

1 **Duration of exposure to multiple antibiotics is associated with increased risk of vancomycin-**
2 **resistant enterococcal bacteraemia: a nested case-control study**

3

4 Theodore GOULIOURIS,^{1,2,3*} Ben WARNE,^{1,3} Edward J. P. CARTWRIGHT,¹ Luke BEDFORD,² Chathika K.
5 WEERASURIYA,³ Kathy E. RAVEN,¹ Nick M. BROWN,² M. Estée TOROK,^{1,2,3} Direk
6 LIMMATHUROTSAKUL,⁴ Sharon J. PEACOCK^{1,3,4,5}

7

8 ¹ Department of Medicine, University of Cambridge, Cambridge, United Kingdom

9 ² Public Health England, Clinical Microbiology and Public Health Laboratory, Cambridge, United
10 Kingdom

11 ³ Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

12 ⁴ Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

13 ⁵ London School of Hygiene & Tropical Medicine, Keppel Street, London, United Kingdom

14

15 ***Corresponding author:** Theodore Gouliouris, Department of Medicine, University of Cambridge,
16 Level 5, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, United Kingdom. Tel: +44 (0)1223
17 336867; Fax: +44 (0)1223 336846; Email: theo.gouliouris@doctors.org.uk

18

19 **Running title: Antibiotic risk factors in VRE bacteraemia**

20

21

22

23

24

25

26

27 **ABSTRACT**

28 **Background.** Vancomycin-resistant enterococcal (VRE) bacteraemia has a high mortality and
29 continues to defy control. Antibiotic risk factors for VRE bacteraemia have not been adequately
30 defined. We aimed to determine the risk factors for VRE bacteraemia focusing on duration of
31 antibiotic exposure.

32 **Methods.** A retrospective matched nested case-control study was conducted amongst hospitalised
33 patients at Cambridge University Hospitals NHS Foundation Trust from 1st January 2006 to 31st
34 December 2012. Cases who developed a first episode of VRE bacteraemia were matched 1:1 to
35 controls by length of stay, year, specialty and ward type. Independent risk factors for VRE
36 bacteraemia were evaluated using conditional logistic regression.

37 **Results.** 235 cases were compared to 220 controls. Duration of exposure to parenteral vancomycin,
38 fluoroquinolones, and meropenem were independently associated with VRE bacteraemia. Compared
39 to patients with no exposure to vancomycin, those who received courses of 1-3 days, 4-7 days, or
40 greater than 7 days had a stepwise increase in risk of VRE bacteraemia (conditional odds ratio (cOR)
41 1.2 (95% confidence interval [CI] 0.4-3.8), 3.8 (95% CI 1.2-11.7), and 6.6 (95% CI 1.9-22.8),
42 respectively). Other risk factors were presence of central venous catheter (cOR 8.7 [95% CI 2.6-
43 29.5]); neutropenia (cOR 15.5 [95% CI 4.2-57.0]); hypoalbuminaemia (cOR 8.5 [95% CI 2.4-29.5]);
44 malignancy (cOR 4.4 [95% CI 1.6-12.0]); gastrointestinal disease (cOR 12.4 [95% CI 4.2-36.8]); or
45 hepatobiliary disease (cOR 7.9 [95% CI 2.1-29.9]).

46 **Conclusions.** Longer exposure to vancomycin, fluoroquinolones, or meropenem was associated with
47 VRE bacteraemia. Antimicrobial stewardship interventions targeting high-risk antibiotics are required
48 to complement infection control procedures against VRE bacteraemia.

49 **INTRODUCTION**

50 Over the last 20 years, vancomycin-resistant enterococci (VRE) have emerged as a major cause of
51 healthcare-associated bacteraemia, disproportionately affecting immunocompromised and critically ill
52 patients. ¹ *Enterococcus faecium* has become responsible for most VRE infections following the global
53 dissemination of a hospital-adapted lineage. ² VRE bacteraemias are associated with increased costs
54 of care, length of stay and mortality compared to vancomycin-susceptible enterococcal (VSE)
55 bacteraemias. ^{3, 4} In contrast to other healthcare-associated infections, rates of VRE bacteraemia
56 have failed to decline in response to a host of generic infection control interventions in different
57 healthcare settings, ⁵⁻⁷ and are even increasing in some countries. ⁸ Consequently, the identification
58 of modifiable risk factors for VRE bacteraemia remains a priority.

59

60 Gut carriage of VRE is a major risk factor for VRE bacteraemia. Bloodstream infection may be
61 preceded by high levels of VRE carriage in the gut. ⁹ In recipients of allogeneic stem cell transplants,
62 this is observed in conjunction with loss of microbiota diversity (particularly anaerobes), a state
63 termed enterococcal dominance. ¹⁰ Exposure to a range of antibiotics increases susceptibility to VRE
64 intestinal colonisation and progression to high-level carriage and bacteraemia, although the effect of
65 individual antibiotics varies at each step of this sequence of events. ¹¹ The rate of progression from
66 carriage to invasive infection is also affected by the comorbidities of the patient population. ¹² Length
67 of stay, adherence to infection control procedures and proximity to VRE colonised patients or a
68 contaminated environment are additional modifiable factors that affect the risk of VRE
69 colonisation.¹³

70

71 A number of studies have identified risk factors for VRE bacteraemia, including haematological
72 malignancy, renal insufficiency, acute severity of illness, immunosuppression/neutropenia,
73 gastrointestinal disease or procedures, and modifiable factors such as antibiotic exposure. ¹⁴⁻²⁴

74 Vancomycin is the antibiotic most commonly implicated, but not all studies agree on its role. Lastly,
75 few studies have quantified the effect of cumulative exposure to individual antibiotics.^{15, 22, 23}

76

77 The aim of this study was to identify modifiable risk factors for VRE bacteraemia, in particular
78 antibiotic exposure, using a nested case-control study design in a centre with high rates of VRE
79 endemicity.

