

1 **Whole-genome sequencing revealed independent emergence of**
2 **vancomycin-resistant *Enterococcus faecium* causing sequential**
3 **outbreaks over three years in a tertiary care hospital**

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24 **Abstract**

25 Vancomycin-resistant *Enterococcus faecium* (VREfm) emerged as an important cause
26 of nosocomial infections worldwide. Previous studies based on molecular typing
27 revealed that VREfm outbreaks are mainly associated with a particular genetic lineage,
28 namely clonal complex 17 (CC17), which harbours either *vanA* or *vanB* gene cluster.
29 The University Hospital of Lausanne faced several VREfm episodes of transmissions
30 between 2014 and 2017. In this study, we used whole-genome sequencing (WGS) to
31 investigate the relatedness of 183 VREfm isolates collected from 156 patients.
32 Sequence type (ST) 17, ST80 and ST117 were the most predominant clones. Based
33 on epidemiological data, 10 outbreaks were identified, which were caused by at least
34 13 distinct genotypes. The majority of isolates involved in outbreaks (91%) differed by
35 only 0 to 3 SNPs. Four outbreaks involved more than one genotype and half of the
36 cases considered as sporadic were possibly linked to an outbreak. By sequencing all
37 isolates, we were able to better understand our local epidemiology of VREfm. The
38 polyclonal structure observed between the different outbreaks strains, the high level of
39 recombination detected in isolates, the time elapsed between admission and the first
40 VREfm detection and the negative screening at admission support the hypothesis of
41 the emergence of new VREfm clones within the hospitalized population.

42

43 **Introduction**

44 Vancomycin-resistant *Enterococcus faecium* (VREfm) colonization and infection
45 represent a major problem mostly in the hospital setting. Since VREfm was first
46 reported in 1988 in the United Kingdom and in France, it emerged worldwide causing
47 outbreaks (1, 2). Patients infected by VREfm are likely to have longer hospitalization
48 stays and exhibit higher mortality rates compared to vancomycin-susceptible infections
49 (3, 4). In addition, asymptomatic VREfm colonized patients can serve as potential
50 sources for transmission and environmental contamination.

51 VREfm belonging to clonal complex 17 (CC17) has been successfully disseminated in
52 hospital settings and became endemic in many countries. *E. faecium* encode
53 resistance to vancomycin through of *van* gene clusters of either type A (*vanA*) or B
54 (*vanB*). Previous studies have shown that VREfm with *vanA* type are widely spread in
55 the United States, Europe, Korea, Africa and South America, while VREfm with *vanB*
56 are predominant in Australia and Singapore (5).

57 Transmission is suspected when two or more VREfm-positive patients are identified in
58 the same unit during an overlapping period. Genotyping of isolates should complement
59 this epidemiological approach, especially when whole-genome sequencing techniques
60 are used, providing greater discrimination between isolates (6-8).

61 Here, we report the findings of a prospective study of several sequential VREfm
62 outbreaks, which occurred between 2014 and 2017 at the University Hospital of
63 Lausanne, Switzerland. Whole genome sequencing (WGS) was used to assess the
64 genetic relatedness among 183 VREfm isolates collected from 156 patients during this
65 period. In addition, we investigated the evolution of VREfm within individual long-term
66 carriers.

67

68 **Materials and methods**

69 **Setting, case definition and infection control measures.** The University Hospital of
70 Lausanne is a 1100-bed tertiary care hospital with 1 to 5-bedrooms, which faced
71 several VRE outbreaks in 2011, 2015 and 2016. A case was defined as a patient
72 colonized/infected with VREfm during a hospitalization stay. All VRE cases were
73 placed on contact isolation. Contact patients (i.e. patients who shared the same room
74 or the same open unit of a new VRE case during at least 24 hours in the last month)
75 were screened and placed on contact isolation until at least 3 rectal samples taken a
76 week apart were found negative for VRE. When one or more contacts were found
77 positive, all patients from the unit were screened on a weekly basis and at discharge
78 until no new case was revealed.

79 **Laboratory.** Screening for VRE was performed by culturing a rectal swab or a stool
80 sample in an enrichment brain–heart infusion with 3.3 mg/L of vancomycin and
81 chromogenic selective agar plates (Biorad, Marnes la Coquette, France). Identification
82 was confirmed with MALDI-TOF mass spectrometer (Bruker, Daltonics, Germany) and
83 the presence of the *van* genes was assessed using the Xpert vanA/vanB rapid test
84 (Cepheid, Sunnyvale, CA, USA). At least one isolate per patient was stored at -80 °C
85 for further analysis.

86 **Epidemiological definition.** Epidemiological links between cases were considered i)
87 between a new case and positive patients identified following the screening of contact
88 patients, ii) new cases identified in the same unit during weekly screening and iii) after
89 reviewing the hospitalisation chart of patients and the detection of links with VRE-
90 positive cases during previous hospitalisation. An outbreak was defined as two or more
91 cases with epidemiological links. A case with no epidemiological links was considered
92 as a single case.

93 **Bacterial isolates.** In this study, we analysed a total of 183 consecutive VREfm
94 isolates. They were collected during successive outbreaks occurring between January
95 2014 and May 2017 at the University hospital of Lausanne. For only 10 patients, the
96 VREfm was detected in a clinical sample, for the others patients, the VRE (n=146)
97 were detected from screening samples (Table S1).

98 **Whole-genome sequencing.** Genomic DNA of isolates was extracted using the
99 Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) according to the
100 manufacturer's instructions. The extracted DNA was quantified by the Qubit double-
101 stranded DNA high-sensitivity (HS) assay kit (Life Technologies, Waltham, MA, USA).
102 Sequencing library preparation was carried out using Nextera XT DNA Library
103 Preparation Kit (Illumina, San Diego, CA, USA) with indexed adapters, following the
104 manufacturer's guidelines, followed by sequencing using version 2 chemistry protocol
105 on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) to generate 150 bp
106 paired-end reads.

