

1 **Gut microbiota and resistome dynamics in intensive care patients receiving selective**
2 **digestive tract decontamination**

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20

21 **ABSTRACT**

22 Background

23 Critically ill patients hospitalized in an Intensive Care Unit (ICU) are at increased risk of acquiring
24 potentially life-threatening infections with opportunistic pathogens. The gut microbiota of ICU
25 patients forms an important reservoir for these infectious agents. To suppress gut colonization with
26 opportunistic pathogens, a prophylactic antibiotic regimen, termed 'Selective decontamination of
27 the digestive tract' (SDD), may be used. SDD has previously been shown to improve clinical outcome
28 in ICU patients, but the impact of ICU hospitalization and SDD on the gut microbiota remains largely
29 unknown. Here, we characterize the composition of the gut microbiota and its antimicrobial
30 resistance genes ('the resistome') of ICU patients during SDD.

31

32 Results

33 During ICU-stay, 30 fecal samples of ten patients were collected. Additionally, feces were collected
34 from five of these patients after transfer to a medium-care ward and cessation of SDD. As a control
35 group, feces from ten healthy subjects were collected twice, with a one-year interval. Gut microbiota
36 and resistome composition were determined using 16S rRNA phylogenetic profiling and nanolitre-
37 scale quantitative PCRs.

38 The microbiota of the ICU patients differed from the microbiota of healthy subjects and was
39 characterized by low microbial diversity, decreased levels of *E. coli* and of anaerobic Gram-positive,
40 butyrate-producing bacteria of the *Clostridium* clusters IV and XIVa, and an increased abundance of
41 Bacteroidetes and enterococci. Four resistance genes (*aac(6')-II*, *ermC*, *qacA*, *tetQ*), providing
42 resistance to aminoglycosides, macrolides, disinfectants and tetracyclines respectively, were
43 significantly more abundant among ICU patients than in healthy subjects, while a chloramphenicol

44 resistance gene (*catA*) and a tetracycline resistance gene (*tetW*) were more abundant in healthy
45 subjects.

46

47 Conclusions

48 The microbiota and resistome of ICU patients and healthy subjects were noticeably different, but
49 importantly, levels of *E. coli* remained low during ICU hospitalization, presumably due to SDD
50 therapy. Selection for four antibiotic resistance genes was observed, but none of these are of
51 particular concern as they do not contribute to clinically relevant resistance. Our data support the
52 ecological safety of SDD, at least in settings with low levels of circulating antibiotic resistance.

53

54

55 **Key words:** Anti-Bacterial Agents; Antibiotic Prophylaxis; Drug Resistance, Microbial; Intensive
56 Care; Microbiome

57 **Background**

58 The human gut microbiota comprises 10^{13} - 10^{14} bacterial cells that belong to hundreds of different
59 species. The gut microbiota plays an important role in numerous metabolic, physiological,
60 nutritional and immunological processes of the human host [1]. In healthy individuals, the gut
61 microbiota mostly consists of bacteria that have a commensal or mutualistic relationship with the
62 human host. Critically patients, however, frequently have an extremely dysbiotic gut microbiota that
63 is characterized by intestinal overgrowth with multi-drug resistant opportunistic pathogens of the
64 phylum Proteobacteria (e.g. *Escherichia coli*) and the genus *Enterococcus*, while the abundance of
65 commensal *Bacteroidetes* and *Firmicutes* is decreased [2–5]. The high levels of aerobic,
66 opportunistic pathogens in the gut during critical illness are likely contributing to the burden of
67 respiratory and bloodstream infections with these organisms in critically ill patients [6].
68 Selective Digestive tract Decontamination (SDD) aims to reduce the risk of nosocomial infections in
69 ICU patients. SDD aims to eradicate opportunistic pathogens from the patients, while minimally
70 impacting commensal bacteria [7]. In SDD a paste containing the antibiotics colistin and tobramycin,
71 and the antifungal amphotericin B, is applied to the oropharynx of ICU patients. The patients also
72 receive a suspension of colistin, tobramycin, and amphotericin B via a nasogastric tube. These
73 antimicrobials are applied from the day of ICU admission until ICU discharge. In addition, a third-
74 generation cephalosporin (usually either cefotaxime or ceftriaxone) is administered intravenously
75 during the first 4 days of ICU stay. SDD lowers patient mortality during ICU stay in settings with a
76 low prevalence of antibiotic resistance and reduces the costs associated with ICU hospitalization
77 [8,9]. Selection of bacteria that are resistant to the antimicrobials used in SDD remains a major
78 concern [10,11], although this is not supported by the results of clinical trials in which conventional
79 culture techniques were used to screen for antibiotic resistance among nosocomial pathogens [12].
80 The patient gut is not only a potential source for opportunistic pathogens, but also forms a large
81 reservoir for antibiotic resistance genes, termed the gut resistome [13–17]. The use of antibiotics

82 may favor the selection for antimicrobial resistance genes (ARGs) among members of the gut
83 microbiota, thus increasing the likelihood of horizontal spread of ARGs between commensals and
84 opportunistic pathogens co-residing in the gut [16]. During the administration of SDD, the gut
85 resistome of patients is monitored by the cultivation of resistant bacteria from rectal swabs or feces,
86 as part of routine diagnostics. However, methods that rely on microbial culture capture only a
87 fraction of the gut resistome, since anaerobic commensals, which are the main reservoir of ARGs in
88 the gut microbiota, are difficult to culture [18–20]. Thus, culture-independent methods are needed
89 to comprehensively assess the impact of antibiotic prophylaxis on the microbiota and resistome of
90 ICU patients.

