



| | |
|------------------|--|
| Title | Diversity changes of microbial communities into hospital surface environments |
| Author(s) | Yano, Rika; Shimoda, Tomoko; Watanabe, Reina; Kuroki, Yasutoshi; Okubo, Torahiko; Nakamura, Shinji; Matsuo, Junji; Yoshimura, Sadako; Yamaguchi, Hiroyuki |
| Citation | Journal of Infection and Chemotherapy, 23(7), 439-445 https://doi.org/10.1016/j.jiac.2017.03.016 |
| Issue Date | 2017-07 |
| Doc URL | http://hdl.handle.net/2115/71154 |
| Rights | © 2017. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/ |
| Rights(URL) | http://creativecommons.org/licenses/by-nc-nd/4.0/ |
| Type | article (author version) |
| File Information | J Infect Chemother 2017.pdf |



[Instructions for use](#)

1 **Diversity changes of microbial communities into hospital surface environments**

2

3

4 Rika Yano^a, Tomoko Shimoda^a, Reina Watanabe^a, Yasutoshi Kuroki^b, Okubo Torahiko^b,

5 Shinji Nakamura^c, Junji Matsuo^b, Sadako Yoshimura^a, Hiroyuki Yamaguchi^{a*}

6

7

8 ^aDepartment of Fundamental Nursing, Faculty of Health Sciences, Hokkaido University,

9 Sapporo, Japan

10 ^bDepartment of Medical Laboratory Science, Faculty of Health Sciences, Hokkaido

11 University, Sapporo, Japan

12 ^cLaboratory of Morphology and Image Analysis, Biomedical Research Center, Juntendo

13 University Graduate School of Medicine, Tokyo, Japan

14

15 *Corresponding author, Department of Medical Laboratory Science, Faculty of Health

16 Sciences, Hokkaido University, N12-W5, Kitaku, Sapporo 060-0812, Japan.

17 Tel/Fax: +81-11-706-3326

18 E-mail address: hiroyuki@med.hokudai.ac.jp

19

20 E-mail:

21 Rika Yano: r-yano@med.hokudai.ac.jp

22 Tomoko Shimoda: tshimoda@hs.hokudai.ac.jp

23 Reina Watanabe: watanabe.reina@takenaka.co.jp

24 Yasutoshi Kuroki: y_k_5_9@hotmail.co.jp

25 Torahiko Okubo: t.okubo@hs.hokudai.ac.jp

26 Sadako Yoshimura: sadako@med.hokudai.ac.jp

27 Shinji Nakamura: shinji-n@juntendo.ac.jp

28 Junji Matsuo: matsuo@hs.hokudai.ac.jp

29 Hiroyuki Yamaguchi: hiroyuki@med.hokudai.ac.jp

30

31 **Running title:** Diversity changes of hospital environmental microbiome

32

33

34 **Abstract**

35 Previous works have demonstrated considerable variability in hospital cleanliness in
36 Japan, suggesting that contamination is driven by factors that are currently poorly
37 controlled. We undertook 16S rRNA sequence analysis to study population structures of
38 hospital environmental microbiomes to see which factor(s) impacted contamination.
39 One hundred forty-four samples were collected from surfaces of three hospitals with
40 distinct sizes (“A”: >500 beds, “B”: 100–500 beds, “C”: <100 beds). Sample locations
41 of two ward types (Surgical and Internal) included patient room (multiple) (4BT),
42 patient overbed table (multiple) (4OT), patient room sink (multiple) (4S), patient room
43 bed table (single) (SBT), patient overbed table (single) (SOT), patient room sink
44 (single) (SS), nurse desk (ND), and nurse wagon (NW). Total DNA was extracted from
45 each sample, and the 50 samples that yielded sufficient DNA were used for further 16S
46 rRNA sequencing of hospital microbiome populations with cluster analysis. The number
47 of assigned bacterial OTU populations was significantly decreased in hospital “C”
48 compared to the other hospitals. Cluster analysis of sampling locations revealed that the
49 population structure in almost all locations of hospital “C” and some locations in the

50 other hospitals was very similar and unusually skewed with a family,
51 *Enterobacteriaceae*. Interestingly, locations included patient area (4OT, 4BT, SBT) and
52 nurse area (ND), with a device (NW) bridging the two and a place (4S and SS) shared
53 between patients or visitors. We demonstrated diversity changes of hospital
54 environmental microbiomes with a skewed population, presumably by medical staff
55 pushing NWs or sinks shared by patients or visitors.

56

57

58 **Keywords:** Hygiene, Nosocomial infection, Hospital cleanliness, 16S rRNA sequence
59 analysis, Microbiome

60

61

62

63 **Introduction**

64 CDC guidelines on controlling hospital-acquired infections (HAIs) prioritize
65 environmental cleanliness, while recommending precautions for contact between
66 patients and medical staff. In particular, the guidelines promote cleanliness of
67 high-contact surfaces such as machines placed around patients [1]. Bacterial
68 contamination of environmental surfaces is the leading cause of HAIs. Although
69 extensive measures to promote cleanliness are routinely undertaken, HAIs are a
70 persistent problem [2-8]. Thus, many hospitals implement monitoring of the
71 effectiveness of cleaning procedures, with visual assessment of surfaces, assessment of
72 residual fluorescent dye after cleaning, determination of aerobic colony-forming units,
73 or detection of ATP on surfaces [9].

