

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

Raven, KE; Gouliouris, T; Parkhill, J; Peacock, SJ; (2017) Genome-based analysis of *Enterococcus faecium* bacteremia associated with recurrent and mixed strain infection. *Journal of clinical microbiology*. ISSN 0095-1137 DOI: <https://doi.org/10.1128/JCM.01520-17>

Downloaded from: <http://researchonline.lshtm.ac.uk/4645977/>

DOI: <https://doi.org/10.1128/JCM.01520-17>

Usage Guidelines:

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

<https://researchonline.lshtm.ac.uk>

1 **Genome-based analysis of *Enterococcus faecium* bacteremia associated with recurrent and**
2 **mixed strain infection**

3

4 Kathy E. Raven,¹# Theodore Gouliouris,^{1,2} Julian Parkhill,³ Sharon J. Peacock.^{1,3,4}

5

6 ¹ Department of Medicine, University of Cambridge, Cambridge, United Kingdom; ²Public Health
7 England, Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital,
8 Cambridge, United Kingdom; ³ Wellcome Trust Sanger Institute, Hinxton, Cambridge, United
9 Kingdom; ⁴ London School of Hygiene and Tropical Medicine, London, United Kingdom

10

11 **Running title: *Enterococcus faecium* recurrent and mixed infection**

12

13 # Address correspondence to Kathy E. Raven, ker37@medschl.cam.ac.uk.

14

15

16

17 **ABSTRACT**

18 Vancomycin-resistant *Enterococcus faecium* (VREfm) bloodstream infections are associated with
19 high recurrence rates. This study used genome sequencing to accurately distinguish the
20 frequency of relapse and reinfection in patients with recurrent *E. faecium* bacteremia, and to
21 investigate strain relatedness in patients with apparent VREfm and vancomycin-susceptible *E.*
22 *faecium* (VSEfm) mixed infection. A retrospective study was performed at the Cambridge
23 University Hospitals NHS Foundation Trust (CUH) between November 2006 and December 2012.
24 We analyzed the genomes of 44 *E. faecium* isolated from 21 patients (26 VREfm from 12
25 patients with recurrent bacteremia, and 18 isolates from 9 patients with putative VREfm/VSEfm
26 mixed infection). Phenotypic antibiotic susceptibility was determined using the Vitek2
27 instrument. Genomes were compared with those for a further 263 *E. faecium* associated with
28 bacteremia in patients at CUH over the same time period. Pairwise comparison of core genomes
29 indicated that 10 (71%) episodes of recurrent VREfm bacteremia were due to reinfection with a
30 new strain, with reinfection being more likely with increasing time between the two positive
31 cultures. The majority (78%) of patients with a mixed VREfm and VSEfm infection had unrelated
32 strains. More than half (59%) of study isolates were closely related to another isolate associated
33 with bacteremia from CUH. This included 60% of isolates associated with re-infection, indicating
34 acquisition in the hospital. This study provides the first high-resolution insights into recurrence
35 and mixed infection by *E. faecium*, and demonstrates that reinfection with a new strain, often
36 acquired from the hospital, is a driver of recurrence.

37 **INTRODUCTION**

38 *Enterococcus faecium* is an important cause of bloodstream infections in critically ill and
39 immunocompromised patients (1), and ranks among the ten most common microorganisms
40 associated with healthcare-associated infections in the United States (US) (2). Bacteremia
41 caused by vancomycin-resistant enterococci is associated with increased mortality, costs of care
42 and rates of recurrence compared to vancomycin-susceptible strains (3–5).

43
44 Despite the establishment of linezolid and daptomycin as mainstay therapeutic agents for
45 vancomycin-resistant *E. faecium* (VREfm) bacteremia since the early 2000s, recurrence remains
46 a common clinical challenge with rates ranging from 3% to 25% (6–8). Following apparent
47 clearance of infection, recurrent bacteremia can be explained by either relapsing infection with
48 the same strain due to a persistent focus of infection, or reinfection with the same or a different
49 strain. Differentiating between these scenarios is clinically relevant; relapsing infection requires
50 investigation and interventions to deal with a persistent focus of infection, whilst reinfection is
51 more likely to be associated with an underlying susceptibility that increases the risk of invasion
52 due to breakdown of host immune defenses. Surprisingly little is known about the relative
53 frequency of relapse versus reinfection in recurrent *E. faecium* bacteremia, with the literature
54 limited to case reports (9–11) and case series predominantly containing patients with
55 *Enterococcus faecalis* infection (12, 13).

