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1 # Microbial communities profiling in intensive care units expose
2 limitations in current sanitary standards

3

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22

23 ## ABSTRACT

24 Hospital-associated infections (HAIs) are the leading cause of morbidity and
25 mortality in intensive care units (ICUs) and neonatal intensive care units (NICUs).
26 Organisms causing these infections are often present on surfaces around the patient.
27 Given that microbiotas may vary across different ICUs, the HAI-related microbial
28 signatures within these units remain underexplored. In this study, we use deep-sequencing
29 analyses to explore and compare the structure of bacterial communities at inanimate
30 surfaces of the ICU and NICU wards of The Medical School Clinics Hospital (Brazil).
31 The data revealed that NICU presents higher biodiversity than ICU and surfaces closest
32 to the patient showed a peculiar microbiota, distinguishing one unit from the other.
33 Several facultative anaerobes or obligate anaerobes HAI-related genera were classified as

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biomarkers for the NICU, whereas *Pseudomonas* was the main biomarker for ICU. Correlation analyses revealed a distinct pattern of microbe-microbe interactions for each unit, including bacteria able to form multi-genera biofilms. Furthermore, we evaluated the effect of concurrent cleaning over the ICU bacterial community. The results showed that, although some bacterial populations decreased after cleaning, various HAI-related genera were quite stable to sanitization, suggesting being well-adapted to the ICU environment. Overall, these results enabled identification of discrete ICU and NICU reservoirs of potentially pathogenic bacteria and provided evidence for the presence of a set of biomarkers that distinguish these units. Moreover, the study exposed the inconsistencies of the routine cleaning to minimize HAI-related genera contamination.

IMPORTANCE

Due to the high impact of HAIs, there is an urgent need for the development of robust policies on microbial surveillance to help guide procedures, improving infection control. To the best of our knowledge, this is the first comprehensive study, using a high-throughput approach, focused on comparing the microbiota peculiarities of the ICU and NICU in one of the largest public hospitals in Brazil. The work highlighted bacteria associated with nosocomial infections, identifying the most potent reservoirs of contamination, and evaluated the microbiota changes related to the cleaning procedure. Therefore, this study contributes to increase the knowledge about (N)ICUs microbiomes and may help to reduce health-care-associated infections, especially in developing countries.

KEYWORDS: ICU cleaning, Intensive care unit, Healthcare-associated infections, NICU biomarkers, Cross-contamination, Polyhexamethylene biguanide.

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68 **INTRODUCTION:**

69 Microbiome refers to the microbial community, and their respective genomes,
70 associated with a particular habitat, including natural or built environments [1]. Natural
71 ecosystems have been well explored; however, not much is known about indoor
72 microbiomes − offices, houses, buildings, hospitals, etc. − where the majority of our life
73 is spent and can have a severe impact on human health. Unlike most indoor environments,
74 intensive care units (ICUs) or neonatal intensive care unit (NICUs) in hospitals are
75 routinely monitored by standard cultivation techniques [2,3]. Nonetheless, conventional
76 cultivation techniques can identify only a tiny proportion of the total bacteria [2,4].
77 Oberauner et al. [2] reported that only 2.5% of the overall bacterial diversity were
78 identified in an ICU microbiome using culture-dependent methods. Culture-independent
79 methods such as next-generation sequencing (NGS) technologies have a tremendous
80 effect on profiling microbiomes. Phylogenetic analyses based on 16S gene diversity have
81 been fundamental to uncover (N)ICU bacterial varieties in depth and at high resolution in
82 space and time, and it can contribute to improving hospital safety.

83 In (N)ICUs, even adopting strict sanitation protocols, many patients are infected
84 with healthcare-associated infections (HAIs), also known as nosocomial infections, a
85 significant public health problem around the world [5–10]. HAIs include diseases that
86 can be associated with surfaces and devices present in hospitals and can spread through
87 health care staff, contaminated surfaces or air droplets. These infections are more frequent
88 in UTIs where outbreaks often originate [11]. HAIs increase deaths (morbidity and
89 mortality), antimicrobial resistance, prolong the duration of hospital stays, and
90 consequentially healthcare costs [12]. The National Healthcare Safety Network of the
91 Centers for Disease Control and Prevention (CDC) has estimated 687,000 HCAIs in U.S.
92 acute care hospitals causing 72,000 deaths, and costs estimated to $97-147 billion
93 annually [13,14]. The most common pathogen causing HAIs are *Clostridium difficile* and
94 'ESKAPE' bacteria (*Enterococcus spp*., *Staphylococcus aureus*, *Klebsiella spp*.,
95 *Acinetobacter spp*., *Pseudomonas aeruginosa*, and Enterobacteriaceae) [14,15]. Many of
96 these bacteria exhibit antimicrobial resistance and can cause infections of the
97 bloodstream, urinary tract, severe pneumonia, and surgical site infection [11,16].

98 Hospital surfaces remain neglected reservoirs for HAI-related bacteria, and strict
99 cleaning protocols have been used as the primary procedure to reduce the risks.
100 Nonetheless, the efficiency of cleaning protocols, usually, has been investigated by

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101 culture-dependent routine techniques. Here, using NGS methodology, we analyzed the
102 differences and similarities between the structure of bacterial communities from the ICU
103 and NICU surfaces of The Medical School Clinics Hospital (Ribeirão Preto, Brazil), one
104 of the biggest hospitals in Latin America, and which has more than 35,000
105 hospitalizations per year and supports a population of four million people. We
106 hypothesized that the microbiota "signature" would vary significantly between ICU and
107 NICU, offering opportunities for targeted spatial biomarkers to improve the combat
108 against HAIs. Furthermore, we tested the impact of the standard cleaning procedure
109 established on the hospital on ICU microbiota, focusing on bacteria associated with
110 nosocomial infections.
111

112 **RESULTS AND DISCUSSION**

113
114 **Microbial profiling of ICU and NICU samples using V4-5 16 rRNA sequencing**

115 In order to compare the microbial community of the ICU and NICU from a clinical
116 hospital in Brazil, we use NGS targeting V4 hypervariable regions within microbial 16S
117 rRNA genes [17]. The intensive care units contained two wards with four beds each
118 (**Fig.1**), where critically ill patients were present. Samples were collected from boxes
119 areas (mattresses, bed rails, monitors, infusion pumps, ventilators, and cufflator), with
120 patients lying down; and also, in common areas (computers-keyboard and mouse, doors
121 handle, hospital cards, medical records, drug stations, and nurse's mobiles). Furthermore,
122 to address the question of how concurrent cleaning impacts the microbial ecosystem of
123 an ICU, samples were sequenced either before or immediately after cleaning.
124 A total of ~1.7 million sequences corresponding to 4.94 Gbp of data from 44
125 samples were generated. The average number of read counts per sample was 34.621,
126 ranging from 33.708 to 34.739. Thus, the data counts were normalized to 33.708 reads.
127 After trimming, the final number of operational taxonomic unit (OTU) consisted of 2054,
128 1586, OTUs for NICU, and ICU, respectively. Rarefaction curves (**Fig. S1**) based on the
129 number of OTUs observed were comparably close to asymptotic for all samples. The cut-
130 off was set to 10,000 sequences per sample whereby the rarefaction curves of all samples
131 reached saturation, indicating the availability of enough covering to represent and
132 compare the microbiome community present within the samples. Chimera and singleton
133 OTU removal was included in the data processing pipeline to prevent overestimated

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134 richness. Bellow, we presented the analysis regarding the microbial composition for each
135 sample and the comparison between the different areas analyzed.
136
137 **Comparative assessment between ICU and NICU microbiota**
138
139 Microbial profiling of the ICU and NICU allowed the identification of nine
140 different bacterial phyla: *Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes,*
141 *Fusobacteria, Cyanobacteria, Deinococcus, Gemmatimonadetes*, and *Euryarchaeota*,
142 while this last was only found in NICU. *Firmicutes* and *Proteobacteria* were the most
143 abundant phyla across all samples, composing 46% and 39% of these bacterial
144 communities, respectively. The over-representation of these phyla agree with previous
145 results obtained for microbial communities found in (N)ICUs inanimate surfaces [2,18–
146 20]. The microbial communities at the genus level (**Fig. 2A**) included sequences of 138
147 and 160 genera, for ICU and NICU respectively, among which a substantial number of
148 organisms are not culturable. For all samples, the relative abundance of Not_Assigned
149 (NA) genera was notably moderated (up to 18%). Gram-positive bacteria were found in
150 higher abundance in both units. Nonetheless, in terms of the number of genera, Gram-
151 negative bacteria were more diverse. The number of strictly aerobic genera were highly
152 represented (50%) followed by facultative anaerobe (36%) and obligatory anaerobic
153 bacteria (14%) for both units (see details in supplemental material). *Bacillus,*
154 *Staphylococcus*, and *Pseudomonas* were the most abundant genera (47% of the total
155 reads) on ICU surfaces, and *Bacillus*, *Propionibacterium* and *Staphylococcus*
156 predominated in NICU (40%). These genera contain many commensal species for
157 humans, although it also includes members associated with nosocomial infections in
158 (N)ICUs. Members from these genera are considered "survival specialists," and can
159 persist for months on dry surfaces [21] or associated with spore or biofilm formation
160 [22,23]. A total of 110 OTUs were found only in ICU and 578 only in NICU, while 1476
161 OTUs were shared between the units (**Fig. S2A**).
162 Analysis of all samples from the care units indicated that NICU samples showed
163 a significantly higher Shannon index ─ a measure of diversity ─ as compared to samples
164 belonging to ICU (Kruskal-Wallis test, p-value < 0.05) (**Fig. 2B**). However, noticeable
165 variation was observed within the sample types (**Fig. S2B**), and computers and doors
166 handle from both units showed the highest diversity among all samples. A higher Shannon
167 index for NICU agrees with the differences in the number of OTUs found in the care

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168 units. The greater diversity in NICU could be explained, in part, due to the higher transit
169 of visitors (e.g., children's parents or relatives) compared with the more restrictive transit
170 in ICU.
171 Beta diversity analysis (**Fig. 2C-D**) of the microbiota for each care unit revealed
172 distinct, but overlapping, profile (ANOSIM, R= 0.3066; p-value < 0.001). A high level
173 of variation among some samples was observed supplemented by less pronounced but
174 distinct variation between ICU/NICU samples closer to the patient (boxes area)
175 (ANOSIM, R= 0.50756; p-value < 0.001) (**Fig. S2C**). Samples from the common area
176 did not show a significant difference (**Fig. S2D**). Boxes area samples from ward A and
177 B, belonging to the same care unit, did not show a significant difference (**Fig. S2E-F**).
178 This analysis suggests that ICU and NICU carry a distinct microbial diversity. Besides, it
179 is also important to remark that more significant differences were observed in the
180 confined area closer to the patients (boxes). These areas are selective environments,
181 where antimicrobial therapies and stringent cleaning protocols are routinely applied.
182
183 **Identification of HAI-related genera in neglected (N)ICU surfaces**
184
185 Evidence suggests that hospital computers (keyboard and mouse) and staff's
186 mobiles may serve as reservoirs for bacteria associated with HAI within the healthcare
187 environment and facilitate the cross-contamination among hospital wards [24–26].
188 Taxonomically, ICU mobiles revealed a far greater abundance of *Acinetobacter,*
189 *Sphingomonas*, and *Brevundimonas* (**Fig. 3A**). These genera are usually found in moist
190 environments and can show a high risk for HAI in immunocompromised patients.
191 Besides, other genera associated with human microflora were also found in high
192 abundances, such as *Lactobacillus* (mouth and vaginal flora) and *Anaerobiospirillum*
193 (human, cat, and dog feces) [27]. NICU mobiles showed a greater abundance of
194 *Fusobacterium, Neisseria, Rothia, Granulicatella, and Streptococcus* (**Fig. 3A**) that are
195 part of the oronasopharynx or skin microflora. However, they can also be associated with
196 severe infections in patients with a weakened immune system. Our data are consistent
197 with previous studies that have reported that although mobiles can work as a repository
198 to opportunistic pathogens, portions of their bacteria are also found on the human
199 microbiome (owner's body) [28].

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200 Computers are indispensable in contemporaneous hospitals, and consequently,
201 keyboard and mouse may be contaminated with dangerous pathogenic bacteria [29,30].
202 Here, we found potential opportunistic genera such as *Kocuria* (present at the skin and
203 oral flora) and *Methylobacterium* in great abundance in ICU computers whereas NICU
204 computers were enriched with *Rothia, Granulicatella*, *Streptococcus, Micrococcus,* and
205 *Prevotella* (**Fig. 3A**). Another important, but generally neglected, potential vector of
206 pathogens are the medical records (aka medical charts), especially those from (N)ICUs
207 [31,32]. ICU medical records were enriched with *Dietzia* and *Flavobacterium*. NICU
208 medical records were similar to NICU computers, except for being more abundant in
209 *Bacteroides* (**Fig. 3A**). Moreover, fecal indicators were detected in a high proportion of
210 NICU medical records (**Fig. S3A**). A hierarchical clustering analysis (**Fig. 3B**) based on
211 the taxonomy of the ICU and NICU samples grouped them into two major clusters. Most
212 of the samples from the same unit were clustered together indicating their similarity.
213 Nonetheless, the microbiota community of ICU mobiles and handles were dispersed:
214 mobiles-ICUab clustered closely with NICU ventilators (and mobiles), while ICU
215 handles clustered with NICU handles group. These samples belonged to a cluster that
216 revealed an almost absent *Bacillus* and higher frequency of *Streptococcus*, among other
217 differences (**Fig. 3A-B**). Medical records were taxonomy similar to computers and also
218 closer to monitors (**Fig. 3B**). Generally, for each unit, samples from surfaces frequently
219 touched by HCW clustered together (**Fig. S4A-B**). These samples showed a higher
220 abundance of skin-associated genera. The effects of these contamination sources for the
221 patients were not part of this study. However, based on a vast literature, it is highly
222 recommended to sensitize healthcare staff to sanitize mobiles, hands, computers and
223 medical records (often neglected) to prevent cross-contamination within the hospital
224 environment.
225
226 **Identification of ICU and NICU bacterial biomarkers**
227
228 Across the ICU and NICU samples, different biogeographical patterns were
229 observed for the different microbiotas. LEfSe analysis was performed to identify the
230 distinguishing genera between ICU and NICU (**Fig. 4A**). LEfSe is a method that allows
231 biomarker discovery most likely to explain differences between groups based on
232 statistical significance, biological consistency and effect relevance [33]. In total, 25
233 genera were identified with LDA scores > 3.0. At the genus level, 11 specific biomarkers

