

1 A CROSS-SECTIONAL STUDY OF *STAPHYLOCOCCUS AUREUS* COLONIZATION AND
2 THE NASAL AND OROPHARYNGEAL MICROBIOMES

3 Authors/ affiliations: Ashley E. Kates, PhD¹; Mark Dalman, PhD²; James C. Torner, PhD¹; Tara
4 C. Smith, PhD²

5 ¹The University of Iowa, Iowa City, Iowa

6 ²Kent State University, Kent, Ohio

7 Corresponding Author: Tara C. Smith, PhD

8 Mailing address: Department of Biostatistics, Epidemiology and Environmental
9 Health Services, College of Public Health, Kent State University, 750 Lowry
10 Hall, Kent, OH 44240

11 Phone number: 330-672-3946

12 Fax number: 330-672-6505

13 Email address: tsmit176@kent.edu

14

15

16

17

18

19

20

21

22

23

24 **Abstract**

25 **Background:** *Staphylococcus aureus* is a frequent cause of both infections globally.

26 Colonization with the organism is known to increase the risk of developing infections and occurs
27 in roughly one third of the general population. While many factors influence colonization, it has
28 been demonstrated other members of the microbiome influence colonization with *S. aureus*.

29 Here, we assessed the nasal and oropharyngeal microbiomes of healthy participants in relation to
30 *S. aureus* colonization in a cross-sectional study using 16s rRNA sequencing of the v1-v3 region.

31 As livestock workers have also been shown to be at an increased risk of carriage, we have also
32 assessed microbiota differences in colonization status in a population of livestock workers.

33 **Results:** In both the nares and oropharynx, there were no microbiota differentially abundant
34 between colonized and non-colonized persons. However, there was a significant difference in the
35 beta diversity (Bray-Curtis distances) between carriers and non-carriers ($P=0.002$). When
36 considering carriage stratified by livestock exposure, there were a number of differences. Most
37 notably, colonized livestock workers had significantly more *Porphyomonas* (2-fold change = -
38 8.54, $P = 0.03$) than the non-colonized livestock workers.

39 **Conclusions:** *S. aureus* is a frequent colonizer of the human upper respiratory tract, including
40 the nares and oropharynx and causes a wide range of infections. Livestock workers are at
41 increased risk for carriage. Interventions such as improving oral hygiene may lead to decreased
42 *S. aureus* carriage by reducing other bacterial species such as *Porphyomonas*. Larger,
43 longitudinal studies are needed to better explore what microorganisms may be associated with *S.*
44 *aureus* colonization.

45

46 Keywords: *Staphylococcus aureus*, microbiome, epidemiology, 16s rRNA, colonization

47 **BACKGROUND**

48 *Staphylococcus aureus* is an important pathogen globally and is traditionally associated
49 with hospital-, community-, and most recently, livestock-associated infections. It is one of the
50 most frequent causes of bacterial infections and has traditionally been the leading cause of skin
51 and soft tissue infections [1]. It has been estimated roughly one third of the general population
52 carry *S. aureus* [2] and 1.5% - 3% carry methicillin-resistant *S. aureus* (MRSA) [3]. While
53 carriage itself is not necessarily harmful to the individual, it is a known risk factor for developing
54 infections [4].

55 Colonization and infection with *S. aureus* has been extensively studied [5-7],
56 predominantly in the hospital [8-10] and community [11, 12] settings; however, it remains
57 unclear why some individuals persistently carry the bacteria while others do not, and why some
58 individuals suffer repeated infections with *S. aureus* despite rounds of antibiotic treatments.
59 Previous studies have shown other bacteria present on the body may decrease the likelihood of
60 carrying *S. aureus* [13-16]. However, previous studies have been primarily based on traditional
61 culture methods which underestimate the complexity of the microbiome.

62 In order to understand how microorganisms what bacteria may be important in preventing
63 *S. aureus* colonization and infection, it is necessary to characterize what bacteria are present in
64 the niches *S. aureus* inhabits. In this study we aim to determine what differences exist between
65 carrier and non-carriers. Additionally, we aimed to determine if there were differences in the
66 microbiota based on carrier status stratified by livestock exposure.

67 **METHODS**

68 ***Study Population***

69 Participants were enrolled into a cross-sectional study between April 2015 and March
70 2016 in Eastern Iowa. Participants were enrolled in their homes by the research team. A portion
71 of the participants were enrolled from a prior, longitudinal study of *S. aureus* colonization.
72 Participants enrolled from that study were selected to provide as even a number of colonized and
73 non-colonized persons as possible. The remaining participants were enrolled from the
74 community in one of 3 ways: through the Iowa Department of Natural Resources animal feeding
75 operations database; a booth at Iowa county fairs; and lastly through snowball sampling where
76 the research team asked already enrolled participants to invite community member they thought
77 might be interested to participate. Eligibility criteria were: 18 years of age, speak English, have
78 not taken antibiotics or inhaled corticosteroids in the prior three months, not had the nasal
79 influenza vaccine in the last month, no active infections of the upper respiratory tract, no
80 hospitalized for greater than 24 hours in the last three months, and did not have HIV/AIDS. We
81 also requested participants not eat, drink, or brush their teeth within one hour of sample
82 collection. The University of Iowa institutional review board approved all study protocols prior
83 to recruitment.

84 After consenting, participants filled out questionnaires assessing demographic
85 characteristics, medical history, and animal contact. Participants provided swabs from their
86 anterior nares and oropharynx using sterile, dry, nylon flocked swabs (Copan Diagnostics,
87 Murrieta, CA) collected by a trained researcher and transported to the University of Iowa Center
88 for Emerging Infectious Diseases for processing. Bacterial DNA was isolated using the MO BIO
89 PowerSoil DNA isolation kit (MO BIO Laboratories Inc, Carlsbad, CA) adapted for swab use by
90 removing the swab head and placing it in the tube during bead beating. Negative controls (kit
91 reagents only) were used for every batch of extractions. Samples were sent for sequencing

92 (including library preparation) to the University of Minnesota Genomics Center. 16s rRNA
93 sequencing of the v1-v3 region was done on the Illumina MiSeq using 2x300 nt reads.

94 **Statistical analysis**

95 Sequences were assessed for quality using FastQC (Babraham Institute, Cambridge, UK)
96 with poor quality reads filtered out (poor quality sequencing reads are defined as sequences with
97 low base quality scores, short reads less than 200bp, reads with uncalled nucleotide bases, or any
98 reads that could not assemble into paired reads). Reads were assembled using FLASh with the
99 following parameters: minimum overlap = 30, maximum overlap = 150, and mismatch = 0.1
100 [17]. Adapters were removed from the merged file using Cutadapt [18]. USEARCH version
101 8.1.1861 and Python version 2.7.12 were used for chimera removal, operational taxonomic unit
102 (OTU) binning, and taxonomy assignment at the genus level. The Ribosomal Database Project
103 (RDP) classifier was used as the reference database. OTUs were grouped together based on 97%
104 similarity. Any species level classification was done using BLAST+2.4.0 and the blastn function.
105 Human-associated OTUs were also removed from the dataset using BLAST+2.4.0 and the blastn
106 function.

107 The primary research question was to assess any difference in the microbiomes of *S.*
108 *aureus* colonized persons compared to non-colonized persons in the nares and oropharynx, In
109 addition to this, we assessed in any differences in the nasal and oropharyngeal microbiomes
110 existed when also considering whether the subject had contact with livestock. This was done by
111 stratifying the colonized and non-colonized persons by livestock contact as well as stratifying
112 those with and without livestock contact by their colonization status.