56
57 *E. faecium* bacteremia is polymicrobial in up to 35% of cases due to mixed infection with other
58 bacterial genera or enterococcal species (14). Apparent mixed infection with VREfm and

59 vancomycin-susceptible *E. faecium* (VSEfm) has been reported in the context of *in-vivo* loss or
60 gain of the *van* transposon by sub-populations of the same strain (15–17). However, whilst
61 carriage of multiple strains of *E. faecium* is presumed to be common (18, 19), the frequency of
62 mixed infection with different *E. faecium* strains is unknown.

63
64 Whole-genome sequencing has demonstrated superior discriminatory power compared to
65 traditional bacterial typing techniques such as pulsed-field gel electrophoresis (PFGE) or
66 multilocus sequence typing (MLST) in epidemiological investigations and in studies of the
67 population structure of *E. faecium* causing bacteremia at a local and national level (20–23).
68 Here, we use genome sequencing to gain a better understanding of *E. faecium* bacteremia.
69 Specifically, we investigated strain relatedness in patients with recurrent VREfm bacteremia,
70 and in patients with apparent mixed bloodstream infection with VREfm and VSEfm. In addition,
71 we compared the findings of genome sequencing with antibiotic resistance profile.

72

73 **RESULTS**

74 A retrospective review of patients at CUH between November 2006 and December 2012
75 identified 231 patients with at least one episode of VREfm bacteremia. Of these, 14 patients had
76 at least one episode of recurrence, giving an estimated recurrence rate of 6.1%. We identified
77 twelve patients that had isolates from at least two episodes of VREfm bacteremia available for
78 whole genome sequencing (see Table 1 for patient summary and Dataset S1 for individual
79 isolate information). Ten patients had one recurrence and two patients had two recurrences of
80 bacteremia. To determine the genetic relatedness of isolates causing recurrence we identified

81 SNPs in the core genome based on mapping to a reference genome. Of the fourteen isolate
82 pairs associated with a recurrent bacteremia, four (from four patients) were closely related (1-7
83 SNPs, median 1.5 SNPs) to the isolate from the previous episode (Figure 1A, Table 1). This
84 finding is highly indicative of relapse (or reinfection) with the same strain, based on a study that
85 reported a genetic distance between *E. faecium* carried by the same person (within-host
86 diversity) of 6 core SNPs (24). By contrast, 10 isolates (from ten patients) were more genetically
87 distant from the isolate from the previous episode (25-368 SNPs, median 258 SNPs), which is
88 consistent with reinfection by a different strain (Figure 1A, Table 1). The two patients with two
89 recurrences of bacteremia had both an episode of relapse (pairwise SNP difference of 2 or 7
90 SNPs, respectively), and an episode of reinfection with a new strain (25 or 309 SNPs,
91 respectively) (Table 1). The SNPs acquired between the first and second isolate for the four
92 genetically related isolate pairs were located in different genes in different patients (Table S1).
93 The median time to first recurrence across the study population was 80 days (range 39 to 1578
94 days), and the second episodes of recurrence occurred 36 and 168 days after the preceding
95 bacteremia. Comparison of the timing of recurrence with the genomic analyses indicated that
96 all isolates from cases of relapse/reinfection with the same strain were isolated within 108 days
97 of each other, whilst recurrences due to reinfection with a different strain were equally likely to
98 occur within 108 days (5/10 episodes) and after 108 days (5/10).

99
100 All twelve study patients with recurrent bacteremia had multiple co-morbidities that predispose
101 to VREfm bacteremia (Table 1, Dataset S1). The most probable source for the bacteremia was
102 defined for each case (Table 1). Mucosal translocation (n=7, with possible concurrent

103 intravascular catheter infection) and intravascular catheters (n=5) were the most common
104 sources of bacteremia for the study patients. There was no clear difference identified between
105 the sources of infection in patients with recurrence due to the same or different strains (Table 1,
106 Table S2). The four episodes of recurrence due to the same strain were associated with
107 presumed persistent intravenous catheter colonization and/or gut carriage (n=3), or failure to
108 eradicate a persistent focus of infection (n=1, chronic pyelonephritis associated with kidney
109 stones) (Table 1, Table S2). A central venous catheter was known to be retained between
110 episodes of bacteremia in 2/10 cases with reinfection with a different strain, meaning that
111 whole genome sequencing was able to refute these as being a persistent focus.