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234 were present in NICU and 6 in ICU. All of them were both highly discriminatory and
235 significantly different (p-value and FDR < 0.05) in term of abundances (**Fig. 4B**). The
236 HAI-related genera *Delftia*, *Streptococcus*, *Haemophilus*, *Gemella*, *Serratia*,
237 *Elizabethkingia*, *Leptotrichia*, *Clostridium_sensu_stricto*, *Chryseobacterium*, and *Vibrio*
238 were biomarkers for NICU. Although most of these genera can be found in the respiratory
239 tract, mouth, vagina, and intestinal tract of healthy adults, they present a high potential
240 for nosocomial infection in neonates. Among these genera, there is a predominance of
241 organisms with low oxygen tolerance (facultative anaerobes or obligate anaerobes).
242 *Pseudomonas* was identified as a biomarker for ICU. It is well known that nosocomial
243 infections caused by *Pseudomonas* are more often in ICUs than in other wards in the
244 hospital [34]. Except for *Streptococcus* and *Leptotrichia*, all these HAI-related genera
245 were found mainly in surfaces closer to the patients (boxes areas). Biomarkers could be
246 used as indicators for the contamination status in a specific area in the hospital. Genera
247 detected as biomarkers suggest that some bacteria can adapt extraordinarily within a
248 particular environment.
249
250 **ICU and NICU microbiotas have well defined community-level structures**
251
252 Community-level relationships among the top 50 abundant bacterial genera were
253 investigated through Pearson's r correlation analysis (**Fig. 5**). Microbial interaction has
254 an essential influence on antibiotic resistance and pathogenicity. In the ICU microbiome
255 (**Fig. 5A**), five distinct clusters (i-v) were detected with significant positive correlations
256 (co-occurrence). These clusters include potentially pathogenic genera such as (i)
257 *Enterobacter*, *Staphylococcus*, *Corynebacterium*, and *Escherichia_Shigella*; (ii) Bacteria
258 associated with outside environment (water, soil, and plants), among which
259 *Pseudomonas*; (iii) *Stenotrophomonas*, *Acinetobacter*, *Sphingomonas*, and
260 *Brevundimonas* (which can also cause co-infection with *Acinetobacter* spp.) [35]. (iv)
261 *Enterococcus*, *Haemophilus*, *Kocuria*, *Dietzia*, *Gemella*, and *Neisseria*; (v) *Micrococcus*,
262 *Fusobacterium*, *Prevotella*, *Delftia*, *Veillonella*, *Granulicatella*, *Rothia*, and
263 *Streptococcus*. Except for *Pseudomonas,* the genera *Thermomonas*, *Bacillus*, and
264 *Pseudoxanthomonas* showed negative correlations with all the five clusters cited above.
265 In the NICU (**Fig. 5B**), we highlighted four (i-iv) clusters containing the following genera
266 associated with nosocomial infections: (i) *Acinetobacter*, *Kocuria*, *Delftia*, and *Dietzia*;
267 (ii) *Staphylococcus*, *Gemella*, and *Haemophilus*; (iii) *Fusobacterium*, *Neisseria*,

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268 *Corynebacterium*, *Rothia*, *Granulicatella*, and *Streptococcus*; (iv) *Enterobacter*,
269 *Enterococcus*, *Sphingomonas*, *Escherichia_Shigella*, and *Serratia*. However, all these
270 clusters revealed a strong negative correlation with *Bacillus*, *Sphingobium*,
271 *Hydrogenophaga*, *Thauera*, *Thermomonas*, and *Gemmobacter*. It is important to note that
272 most of these bacterial genera are known players in biofilms formation, including
273 synergic multi-genera biofilms, on various hospital dry surfaces [36,37]. Biofilms matrix
274 is a resistance mechanism that could stabilize a bacteria community in a selective
275 environment such as (N)ICUs [38].
276 In order to verify whether the most prevalent potentially pathogenic genera
277 identified in the ICU and NICU correlate with infected patients, 108 bacterial strains were
278 isolated. Following standard cultivation, these strains were isolated from blood,
279 bronchoalveolar lavage, peritoneal, cerebrospinal and ascitic fluids of hospitalized
280 patients. All these isolates were identified, at the species level, by selective media,
281 morphological features, and Vitek 2 rapid identification system and distributed among 12
282 genera. These strains comprised the genera *Klebsiella*, *Acinetobacter*, *Stenotrophomonas*,
283 *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Burkholderia*,
284 *Cupriavidus*, *Morganella*, and *Ralstonia*. The most common culture-dependent isolates
285 matched with the most abundant HAI-related genera found in the sequencing data (**Fig.**
286 **S5A-C**). This correlation shows that potentially pathogenic organisms, even when found
287 in abundance <10% in sequencing, may be predominant in hospital infections. The
288 majority of the isolates obtained belonged to *Staphylococcus*, which was the second more
289 abundant Gram-positive genus found in the sequencing. *Staphylococcus* already is
290 described as one of the most common genera found in hospitals [39].
291
292 **Investigation of ICU microbial community profiling reveals substantial variation on**
293 **the efficiency of the cleaning procedures**
294
295 Cleaning procedures at ICUs are an important practice to prevent HAI-related
296 bacteria spreading [39]. Although the protocols may vary between hospitals, concurrent
297 cleaning procedures involved strict disinfection and sterilization of patient supplies and
298 equipment during hospitalization. Here, the antimicrobial solution used for daily ICU
299 cleaning contained the cationic polymer polyhexamethylene biguanide (PHMB). A recent
300 model suggests that PHMB enter bacterial cells and condenses chromosomes, inhibiting
301 cell division [40]. Thus, in order to investigate how concurrent cleaning affects the ICU

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302 microbiome, samples from surfaces near patients were sequencing and analyzed either
303 before or immediately after cleaning. The microbial communities at genus level included
304 sequences of 117 and 94 genera, for before and after cleaning respectively (**Fig. 6A**).
305 Seven percent of the OTUs could not be classified to genera level (NA). These
306 unclassified groups had higher relative abundance in cufflator-ICUab (35%). Samples
307 after cleaning showed a slight but significant decrease in the diversity (Kruskal-Wallis
308 test, p-value < 0.05) (**Fig. 6B**). However, noticeable variation was observed within the
309 sample types (**Fig. S6A**). Beta diversity analysis revealed distinct, but overlapping,
310 profile (R= 0.091961; p-value < 0.05) (**Fig. 6C**). Most of the samples from ICU ward-A
311 after cleaning clustered separately from the rest of the surfaces. Quite remarkably, these
312 differences in diversity after cleaning reveal that the procedure did not have the same
313 effect on all surfaces. Although it is known that different microbiomes may exert different
314 effects on cleaning [37], this was not the case, since no significant difference between
315 room A and B was observed prior to cleaning. Therefore, differences in the effect of
316 cleanliness on diversity could be explained, in part, by a lack of standardization in the
317 protocol.
318 The samples either before or after cleaning were inhabited by high relative
319 abundances (~65%) of *Bacillus*, *Pseudoxanthomonas*, *Thermomonas*, *Staphylococcus*,
320 *Castellaniella*, and *Acinetobacter*. Core microbiome analysis showed that 19 genera were
321 shared in 80% of all samples (before and after) at the minimum detection threshold of
322 0.001% relative abundance (**Fig. 6D**). Most notably, the most abundant genera were also
323 clearly most prevalent in the core microbiome before and after cleaning. Gram-positive
324 bacteria were found in higher abundance (before ─ 53%; after ─ 51%, respectively),
325 showing 45 different genera before and 30 after cleaning (33% less). Furthermore, Gram-
326 negative bacteria revealed higher diversity, with 72 genera before and 64 after cleaning
327 (11% less). Most of the genera absent after cleaning showed very low abundance (<
328 0.05%) before cleaning. The HAI-related organism *Chryseobacterium*, and
329 *Clostridium_XI* are among the genera absent (or extremely low) after cleaning. Besides
330 these absent genera, using the statistical parameters p-value and FDR < 0.05, no other
331 analyzed genera showed a significant difference between the average abundance
332 calculated for all samples before and after cleaning. However, the HAI-related genera
333 *Comamonas*, *Pseudomonas*, *Enterobacter*, *Kocuria*, *Ralstonia*, and *Delftia* showed a
334 decrease, while *Leptotrichia*, *Streptococcus*, and *Acinetobacter* presented an increase on
335 average abundance ≥two-fold after cleaning (**Fig. S7A**). Curiously, cleaning efficiency

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336 was notably variable among the samples (**Fig. S7B**). Previous studies have shown that
337 even with strict cleaning procedures, HAI-related genera, such as *Staphylococcus*,
338 *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterococcus*, *Escherichia*, and *Enterobacter*,
339 are generally found on the surface of the ICU devices [41–45]. To examine more deeply
340 the cleaning effect among the samples, a heatmap of the top 45 genera is illustrated in
341 **Fig. 7A**. The cleaning efficiency was not the same through the samples and wards. Some
342 genera showed a tendency to decrease after cleansing, such as *Enterococcus*,
343 *Enterobacter*, *Staphylococcus*, *Burkholderia*, *Comamonas*, *Pseudomonas*, and *Delftia*.
344 However, others increased in one ward and dropped in the other, such as
345 *Corynebacterium* and *Acinetobacter* (increased for ward-A and decreased for ward-B) or
346 *Prevotella* and *Novosphingobium* (decrease for ward-A and increase for ward-B).
347 Moreover, there were genera that revealed an tremendous increasing after
348 cleaning in some specific surfaces, such as *Stenotrophomonas* (mattresses-ICUaA and
349 rails-ICUbA), *Methylobacterium* (monitors-ICUbA), *Bacteroides*, *Neisseria*, and
350 *Streptococcus* (rails-ICUaA), *Acinetobacter* and *Escherichia* (ventilators-ICUaA),
351 *Dietzia* (monitors-ICUaA), *Delftia* (pumps-ICUbA), *Novosphingobium* and *Tepidimonas*
352 (ventilators-ICUbA). Fecal indicators were detected in higher abundance after cleaning
353 on bed rails (mainly on Rails-ICUaA) **(Fig. S3A)**. These results reveal that cleaning was
354 inconsistent and, in some cases, increased the load of specific genera. Previous studies
355 have shown that hands are one of the primary vectors of HAI-related bacterial cross-
356 contamination [46,47], mainly because of the variable compliance on hands hygiene and
357 gloves changing after touching surfaces near to the patients [48]. Besides, disinfectant
358 solutions and wipes used for hospital cleaning also can be a vital source of pathogen
359 transfer and inconsistency in surfaces cleaning, even when standard protocols are
360 followed [49]. Furthermore, other factors to be considered is the low efficiency of PHMB-
361 based products in relation to contaminations by wound secretions or urine containing a
362 massive load of bacteria [50], and a possible discrepancy in the cleaning procedure
363 performed by different nurses. Based on hierarchical clustering analysis, before cleaning
364 (**Fig. 7B**) most of the samples with the same functionality, but from different wards, were
365 clustered together indicating their similarity. Nonetheless, the microbiota community
366 after cleaning (**Fig. 7C**) revealed a higher dispersion among the samples. We speculate
367 that cleaning could be a way of spreading colonizing genera from one surface to another,
368 but that over time there may be a reestablishment of the microbial community related to
369 a specific sample.

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371 **Cleaning procedures generates substantial rearrangements in the community-level**
372 **structures**

373 To investigate the changes in the microbial community structure before and after
374 cleaning the correlation coefficients among the top 50 genera was analyzed (**Fig. 8A-B**).
375 For the microbiome before cleaning, four distinct clusters (i-iv) were detected with
376 significant positive co-occurrence (**Fig. 8A**). These clusters include potentially
377 pathogenic genera such as (i) *Enterococcus*, *Escherichia_Shigella*, *Stenotrophomonas*,
378 *Enterobacter*, *Staphylococcus*, *Acinetobacter*, and *Corynebacterium*; (ii) *Dietzia*,
379 *Streptococcus*, and *Veillonella*; (iii) *Sphingomonas*, *Neisseria*, and *Methylobacterium*;
380 (iv) *Burkholderia*, *Pseudomonas*, *Ralstonia*, and *Comamonas*. The environmental genus
381 *Belnapia* showed negative correlations with all the genera cited above.

382 After cleaning, six clusters (i-vi) are presented (**Fig. 8B**) containing highlighted
383 genera associated with nosocomial infections: (i) *Stenotrophomonas* and other
384 environmental genera; (ii) *Veillonella*, *Morganella*, *Streptococcus*, *Acinetobacter*,
385 *Granulicatella*, *Comamonas*, *Corynebacterium*, *Staphylococcus*, *Haemophilus*, and
386 *Neisseria*; (iii) *Methylobacterium*, *Escherichia*, and *Sphingomonas*. (iv) *Prevotella* and
387 other genera related to low oxygen tolerance or vaginal microbiome [51]; (v)
388 *Pseudomonas*, *Enterococcus*, *Pantoea*, and *Burkholderia*; (vi) *Enterobacter*, *Delftia*, and
389 *Novosphingobium*. However, most of these HAI-related genera revealed a strong negative
390 correlation with *Pseudoxanthomonas* (except *Delftia* and *Novosphingobium*). The
391 correlation data showed a predominance of Proteobacteria among most of the clusters.
392 Proteobacteria are predominant in the skin of the forearm [52] and are highly associated
393 with biofilms formation on the surface of devices used on ICUs [36]. Several genera
394 relationships were quite stable to disinfection stress because it was found clustered both
395 before and after cleaning. In all the clusters were found genera associated with species
396 able to form biofilms. Genera associated with xenobiotic metabolism were found among
397 the clusters i-iv, and ii-v before and after cleaning, respectively [53,54]. After cleaning it
398 was noticed a redistribution of some genera in new clusters. For example, a more
399 extensive cluster involving ten HAI-related genera (ii) was formed after cleaning, this
400 cluster included a mixture of several genera found in clusters i-iv before cleaning.
401 Although this cluster analysis is useful to visualize the dynamics of microbiotas with the
402 cleaning efficiency, further studies will be required to understand the exact changes in the
403 microbe-microbe interactions underlying the differences observed across time.