113 R version 3.3.1 was used for all statistical analyses and plot generation using the
114 following packages: phyloseq [19], vegan [20], DESeq2 [21], and ampvis [22]. Alpha diversity

115 was assessed using the Inverse Simpson diversity index [23] and beta diversity was assessed
116 using the Bray-Curtis dissimilarity measure [24]. Principal coordinates analysis (PCoA) was
117 used to visualize beta diversity. Permutational multivariate analysis of variance
118 (PERMANOVA), through the vegan package, was used to assess diversity differences between
119 groups. PERMANOVA was chosen because it does not assume any distribution, unlike
120 parametric tests [25]. The DESeq2 and ampvis packages were used to assess microbiota
121 differences between groups. Results were considered significant if the P was less than 0.05.

122 RESULTS

123 *Participant demographics*

124 Fifty-nine participants were enrolled into the study, 37 of which were colonized in either
125 the nares, oropharynx, or both. Thirty participants were colonized in the nares and seven in the
126 oropharynx. The average age of participants was 54.6 years (range: 28-85 years) and 69.5% were
127 male. Fifty-eight participants identified as Caucasian (98.3%) and five identified as another race
128 (8.5%). Twenty-six participants had contact with one or more types of livestock. Animal types
129 included swine (n=18), cattle (n=12), poultry (n=4), sheep (n=4), horses (n=2) and goats (n=1).

130 *Microbiota comparisons by colonization status*

131 There was no significant difference in inverse Simpson's diversity index between
132 colonized and non-colonized persons in either the nares ($P = 0.376$) or the oropharynx ($P =$
133 0.728) (Figure 1). The PCoA of the Bray-Curtis distances for all samples is shown in Figure 2.
134 The samples cluster by both colonization status ($P > 0.001$) and sample type ($P > 0.001$). When
135 considering only the nasal samples, the significant difference between the colonized and non-
136 colonized persons remained ($P = 0.002$) (Figure 2b); however, there was no difference between
137 the colonized and non-colonized clusters in the oropharyngeal samples ($P = 0.899$) (Figure 2c).

138 Figure 3 shows the relative abundances of all OTUs in the colonized and non-colonized
139 persons in the nares and oropharynx. There is a great deal of similarity between colonized and
140 non-colonized person's microbiota in the nasal samples, though there are several OTUs that are
141 abundant in a greater number of colonized samples. The oropharyngeal microbiomes of
142 colonized persons and non-colonized persons look very similar. OTUs belonging to the
143 Firmicutes and Actinobacteria phyla dominate the microbiomes of the colonized and non-
144 colonized persons; however, colonized persons have a greater amount of OTUs belonging to the
145 Firmicutes phylum.

146 In the nasal microbiome, there was only one differential OTU, *S. aureus*. While the other
147 *Staphylococcus* species were not significantly more abundant in the colonized persons, colonized
148 persons did have more *Staphylococcus* OTUs compared to those not colonized with *S. aureus*.
149 Other *Staphylococcus* species present were *S. epidermidis*, *S. cohnii*, *S. lentus*, *S. hominis*, *S.*
150 *lugdunensis*, and *S. massiliensis*. *S. epidermidis* was the most prevalent non-*aureus*
151 *Staphylococcus* species and was present in 100% (n=30) of the *S. aureus* colonized nasal
152 samples compared to 89.6% (n=26) in the non-colonized samples. *S. cohnii* was the second most
153 prevalent at 76.7% (n=23) of *S. aureus* colonized persons and 62.1% (n=18) of non-colonized
154 samples. *S. homini* was prevalent in 60% (n=18) of colonized persons and 55.2% (n=16) of non-
155 colonized persons. The largest difference between colonized and non-colonized persons was with
156 *S. lentus* at 20% (n=6) in the colonized compared to 6.9% (n=2) in the non-colonized. Colonized
157 persons also had slightly higher amounts of Proteobacteria (*Haemophilus* and an unclassified
158 genus), Actinobacteria (*Rothia*), and Bacteroidetes (*Prevotella*). Non-colonized persons had
159 higher amounts of Actinobacteria (*Corynebacterium*) and Cyanobacteria/Chloroplast
160 (*Streptophyta*) compared to the colonized (Figure 4a).

161 There were no OTUs significantly different between the colonized and non-colonized
162 persons in the oropharynx. Many of the most abundant OTUs were similarly abundant in both
163 the colonized and non-colonized persons. *Saccharibacteria genera incertae sedis* was slightly
164 more abundant in the oropharynx of colonized persons. Bacteroidetes (*Porphyomonas*) was
165 slightly more abundant among non-colonized persons (Figure 4b). Of the *Staphylococcal*
166 species, *S. epidermidis* was the most prevalent and was in 100% (n=7) in the oropharynx of
167 colonized persons compared to 35.6% (n=21) of the non-colonized persons. *S. cohnii* was present
168 in the oropharynx of 42.9% (n=3) of the colonized persons and 28.6% (n=2) of the non-
169 colonized persons.

170 **Colonization stratified by livestock contact**

171 The Inverse Simpson's diversity index was significantly greater for livestock workers in
172 the nares ($P = 0.0015$) though not different in the oropharynx ($P = 0.571$); however, there was no
173 difference in livestock exposure based on colonization status in either the nares ($P = 0.815$) or
174 the oropharynx ($P = 0.484$) (Figure 5a, b). The ordination plots of colonization status and
175 livestock exposure in the nares show the samples significantly cluster by colonization status ($P =$
176 0.001) and livestock exposure ($P = 0.001$) (Figure 6a); however, there are no significant clusters
177 in the oropharyngeal samples (Figure 6b). When considering only colonized persons, samples
178 associated with livestock exposure clustered separately from those without livestock exposure (P
179 = 0.004). Samples associated with livestock exposure cluster together in the non-colonized
180 persons ($P = 0.021$) (Figure 6c, d).

181 The most abundant OTUs in the nasally colonized persons belonged to the Firmicutes,
182 Actinobacteria, and Proteobacteria. Fifteen OTUs were significantly differentially abundant
183 between *S. aureus* colonized livestock workers and non-livestock workers. The majority of the

184 significantly differential OTUs belonged to the Firmicutes phylum (Figure 7a). In the nasally
185 non-colonized persons, Actinobacteria was the most abundant phylum followed by the
186 Firmicutes, and Proteobacteria. Fewer differences were seen in abundance by livestock contact
187 compared to the colonized persons. Seven OTUs were significantly more abundant in the non-
188 colonized livestock workers compared to the non-colonized non-livestock workers and all
189 belonged to the Firmicutes phylum (Figure 7b).

190 Livestock workers oropharyngeally colonized with *S. aureus* had less *Streptococcus*,
191 *Prevotella*, *Heamophilus*, *Campylobacter* and *Veillonella* than oropharyngeally colonized non-
192 livestock workers and had greater abundances of *Rothia*, *Fusobacterium*, *Neisseria*, and
193 *Lachnoanerobaculum* than non-livestock workers. *Alloprevotella* was the only significantly
194 differentially abundant OTU and was more abundant in oropharyngeally colonized, non-
195 livestock workers (2-fold change: 5.17, adjusted $P = 0.0006$). Among the oropharyngeally non-
196 colonized persons, the abundances between livestock workers and those without livestock
197 contact were very similar and there were not significantly differentially abundant OTUs.

198 When considering livestock exposure stratified by colonization status, there were no
199 differences in OTUs between the colonized and non-colonized livestock workers with the
200 exception of *S. aureus* in the nares. The same was true for those without livestock exposure in
201 the nares. However, in the oropharynx, colonized livestock workers had significantly more
202 *Porphyromonas* (2-fold change = -8.54, $P = 0.03$) than the non-colonized livestock workers.
203 Additionally, non-colonized livestock workers had significantly more *Atopobium* (2-fold change
204 = 6.25, $P = 0.004$) compared to colonized livestock workers. There were no differences in the
205 microbiomes of colonized persons with no livestock contact compared to non-colonized persons
206 without livestock contact.

