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

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ABSTRACT

Background: How mupirocin affects the human nasal microbiota over time remains uncharacterized.

Methods: We repeatedly sampled the anterior nares of four healthy staphylococcal carriers before and after mupirocin use. By sequencing bacterial 16S ribosomal cDNA, we characterized sequential changes in the carriage status, the nasal microbiota, and the hosts' antimicrobial peptide expression up to 90 days after decolonization.

Results: Before mupirocin use, the nasal microbiota differed by the initial, culture-based staphylococcal carriage status, with *Firmicutes* (54.1%) being the most predominant in carriers and *Proteobacteria* (75.8%) in the only noncarrier. The nasal microbiota became less diverse (Shannon diversity: 1.33, 95% confidence interval [CI]: 1.06-1.54) immediately after decolonisation than that before decolonisation (1.78, 95%CI: 0.58-1.93). Based on results of differential abundance analysis, *Firmicutes* were significantly enriched ($\log_2$ fold changes ≥ 4, Benjamini-Hochberg adjusted $P < .01$) while *Actinobacteria*, particularly *Corynbebacterium*, were relatively depleted in samples from staphylococcal carriers. Results of nonmetric multidimensional scaling (NMDS) and constrained correspondence analysis (CCA) also suggested that the initial staphylococcal carriage status, human neutrophil peptide 1 levels, and sampling times were major contributors to the between-community dissimilarities (*P* for marginal permutation test: .014) though the significance attenuated when within-group correlation was considered (*P* for blocked permutation test: .047).

Conclusion: These findings suggest that large-scale investigations on antibiotic effects on the human nasal microbiota are warranted.

Keywords: Nasal carriage, Staphylococcus aureus, Decolonization,

Microbiota/Microbiome, Antimicrobial peptides

INTRODUCTION

In the U.S., 80% of *S. aureus* infections occurred in the healthcare setting. Among surgical patients and patients newly-admitted to the intensive care unit,[1] nasal staphylococcal carriage has been consistently shown to increase infection risks by 2-12 folds at surgical sites (SSI), in the bloodstream, or at catheter exit sites.[2] While targeted decolonization with nasal mupirocin calcium ointment, either alone or in a bundle, can effectively reduce *S. aureus*-associated SSIs among carriers,[3] there has been concerns about a potential increase in bacterial resistance upon prolonged or widespread implementation of antibiotic decolonization.[4]

In 2009, Ammerlaan and colleagues reviewed 23 randomized controlled trials that assessed the comparative effectiveness of eradicating methicillin-resistant *S. aureus* (MRSA) carriage by using oral antibiotics, topical antimicrobial agents, or both.[5] Although the estimated success rate of eradication was 90% one week after mupirocin decolonization, there was 1% incidence of treatment-associated resistance to mupirocin among 12 studies included by Ammerlaan et al.[5] In another recently published meta-analysis, universal and targeted decolonization appeared to be similarly effective on reducing *S. aureus* infections in non-surgical healthcare settings.[6] However, the authors noted that 5 out of 14 studies reported the emergence of mupirocin resistance.[6] In still another systemic review, Grothe and colleagues also cautioned the development of drug resistance in specific patients populations for whom repeated or prolong decolonization may be clinically indicated.[7]

Cumulative evidence from animal models and clinical studies have suggested that, the human microbiota constitutes an essential microenvironment that may promote or inhibit colonization of certain bacterial species by inter-species or host-pathogen interactions.[8] Longitudinal observations of infants exposed to antibiotics revealed that the resulting dysbiosis of the gut microbiota could be long-lasting and might predispose to the later development of allergic or atopic phenotypes, obesity, and other autoimmune disease.[9] In adults, repeated exposure to antibiotics would eventually lead to a stable yet different-from-baseline microbiome in the distal gut; the functional consequences remained to be determined.[10]

While staphylococcal carriers are known to harbour a characteristically distinctive microbial profile in the anterior nares as compared to noncarriers,[11,12] there is a paucity of literature on how antibiotics affect the human nasal microbiota as a whole. Understanding the impacts of antibiotics on the nasal microbiome and how the host's local immunity reacts to the perturbed microenvironment, may shed new light on the continual debate on universal versus targeted decolonization policies.[4] Therefore, we sought to characterize changes in the microbial composition and abundance of the nasal microbiota by following healthy staphylococcal carriers up to three months after mupirocin decolonization. We also aimed to determine whether host's local antimicrobial responses would change in response to the perturbed microbial community by short-term use of mupirocin.

METHODS

Study design and data collection

The current study extended from a parent cohort study on healthy staphylococcal carriers, of whom the enrolment and follow-up procedures were described elsewhere.[13] Briefly, we sampled the anterior nares of female carriers twice per week for six consecutive menstrual cycles. Four of 15 participants requested for a standard course of topical decolonization at the end of the study, at which time (April 2015) there were no clinical consensus on screening or decolonising staphylococcal carriage in Taiwan.

After obtaining written informed consent, we sampled anterior nares of each participant before (T0) and after a five-day course of mupirocin at 3, 17, 31, 60 and 90 days (T1-T5). The decolonization strategy was in accordance to the clinical guidelines issued by the Infectious Diseases Society of America.[14] At each sampling visit, the research assistant used one Copan® culture swab for culture-based identification of *S. aureus*; the other sterilized cotton swab for microbial 16S ribosomal RNA gene sequencing; still another sterile cotton swab for gene expression quantification. The sampling order of the three swabs remained the same for all subjects throughout the observation period. The Institutional Review Board at Chang Gung Medical Foundation reviewed and approved the addendum study protocol and the consent form.

Laboratory procedures

Microbiology study. According to the current guideline, we performed microbial isolation within 48 hours of swab collection; MRSA isolates were determined by results

of the disk diffusion method that demonstrated resistance to both Penicillin and Cefoxitin.[15,16]

RNA extraction and transcript quantification. We stored nasal swabs at -80°C before using TRIzol Reagent® to extract total RNA. To quantify expression levels of selected host antimicrobial peptides (AMP) including HNP1 (*DEFA1*),[17] RNase 7 (*RNASE7*),[18] and HBD3 (*DEFB103A*),[19] we used the reversely-transcribed cDNA as a template for RT-qPCR using Roche Light Cycler 480®. In accordance with the literature, we chose glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as the reference gene,[20] and median $\log_2$-fold changes (with inter-quartile range [IQR]) for statistical comparisons using median regression models with robust variance estimation in Stata (version 13) to account for repeated measures.[21]