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404

405 **Conclusions**

406 The relevance of spatial composition of the microbial communities within a
407 hospital is unclear. To our knowledge, this is the first study using deep sequencing of
408 inanimate surfaces samples to develop a spatial assessment of the microbial community
409 in ICU and NICU wards within the same hospital. In this comprehensive study, we
410 observed a peculiar spatial structure between ICU and NICU microbiota in one of the
411 largest hospitals in Brazil. The data revealed that among the samples analyzed, NICU
412 presents higher biodiversity than in the ICU. Genera considered "survival specialists" are
413 among the most persistent and abundant in both units. Areas closest to the patient hold
414 more specific microbiota, distinguishing one unit from other. Most of the genera found
415 in both units are present on the healthy human microbiome, suggesting that the most likely
416 vectors of contamination are hospital staff and patients. Most of these genera can also be
417 associated with nosocomial infection, especially for patients in (N)ICU. Devices
418 commonly used, but generally neglected, such as mobile phones, computers, and medical
419 charts are enriched with HAI-related genera (e.g., *Acinetobacter*, *Fusobacterium*,
420 *Kocuria*, *Rothia*, and *Dietzia*). For the samples analyzed in the present study, some
421 facultative anaerobes or obligate anaerobes genera were classified as biomarkers for the
422 NICU (e.g., *Serratia* and *Clostridium*), whereas *Pseudomonas* as a biomarker for ICU.
423 Correlation analyses revealed a distinct pattern of microbe-microbe interactions for each
424 unit, including several bacteria able to form multi-genera biofilms. Cultivation-dependent
425 results showed a positive correlation between the most abundant HAI-related genera
426 identified by sequencing with infections found in the hospital. According, our data
427 showed similarity with previous studies and can help to define soon what constitutes a
428 "typical" microbiome in the ICU and NICU environments. The ability to identify HAI-
429 related genera that are spatially concentrated in a hospital ward may influence the future
430 use of improved tools and protocols for infection control.

431 Furthermore, we evaluated the effect of concurrent cleaning over the ICU bacterial
432 community. Cleaning showed a slight decrease in diversity. Several genera were quite
433 stable to disinfection, suggesting being well-adapted to the ICU environment. In general,
434 the cleaning procedure was inconsistent. Potential influencing factors from the
435 unsatisfactory cleaning include low efficiency of the biocide used, bacteria well-adapted
436 to daily cleaning, disinfectant solutions and wipes contaminated, and variable compliance

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437 on hands hygiene and cleaning procedure. Therefore, this type of analysis can be used for
438 designing better strategies for cleaning procedures. In conclusion, we demonstrate here
439 how NGS could be used for monitoring potential contamination sources in (N)ICU units
440 and to evaluate existent decontamination protocols stablished in these unities. We
441 highlight that similar approaches, while still very costly, could be implemented for
442 periodic monitoring of microbial profiles in clinical hospitals to help reducing potential
443 secondary infections.
444

445 **MATERIALS AND METHODS**

446

447 *Sample collection and DNA extraction*

448 A total of 158 samples were collected from the ICU and NICU at The Medical
449 School Clinics Hospital (Ribeirão Preto, Brazil) by a single investigator from September
450 to October 2018. The intensive care units contained two wards with four beds each, where
451 critically ill patients from all medical specialties are treated. Samples from NICU were
452 collected only before the concurrent cleaning, while from ICU samples were collected
453 either before or immediately after cleaning. During sampling, all employees and devices
454 of the ICU/NICU were in full operation. Boxes with patients lying down were swabbed
455 on the surfaces of mattress, bed rail, monitors, infusion pumps, ventilator, and cufflator
456 (when present). In common areas of the ICU/NICU, computer keyboard and mouse, doors
457 handle, hospital cards, medical records, drug station, and nurse's mobiles were also
458 swabbed. All sampling locations and their characteristics are given in Fig.1 and Table 1.
459 The following code was used to name the samples: Samples-Unit (ICU or NICU) ward
460 (a, b or ab) A (after cleaning), e.g., Monitors-ICUaA Samples were collected using sterile
461 swabs (Absorve®, Jiangsu, China) premoistened with sterile Amies media [55]. The
462 swabs were streaked across a 400-cm^2 area in four different directions with firm
463 movements for 2 minutes; swabs were rotated to ensure full contact of all parts of the
464 swab tip and the surface. After a surface was sampled, the swab was immediately placed
465 into sterile 15-ml Falcon tubes containing 1 mL of sterile Amies media and stored in a
466 4°C cooler until returning to the laboratory. In the laboratory, due to extremely low
467 biomass, samples from a similar source and the same ward were pooled together −, e.g.,
468 four monitors from NICU ward A is a pool, and four monitors from NICU ward B another
469 pool− generating 43 pooled samples. Then, the samples were concentrated to 500 µL by

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470 centrifugation (10000 g / 20min), and DNA was extracted using the MoBio Powersoil
471 DNA isolation kit, then stored in a -80°C freezer until further processing.
472
473 *Concurrent cleaning procedures in the ICU*
474
475 At the beginning of each 24-h shift, a registered nurse washed his or her hands,
476 put on nonsterile gloves, and wiped Boxes surfaces (mattress, bed rail, computer touch
477 screens, monitors, infusion pumps, ventilator, and cufflator) with 1% polyhexamethylene
478 biguanide (PHMB) solution on a soft wipe.
479
480 *Sequencing and diversity analysis*
481 The DNA concentrations were measured fluorometrically (Qubit® 3.0, kit Qubit®
482 dsDNA Broad Range Assay Kit, Life Technologies, Carlsbad, CA, USA). DNA integrity
483 was determined by agarose gel electrophoresis using a 0.8% (w/v) gel, and subsequent
484 staining with SYBR Safe DNA Gel Stains (Invitrogen, Carlsbad, CA, USA). A PCR was
485 employed to amplify the V4 regions of the 16S ribosomal RNA gene 16S rRNA for
486 bacteria [17]. Each PCR reaction mixture contained 20 ng of metagenomic DNA, 10 µM
487 of each forward and reverse primers, 1.25 mM of magnesium chloride, 200 µM of dNTP
488 mix (Invitrogen, Carlsbad, CA, USA), 1.0 U Platinum Taq DNA polymerase high fidelity
489 (Invitrogen, Carlsbad, CA, USA), high fidelity PCR buffer [1X], and milli-Q water.
490 Reactions were held at 95 °C for 3 min, with amplification proceeding for 30 cycles at 95
491 °C for 30 s, 53.8 °C for 30 s, and 72 °C for 45 s; a final extension of 10 min at 72 °C was
492 added to ensure complete amplification. The expected fragment length of PCR products
493 was verified by agarose gel (1%) electrophoresis, and the amplicon size was estimated by
494 comparison with a 1 kb plus DNA ladder (1 kb plus DNA ladder, Invitrogen, Carlsbad,
495 CA, USA). The PCR fragments were purified using the Zymoclean™ Gel DNA Recovery
496 kit following the manufacturer's instructions. Sequencing was performed using the Miseq
497 Reagent kit v3 2 x 300 bp.
498 All sequences data were processed, removing adapters using Scythe 0.991
499 (https://github.com/vsbuffalo/scythe) and Cutadapt 1.7.1 [56]. Sequence trimming was
500 carried out by selecting sequences over 200 bp in length with an average quality score
501 higher than 20 based on Phred quality, and duplicate reads were removed using the
502 Prinseq program [57]. The QIIME software package version 1.9.1 was used to filter reads
503 and determine Operational Taxonomic Units (OTUs) as described in Caporaso et al.

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504 (2010). The Usearch algorithm was used to cluster the reads OTUs with a 97% cutoff,
505 and to assign taxonomy using the Ribosomal Database Project (RDPII) version 10 [58].
506 Bacterial sequences were de-noised, and suspected chimeras were removed using the
507 OTU pipe function within QIIME. Sequence data were summarized at the phylum, class,
508 and family levels; Also, Alpha_diversity.py in QIIME was used to calculate ACE, Chao1,
509 Shannon, and Simpson indices. Principal coordinate analyses (PCoA) were conducted to
510 evaluate differences in community structure among experimental groups (β-diversity).
511 For further statistical analysis and visualization, OTU table with taxa in plain
512 format and metadata file were uploaded to the MicrobiomeAnalyst tool (available at
513 http://www.microbiomeanalyst.ca) [59]. Shallow abundant features were filtered using
514 options; minimum count 4, low-count filter based on 20% prevalence in samples. For
515 comparative analyses, a low variance filter was applied based on Inter-quantile range and
516 removing the 10% lowest features. Data were rarefied to the minimum library size and
517 normalized using total sum scaling (TSS) before any statistical comparisons [60].
518
519
520 **Availability of supporting data**
521 The nucleotide sequences obtained in the present study have been deposited in the
522 GenBank database under the Accession number PRJNA541082.
523
524 **Competing interests**
525 The authors declare that no non-financial conflicts of interest exist.
526
527 **Ethics approval and consent to participate**
528 No specific permissions or ethics approval were required for this study with inanimate
529 surfaces.
530
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538 **Author Contributions**

539 LFR, RSR, and MEG conceived of the project. LFR, LFCR, MGM, and GGG organized
540 the sample collections. LFR conducted the nucleic acid extractions. EML and LTK
541 conducted the MiSeq library preparations and provided the bioinformatics support, and
542 LFR contributed to the data analysis. LFR, RSR, and MEG wrote the final manuscript.
543 All authors have read and approved the manuscript.

544

545 **References**

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547 1. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal.
548 Microbiome. 2015; doi:10.1186/s40168-015-0094-5
549 2. Oberauner L, Zachow C, Lackner S, Högenauer C, Smolle KH, Berg G. The
550 ignored diversity: Complex bacterial communities in intensive care units revealed
551 by 16S pyrosequencing. Sci Rep. 2013; doi:10.1038/srep01413
552 3. Daneman N, Stukel TA, Ma X, Vermeulen M, Guttmann A. Reduction in
553 Clostridium difficile infection rates after mandatory hospital public reporting:
554 Findings from a longitudinal cohort study in Canada. PLoS Med. 2012;
555 doi:10.1371/journal.pmed.1001268
556 4. Staley JT, Konopka A. Measurement of in Situ Activities of Nonphotosynthetic
557 Microorganisms in Aquatic and Terrestrial Habitats. Annu Rev Microbiol. 2003;
558 doi:10.1146/annurev.mi.39.100185.001541
559 5. Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, et
560 al. Burden of endemic health-care-associated infection in developing countries:
561 Systematic review and meta-analysis. Lancet. 2011; doi:10.1016/S0140-
562 6736(10)61458-4
563 6. Calfee DP. Crisis in Hospital-Acquired, Healthcare-Associated Infections. Annu
564 Rev Med. 2011; doi:10.1146/annurev-med-081210-144458
565 7. WHO. Report on the Burden of Endemic Health Care-Associated Infection
566 Worldwide. In: http://whqlibdoc.who.int/publications. 2011.
567 doi:http://whqlibdoc.who.int/publications/2011/9789241501507_eng.pdf
568 8. Teerawattanapong N, Panich P, Kulpokin D, Na Ranong S, Kongpakwattana K,
569 Saksinanon A, et al. A systematic review of the burden of multidrug-resistant
570 healthcare-associated infections among intensive care unit patients in southeast
571 asia: The rise of multidrug-resistant acinetobacter baumannii. Infection Control
572 and Hospital Epidemiology. 2018. doi:10.1017/ice.2018.58
573 9. Walter J, Haller S, Quinten C, Kärki T, Zacher B, Eckmanns T, et al. Healthcare-
574 associated pneumonia in acute care hospitals in European union/European
575 economic area countries: An analysis of data from a point prevalence survey, 2011
576 to 2012. Eurosurveillance. 2018; doi:10.2807/1560-7917.ES.2018.23.32.1700843
577 10. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health
578 care-associated infections: AMeta-analysis of costs and financial impact on the US
579 health care system. JAMA Intern Med. 2013;
580 doi:10.1001/jamainternmed.2013.9763
581 11. Agaba P, Tumukunde J, Tindimwebwa JVB, Kwizera A. Nosocomial bacterial
582 infections and their antimicrobial susceptibility patterns among patients in
583 Ugandan intensive care units: A cross sectional study. BMC Res Notes. 2017;

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584 doi:10.1186/s13104-017-2695-5
585 12. CDC. Healthcare-asociated infections [Internet]. Available:
586 https://www.cdc.gov/hai/index.html
587 13. CDC. HAI Hospital Prevalence Survey [Internet]. Available:
588 https://www.cdc.gov/hai/data/portal/index.html
589 14. Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al.
590 Multistate Point-Prevalence Survey of Health Care–Associated Infections. N Engl
591 J Med. 2014; doi:10.1056/NEJMoa1306801
592 15. Magill SS, O'Leary E, Janelle SJ, Thompson DL, Dumyati G, Nadle J, et al.
593 Changes in Prevalence of Health Care–Associated Infections in U.S. Hospitals. N
594 Engl J Med. 2018; doi:10.1056/NEJMoa1801550
595 16. Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial
596 infections. Trends in Molecular Medicine. 2015.
597 doi:10.1016/j.molmed.2015.03.005
598 17. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh
599 PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences
600 per sample. Proc Natl Acad Sci. 2011;108: 4516–4522.
601 doi:10.1073/pnas.1000080107
602 18. Poza M, Gayoso C, Gómez MJ, Rumbo-Feal S, Tomás M, Aranda J, et al.
603 Exploring Bacterial Diversity in Hospital Environments by GS-FLX Titanium
604 Pyrosequencing. PLoS One. 2012; doi:10.1371/journal.pone.0044105
605 19. Bokulich NA, Mills DA, Underwood MA. Surface microbes in the neonatal
606 intensive care unit: Changes with routine cleaning and over time. J Clin Microbiol.
607 2013; doi:10.1128/JCM.00898-13
608 20. Brooks B, Firek BA, Miller CS, Sharon I, Thomas BC, Baker R, et al. Microbes in
609 the neonatal intensive care unit resemble those found in the gut of premature
610 infants. Microbiome. 2014; doi:10.1186/2049-2618-2-1
611 21. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on
612 inanimate surfaces? A systematic review. BMC Infectious Diseases. 2006.
613 doi:10.1186/1471-2334-6-130
614 22. Achermann Y, Goldstein EJC, Coenye T, Shirtliffa ME. Propionibacterium acnes:
615 From Commensal to opportunistic biofilm-associated implant pathogen. Clin
616 Microbiol Rev. 2014; doi:10.1128/CMR.00092-13
617 23. Majed R, Faille C, Kallassy M, Gohar M. Bacillus cereus Biofilms-same, only
618 different. Frontiers in Microbiology. 2016. doi:10.3389/fmicb.2016.01054
619 24. Badr RI, Badr HI, Ali NM. Mobile phones and nosocomial infections. Int J Infect
620 Control. 2012; doi:10.3396/ijic.v8i2.014.12
621 25. Brady RRW, Verran J, Damani NN, Gibb AP. Review of mobile communication
622 devices as potential reservoirs of nosocomial pathogens. Journal of Hospital
623 Infection. 2009. doi:10.1016/j.jhin.2008.12.009
624 26. Brady RR, Hunt AC, Visvanathan A, Rodrigues MA, Graham C, Rae C, et al.
625 Mobile phone technology and hospitalized patients: A cross-sectional surveillance
626 study of bacterial colonization, and patient opinions and behaviours. Clin
627 Microbiol Infect. 2011; doi:10.1111/j.1469-0691.2011.03493.x
628 27. Malnick H, Williams K, Phil-Ebosie J, Levy AS. Description of a medium for
629 isolating Anaerobiospirillum spp., a possible cause of zoonotic disease, from
630 diarrheal feces and blood of humans and use of the medium in a survey of human,
631 canine, and feline feces. J Clin Microbiol. 1990;
632 28. Meadow JF, Altrichter AE, Green JL. Mobile phones carry the personal
633 microbiome of their owners. PeerJ. 2014; doi:10.7717/peerj.447