74 Previous assessments of hospital cleanliness in Japan, using ATP bioluminescence
75 and stamp agar methods, revealed bacterial contamination to be highly variable and
76 independent of time or ward type [10]. No significant relationship was observed
77 between the number of patients or medical personnel in the hospital and organic or
78 microbiological contamination [10]. However, the physical properties of the surfaces

79 that were sampled had a strong effect on contamination [11]. These findings suggest
80 that numerous currently uncontrolled factors contribute to HAIs in Japanese hospitals
81 [10-11].

82 Amplicon sequencing, targeting the hypervariable region of the bacterial
83 small-subunit ribosomal RNA gene (16S rRNA), is currently a widely used technique to
84 study environmental microbiomes. Data can be used quantitatively and qualitatively to
85 compare environments, as well as provide direct diagnostic information that can inform
86 therapeutic decisions [12, 13]. Microbiome analysis also has been applied in a wide
87 range of research fields, including medicine, as well as ecology of indoor and marine
88 environments and urban public spaces such as subways [14-18]. Thus, microbiome
89 analysis is likely to provide pertinent data in attempts to understand the factors
90 controlling hospital cleanliness and HAIs.

91 In the present study, we undertook amplicon sequencing to visualize and compare
92 microbial population structures in three different hospitals, which differed in bed
93 numbers.

94

95

96 **Materials and methods**

97 *Hospitals*

98 Because of their different sizes, three hospitals were selected, which have distinct
99 numbers of beds (hospital “A”: >500 beds, hospital “B”: 100–500, hospital “C”: <100).
100 Both hospitals “A” and “B” are general hospitals with 30 and 21 clinical departments,
101 respectively, while hospital “C” specializes mainly in lung cancer treatment (including
102 antibiotic treatment to prevent infectious diseases after surgery) with only 5 clinical
103 departments. Because of distinct treatment policies, “Surgical” and “Internal” wards
104 were selected for sampling locations. For each of the wards, mean hospitalization
105 periods (days)/patient numbers are as follows: hospital “A”: 24 (Surgical 33.2, Internal
106 14.8)/49.5 (Surgical 50.1, Internal 48), hospital “B”: 15 (Surgical 16.3, Internal
107 13.7)/34.7 (Surgical 36.9, Internal 32.5), and hospital “C”: 12.3 (Surgical 10.8, Internal
108 13.7)/21.1 (Surgical 18.5, Internal 23.4). These hospitals are located in the
109 Hokkaido-Tohoku area in Japan. Hospital features with relative ratios of patients with
110 lung cancer are summarized in Table 1. The numbers of patients with lung cancer were

111 obtained from data published as the hospital index, and the relative ratio of patients with
112 lung cancer among all patients was then estimated. Cleaning was similar in all hospitals
113 and followed CDC guidelines [1]. Cleaning was performed daily in several locations, as
114 shown in Fig. 1, according to the following protocol. In brief, floors were cleaned with
115 disinfectant (Fig. 1, asterisks). The overbed table or bed frame was wiped with detergent
116 and then treated with disinfectant (Fig. 1, arrowheads). The handrail or window frame
117 was also cleaned with detergent only (Fig. 1, arrows).

118

119 *Sampling locations and swab collection*

120 A total of 144 samples were collected from hospital environments. As mentioned above,
121 sampling locations belonging to two ward types [Surgical (Su) and Internal (In)]
122 included patient room (multiple) (4BT), patient overbed table (multiple) (4OT), patient
123 room sink (multiple) (4S), patient room bed table (single) (SBT), patient overbed table
124 (single) (SOT), patient room sink (single) (SS), nurse desk (ND), and nurse wagon
125 (NW). Samples were collected from these environmental surfaces on weekday mornings.
126 Sterile cotton swabs dipped in sterile saline were wiped over 400 cm² at each location.

127 To prevent contamination from hands, disposable sterilized plastic gloves were used for
128 all sample collections.

129

130 *DNA extraction*

131 Each cotton swab was intensely vortexed in sterile saline, and the resulting suspension
132 was then centrifuged, yielding pellets that were used for total genomic DNA extraction.

133 Extraction was performed using the QIAamp DNA kit (Qiagen, Valencia, CA, USA),

134 according to the manufacturer's instructions. DNA was eluted in 50 µl of the elution

135 buffer supplied with the kit, and stored at -20 °C until use. In addition, all laboratory

136 procedures were conducted in a safety cabinet with filtered airflow to prevent

137 cross-contamination.

138

139 *Quality checking of extracted DNA*

140 The quality of extracted DNA from each sample ($n=144$) was confirmed by PCR

141 amplification using specific primers that target the 16S rRNA gene of *Mycobacterium*

142 sp., a species that inhabits most indoor and outdoor environments, including pond water

143 and soil [18]. Band densities of electrophoresed PCR samples on 2 % agarose gels were
144 quantified using ImageJ software. DNA samples from which *Mycobacterium* sp. 16S
145 rRNA could not be PCR amplified ($n=94$) were omitted from the following amplicon
146 sequence analysis because of insufficient DNA concentrations.