80

81 **METHODS**

82 **Study Setting, Design and Participants**

83 A retrospective matched nested case-control study was conducted amongst hospitalised patients at
84 Cambridge University Hospitals NHS Foundation Trust (CUH) in the United Kingdom (UK) from 1st
85 January 2006 to 31st December 2012. This tertiary referral teaching hospital has 1,170 beds, 340,000
86 occupied-bed-days per year, and a range of specialties including hepatology and hepatobiliary
87 surgery, solid organ transplantation (kidney, liver, pancreas and small bowel/multivisceral), adult
88 haematopoietic stem cell transplantation, paediatric haemato-oncology, and general and
89 neurocritical intensive care units (ICU). CUH has reported the highest number of VRE bacteraemias in
90 England in the national mandatory surveillance scheme from 2003-2012 (426/6246, or 7% of national
91 total out of 161 hospital Trusts). An active antimicrobial stewardship programme was in place
92 throughout the duration of the study, including prescribing guidelines and regular antimicrobial
93 rounds. Infection control practices targeting VRE did not change during the study period, however a
94 line care bundle was implemented during 2006 and a deep clean programme in 2007. Vancomycin
95 and teicoplanin susceptibility was determined by disk diffusion using British Society of Antimicrobial
96 Chemotherapy breakpoints ([http://www.bsac.org.uk/wp-content/uploads/2012/02/BSAC-](http://www.bsac.org.uk/wp-content/uploads/2012/02/BSAC-Susceptibility-testing-version-143.pdf)
97 [Susceptibility-testing-version-143.pdf](http://www.bsac.org.uk/wp-content/uploads/2012/02/BSAC-Susceptibility-testing-version-143.pdf)). Cases and controls were identified using the diagnostic
98 laboratory information system and the hospital electronic database, respectively. Cases were
99 consecutive inpatients with their first episode of VRE bacteraemia during the study period. Patients

100 with presumed contaminated blood cultures (single positive sets not necessitating the use of
101 targeted antibiotic therapy for symptom and bacteraemia resolution at the clinicians' discretion)
102 were excluded (Supplementary Table 1). Controls were matched to cases in a 1:1 ratio for the
103 following: (i) duration of stay (matched to cases based on time from admission to day that positive
104 blood culture was taken); (ii) year of admission; (iii) specialty; and (iv) ward type defined as general
105 adult, adult ICU, or paediatric ward. Specialty and ward type were treated as time-varying variables
106 and matched at the day of the bacteraemia. Matching for year of admission was chosen to minimise
107 potential confounding that may arise due to changes in antimicrobial prescribing or infection control
108 practices during the study period. Matching for specialty and ward type was used to account for
109 underlying comorbidities that predispose to VRE infections and for changes in local unit VRE
110 prevalence. Cases could serve as controls before becoming a case, and controls could serve as
111 controls more than once. ²⁵

112

113 **Covariates**

114 Demographic, epidemiological and clinical information were selected for inclusion based on a
115 literature review of risk factors for VRE bacteraemia, and extracted from paper and electronic patient
116 records. These included duration of hospital stay and prior ICU stay at CUH up to point of matching,
117 in-patient transfer from another hospital at the start of the current admission, and cumulative length
118 of stay in all wards and high-risk wards at CUH over the year prior to current admission. High-risk
119 wards were those associated in the literature with increased risk of VRE colonisation and invasive
120 disease (adult and paediatric haemato-oncology, solid organ transplant, nephrology, hepatology and
121 ICU). ²⁶ Mortality at 30 days was determined from hospital records or from an on-line national
122 database (NHS Spine). Usage data for all antibacterial and antifungal agents (including treatment and
123 prophylactic doses) was collected for 30 days prior to matching from paper local and referring
124 hospital records and drug charts. Cut-offs for duration of antimicrobial exposure were chosen at 3
125 days and 7 days in line with current antimicrobial stewardship recommendations where indication

126 for continuing antibiotics should be reviewed at 48-72 h and prolonged courses beyond one week are
127 discouraged in the absence of a clear indication.²⁷

128
129 Clinical parameters and comorbidities recorded at the time of matching included use of gastric acid
130 suppressing medication; presence of central venous catheter (CVC); neutropenia (neutrophil count
131 $<500 \times 10^6/L$); immunosuppression (other than neutropenia); hypoalbuminaemia (albumin <30 g/dL);
132 solid organ or haematological malignancy; solid organ or haematopoietic stem cell transplantation;
133 liver cirrhosis; gastrointestinal disease; and hepatobiliary disease. VRE carriage was defined as
134 growth of VRE from any clinical culture in the 12 months prior to matching. An additional list of
135 candidate variables and definitions is provided in Supplementary material.

136

137 **Statistical methods**

138 Our primary analysis examined the association between exposure to antimicrobials and the
139 subsequent development of VRE bacteraemia. We used univariable and multivariable logistic
140 regression models conditioned on the matched variable to estimate conditional odds ratios (cORs)
141 and 95% confidence intervals (CIs) for the association between independent factors and the
142 development of VRE bacteraemia. Variables were evaluated in a multivariable model if differences
143 between cases and controls on univariable analysis showed a *P* value less than 0.2. The final
144 multivariable model was built using Hosmer and Lemeshow's purposeful selection.²⁸ Statistical
145 analyses were performed using the Stata 12.1 software package (Stata Corp., USA).

146

147 **Ethics statement**

148 The study was approved by the local Research Ethics Committee (reference 13/EE/0044) and by the
149 CUH Research and Development Department (reference A092807).

150

151 **RESULTS**

152 We identified 295 patients with 331 episodes of VRE bacteraemia from 1st January 2006 to 31st
153 December 2012 (average annual incidence of 12.8/100,000 occupied-bed-days) (Figure 1). These
154 originated from a base of 218,223 patients that had 380,242 overnight admissions from 5th
155 November 2005 to 31st December 2012. Twelve patients could not be assessed due to missing paper
156 records and 38 were excluded as their positive blood cultures were deemed contaminants by the
157 treating doctors. Of the remaining 245 patients, 235 defined as cases were successfully matched to
158 220 controls. Eight cases also served as controls before becoming a case, and seven controls were
159 matched to cases more than once, resulting in 235 paired comparisons.