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634 29. Jones HW. Bacterial contamination of keyboards: Efficacy and functional impact
635 of disinfectants - Commentary. Obstetrical and Gynecological Survey. 2007.
636 doi:10.1097/01.ogx.0000253752.51984.e7
637 30. Bures S, Fishbain JT, Uyehara CFT, Parker JM, Berg BW. Computer keyboards
638 and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit.
639 Am J Infect Control. 2000; doi:10.1067/mic.2000.107267
640 31. Teng S-O, Lee W-S, Ou T-Y, Hsieh Y-C, Lee W-C, Lin Y-C. Bacterial
641 contamination of patients' medical charts in a surgical ward and the intensive care
642 unit: impact on nosocomial infections. J Microbiol Immunol Infect. 2009;
643 32. Chen KH, Chen LR, Wang YK. Contamination of medical charts: An important
644 source of potential infection in hospitals. PLoS One. 2014;
645 doi:10.1371/journal.pone.0078512
646 33. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al.
647 Metagenomic biomarker discovery and explanation. Genome Biol. 2011;
648 doi:10.1186/gb-2011-12-6-r60
649 34. Donowitz LG, Wenzel RP, Hoyt JW. High risk of hospital-acquired infection in
650 the ICU patient. Crit Care Med. 1982; doi:10.1097/00003246-198206000-00001
651 35. Ryan MP, Pembroke JT. Brevundimonas spp: Emerging global opportunistic
652 pathogens. Virulence. 2018; doi:10.1080/21505594.2017.1419116
653 36. Perez E, Williams M, Jacob JT, Reyes MD, Tejedor SC, Steinberg JP, et al.
654 Microbial biofilms on needleless connectors for central venous catheters:
655 Comparison of standard and silver-coated devices collected from patients in an
656 acute care hospital. J Clin Microbiol. 2014; doi:10.1128/JCM.02220-13
657 37. Hu H, Johani K, Gosbell IB, Jacombs ASW, Almatroudi A, Whiteley GS, et al.
658 Intensive care unit environmental surfaces are contaminated by multidrug-resistant
659 bacteria in biofilms: Combined results of conventional culture, pyrosequencing,
660 scanning electron microscopy, and confocal laser microscopy. J Hosp Infect. 2015;
661 doi:10.1016/j.jhin.2015.05.016
662 38. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious
663 biofilms. Trends in Microbiology. 2005. doi:10.1016/j.tim.2004.11.010
664 39. Mora M, Mahnert A, Koskinen K, Pausan MR, Oberauner-Wappis L, Krause R, et
665 al. Microorganisms in confined habitats: Microbial monitoring and control of
666 intensive care units, operating rooms, cleanrooms and the international space
667 station. Frontiers in Microbiology. 2016. doi:10.3389/fmicb.2016.01573
668 40. Chindera K, Mahato M, Kumar Sharma A, Horsley H, Kloc-Muniak K,
669 Kamaruzzaman NF, et al. The antimicrobial polymer PHMB enters cells and
670 selectively condenses bacterial chromosomes. Sci Rep. 2016;
671 doi:10.1038/srep23121
672 41. Myers MG. Longitudinal evaluation of neonatal nosocomial infections: association
673 of infection with a blood pressure cuff. Pediatrics. 1978;
674 42. Livornese LL, Dias S, Samel C, Romanowski B, Taylor S, May P, et al. Hospital-
675 acquired infection with vancomycin-resistant Enterococcus faecium transmitted by
676 electronic thermometers. Ann Intern Med. 1992; doi:10.7326/0003-4819-117-2-
677 112
678 43. Marinella MA. The stethoscope. A potential source of nosocomial infection? Arch
679 Intern Med. 2003; doi:10.1001/archinte.157.7.786
680 44. Schabrun S, Chipchase L, Rickard H. Are therapeutic ultrasound units a potential
681 vector for nosocomial infection? Physiother Res Int. 2006; doi:10.1002/pri.329
682 45. Safdar N, Drayton J, Dern J, Warrack S, Duster M, Schmitz M. Telemetry leads
683 harbor nosocomial pathogens. Int J Infect Control. 2012;

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684 doi:10.3396/ijic.v8i2.012.12
685 46. Agodi A, Barchitta M, Cipresso R, Giaquinta L, Romeo MA, Denaro C.
686 Pseudomonas aeruginosa carriage, colonization, and infection in ICU patients.
687 Intensive Care Med. 2007; doi:10.1007/s00134-007-0671-6
688 47. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of
689 hospital surfaces in the transmission of emerging health care-associated pathogens:
690 Norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control.
691 2010; doi:10.1016/j.ajic.2010.04.196
692 48. Ali S, Wilson APR. Effect of poly-hexamethylene biguanide hydrochloride
693 (PHMB) treated non-sterile medical gloves upon the transmission of Streptococcus
694 pyogenes, carbapenem-resistant E. coli, MRSA and Klebsiella pneumoniae from
695 contact surfaces. BMC Infect Dis. 2017; doi:10.1186/s12879-017-2661-9
696 49. Ramm L, Siani H, Wesgate R, Maillard JY. Pathogen transfer and high variability
697 in pathogen removal by detergent wipes. Am J Infect Control. 2015;
698 doi:10.1016/j.ajic.2015.03.024
699 50. Hedin G, Rynbäck J, Loré B. Reduction of bacterial surface contamination in the
700 hospital environment by application of a new product with persistent effect. J Hosp
701 Infect. 2010; doi:10.1016/j.jhin.2010.02.007
702 51. Younes JA, Lievens E, Hummelen R, van der Westen R, Reid G, Petrova MI.
703 Women and Their Microbes: The Unexpected Friendship. Trends in Microbiology.
704 2018. doi:10.1016/j.tim.2017.07.008
705 52. Grice EA, Segre JA. The skin microbiome. Nature Reviews Microbiology. 2011.
706 doi:10.1038/nrmicro2537
707 53. Haiser HJ, Turnbaugh PJ. Developing a metagenomic view of xenobiotic
708 metabolism. Pharmacological Research. 2013. doi:10.1016/j.phrs.2012.07.009
709 54. Das A, Srinivasan M, Ghosh TS, Mande SS. Xenobiotic metabolism and gut
710 microbiomes. PLoS One. 2016; doi:10.1371/journal.pone.0163099
711 55. Amies CR. A modified formula for the preparation of Stuart's Transport Medium.
712 Can J Public Heal. 1967;
713 56. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing
714 reads. EMBnet.journal. 2011;17: 10. doi:10.14806/ej.17.1.200
715 57. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic
716 datasets. Bioinformatics. 2011;27: 863–864. doi:10.1093/bioinformatics/btr026
717 58. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal
718 Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids
719 Res. 2014;42: D633–D642. doi:10.1093/nar/gkt1244
720 59. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. MicrobiomeAnalyst:
721 A web-based tool for comprehensive statistical, visual and meta-analysis of
722 microbiome data. Nucleic Acids Res. 2017; doi:10.1093/nar/gkx295
723 60. Paulson JN, Colin Stine O, Bravo HC, Pop M. Differential abundance analysis for
724 microbial marker-gene surveys. Nat Methods. 2013; doi:10.1038/nmeth.2658
725 61. I. B. Clinical review: Bacteremia caused by anaerobic bacteria in children. Crit
726 Care. 2002;
727 62. Cosseau C, Romano-Bertrand S, Duplan H, Lucas O, Ingrassia I, Pigasse C, et al.
728 Proteobacteria from the human skin microbiota: Species-level diversity and
729 hypotheses. One Heal. 2016; doi:10.1016/j.onehlt.2016.02.002
730 63. Palmer RJ. Composition and development of oral bacterial communities.
731 Periodontol 2000. 2014; doi:10.1111/j.1600-0757.2012.00453.x
732 64. Mo S, Wei L, Chen H, Li R, Li S, Luo G. A chinese case of prevotella intermedia
733 and streptococcus constellatus intracranial mixed infection. Metab Brain Dis.

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734 2018; doi:10.1007/s11011-017-0142-x
735 65. Rovery C, Etienne A, Foucault C, Berger P, Brouqui P. Veillonella
736 montpellierensis endocarditis. Emerg Infect Dis. 2005;
737 doi:10.3201/eid1107.041361
738 66. Zaninetti-Schaerer A, Van Delden C, Genevay S, Gabay C. Total hip prosthetic
739 joint infection due to Veillonella species [2]. Joint Bone Spine. 2004.
740 doi:10.1016/j.jbspin.2003.10.019
741 67. Liu JW, Wu JJ, Wang LR, Teng LJ, Huang TC. Two fatal cases of Veillonella
742 bacteremia [2]. European Journal of Clinical Microbiology and Infectious
743 Diseases. 1998. doi:10.1007/BF01584370
744 68. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The
745 composition of the gut microbiota throughout life, with an emphasis on early life.
746 Microb Ecol Health Dis. 2015; doi:10.3402/mehd.v26.26050
747 69. Layton A, McKay L, Williams D, Garrett V, Gentry R, Sayler G. Development of
748 Bacteroides 16S rRNA gene taqman-based real-time PCR assays for estimation of
749 total, human, and bovine fecal pollution in water. Appl Environ Microbiol. 2006;
750 doi:10.1128/AEM.01036-05
751 70. Falagas ME, Siakavellas E. Bacteroides, Prevotella, and Porphyromonas species:
752 A review of antibiotic resistance and therapeutic options. International Journal of
753 Antimicrobial Agents. 2000. doi:10.1016/S0924-8579(99)00164-8
754 71. Woodford N, Livermore DM. Infections caused by Gram-positive bacteria: a
755 review of the global challenge. J Infect. 2009; doi:10.1016/S0163-4453(09)60003-
756 7
757 72. Prabaker K, Weinstein RA. Trends in antimicrobial resistance in intensive care
758 units in the United States. Current Opinion in Critical Care. 2011.
759 doi:10.1097/MCC.0b013e32834a4b03
760 73. de Kraker MEA, Davey PG, Grundmann H. Mortality and hospital stay associated
761 with resistant Staphylococcus aureus and Escherichia coli bacteremia: Estimating
762 the burden of antibiotic resistance in Europe. PLoS Med. 2011;
763 doi:10.1371/journal.pmed.1001104
764 74. Macvane SH. Antimicrobial Resistance in the Intensive Care Unit. J Intensive Care
765 Med. 2017; doi:10.1177/0885066615619895
766 75. Celandroni F, Salvetti S, Gueye SA, Mazzantini D, Lupetti A, Senesi S, et al.
767 Identification and pathogenic potential of clinical Bacillus and Paenibacillus
768 isolates. PLoS One. 2016; doi:10.1371/journal.pone.0152831
769 76. Adler A, Gottesman G, Dolfin T, Arnon S, Regev R, Bauer S, et al. Bacillus species
770 sepsis in the neonatal intensive care unit. J Infect. 2005;
771 doi:10.1038/ncpgasthep0256
772 77. Van Der Zwet WC, Parlevliet GA, Savelkoul PH, Stoof J, Kaiser AM, Van Furth
773 AM, et al. Outbreak of Bacillus cereus infections in a neonatal intensive care unit
774 traced to balloons used in manual ventilation. J Clin Microbiol. 2000;
775 78. Turabelidze G, Gee JE, Hoffmaster AR, Manian F, Butler C, Byrd D, et al.
776 Contaminated ventilator air flow sensor linked to bacillus cereus colonization of
777 Newborns. Emerg Infect Dis. 2013; doi:10.3201/eid1905.120239
778 79. Cogen AL, Nizet V, Gallo RL. Skin microbiota: A source of disease or defence?
779 Br J Dermatol. 2008; doi:10.1111/j.1365-2133.2008.08437.x
780 80. Lindsay JA. Staphylococci. Molecular Typing in Bacterial Infections. 2013.
781 doi:10.1007/978-1-62703-185-1_23
782 81. Chessa D, Ganau G, Mazzarello V. An overview of staphylococcus epidermidis
783 and staphylococcus aureus with a focus on developing countries. J Infect Dev

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784 Ctries. 2015; doi:10.3855/jidc.6923
785 82. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin
786 Microbiol Rev. 2014; doi:10.1128/CMR.00109-13
787 83. Fischetti VA, Ryan P. Streptococcus. Practical Handbook of Microbiology, Third
788 Edition. 2015. doi:10.1201/b17871
789 84. Esfahani B, Basiri R, Mirhosseini S, Moghim S, Dolatkhah S. Nosocomial
790 Infections in Intensive Care Unit: Pattern of Antibiotic-resistance in Iranian
791 Community. Adv Biomed Res. 2017; doi:10.4103/2277-9175.205527
792 85. Patterson MJ. Streptococcus. In: Baron's Med Microbiol, editor. Medical
793 Microbiology. University of Texas Medical Branch; 1996.
794 86. Li S, Huang J, Chen Z, Guo D, Yao Z, Ye X. Antibiotic prevention for maternal
795 group B streptococcal colonization on neonatal GBS-related adverse outcomes: A
796 meta-analysis. Frontiers in Microbiology. 2017. doi:10.3389/fmicb.2017.00374
797 87. Spanu T, De Angelis G, Cipriani M, Pedruzzi B, D'Inzeo T, Cataldo MA, et al. In
798 Vivo Emergence of Tigecycline Resistance in Multidrug-Resistant Klebsiella
799 pneumoniae and Escherichia coli . Antimicrob Agents Chemother. 2012;
800 doi:10.1128/aac.00234-12
801 88. Yue DM, Song C, Zhang B, Liu Z, Chai J, Luo Y, et al. Hospital-wide comparison
802 of health care-associated infection among 8 intensive care units: A retrospective
803 analysis for 2010-2015. Am J Infect Control. 2017; doi:10.1016/j.ajic.2016.10.011
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834 **Table 1. Essential characteristics and localization of the sequenced samples.**
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Sample ID	Sample source	Care unit	Ward	Cleaning
Pumps-ICUa	Pump	ICU	A	−
Mattresses-ICUa	Mattress	ICU	A	−
Rails-ICUa	Rail	ICU	A	−
Monitors-ICUa	Monitor	ICU	A	−
Ventilators-ICUa	Ventilator	ICU	A	−
Pumps-ICUb	Pump	ICU	B	−
Mattresses-ICUb	Mattress	ICU	B	−
Rails-ICUb	Rail	ICU	B	−
Monitors-ICUb	Monitor	ICU	B	−
Ventilators-ICUb	Ventilator	ICU	B	−
MedicalRecords-ICUab	Medical record	ICU	AB	−
Cards-ICUab	Card	ICU	AB	−
Mobiles-ICUab	Mobiles	ICU	AB	−
Handles-ICUab	Handle	ICU	AB	−
DrugStations-ICUab	Drug station	ICU	AB	−
Computers-ICUab	Computer	ICU	AB	−
Pumps-ICUaA	Pump	ICU	A	+
Mattresses-ICUaA	Mattress	ICU	A	+
Rails-ICUaA	Rail	ICU	A	+
Monitors-ICUaA	Monitor	ICU	A	+
Ventilators-ICUaA	Ventilator	ICU	A	+
Pumps-ICUbA	Pump	ICU	B	+
Mattresses-ICUbA	Mattress	ICU	B	+
Rails-ICUbA	Rail	ICU	B	+
Monitors-ICUbA	Monitor	ICU	B	+
Ventilators-ICUbA	Ventilator	ICU	B	+
Cufflators-ICUabA	Cufflator	ICU	AB	+
Pumps-NICUa	Pump	NICU	A	−
Mattresses-NICUa	Mattress	NICU	A	−
Rails-NICUa	Rail	NICU	A	−
Monitors-NICUa	Monitor	NICU	A	−
Ventilators-NICUa	Ventilator	NICU	A	−
Pumps-NICUb	Pump	NICU	B	−
Mattresses-NICUb	Mattress	NICU	B	−
Rails-NICUb	Rail	NICU	B	−
Monitors-NICUb	Monitor	NICU	B	−
Ventilators-NICUb	Ventilator	NICU	B	−
Mobiles-NICUab	Mobiles	NICU	AB	−
Cards-NICUab	Card	NICU	AB	−
Handles-NICUab	Handle	NICU	AB	−
MedicalRecords-NICUab	Medical record	NICU	AB	−
DrugStations-NICUab	Drug station	NICU	AB	−
Computers-NICUab	Computer	NICU	AB	−