112
113 The retrospective review of CUH patients also identified nine patients with putative mixed
114 bloodstream infection with VREfm and VSEfm for whom both isolates were available for whole
115 genome sequencing (Table 1). Pairwise core genome comparison of the 9 VREfm/VSEfm pairs
116 revealed that 7/9 (78%) patients had isolate pairs that were genetically distinct (median 217
117 SNPs, range 70-381 SNPs) (Figure 1B), which is consistent with true mixed-strain infection. The
118 most common source of infection for patients with true mixed infection was an intravascular
119 catheter (4/7, 57%). The remaining 2 patients had isolates that were identical at the core
120 genome level. Further analysis of the genetic content between these 2 pairs through
121 comparison to the ResFinder database confirmed the variable presence of the *vanRSHWXYZ*
122 genes, which encode vancomycin resistance. There was insufficient sequence adjacent to the
123 *vanA* transposon in the genome assemblies to identify the genetic location of these genes, so
124 differences in gene content between the VREfm and VSEfm in each pair were assessed. In one

125 isolate pair (Patient 16) the *van* genes had been lost together with 21 genes, including 7 genes
126 best matched to a plasmid (based on BLAST), suggesting they may have been lost/gained
127 together with part of a plasmid (Dataset S2). Two genes labelled as *tetM* and *ermB* were lost
128 alongside the *van* genes in this patient, but both isolates retained a copy of *tetM* and *ermB* and
129 so this may not have affected the wider antibiotic resistance phenotype (Figure 2). In the
130 second isolate pair (Patient 21) an additional 14 genes had been lost with the *van* genes
131 including five genes located adjacent in the genome (Dataset S2) suggesting that *vanA* was not
132 gained/lost as part of a plasmid but may have moved as part of a smaller transposable element.

133
134 We evaluated whether the phenotypic antibiotic resistance profile (antibiogram) to 11 drugs
135 (excluding glycopeptides) could be used to distinguish between genetically related and distinct
136 strains from the same patient (Figure 2). Of the 6 isolate pairs that were closely related in the
137 two study collections, four had identical antibiograms, one varied by tetracycline resistance
138 associated with gain/loss of the *tetM* gene, and one varied between susceptible and
139 intermediate resistance to quinupristin-dalfopristin (Figure 2). Of the 17 strain pairs that were
140 genetically different, three had identical antibiograms and the remainder had between 1 and 5
141 (median 2) differences (Figure 2). The most variable antibiotic was tetracycline (10/17 pairs),
142 followed by high-level resistance to streptomycin (n=7), and nitrofurantoin (n=6). Since 3/7
143 identical antibiograms (43%) belonged to genetically distinct strains, this suggests that
144 antibiogram does not reliably distinguish between genetically related and distinct strains from
145 the same patient. However, more than one change in the antibiogram was only identified in
146 genetically distinct strains (12/17 genetically distinct pairs).

147
148 The high rate of true mixed VREfm and VSEfm infection and recurrence with a new strain
149 indicates carriage of multiple lineages or the acquisition of new strains over time. Healthcare
150 settings are associated with the acquisition of *E. faecium*, and so we investigated CUH as a
151 putative source by combining the 44 study *E. faecium* genomes with a further 263 *E. faecium*
152 genomes associated with bloodstream infection in 263 patients at the same hospital over the
153 same time period (2006-2012) (Figure S1). Taken together, 26 of the 44 study isolates were
154 closely related to at least one CUH isolate (0-8 SNPs, median 3), including 3 isolates that were
155 closely related to an isolate from another study patient. This included isolates from 6/7 patients
156 infected with different VREfm and VSEfm strains and 6/10 patients with recurrence caused by
157 different strains. The remaining isolates were between 12 and 86 SNPs (median 33 SNPs) from
158 the closest genetic match.

159
160 **DISCUSSION**

161 This study represents the first use of whole genome sequencing in the context of *E. faecium*
162 bacteremia to investigate the relative rates of relapse and reinfection in recurrent infections,
163 and to study mixed infection with VREfm and VSEfm. Although rates of recurrence vary in the
164 literature, the estimated rate of 6.1% identified at CUH is within the range of those reported
165 previously (6–8).

166
167 We found that the majority of patients in our study had a recurrent VREfm bacteremia caused
168 by reinfection with a new strain. This finding supports that of Cheng *et al.* which found

169 reinfection to be responsible for ~70% of recurrence based on PFGE, although the study focused
170 primarily on *E. faecalis* (12). These reinfections could either be due to persistent carriage of a
171 genetically distinct strain, or reinfection with a newly acquired strain. We found that at least
172 60% of reinfections were caused by isolates that were genetically closely related to another
173 bacteremia isolate from CUH, suggesting cross-transmission in the hospital. Additionally, the
174 rates of cross-transmission found in this study are likely to be an underestimate, since
175 asymptomatic gut carriage and the environment represent a large reservoir of VREfm and were
176 not sampled in this study. These findings suggest that the emphasis on preventing recurrent
177 VREfm bacteremia should be on infection control and minimizing periods of susceptibility to
178 infection. Further studies will be required to elucidate the role of the environment, staff and
179 patients as sources for these hospital acquisitions to improve infection control.