107 **Sequence data analysis and core-genome phylogeny.** *In silico* multi-locus
108 sequence typing (MLST) analysis and identification of STs were performed using
109 SRST2 pipeline (9). Sequence reads of the 183 VREfm were mapped to the VREfm
110 reference genome AUS004 (GenBank accession number: NC-017022) using Snippy
111 version 3.1 pipeline (<https://github.com/tseemann/snippy>). Furthermore, Snippy used
112 BWA-MEM version 0.7.15 (10) for aligning reads against the reference genome, while
113 Freebayes version 1.0.2 (11) was used for variant calling. Single nucleotide
114 polymorphisms (SNPs) were identified based on the following parameters: first, a
115 minimum read coverage of 10 x, a minimum base quality score of 30, and a minimum
116 proportion for variant evidence of 0.9. SNPs found within the core-genome of the 183
117 VREfm isolates (1,755,155 bp in size) were included, while SNPs located within mobile

118 genetic elements and repetitive regions were excluded. Briefly, mobile genetic
119 elements such as insertion sequences, transposons, and conjugative plasmids were
120 identified from the reference genome annotations AUS004 (GenBank accession
121 numbers: NC-017022) and the repeat-match algorithm that is implemented in MUMmer
122 package version 3.23 (12), while putative prophages and repetitive regions were
123 identified using PHAST (phast.wishartlab.com). Gubbins version 2.2.2 (13) with default
124 settings was used to identify and exclude regions of high SNPs densities and
125 suspected to undergo recombination events based on the isolates' phylogeny.
126 Subsequently, the final alignment of non-recombinant core genome SNPs was
127 generated and used to construct a maximum likelihood phylogenetic tree using PhyML,
128 which was visualized with the Seaview software version 4.6 (14).

129 A cluster was defined as a group of isolates showing a high degree of similarity based
130 on SNPs analysis, suggesting they belong to the same chain of transmission.

131 **Detection of antibiotic resistance and virulence genes.** The determination of
132 acquired resistance and putative virulence genes were performed through mapping
133 and from de novo assembled using both SRST2 and ARIBA (rapid antimicrobial
134 resistance genotyping directly from sequencing reads)
135 (<http://www.biorxiv.org/content/early/2017/03/18/118000>) with default settings,
136 respectively. Furthermore, the public databases ARG-ANNOT
137 (<http://en.mediterranee-infection.com/article.php?laref=283&titre=arg-annot>) and
138 CARD (15) were used as a reference for detecting the antimicrobial resistance
139 determinants, while the VFDB database (16) was used for identifying the virulence
140 factor genes. To study the genetic variation within *vanA* and *vanB* transposons among
141 the investigated isolates, sequence reads were mapped against *Tn1549* (ENA

142 accession number M97297) and Aus0004 (2835430-2869240 bp) reference genomes,
143 respectively, using the Snippy pipeline version 3.1.

144

145 **Results**

146 From January 2014 to May 2017, 156 patients were found infected/colonized with
147 VREfm; the majority being hospitalized in the visceral surgery ward (n=76), the medical
148 ward (n=33), the septic surgical ward (n=25) and the intensive care units (n=10). A
149 total of 183 VREfm isolates were sequenced (one per patient for 146 patients, 2-17
150 isolates for 10 patients). Outbreaks were defined on epidemiological links as described
151 in Material and Methods. During the study period, a total of ten outbreaks were
152 recorded (Figure 1 and Table S1). The median age of VREfm positive patients was 66
153 years (range 1 to 96). The median number of days between patients' admission and
154 first detection of VREfm was 25 days (range 0 to 280). Moreover, temporary universal
155 VRE screening of patients at admission in the surgical unit during six weeks revealed
156 only one VREfm case out of 187, suggesting intra-hospital emergence of vancomycin-
157 resistance in *E. faecium* instead of importation.

158 **Population structure of VREfm within the hospital setting**

159 *In silico* MLST analysis revealed eleven different STs among the 156 patients: ST80
160 (n = 77), ST117 (n = 36) and ST17 (n = 30) were the most prevalent STs, while few
161 isolates were assigned to ST203 (n = 3), ST18 (n = 3), and ST192 (n = 2). In addition,
162 ST82, ST182, ST412 and ST721 were represented each by a single isolate, and one
163 remaining isolate had a novel ST (not assigned to any of the previously published STs)
164 (Table S1).

165 **Recombination**

166 Using Gubbins, a high rate of recombination events was detected, especially among
167 VREfm isolates that belongs to ST80 (216 recombination events) and ST117 (95
168 recombination events) (Figure S1). These recombination events were distributed over
169 the entire genomes of both ST80 and ST117 and varied broadly in size between 19 bp
170 to 109,812 bp and between 31 bp to 93,428 bp, respectively. The majority of these
171 recombination events were due to the acquisition of insertion sequences that encode
172 for antimicrobial resistance and phosphotransferase system (PTS). Consequently, the
173 entire SNPs detected within the identified recombinant sequences were excluded from
174 the final core-genome SNPs alignment that was used to build the phylogeny of the
175 investigated VREfm isolates.

176 **Phylogenetic analysis**

177 The phylogenetic analysis based on core genome SNPs of the 183 VREfm revealed
178 that isolate H32990 (ST82) was distantly related to the remaining 182 isolates and,
179 therefore, was used as an outgroup to root the phylogenetic tree. Three major
180 phylogenetic clades were identified, namely, clade ST117, clade ST17 and clade
181 ST80-ST18 (Figure 2), which were correlated to the different successive VREfm
182 outbreaks, suggesting clonal disseminations of different VREfm clones within the
183 hospital. The monophyletic clade ST17 corresponded to the first and second outbreaks
184 and consisted of 51 ST17 VREfm isolates with a median SNP difference of zero (range
185 0-2), suggesting direct transmission between patients.

186 In contrast, clade ST80-ST18 was subdivided into six distinct clusters (ST80-A, ST80-
187 B, ST80-C, ST80-D, ST80-E and ST18), which represented different lineages
188 introduced into the hospital on different occasions (Figure 2).