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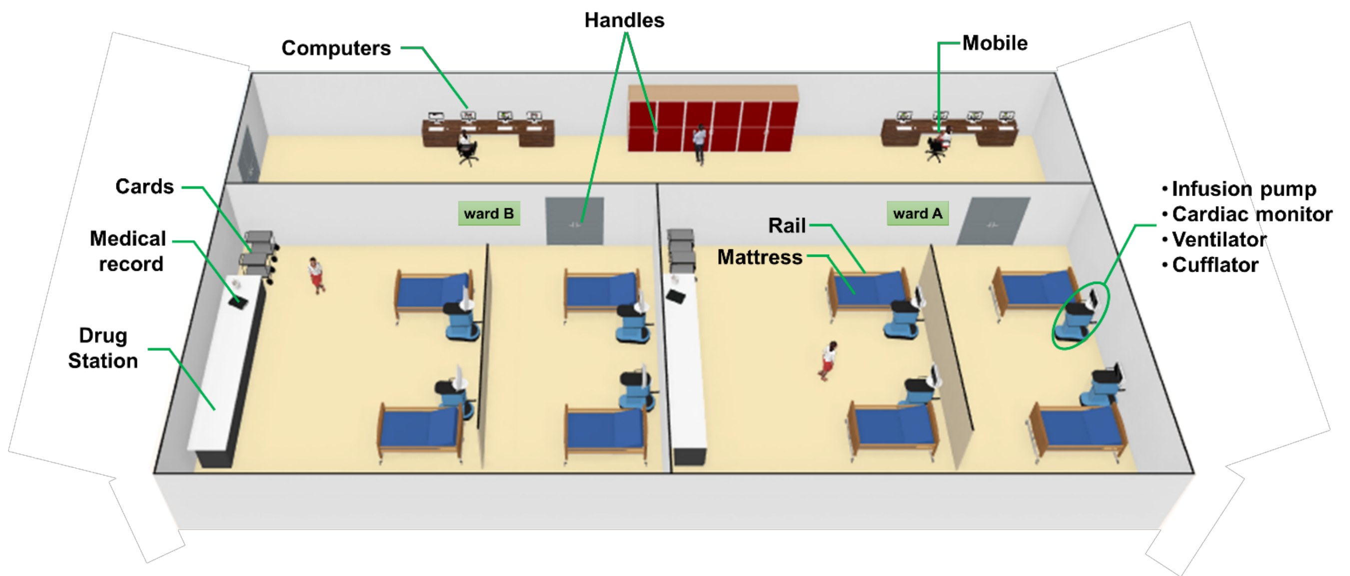
839 **Figure 1**



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841 **Figure 1. 3D-rendered-model showing each sampling site of the (Neonatal) intensive care unit**
842 **((N)ICU).** ICU and NICU are on different floors at the hospital but have a similar arrangement of wards and
843 devices in general. A detailed explanation of each sample is shown in Table 1.
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Figure 2

Figure 2. The ICU and NICU bacteria microbiota profile. (**A**) The relative abundance of bacterial genera within the top 379 OTUs among the two units. Colors correspond to the bacterial genera in the legend. Rectangles represent specific genera organized in order of abundance. Sequencing results are presented for each sample clustered using Usearch algorithm with a 97% cutoff. NA (Not_Assigned) represents sequences reads that were not assigning an accurate taxonomic label at the genus level but assigned at the higher taxonomic level. (**B**) Alpha diversity at OTU level at ICU (red, n=16) and NICU (cyan, n=16) calculated using Shannon index (Kruskal-Wallis test, p-value < 0.05). For each box plot herein forward, the line within the box and the black diamond represent the median and mean, respectively. The bottom and top boundaries of each box indicate the first and third quartiles (the 25th and 75th percentiles), respectively. The whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR). Two- (**C**) and three-dimensional (**D**) principal coordinate analysis (PCoA) plot based on Jensen-Shannon distances between bacterial communities associated with ICU and NICU areas (ANOSIM, R= 0.3066; p-value < 0.001). Samples are shown as single dots. Divergence at OTU level was computed on Total sum scaling–normalized (TSS-normalized) datasets.

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879 **Figure 3**

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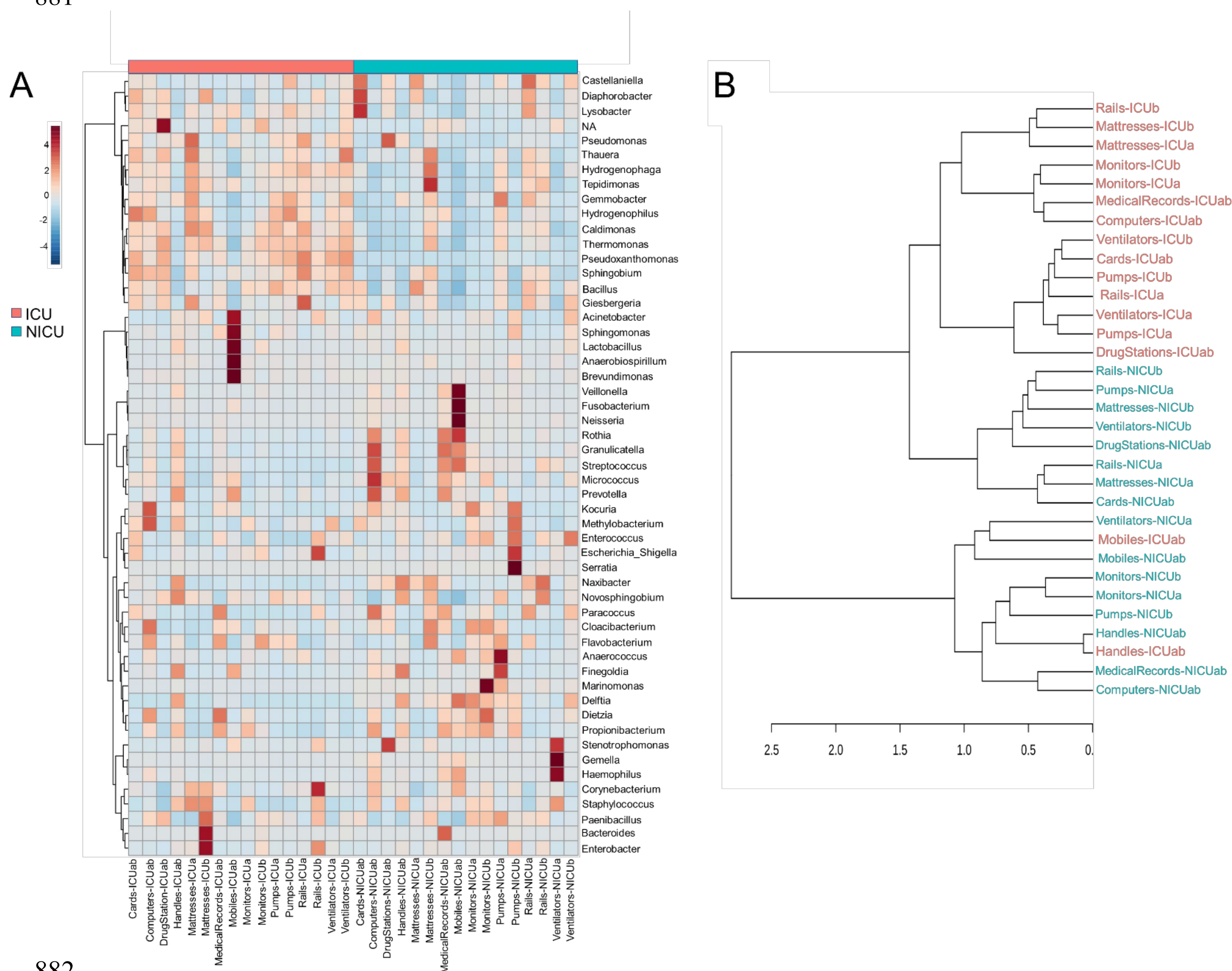

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884 **Figure 3. Clustering analysis of the ICU and NICU**. (**A**) Heatmap, and hierarchical clustering of
885 the main genera associated with ICU and NICU samples. The heatmap shows the relative abundance
886 of the top 52 bacterial genera (rows) in each sample (columns). Hierarchical clustering is based on
887 Ward Clustering algorithm and Euclidean Distance measure to generate the hierarchical tree. The
888 color bar indicates the range of the relative abundance. (**B**) Dendrogram showing the similarities
889 between ICU and NICU samples. The dendrogram was created using the Jaccard index as distance
890 measure and Ward's clustering algorithm.
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897 **Figure 4**

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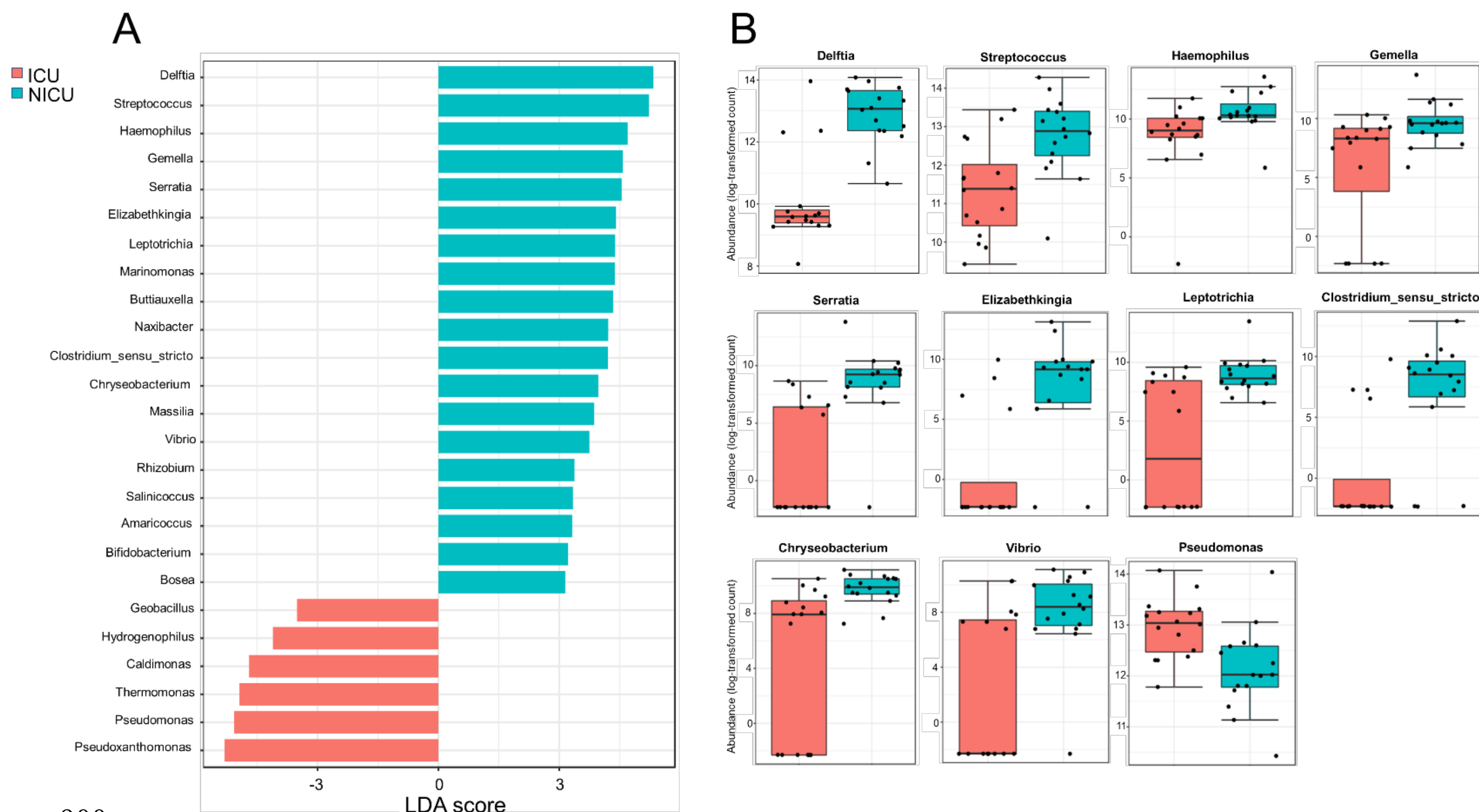

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902 **Figure 4. Significant differences between ICU and NICU**. (**A**) Taxonomic biomarkers for ICU and
903 NICU. Linear Discriminant Analysis (LDA) combined with Effect Size (LEfSe) indicate significant
904 differences at the genus level that enable discrimination between the ICU and NICU samples ($p <$
905 0.05). Only those genera with log LDA score >3 are ultimately considered. (**B**) Boxplot of relative
906 abundance (log scale) of the eleven HAI-related bacterial genera with significant differences between
907 ICU (red, n=16) and NICU (cyan, n=16). The difference was calculated using Mann-
908 Whitney/Kruskal-Wallis test (p-value and FDR < 0.05).