180
181 The results of this study suggest that recurrence with the same strain may be related to time.
182 Episodes of recurrence with the same strain were only identified up to 108 days apart, which
183 concurs with findings by Baran *et al.* based on *E. faecalis* and *E. faecium* from a total of three
184 patients (13). In contrast to our findings for same-strain recurrence, our study showed that
185 bacteremic episodes due to reinfections with a distinct strain were roughly equally likely to
186 occur within and after 108 days of each other, and as early as 57 days apart. Further work will
187 be required using larger sample sizes from multiple centers to determine whether there is a
188 true relationship between the relatedness of *E. faecium* strains causing recurrence and the time
189 between episodes.

190

191 We also identified that the majority of patients with mixed VREfm and VSEfm bacteremia were
192 infected with two genetically distinct strains. This differs from the finding by Cardenas et al. that
193 four patients had closely related VREfm and VSEfm strains associated with bacteremia based on
194 MLST (16). The true mixed infections in our study frequently varied in antibiotic resistance
195 profiles. Whilst most cases of mixed VREfm and VSEfm would be detected during routine disc
196 susceptibility testing, this variation in antibiotic resistance profiles could complicate treatment
197 in cases that go undetected. Although the numbers are low in our study, it was interesting to
198 note that true mixed VREfm and VSEfm infections were commonly suspected to originate from
199 an intravascular source, suggesting that central venous catheters may become colonized with
200 multiple strains of *E. faecium*.

201
202 The results of our study suggest that antibiogram lacks accuracy in predicting the genetic
203 relatedness of strains. The utility of antibiograms for determining the relatedness of *E. faecium*
204 has not previously been evaluated, but our finding that a pair of identical strains could vary in
205 their resistance to antibiotics is consistent with the fact that *E. faecium* has a highly mobile
206 genome, with many resistance genes carried on mobile genetic elements.

207
208 Our study may have implications on future evaluation of VREfm treatment efficacy. There are
209 currently no randomized controlled trials to define the optimal antibiotic for the treatment of
210 VREfm bacteremia. Current knowledge is based on retrospective observational studies
211 comparing linezolid to daptomycin, where recurrent infection is often defined as one of the
212 outcome measures in the absence of bacterial typing results (6, 8, 25). These studies imply that

213 early recurrence (often assessed at 30 or 60 days after treatment completion) is caused by true
214 relapse (6, 8), or rely on phenotype such as identical antibiograms to infer relapse (25). Our
215 results show that in the absence of prospective randomized studies or bacterial genotyping, one
216 needs to question whether a recurrent infection is indeed due to ineffective therapy as opposed
217 to underlying confounding from patient related factors conferring increased susceptibility to
218 reinfection. Future studies should address this issue.

219
220 This study has several limitations. We did not sequence multiple colonies from the same sample
221 to assess diversity, meaning that apparent reinfections could have been mixed infections at the
222 outset. The study samples were retrieved from frozen stock and it is not possible to know
223 whether these were originally created from a single or multiple colonies. It is not possible to
224 differentiate between relapse and reinfection by the same strain, introducing an element of
225 uncertainty into our classification. The true level of mixed infection will be higher than we
226 report here, since we only assessed patients with a VREfm and VSEfm mixed infection and
227 analysed one colony of each from every bacteremic episode. Finally, the rate of recurrence
228 identified here may be an underestimate since repeat cultures were not taken systematically.

229
230 In conclusion, the findings of this study show that the majority of VREfm recurrences and mixed
231 VREfm and VSEfm infections are due to different strains, and that antibiogram lacks accuracy in
232 determining genetic relatedness. This has important implications for infection control as it
233 highlights the importance of reducing cross-transmission in vulnerable patient groups.

234

235 **MATERIALS AND METHODS**

236 A retrospective study was conducted at the Cambridge University Hospitals NHS Foundation
237 Trust (CUH), a tertiary referral center in the United Kingdom with 1,170 beds and 350,000
238 occupied-bed-days per year. The rate of vancomycin resistance in *E. faecium* bacteremia
239 isolates at CUH is high (>60%), approaching rates reported in the US (2) and historical data from
240 2001 suggests that 32.6% of patients at CUH in high-risk wards carry VRE (either *E. faecium* or *E.*
241 *faecalis*) (26).

242
243 All patients with VREfm bloodstream infection between November 2006 and December 2012
244 were identified using the diagnostic microbiology laboratory database. These cases were
245 evaluated to identify all patients with (i) recurrence of VREfm bloodstream infection, and/or (ii)
246 putative mixed VREfm and VSEfm bloodstream infection. Recurrence was defined as a blood
247 culture that was positive for VREfm taken >30 days after the index culture from a patient with
248 intervening negative blood cultures and/or resolution of clinical signs of infection. Putative
249 mixed VREfm and VSEfm infection was defined as the isolation of VREfm and VSEfm from the
250 same blood culture or different cultures taken within 48 h of the index sample.