160

161 The demographic, clinical and microbiological characteristics of 235 cases and 220 controls are
162 shown in Table 1. Comparison between the two groups confirmed effective matching for age,
163 gender, speciality, ward type, year, and length of stay. Thirty cases were younger than 16 years, and
164 of the adult patients 55 (27%) were located in an ICU at the onset of infection. VRE bacteraemia
165 occurred in cases a median of 16 days following admission to CUH. *E. faecium* accounted for 91% and
166 the VanA phenotype (resistance to both vancomycin and teicoplanin) for 87% of bacteraemias. The
167 crude (all-cause) mortality at 30 days was higher in cases compared to controls (34% versus 13%).
168 Only 2 deaths occurred in the paediatric population, both of which were cases.

169

170 A univariable analysis was performed to identify risk factors associated with VRE bacteraemia (Table
171 2 and Supplementary Table 2). This demonstrated associations with the following: cumulative length
172 of stay on high-risk wards at CUH during the year preceding the current admission; in-patient
173 transfer from another hospital; gastric acid suppression therapy; presence of CVC; neutropenia; solid
174 organ tumour; severe renal failure; gastrointestinal disease; hepatobiliary disease; diabetes with end-
175 organ damage; and hypoalbuminaemia.

176

177 The univariable analysis also examined the association between VRE bacteraemia and antibiotic use
178 (Table 3 and Supplementary Table 2). Both groups had high rates of overall exposure to antibiotics in
179 the preceding 30 days, but cases received antibiotics more often and for longer durations. The
180 commonest antibiotics prescribed in both groups were intravenous (IV) vancomycin, meropenem,
181 fluoroquinolones, piperacillin-tazobactam and metronidazole. We found an association with VRE
182 bacteraemia for cumulative antibiotic duration over the prior 30 days, and for exposure to IV
183 vancomycin, meropenem, cephalosporins, fluoroquinolones, aminoglycosides, penicillins, and
184 antifungals. The duration of exposure to IV vancomycin, meropenem, fluoroquinolones,
185 cephalosporins, and antifungals was also associated with VRE bacteraemia.

186

187 Factors that were significant in the univariable analysis were then used in a multivariable analysis to
188 define independent risk factors for VRE bacteraemia (Table 4). After adjustment for comorbidities,
189 when compared to patients who did not receive any IV vancomycin, those exposed for 1-3 days, 4-7
190 days, or more than 7 days had a stepwise increase in risk for developing VRE bacteraemia (cOR of 1.2
191 (95% CI 0.4-3.8), 3.8 (95% CI 1.2-11.7), and 6.6 (95% CI 1.9-22.8), respectively). Similar stepwise
192 increases in cORs were observed for fluoroquinolones and meropenem. Additional risk factors
193 independently associated with an increased risk for VRE bacteraemia were: presence of CVC (cOR 8.7
194 [95% CI 2.6-29.5]); neutropenia (cOR 15.5 [95% CI 4.2-57.0]); hypoalbuminaemia (cOR 8.5 [95% CI
195 2.4-29.5]); solid organ tumour (cOR 4.4 [95% CI 1.6-12.0]); gastrointestinal disease (cOR 12.4 [95% CI
196 4.2-36.8]); and hepatobiliary disease (cOR 7.9 [95% CI 2.1-29.9]).

197

198 **DISCUSSION**

199 In this study, we found that receiving IV vancomycin, fluoroquinolones, or meropenem was each
200 associated with VRE bacteraemia. We also observed that the risk increased considerably when the
201 duration of antibiotic exposure was longer than 72 hours and 7 days for each of these three agents,
202 and that the effect was independent of other risk factors. To our knowledge, our study is the largest

203 to investigate risk factors for VRE bacteraemia and the first to have been performed in the UK, in a
204 setting with high levels of VRE endemicity similar to the situation in the United States. These results
205 not only demonstrate an association for these high-risk antibiotics, but also provide a clinically
206 important message, encouraging the discontinuation of these agents within 48-72 hours of initiation
207 when appropriate to minimise the risk of VRE bacteraemia.

208

209 The multivariable model identified previously reported markers of disease severity that predispose to
210 VRE bacteraemia (hypoalbuminaemia, neutropenia, and gastrointestinal disease).^{16, 18, 23} It also
211 identified hepatobiliary disease as an independent risk factor, which has not previously been
212 distinguished from gastrointestinal disease. These conditions are likely to predispose a colonised
213 patient to invasive disease through gut or biliary translocation and suggest that patients who develop
214 VRE bacteraemia represent a subgroup of patients with more significant comorbidities than matched
215 controls on same wards and specialties, irrespective of length of stay or nursing in ICU. The
216 association between a CVC and VRE bacteraemia has been reported previously, and could represent
217 a marker of severity of illness or a potential portal for infection.¹⁸

218

219 The role of vancomycin in promoting VRE acquisition is controversial and reported associations, or
220 lack thereof, could be explained by study design. A meta-analysis of early studies investigating the
221 role of vancomycin in hospital-acquired VRE colonisation or infection attributed strong associations
222 to confounding by length of stay, control group selection and publication bias.²⁹ This goes against
223 human experimental evidence where administration of glycopeptides orally led to gastrointestinal
224 selection of VRE.³⁰ Two studies of VRE bacteraemia using controls without enterococcal bacteraemia
225 have implicated vancomycin exposure as an independent risk factor,^{31, 32} but two further recent
226 studies with adequate sample size failed to demonstrate this effect.^{18, 23} Both of the latter studies
227 were conducted in Australia where vancomycin resistance was predominantly mediated by the *vanB*

228 operon. ⁶ This contrasts with the CUH and UK epidemiology where vancomycin resistance in VRE
229 bacteraemia is predominantly mediated by *vanA*. ^{33, 34}