91 Here, we used the 16S ribosomal RNA (rRNA) gene-targeted Human Intestinal Tract Chip (HITChip)
92 and nanolitre-scale quantitative PCR (qPCR) targeting 81 ARGs, to determine the dynamics of gut
93 microbiota composition and resistome in patients receiving SDD during ICU hospitalization. We
94 contrast these findings in ICU patients with the composition of the microbiota and resistome of
95 healthy subjects.

96

97 **Methods**

98 **Study population**

99 All included patients ($n = 10$) were acutely admitted to the ICU of the University Medical Center
100 Utrecht from the community and had not been hospitalized in the previous six months, with the
101 exception of patient 105 who was hospitalized for 5 days prior to transfer to the ICU and start of
102 SDD. None of the patients were treated with antibiotics in six months prior to ICU hospitalization.
103 All patients received SDD from the start of ICU stay until ICU discharge. SDD consists of 1000 mg of
104 cefotaxime intravenously four times daily for four days, an oropharyngeal paste containing
105 polymyxin E, tobramycin and amphotericin B (each in a 2% concentration) and administration of a
106 10 mL suspension containing 100 mg polymyxin E, 80 mg tobramycin and 500 mg amphotericin B
107 via a nasogastric tube, four to eight times daily throughout ICU stay. All patients received additional
108 antibiotics during ICU stay. Fecal samples of patients were collected by nursing staff upon
109 defecation and stored at 4°C for 30 min to 4 h, after which the samples were transferred to -80°C.
110 Seven patients included here (patient numbers: 105, 108, 120, 163, 164, 165 and 169) were also
111 included in a previous study where the dynamics of two aminoglycoside resistance genes in the gut
112 microbiota of ICU patients was studied [20]. A total of 30 fecal samples were collected during ICU
113 stay. Five additional fecal samples were collected after transfer to a medium care ward and
114 cessation of SDD. Fig. S1 includes detailed information on sampling time points and antibiotic usage
115 of the ICU patients in this study.

116 Routine surveillance for colonization with aerobic Gram-negative bacteria in ICU patients was
117 performed through culturing of rectal swabs on sheep blood agar and MacConkey agar. All
118 suspected Gram-negative colonies were analyzed by Maldi-TOF for species identification. Antibiotic
119 resistance phenotypes were determined using the Phoenix system (BD, Franklin Lakes, NJ, USA).

120 Fecal samples of healthy subjects were collected as part of the ‘Cohort study of intestinal
121 microbiome among Irritable Bowel Syndrome patients and healthy individuals’ (CO-MIC) study at
122 two time-points with a one-year interval between sampling. None of the individuals in this cohort
123 received antibiotics. All included patients and healthy subjects were adults.

124

125 **Gut microbiota profiling by HITChip**

126 The HITChip is a validated phylogenetic array produced by Agilent Technologies (Palo Alto, CA) and
127 developed at Wageningen University, The Netherlands [21,22]. It contains over 4,800
128 oligonucleotides targeting the V1 and the V6 region of the 16S rRNA gene from 1,132 microbial
129 phylotypes present in the human gut [21]. DNA from fecal samples was isolated as previously
130 described [23]. The full-length 16S rRNA gene was amplified from fecal DNA, and PCR products
131 were further processed and hybridized to the microarrays as described previously [24]. Data
132 analyses were performed using R (www.r-project.org), including the microbiome package
133 (<https://github.com/microbiome>). Bacterial associations in the different patient groups and healthy
134 subjects were assessed using Principal Component Analysis (PCA) as implemented in CANOCO 5.0
135 [25]. Statistical testing for the differences in microbiota composition between ICU patients and
136 healthy subjects was performed by the non-parametric Mann-Whitney U test. All P-values were
137 corrected for false discovery rate (FDR) by the Benjamini and Hochberg method [26], and corrected
138 P-values (q) below 0.05 were considered significant.

139

140 **Quantification of *E. coli* by qPCR**

141 qPCRs for the quantification of *E. coli* were performed with primers that were previously described
142 [27], using serial dilutions of genomic DNA of *E. coli* DH5 α to generate a standard curve. The

143 quantification of 16S rRNA was performed with primers described in [28]. The PCR conditions were
144 identical to the qPCR conditions for the detection of *mcr-1* (described above).

145

146 **qPCR analysis of antibiotic resistance genes**

147 qPCR analysis was performed using the 96.96 BioMark™ Dynamic Array for Real-Time PCR
148 (Fluidigm Corporation, San Francisco, CA), according to the manufacturer's instructions, with the
149 exception that the annealing step in the PCR was held at 56°C. Fecal DNA was first subjected to 14
150 cycles of Specific Target Amplification using a 0.18 μM mixture of all primer sets, excluding the 16S
151 rRNA primer sets, in combination with the Taqman PreAmp Master Mix (Applied Biosystems),
152 followed by a 5-fold dilution prior to loading samples onto the Biomark array for qPCR. Thermal
153 cycling and real-time imaging was performed on the BioMark instrument, and Ct values were
154 extracted using the BioMark Real-Time PCR analysis software.