147

148 *Amplicon sequence analysis*

149 Analysis of samples that passed the quality check ($n=50$) was performed according to
150 the following general protocols. First, DNA amplicons were amplified by specific
151 primer sets that target the V3–V4 region of SSU rRNA (supplied by Hokkaido System
152 Science Co., Ltd.); PCR products were resolved on 2 % agarose gels and then purified.
153 After indexing PCR adaptor sequences had been added to the amplicons, a DNA library
154 for each sample was constructed, with a constant volume of amplicon solution (*i.e.*,
155 concentrations were not adjusted), to permit comparisons between samples of read
156 numbers produced by the Illumina 16S Metagenomic Sequencing Library Preparation
157 kit (Illumina). Libraries were sequenced on the MiSeq Illumina sequencer platform,
158 producing 300-bp paired-end reads. QIIME was used to process raw reads, with quality

159 scores >30, and perform OTU (Operational Taxonomic Units) clustering. OTU
160 annotation was based on BLAST analysis with a baseline of >90 % similarity.
161 Metagenomic analysis including quality filtering, OTU production, taxonomic
162 classification, and phylogeny generation was all conducted by Hokkaido System
163 Science Co., Ltd. Cluster analysis was performed by using Cluster 3.0 for Mac OS X (C
164 Clustering Library 1.52). Phylogenetic trees generated from aligned population
165 structures were constructed and then visualized in Java TreeViewX (version 0.5.0).

166

167 *Ethical considerations*

168 Before samples were collected, the research design was explained to medical staff, and
169 informed consent was obtained orally from medical staff. Privacy and confidentiality of
170 personal information was protected according to the Helsinki Declaration [19]. Both the
171 Ethics Committee of Hokkaido University and the ethics review committee of each
172 hospital approved this study.

173

174 *Statistical analysis*

175 Comparisons of assigned numbers into OTU units among hospitals were assessed by
176 single-factor ANOVA. A p value of <0.05 was considered to be statistically significant.
177 All calculations were executed in Excel for Mac (2001) with Statcel3C.

178

179

180 **Results**

181 *Validity of sampling and analysis*

182 As described in the protocol outlined in Fig. 2, amplicon sequencing using Illumina
183 MiSeq was undertaken to compare microbiomes on surfaces in three hospitals (“A”,
184 “B”, and “C”) with distinct bed numbers. A total of 144 samples were randomly
185 collected from various hospital environmental surfaces, according to a general swabbing
186 protocol (see Methods). Based on the ability to PCR-amplify *Mycobacterium* 16S rRNA,
187 50 samples were selected for next-generation sequencing targeting the hypervariable
188 (V3–V4) region of the bacterial 16S rRNA gene. Illumina sequencing yielded 8,948,588
189 sequences in total, with a mean of 178,971.76 sequences per sample and a mean
190 sequence length of 419 bp. Sequence cleanup was performed using QIIME, creating

191 115,573 OTUs with a mean of 2,311 OTUs per sample. These data with sampling
192 information are deposited as supplementary data (Online Resource 1).

193

194 *Hospital microbiome diversity and population structure*

195 Microbial diversity was first compared among the three hospitals. Analyses of OTU
196 numbers revealed considerable diversity in each hospital, with 543 species in 23 phyla
197 in hospital “A”, 519 species in 20 phyla in hospital “B”, and 483 species in 17 phyla in
198 hospital “C”. Hospital “C” was significantly less diverse than hospitals “A” and “B”
199 ($p=0.0063$) (Fig. 3). Population structure analysis among hospitals also showed that
200 species in family *Enterobacteriaceae* accounted for 78.49 % of all OTUs identified only
201 in the samples of hospital “C” (data not shown). Thus, in contrast to the population
202 structure of samples from hospitals “A” and “B”, hospital “C” appeared to show a
203 highly skewed population. Data were also analyzed by sampling location to understand
204 the factors responsible for the unique population structure with less diversity found in
205 samples from hospital “C”. Cluster analysis among sampling locations revealed that the
206 population structure in almost all locations of hospital “C” and some locations in the

207 other hospitals formed a cluster, being very similar and unusually skewed with a single
208 family, *Enterobacteriaceae* (Fig. 4, highlighted with an arrow). The locations included
209 patient area (4OT, 4BT, SBT) and nurse area (ND), with a device (NW) bridging the
210 locations and a place (4S and SS) shared between patients or visitors (Table 2).

211

212

213 **Discussion**

214 Since microbial contamination of the hospital environment likely contributes to the
215 spread of HAIs, surface disinfection and ward cleaning are typically prioritized to
216 maintain a clean clinical environment [19-23]. However, whether environmental
217 contamination has a role in the transmission of HAIs is poorly understood. Moreover,
218 there is little accurate epidemiological evidence based on metagenomic analysis to
219 support the belief that hospital cleanliness can specifically reduce the incidence of HAIs
220 [19-23].

221 Comparison among assigned numbers into OTU units of hospitals revealed
222 dramatic differences in diversity among hospitals, with hospital “C” being less diverse,

223 which specializes in cancer chemotherapy (see Table 1). Although several other factors
224 (e.g., hospitalization periods, patient numbers, or cleaning protocol) than the use of
225 chemotherapy may be responsible, chemotherapy may lead to altered microbial
226 diversity. In fact, since cancer chemotherapy typically leads to physiological changes of
227 the human gut, with considerable alteration of the intestinal microbiome [24, 25], it
228 cannot be denied that microbial diversity may be the result of the influence of specific
229 drugs on patients' microbiomes, presumably with environmental spread of fecal bacteria
230 via toilets. Further studies, in particular gut microbiome analysis of patients, are needed
231 to clarify this association.