230

231 Carbapenem use has only been implicated as an independent risk factor of VRE compared to VSE
232 bacteraemia in one published study. ¹⁴ However, since VRE was predominantly caused by ampicillin-
233 resistant VRE *faecium* and VSE by ampicillin-susceptible VSE *faecalis*, the effect could have been
234 previously overestimated. ¹⁴ This is particularly the case as imipenem, which was the carbapenem
235 used in this study, has higher efficacy against ampicillin-susceptible enterococci compared to other
236 carbapenems. Our study supports the independent association of carbapenem use (meropenem)
237 with VRE bacteraemia. Carbapenems have anaerobic activity which could promote VRE
238 colonisation.³⁵ A number of investigators have reported that antibiotics with anaerobic activity
239 predispose to VRE colonisation, ⁹ but definitions of this group of antibiotics have not been applied
240 consistently in the literature. Interestingly, piperacillin-tazobactam, an antibiotic with similar
241 spectrum of activity to meropenem including anaerobic, was not associated with VRE bacteraemia
242 here. This is consistent with murine experiments where administration of piperacillin-tazobactam
243 was protective against the establishment of high-level VRE colonisation, ³⁶ and with some
244 observational studies where antimicrobial stewardship interventions involving replacement of
245 cephalosporin use with piperacillin-tazobactam resulted in reduction of VRE colonisation. ¹¹
246 However, this effect was not noted by other investigators. ³⁷ It is possible that meropenem was
247 preferentially used in sicker patients in our study or that the lack of observed association with
248 piperacillin-tazobactam was due to insufficient power. The impact of switching therapy from
249 meropenem to piperacillin-tazobactam on the acquisition of VRE infection merits further
250 investigation.

251

252 Fluoroquinolone use has often been identified as a risk factor for VRE bacteraemia on univariable
253 analysis but not following adjustment for other factors. ^{14, 18, 21, 23} In a meta-analysis of ten studies

254 reported by Harbarth *et al.*,³⁸ fluoroquinolone use was associated with VRE colonisation or infection
255 (pooled OR 2.33, 95% CI 1.5-3.61). In a recent prospective observational study Sanchez-Diaz *et al.*
256 showed that long-term prophylaxis with levofloxacin in neutropenic haemato-oncology patients led
257 to intestinal overgrowth of hospital-adapted clones of *E. faecium*.³⁹

258

259 Placing our findings into the context of the published literature, longer courses of fluoroquinolones
260 and meropenem may promote gut colonisation with hospital-adapted strains of *E. faecium* (VSE or
261 VRE depending on local epidemiology). Plausibly, in settings where VRE is endemic, IV vancomycin
262 could shift the balance of the gut population and/or invasive isolates from VSE to VRE⁴⁰ thus
263 increasing the risk of VRE bacteraemia in susceptible patients. Gastrointestinal or hepatobiliary
264 insults or presence of a CVC could constitute portals of entry for the infection in heavily colonised
265 patients, particularly those with neutropenia.

266

267 This study has a number of limitations. It was conducted in a single centre with high endemicity of
268 VRE and so the findings may not apply to other settings, particularly those that utilise active
269 screening programmes for VRE. However, the infection control practices in our hospital are typical
270 for the UK and the study included patients from all high-risk groups including adult and paediatric
271 populations. Thirty-eight patients with positive blood cultures for VRE were excluded as
272 contaminants based on contemporaneous clinical assessment. These patients had a comparable 30-
273 day mortality to the control population (8%) and repeat blood cultures performed in 33 patients
274 (87%) were negative in the absence of treatment supporting their exclusion. Cases and controls
275 differed in the duration of prior hospitalisations both at CUH and elsewhere, and despite adjusting
276 for these in the model, there could be residual confounding. We did not adjust for the Charlson
277 comorbidity index as this score is not applicable to children, but analysed its individual components
278 instead. Also, we did not adjust for acute severity of illness using the Pitt bacteraemia or other
279 scores, as we could not ascertain whether the observed score was a cause or an effect of the

280 bacteraemia. We opted against using a case-case-control design which has been advocated for
281 studies of antibiotic resistance to enable distinction between risk factors predisposing to infection by
282 a particular organism as opposed to those specific to its resistance marker.⁴¹ This decision was made
283 because VRE bacteraemia tends to occur later in the course of hospitalisation than VSE bacteraemia,
284^{18, 23} so accounting for markers of hospital exposure related to length of stay would not have been
285 possible. Consequently, some of the findings should be interpreted as potentially predisposing to
286 both VSE and VRE bacteraemia rather than just VRE as explained above.

287

288 In conclusion, this study identified longer duration of exposure to vancomycin, fluoroquinolones or
289 meropenem as independent risk factors for VRE bacteraemia. Antibiotic formulary interventions
290 have not been proven to be effective in reducing VRE bacteraemia but are generally accepted as part
291 of a response to curb resistant pathogens, in addition to infection control interventions such as hand
292 washing and improved cleaning protocols.^{42, 43} This study suggests that targeting the use of a single
293 antibiotic in an endemic setting is unlikely to impact significantly on rates of VRE bacteraemia.
294 Instead, a combination of approaches including antimicrobial stewardship focusing on limiting the
295 duration of high-risk antibiotics in addition to infection control interventions would be required to
296 curb the rates of VRE bacteraemia.

297

298 **ACKNOWLEDGEMENTS**

299 We thank Lois Chaparadza for assistance with data retrieval and Carol Churcher for administrative
300 support.

301

302 **FUNDING**

303 TG is a Wellcome Trust Research Training Fellow (grant number: 103387/Z/13/Z) and has received
304 support by Public Health England. BW is an Academic Clinical Fellow supported by the National
305 Institute for Health Research. MET is a Clinician Scientist Fellow supported by the Academy of

306 Medical Sciences and the Health Foundation, and is also supported by the National Institute for
307 Health Research Cambridge Biomedical Research Centre. This work was also supported by the Health
308 Innovation Challenge Fund (WT098600, HICF-T5-342), a parallel funding partnership between the
309 Department of Health and Wellcome Trust. The views expressed in this publication are those of the
310 author(s) and not necessarily those of the Department of Health or Wellcome Trust.