251
252 Fourteen patients fulfilled the criterion for recurrence and 10 patients fulfilled the criterion for
253 putative mixed VREfm and VSEfm infection, with no overlap of cases between the two. Seven of
254 the patients had mixed infection with other bacterial species, as shown in Dataset S1. Cross-
255 referencing these 24 patients with the bacterial freezer archive identified 44 isolates from 21
256 patients (12 patients with recurrence and 9 patients with mixed VREfm and VSEfm infection),

257 who were the basis for this study. Clinical data for the 21 cases were collected from paper and
258 computerized medical records using a standardized proforma, including the suspected focus of
259 infection, underlying comorbidities, and dates of positive and negative blood cultures.
260 Neutropenia was defined as a polymorphonuclear leukocyte count of less than 500/ μ l within 24
261 hours of the onset of bacteremia. The focus of infection was defined based on clinical,
262 radiological and microbiological features. Bacteremia was determined to be secondary to an
263 intravascular device if i) a positive intravascular catheter tip semi-quantitative culture yielded
264 more than 15 cfu of *E. faecium* with identical antibiogram as the blood culture isolate (definite),
265 or ii) if no other focus of infection was identified in the presence of an intravascular catheter,
266 and/or clinical signs of sepsis improved after line removal (probable). For neutropenic patients
267 with no definite clinical focus, mucosal translocation was presumed to be the origin of the
268 bacteremia based on the Centers for Disease Control and Prevention definition
269 (http://www.cdc.gov/nhsn/pdfs/pscmanual/4psc_clabscurrent.pdf). In cases of recurrent
270 infection, a focus was considered persistent if there was an unresolved deep source of infection
271 or if a potentially infected intravascular catheter was not removed between episodes of
272 bacteremia. Ethical approval for the study was obtained from the local Research Ethics
273 Committee (reference no. 13/EE/0044) and the need for informed consent was waived.

274
275 Twenty-one of the 44 isolates had been sequenced previously (27). For the 23 new *E. faecium*
276 isolates sequenced here, bacteria were cultured on Columbia Blood Agar (CBA, Oxoid) for 48
277 hours at 37°C in air. Phenotypic antimicrobial susceptibility testing for all 44 isolates was
278 performed using the Vitek2 instrument (bioMérieux, Marcy l'Etoile, France) with the AST-P607

279 card. DNA was extracted using the QIAextractor (QIAGEN) and sequencing performed on an
280 Illumina HiSeq2000. Sequence reads were assembled using Velvet and annotated using Prokka.
281 The pangenome was estimated using Roary (28) with a 98% ID cut-off. The *van* gene in the two
282 patients with genetically related VREfm and VSEfm was extracted from the Roary pan genome
283 and compared to the *vanA* gene extracted from a *vanA* transposon (accession number M97297)
284 and the *vanB* gene extracted from Aus0004 (accession number CP003351) using BLAST. The
285 presence of antibiotic resistance genes was determined using an in-house curated version of the
286 ResFinder database (genes listed in Dataset S3) (29) and ARIBA ([https://github.com/sanger-](https://github.com/sanger-pathogens/ariba/wiki)
287 [pathogens/ariba/wiki](https://github.com/sanger-pathogens/ariba/wiki)).

288
289 Sequence data for an additional 263 *E. faecium* associated with bloodstream infection in 263
290 patients at CUH between November 2006 and December 2012 and belonging to the hospital-
291 adapted clone of Clade A based on whole genome sequence analysis were taken from Raven et
292 al. (27). These 263 genomes together with the 44 study genomes were mapped to *E. faecium*
293 Aus0004 (ENA accession number CP003351) using SMALT
294 (<http://www.sanger.ac.uk/science/tools/smalt-0>). Mobile genetic elements (identified based
295 on annotation and PHAST (30)) and recombination events (identified using Gubbins (31)) were
296 removed to identify the core genome. A maximum likelihood tree was created using RAXML
297 based on single nucleotide polymorphisms (SNPs) in the core genome. Pairwise SNP differences
298 were calculated based on SNPs in the core genome.

299
300 Accession numbers are listed in Dataset S1.

301
302 **Acknowledgements**
303 We thank the Wellcome Trust Sanger Institute library construction, sequence and core
304 informatics teams, the staff at the Cambridge Public Health England Clinical Microbiology and
305 Public Health Laboratory, Hayley Brodrick, Kim Judge and Elizabeth Blane for technical support,
306 and Estee Török for support with the ethical approval. We thank Dr Nick Brown (Cambridge
307 Clinical Microbiology and Public Health Laboratory) for generous provision of isolates. This study
308 was supported by grants from the Health Innovation Challenge Fund (WT098600, HICF-T5-342),
309 a parallel funding partnership between the Department of Health and Wellcome Trust. The
310 views expressed in this publication are those of the author(s) and not necessarily those of the
311 Department of Health or Wellcome Trust. This project was also funded by a grant awarded to
312 the Wellcome Trust Sanger Institute (098051). TG is a Wellcome Trust Clinical Research Training
313 Fellow (103387/Z/13/Z). JP and SJP are paid consultants to Specific Technologies.