155

156 **Target selection, primer design and primer validation**

157 The primer set used in the qPCR assays covered 81 antimicrobial resistance genes (ARGs) of 14
158 resistance gene classes (Table S1). Primers were designed for the ARGs that are most commonly
159 detected in the gut microbiota of healthy individuals [14,15] and clinically relevant ARGs, including
160 genes encoding extended spectrum β-lactamases (ESBLs), carbapenemases, and proteins involved
161 in vancomycin resistance. We also included 10 genes encoding transposases, and a gene encoding
162 an integrase as representatives of mobile genetic elements [29]. Seven of these genes were detected
163 by qPCR but no significant differences could be observed between patients and healthy subjects
164 (data not shown) and these are not further discussed in our manuscript. Primer design was
165 performed using Primer3 [30] with its standard settings with a product size of 80 – 120 bp and a

166 primer melting temperature of 60°C. The universal primers for 16S rRNA genes were previously
167 described by Gloor *et al.* [28]. Forward and reverse primers were evaluated *in silico* for cross
168 hybridization using BLAST [31] and were cross-referenced against ResFinder [32] to ensure the
169 correct identity of the targeted genes. All primers that aligned with more than 10 nucleotides at
170 their 3' end to another primer sequence were discarded and redesigned. Additionally, all primer
171 sets were aligned to all resistance genes that were targeted in this PCR analysis to test for cross
172 hybridisation with genes other than the intended target resistance gene. Primers that aligned with
173 more than 10 nucleotides at their 3' end sequence with a non-target resistance gene were discarded
174 and redesigned. A reference sample consisting of pooled fecal DNA from different patients was
175 loaded in a series of 4-fold dilutions and was used for the calculation of primer efficiency. All
176 primers whose efficiency was experimentally determined to be between 80% and 120% were used
177 to determine the normalized abundance of the target genes. The detection limit on the Biomark
178 system was set to a CT value of 20, as recommended by the manufacturer. In addition, to assess
179 primer specificity we performed melt curve analysis using the Fluidigm melting curve analysis
180 software (<http://fluidigm-melting-curve-analysis.software.informer.com/>). All PCRs were
181 performed in triplicate and sample-primer combinations were only included in the analysis when all
182 triplicate reactions resulted in a CT-value below the detection limit.

183 After completion of the nanolitre-scale qPCR assays, the transferable colistin resistance gene *mcr-1*
184 was described [33]. To detect and quantify *mcr-1*, we developed primers (qPCR-mcr1-F: 5'-
185 TCGGACTCAAAGGCGTGAT-3' and qPCR-mcr1-R: 5'-GACATCGCGGCATTCGTTAT-3') for use in a
186 standard qPCR assay. The *mcr-1* gene was synthesized based on the sequence described in [33] by
187 Integrated DNA Technologies (Leuven, Belgium) and used as a positive control in our assays. The
188 qPCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Leusden,
189 The Netherlands) and a StepOnePlus instrument (Applied Biosystems, Nieuwekerk a/d IJssel, The

190 Netherlands) with 5 ng DNA in the reaction and the following program: 95°C for 10 min, and
191 subsequently 40 cycles of 95°C for 15 sec, 56°C for 1 min.

192

193 **Calculation of normalized and cumulative abundance**

194 Normalized abundance of resistance genes was calculated relative to the abundance of the 16S
195 rRNA gene ($CT_{ARG} - CT_{16S\ rRNA}$), resulting in a log₂-transformed estimate of ARG abundance.

196 Statistical analysis was performed using GraphPad Prism (La Jolla, CA). Visualization of the qPCR

197 data in the form of a heat map was performed using Microsoft Excel. Statistical testing of the

198 differences in the abundance of resistance genes in ICU patients versus healthy subjects was

199 performed with the non-parametric Mann-Whitney U test with a False Discovery Rate (Benjamini

200 Hochberg) < 0.05 to correct for multiple testing.

201

202 **Results**

203

204 **Microbiota dynamics in ICU patients and healthy subjects**

205 Global changes in the gut microbiota of healthy subjects and ICU patients were visualized by
206 Principal Component Analysis (Fig. 1A). The microbiota profiles of healthy subjects clustered
207 together, indicating that they had stable and broadly comparable microbiota profiles, which were
208 clearly distinct from the microbiota profiles of patients during and after ICU stay. These profiles
209 covered a larger area in the PCA plot, indicating that the differences in the microbiota composition
210 of patients were larger than in healthy subjects. Notably, the microbiota of several ICU patients (e.g.
211 #120, #169, #180, #43) was already distinct from the microbiota of the healthy subjects in the first
212 days of ICU hospitalization, indicating that ICU hospitalization has a rapid effect on the microbiota.
213 The diversity of the microbiota, as quantified by Shannon's diversity index, was significantly lower
214 in ICU patients compared to healthy subjects (5.90 ± 0.20 vs 5.19 ± 0.46 , respectively; $P < 0.001$,
215 Student's t-test). The diversity of the microbiota of ICU patients was highly dynamic (Fig. 1B).
216 Several patients (#108, #163, #164, #165 and #169) experienced a rapid loss of diversity in the
217 first days of ICU stay. In contrast, the diversity of the microbiota was more stable in healthy subjects
218 when comparing samples that were collected one year apart (Fig. 1C). Compared to healthy
219 subjects, the microbiota of patients during ICU hospitalization was characterized by a significantly
220 higher abundance in the taxa Bacteroidetes and Bacilli: *Enterococcus* and a lower abundance of the
221 taxa *Clostridium* cluster IV and XIVa (Fig. 1D).