189 With exception of three isolates, all VREfm ST117 isolates were grouped into the same
190 clade, which is subdivided into four distinct clusters (ST117-A, ST117-B, ST117-C and

191 ST117-D). Pairwise SNPs distance analysis revealed that isolates within each cluster
192 of clades ST80-ST18 and clade ST117 were closely related with a median SNP
193 difference of 0-2 SNPs (range 0-5), with exception of cluster ST117-D that had a range
194 of 0 to 51 SNPs. This wide range of SNPs was due to isolate H32231, which is single
195 locus variant from ST117 and differed by 49 to 51 SNPs compared to the remaining
196 isolates within this cluster. Noteworthy, the inter-clades/clusters median SNPs
197 differences were considerably high with up to 50 SNPs (range 2-82).
198 Seventeen patients carried an isolate that was not closely related to another isolate
199 (unique genotypes).

200 **Comparison between epidemiological and genotyping data**

201 The relation between outbreaks (documented based on epidemiological data) and
202 genetic clusters (defined based on genomic data) is shown in Figure 3. This figure is
203 highly informative. First, among the 129 cases involved in outbreaks, 109 (91%)
204 belonged to the same genetic clusters. The remaining 20 cases (16%) could be
205 excluded from the transmission chains because they carried an isolate genetically
206 different. In four of the ten outbreaks, patients with a unique genotype or belonging to
207 another cluster were observed, excluding these cases from the transmission chain.
208 More interesting, on one occasion, a small outbreak (#1, Figures 1 and 3) was followed
209 two months later by a larger outbreak (#2), suggesting the resurgence of transmission
210 that was considered under control. On the other hand, among the 27 cases with no
211 epidemiological link with other cases (singles), only 12 carried a unique VRE genotype,
212 the other 15 carried isolates genetically highly similar to isolates from other cases,
213 strongly suggesting they were associated to some putative chains of transmission. We
214 also observed patients hospitalized at different times, with no obvious epidemiological
215 link, but carrying isolates belonging to the same cluster and therefore putative

216 transmission (ST80-A, ST117-C, ST117-D and ST117-E). The most striking example
217 is cluster ST117-D which was first observed in a single case in March 2016, followed
218 by 5 cases between October 2016 and January 2017 that were detected during weekly
219 screenings performed to control outbreak #8 (cluster ST80-D), and a last case in May
220 2017. These observations strongly suggest the existence of undetected carriers.

221 **Genetic characterisation of VRE isolates**

222 In this study, the antimicrobial resistance genes detected in the 183 VREfm isolates
223 genomes are summarized in Table S2. The majority harboured the *vanA* gene (91.3%),
224 whereas the remaining 8.7% carried the *vanB* gene. In addition, the presence or
225 absence of antimicrobial resistance genes was consistent within each cluster (Table
226 S2).

227 To estimate the number of different origins of the *van* genes in our isolates' collection,
228 the genetic contents of *van* transposon were investigated. Sixteen isolates carried the
229 *vanB* genes and constituted clusters ST18, ST117-A and ST117-B. Mapping the *vanB*
230 transposon of these isolates against the *vanB* transposon of the reference genome
231 AUS004 (GenBank accession number: NC-017022) grouped the isolates into three
232 distinct clusters with 8 to 11 SNPs differences among them. The tree topology agreed
233 with the core-genome SNPs phylogeny (Figure S2). Similar analysis was performed
234 with the *vanA* transposon, showing a limited diversification (zero to five SNPs) and no
235 correlation with clusters (data not shown).

236 Several genes conferring resistance to various aminoglycosides were detected among
237 the isolates collection. Most of these genes were associated with particular
238 phylogenetic clusters. For example, all 33 isolates belonging to cluster ST80-D lacked
239 the high-level gentamicin resistance *aac(6')-aph(2'')* gene, which was detected in 47%
240 (86/183) of the isolates. Similarly, isolates from clusters ST80-C and ST80-D lacked

241 both *sat4* and *aph(3')-IIIa* genes, while ST17 isolates lacked the *ant(6')-Ia* gene that
242 mediate only high-level streptomycin resistance; these genes were detected in 47.5%
243 (87/183), 53% (97/183) and 67% (128/183) of the investigated isolates, respectively.
244 The *msrC*, *ermA*, *ermB*, and *ermC* genes that confer resistance to macrolides were
245 detected in 100% (183/183), 0.5 % (1/183), 98% (180/183) and 4.9% (9/183) of the
246 investigated VREfm isolates, respectively. The *dfp* gene, that encodes resistance to
247 trimethoprim, was found in 46.5% (85/183) of the isolates. Of note, all isolates
248 belonging to clade ST17 and cluster ST117-D lacked this gene.
249 Interestingly, mutations in *liaS* gene and/or *liaR* conferring resistance to daptomycin
250 were detected in 20% (37/183) of isolates, which all but one belonged to ST80 and
251 were mainly located in clusters ST80-B and ST80-D.
252 Similarly, screening all the 183 VREfm for putative virulence factors genes using the
253 Virulence Factor Database (VFDB) (17) revealed that all isolates but two harboured
254 the *acm* gene. In addition, clusters ST80-B and ST80-D isolates lacked pilin gene
255 cluster, while the *ecbA* gene was present in > 50% of isolates but was absent from
256 ST17 isolates (Table S2).

257 **In-host evolution**

258 To investigate the in-host diversity and the evolution of VREfm within host, we analysed
259 36 isolates that were sampled between 0 to 391 days apart from nine patients (P003
260 [n = 2], P008 [n = 2], P009 [n = 17], P020 [n = 2], P025 [n = 2], P033 [n = 5], P037 [n
261 = 2], P089 [n = 2] and P159 [n = 2]). For Patient P009, on two occasions, different
262 colonies from the same sample were isolated for WGS (P009-02 to P009-05; and
263 P009-08 to P009-16, Figure 2). For six patients, phylogenetic analysis revealed
264 evidence of restricted numbers of in-host variations: isolates sampled up to 391 days
265 apart differed by only 0 to 3 SNPs. Evidence for multiple colonization was found in

266 patients P033 and P089. Over a period of 370 days, P033 was simultaneously
267 colonized by two different genotypes ST80-A and ST80-B which differed by a mean of
268 20 SNPs. The two isolates from P089 were obtained 182 days apart and belonged to
269 two different genotypes (unique and ST117-C; 42 SNP differences), suggesting
270 successive colonization by different genotypes.