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Figure 5

Figure 5. Co-occurrence and co-exclusion analysis of the bacterial genera. Heatmap showing Pearson's r correlation coefficients among the top 50 abundant bacterial genera from the (**A**) ICU and (**B**) NICU. The correlation values ranged from −1.00 (blue) to 1.00 (red). Each square represents the Pearson's r correlation coefficient between the genera of the column with that of the row. Self-self-correlations are identified in brown.

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943 **Figure 6**

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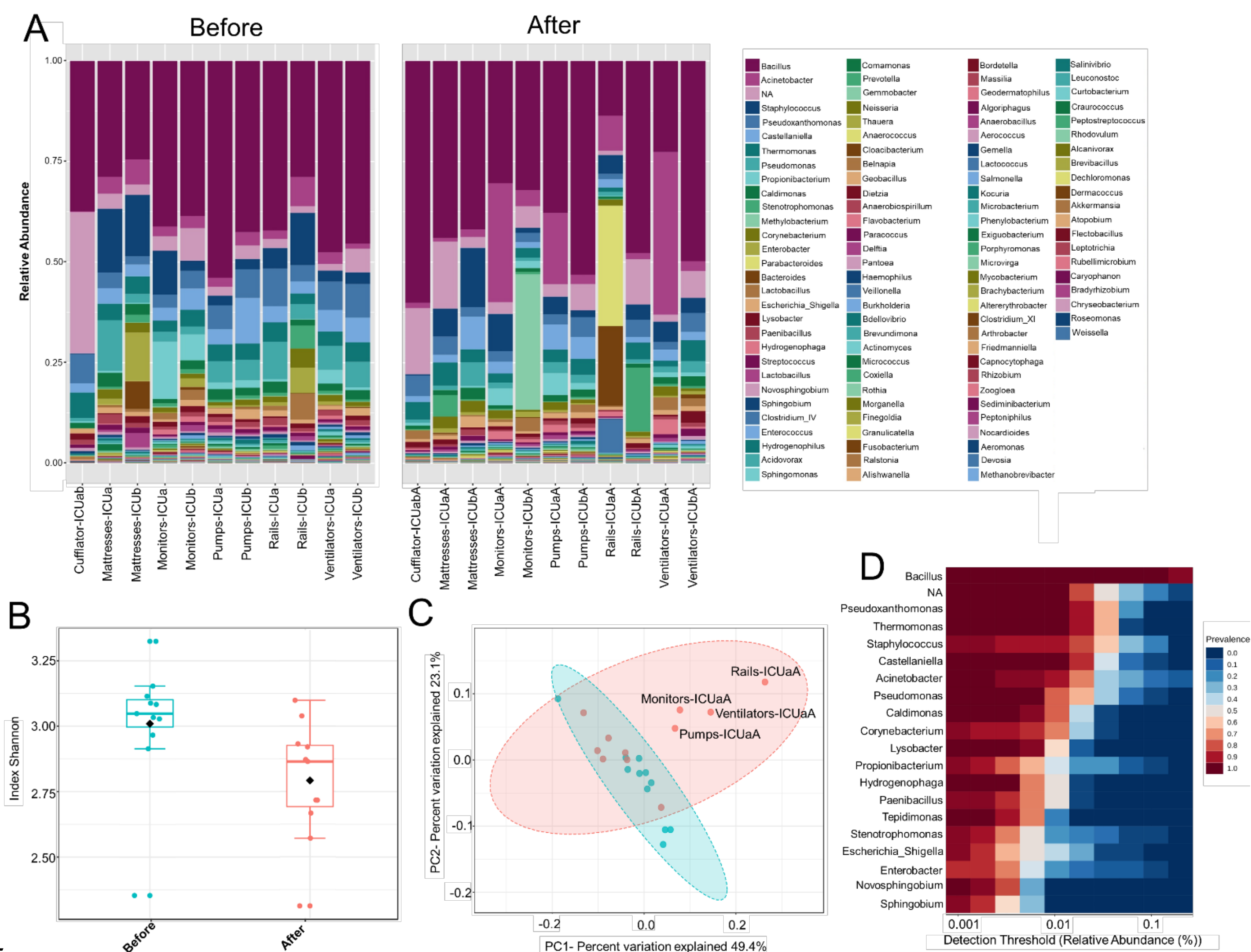

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948 **Figure 6. ICU bacteria microbiota profile before and after cleaning**. (**A**) Relative bacterial
949 abundance at the genus level. Sequencing results are showed for each sample surface clustered using
950 Usearch algorithm with a 97% cutoff. Only genera with abundance > 1.0% were plotted. (**B**) Alpha
951 diversity at OTU level, before (red, n=11) and after cleaning (cyan, n=11) calculated using Shannon
952 index (Kruskal-Wallis test, p-value < 0.05). (**C**) PCoA plot based on Jensen-Shannon distances
953 between bacterial communities associated with cleaning (ANOSIM, R= 0.091961; p-value = 0.039).
954 Samples are shown as single dots. Divergence at OTU level was computed on Total sum scaling–
955 normalized (TSS-normalized) datasets. (**D**) Core microbiome analysis based on relative abundance
956 and sample prevalence of bacterial genus before and after cleaning.
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961 **Figure 7**

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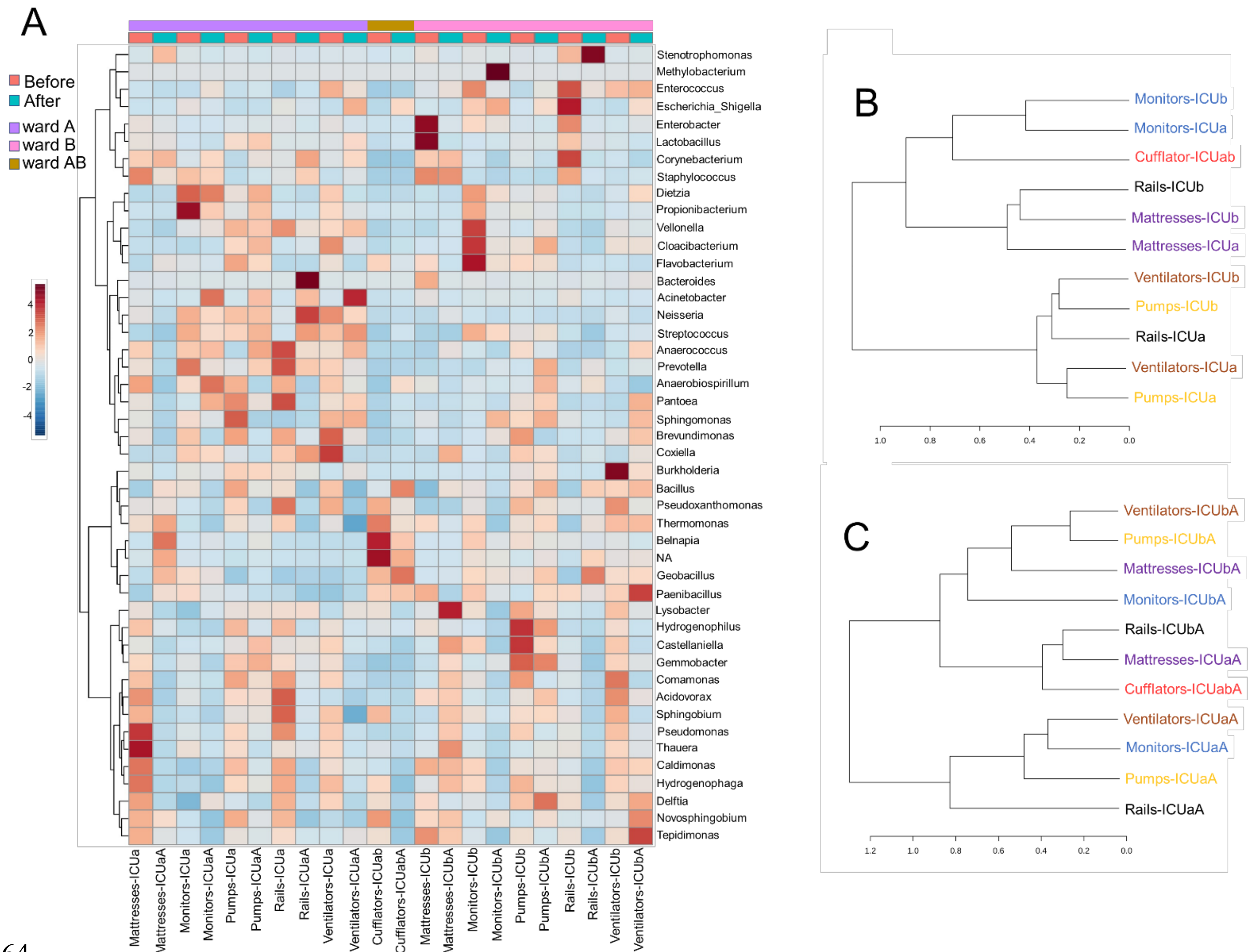

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966 **Figure 7. Clustering analysis of the ICU samples before and after cleaning. (A)** Heatmap of the
967 main genera associated with ICU samples before and after cleaning. The heatmap shows the relative
968 abundance of top 45 bacterial genera (rows) in each sample (columns). The color bar indicates the
969 range of the relative abundance. Dendrogram showing the similarities between samples **(B)** before
970 and **(C)** after cleaning. The dendrogram was created using the Jaccard index as distance measure and
971 Ward's clustering algorithm.

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Figure 8

Figure 8. Co-occurrence and co-exclusion analysis of the bacterial genera. Heatmap showing Pearson's r correlation coefficients among the top 50 abundant bacterial genera from the (**A**) ICU before and (**B**) ICU after cleaning. The correlation values ranged from −1.00 (blue) to 1.00 (red). Each square represents the Pearson's r correlation coefficient between the genera of the column with that of the row. Self-self-correlations are identified in brown.

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1002 # Supplementary material

1003 ## Figure S1

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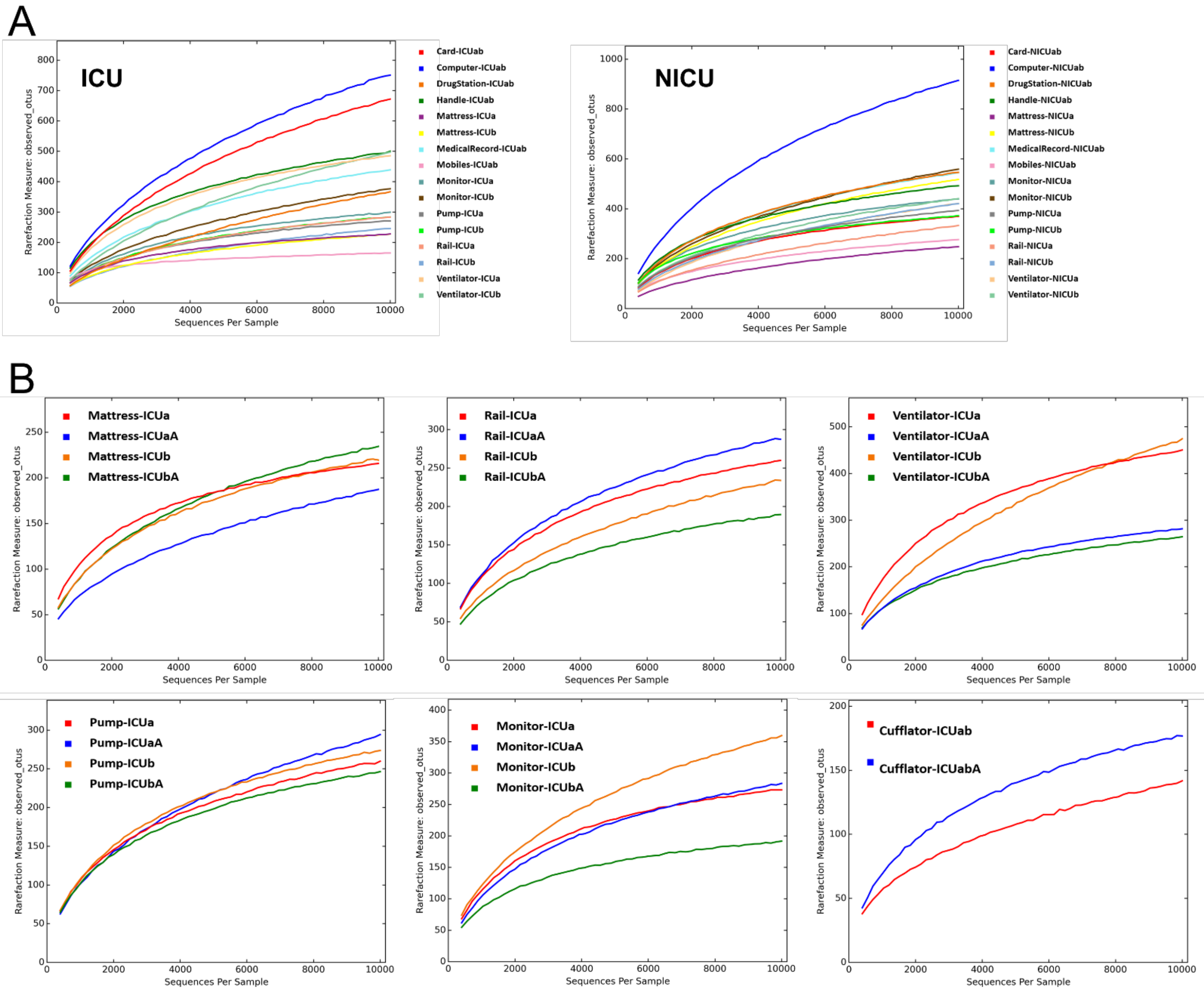

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1006 **Figure S1. Rarefaction curve showing the relationship between the sequencing per sample and the**
1007 **number of OTUs that these reads represent. (A)** ICU and NICU, and **(B)** ICU before and after cleaning.
1008 Sequences were rarefied with 33.708 read counts per sample.
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Figure S2