314
315
316
317
318
319
320
321
322

323 **REFERENCES**

- 324 1. Arias CA, Murray BE. 2013. The rise of the *Enterococcus*: beyond vancomycin resistance.
325 Nat Rev Microbiol 10:266–278.
- 326 2. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B,
327 Fridkin S. 2013. Antimicrobial-resistant pathogens associated with healthcare-associated
328 infections: summary of data reported to the National Healthcare Safety Network at the
329 Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol
330 34:1–14.
- 331 3. Salgado CD, Farr BM. 2003. Outcomes associated with vancomycin-resistant enterococci:
332 a meta-analysis. Infect Control Hosp Epidemiol 24:690–698.
- 333 4. Prematunge C, MacDougall C, Johnstone J, Adomako K, Lam F, Robertson J, Garber G.
334 2015. VRE and VSE bacteremia outcomes in the era of effective VRE therapy: a systematic
335 review and meta-analysis. Infect Control Hosp Epidemiol 37:26–35.
- 336 5. Linden PK, Pasculle AW, Manez R, Kramer DJ, Fung JJ, Pinna AD, Kusne S. 1996.
337 Differences in outcomes for patients with bacteremia due to vancomycin-resistant
338 *Enterococcus faecium* or vancomycin-susceptible *E. faecium*. Clin Infect Dis 22:663–670.
- 339 6. Britt NS, Potter EM, Patel N, Steed ME. 2015. Comparison of the effectiveness and safety
340 of linezolid and daptomycin in vancomycin-resistant enterococcal bloodstream infection:
341 a national cohort study of veterans affairs patients. Clin Infect Dis 61:871–878.
- 342 7. Shukla BS, Shelburne S, Reyes K, Kamboj M, Lewis JD, Rincon SL, Reyes J, Carvajal LP,
343 Panesso D, Sifri CD, Zervos MJ, Pamer EG, Tran TT, Adachi J, Munita JM, Hasbun R, Arias
344 CA. 2016. Influence of minimum inhibitory concentration in clinical outcomes of

- 345 *Enterococcus faecium* bacteremia treated with daptomycin: is it time to change the
346 breakpoint? Clin Infect Dis 62:1514–1520.
- 347 8. Twilla JD, Finch CK, Usery JB, Gelfand MS, Hudson JQ, Broyles JE. 2012. Vancomycin-
348 resistant *Enterococcus* bacteremia: An evaluation of treatment with linezolid or
349 daptomycin. J Hosp Med 7:243–248.
- 350 9. Elsner H., Drews D, Burdelski M, Kaufers PM. 1997. Recurrent septicemias with
351 *Enterococcus faecium*. Infection 25:127–128.
- 352 10. Papp L, McNeeley DF, Projan SJ, Bradford PA, Frost A, Nesin M. 2003. Recurrent episodes
353 of shock-like syndrome caused by the same strain of vancomycin-resistant *Enterococcus*
354 *faecium* in a pediatric patient. Microb Drug Resist 9:307–312.
- 355 11. Roghmann M-C, Qaiyumi S, Johnson JA, Schwalbe R, Morris JG. 1997. Recurrent
356 vancomycin-resistant *Enterococcus faecium* bacteremia in a leukemia patient who was
357 persistently colonized with vancomycin-resistant enterococci for two years. Clin Infect Dis
358 24:514–515.
- 359 12. Cheng AC, Murdoch DR, Harrell LJ, Barth Reller L. 2005. Clinical profile and strain
360 relatedness of recurrent enterococcal bacteremia. Scand J Infect Dis 37:642–646.
- 361 13. Baran J, Riederer KM, Ramanathan J, Khatib R. 2001. Recurrent vancomycin-resistant
362 *Enterococcus* bacteremia: prevalence, predisposing factors, and strain relatedness. Clin
363 Infect Dis 32:1381–1383.
- 364 14. Cheah ALY, Spelman T, Liew D, Peel T, Howden BP, Spelman D, Grayson ML, Nation RL,
365 Kong DCM. 2013. Enterococcal bacteraemia: factors influencing mortality, length of stay
366 and costs of hospitalization. Clin Microbiol Infect 19:E181-9.