Targeted amplicon preparation and sequencing. We used the primer pair 27F-534R for targeted amplification and pair-end sequencing of the variable regions V1 to V3 of 16S ribosomal cDNA [22] using Illumina MiSeq® (2x300bp). There were totally 1,958,527 sequences obtained, with an average of 81,605 sequence reads per sample (Supplementary Table S1). We employed the 'closed_reference' approach for taxonomy assignment against the Greengenes database (Version 13.8) [23] via the UCLUST algorithm [24] at a threshold of 97% similarity. After quality checking procedures according to the QIIME pipeline (version 1.9.1),[25] we performed additional data management and subsequent statistical analysis in R (Version 3.2.4). [26]

Statistical analysis

Community richness, evenness and diversity. We estimated and compared relative abundance of the top 20 dominant genera and of the most-affected community members (changes in relative abundance ≥ 2%) by carrier type over time. Similarly, we compared changes in the microbial structure using ecological metrics: *alpha-diversity estimates*, including the observed number of operational taxonomy units (OTU) and the Chao1 index; *beta-diversity estimates*, including Shannon diversity ($D_{Shannon}$) and Simpson diversity ($D_{Simpson}$) indices for quantifying community richness and diversity simultaneously.[27] We also calculated Shannon evenness ($E_{Shannon}$) and Simpson evenness ($E_{Simpson}$) measures to quantify richness-independent evenness of component members (the Supplementary File).[28] We applied Mann-Whitney U test; Wilcoxon signed rank test or median regression models with robust variance estimation whenever appropriate. In sensitivity analysis, we computed the above-mentioned indices on rarefied data using coverage (sequence reads)-based rarefaction at a subsampling depth of 41,698 reads (without replacement).[27,28]

Community similarity and dissimilarity. To evaluate between-community similarity over time, we quantified proportions of shared and (sample-) unique OTUs at each sampling time.[29] We also identified major OTUs (≥ 5% in each sample) concurrently shared by all participants. We employed the Morisita-Horn similarity (overlap) index (SI) to quantify pairwise, OTU-by-OTU shared information among all participants or by pre-treatment carriage status at each visit.[28]

We applied differential expression analysis to identify OTUs of differential abundance that were associated with staphylococcal carriage status or with levels of AMPs. We

chose Wald test to compare geometric mean-normalized count data and specified the significance level of Benjamini-Hochberg adjusted P-values at 0.01.[30,31] We further performed nonmetric multidimensional scaling (NMDS) to determine "environmental" variables that could explain for between-sample dissimilarities, which was estimated by the complementary of the Morisita-Horn index ('1- Morisita-Horn SI').[28]

Covariates considered included sample-level factors (different sampling times, pre-versus post-treatment, days since the start or completion of mupirocin use); subject characteristics (pre-treatment carriage status); relative levels of AMPs at each visit. We then constrained the analysis on community dissimilarities that could be best explained by selected host characteristics using constrained correspondence analysis (CCA).[27] We considered a two-tailed, statistical significance at 0.05 unless otherwise specified; p-values were based on 1000 permutations.

RESULTS

The included participants aged between 22 and 42 years, and have spent at least one year at the relevant full-time work (Table 1). Overall, we collected 24 nasal swabs, with six from each participant. Before mupirocin decolonization (T0), three women carried *S. aureus* (75%) based on culture results. Despite transient detections of *S. aureus* after decolonization at 19, 33 and 62 days (Table 1), the clearance rate of staphylococcal carriage at post-treatment 90 days (T5) was 100% (Table 1).

Table 2 shows results of relative quantities of HBD3 and HNP1 at each sampling time. Levels of RNase7 were undetectable in multiple samples and thus not shown. While there were no differences in HBD3 levels by staphylococcal carriage (*P*: .439), there was a 1.59-fold (95% CI: 1.08-2.36) change in HBD3 level over time (*P* for trend: .022, Table 2). By contrast, HNP1 levels did not show any temporal pattern; yet HNP1 levels were generally lower in carriers than in the noncarrier, without (*P*: .033) or with (*P*: .016) adjustment for sampling time (Table 2).

Community structure of the nasal microbiome

Before decolonization, the dominant phylum was *Proteobacteria*, mostly of unclassified *Oxalobacteraceae* (73.0%) in the only noncarrier (column A at T0, Figure 1) whereas Firmicutes were predominant colonizing microbes in carriers (columns B-D, Figure 1). Immediately following decolonization (T1), Gram-negative *Oxalobacteraceae* took dominance in all subjects with a relative abundance ranging from 49.8% to 93.7% before *Actinobacteria* (mainly *Corynebacterium*) and *Firmicutes* (unclassified *Bacillaceae* and *Staphylococcus*) gradually re-emerged in the noncarrier and carriers, respectively (Figure 1). The re-appearance of *Oxalobacteraceae*, *Bacillaceae*, and *Staphylococcus* was particularly evident in carriers (Figure 2b) but not in the only noncarrier (Figure 2a) when examining the most-affected OTUs, of which the relative abundance changed at least 2% comparing post-treatment 90 days (T5) to the baseline (T0).

Community richness, evenness, and diversity

Table 3 shows estimates for the compositional diversity of the nasal microbiota for all 24 samples as a whole and by sample characteristics. Overall, there were 341 unique OTUs in 24 swab samples. The overall diversity estimate for the assemblage of 24 nasal microbiota under study was within two standard errors (SE) of the Chao1 index (361.6, SE: 10.5), suggesting that the number of "unobserved" OTUs associated with possible under-sampling might be negligible. When using rarefied data, richness estimates ranked the community samples comparably (Supplementary Figure S1) and diversity estimates also followed a similar pattern of changes over time (Supplementary Table S3).

Nevertheless, there were substantial between-sample variations in the microbiota composition as suggested by the large difference in the observed OTUs (341 vs. 95.5) and the Chao1 index (361.6 vs. 123.8) between the total assemblage and the median estimate of a single sample (Table 3). The observation that the within-sample 'species' diversity as quantified by $D_{Shannon}$ (median: 1.69, IQR: 1.20-1.87) and $D_{Simpson}$ (median: 0.66, IQR: 0.46-0.76) represented only 50.2% and 56.1% of the overall diversity (2.39 and 0.82, respectively) further highlighted the wide, sample-to-sample variation (Figures 3a & 3b).

As shown in Figure 3a, post-treatment community samples showed a reduction in richness (observed OTU: 93; Chao1: 116.4), diversity ($D_{Shannon}$: 1.57; $D_{Simpson}$: 0.64), or evenness ($E_{Shannon}$: 0.35; $E_{Simpson}$: 0.006); yet the reduction was transient and the differences were not statistically significant after correcting for within-group correlation

(Table 3, Supplementary Table S4). Although carriers demonstrated a comparable community richness to that in the noncarrier (Table 3, Figure 3b), an increased evenness ($E_{Shannon}$: 0.38 vs. 0.23, *P*< .001) and diversity measures ($D_{Shannon}$: 1.75 vs. 1.08, *P*: .003) appeared to be associated with the initial staphylococcal carriage status (Table 3).