222 We performed quantitative PCRs to accurately determine the abundance of *E. coli*, one of the
223 primary targets of SDD, in the gut microbiota of patients and healthy subjects (Fig. 2). The
224 abundance of *E. coli* in samples of ICU patients was lower compared to the healthy subjects ($p =$
225 0.001 ; Mann-Whitney U test). Notably, upon cessation of SDD and transfer to a medium-care ward,

226 the abundance of *E. coli* rebounded in one patient (#105) to levels surpassing those found in healthy
227 individuals.

228 During ICU stay, routine surveillance by conventional microbiological culture was performed on all
229 patients. *E. coli* could be cultured from six out of 73 rectal swabs that were collected during the
230 patients' ICU-stay. Five *E. coli* positive rectal swabs, of patients #43, #105, #108, #163, and #169,
231 were collected within one day of ICU admission, while the sixth positive swab (of patient #165) was
232 collected after nine days of ICU-stay. In addition, an *E. coli* strain from patient #105 with an ESBL-
233 producing and tobramycin-resistant phenotype was isolated after ICU discharge, while the patient
234 was in a medium-care ward. The *E. coli* strains isolated during ICU stay were susceptible to
235 cephalosporins and aminoglycosides. All *E. coli* strains were susceptible to colistin.

236

237 **Resistome dynamics in ICU patients and healthy subjects**

238 A total of 46 unique ARGs conferring resistance to 12 different classes of antimicrobials were
239 detected in the DNA isolated from fecal samples of hospitalized patients and healthy subjects (Fig.
240 S2). The number of detected resistance genes per sample ranged between 6 and 38. Eleven
241 resistance genes were detected in >80% of healthy subjects and critically ill patients. This highly
242 prevalent set of resistance genes included tetracycline resistance genes (*tetO*, *tetQ*, *tetM*, *tetW*), two
243 aminoglycoside resistance genes (*aph(3')-III* and an *aadE*-like gene), the bacteroidal β -lactam
244 resistance gene *cblA*, and the macrolide resistance gene *ermB*.

245 Genes associated with major antibiotic resistance threats, including those identified by the Centers
246 for Disease Control, were relatively rare. Genes encoding for extended-spectrum beta-lactamases
247 (ESBLs) were not detected in healthy subjects. In two ICU patient samples (#105C and #108C),
248 however, the ESBL genes *bla_{CTX-M}* and *bla_{DHA}*, respectively, could be detected. Sample #105C was

249 collected after ICU discharge and cessation of SDD, while sample #108C was collected after 12 days
250 of ICU hospitalization and SDD treatment. The carbapenemase *bla_{KPC}* was detected in a single patient
251 (patient #180), but only in the first sample (collected after 5 days in the ICU) and not in the second
252 sample, which was collected after 16 days of ICU hospitalization. No other ESBL- or carbapenemase-
253 producing strains were isolated from the patients during ICU hospitalization. Other Enterobacterial
254 β -lactamases were found to be widespread in our resistome analysis. The *bla_{AMPC}* β -lactamase was
255 present in 37% of samples, with nine of ten patients and eight of ten healthy subjects having
256 detectable levels of *bla_{AMPC}* at one or more sampling points. The *bla_{TEM}* β -lactamase was present in
257 26% of samples, corresponding with five of ten patients and four of ten healthy subjects in which
258 this gene was detectable at one or more sampling points. None of the samples were positive for the
259 carbapenemases *bla_{NDM}* and *bla_{oxa}*, or the transferable colistin resistance gene *mcr-1* (data not
260 shown). Among resistance genes that are associated with Gram-positive pathogens, the
261 staphylococcal methicillin resistance gene *mecA* was detected in 13 samples from eight of ten
262 patients, but not in samples of healthy subjects. The vancomycin resistance gene *vanB* was present
263 in 5 samples from three of ten patients and six samples from four of ten healthy subjects.

264 A comparison of the abundance of individual ARGs in samples that were collected during ICU stay,
265 versus samples from healthy subjects, revealed that four ARGs (*aac(6')-II*, *ermC*, *qacA*, *tetQ*) were
266 significantly more abundant in ICU patients, while two ARGs (*catA* and *tetW*) were significantly
267 more abundant in healthy individuals (Fig. 3).

268

269 Discussion

270

271 Current guidelines in the Netherlands recommend topical antibiotic decontamination in ICU
272 patients with an expected ICU stay of two days or longer. Yet, the original claim that these
273 interventions do not affect harmless anaerobic intestinal bacteria [7], has recently been questioned
274 [20,34]. While culture-based studies did not demonstrate selection for antibiotic-resistant
275 opportunistic pathogens during SDD-treatment [9,12,35,36], concerns remain that selection for
276 antibiotic resistance genes occurs in the gut microbiota of patients.

277 The current study describes the dynamics of the gut microbiota of ICU-patients receiving SDD
278 during ICU-stay towards dysbiosis, i.e. the perturbation of the complex commensal communities of
279 the gut microbiota of healthy humans [37]. The gut microbiota of ICU patients was characterized by
280 a low diversity, the increased abundance of enterococci and lower abundance of anaerobic Gram-
281 positive, butyrate-producing bacteria of the *Clostridium* clusters IV and XIVa. These findings expand
282 on previous findings of selection for Gram-positive cocci [12,20,38,39] and depletion of *F.*
283 *prausnitzii* during SDD [34]. In addition, we were able to demonstrate that the abundance of *E. coli*
284 was significantly lower in ICU-patients than in healthy individuals. The suppression of *E. coli* in the
285 SDD-treated ICU patients starkly contrasts with other studies in critically ill patients not receiving
286 SDD, in which high-level *E. coli* gut colonization is a common event [2,3,5]. This observation further
287 supports previous studies which found that SDD is successful in suppressing outgrowth of *E. coli* in
288 the gut microbiota of ICU patients [8,9], corresponding to the original aim of SDD [7].