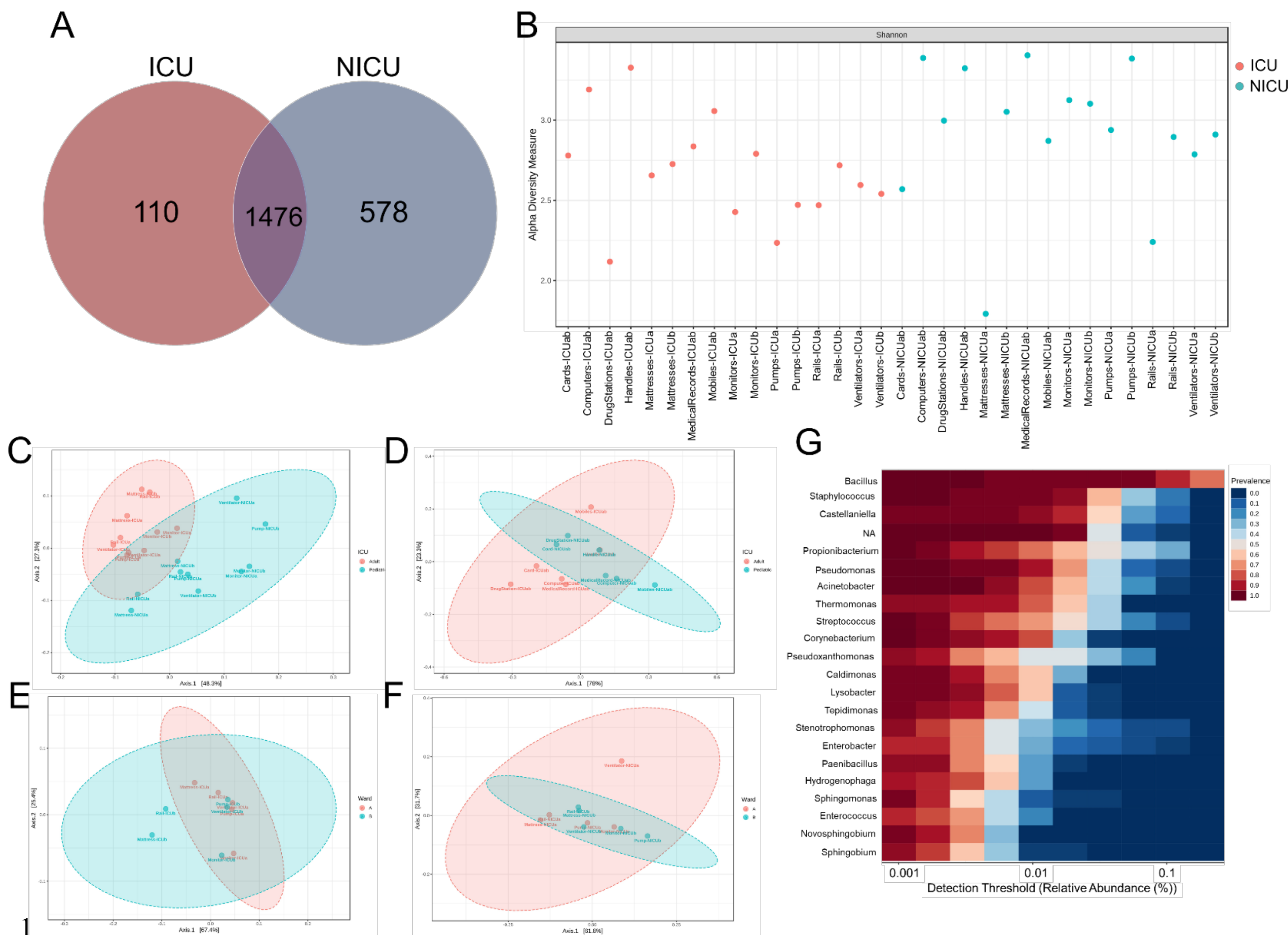

Figure S2. (**A**) Venn diagram showing shared and unique OTUs. (**B**) Alpha diversity at the OTU level for each sample at ICU (red) and NICU (cyan) calculated using Shannon index (Kruskal-Wallis test, p-value < 0.05). PCoA plot based on Jensen-Shannon distances between bacterial communities associated with (**C**) ICU and NICU boxes areas (ANOSIM, R= 0.50756; p-value < 0.001); (**D**) ICU and NICU common areas (ANOSIM, R= 0.14074; p-value = 0.116); (**E**) ICU wards (ANOSIM, R= 0.124; p-value = 0.177); (**F**) NICU wards (ANOSIM, R= -0.02; p-value = 0.52). Samples are shown as single dots. (**G**) Core microbiome analysis based on relative abundance and sample prevalence of bacterial genus in ICU and NICU. Divergence at OTU level was computed on Total sum scaling–normalized (TSS-normalized) datasets.

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1039 **Figure S3**

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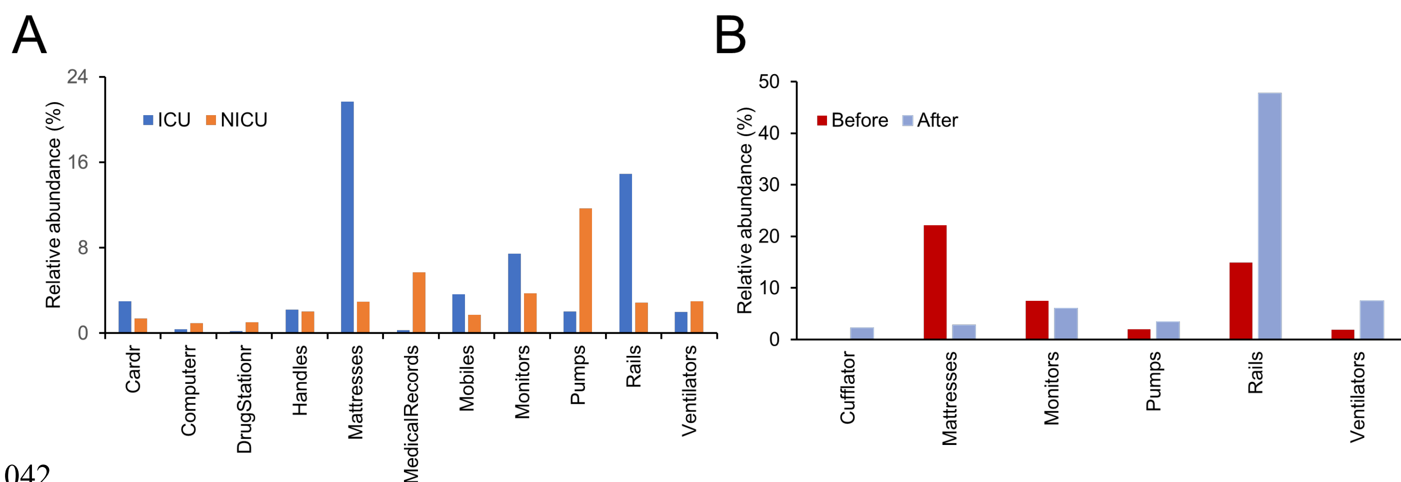

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1044 **Figure S3.** The abundance of fecal indicators in **(A)** ICU/NICU and **(B)** ICU before/after cleaning.

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1071 **Figure S4**

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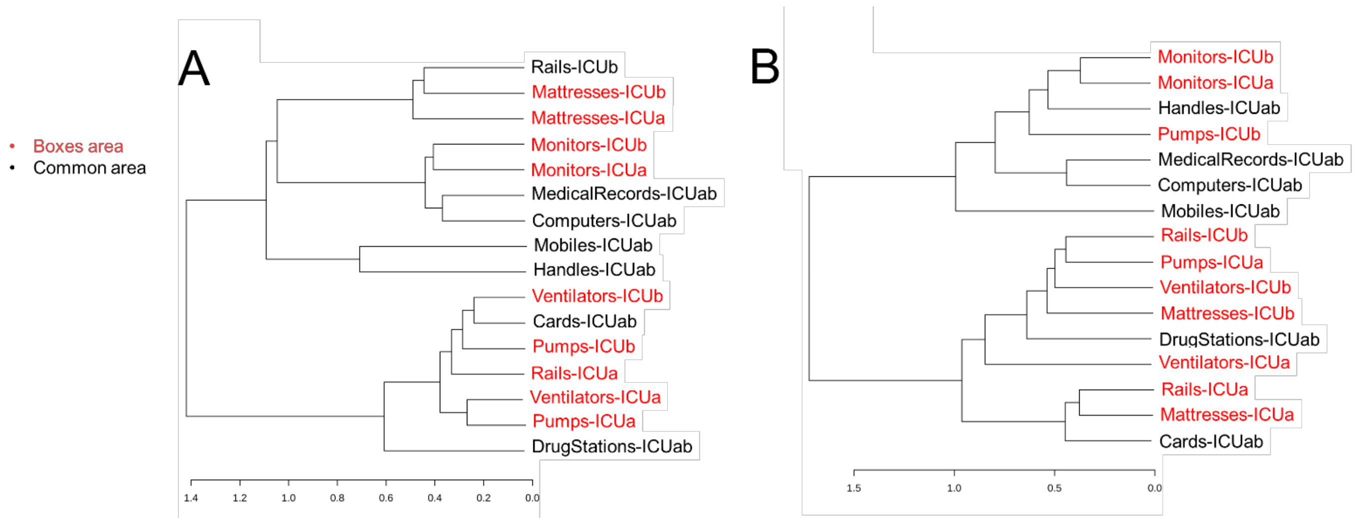

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1076 **Figure S4. Dendrogram showing the similarities between samples**. **(A)** ICU; **(B)** NICU. The
1077 dendrogram was created using the Jaccard index as distance measure and Ward's clustering algorithm.
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1105 **Figure S5**

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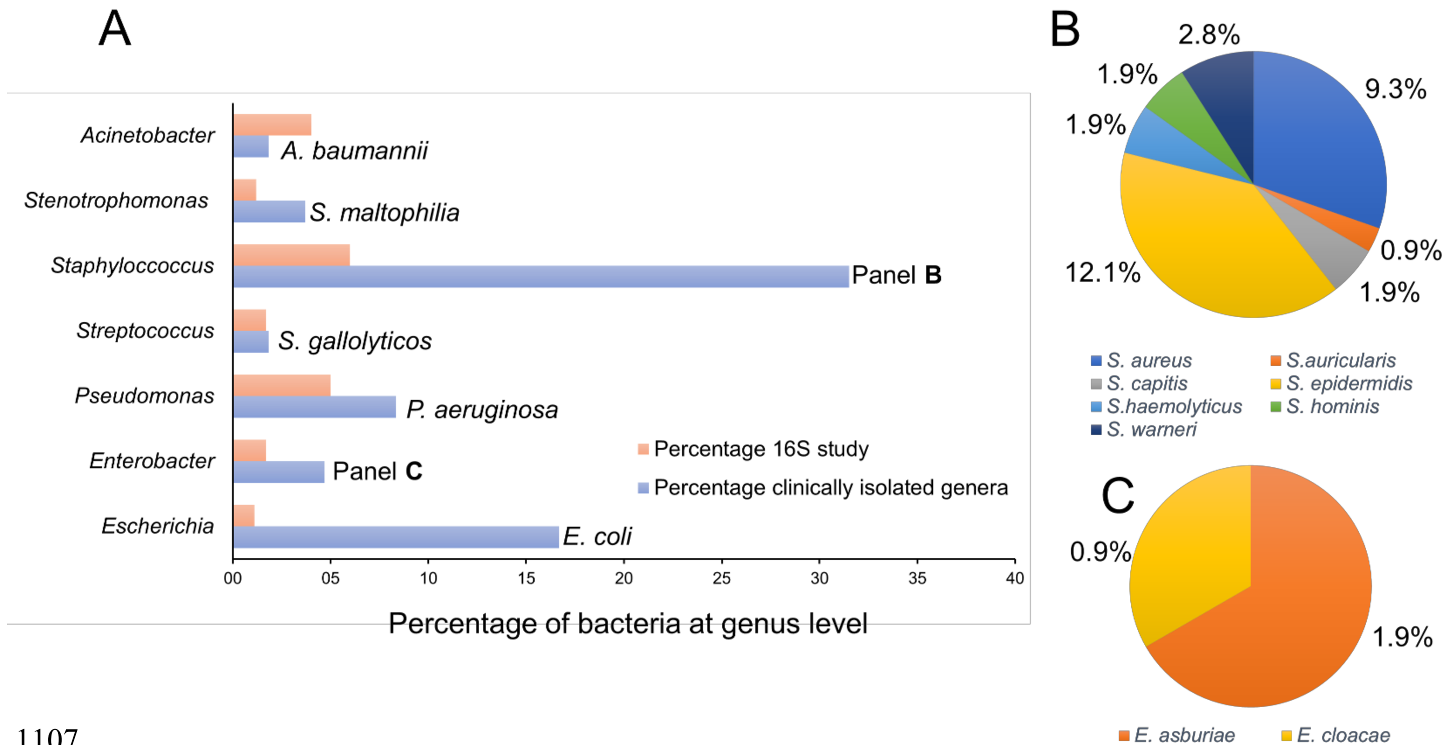

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1109 **Figure S5. Percentage of identified bacteria at the genus level from the 16S analysis and isolated**
1110 **bacteria from clinical samples.** (**A**) A total of 108 bacterial strains (gathered in 12 different genera)
1111 isolated from blood, bronchoalveolar lavage, peritoneal, cerebrospinal and ascitic fluids of hospitalized
1112 patients in the ICU were evaluated. In the graph are represented only the seven most abundant genera
1113 from the 16S amplicon study (considering up to 4% of all quality sequences). Data at the level of
1114 species are presented just for the bacteria isolated from clinical samples. Percentage of species
1115 belonging to the genera *Staphylococcus* (**B**) and *Enterobacter* (**C**), concerning the 108 bacterial strains
1116 isolated. For the other genera, single species were identified among biological samples, as presented
1117 in panel A.
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1133 **Figure S6**

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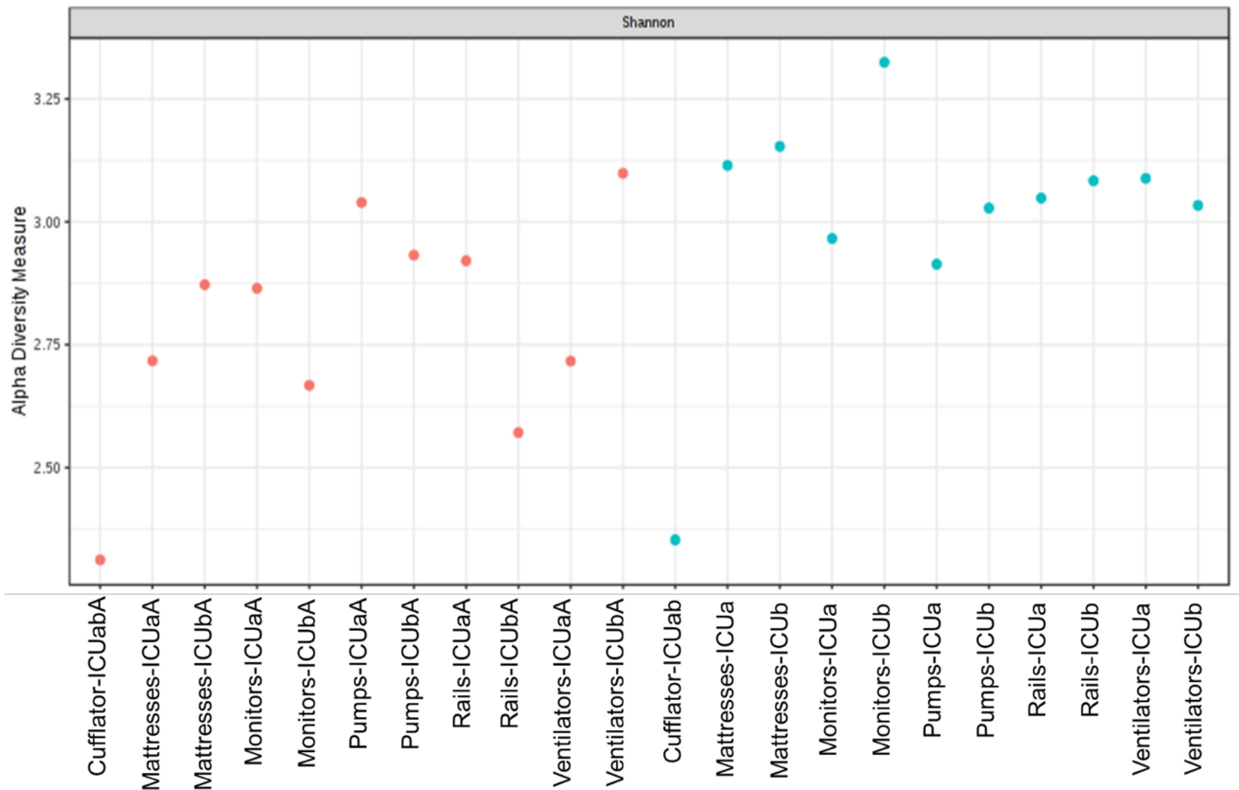

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1139 **Figure S6**. Alpha diversity at OTU level for each sample at ICU before (red) and after cleaning (cyan)
1140 calculated using Shannon index (Kruskal-Wallis test, p-value < 0.05).