311

312 **TRANSPARENCY DECLARATIONS**

313 The authors declare no conflicts of interest.

314

315 **References**

- 316 1. Werner G, Coque TM, Hammerum AM *et al.* Emergence and spread of vancomycin resistance
317 among enterococci in Europe. *Euro Surveill* 2008; **13**: 1-11.
- 318 2. Lebreton F, van Schaik W, McGuire AM *et al.* Emergence of epidemic multidrug-resistant
319 *Enterococcus faecium*. *mBio* 2013; **4**: e00534-13.
- 320 3. Butler AM, Olsen MA, Merz LR *et al.* Attributable costs of enterococcal bloodstream
321 infections in a nonsurgical hospital cohort. *Infect Control Hosp Epidemiol* 2010; **31**: 28-35.
- 322 4. Prematunge C, MacDougall C, Johnstone J *et al.* VRE and VSE bacteremia outcomes in the era
323 of effective VRE therapy: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2016;
324 **37**: 26-35.
- 325 5. Gouliouris T, Peacock SJ. The rise and fall of mandatory surveillance for glycopeptide-
326 resistant enterococcal bacteraemia in England. *J Infect* 2014; **68**: 401-3.
- 327 6. Howden BP, Holt KE, Lam MMC *et al.* Genomic insights to control the emergence of
328 vancomycin-resistant enterococci. *mBio* 2013; **4**: e:00412-13.
- 329 7. Sievert DM, Ricks PP, Edwards JR *et al.* Antimicrobial-resistant pathogens associated with
330 healthcare-associated infections: summary of data reported to the National Healthcare Safety
331 Network at the Centers for Disease Control and Prevention, 2009–2010. *Infect Control Hosp*
332 *Epidemiol* 2013; **34**: 1-14.
- 333 8. Gastmeier P, Schröder C, Behnke M *et al.* Dramatic increase in vancomycin-resistant
334 enterococci in Germany. *J Antimicrob Chemother* 2014; **69**: 1660-4.
- 335 9. Donskey CJ, Chowdhry TK, Hecker MT *et al.* Effect of antibiotic therapy in the density of
336 vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med* 2000; **343**: 1925-
337 32.
- 338 10. Taur Y, Xavier JB, Lipuma L *et al.* Intestinal domination and the risk of bacteremia in patients
339 undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2012; **55**: 905-14.

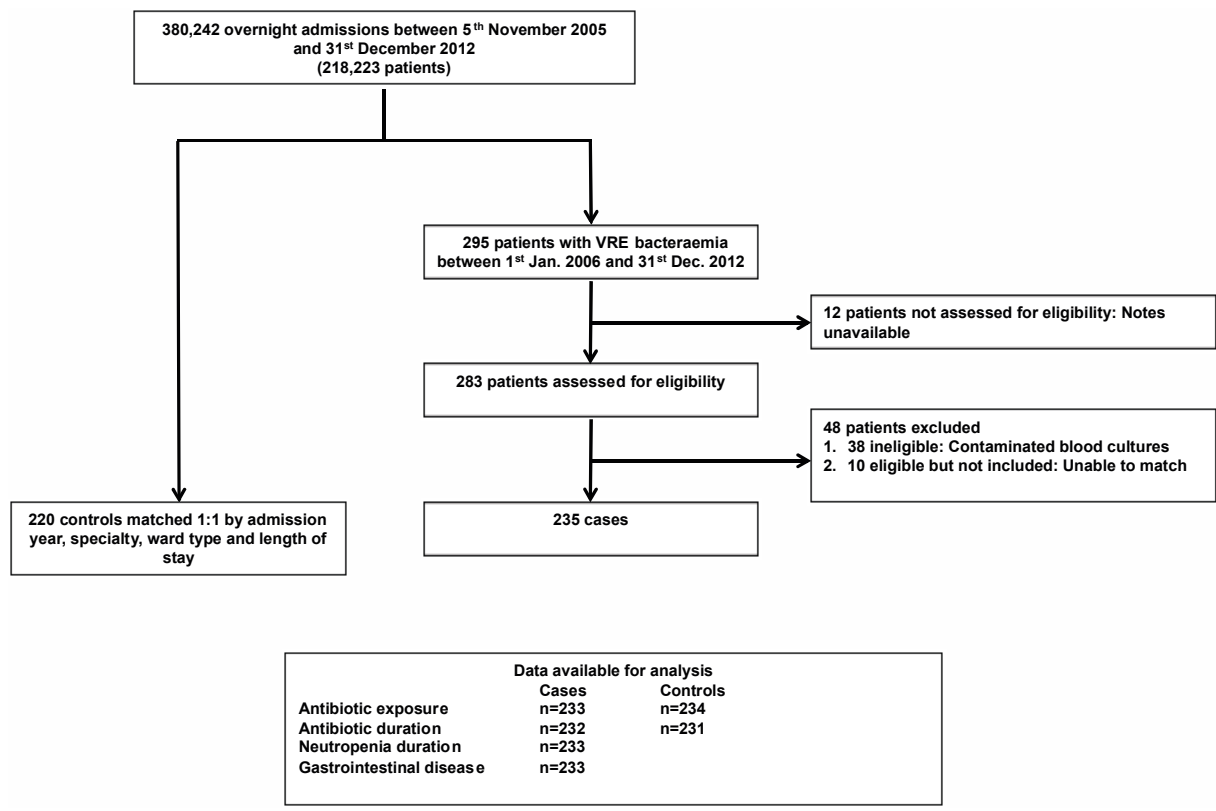
- 340 11. Patel R. Clinical impact of vancomycin-resistant enterococci. *J Antimicrob Chemother* 2003;
341 **51**: 13iii-21.
- 342 12. Salgado CD. The risk of developing a vancomycin-resistant *Enterococcus* bloodstream
343 infection for colonized patients. *Am J Infect Control* 2008; **36**: S175.e5-8.
- 344 13. Bonten MJM, Slaughter S, Amberg AW *et al*. The Role of “colonization pressure” in the
345 spread of vancomycin-resistant enterococci. *Arch Intern Med* 1998; **158**: 1127-32.
- 346 14. Bhavnani SM, Drake JA, Forrest A *et al*. A nationwide, multicenter, case-control study
347 comparing risk factors, treatment, and outcome for vancomycin-resistant and -susceptible
348 enterococcal bacteremia. *Diagn Microbiol Infect Dis* 2000; **36**: 145-58.
- 349 15. Haas EJ, Zaoutis TE, Prasad P *et al*. Risk factors and outcomes for vancomycin-resistant
350 *Enterococcus* bloodstream infection in children. *Infect Control Hosp Epidemiol* 2010; **31**: 1038-42.
- 351 16. Kang Y, Vicente M, Parsad S *et al*. Evaluation of risk factors for vancomycin-resistant
352 *Enterococcus* bacteremia among previously colonized hematopoietic stem cell transplant patients.
353 *Transpl Infect Dis* 2013; **15**: 466-73.
- 354 17. Lautenbach E, Bilker WB, Brennan PJ. Enterococcal bacteremia: risk factors for vancomycin
355 resistance and predictors of mortality. *Infect Control Hosp Epidemiol* 1999; **20**: 318-23.
- 356 18. Peel T, Cheng AC, Spelman T *et al*. Differing risk factors for vancomycin-resistant and
357 vancomycin-sensitive enterococcal bacteraemia. *Clin Microbiol Infect* 2012; **18**: 388-94.
- 358 19. Peset V, Tallón P, Sola C *et al*. Epidemiological, microbiological, clinical, and prognostic
359 factors of bacteremia caused by high-level vancomycin-resistant *Enterococcus* species. *Eur J Clin*
360 *Microbiol Infect Dis* 2000; **19**: 742-9.
- 361 20. Shay DK, Maloney SA, Montecalvo M *et al*. Epidemiology and mortality risk of vancomycin-
362 resistant enterococcal bloodstream infections. *J Infect Dis* 1995; **172**: 993-1000.
- 363 21. Vergis EN, Hayden MK, Chow JW *et al*. Determinants of vancomycin resistance and mortality
364 rates in enterococcal bacteremia. *Ann Intern Med* 2001; **135**: 484-92.

