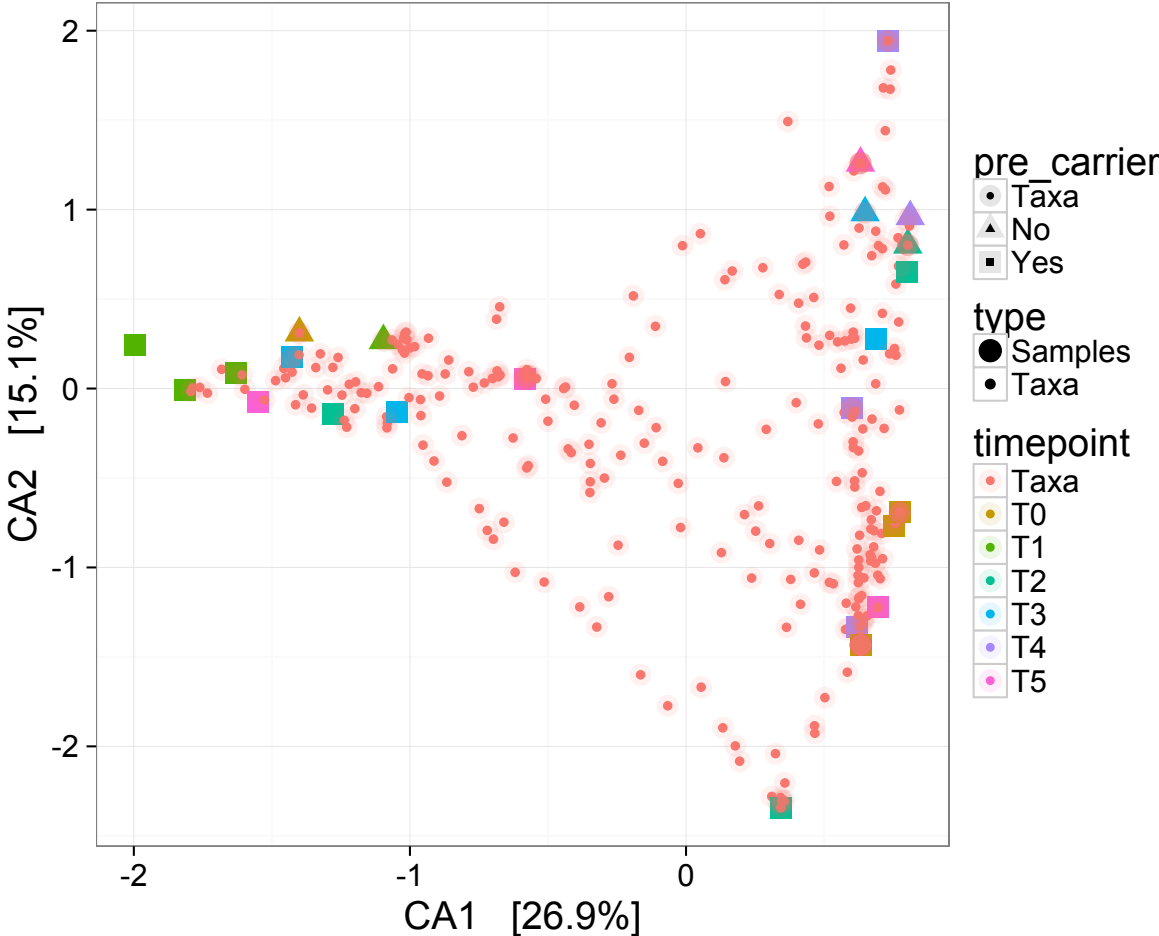
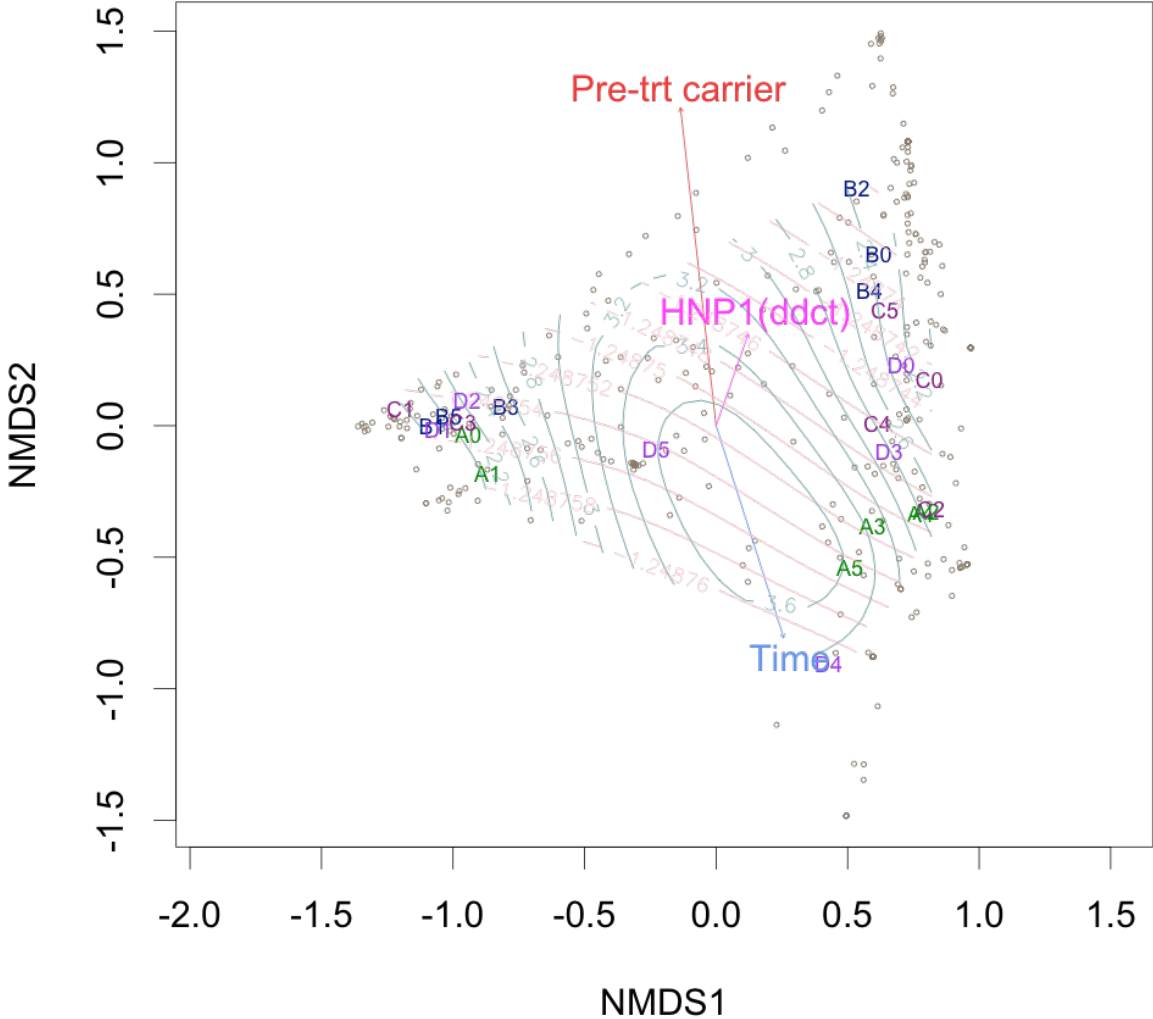


Figure S4. Samples clustered by two major axes using nonmetric multidimensional scaling (NMDS) methods, overlaid with "environmental" variables including staphylococcal carriage status before decolonisation ("Pre-trt carrier"), antimicrobial HNP1 levels, and sampling time (as a continuous covariate).



REFERENCES

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2. Chao A, Ma KH, Hsieh TC. SpadeR: Species Prediction and Diversity Estimation with R. R package version 0.1.0., **2015**.
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