207 **DISCUSSION**

208 Understanding the ecologic niche *S. aureus* lives in is an important step in understanding
209 the biologic factors associated with colonization and infection prevention. Here we have
210 compared the microbiomes of the anterior nares and oropharynx and their relation to *S. aureus* in
211 59 healthy Iowans. Our population was predominantly male. This was because an aim of this
212 study was to assess livestock workers. In the US, the majority of livestock workers are older
213 males [26]. Male sex has been associated with increased risk of *S. aureus* carriage in non-
214 hospitalized patients [27]. *S. aureus* colonization occurred in the nares of 30 participants and the
215 oropharynx of seven participants. While were unable to identify any OTUs significantly
216 associated with either *S. aureus* presence or absence, there were several differences between
217 carriers and non-carriers, particularly in the nasal microbiome.

218 *S. aureus* nasal carriers' microbiomes were significantly different from the non-carriers
219 with regard to beta diversity ($P = 0.002$), though no individual OTUs were identified as
220 differential between the groups. However, colonized individuals have more Firmicutes compared
221 to the non-colonized participants while the non-colonized participants have more Actinobacteria.
222 This is similar to other studies which have found strong, significant inverse correlations between
223 the presence of Firmicutes and Actinobacteria in the nares [28] and have found communities
224 dominated by Actinobacteria – particularly *Propionibacterium* and *Corynebacterium* – have
225 fewer staphylococci [29]. While the differences were not significant, we also found
226 *Corynebacterium* to be more frequently present in non-carriers compared to carriers; however,
227 *Propionibacterium* was very similar between the two groups. *Corynebacterium* is one of the
228 most consistently negatively-associated genera with regard to *S. aureus* colonization [28-32].

229 *Corynebacterium* has been shown to inhibit the growth of *S. aureus* in culture, specifically with
230 regard to *C. pseudodiphtheriticum* [30].

231 Several other studies have found *S. epidermidis* to be both positively [30, 33] and
232 negatively associated with *S. aureus* colonization [29]. Here, we observed *S. epidermidis* to be
233 present 100% of the time when *S. aureus* was present in both the nares and the oropharynx. *S.*
234 *epidermidis* was the most prevalent *Staphylococcus* species present in 56 of the 59 nasal samples
235 and 28 of the oropharyngeal samples. The study by Frank et al. that identified *S. epidermidis* as
236 being negatively associated with *S. aureus* carriage ($P = 0.004$) was done on primarily patients in
237 intensive care units (ICUs) (n= 42) and only five non-hospitalized participants. It has been
238 shown ICU stays can change the composition of the microbiome [34] with large changes to the
239 ratios of Bacteroidetes and Firmicutes being observed in the gut microbiome [35]. It is possible
240 the ICU environment is affecting the relationship between *S. aureus* and *S. epidermidis* in the
241 Frank et al. study. It has also been hypothesized *S. epidermidis* will only inhibit *S. aureus*
242 colonization when *S. epidermidis* is able to express a serine protease, *esp* [36] and the positive
243 association between *S. aureus* and *S. epidermidis* is only observed when the majority of the *S.*
244 *epidermidis* strains present in the microbiome do not express *esp*. However, a recent study
245 demonstrated while *esp* was ubiquitous in all *S. epidermidis* strains tested, there was no
246 correlation with *S. aureus* inhibition [37]. Future studies are need comparing hospitalized
247 patients to healthy persons to better understand the association between the two microorganisms.

248 In the oropharynx, we observed a great deal of genus richness which is consistent with
249 other studies [28]. As with prior studies, we observed the oropharynx to be dominated by
250 Firmicutes as well as a high prevalence of Bacteroidetes and Proteobacteria [38]. Our inability to

251 see any differences in the oropharyngeal microbiomes between carriers and non-carriers is
252 possibly due to the small number of oropharyngeally colonized individuals (n=7).

253 When comparing the colonized individuals, colonized livestock workers had twenty-four
254 OTUs that were significantly more abundant than in the nasal microbiota of their colonized, non-
255 livestock worker counterparts. Almost all of these OTUs belonged to the Firmicutes phylum and
256 many have been found to be associated with livestock contact. It is possible that at least a portion
257 of livestock workers identified as *S. aureus* carriers are not truly colonized, only contaminated,
258 and that while they are continuously in the presence of aerosolized *S. aureus*, *S. aureus* is unable
259 to adhere to the epithelial cells. van Cleef et al, showed up to 94% of those testing positive after
260 working around livestock would test negative within 24 hours of the exposure [39]. As our study
261 was cross-sectional, it was not possible for us to determine the duration of colonization for those
262 participants with livestock exposure. We were able to assess how long it had been since the
263 livestock workers last contact with livestock. For swine and cattle, it had been around 24-30
264 hours since last contact, so it is likely any *S. aureus* presence is true colonization, not
265 contamination.

266 We also assessed the differences between livestock workers who were colonized and not
267 colonized in the nares and oropharynx to assess any microbial differences that may influence
268 whether a livestock worker was a carrier or not. While there were no significant differences in
269 the nares, colonized livestock workers were significantly more likely to carry *Porphyomonas*,
270 specifically *P. gingivalis* in the oropharynx while non-colonized livestock workers were more
271 likely to carry *Atopobium* in the oropharynx. Studies have shown in the presence of *P. gingivalis*,
272 *Streptococcus* spp. (which was significantly more abundant in colonized livestock workers) and
273 other members of the oropharyngeal microbiome will aggregate with *S. aureus*. Meaning even if

274 *S. aureus* is a contaminant instead of a true colonizing species in the oropharynx, when *P.*
275 *gingivalis* is present, *S. aureus* may adhere to the biofilms created by the other microorganisms.
276 *P. gingivalis* acts as a ‘coaggregation bridge’ mediating the attachment between two species that
277 would not otherwise aggregate [40]. Additionally, the presence of *P. gingivalis* in the oral cavity
278 is associated with poor oral health and hygiene [41]. In our study, livestock workers were
279 significantly less likely to brush their teeth daily ($P < 0.001$, data not shown) which has been
280 linked to poor oral health and gingivitis [42]. However, we were not able to identify an
281 association between oral hygiene and any differences in the microbiota in our study, likely due to
282 small sample sizes and an inadequate measure of oral hygiene. While this observation requires a
283 great deal of further study to better understand and quantify the relationship between *P.*
284 *gingivalis* and *S. aureus* as our numbers are small, the improved oral hygiene may help reduce
285 oropharyngeal *S. aureus* colonization.

286 This study was cross-sectional. As such, we were unable to assess any change in the
287 microbiome and how those changes may impact *S. aureus* colonization. It’s also highly possible
288 some of the intermittent *S. aureus* colonizers were mistakenly classified as not colonized [4]
289 which could mask any differences in the microbiomes.

290 CONCLUSIONS

291 The microbiome likely plays a crucial role in *S. aureus* colonization, and while we were
292 unable to identify any differential species, we did observe a difference in the beta diversity
293 between the colonized and non-colonized subjects. There is a great deal of difference between
294 colonized livestock workers and non-livestock workers as well as differences between colonized
295 and non-colonized livestock workers, specifically, *P. gingivalis*. Improving oral hygiene may
296 help to reduce oropharyngeal carriage of *S. aureus* and MRSA in livestock workers.

297 Longitudinal studies on larger populations are needed to better characterize the relationship
298 between the microbiome and *S. aureus* carriage. Understanding community compositions is a
299 necessary start understanding colonization, but does not provide a full picture of the relationship
300 between the microbiome and colonization with pathogenic organisms such as *S. aureus*. Studies
301 assessing the potential interactions between the host, the microbiome, and *S. aureus* are needed
302 in order to successfully manipulate the microbiome to inhibit *S. aureus* colonization.

303 **Funding**

304 This publication was supported in part by Grant Number 5 U54 OH007548-11 from
305 CDC-NIOSH. Its contents are solely the responsibility of the authors and do not necessarily
306 represent the official views of the CDC, NIOSH, or the Great Plains Center for Agriculture
307 Health. The funding organization had no influence on the study design, data analysis,
308 interpretation of data, or writing of the manuscript.