289 Notably, levels of *E. coli* increased again after ICU-discharge in two of patients, reaching levels in the
290 gut similar to, or even surpassing, those in healthy individuals. These findings suggest that a rapid
291 regrowth or recolonization of the intestinal tract by *E. coli*, and possibly other aerobic Gram-
292 negative bacteria, occurs upon cessation of prophylactic antibiotic therapy. It remains to be

293 determined whether rapid post-ICU recolonization by *E. coli* increases the risk for infections with
294 this bacterium. Yet, in the only prospective evaluation on the post-ICU effects of SDD, the
295 implementation of SDD was not associated with higher infection rates after ICU discharge [40].

296 The qPCR-based analysis of the resistome confirms previous metagenomic studies, in showing that
297 tetracycline and aminoglycoside resistance genes and bacteroidal β -lactamases are widespread in
298 the human intestinal microbiota [14,15,18,20]. Four resistance genes (*aac(6')-II*, *ermC*, *qacA*, *tetQ*)
299 were significantly more abundant among ICU patients than in healthy subjects. The *aac(6')-II* gene is
300 a specific chromosomal marker for the nosocomial pathogen *Enterococcus faecium* and provides
301 low-level resistance to aminoglycosides [41]. Its high abundance in ICU patients is in line with the
302 increased levels of enterococci in the microbiota of the patients. The increased abundance of the
303 macrolide resistance gene *ermC* may have been selected for by the use of low doses of the macrolide
304 erythromycin, which was used as an agent to accelerate gastric emptying during ICU stay in six
305 patients. The increased abundance of *tetQ* in the gut microbiota of ICU patients may reflect the
306 higher abundance of Bacteroidetes in ICU patients versus healthy subjects, as *tetQ* is widely
307 distributed on conjugative transposons in this phylum [42]. Finally, the *qacA* gene confers resistance
308 to a number of disinfectants, including the biguanidine compound chlorhexidine and the quaternary
309 ammonium compound benzalkonium chloride [43,44]. Disinfectants are widely used in ICUs as
310 cleaning and infection control agents [45] and their use could select for *qacA* in the gut microbiota of
311 patients.

312 Two resistance genes (*catA* and *tetW*) were more abundant in healthy individuals than in ICU
313 patients. There is currently little information on the distribution of the *catA* gene among bacteria
314 associated with the human gut microbiota, but the gene was frequently found in human faeces in a
315 recent study set in low-income human habitats [46]. The tetracycline resistance gene *tetW* is
316 present in Gram-positive anaerobic gut commensals [47]. The gut bacteria that *catA* and *tetW* are
317 associated with, most likely members of *Clostridium* clusters IV and XIVa, are likely depleted during

318 ICU-stay and SDD-treatment.

319 Although SDD improves survival of ICU-patients, its use remains controversial due to the perceived
320 risk for selection of antibiotic resistance among bacteria that populate the patient gut. In this study,
321 we were not able to include an ICU control group that was not treated with SDD, as this would be a
322 breach of clinical guidelines for ICU-patients in our country. It is notable, however, that we did not
323 find selection for high-risk antibiotic resistance genes (like ESBLs, carbapenemases or vancomycin
324 resistance genes) in SDD-treated patients. The increased abundance of the resistance genes *aac(6')*-
325 *li*, *ermC*, *qacA* and *tetQ* in SDD-treated ICU patients in our study is – in our opinion - of limited
326 concern. The first three resistance genes contribute to resistance in enterococci, either to relatively
327 low concentrations of antibiotics (*aac(6')*-*li*) or to classes of antimicrobials that are of limited
328 relevance for the treatment of enterococcal infections (*ermC* and *qacA*). The *tetQ* gene provides
329 resistance to tetracyclines in Bacteroidetes, but this class of antibiotics is scarcely used for the
330 treatment of anaerobic infections [48]. Our data further support the ecological safety of SDD, at least
331 in settings with low levels of circulating antibiotic resistance, as it does not lead to selection for
332 clinically relevant antibiotic resistance phenotypes [36].

333 In addition, our study illustrates the expediency of using culture-independent methods to monitor
334 the presence and abundance of antibiotic resistance genes. The qPCR platform used here enables
335 the rapid detection and quantification of antibiotic resistance genes in the gut microbiota of
336 patients. The detection of high-risk antibiotic resistance genes (encoding e.g. ESBLs,
337 carbapenemases or vancomycin resistance proteins) in the resistome of patients may lead to the
338 implementation of targeted antibiotic therapy or infection control measures to minimize the risk
339 for selection and spread of these resistance genes.

340

341 **Declarations**

342 **Ethics approval and consent to participate**

343 The protocol for the ICU patient arm of this study was reviewed and approved by the institutional
344 review board of the University Medical Center Utrecht (Utrecht, The Netherlands) under number
345 10/0225. Informed consent for faecal sampling during hospitalization was waived. The protocol for
346 the feces collection of healthy subjects, including informed consent, was reviewed and approved by
347 the Ethics Committee of Gelderse Vallei Hospital (Ede, The Netherlands).