271 Evidence of in-host recombination was found in patients P037 and P089. After
272 excluding the SNPs located within recombinant genomic regions, four (P037) and 42
273 (P089) SNPs differences were detected among the repetitive isolates from these
274 patients.

275 **Infection control**

276 These outbreaks led to reinforcement of infection control measures in units with VRE
277 cases, including information, training and observations of health care workers.
278 Procedures for disinfection of the patient's environment were reinforced, and additional
279 staff was dedicated to this task. Weekly screenings by culture of all patients were
280 performed until two weeks passed without any new case. In addition, patients in units
281 with recurrent VRE outbreaks were screened on a weekly basis for several months.
282 We also transformed some of the 5-bed rooms into 2-bed rooms with sanitation. Since
283 the introduction of these infection control measures, a marked decrease in new cases
284 has been observed (N=16, including only three small outbreaks of 2, 2 and 3 cases)
285 during the year following this study.

286

287 **Discussion:**

288 Using WGS analysis, we prospectively investigated the epidemiology of VRE_{fm} at the
289 University hospital of Lausanne over a period of three years. Among the 156 studied
290 patients, thirteen clusters (genotypes) of genetically highly related isolates were

291 involved in the sequential outbreaks, suggesting several independent introductions of
292 VRE isolates into the hospital followed by direct or indirect transmission. These findings
293 are supported by previous studies, which revealed the emergence of several VREfm
294 clones within hospitals through multiple independent introductions followed by intra-
295 hospital transmissions (6, 7, 18, 19).

296 Our results showed that 91% of the cases involved in outbreaks were confirmed by
297 WGS results. Moreover, isolates of the same outbreak differed by only 0 to 3 SNPs,
298 which is in agreement with the estimated molecular clock rate of ~10 SNPs per genome
299 per year (19). Conversely, based on WGS results, we were able to completely exclude
300 patients from a chain of transmission when they were harbouring a different genotype.
301 Moreover, WGS highlighted the co-circulation of several genotypes in some wards. For
302 example, during outbreaks #3 and #9 (four patients each), only one transmission was
303 confirmed, whereas during outbreak#7, two genotypes (ST80D and ST117-D) were
304 spreading concomitantly in the ward.

305 Another added value of WGS analysis lies in its very high discriminatory power, which
306 enables to cluster patients for whom no apparent epidemiological link was recorded.
307 For example, WGS linked the first large outbreak in January 2015 to two
308 epidemiologically unsuspected cases in November 2014, which highly suggests the
309 presence of undetected cases between both episodes. This is corroborated in our
310 study by the presence of several patients hospitalized at different times, with no
311 obvious epidemiological link, but carrying isolates belonging to the same cluster. The
312 hypothesis of a persistent contamination of the environment was considered. Several
313 other factors contributing to the spread of VREfm were also identified: i) multiple
314 movements of patients within the hospital, ii) five-bed rooms and open units, iii) lack of
315 individual toilet facility, iii) suboptimal disinfection of the environment. Interestingly,

316 VREfm cases transferred from a foreign hospital, for whom contact precautions were
317 taken since admission, had a unique genotype, suggesting they were not the source
318 of transmission to other patients.

319 Investigating the in-host evolution of VREfm revealed that most patients carried
320 isolates with only 0 to 3 SNP differences despite long period of carriage (up to 391
321 days). Analysing various repetitive isolates from the same sample showed a small
322 number of SNP difference emphasising that patients harbour a collection of VREfm
323 isolates that have evolved independently following the first colonization/infection event.
324 Conversely, different genotypes were observed for two patients, suggesting
325 colonization on different occasions from various sources. These findings are consistent
326 with previous studies that suggested the carriage of several VREfm lineages by
327 patients (7, 20). Hence, a larger number of sequenced isolates per sample and per
328 patient are required to better characterize the in-host population dynamic of VREfm.
329 Of note, recombination between patient's isolates was observed, highlighting the
330 necessity to consider this diversification process when analysing SNP similarity
331 between isolates.

332 The diversity of genotypes within our population of patients raised the question of their
333 emergence. Van Hal *et al.* (21) hypothesised that the emergence of new clone is a
334 result of continuous recombination. Our study supports this hypothesis since a high
335 recombination rate was observed among the investigated isolates. Moreover, recent
336 studies showed that resistance to vancomycin is repeatedly introduced in the *E.*
337 *faecium* population (7, 19). A limitation of these studies was that only blood culture
338 isolates were included and carriage isolates were missing. Our study includes carriage
339 isolates and we identified various resistance patterns that were lineage specific,
340 emphasizing the role of horizontal gene transfer in the emergence of new genotypes.

341 However, the limitation of our study to investigate this hypothesis resides in the lack of
342 sequencing isolates of *E. faecium* susceptible to vancomycin. Together, these findings
343 suggest the common *de novo* emergence of VREfm. The time elapsed between
344 patients' admission and first VREfm detection (median 26 days) and the fact that only
345 one patient out of 187 was positive for VREfm at admission in a unit with recurrent
346 outbreaks suggest that the emergence occurred within the hospitalized patients.
347 Therefore, control measures should focus on (i) the prevention of VREfm emergence
348 with an effective antibiotic stewardship program and (ii) a bundle of infection control
349 actions to prevent cross transmission, particularly with early identification of cases by
350 repeated screening throughout the hospitalization as well as prompt implementation of
351 contact precautions.

352 In conclusion, WGS of all VREfm isolates enable us to better understand our local
353 epidemiology. Of interest, sporadic cases were often found to be related to a past or
354 future outbreak. The polyclonal structure observed between the different outbreaks,
355 the high level of recombination detected in studied isolates, the time elapsed between
356 admission and the first VREfm detection and the negative screening at admission
357 support the hypothesis of the emergence of new VREfm clones within hospitalized
358 patients.