Community similarity and compositional differences over time

Figure 4a reveals proportions of sample-unique and shared OTUs (the 'core' microbiota) for all participants at T0. The proportion of shared OTUs before decolonization (T0) (20.1%) remain relatively unchanged over time (*P* for trend: .411; Table 4) whereas the Morisita-Horn overlap estimate increased drastically over time (0.927, 95%CI: 0.925-0.929; 151%), representing a substantial dissimilarity between carriers and the noncarrier after decolonization (-46%; Table 4).

Results of differential abundance analysis further showed that *Firmicutes* and *Actinobacteria* were two major phyla that were enriched and depleted in carriers' samples, respectively, as compared to those from the noncarrier (Figure 4b). A higher level of HBD3 was also associated with a decreased abundance of *Corynebacterium kroppenstedtii* (log2 fold change: -1.31, *P*< .001) while there was a 1.40-fold enrichment in (log2-counts of) *Corynebacterium kroppenstedtii* (1.40, *P*< .001) and a 0.7-fold depletion in unclassified *Staphylococci* (-0.71, *P*: .004) in samples with a higher HNP1 level (Supplementary Table S5.)

Community dissimilarity and host factors

Using either CCA or NMDS on dissimilarity metrics, we found that the first two axes similarly grouped microbiota samples into two major clusters (Supplementary Figures S3 & S4). Based on eigenvalues partitioning, we found that the pre-treatment staphylococcal carriage status (14.1%), fold changes in HNP1 levels (9.7%), and sampling time (7.6%) could explain a maximum of 41% of the total data variation. Figure 5 displays results of CCA in the first two axes with overlaid arrows that were perpendicular to a hypothetical plane optimally separating OTUs/ samples by individual host characteristic. As compared to a null model, the multivariable-constrained model was statistically significant according to the permutation test (*P*: .014), yet the significance attenuated when within-group correlation was accounted for (*P*: .047).

DISCUSSION

Despite of a small sample size, we took advantage of the serial sampling scheme in characterizing the temporal dynamics in the nasal microbiome after mupirocin decolonization. Mupirocin initially reduced the microbial diversity up to 60%-75% of the pre-treatment level before a noticeable resurgence of the community richness as early as by post-decolonization day 30 (T2). Although mupirocin has a well-recognized antimicrobial activity against staphylococci and streptococci,[32] its effects against the nasal commensals as a whole remain less characterised. By examining the nasal microbiota longitudinally, we noted that mupirocin appeared to suppress a broad range of resident commensals, including *Oxalobacteracea* family, *Corynebacteria*, and *P. acnes* (Supplementary Table S2), effectively disrupting the ecological balance of the

nasal microbiota in qualitatively distinctive ways beyond what diversity metrics could have revealed.

We also observed that, how mupirocin changed the microbial landscape generally depended on the initial staphylococcal carriage status. This finding was consistent with previous studies on how oral antibiotics could perturb the human gut microbiome.[33] Raymond and colleagues followed a cohort of 18 participants and six control subjects after a seven-day course of oral cefprozil (500mg) twice per day.[34] By comparing stool samples collected before (day 0) and after treatment (day 7 and day 90), the investigators concluded that the administered antibiotic had a predictable effect on all participants' gut microbiota by their faecal microbiome on day 0.[34]

The current study also revealed a possible instance of inter-species competition between *Staphylococcus* and *Corynebacterium kroppenstedtii* (*C. kroppenstedtii*) (Figure 4b, Supplementary Table S2). Possible inter-species competitions between *S. aureus* and *Corynebacteria* among healthy volunteers were not unheard of in culture-based investigations.[35] Recently, Yan and colleagues showed that *Corynebacterium accolens* (*C. accolens*) was likely to co-exist with *S. aureus* while *Corynebacterium pseudodiphtherium* and *S. aureus* were rarely found together in the nasal microbiota of healthy individuals.[36]

Investigators have shown that phenol-soluble modulins secreted by murine *Staphylococcus epidermis* (*S. epidermis*) could suppress the invasion of *S. aureus* and

reduced the survival of Group A Streptococcus on mouse skin.[37] Intriguingly, we noted that higher levels of HBD3 and HNP1 were associated with the depletion and the enrichment of *C. kroppenstedtii*, respectively (Supplementary Table S5), suggesting a potential role of the host immunity in determining the co-existent or mutually exclusive partnership among community members. We also found temporal variations in HBD3 levels, but not in HNP1, after mupirocin decolonization (Table 2); these findings were in line with the literature supporting our hypothesis that HNP1 is constitutively produced by skin-resident neutrophils whereas the expression of HBD3 responds to exogenous stimuli to keratinocytes.[17,20,38]

Additionally, the observation that HNP1 levels were substantially higher in the (only) noncarrier than those in the carriers (Table 2) might be explained by a defective or down-regulated recruitment of neutrophils by staphylococcal carriers, possibly an immuno-modulating effect resulting from the host-pathogen interaction.[39] Although an early study by Cole et al. has suggested the otherwise,[40] we could infer that the abundance and the lack of *S. aureus* was possibly correlated with the local antimicrobial levels of HBD3 and HNP1 based on the observed associations between *C. kroppenstedtii* and expressed AMP levels (Supplementary Table S5).

Limitations

The current pilot study was not well-powered and was mostly descriptive in nature. The large between-individual variation and the small number of participants rendered the between-group comparisons by the initial carriage status of limited power. The fact that

all study subjects were healthcare workers who routinely observed universal precautions at work also limited the generalizability of our findings to healthy carriers in other settings. However, with repeated measures from the same individual, we have gained efficiencies while making intra-person (or intra-group) comparisons.

While the study findings suggested a distinction between sample microbiota from participants with or without *S. aureus* before decolonization, we cautioned the interpretation of statistical comparisons by the initial carriage status as estimates associated with non-carriage were obviously under-estimated. Regardless, the current study contributed to the field by providing empirical estimates for future sample size and effect size calculation. Formal evaluations are required to better characterize mupirocin effects on the human nasal microbiome in absolutely noncarriers, intermittent as well as (strictly) persistent carriers.[13]

Conclusions

In a small group of healthy staphylococcal carriers, we found short-term yet pervasive effects of mupirocin decolonization on the ecology of the nasal microbiota. We also identified host immune correlates that were associated with substantial changes in the relative abundance of not only *S. aureus* but also of the majority of the microbial community after mupirocin use. These observations though preliminary suggested that larger-scale investigations are warranted to better understand how antibiotics may affect the second human genome and the host-pathogen interactions.