309 **Acknowledgments**

310 The authors would like to thank Dr. Patrick Breheny for his invaluable guidance as well
311 as the University of Minnesota Genomics Center for conducting the sequencing.

312

313

314

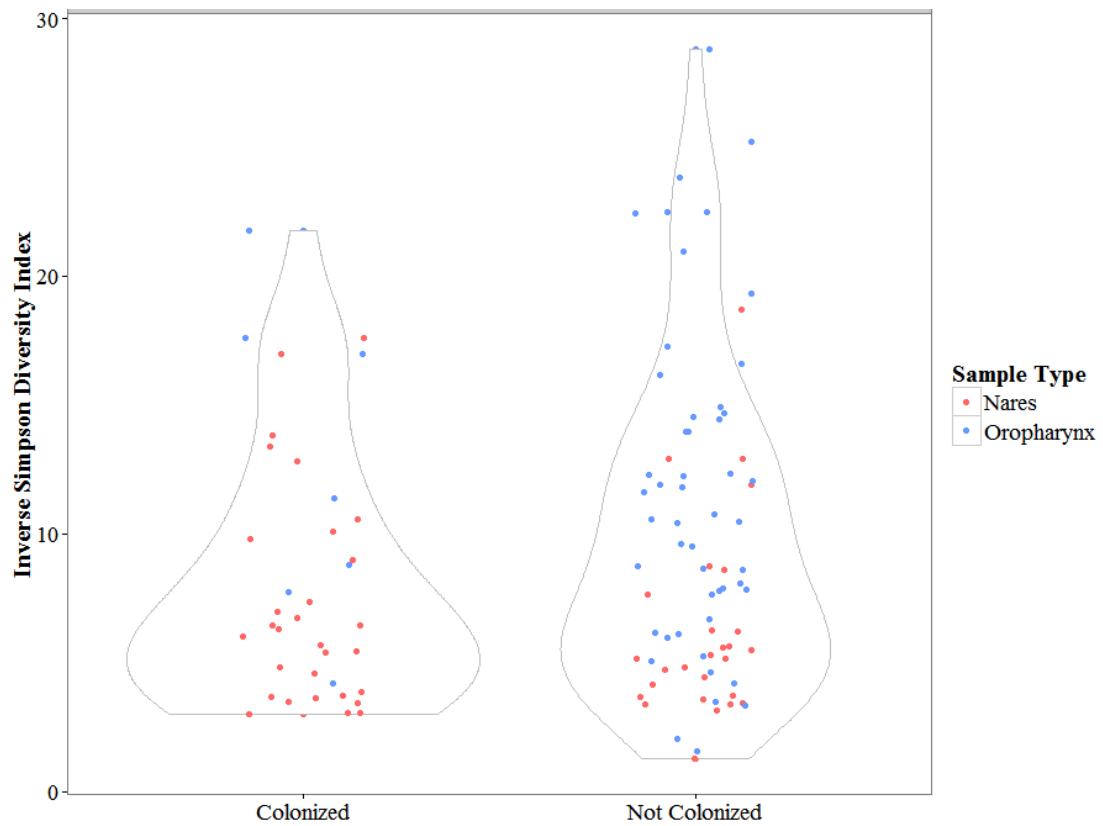
315

316

317

318

319 **FIGURES**



332 Figure 1: Alpha diversity of the colonized and non-colonized participants in the nares and
333 oropharynx.

334

335

336

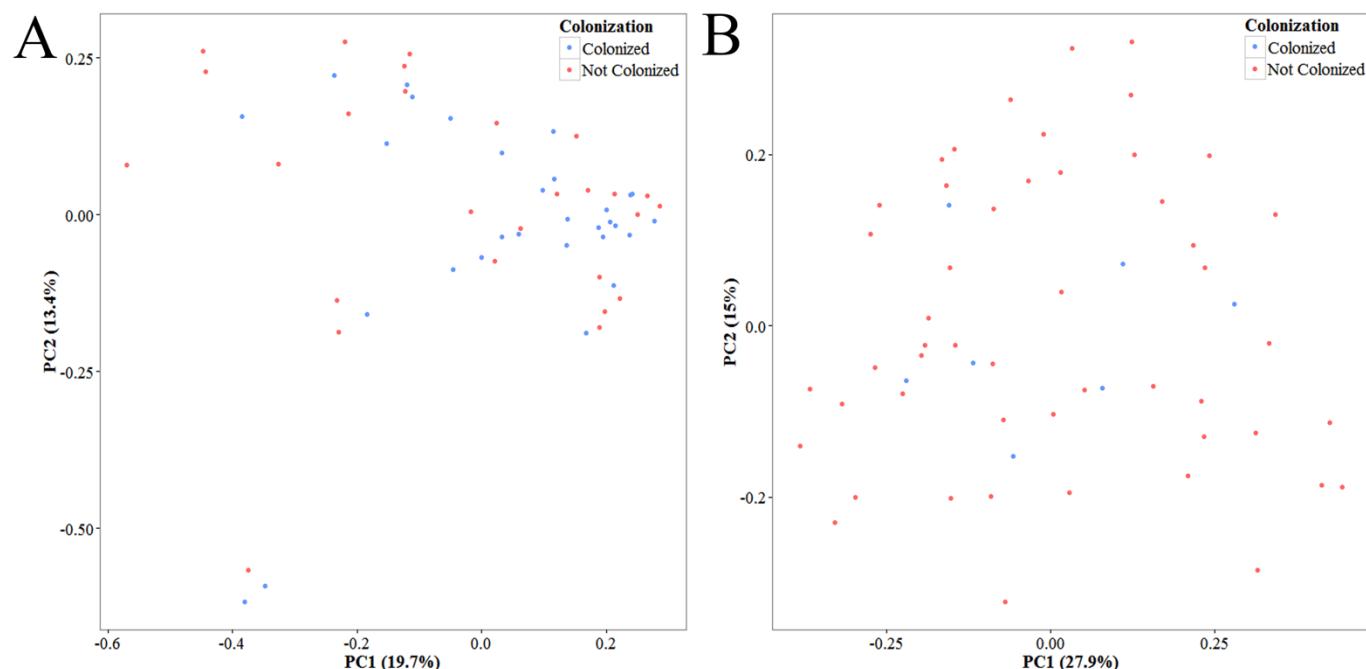
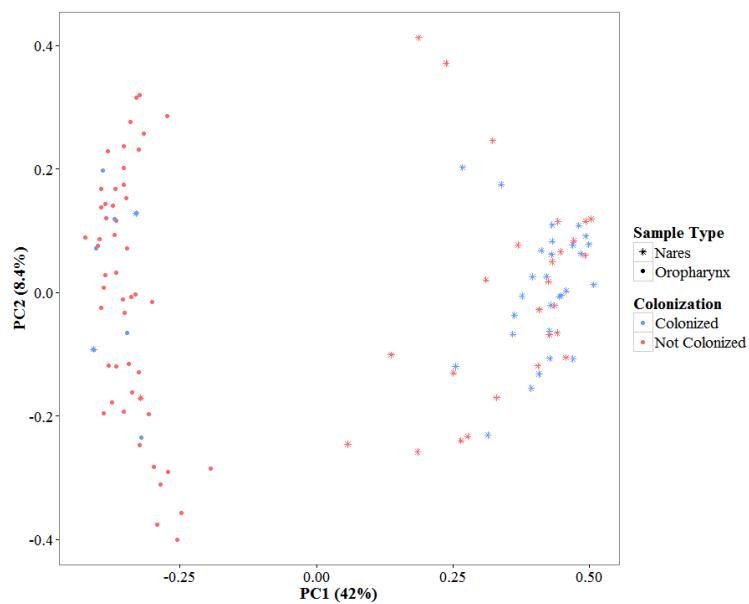
337