232 Furthermore, cluster analysis of sampling locations showed a cluster consisting of
233 samples from hospital "C" and samples from some locations of the other hospitals. The
234 results indicated that microbial population structures in these locations were very
235 similar and unusually skewed with a single family *Enterobacteriaceae* (unclassified
236 genera), members of which inhabit the human gut and can be associated with
237 antibiotic-resistant HAIs, spreading via the fecal-oral route [1-8, 21-25]. Interestingly,
238 the locations included patient area (4OT, 4BT, SBT) and nurse area (ND), with a device

239 (NW) bridging the locations and a place (4S or SS) shared between patients or visitors.

240 Undoubtedly, washing hands with disinfectant is critical to reduce the prevalence of

241 nosocomial infections [26]. Hence, CDC guidelines promote not only cleanliness of

242 high-contact surfaces such as machines placed around patients, but also careful

243 handwashing with disinfectants for nurses after treating each patient [1, 26]. However,

244 we found “sink” as well as “nurse wagon” in the cluster. Although the exact reason for

245 the unusually skewed cluster of *Enterobacteriaceae* remains to be clarified, it is likely

246 that unaware harmful behaviors of nurses while treating patient wastes or cleaning sinks

247 shared by patients or visitors result in the spread of bacteria originating from patients or

248 sinks to hospital wards. A recent study revealed that handwashing sinks were a possible

249 reservoir for KPC-2-producing *Klebsiella oxytoca* with a risk of hospital outbreak. Such

250 bacteria could persistently survive in the overflow trap of the sink and unknowingly be

251 transferred to individuals [26], even though cleaning according to CDC guidelines is

252 properly performed with disinfectant. Additionally, biofilms formed on the overflow

253 trap of the sink may facilitate bacterial survival [27]. Taken together, medical staff

254 pushing NW and sinks shared by patients or visitors likely not only changes the

255 microbiota in hospital environments, but also spreads bacteria present in patient wastes
256 or the overflow trap of sinks to the hospital ward.

257 It may be unexpected that most of the OTUs identified from hospital “C” and
258 some samples from the other hospitals should belong to Gram-negative
259 *Enterobacteriaceae*, which have fragile cell walls. However, *Escherichia coli* can
260 survive on stainless steel surfaces without any nutrients for at least 100 days [28],
261 suggesting that Gram-negative bacteria can in fact remain viable for a long time on a
262 wide range of artificial surfaces. Although the exact reason why NWs became
263 contaminated with Gram-negative *Enterobacteriaceae* regardless of constant daily
264 cleaning remains unknown, there could be unrecognized factors responsible for
265 contamination on NW surface, which may be a significant factor determining the
266 relationships between hospital contamination and HAIs as well as contaminated sinks.

267 In conclusion, we demonstrated diversity changes of hospital environmental
268 microbiomes with a skewed population, presumably by medical staff pushing NWs and
269 sinks shared between patients or visitors. This factor may help to explain variability in
270 the relationship between hospital contamination and HAIs.

271

272

273 **Supporting Information**

274 **Online Resource 1.** Sample information (sampling location, ward type, hospital) and

275 basic data obtained from 16S rRNA amplicon sequencing. Sample numbers, $n=50$.

276

277

278 **Declaration of interest**

279 **Consent for publication**

280 Not applicable.

281

282

283 **Availability of data and materials**

284 The datasets generated during and/or analyzed during the current study are available

285 from the corresponding author upon reasonable request.

286

287

288 **Conflict interest**

289 The authors declare that they have no conflict interests.

290

291

292 **Funding**

293 This work was funded by the Japan Society for the Promotion of Science (JSPS),

294 Grant-in-Aid for Challenging Exploratory Research (No. 25670902 to RY).

295

296

297 **Authors' contributions**

298 The project was conceived by RY, TS, RW, SY, and HY. The samples were collected by

299 RY and TS. All laboratory work was conducted by RY, TS, YK, SN, TO, and JM.

300 Bioinformatics analysis was conducted by HY. The draft paper was critically edited by

301 RY, TS, TO, JM, and HY. RY and HY wrote the paper.

302

303

304 **Acknowledgements**

305 We thank the staff members of the Departments of Medical Laboratory Science and

306 Nursing, Faculty of Health Sciences, Hokkaido University, for their assistance

307 throughout this study.

308

309 **References**

- 310 [1] Siegel JD, Rhinehart E, Jackson M, Chiarello L. The Healthcare Infection Control
311 Practices Advisory Committee: Management of Multidrug-Resistant Organisms In
312 Healthcare Settings. Atlanta: Centers for Disease Control and Prevention. 2006.
- 313 [2] Samore MH, Venkataraman L, DeGirolami PC, Arbeit RD, Karchmer AW. Clinical
314 and molecular epidemiology of sporadic and clustered cases of nosocomial
315 *Clostridium difficile* diarrhea. Am J Med 1996; 100: 32-40.
- 316 [3] Hayden MK, Bonten MJ, Blom DW, Lyle EA, van de Vijver DA, Weinstein RA.
317 Reduction in acquisition of vancomycin-resistant *Enterococcus* after enforcement of
318 routine environmental cleaning measures. Clin Infect Dis 2006; 42: 1552-60.
- 319 [4] Hayden MK, Blom DW, Lyle EA, Moore CG, Weinstein RA. Risk of hand or glove
320 contamination after contact with patients colonized with vancomycin-resistant
321 *Enterococcus* or the colonized patient's environment. Infect Control Hosp Epidemiol
322 2008; 29: 149-54.
- 323 [5] Martinez JA, Ruthazer R, Hansjosten K, Barefoot L, Snyderman DR. Role of

324 environmental contamination as a risk factor for acquisition of vancomycin-resistant
325 enterococci by in patients treated in a medical intensive care unit. Arch Intern Med
326 2003; 163: 1905-12.