Availability of supporting data

The data sets supporting the results of this article are included with the article and its additional file (upon acceptance for publication).

Competing interests

The authors declared that they have no competing interests.

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Authors' contributions

SL conceived and designed the study, performed statistical analysis, and drafted the manuscript. YT helped manage the 16S rDNA sequence data and contributed to data analysis, results interpretation, and manuscript preparation. YL performed the laboratory work and assisted with the manuscript preparation. KC and CC participated in data interpretation and reviewed the manuscript. YH supervised the laboratory work,

contributed to data interpretation, reviewed the manuscript. LYC involved in the study design, data analysis, and the manuscript drafting. All authors read and approved the final version of the manuscript.

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Tables

Table 1. Characteristics and nasal carriage status of participants

Case no.	Age (years)	BMI (Kg/m^2)	Time at work (years)	Rhinitis history	Carriage status at FU	Carriage status before and after mupirocin use[a]					
						T0	T1	T2	T3	T4	T5
						Days before	Days after				
					(% FU visits)	-1 (-6, -1)	4 (3, 6)	19 (17, 22)	33 (30, 36)	62 (57, 66)	92 (88, 97)
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 2. Fold changes in quantities of antimicrobial peptide transcripts, HBD3 and HNP1, relative to the baseline (T0) by pre-treatment carriage status

AMP	**All (n=24)**		Noncarrier (n=6)	Carrier (n=18)	
HBD3	Median	(IQR)		Median	(IQR)
T1	2.91	(0.59, 7.52)	4.76	1.07	(0.11, 10.3)
T2	2.30	(1.83, 2.55)	1.52	2.48	(2.13, 2.61)
T3	10.7	(5.35, 23.9)	3.65	14.3	(7.06, 33.6)
T4	4.14	(1.23, 33.4)	0.95	6.77	(1.52, 59.9)
T5[a]	6.17	(1.75, 12.9)	1.41	10.3	(2.08, 15.5)[b]
HNP1					
T1	0.67	(0.13, 1.36)	1.61	0.22	(0.04, 1.11)
T2	0.36	(0.22, 0.84)	1.27	0.32	(0.12, 2.22)
T3	0.64	(0.17, 2.48)	4.00	0.32	(0.02, 3.33)
T4	0.20	(0.09, 1.95)	3.68	0.17	(0.01, 4.44)
T5[c]	0.87	(0.41, 2.00)	3.02	0.76	(0.06, 5.55)[d]

Abbreviations: AMP, antimicrobial peptide; IQR, interquartile range; HBD3, human beta-defensin 3; HNP1, human neutrophil peptide 1

a. P for (temporal) trend: 0.022

b. No statistical difference by carriage status, with (P: 0.497) or without (P: 0.439) adjusting for study visit

c. P for trend: 0.141

d. Statistical difference by carriage status, with (P: 0.016) or without (P: 0.033) adjustment for study visit.

Table 3. Diversity estimates for the community composition of the nasal microbiota: overall, by sampling time, and by pre-treatment staphylococcal carriage status, median (IQR)

	No. Samples	Community richness		Community diversity		Community evenness	
		Observed OTU	Chao1	Shannon	Simpson	Shannon evenness	Simpson evenness (E-03)
Overall	24	341	361.6±10.5[a]	2.39	0.82	0.41	2
Per sample		95.5 (80.0-116.5)	123.8 (107.9-140)	1.69 (1.20-1.87)	0.66 (0.46-0.76)	0.35 (0.27-0.40)	6 (5-7)
Decolonisation status							
Before (T0)	4	120.5 (106.0-147.3)	140.3 (125.0-171.1)	1.78 (0.58-1.93)	0.76 (0.67-0.78)	0.37 (0.29-0.41)	5 (4-6)
After (T1-T5)	20	93 (77-103)	116.4 (106.3-135.6)	1.57 (1.24-1.83)	0.64 (0.46-0.71)	0.35 (0.27-0.40)	6 (5-8)
Pre-treatment staphylococcal carriage[b]							
Negative	6	116.5 (103.8-120.3)	143.5 (128.4-151.2)	1.08 (1.02-1.51)	0.50 (0.44-0.67)	0.23 (0.20-0.34)	5 (4-6)
Positive	18	89.5 (77.0-97.5)	113.1 (105.2-125.9)	1.75 (1.38-1.90)[c]	0.67 (0.54-0.78)	0.38 (0.31-0.40)[c]	7 (6-8)[c]

Abbreviations: OTU, operational taxnomony unit; S. aureus, Staphylococcus aureus; SE, standard error

a. mean±SE

b. P-values > 0.05 for Wilcoxon rank-sum tests comparing T0 samples by carrier type

c. P-values <0.05 comparing all samples by pre-treatment carriage status using time-adjusted median regression models with robust variance estimation to account for repeated measures

Table 4. Community similarity estimates based on shared membership and community overlapping by sampling time and by the initial staphylococcal carriage status

	T0	T1	T2	T3	T4	T5
Overall						
Observed OTUs, N	254	173	186	177	204	185
Shared OTUs,%	20.1%	17.3%	18.8%	20.3%	15.7%	18.9%
Morisita-Horn SI	0.370	0.927	0.361	0.456	0.550	0.339
(SE)	(0.002)	(0.001)	(0.001)	(0.001)	(0.002)	(0.001)
% change	(Reference)	151%	-2%	23%	49%	-8%
Between-group						
Observed OTUs, N						
Carriers, pooled	243	136	133	139	180	146
Noncarrier	100	121	147	115	99	118
Shared OTUs,%[a]						
Carriers, pooled	**36.6%**	61.8%	70.7%	55.4%	**41.7%**	**54.1%**
Noncarrier	**89.0%**	69.4%	63.9%	67.0%	**75.8%**	**66.9%**
Morisita-Horn SI	0.851	0.863	0.756	0.466	0.618	0.463
(SE)	(0.023)	(0.002)	(0.002)	(0.002)	(0.002)	(0.002)
% change	(Reference)	1%	-11%	-45%	-27%	-46%

Abbreviations: OTU, operational taxonomy unit; SI, similarity index; SE, standard error

a. Two-sample proportional test showed significant differences in proportions of shared OTUs by the initial carriage status at T0 (P<.001), T4 (P<.001), and T5 (P=.034).