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360

361 **Note**

362 Current affiliation of Mohamed H.H Abdelbary: Division of Oral Microbiology and
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365 **Compliance with Ethical Standards**

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369 **Conflict of Interest**

370 The authors declare no conflict of interest.

371 **Ethical approval**

372 All procedures performed in this study involving human participants were in
373 accordance with the ethical standards of the regional and national research committee
374 and with the 1964 Helsinki declaration and its later amendments or comparable ethical
375 standards.

376 **Informed consent**

377 For this type of study, informed consent is not required.

378

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436 transition to bloodstream infection in immunocompromised patients. *Genome*
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441 *Microbial genomics.* 2016;2(1).

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444

445 **Figure legends**

446

447 **Figure 1.** Incidence of VREfm cases and outbreaks identified with epidemiological
448 data.

449

450 **Figure 2.** Maximum likelihood tree based on core-genome SNPs in the 182
451 vancomycin-resistant *Enterococcus faecium* isolates collected in 156 patients from
452 January 2014 to May 2017. MLST sequence type, isolate number and patient number
453 are indicated. Isolates carrying the *vanA* gene are in black and *vanB* in red. A "\$"
454 indicates isolates retrieved at admission of patients transferred from a foreign hospital.

455

456 **Figure 3.** Distribution of the different genetic clusters within outbreaks defined by
457 epidemiological data

458

459

460 **Supplementary materials**

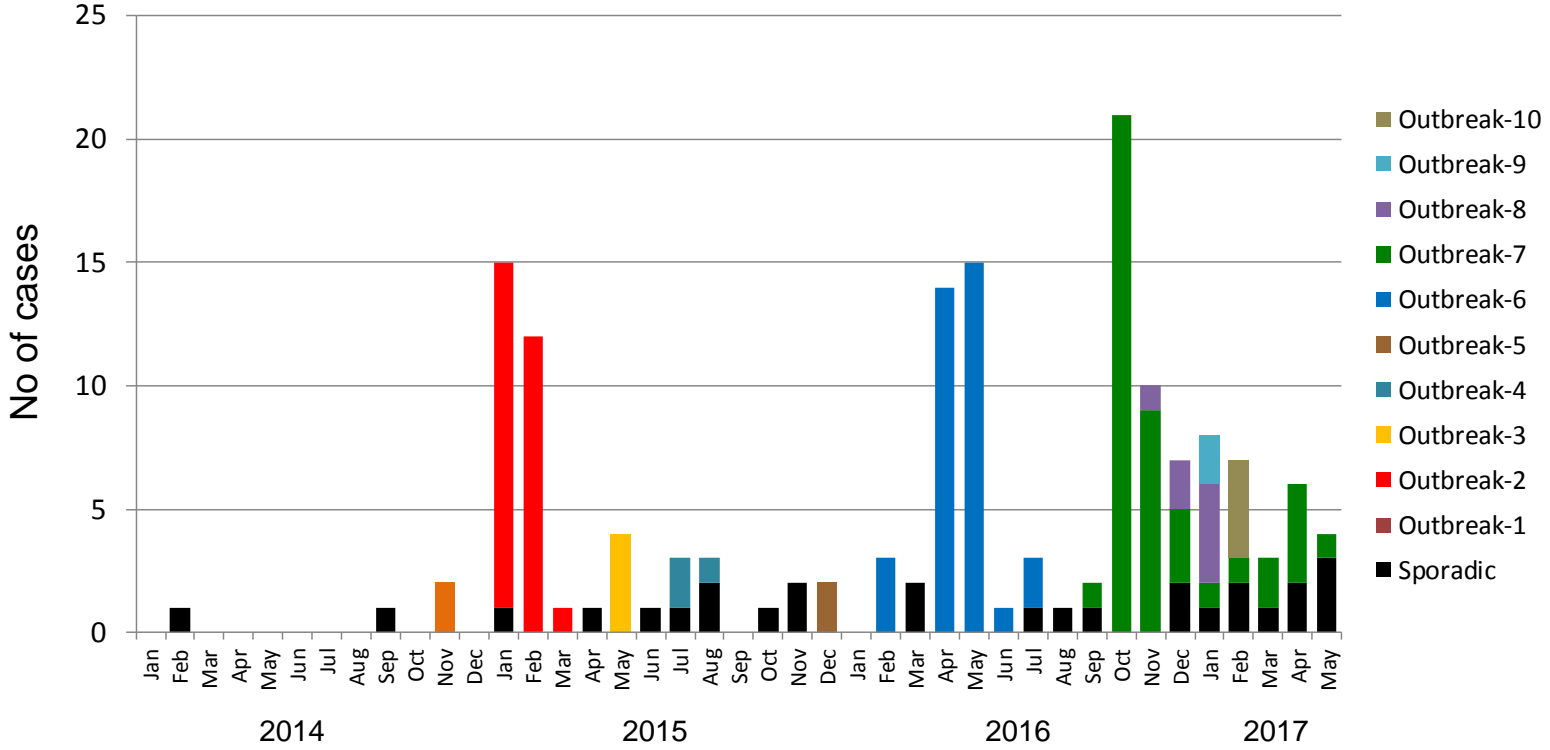
461 **Figure S1.** Phylogenetic tree of the 183 vancomycin-resistant *Enterococcus faecium*
462 isolates inferring recombination events using Gubbins. Red blocks represent regions
463 of high SNPs density detected in multiple isolates, while blue blocks represent high
464 SNPs density only found in a single isolate.

465 **Figure S2.** Phylogenetic tree reconstruction of 16 VREfm isolates based on core SNPs
466 in *vanB Tn1549*.