327 [6] Huang SS, Datta R, Platt R. Risk of acquiring antibiotic-resistant bacteria from prior
328 room occupants. Arch Intern Med 2006; 166: 1945-51.

329 [7] Goodman ER, Platt R, Bass R, Onderdonk AB, Yokoe DS, Huang SS. Impact of an
330 environmental cleaning intervention on the presence of methicillin-resistant
331 *Staphylococcus aureus* and vancomycin-resistant enterococci on surfaces in intensive
332 care unit rooms. Infect Control Hosp Epidemiol 2008; 29: 593-9.

333 [8] Wu HM, Fornek M, Schwab KJ, Chapin AR, Gibson K, Schwab E. A norovirus
334 outbreak at a long term-care facility: the role of environmental surface
335 contamination. Infect Control Hosp Epidemiol 2005; 26: 802-10.

336 [9] Boyce JM. Environmental contamination makes an important contribution to
337 hospital infection. J Hosp Infect 2007; 65(Suppl. 2): 50-4.

338 [10] Watanabe R, Shimoda T, Yano R, Hayashi Y, Nakamura S, Matsuo J, et al.
339 Visualization of hospital cleanliness in three Japanese hospitals with a tendency

340 toward long-term care. *BMC Res Notes*, 2014; 7: 121.

341 [11] Shimoda T, Yano R, Nakamura S, Yoshida M, Matsuo J, Yoshimura S, et al. ATP
342 bioluminescence values are significantly different depending upon material surface
343 properties of the sampling location in hospitals. *BMC Res Notes* 2015; 8: 807.

344 [12] Oulas A, Pavludi C, Polymenakou P, Pavlopoulos GA, Papanikolaou N, Kotoulas
345 G, et al. Metagenomics: tools and insights for analyzing next-generation sequencing
346 data derived from biodiversity studies. *Bioinform Biol Insights* 2015; 9: 75-88.

347 [13] Vincent AT, Derome N, Boyle B, Culley AI, Charette SJ. Next-generation
348 sequencing (NGS) in the microbiological world: How to make the most of your
349 money. *J Microbiol Methods* 2016; pii: S0167-7012(16)30031-8.

350 [14] Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, et al. Characterization
351 of the gut Microbiome using 16S or shotgun metagenomics. *Front Microbiol* 2016;
352 7:459.

353 [15] Huang B, Fettweis JM, Brooks JP, Jefferson KK, Buck GA. The changing
354 landscape of the vaginal microbiome. *Clin Lab Med* 2014; 34:747-61.

355 [16] Prussin AJ, Marr LC, Bibby KJ. Challenges of studying viral aerosol

356 metagenomics and communities in comparison with bacterial and fungal aerosols.
357 FEMS Microbiol Lett 2014; 357:1-9.

358 [17] Bik HM. Deciphering diversity and ecological function from marine metagenomes.
359 Biol Bull 2014, 227: 107-16.

360 [18] Afshinnekoo E, Meydan C, Chowdhury S, Jaroudi D, Boyer C, Bernstein N, et al.
361 Geospatial resolution of human and bacterial diversity with city-scale metagenomics.
362 Cell Syst 2015; 1: 72-7.

363 [19] Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination
364 due to methicillin-resistant *Staphylococcus aureus*: possible infection control
365 implications. Infect Control Hosp Epidemiol 1997; 18: 622-7.

366 [20] Dancer SJ. How do we assess hospital cleaning? A proposal for microbiological
367 standards for surface hygiene in hospitals. J Hosp Infect 2004; 56: 10-5.

368 [21] Dancer SJ. Importance of the environment in methicillin-resistant *Staphylococcus*
369 *aureus* acquisition: the case for hospital cleaning. Lancet Infect Dis 2008; 8: 101-13.

370 [22] Dancer SJ, White LF, Lamb J, Girvan EK, Robertson C. Measuring the effect of
371 enhanced cleaning in a UK hospital: a prospective cross-over study. BMC Med 2009;

372 7: 28.

373 [23] N.P.S.A. The National Specifications for Cleanliness in the NHS: A Framework for
374 Setting and Measuring Performance Outcomes, London, The National Patient Safety
375 Agency. 2007.

376 [24] Chase D, Goulder A, Zenhausem F, Monk B, Herbst-Kralovetz M. The vaginal and
377 gastrointestinal microbiomes in gynecologic cancers: a review of applications in
378 etiology, symptoms and treatment. *Gynecol Oncol* 2015; 1381: 190-200.

379 [25] Ciorba MA, Hallemeier CL, Stenson WF, Parikh PJ. Probiotics to prevent
380 gastrointestinal toxicity from cancer therapy: an interpretive review and call to action.
381 *Curr Opin Support Palliat Care* 2015; 9: 157-62.