Figure legends

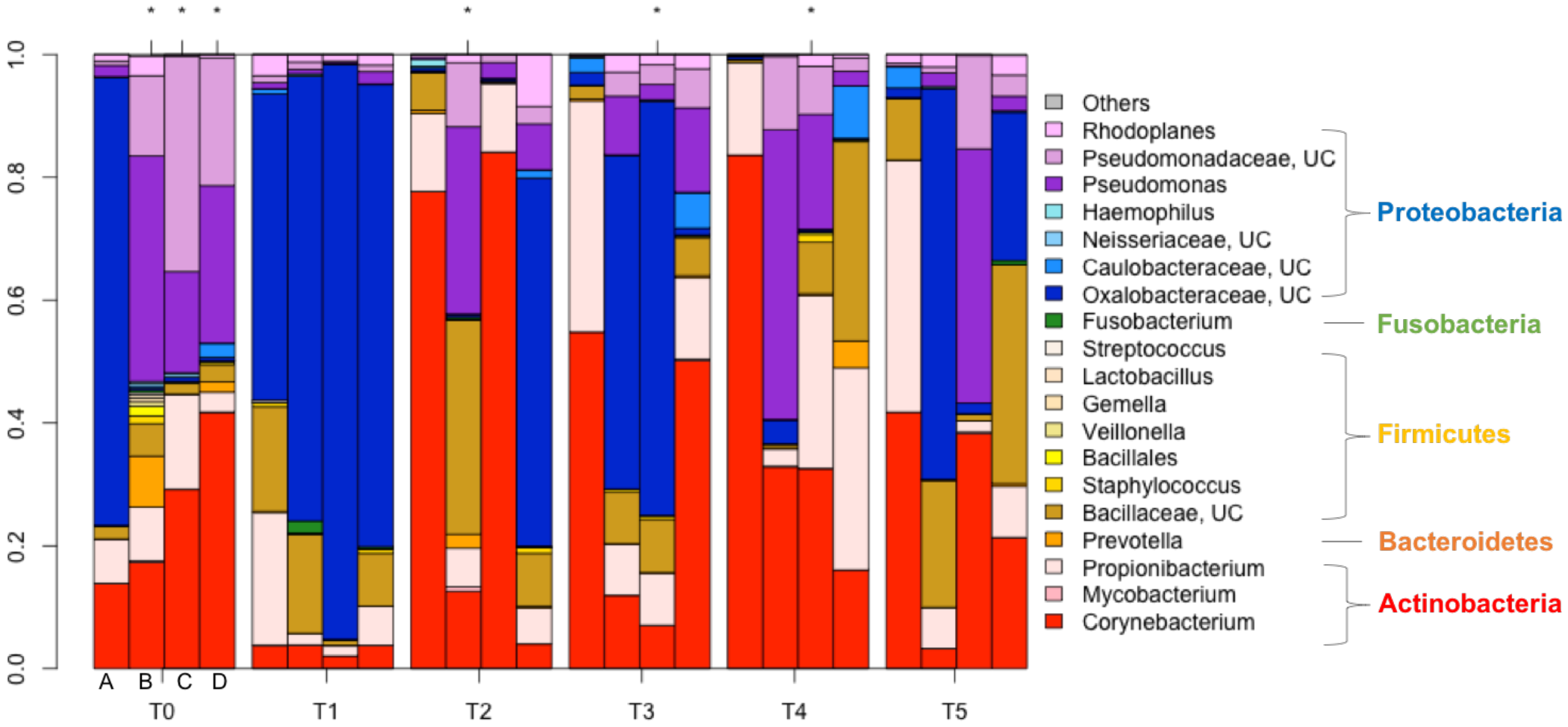
Figure 1. Relative abundance of dominant genera and culture results for *S. aureus* (*) in indivisual study subject (A-D) at each sampling time (T0-T5). (UC=unclassified)

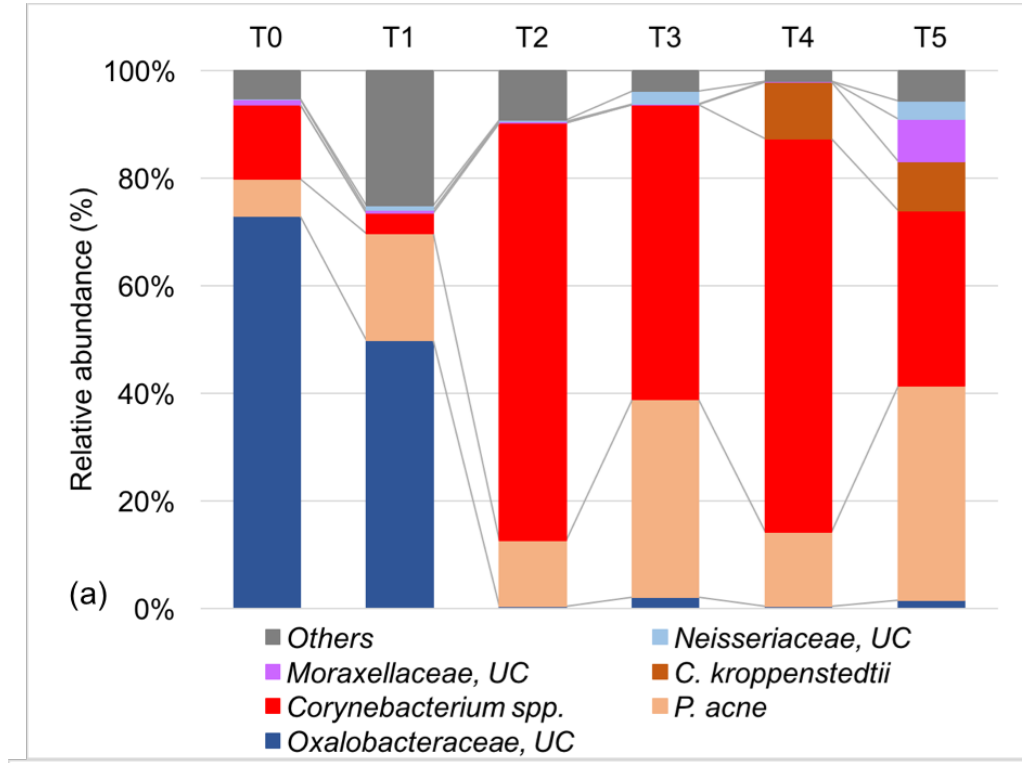
Figure 2. Temporal dynamics of most-changed OTUs (≥ 2%) in the noncarrier (a) and carriers (b). (UC=unclassified)