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1160 **Figure S7**

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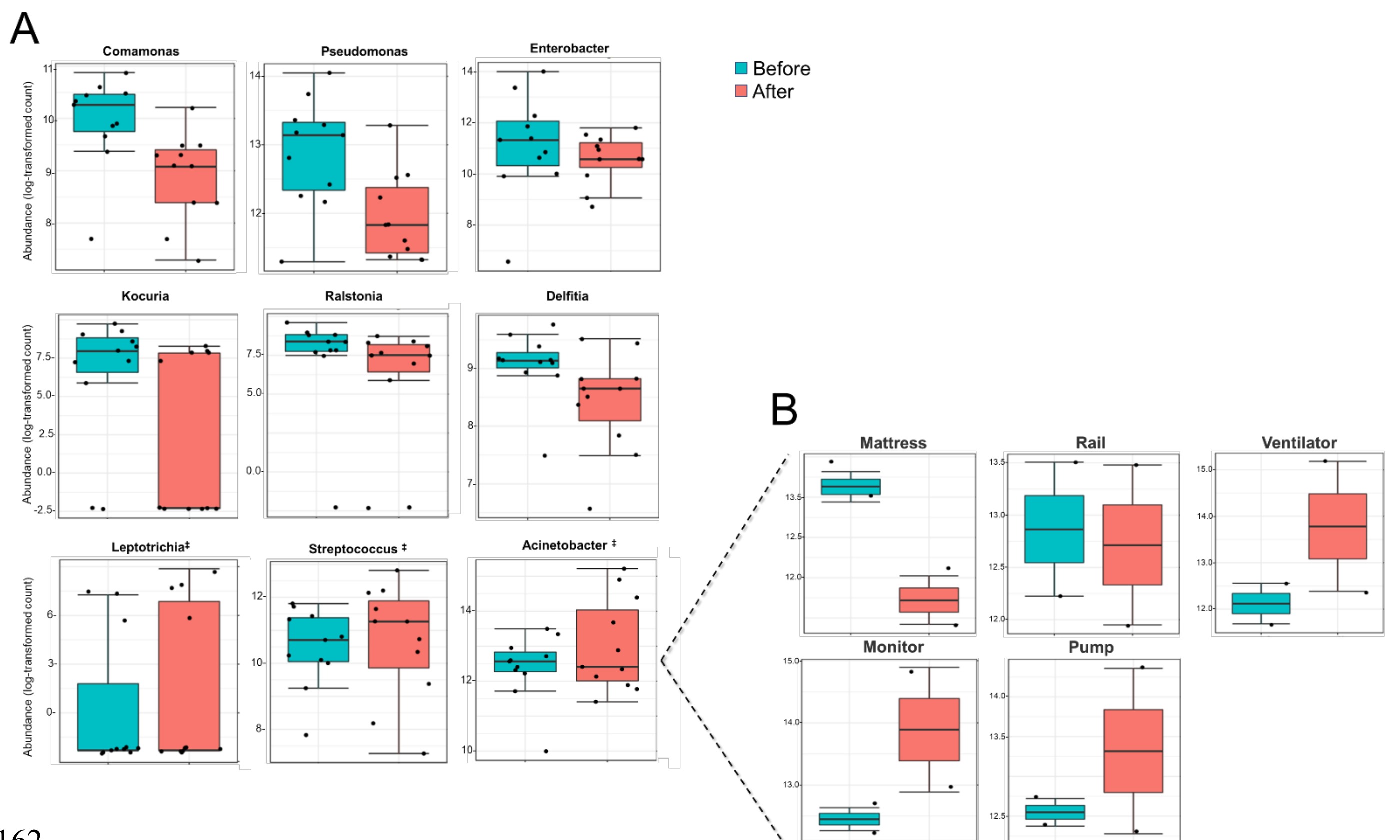

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1164 **Figure S7**. (**A**) Boxplot of relative abundance (log scale) of the genera HAI-related before (cyan) and
1165 after (red) cleaning. The difference was calculated using Mann-Whitney/Kruskal-Wallis test (FDR
1166 ≥0.3). ‡Genera with higher abundance after cleaning. (**B**) Differential relative abundance for
1167 *Acinetobacter* across all the ICU samples.
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1186 # Supplementary text

1187 *Oxygen tolerance*

1188 Most of the samples contained a mixture of organisms with various degrees of
1189 oxygen tolerance. The number of strictly aerobic genera were highly represented (50%)
1190 followed by facultative anaerobe (36%) and obligatory anaerobic bacteria (14%) for both
1191 units. Infections caused by anaerobic bacteria are often underestimated, due to the
1192 difficulty to isolate and identify these microorganisms. The use of unspecific therapy
1193 against these infections may cause clinical failures [61]. Most abundant anaerobic
1194 organisms in ICU were, on decrescent order, *Propionibacterium*, *Bacteroides*, and
1195 *Prevotella*, whereas for NICU were *Propionibacterium*, *Prevotella*, and *Veillonella*.
1196 *Propionibacterium* is a human skin-associated genus [62], while *Prevotella* and
1197 *Veillonella* are part of the healthy microflora in the oral cavity and vaginal [51,63].
1198 However, many species of *Prevotella*, and *Veillonella* genera are pathogens that cause
1199 oral or respiratory diseases [64], as well as meningitis [64]. *Veillonella* has also been
1200 involved in prosthetic cardiac valve or joint infections [65,66] and fatal sepsis [67].
1201 *Bacteroides* species are usually part of the gastrointestinal microbiota [68], and they make
1202 a significant portion of the fecal bacterial population [69]. Among all anaerobic bacteria,
1203 *Bacteroides*, *Prevotella*, and *Veillonella* are the most frequently isolated in clinical
1204 samples of infection [70].

1205

1206 *Gram-positive bacteria*

1207 Gram-positive bacteria were found in higher abundance in both units (Gram-
1208 positive and Gram-negative at ICU ─ 49% and 46%; NICU ─ 52% and 44.5%,
1209 respectively; ~5% were Gram-variable). Nonetheless, in terms of the number of genera,
1210 Gram-negative bacteria were predominant in both ICU (70%) and NICU (66%). Over the
1211 last decades, the focus of infection control centers was targeting Gram-positive pathogens
1212 due to their high rate of morbidity and mortality [71]. However, the incidence of
1213 infections in UTIs caused by Gram-negative bacteria has been rising alarmingly,
1214 requiring a better understanding of hospital microbiomes [72–74].

1215 The five more abundant Gram-positive genera found in both ICU and NICU
1216 samples were, in order of decreasing abundance, *Bacillus*, *Staphylococcus*,
1217 *Propionibacterium*, *Streptococcus*, and *Corynebacterium*. Core microbiome analysis was
1218 performed combining samples from both ICU and NICU. In total, 21 genera were shared

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1219 in 80% of all samples at the minimum detection threshold of 0.001% relative abundance
1220 (**Fig. S2G**). The most abundant Gram-positive genera were also among the top 10 more
1221 prevalent. Most notably, *Bacillus* was the most prevalent genus in the core microbiome
1222 for both care units. *Bacillus* was also most abundant in ICU (36%) and NICU (26%)
1223 (**Fig. 2A**), mainly in the boxes area. To examine more deeply the bacterial community
1224 variations among the samples, a heatmap of the top 52 genera is illustrated in **Fig. 3A**.
1225 Accordingly, the ICU pumps and NICU mattresses contained the highest abundance of
1226 the total reads (~6%) (**Fig. 3A**). The identification of the *Bacillus* genus in hospital
1227 samples is often considered clinically safe since it is ubiquitous in the environment.
1228 However, recently outbreaks of severe and lethal *Bacillus* infections have been widely
1229 reported, especially diseases related with *Bacillus cereus* at NICUs [75,76]. Several of
1230 these infections resulted of contamination of respiratory equipment [76–78]. Therefore,
1231 contamination with this genus should not be routinely neglected.

1232 Several clinical and metagenomic studies have described Gram-positive bacteria
1233 as a highly frequent colonizer of the skin [52,79]. The skin-associated genera
1234 *Staphylococcus*, *Propionibacterium*, *Streptococcus*, and *Corynebacterium*, were found in
1235 high abundance in surfaces frequently touched by hands of healthcare workers (HCW)
1236 such as, in order of decreasing abundance, computers, door handles, medical records,
1237 monitors, and mobiles (**Fig. 3A**). Most of the bacteria of these genera are harmless but
1238 may become opportunistic pathogens for immunocompromised patients [39]. Moreover,
1239 a high abundance of these genera was also observed in ventilators and pumps, suggesting
1240 that skin contact may be an essential source of contamination. *Staphylococcus* was found
1241 in all samples with a total abundance of 6% for both ICU and NICU. In the boxes area,
1242 this genus was more present on mattresses in ICU and ventilators in NICU (**Fig. 3A**).
1243 *Staphylococcus* can be found on the skin or in the nose of healthy patients causing no
1244 disease or only minor skin infections. However, several species can be deadly when
1245 invading bloodstream, joints, bones, lungs or heart [80]. *Staphylococcus aureus* is the
1246 most pathogenic and well-established species in the hospital environment.

1247 Furthermore, coagulase-negative species such as *S. epidermidis*, *S. sciuri*, and *S.*
1248 *haemolyticus* are also an emerging problem in UTIs [80–82]. *Streptococcus* was found in
1249 all samples with a total abundance of 1.7% and 5% for ICU and NICU, respectively. In
1250 the boxes area, this genus was more present on monitors for both ICU and NICU (**Fig.**
1251 **3Aa**). Nosocomial infections with *Streptococcus spp.* are often associated with
1252 respiratory or skin diseases [83] and cause long days of hospitalization [84]. Species such

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1253 as *S. pneumoniae* is the first most common cause of fatal bacterial pneumonia in
1254 developing countries with high morbidity in children [85]. Group B *Streptococcus*, a
1255 commensal bacterium, is the leading cause of death from early-onset infections in the
1256 neonate [86].

1257 Other Gram-positive genera related to nosocomial infection were found but in
1258 intermediate abundance (0.5-1%), as, e.g., *Gemella*, *Enterococcus*, and *Clostridium*.
1259 These genera were present mainly in NICU being more abundant in ventilators (**Fig.**
1260 **3Aa**). However, they were also found in pumps (*Enterococcus)*, door handles
1261 (*Clostridium*) and computers (*Gemella*). A very low abundance (≤0.1%) of these genera
1262 was observed in ICU samples, e.g., room cards).

1263

1264 *Gram-negative bacteria*

1265 In the last decade, Gram-negative strains have gotten attention in their ability to
1266 spread their antibiotic resistance in hospital environments [87]. New molecular protocols,
1267 such as NGS, have allowed identifying emerging threats associated with nosocomial
1268 infections and multidrug-resistant [16]. An analysis involving eight ICUs reported that
1269 Gram-negative organisms were the principal responsible for HAI [88]. Here, the five
1270 more abundant Gram-negative genera found in ICU samples were, in order of decreasing
1271 abundance, *Pseudomonas*, *Pseudoxanthomonas*, *Castellaniella*, *Acinetobacter*,
1272 *Thermomonas*. Whereas for NICU were *Castellaniella*, *Delftia*, *Acinetobacter*,
1273 *Stenotrophomonas*, *Pseudomonas*. Previous studies have reported *Acinetobacter* and
1274 *Pseudomonas* as typical Gram-negative genera on (N)ICU surfaces [39].

1275 *Pseudomonas*, *Acinetobacter*, *Delftia*, and *Stenotrophomonas* are known for their
1276 facultative pathogenic nature and for being nosocomial bacteria. *Pseudomonas*
1277 constituted 5%, and 2.6% of the bacterial community at ICU and NICU, respectively. The
1278 ICU mattresses and NICU drug station contained the highest abundance (**Fig. 3A**).
1279 *Acinetobacter* was found in all samples with a total abundance of 4% and 3% for ICU
1280 and NICU, respectively. *Acinetobacter* was more frequent on ICU mobiles. In the boxes
1281 area, this genus was more present on ICU mattresses and NICU monitors (**Fig. 3A**).
1282 *Delftia* showed an abundance of 1% at ICU and 5.5% at NICU, being more frequent on
1283 mobiles and NICU monitors (**Fig. 3A**). *Stenotrophomonas* showed an abundance of 1.2%
1284 and 2.7% for ICU and NICU, respectively. This genus was more present on NICU drug
1285 station and ventilators (**Fig. 3A**). *Enterobacter* and *Escherichia_Shigella* were also found
1286 in high abundance in both units, mainly in pumps (NICU), and ICU bed matrasses and

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1287 rails (**Fig. 3Aa**). In general, fecal indicators (*Enterobacter, Escherichia, Bacteroides,*
1288 *Anaerobiospirillum, and Parabacteroides*) were more frequent in bed matrasses and rails
1289 for ICU, and pumps for NICU (**Fig. S3Aa**).

1290 Others Gram-negative HAI-related genera such as *Elizabethkingia* (bed rails),
1291 *Neisseria* (mobiles), *Haemophilus* (ventilators), *Leptotrichia* (mobiles), and *Serratia*
1292 (pumps) were primarily present only in NICU. *Prevotella* and *Sphingomonas* were found
1293 in both units in intermediate abundance.
1294