382 [26] Won SP, Chou HC, Hsieh WS, Chen CY, Huang SM, Tsou KI, et al. Handwashing
383 program for the prevention of nosocomial infections in a neonatal intensive care unit.
384 *Infect Control Hosp Epidemiol* 2004; 25:742-6.

385 [27] Vickery K, Deva A, Jacombs A, Allan J, Valente P, Gosbell IB. Presence of biofilm
386 containing viable multiresistant organisms despite terminal cleaning on clinical
387 surfaces in an intensive care unit. *J Hosp Infect* 2012; 80: 52-5.

388 [28] Warnes SL, Highmore CJ, Keevil CW. Horizontal transfer of antibiotic resistance
389 genes on abiotic touch surfaces: implications for public health. MBio 2012; 3:
390 e00489-12.
391
392
393
394

395

396 **Figure legends**

397 **Fig. 1.** Daily cleaning places in hospital aisles and patient rooms. Asterisks indicate
398 floors cleaned with disinfectant. Arrowheads indicate overbed tables or bed frames
399 wiped with detergent followed by treatment with disinfectant. Arrows indicate handrails
400 or window frames cleaned with detergent only

401

402 **Fig. 2.** Flowchart illustrating experimental protocols for sampling, quality checks and
403 sample selection, Illumina sequencing, and population analysis

404

405 **Fig. 3.** Differences in assigned numbers into OTU units (microbiome populations)
406 among hospitals. Asterisks indicate statistically significant differences ($p < 0.05$),
407 estimated by single-factor ANOVA

408

409 **Fig. 4.** Cluster analysis of sampling locations shows population structures in almost all
410 locations of hospital “C” and some locations in the other hospitals clustered together
411 and were unusually skewed with *Enterobacteriaceae*

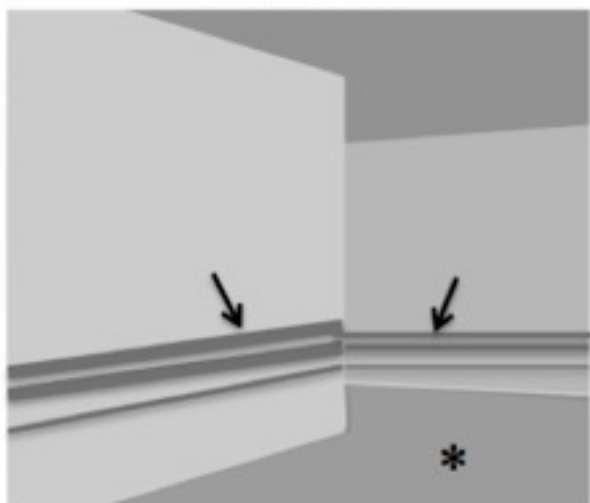
Table 1 Hospital's size and summary

| Hospital (Speciality/Total bed number) | Number of clinical department | Ward | Bed number per ward | Average hospitalization periods (Days) | Patient numbers per ward | Relative ratio of patients with lung cancer¹ |
|---|--|----------------------|--------------------------------|---|---|--|
| A (General hospital/500-1,000) | 30 | Surgical Internal | 60 60 | 33.2 14.8 | 50.1 48 | 1 1 |
| B (General hospital/100-500) | 20 | Surgical Internal | 46 40 | 16.3 13.7 | 36.9 32.5 | 0.3 0.5 |
| C (Lung cancer treatment/less than 100) | 5 | Surgical Internal | 24 25 | 10.8 13.7 | 18.5 23.4 | 4.5 2.4 |

¹ratio vs. Hospital A: patient numbers with lung cancer were obtained from data published as hospital index