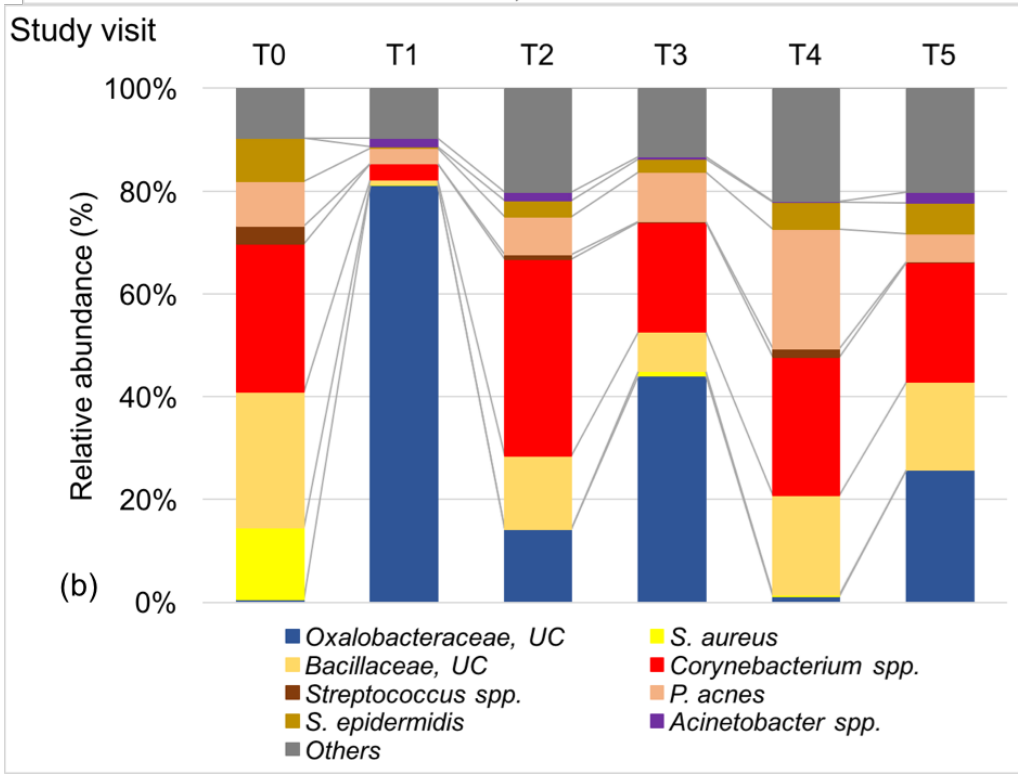
Figure 3. Alpha diversity measures at each sampling time (a) and by the initial staphylococcal carriage status (b). (pre_carrier=initial carriage status)

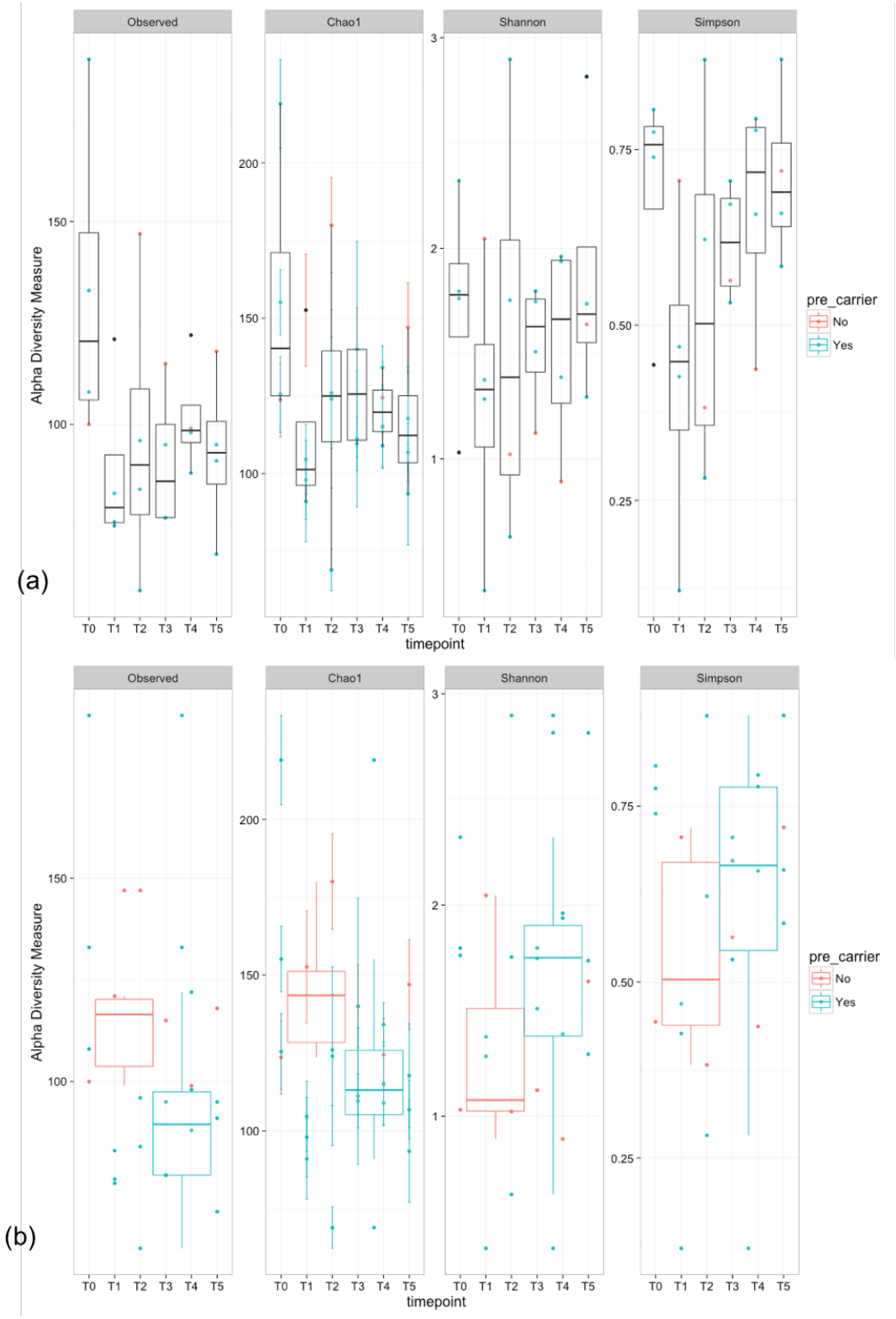
Figure 4. (a) Venn diagram showing relative abundance of sample-specific and shared community members at the pre-treatment visit (T0). (b) Results of differential expression analysis showing specific OTUs that were substantially enriched or depleted in samples collected from carriers versus the noncarrier. (NA=unclassified)