338

339

340

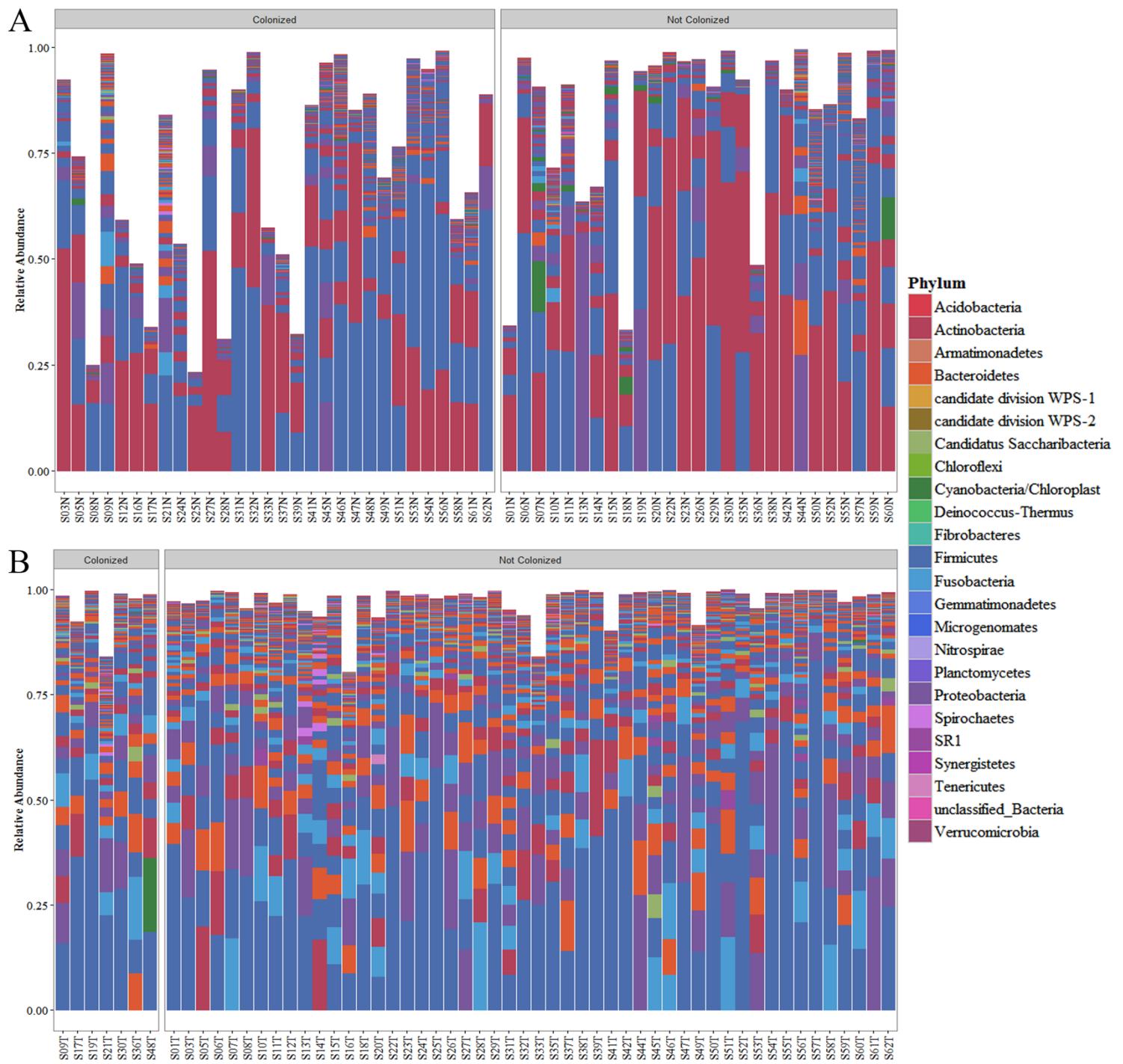
341



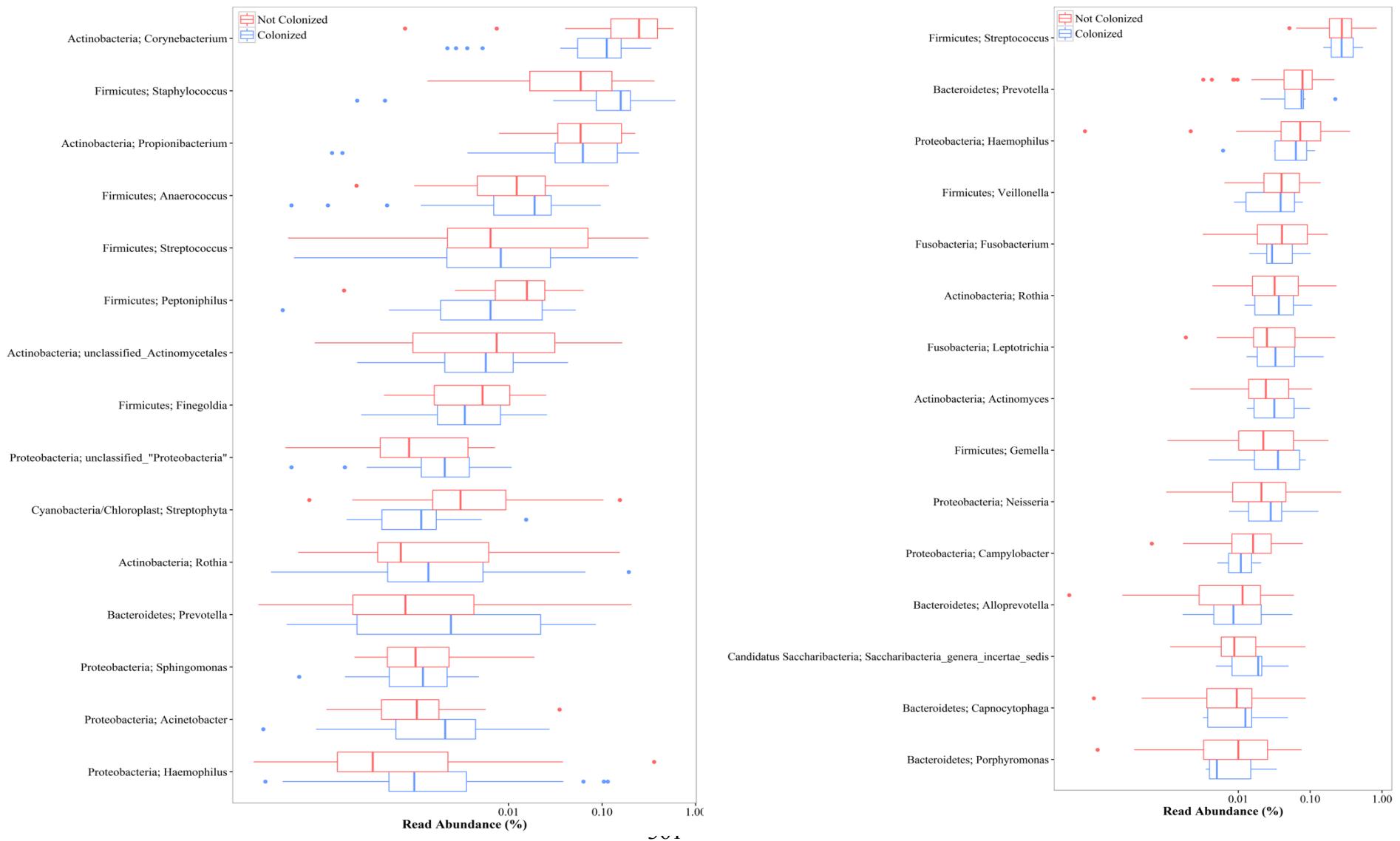
350 Figure 2: Principal coordinates analysis by colonization status. (a) Ordination based on the Bray-
351 Curtis dissimilarities of the nasal and oropharyngeal sample microbiomes. (b) Ordination based
352 on the Bray-Curtis dissimilarities of the nasal sample microbiomes. (c) Ordination based on the
353 Bray-Curtis dissimilarities of the oropharyngeal sample microbiomes. PC1 and PC2 = principal
354 coordinates 1 and 2, respectively.
355