Figure 1

Hospital aisle



Hospital room

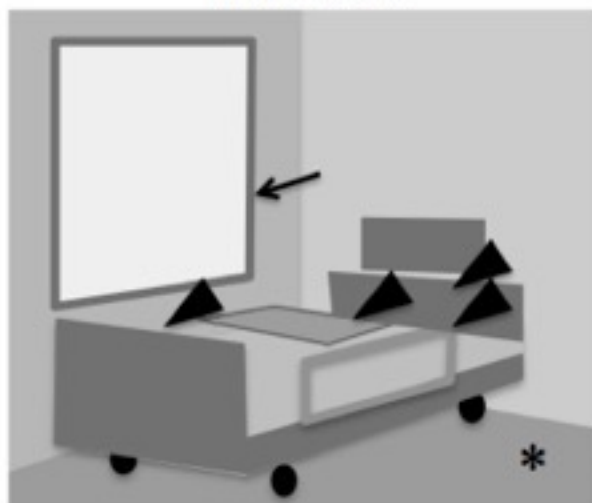


Figure 2

Quality check for sample DNAs and sample selection

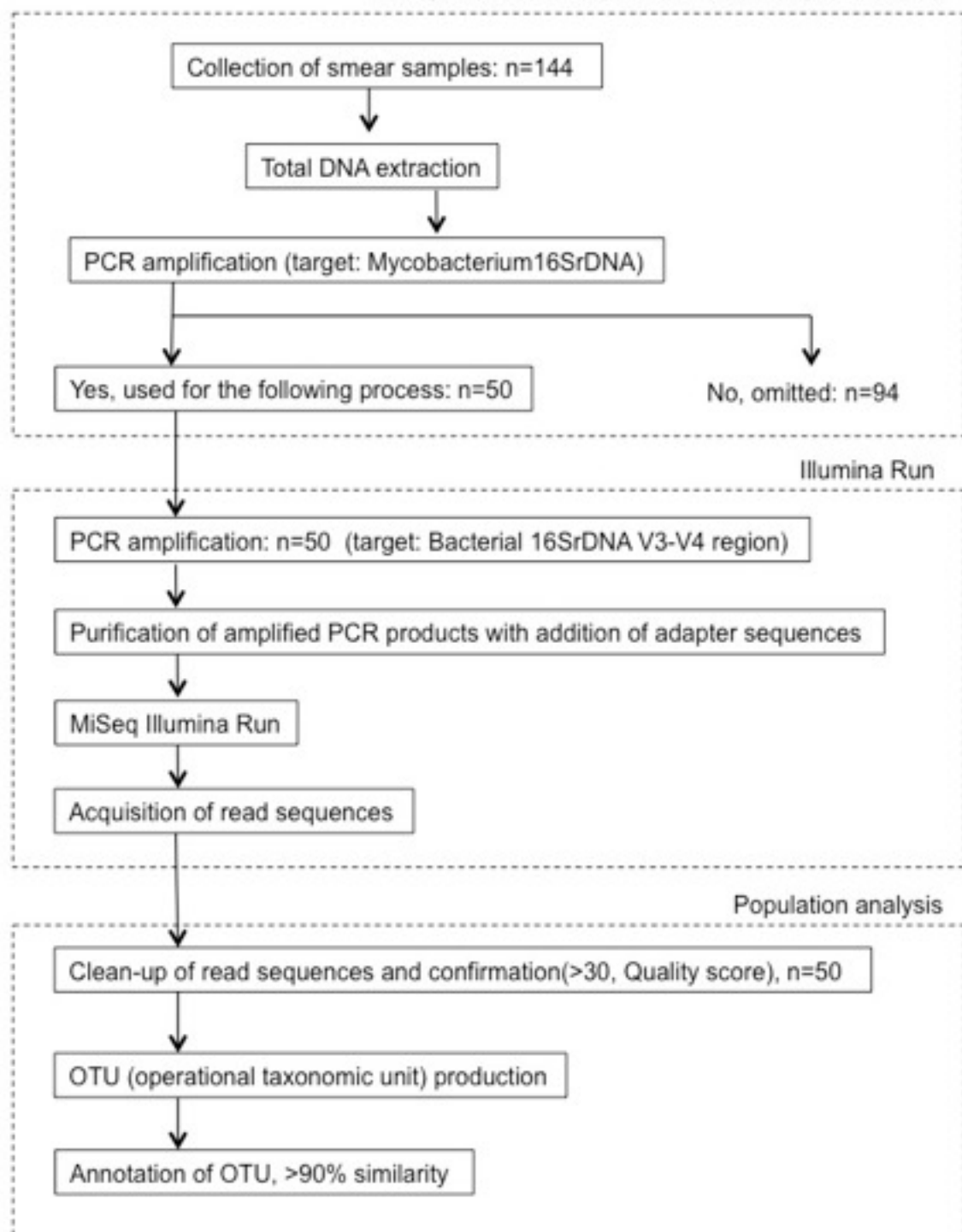
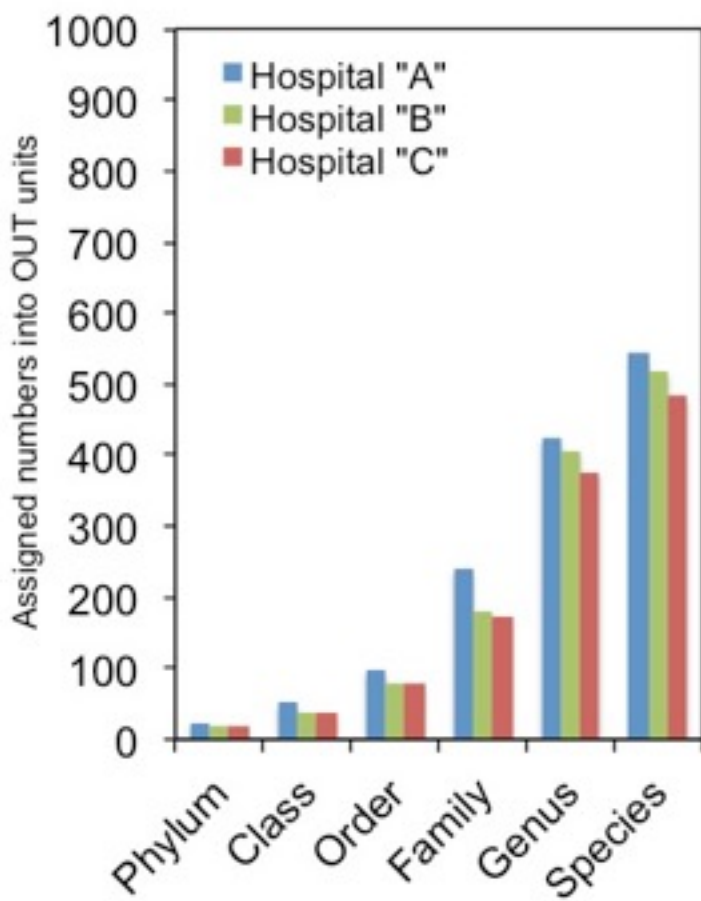