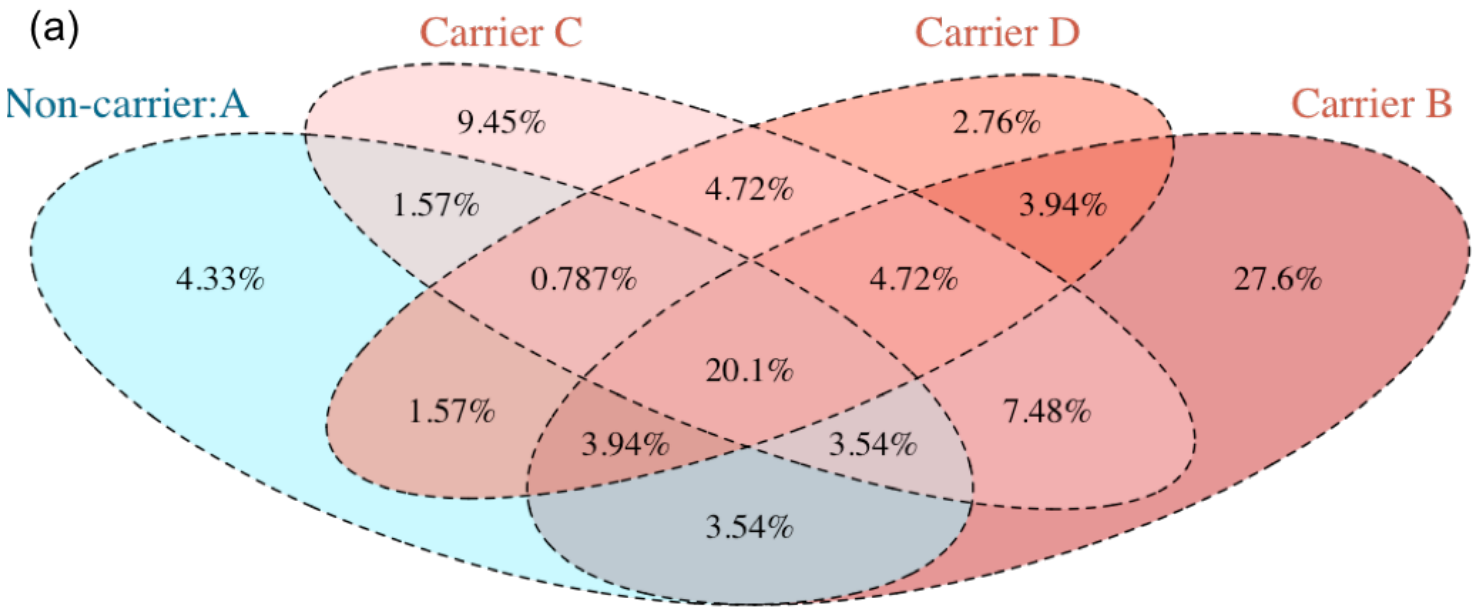
Figure 5. Results of multivariable-constrained correspondence analysis (CCA) showing the first two axes in grouping OTUs and samples by host factors, including the initial staphylococcal carriage status ("Pre-trt carrier"), levels of antimicrobial HNP1, and sampling times (as a categorical covariate). Letters denote individual study subjects and numbers specify sampling times, all corresponding to Figure 1.

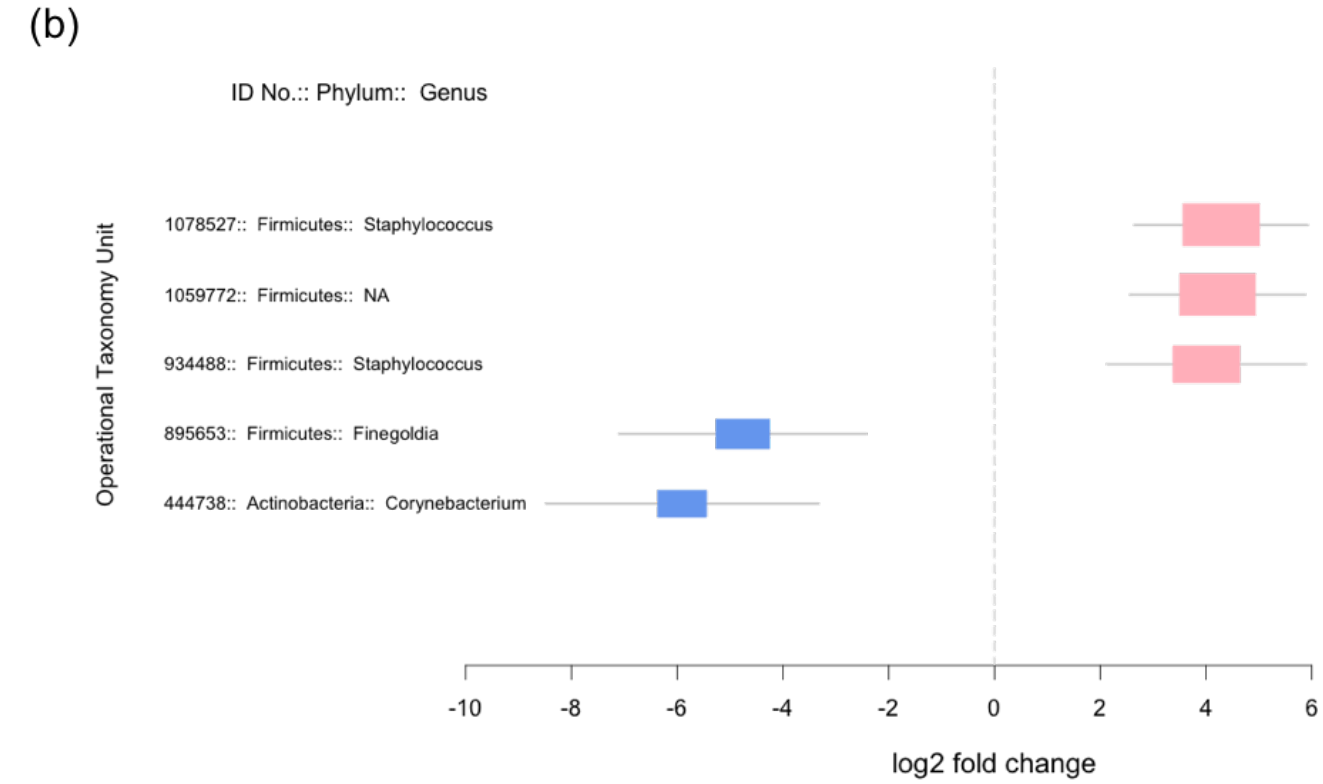

* * *
*
*
*
1.0
0.8
0.6
0.4
0.2
0.0
A B C D
T0
T1
T2
T3
T4
T5
Others
Rhodoplanes
Pseudomonadaceae, UC
Pseudomonas
Haemophilus
Neisseriaceae, UC
Caulobacteraceae, UC
Oxalobacteraceae, UC
Fusobacterium
Streptococcus
Lactobacillus
Gemella
Veillonella
Bacillales
Staphylococcus
Bacillaceae, UC
Prevotella
Propionibacterium
Mycobacterium
Corynebacterium
Proteobacteria
Fusobacteria
Firmicutes
Bacteroidetes
Actinobacteria