356

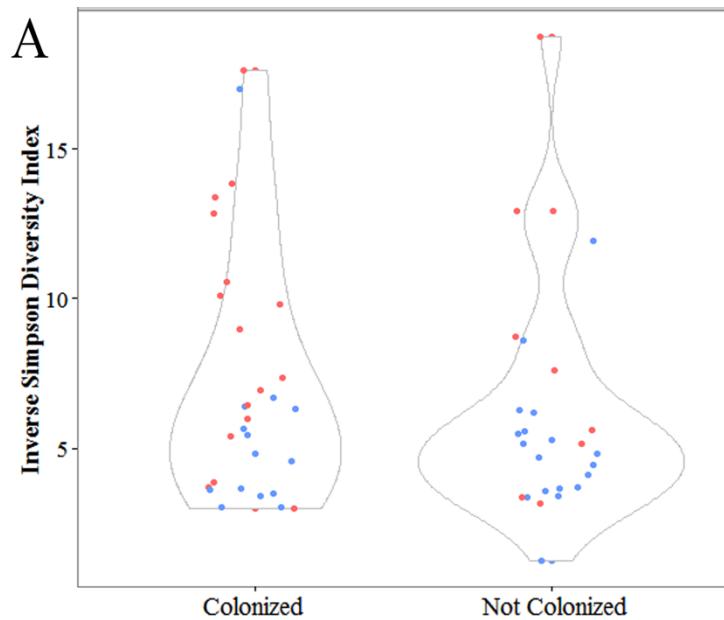
357



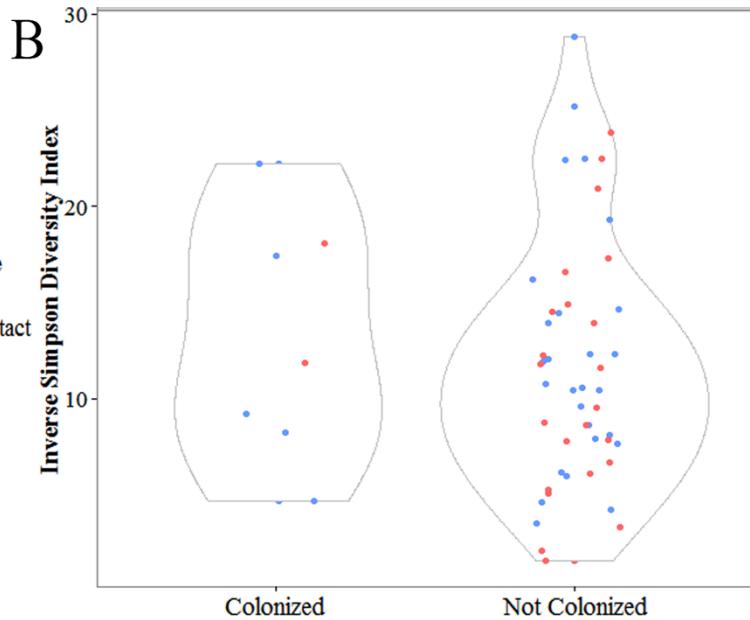
358 Figure 3: Relative abundances of bacterial phyla between colonized and non-colonized
 359 participants in the (a) nares and (b) oropharynx.
 360



362 Figure 4: Top 15 most abundant organisms in the (a) nares and (b) oropharynx.



Livestock Exposure
● Livestock Contact
● No Livestock Contact



363

364 Figure 5: Inverse Simpson Diversity index by colonization status and livestock exposure in the (a) nares and (b) oropharynx.

365

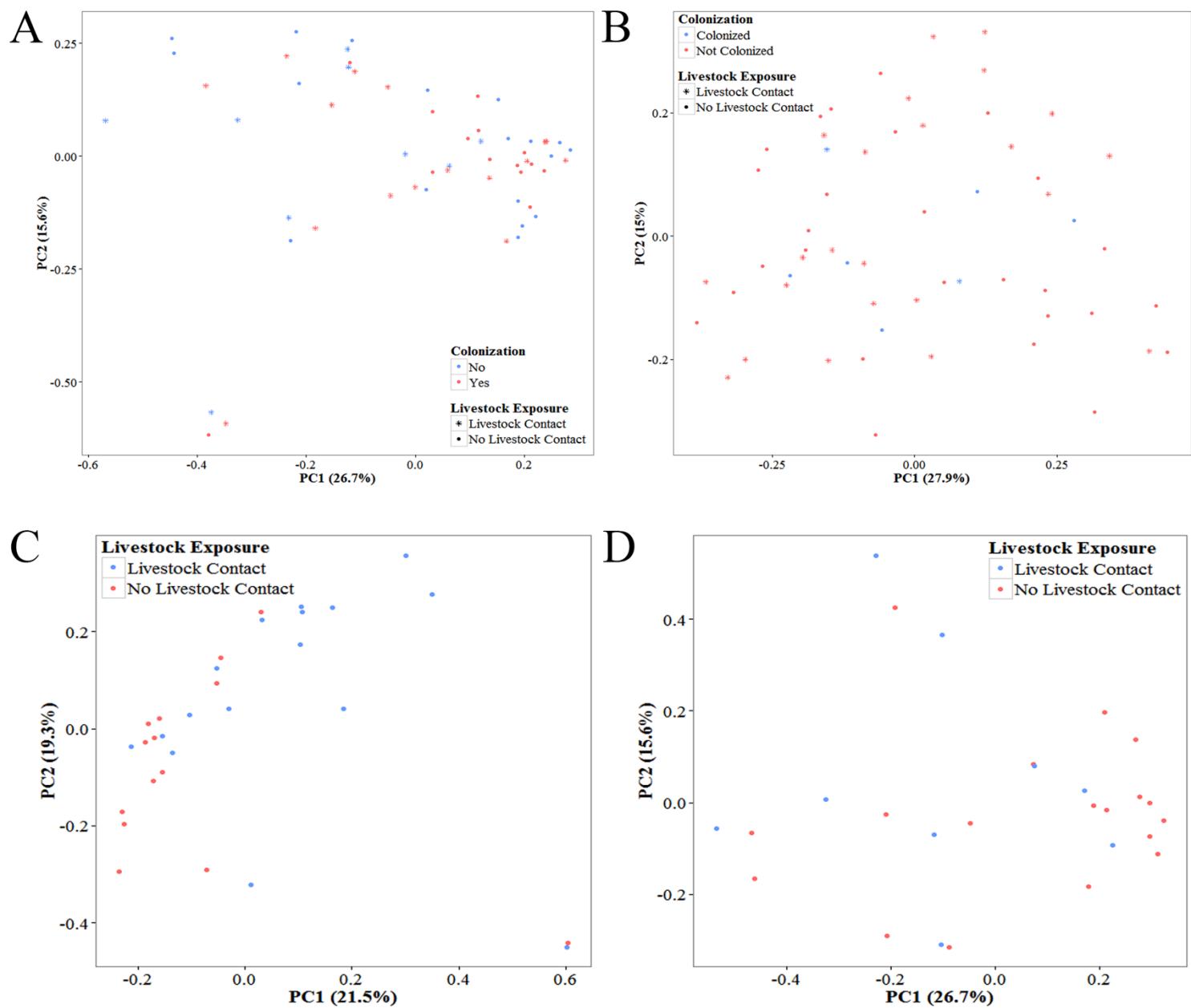
366

367

368

369

370



371 Figure 6: Principal coordinates analysis by colonization status and livestock exposure in the
372 nares and oropharynx. (a) Ordination based on the Bray-Curtis dissimilarity of the all nasal
373 samples. (b) Ordination based on the Bray-Curtis dissimilarity of all oropharyngeal samples.
374 (c) Ordination based on the Bray-Curtis dissimilarity of the colonized nasal sample
375 microbiomes. (d) Ordination based on the Bray-Curtis dissimilarity of the non-colonized nasal
376 sample microbiomes. PC1 and PC2 = principal coordinates 1 and 2, respectively.
377