Figure 3



$p=0.0063^*$

Among the hospital "A"- "C"

Among phylum-species $p<0.0001^*$

Figure 4

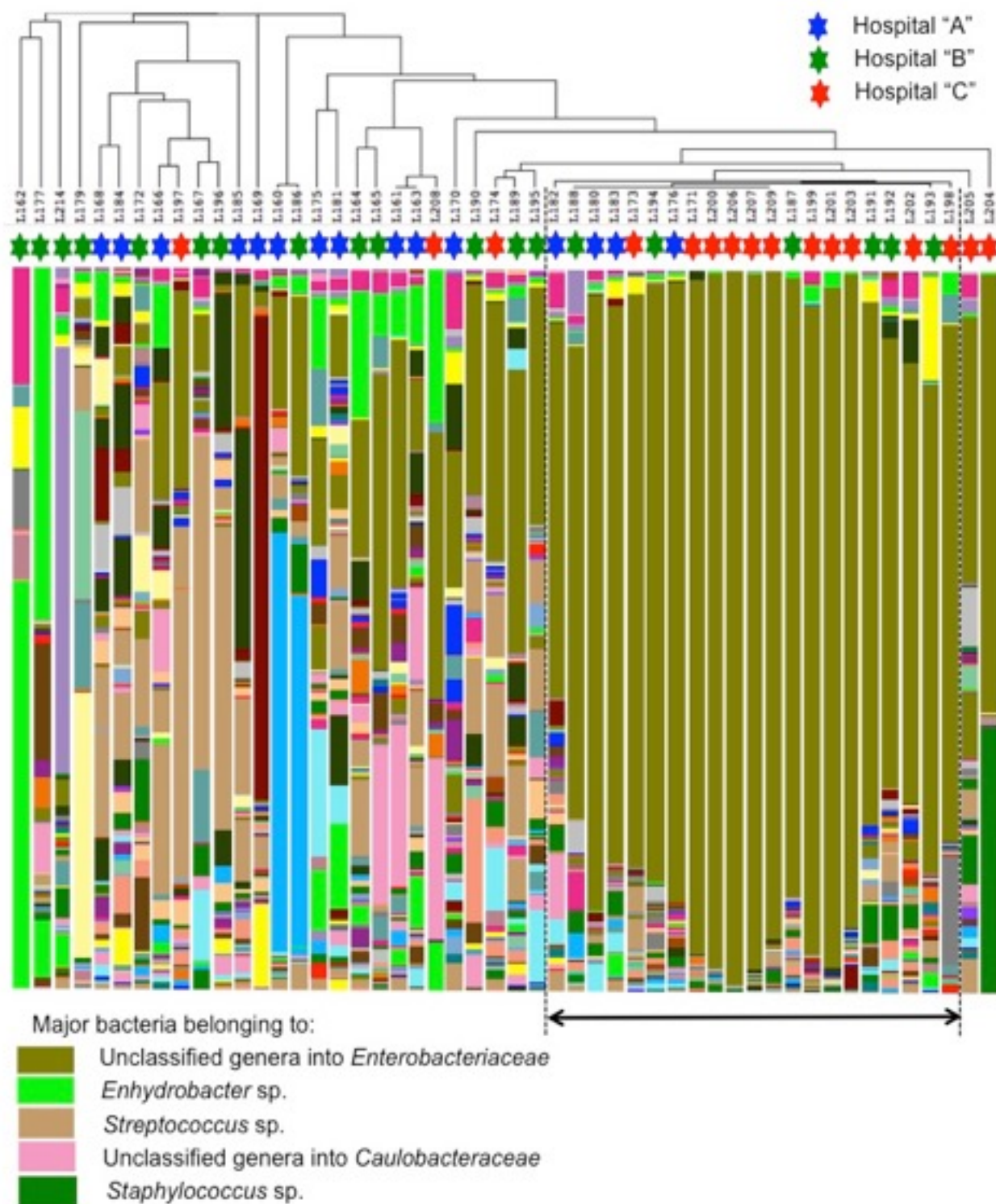


Table 2 Hospital location and ward type of the samples being more similar to each other in a cluster

| Hospital | Sample number | Sampling location ¹ | Ward type ² |
|----------|---------------|--------------------------------|------------------------|
| A | L176 | SBT | Su |
| A | L180 | 4OT | Su |
| A | L182 | NW | Su |
| A | L183 | ND | Su |
| B | L187 | SBT | Su |
| B | L188 | 4OT | Su |
| B | L191 | NW | In |
| B | L192 | 4BT | In |
| B | L193 | 4S | In |
| B | L194 | 4OT | In |
| C | L171 | NW | Su |
| C | L173 | SS | Su |
| C | L198 | 4BT | Su |
| C | L199 | SBT | Su |
| C | L200 | ND | Su |
| C | L201 | 4OT | Su |
| C | L202 | SBT | Su |
| C | L203 | 4S | Su |
| C | L206 | SS | In |
| C | L207 | 4OT | In |
| C | L209 | 4BT | In |

¹patient room (multiple), 4BT); patient overbed table (multiple), 4OT); patient room sink (multiple), 4S; patient room bed table (single), SBT; patient overbed table (single), SOT); patient room sink (single), SS); nurse desk, ND); nurse wagon (NW). ²Surgical, Su; Internal, In.