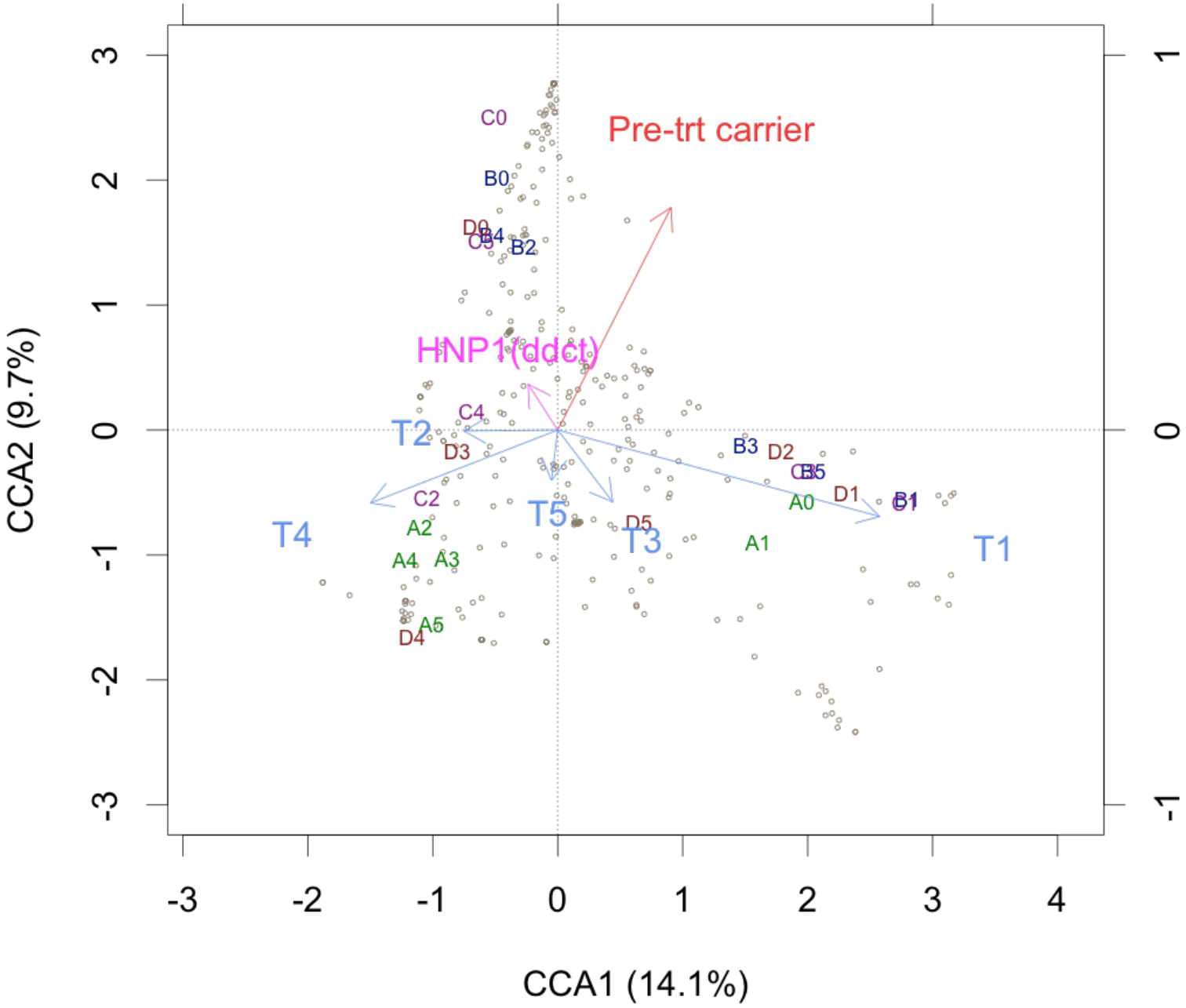

T0
T1
T2
T3
T4
T5
100%
80%
60%
40%
20%
0%
Relative abundance (%)
(a)
Others
Neisseriaceae, UC
Moraxellaceae, UC
C. kroppenstedtii
Corynebacterium spp.
P. acne
Oxalobacteraceae, UC


Study visit
T0
T1
T2
T3
T4
T5
100%
80%
60%
40%
20%
0%
Relative abundance (%)
(b)
Oxalobacteraceae, UC
S. aureus
Bacillaceae, UC
Corynebacterium spp.
Streptococcus spp.
P. acnes
S. epidermidis
Acinetobacter spp.
Others


(a)
Observed
Chao1
Shannon
Simpson
Alpha Diversity Measure
timepoint
T0 T1 T2 T3 T4 T5
pre_carrier
No
Yes
(b)
Observed
Chao1
Shannon
Simpson
Alpha Diversity Measure
timepoint
T0 T1 T2 T3 T4 T5
pre_carrier
No
Yes


(a)
Carrier C
Carrier D
Non-carrier:A
Carrier B
9.45%
2.76%
1.57%
4.72%
3.94%
4.33%
0.787%
4.72%
27.6%
20.1%
1.57%
7.48%
3.94%
3.54%
3.54%


(b)
ID No.:: Phylum:: Genus
Operational Taxonomy Unit
1078527:: Firmicutes:: Staphylococcus
1059772:: Firmicutes:: NA
934488:: Firmicutes:: Staphylococcus
895653:: Firmicutes:: Finegoldia
444738:: Actinobacteria:: Corynebacterium
-10
-8
-6
-4
-2
0
2
4
6
log2 fold change


Pre-trt carrier
HNP1(ddct)
T1
T2
T3
T4
T5
C0
B0
D0
C5
B4
B2
C4
D3
C2
A2
A4
A3
A5
D4
B3
D2
C3
B5
D1
A0
B1
D5
A1
CCA1 (14.1%)
CCA2 (9.7%)
-3
-2
-1
0
1
2
3
4