- 367 15. Reuter S, Ellington MJ, Cartwright EJP, Köser CU, Török ME, Gouliouris T, Harris SR, Brown
368 NM, Holden MTG, Quail M, Parkhill J, Smith GP, Bentley SD, Peacock SJ. 2014. Rapid
369 bacterial whole-genome sequencing to enhance diagnostic and public health
370 microbiology. *JAMA Intern Med* 173:1397–1404.
- 371 16. Cárdenas AM, Andreacchio KA, Edelstein PH. 2014. Prevalence and detection of mixed-
372 population enterococcal bacteremia. *J Clin Microbiol* 52:2604–2608.
- 373 17. Arias CA, Torres HA, Singh K V, Panesso D, Moore J, Wanger A, Murray BE. 2007. Failure
374 of daptomycin monotherapy for endocarditis caused by an *Enterococcus faecium* strain
375 with vancomycin-resistant and vancomycin-susceptible subpopulations and evidence of
376 *in vivo* loss of the *vanA* gene cluster. *Clin Infect Dis* 45:1343–1346.
- 377 18. Bonten MJM, Hayden MK, Nathan C, Rice TW, Weinstein RA. 1998. Stability of
378 vancomycin-resistant enterococcal genotypes isolated from long-term-colonized patients.
379 *J Infect Dis* 177:378–382.
- 380 19. Tremlett CH, Brown DFJ, Woodford N. 1999. Variation in structure and location of *vanA*
381 glycopeptide resistance elements among enterococci from a single patient variation in
382 structure and location of *vanA* glycopeptide resistance elements among enterococci from
383 a single patient. *J Clin Microbiol* 37:37–40.
- 384 20. Howden BP, Holt KE, Lam MMC, Seemann T, Ballard S, Coombs GW, Tong SYC, Grayson
385 ML, Johnson PDR, Stinear TP. 2013. Genomic insights to control the emergence of
386 vancomycin-resistant enterococci. *MBio* 4:1–9.
- 387 21. van Hal SJ, Ip CLC, Ansari MA, Wilson DJ, Espedido BA, Jensen SO, Bowden R. 2016.
388 Evolutionary dynamics of *Enterococcus faecium* reveals complex genomic relationships

389 between isolates with independent emergence of vancomycin resistance. *Microb*
390 *Genomics* 2.

391 22. Raven KE, Reuter S, Reynolds R, Brodrick HJ, Russell JE, Török ME, Parkhill J, Peacock SJ.
392 2016. A decade of genomic history for healthcare-associated *Enterococcus faecium* in the
393 United Kingdom and Ireland. *Genome Res* 26:1388–1396.

394 23. Pinholt M, Lerner-Svensson H, Littauer P, Moser CE, Pedersen M, Lemming LE, Ejlersen T,
395 Søndergaard TS, Holzkecht BJ, Justesen US, Dzajic E, Olsen SS, Nielsen JB, Worning P,
396 Hammerum AM, Westh H, Jakobsen L. 2015. Multiple hospital outbreaks of *vanA*
397 *Enterococcus faecium* in Denmark, 2012–13, investigated by WGS, MLST and PFGE. *J*
398 *Antimicrob Chemother* 70:2474–82.

399 24. Brodrick HJ, Raven KE, Harrison EM, Blane B, Reuter S, Török ME, Parkhill J, Peacock SJ.
400 2016. Whole-genome sequencing reveals transmission of vancomycin-resistant
401 *Enterococcus faecium* in a healthcare network. *Genome Med* 8:4.

402 25. Mave V, Garcia-diaz J, Islam T, Hasbun R. 2009. Vancomycin-resistant enterococcal
403 bacteraemia: is daptomycin as effective as linezolid? *J Antimicrob Chemother* 64:175–
404 180.

405 26. Goossens H, Jabes D, Rossi R, Lammens C, Privitera G, Courvalin P. 2003. European survey
406 of vancomycin-resistant enterococci in at-risk hospital wards and in vitro susceptibility
407 testing of ramoplanin against these isolates. *J Antimicrob Chemother* 51 Suppl 3:iii5-12.

408 27. Raven KE, Gouliouris T, Brodrick H, Coll F, Brown NM, Reynolds R, Reuter S, Török ME,
409 Parkhill J, Peacock SJ. 2017. Complex routes of nosocomial vancomycin-resistant
410 *Enterococcus faecium* transmission revealed by genome sequencing. *Clin Infect Dis*

411 64:15–17.

412 28. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D,
413 Keane JA, Parkhill J. 2015. Roary: Rapid large-scale prokaryote pan genome analysis.
414 *Bioinformatics* 31:3691–3.

415 29. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM,
416 Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob*
417 *Chemother* 67:2640–4.

418 30. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool.
419 *Nucleic Acids Res* 39:W347-52.

420 31. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR.
421 2014. Rapid phylogenetic analysis of large samples of recombinant bacterial whole
422 genome sequences using Gubbins. *Nucleic Acids Res* 43:e15.