348

349 **Consent for publication**

350 Not applicable

351

352 **Availability of data and material**

353 The datasets used and/or analysed during the current study available from the corresponding
354 author on request.

355

356 **Competing interests**

357 W.v.S. is a consultant for Vedanta Biosciences.

358

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365 **Authors' contributions**

366 RJLW, MJMB, MWJvP, HS and WvS designed the study. EB, MSvM, EANO, MWJvP, HS and WvS
367 supervised collection of fecal samples. EB, TdJBG, EAMM and JCB performed experiments to map the
368 gut resistome and phylogenetic composition of the microbiome. SF, WAAdSP, LL and JRB
369 contributed bioinformatic analyses. The manuscript was written by EB, TdJBG, SF, RJLW, MJMB,
370 MWJvP, HS and WvS and approved by all authors.

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378

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503 **Figure legends**

504 **Figure 1. Dynamics of gut microbiota composition and diversity in ICU patients and healthy**
505 **subjects.** (A) Principal Component Analysis (PCA) of gut microbiota composition of ICU patients.
506 Dashed symbols indicate fecal samples collected after ICU discharge and continued hospitalization
507 in a medium-care ward. Fecal samples that were collected in the first 5 days of ICU hospitalization
508 are indicated by a black line around the symbol. Fecal samples of healthy subjects were collected at
509 two time-points with one-year interval, indicated with black and grey circles, respectively. (B)
510 Diversity (Shannon index) of the microbiota of ICU patients. Double lines indicate hospitalization in
511 a medium-care ward. (C) Diversity (Shannon index) of the microbiota of healthy subjects. (D) Gut
512 microbiota composition of patients and healthy subjects. Stacked bar charts represent the
513 abundance of different major taxa in the gut microbiota of ICU patients and healthy subjects. Among
514 Bacilli, the genus *Enterococcus* has been highlighted, as SDD has previously been shown to select for
515 colonization with enterococci [38,39]. Fecal samples that were collected after ICU discharge and
516 during medium-care hospitalization are indicated by grey triangles. Statistically significant
517 differences of the abundance of taxa in the gut microbiota of patients during ICU hospitalization and
518 healthy subjects are indicated in the legend (*: $q < 0.05$; **: $q < 0.01$; ***: $q < 0.001$; Mann-Whitney U
519 test with Benjamini-Hochberg correction for multiple testing).

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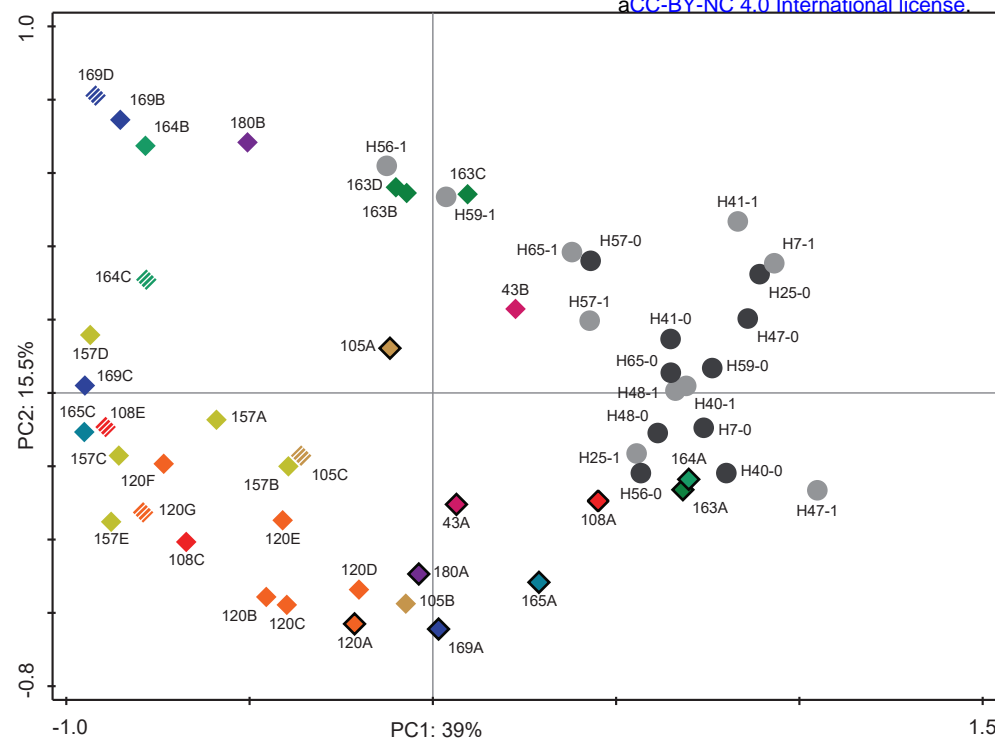
521 **Figure 2. Abundance of *E. coli* in the gut microbiota of ICU patients and healthy subjects.**

522 Quantification of *E. coli* 16S rRNA gene copies relative to total 16S rRNA gene copies, performed by
523 qPCR with three technical replicates. Error bars indicate standard deviation. Samples are ordered
524 by time of sampling during ICU stay. The color coding of the samples is unique for each patient and
525 is identical to Fig 1. Statistical testing was performed with the Mann-Whitney U test.