- 365 22. Zaas AK, Song X, Tucker P *et al.* Risk factors for development of vancomycin-resistant
366 enterococcal bloodstream infection in patients with cancer who are colonized with vancomycin-
367 resistant enterococci. *Clin Infect Dis* 2002; **35**: 1139-46.
- 368 23. Cheah AL, Peel T, Howden BP *et al.* Case-case-control study on factors associated with vanB
369 vancomycin-resistant and vancomycin-susceptible enterococcal bacteraemia. *BMC Infect Dis* 2014;
370 **14**: 353.
- 371 24. Montecalvo MA, Horowitz H, Gedris C *et al.* Outbreak of vancomycin- , ampicillin- , and
372 aminoglycoside- resistant *Enterococcus faecium* bacteremia in an adult oncology unit. *Antimicrob*
373 *Agents Chemother* 1994; **38**: 1363-7.
- 374 25. Essebag V, Genest J, Jr., Suissa S *et al.* The nested case-control study in cardiology. *Am Heart*
375 *J* 2003; **146**: 581-90.
- 376 26. Cookson BD, Macrae MB, Barrett SP *et al.* Guidelines for the control of glycopeptide-resistant
377 enterococci in hospitals. *J Hosp Infect* 2006; **62**: 6-21.
- 378 27. Ashiru-Oredope D, Sharland M, Charani E *et al.* Improving the quality of antibiotic prescribing
379 in the NHS by developing a new Antimicrobial Stewardship Programme: Start Smart-Then Focus. *J*
380 *Antimicrob Chemother* 2012; **67 Suppl 1**: i51-63.
- 381 28. Hosmer DL, S. *Applied logistic regression*. New York: Wiley, 2000.
- 382 29. Carmeli Y, Samore MH, Huskins C. The association between antecedent vancomycin
383 treatment and hospital-acquired vancomycin-resistant enterococci: a meta-analysis. *Arch Intern Med*
384 1999; **159**: 2461-8.
- 385 30. Van der Auwera P, Pensart N, Korten V *et al.* Influence of oral glycopeptides on the fecal flora
386 of human volunteers: selection of highly glycopeptide-resistant enterococci. *J Infect Dis* 1996; **173**:
387 1129-36.
- 388 31. Furtado GHC, Mendes RE, Pignatari ACC *et al.* Risk factors for vancomycin-resistant
389 *Enterococcus faecalis* bacteremia in hospitalized patients: an analysis of two case-control studies. *Am*
390 *J Infect Control* 2006; **34**: 447-51.

- 391 32. Worth LJ, Thursky KA, Seymour JF *et al.* Vancomycin-resistant *Enterococcus faecium* infection
392 in patients with hematologic malignancy: patients with acute myeloid leukemia are at high-risk. *Eur J*
393 *Haematol* 2007; **79**: 226-33.
- 394 33. Raven KE, Reuter S, Gouliouris T *et al.* Genome-based characterization of hospital-adapted
395 *Enterococcus faecalis* lineages. *Nat Microbiol* 2016; **1**: pii:15033.
- 396 34. Raven KE, Gouliouris T, Brodrick H *et al.* Complex routes of nosocomial vancomycin-resistant
397 *Enterococcus faecium* transmission revealed by genome sequencing. *Clin Infect Dis* 2017; **64**: 886-93.
- 398 35. Padiglione AA, Wolfe R, Grabsch E *et al.* Risk factors for new detection of vancomycin-
399 resistant enterococci in acute-care hospitals that employ strict infection control procedures.
400 *Antimicrob Agents Chemother* 2003; **47**: 2492-8.
- 401 36. Rice LB. The complex dynamics of antimicrobial activity in the human gastrointestinal tract.
402 *Trans Am Clin Climatol Assoc* 2013; **124**: 123-32.
- 403 37. Paterson DL, Muto CA, Ndirangu M *et al.* Acquisition of rectal colonization by vancomycin-
404 resistant *Enterococcus* among intensive care unit patients treated with piperacillin-tazobactam
405 versus those Receiving cefepime-containing antibiotic regimens. *Antimicrob Agents Chemother* 2008;
406 **52**: 465-9.
- 407 38. Harbarth S, Cosgrove S, Carmeli Y. Effects of antibiotics on nosocomial epidemiology of
408 vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 2002; **46**: 1619-28.
- 409 39. Sánchez-Díaz AM, Cuartero C, Rodríguez JD *et al.* The rise of ampicillin-resistant *Enterococcus*
410 *faecium* high-risk clones as a frequent intestinal colonizer in oncohaematologic neutropenic patients
411 on levofloxacin prophylaxis: a risk for bacteraemia? *Clin Microbiol Infect* 2015; **22**: 59.e1-.e8.
- 412 40. Moradigaravand D, Gouliouris T, Blane B *et al.* Within-host evolution of *Enterococcus faecium*
413 during longitudinal carriage and transition to bloodstream infection in immunocompromised
414 patients. *Genome Med* 2017; **9**: 119.