378

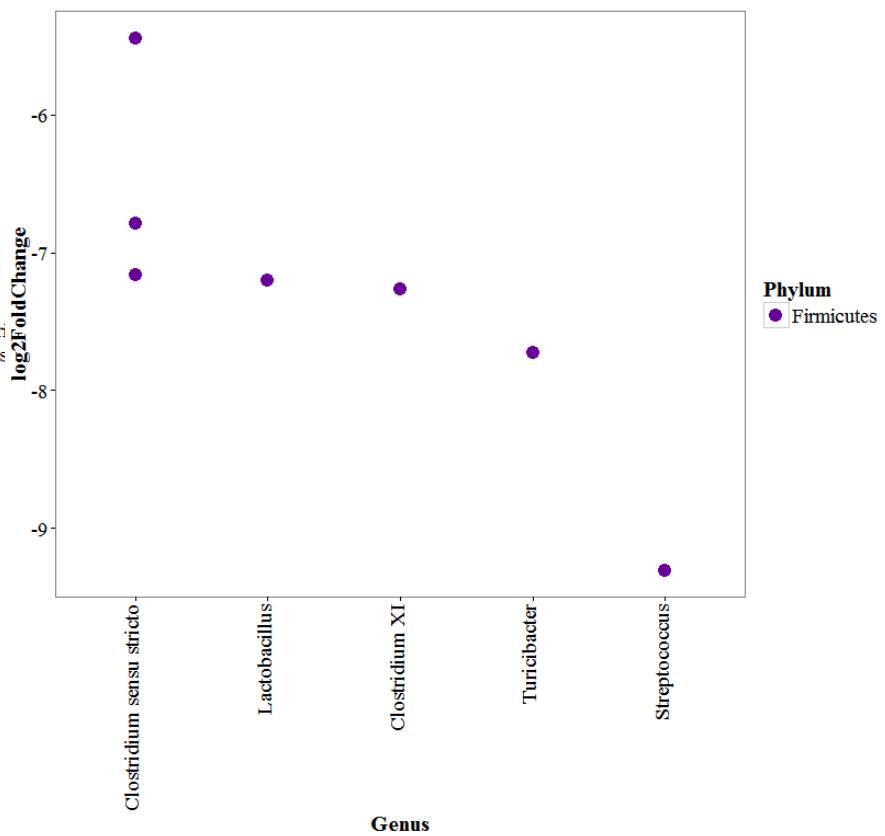
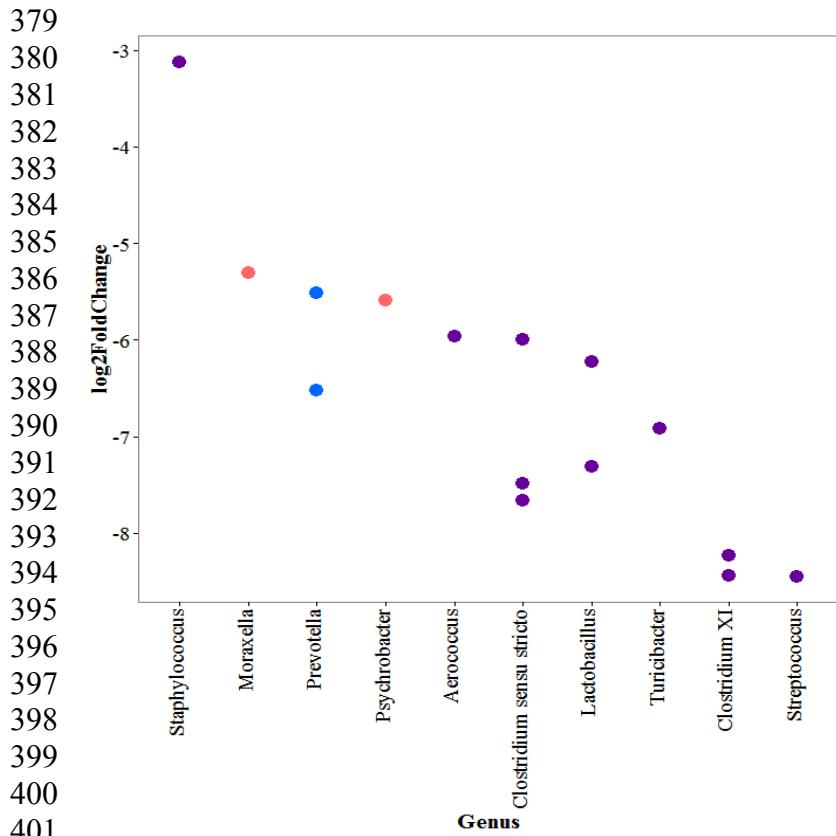


Figure 7: Differentially abundant organisms among colonized and non-colonized persons by livestock exposure. (a) Log 2-fold change of the significantly differentially abundant OTUs between colonized livestock workers and colonized persons with no livestock contact in the nares (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in colonized livestock workers and positive values represent OTUs significantly more abundant in colonized, non-livestock workers. (b) Log 2-fold change of the significantly differentially abundant OTUs between non-colonized livestock workers and non-colonized persons with no livestock contact (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in non-colonized livestock workers and positive values represent OTUs significantly more abundant in non-colonized, non-livestock workers.

412 **REFERECEES**

413 1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr.: **Staphylococcus**
414 **aureus infections: epidemiology, pathophysiology, clinical manifestations, and**
415 **management.** *Clinical microbiology reviews* 2015, **28**(3):603-661.

416 2. Bischoff WE, Wallis ML, Tucker KB, Reboussin BA, Sherertz RJ: **Staphylococcus**
417 **aureus nasal carriage in a student community: prevalence, clonal relationships, and**
418 **risk factors.** *Infection control and hospital epidemiology : the official journal of the*
419 *Society of Hospital Epidemiologists of America* 2004, **25**(6):485-491.

420 3. Dulan M, Peters C, Schablon A, Nienhaus A: **MRSA carriage among healthcare**
421 **workers in non-outbreak settings in Europe and the United States: a systematic**
422 **review.** *BMC infectious diseases* 2014, **14**:363.

423 4. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA,
424 Nouwen JL: **The role of nasal carriage in Staphylococcus aureus infections.** *The*
425 *Lancet Infectious diseases* 2005, **5**(12):751-762.

426 5. Karanika S, Kinamon T, Grigoras C, Mylonakis E: **Colonization With Methicillin-**
427 **resistant Staphylococcus aureus and Risk for Infection Among Asymptomatic**
428 **Athletes: A Systematic Review and Metaanalysis.** *Clinical infectious diseases : an*
429 *official publication of the Infectious Diseases Society of America* 2016, **63**(2):195-204.

430 6. Safdar N, Bradley EA: **The risk of infection after nasal colonization with**
431 **Staphylococcus aureus.** *Am J Med* 2008, **121**(4):310-315.

432 7. Nair R, Ammann E, Rysavy M, Schweizer ML: **Mortality among patients with**
433 **methicillin-resistant Staphylococcus aureus USA300 versus non-USA300 invasive**
434 **infections: a meta-analysis.** *Infection control and hospital epidemiology : the official*
435 *journal of the Society of Hospital Epidemiologists of America* 2014, **35**(1):31-41.

436 8. McKinnell JA, Miller LG, Eells SJ, Cui E, Huang SS: **A systematic literature review**
437 **and meta-analysis of factors associated with methicillin-resistant Staphylococcus**
438 **aureus colonization at time of hospital or intensive care unit admission.** *Infection*
439 *control and hospital epidemiology : the official journal of the Society of Hospital*
440 *Epidemiologists of America* 2013, **34**(10):1077-1086.