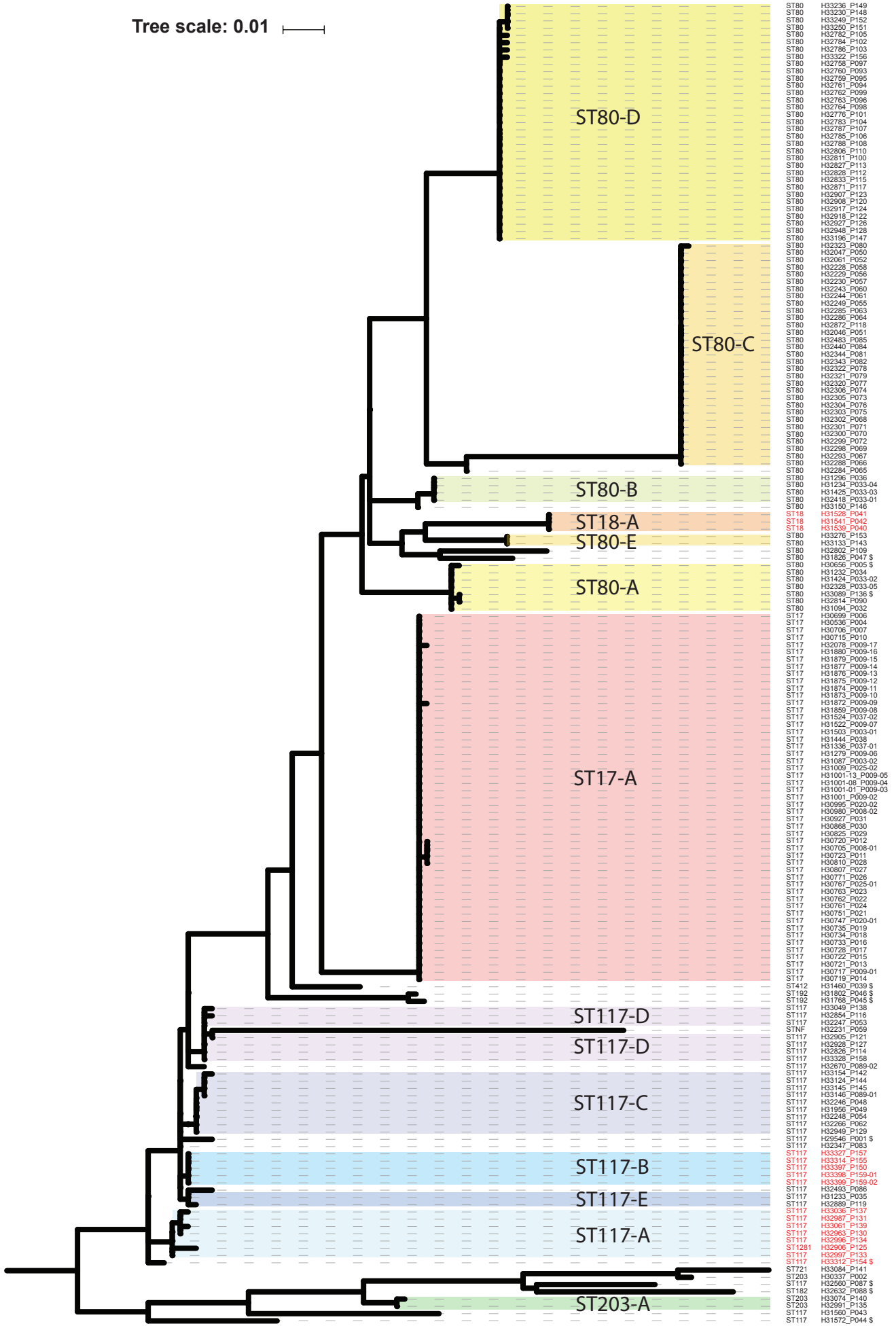
467 **Table S1.** Patient's epidemiological data.

468 **Table S2.** Entire resistance and virulence factors detected in the 183 VREfm isolates.