- 415 41. Kaye KS, Harris AD, Samore M *et al.* The case-case-control study design: addressing the
416 limitations of risk factor studies for antimicrobial resistance. *Infect Control Hosp Epidemiol* 2005; **26**:
417 346-51.
- 418 42. De Angelis G, Cataldo MA, De Waure C *et al.* Infection control and prevention measures to
419 reduce the spread of vancomycin-resistant enterococci in hospitalized patients: a systematic review
420 and meta-analysis. *J Antimicrob Chemother* 2014; **69**: 1185-92.
- 421 43. Anderson DJ, Chen LF, Weber DJ *et al.* Enhanced terminal room disinfection and acquisition
422 and infection caused by multidrug-resistant organisms and *Clostridium difficile* (the Benefits of
423 Enhanced Terminal Room Disinfection study): a cluster-randomised, multicentre, crossover study.
424 *Lancet* 2017; **389**: 805-14.
- 425

426 **Figure 1. Selection of study population for nested case-control comparison of risk factors for**
 427 **vancomycin-resistant enterococcal (VRE) bacteraemia.**



428
 429

430 **Table 1. Demographic, clinical and microbiological characteristics of cases and controls**

Characteristic	Cases: Patients with VRE bacteraemia (n=235)	Controls: Patients without VRE bacteraemia (n=220)	P value
Age (years), median (IQR)	56.6 (39.0-66.7)	57.8 (42.2-69.5)	0.34
Male	145 (61.7)	127 (57.7)	0.47
Year of admission			
2005	2 (0.9)	2 (0.9)	N/A
2006	34 (14.5)	33 (15.0)	
2007	42 (17.9)	39 (17.7)	
2008	30 (12.8)	29 (13.2)	
2009	39 (16.6)	33 (15.0)	
2010	31 (13.2)	29 (13.2)	
2011	25 (10.6)	25 (11.4)	
2012	32 (13.6)	30 (13.6)	
Ward at time of bacteraemia			
Adult general	150 (63.8)	141 (64.1)	N/A
Adult ICU	55 (23.4)	53 (24.1)	
Paediatric	30 (12.8)	26 (11.8)	
Time from admission to bacteraemia (cases) and matching (controls) (days), median (IQR) ^a	16 (9-31)	16 (8-31)	N/A
Lead specialty type at time of matching ^b			
Adult haematology	67 (28.5)	57 (25.9)	N/A
Adult oncology	4 (1.7)	4 (1.7)	
Adult medicine	66 (28.1)	65 (29.6)	
Adult solid organ transplant	31 (13.2)	31 (14.1)	
Adult surgery	37 (15.7)	37 (16.8)	
Paediatric haemato-oncology	30 (12.8)	26 (11.8)	
<i>Enterococcus faecalis</i>	17 (7.2)		
<i>Enterococcus faecium</i>	214 (91.1)		
Other enterococcal species ^c	4 (1.7)		
VanA	202/232 (87.1)		
Death within 30 days of matching	79 (33.6)	28 (12.7)	<0.001 ^d

431

432 Data are presented as number (%) unless indicated otherwise.

433 Abbreviations: ICU, intensive care unit; IQR, interquartile rate; VRE, vancomycin-resistant
 434 enterococcus.

435 N/A, not applicable: these variables were used to match cases and controls.

436 ^a The onset of bacteraemia for 27 of 235 cases was within 2 days post hospital admission; for 25 of 27
437 of these cases, there was healthcare contact in the preceding 3 months.

438 ^b Lead specialties included 18 different options used for matching, grouped under six categories in
439 this table. For full list of specialties see Supplementary material.

440 ^c Other species were *Enterococcus raffinosus* (1), mixed *E. faecalis* and *E. faecium* (1), mixed *E.*
441 *faecium* and *E. raffinosus* (1), and one unspciated. Both *E. raffinosus* isolates were phenotypically
442 VanA.

443 ^d Fisher's exact test

Table 2: Risk factors for vancomycin-resistant enterococcal bacteraemia

Variable	Cases (n=235)	Controls (n=235)	Crude cOR (95% CI)	P value
Comorbidities				
Solid organ tumour	60 (25.5)	42 (17.9)	1.7 (1.0-2.8)	0.03
Haematological malignancy	94 (40.0)	86 (36.6)	1.6 (0.8-3.2)	0.17
Neutropenia	89 (37.9)	38 (16.2)	6.7 (3.3-13.4)	<0.001
Severe renal failure on admission	15 (6.4)	6 (2.6)	3.3 (1.1-10.0)	0.04
Liver cirrhosis	29 (12.3)	24 (10.2)	2.0 (0.7-5.9)	0.21
Gastrointestinal disease	67/233 (28.8)	25 (10.6)	4.6 (2.5-8.6)	<0.001
Hepatobiliary disease	41 (17.5)	22 (9.4)	3.4 (1.5-7.4)	0.003
Diabetes (with end-organ damage)	16 (6.8)	5 (2.1)	4.7 (1.3-16.2)	0.02
Hypoalbuminaemia	209 (88.9)	150 (63.8)	8.4 (4.0-17.4)	<0.001
Clinical exposures				
Gastric acid suppression	203 (86.4)	183 (77.9)	1.9 (1.1-3.2)	0.01
Central venous catheter	197 (83.8)	153 (65.1)	5.4 (2.8- 10.6)	<0.001
Immunosuppression (other than neutropenia)	146 (62.1)	143 (60.9)	1.2 (0.6-2.1)	0.65
Abdominal procedures	85 (36.2)	71 (30.2)	1.6 (1.0-2.8)	0.07
Prior microbiology				
VRE grown from clinical sample within 1 year prior to matching	38 (16.2)	25 (10.6)	1.6 (0.9-2.6)	0.09
Hospital exposure				
Cumulative length of stay at CUH within 1 year of current admission (all wards)				
0 days	88 (37.4)	101 (43.0)	1.0	0.16
1-14 days	43 (18.3)	49 (20.9)	1.1 (0.6-1.8)	
>14 days	104 (44.3)	85 (36.2)	1.5 (1.0-2.4)	
Cumulative length of stay at CUH within 1 year of current admission (high-risk wards) ^a				
0 days	121 (51.5)	142 (60.4)	1.0	0.05
1-14 days	33 (14.0)	30 (12.8)	1.5 (0.8-2.7)	
>14 days	81 (34.5)	63 (26.8)	1.8 (1.1-3.0)	
ICU stay current admission	87 (37.0)	77 (32.8)	1.6 (0.9-2.9)	0.14
Transfer from another hospital	65 (27.7)	44 (18.7)	1.7 (1.1-2.6)	0.02