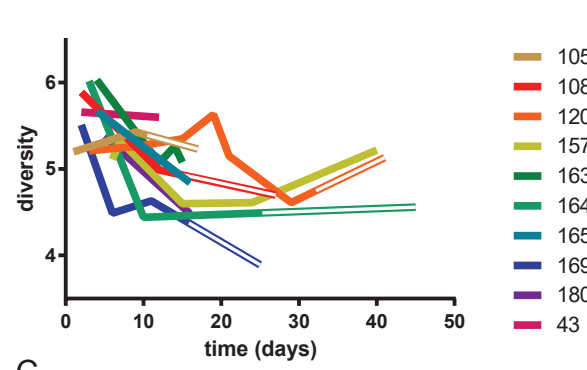
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527 **Figure 3. Antimicrobial resistance genes present at significantly higher or lower levels in the**
528 **microbiota of ICU patients, compared to healthy subjects.** ARGs that are present at significantly
529 higher (*aac(6)-li*, *ermC*, *qacA*, and *tetQ*) or lower (*catA* and *tetW*) abundance in ICU patients,
530 compared to healthy subjects, are shown. Testing for statistically significant differences was
531 performed by the Mann-Whitney U test, with Benjamini-Hochberg correction for multiple testing (*
532 = $q < 0.05$; ** = $q < 0.01$). The horizontal line denotes the median value. The detection limit of the
533 qPCR assay is indicated with the dashed line.

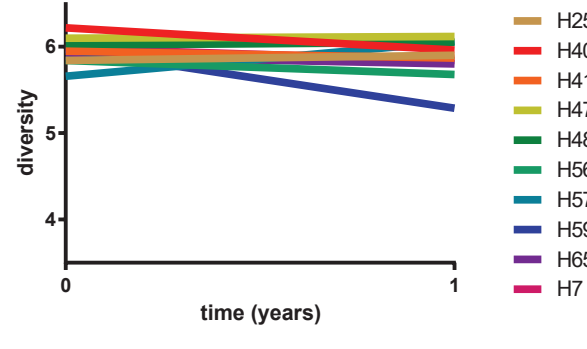
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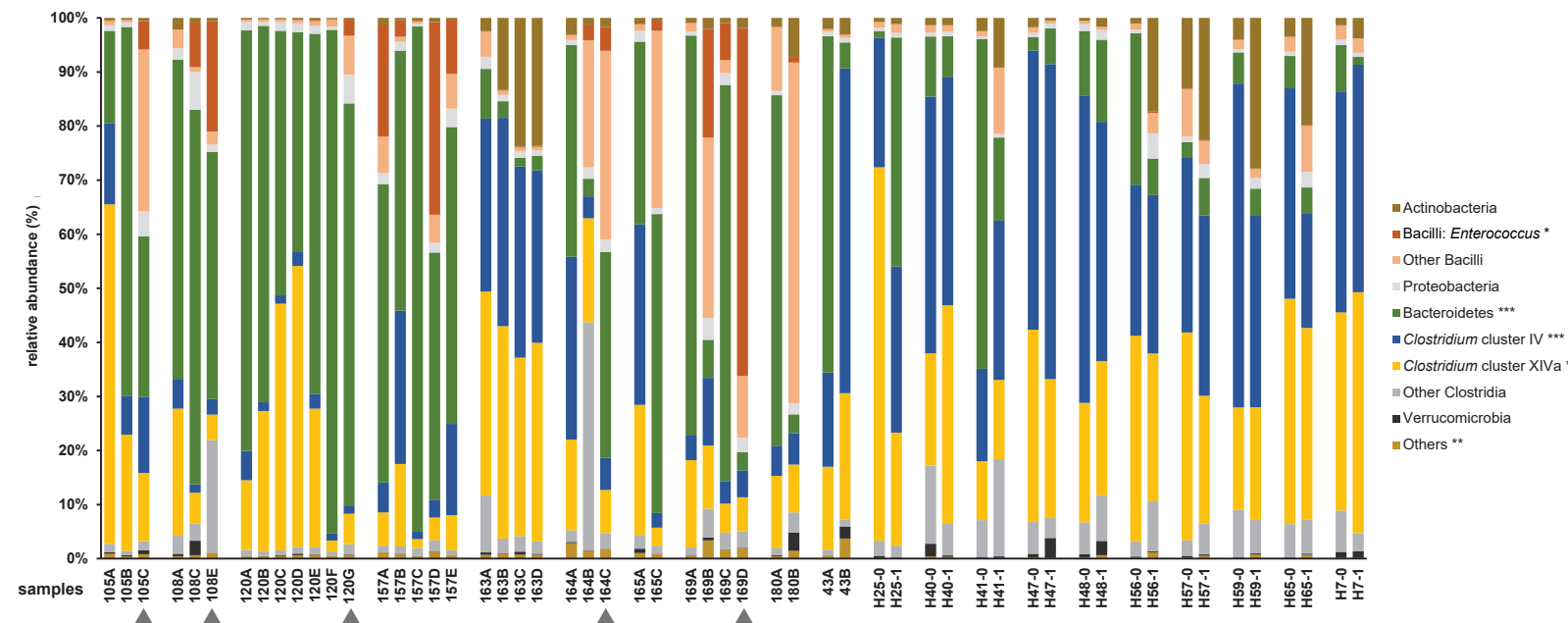
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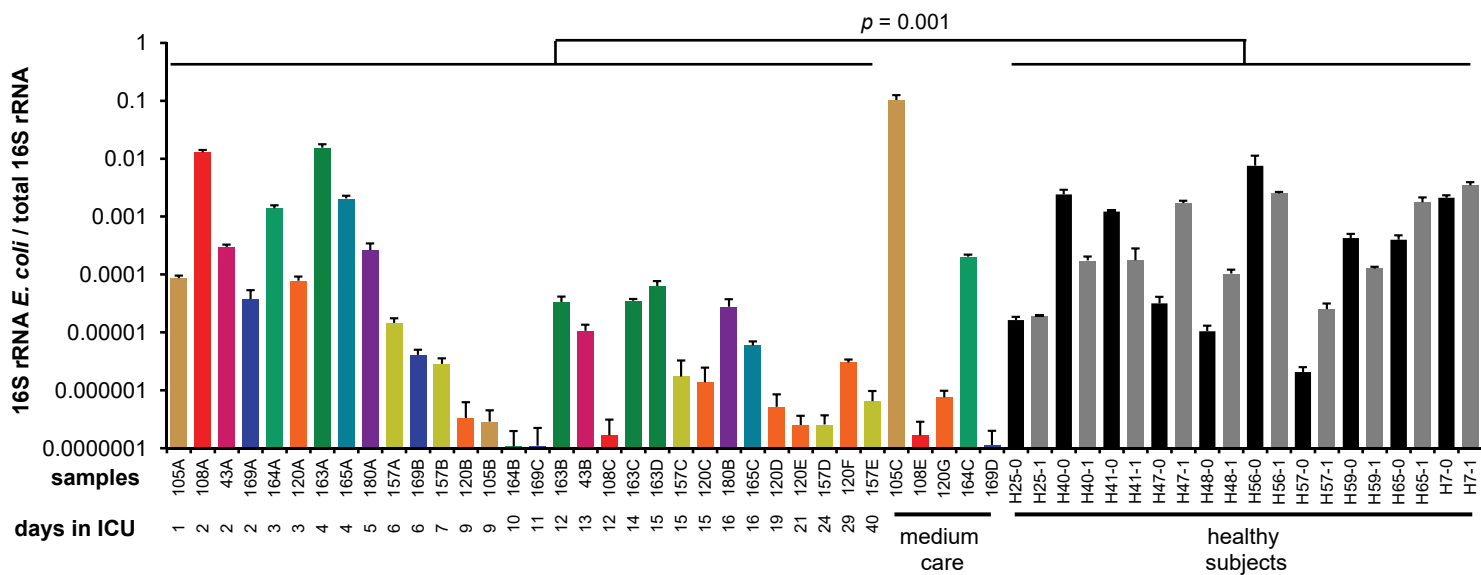