423

424

425

426 **FIGURE LEGENDS**

427 **Figure 1. Genetic relatedness of isolates from the same patient with recurrent bacteremia (A),**
428 **or mixed infection with VREfm and VSEfm (B).** A) Right hand side: Graph showing the pairwise
429 core genome SNP difference between *E. faecium* isolates cultured from the same patient more
430 than 30 days apart, and timing of episodes. Red indicates isolate pairs that were closely related
431 (1-7 SNPs) based on genome sequence data. Left hand side: expansion of the area of the graph
432 under 100 SNPs. B) Graph showing the pairwise core genome SNP difference between VREfm
433 and VSEfm isolates cultured from the same patient within 48 hours.

434
435 **Figure 2. Comparison between strain similarity by genome sequence data and antibiogram.**
436 Left hand side indicates the strain relatedness based on genome sequence data, and the
437 patient. Two patients with both relapse and reinfection occur twice in the list. Middle block
438 indicates changes in antibiotic resistance (red, defined as a change from susceptible to resistant;
439 green, defined as a change from resistant to susceptible; white, no change) between isolates
440 from the same patient.

441 **Table 1. Clinical and isolate details for the patient cohort.**

	Pt	Age **	Gender	Co-morbidities ***	Number of isolates	Presumptive source of infection			Year of first isolate	Number of days		Number of SNPs#			Interpretation
						1 st	2 nd	3 rd		1 st to 2 nd	2 nd to 3 rd	1 st to 2 nd	2 nd to 3 rd	1 st to 3 rd	
Recurrence	1	67	Male	SOM	3	Urinary	Urinary	Urinary	2010	54	36	25	2	25	Reinfection/Relapse
	2	50	Male	HM, SCT	2	MT/IV	MT/IV	-	2007	1578	-	234	-	-	Reinfection
	3*	56	Male	HM	2	MT/IV	MT/IV	-	2009	61	-	268	-	-	Reinfection
	4*	24	Female	Congenital neutropenia	3	IV	MT/IV	MT/IV	2012	108	168	7	311	309	Relapse/Reinfection
	5	44	Female	SOT, ESRD, DM	2	IV	Unknown	-	2009	777	-	299	-	-	Reinfection
	6	42	Female	Alcoholic liver disease	2	IV	IV	-	2009	215	-	282	-	-	Reinfection
	7	13	Male	HM, SCT, ESRD	2	MT/IV	IV	-	2007	1484	-	232	-	-	Reinfection
	8	41	Female	HM, SCT, ESRD	2	MT/IV	MT/IV	-	2011	57	-	64	-	-	Reinfection
	9	10	Female	HM	2	IV	MT/IV	-	2010	80	-	1	-	-	Relapse
	10	0	Male	HM	2	IV	MT/IV	-	2010	59	-	348	-	-	Reinfection
	11	15	Female	HM	2	MT/IV	MT/IV	-	2010	39	-	1	-	-	Relapse
	12	39	Male	HM	2	MT/IV	MT/IV	-	2012	104	-	282	-	-	Reinfection
Mixed VREfm & VSEfm	13	59	Female	SOM, HM	2	IV	-	-	2012	2	-	329	-	-	Genetically distinct
	14	72	Male	SOM, HM	2	MT	-	-	2012	0	-	119	-	-	Genetically distinct
	15	62	Male	HM, SCT	2	IV	-	-	2011	0	-	217	-	-	Genetically distinct
	16	50	Male	HM, SCT	2	MT/IV	-	-	2009	0	-	0	-	-	Genetically related
	17	56	Male	HM	2	IV	-	-	2008	0	-	18	-	-	Genetically distinct
	18	59	Male	SOM	2	IA - biliary	-	-	2008	0	-	381	-	-	Genetically distinct
	19	63	Male	HM, SOT	2	IV	-	-	2009	1	-	203	-	-	Genetically distinct
	20	19	Male	HM, SCT	2	MT/IV	-	-	2010	0	-	154	-	-	Genetically distinct
	21	48	Female	ESRD, LC	2	Lung and IA	-	-	2010	0	-	0	-	-	Genetically related

442 * Patients with recurrence with a different strain for whom a central venous catheter was retained between episodes of bacteremia.

443 ** Age at time of first bacteremia

444 *** Co-morbidities identified across all episodes of bacteremia in the study (breakdown by bacteremic episode in Dataset S1)

445 # Number of SNPs based on mapping to a reference genome (*E. faecium* Aus0004)

446 Abbreviations: Pt = patient, SOM = solid organ malignancy, HM = hematological malignancy, SCT = stem cell transplant, SOT = solid organ transplant, ESRD =

447 end stage renal disease, DM = diabetes mellitus, LC = liver cirrhosis, MT = mucosal translocation, IV = intravascular, IA = intra-abdominal