Data are presented as number (%) of patients unless indicated otherwise.

Abbreviations: CI, confidence intervals; cOR, conditional odds ratio; CUH, Cambridge University Hospitals; ICU, intensive care unit; IQR, interquartile rate; IV, intravenous; PO, per os (oral administration); VRE, vancomycin-resistant enterococcus.

^a High-risk wards included: adult and paediatric haemato-oncology, solid organ transplant, nephrology, hepatology and ICU.

Table 3: Association between antimicrobial exposure and vancomycin-resistant enterococcal bacteraemia

Antimicrobial		Cases (n=235)	Controls (n=235)	Crude cOR (95% CI)	P value
Vancomycin (IV)		169/233 (72.5)	123/234 (52.6)	3.3 (2.0-5.4)	<0.001
Vancomycin (PO)		10/233 (4.3)	8/234 (3.4)	1.3 (0.5-3.2)	0.64
Cephalosporins		33/233 (14.2)	18/234 (7.7)	2.2 (1.1-4.2)	0.02
Fluoroquinolones		144/234 (61.5)	100/234 (42.7)	2.5 (1.6-3.8)	<0.001
Amoxicillin-clavulanic acid		36/233 (15.5)	31/234 (13.3)	1.2 (0.7-2.1)	0.49
Piperacillin-tazobactam		74/233 (31.8)	63/234 (26.9)	1.4 (0.8-2.3)	0.21
Meropenem		157/233 (67.4)	109 (46.4)	2.8 (1.8- 4.3)	<0.001
Metronidazole		70/234 (29.9)	54/234 (23.1)	1.5 (1.00-2.4)	0.07
Aminoglycosides		53/233 (22.8)	35/234 (15.0)	2.00 (1.1-3.3)	0.02
Penicillins		50/233 (21.5)	36/234 (15.4)	1.8 (1.0-3.0)	0.05
Macrolides		31/233 (13.3)	42 (17.9)	0.7 (0.4-1.2)	0.16
Antifungals		167/233 (71.7)	128 (54.5)	3.0 (1.8-5.1)	<0.001
Antimicrobial Duration					
Vancomycin IV duration	None	64/233 (27.5)	111/234 (47.4)	1.0	<0.001
	1 to 3 days	28/233 (12.0)	40/234 (17.1)	1.5 (0.8-2.9)	
	4 to 7 days	49/233 (21.0)	37/234 (15.8)	3.2 (1.6-6.0)	
	>7 days	92/233 (39.5)	46/234 (19.7)	5.4 (2.9-10.0)	
Fluoroquinolone duration	None	90/232 (38.8)	134/233 (57.5)	1.0	<0.001
	1 to 3 days	32/232 (13.8)	27/233 (11.6)	2.0 (1.1-3.7)	
	4 to 7 days	39/232 (16.8)	25/233 (10.7)	2.6 (1.4-4.8)	
	>7 days	71/232 (30.6)	47/233 (20.2)	2.7 (1.6-4.7)	
Meropenem duration	None	76/233 (32.6)	126/231 (54.6)	1.0	<0.001
	1 to 3 days	27/233 (11.6)	18/231 (7.8)	2.6 (1.3-5.4)	
	4 to 7 days	39/233 (16.7)	35/231 (15.2)	2.1 (1.2-3.8)	
	>7 days	91/233 (39.1)	52/231 (22.5)	4.3 (2.4-7.7)	

Data are presented as number (%) of patients unless indicated otherwise.

Abbreviations: CI, confidence intervals; cOR, conditional odds ratio; CUH, Cambridge University Hospitals; ICU, intensive care unit; IQR, interquartile rate; IV, intravenous; PO, per os (oral administration); VRE, vancomycin-resistant enterococcus.

Table 4: Independent risk factors associated with vancomycin-resistant enterococcal bacteraemia

Variables		Adjusted cOR (95% CI)	P value
Vancomycin IV duration (days)	None	1.0	0.004
	1 to 3	1.2 (0.4-3.8)	
	4 to 7	3.8 (1.2-11.7)	
	>7	6.6 (1.9-22.8)	
Fluoroquinolone duration (days)	None	1.0	<0.001
	1 to 3	1.3 (0.4-3.7)	
	4 to 7	4.5 (1.6-12.9)	
	>7	6.9 (2.4-20.0)	
Meropenem duration (days)	None	1.0	0.03
	1 to 3	1.8 (0.5-6.4)	
	4 to 7	2.3 (0.8-6.3)	
	>7	3.5 (1.3-10.0)	
Central venous catheter		8.7 (2.6-29.5)	0.001
Neutropenia		15.5 (4.2-57.0)	<0.001
Hypoalbuminaemia		8.5 (2.4-29.5)	0.001
Solid organ tumour		4.4 (1.6-12.0)	0.003
Hepatobiliary disease		7.9 (2.1-29.9)	0.002
Gastrointestinal disease		12.4 (4.2-36.8)	<0.001

Note: Only risk factors found to be statistically significant on multivariable analysis are shown.

Abbreviations: CI, confidence interval; cOR, conditional odds ratio; IV, intravenous.