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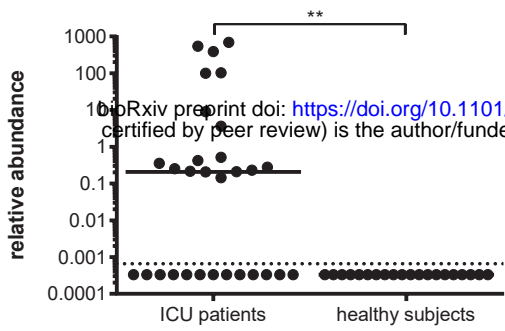


D

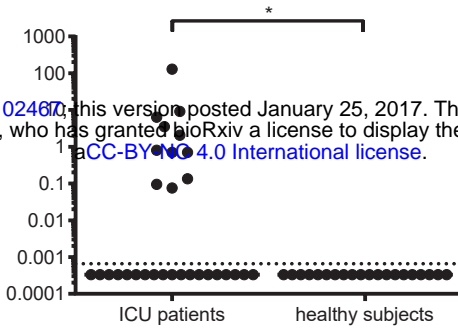




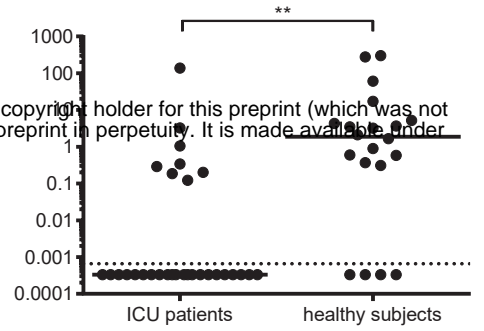
aac(6)-li



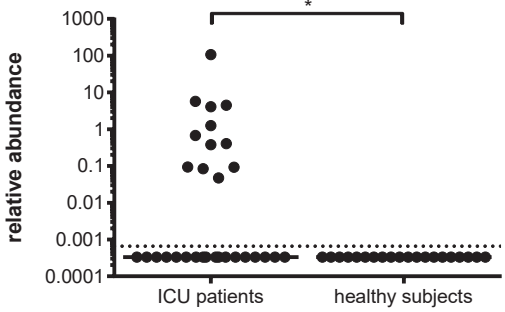
ermC



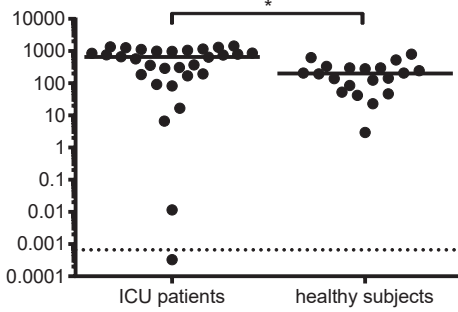
catA



qacA



tetQ



tetW