Figure 1

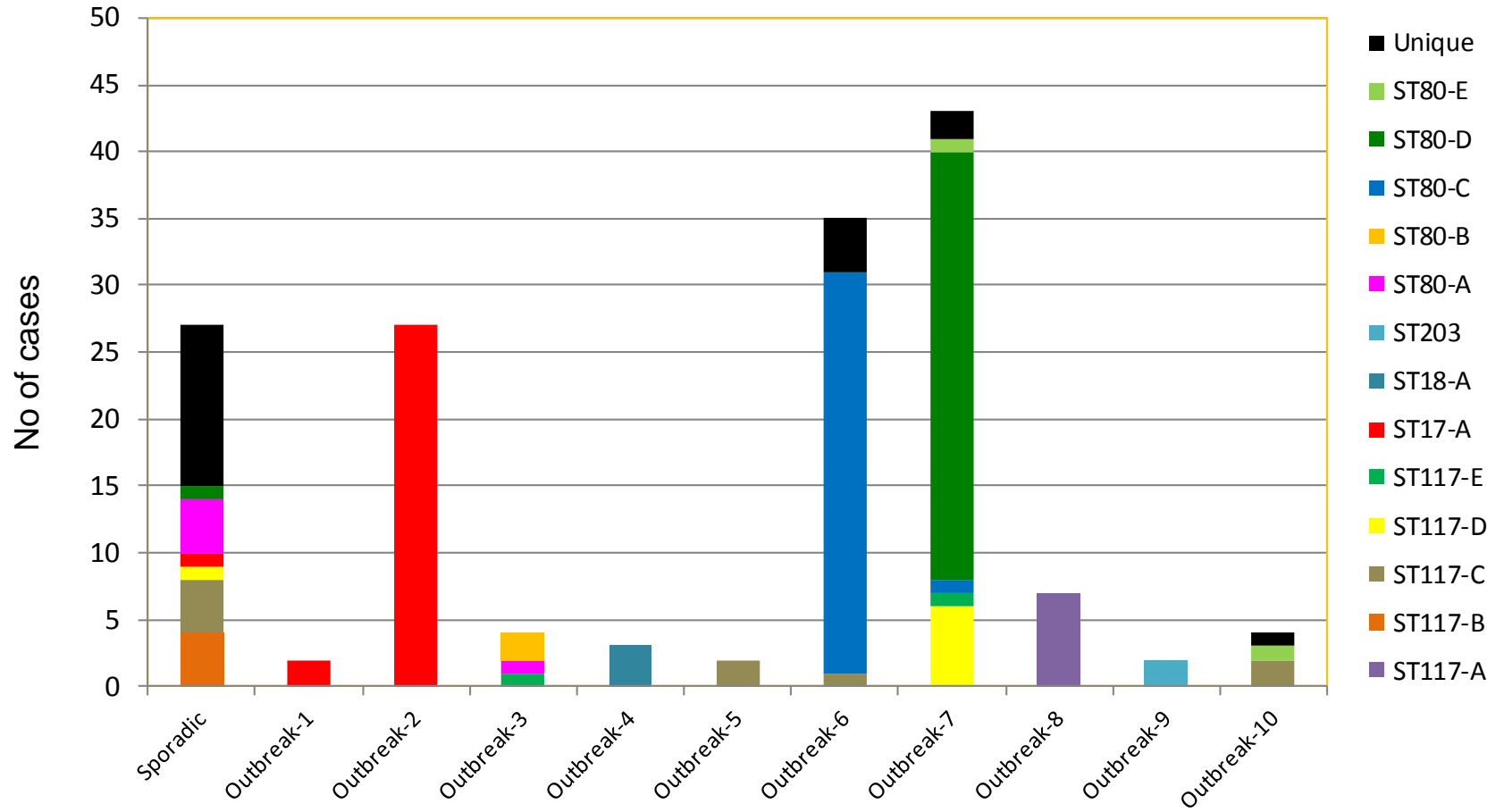


Tree scale: 0.01



ST80 H33236_P149
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ST80 H33249_P152
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ST80 H32752_P105
ST80 H32784_P102
ST80 H32786_P103
ST80 H33322_P156
ST80 H32758_P097
ST80 H32760_P093
ST80 H32759_P095
ST80 H32761_P094
ST80 H32762_P099
ST80 H32763_P096
ST80 H32764_P098
ST80 H32776_P101
ST80 H32783_P104
ST80 H32787_P107
ST80 H32785_P106
ST80 H32788_P108
ST80 H32806_P110
ST80 H32811_P100
ST80 H32827_P113
ST80 H32828_P112
ST80 H32833_P115
ST80 H32871_P117
ST80 H32907_P123
ST80 H32906_P120
ST80 H32917_P124
ST80 H32918_P122
ST80 H32927_P126
ST80 H32948_P128
ST80 H33196_P147
ST80 H32323_P080
ST80 H32304_P080
ST80 H32061_P052
ST80 H32228_P058
ST80 H32225_P056
ST80 H32230_P057
ST80 H32243_P060
ST80 H32244_P061
ST80 H32249_P055
ST80 H32285_P063
ST80 H32286_P064
ST80 H32872_P118
ST80 H32046_P051
ST80 H32463_P085
ST80 H32440_P084
ST80 H32344_P081
ST80 H32343_P082
ST80 H32322_P078
ST80 H32321_P079
ST80 H32320_P077
ST80 H32306_P074
ST80 H32305_P073
ST80 H32304_P076
ST80 H32303_P075
ST80 H32302_P068
ST80 H32301_P071
ST80 H32300_P070
ST80 H32299_P072
ST80 H32298_P069
ST80 H32293_P067
ST80 H32286_P066
ST80 H32284_P065
ST80 H31296_P036
ST80 H31234_P033-04
ST80 H31425_P033-03
ST80 H32418_P033-01
ST80 H3150_P146
ST118 H3152_P041
ST118 H31541_P042
ST118 H31539_P043
ST80 H33270_P153
ST80 H33133_P143
ST80 H32802_P101
ST80 H31825_P041 \$
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ST80 H31424_P033-02
ST80 H32328_P033-05
ST80 H33089_P136 \$
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ST80 H31094_P032
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ST117 H30336_P004
ST117 H30706_P007
ST117 H30715_P010
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ST117 H31336_P037-01
ST117 H31273_P009-06
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ST117 H33396_P159-01
ST117 H33395_P159-02
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ST117 H31580_P044 \$
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Figure 3