441 9. Ziakas PD, Anagnostou T, Mylonakis E: **The prevalence and significance of**
442 **methicillin-resistant Staphylococcus aureus colonization at admission in the general**
443 **ICU Setting: a meta-analysis of published studies.** *Critical care medicine* 2014,
444 **42**(2):433-444.

445 10. Glick SB, Samson DJ, Huang ES, Vats V, Aronson N, Weber SG: **Screening for**
446 **methicillin-resistant Staphylococcus aureus: a comparative effectiveness review.**
447 *American journal of infection control* 2014, **42**(2):148-155.

448 11. Salgado CD, Farr BM, Calfee DP: **Community-acquired methicillin-resistant**
449 **Staphylococcus aureus: a meta-analysis of prevalence and risk factors.** *Clinical*
450 *infectious diseases : an official publication of the Infectious Diseases Society of America*
451 **2003, 36**(2):131-139.

452 12. Dukic VM, Lauderdale DS, Wilder J, Daum RS, David MZ: **Epidemics of community-**
453 **associated methicillin-resistant Staphylococcus aureus in the United States: a meta-**
454 **analysis.** *PloS one* 2013, **8**(1):e52722.

455 13. Shinefield HR, Ribble JC, Boris M, Eichenwald HF, Aly R, Maibach H: **Bacterial**
456 **interference between strains of *S. aureus***. *Annals of the New York Academy of Sciences*
457 1974, **236**(0):444-455.

458 14. Dall'Antonia M, Coen PG, Wilks M, Whiley A, Millar M: **Competition between**
459 **methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares**. *The*
460 *Journal of hospital infection* 2005, **61**(1):62-67.

461 15. Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, Derazne E, Rahav G,
462 Rubinstein E: **Association between carriage of *Streptococcus pneumoniae* and**
463 ***Staphylococcus aureus* in Children**. *Jama* 2004, **292**(6):716-720.

464 16. Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke HC, Verbrugh
465 HA, Hermans PW: **Colonisation by *Streptococcus pneumoniae* and *Staphylococcus***
466 ***aureus* in healthy children**. *Lancet* 2004, **363**(9424):1871-1872.

467 17. Magoc T, Salzberg SL: **FLASH: fast length adjustment of short reads to improve**
468 **genome assemblies**. *Bioinformatics (Oxford, England)* 2011, **27**(21):2957-2963.

469 18. Martin M: **Cutadapt removes adapter sequences from high-throughput sequencing**
470 **reads**. *EMBnetjournal* 2011, **17**(1).

471 19. McMurdie PJ, Holmes S: **phyloseq: an R package for reproducible interactive**
472 **analysis and graphics of microbiome census data**. *PLoS one* 2013, **8**(4):e61217.

473 20. vegan: **Community Ecology Package**. R package version 2.4-0 [<https://CRAN.R-project.org/package=vegan>]

475 21. Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion**
476 **for RNA-seq data with DESeq2**. *Genome biology* 2014, **15**(12):550.

477 22. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH: **Back to Basics--The**
478 **Influence of DNA Extraction and Primer Choice on Phylogenetic Analysis of**
479 **Activated Sludge Communities**. *PLoS one* 2015, **10**(7):e0132783.

480 23. Simpson EH: **Measurement of Diversity**. *Nature* 1949, **163**:688.

481 24. Bray JR, Curtis JT: **An ordination of upland forest communities of southern**
482 **Wisconsin**. *Ecological Monographs* 1957, **27**:325-349.

483 25. McArdle BH, Anderson MJ: **Fitting Multivariable Models to Community Data: A**
484 **comment on Distance-based Redundancy Analysis**. *Ecology* 2001, **82**(1):290-297.

485 26. **2012 Census of Agriculture Highlights: Farm Demographics - U.S. Farmers by**
486 **Gender, Age, Race, Ethnicity, and more**
487 [https://www.agcensus.usda.gov/Publications/2012/Online_Resources/Highlights/Farm_Demographics/Highlights_Farm_Demographics.pdf]

489 27. Harbarth S, Sax H, Fankhauser-Rodriguez C, Schrenzel J, Agostinho A, Pittet D:
490 **Evaluating the probability of previously unknown carriage of MRSA at hospital**
491 **admission**. *Am J Med* 2006, **119**(3):275.e215-223.

492 28. Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R: **Comparative**
493 **analyses of the bacterial microbiota of the human nostril and oropharynx**. *mBio*
494 2010, **1**(3).

495 29. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR: **The human nasal**
496 **microbiota and *Staphylococcus aureus* carriage**. *PLoS one* 2010, **5**(5):e10598.

497 30. Yan M, Pamp SJ, Fukuyama J, Hwang PH, Cho DY, Holmes S, Relman DA: **Nasal**
498 **microenvironments and interspecific interactions influence nasal microbiota**
499 **complexity and *S. aureus* carriage**. *Cell host & microbe* 2013, **14**(6):631-640.

500 31. Kelly BJ, Bushman FD, Collman RG: **Nasal microbiome features of *Staphylococcus aureus* colonization and infection during critical illness.** In: *ASM Microbe 216; Boston, MA.* 2016.

501 32. Uehara Y, Nakama H, Agematsu K, Uchida M, Kawakami Y, Abdul Fattah AS, Maruchi N: **Bacterial interference among nasal inhabitants: eradication of *Staphylococcus aureus* from nasal cavities by artificial implantation of *Corynebacterium* sp.** *The Journal of hospital infection* 2000, **44**(2):127-133.

502 33. Kaspar U, Kriegeskorte A, Schubert T, Peters G, Rudack C, Pieper DH, Wos-Oxley M, Becker K: **The culturome of the human nose habitats reveals individual bacterial fingerprint patterns.** *Environmental microbiology* 2016, **18**(7):2130-2142.

503 34. Iapichino G, Callegari ML, Marzorati S, Cigada M, Corbella D, Ferrari S, Morelli L: **Impact of antibiotics on the gut microbiota of critically ill patients.** *Journal of medical microbiology* 2008, **57**(Pt 8):1007-1014.

504 35. Ojima M, Motooka D, Shimizu K, Gotoh K, Shintani A, Yoshiya K, Nakamura S, Ogura H, Iida T, Shimazu T: **Metagenomic Analysis Reveals Dynamic Changes of Whole Gut Microbiota in the Acute Phase of Intensive Care Unit Patients.** *Digestive diseases and sciences* 2016, **61**(6):1628-1634.

505 36. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, Agata T, Mizunoe Y: ***Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization.** *Nature* 2010, **465**(7296):346-349.

506 37. Fredheim EG, Flaegstad T, Askarian F, Klingenberg C: **Colonisation and interaction between *S. epidermidis* and *S. aureus* in the nose and throat of healthy adolescents.** *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2015, **34**(1):123-129.

507 38. Simor AE, Phillips E, McGeer A, Konvalinka A, Loeb M, Devlin HR, Kiss A: **Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization.** *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007, **44**(2):178-185.

508 39. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, Kluytmans JA: **Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves.** *J Clin Microbiol* 2011, **49**(3):1030-1033.

509 40. Kamaguchi A, Nakayama K, Ichiyama S, Nakamura R, Watanabe T, Ohta M, Baba H, Ohyama T: **Effect of *Porphyromonas gingivalis* vesicles on coaggregation of *Staphylococcus aureus* to oral microorganisms.** *Current microbiology* 2003, **47**(6):485-491.

510 41. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ: **Prevalence of *Porphyromonas gingivalis* and periodontal health status.** *J Clin Microbiol* 1998, **36**(11):3239-3242.

511 42. Barenie JT, Leske GS, Ripa LW: **The effect of toothbrushing frequency on oral hygiene and gingival health in school children: reassessment after two and one-half years.** *Journal of public health dentistry* 1976, **36**(1):9-16.

512 543