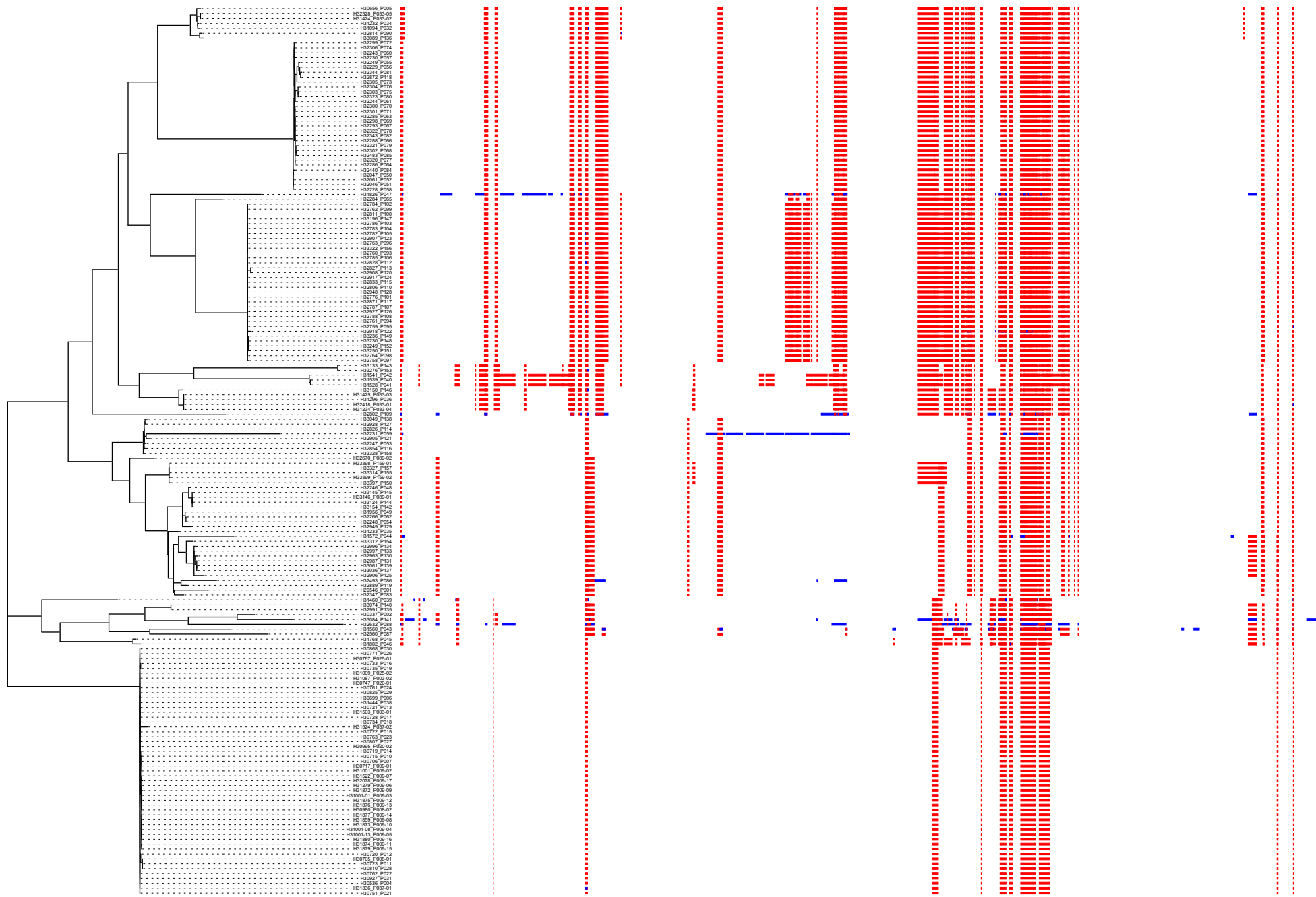


Figure S1. Phylogenetic tree of the 183 vancomycin-resistant *Enterococcus faecium* isolates inferring recombination events using Gubbins. Red blocks represent regions of high SNPs density detected in multiple isolates, while blue blocks represent high SNPs density only found in a single isolate.

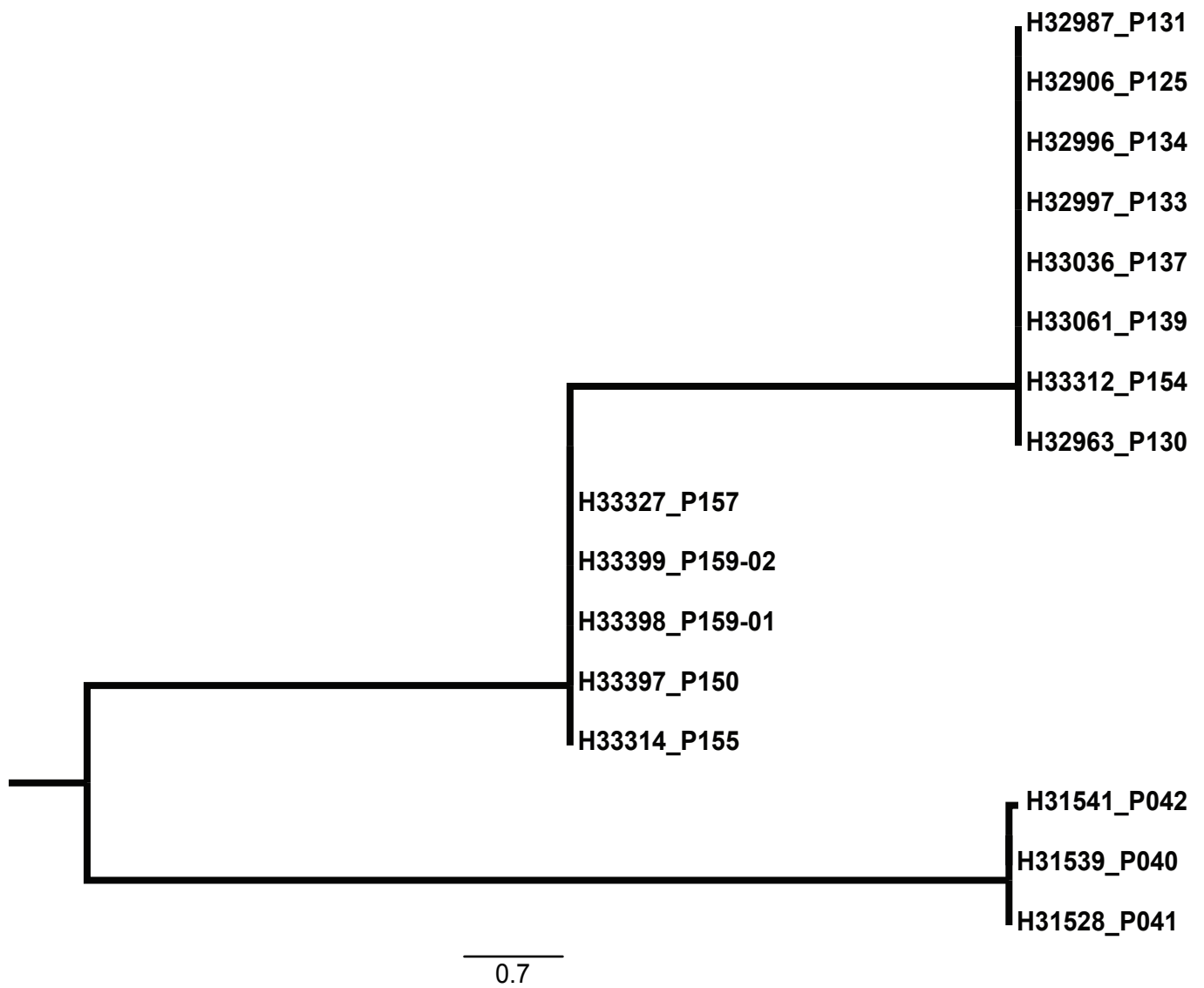


Figure S2. Phylogenetic tree reconstruction of 16 VREfm isolates based on core SNPs in vanB Tn1549.