



HAL
open science

Investigation of a vanA linezolid- and vancomycin-resistant *Enterococcus faecium* outbreak in the Southwest Indian Ocean (Reunion Island)

L. Kamus, G. Auger, K. Gambarotto, J. Houivet, M. Ramiandrisoa, S. Picot, N. Lugagne-Delpon, M.C. Jaffar Bandjee, A. Zouari, Aurélien Birer, et al.

► To cite this version:

L. Kamus, G. Auger, K. Gambarotto, J. Houivet, M. Ramiandrisoa, et al.. Investigation of a vanA linezolid- and vancomycin-resistant *Enterococcus faecium* outbreak in the Southwest Indian Ocean (Reunion Island). *International Journal of Antimicrobial Agents*, 2022, 60 (5-6), pp.106686. 10.1016/j.ijantimicag.2022.106686 . hal-03932362

HAL Id: hal-03932362

<https://hal.science/hal-03932362v1>

Submitted on 19 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Short Communication

Investigation of a *vanA* linezolid- and vancomycin-resistant *Enterococcus faecium* outbreak in the Southwest Indian Ocean (Reunion Island)

Laure Kamus^{a,#}, Gabriel Auger^{b,#}, Karine Gambarotto^c, Julien Houivet^c, Mahery Ramiandrisoa^d, Sandrine Picot^e, Nathalie Lugagne-Delpon^c, Marie-Christine Jaffar-Bandjee^a, Asma Zouari^b, Aurélien Birer^b, Sophie Nogues^b, Anaïs Collet^b, Maxime Lecourt^b, Olivier Belmonte^a, Vincent Cattoir^{b,#}, Guillaume Miltgen^{a,f,g,#,*}

^a Laboratoire de Bactériologie, CHU Félix Guyon, Saint-Denis, La Réunion, France

^b Laboratoire de Bactériologie, CHU Pontchaillou, Rennes, France. CNR de la Résistance aux antibiotiques (laboratoire associé « Entérocoques »)

^c Service d'hygiène hospitalière, CHU Félix Guyon, Saint-Denis, La Réunion, France

^d Laboratoires Cerballiance, Le Port, La Réunion, France

^e Laboratoire de Microbiologie, CHU Sud Réunion, Saint-Pierre, La Réunion, France

^f UMR Processus Infectieux en Milieu Insulaire Tropical (PIMIT), CNRS 9192, INSERM U1187, IRD 249, Université de La Réunion, Saint-Denis, La Réunion, France

^g Centre Régional en Antibiothérapie (CRAtb) de La Réunion



ARTICLE INFO

Article history:

Received 29 June 2022

Accepted 17 October 2022

Editor: Professor Geoffrey Coombs

Keywords:

Enterococcus faecium

Linezolid and vancomycin resistance

Outbreak

vanA

optrA

ST761

Reunion Island

ABSTRACT

Introduction: Dual resistance to linezolid and glycopeptides is a milestone reached by certain extensively drug-resistant (XDR) enterococci. This paper describes the molecular and epidemiological investigations of a linezolid-resistant and vancomycin-resistant *Enterococcus faecium* (*E. faecium*) (LVREf) outbreak in the French overseas territory of Reunion Island (Indian Ocean).

Methods: All vancomycin-resistant *Enterococcus* (VRE) isolates detected on Reunion Island between 2015 and 2019 were included in the study. The VRE isolates were phenotypically characterised and genetically explored by whole-genome sequencing (WGS).

Results: Sixteen vancomycin-resistant *E. faecium* (VREf) isolates were retrieved between 2015 and 2019. Seven isolates obtained in 2019 were involved in the outbreak. These seven LVREf isolates from the 2019 outbreak at the University Hospital of Reunion Island (UHRI) were suspected to be related to a linezolid-susceptible VREf strain imported from India. An epidemiological link was highlighted for six of the seven outbreak cases. All the LVREf outbreak isolates were obtained from rectal swabs (colonisation) and resistant to vancomycin (MIC > 128 mg/L) and linezolid (MIC 8–32 mg/L); one isolate was also resistant to daptomycin (MIC 8 mg/L). The seven outbreak isolates were positive for the *vanA* and *optrA* genes and belonged to ST761.

Conclusions: These results argue for the strict application of control and prevention measures for VRE clones at high risk of spread, particularly in areas such as Reunion Island where the risk of importation from the Indian subcontinent is high. The regional spread of *optrA* linezolid-resistance genes in VRE isolates is a matter of concern, due to possibility of treatment failure.

© 2022 The Author(s). Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

* Corresponding author: Laboratoire de Bactériologie, CHU Félix Guyon, Allée des Topazes, 97400 Saint-Denis, La Réunion, France.

E-mail address: guillaume.miltgen@chu-reunion.fr (G. Miltgen).

These authors contributed equally to the study

1. Introduction

Enterococci are facultative, anaerobic, Gram-positive, commensal bacteria of the human and animal intestinal microbiota; they are a leading cause of nosocomial infections like urinary tract/intra-abdominal infections, bacteraemia and endocarditis. Un-

der the selection pressure exerted by antibiotics, vancomycin-resistant enterococci (VRE) have increasingly been reported worldwide during the last two decades [1,2]. These multidrug-resistant (MDR) pathogens – especially *vanA*-positive *Enterococcus faecium* (*E. faecium*) – can cause nosocomial outbreaks associated with higher costs and healthcare-associated morbidity and mortality [3,4]. There are very few alternatives to vancomycin for treating enterococcal infections, the principal options being linezolid (oxazolidinone) or daptomycin (cyclic lipopeptide) [5,6]. Dual resistance to both vancomycin and linezolid has recently been described in some clinical isolates of *E. faecium*, constituting a milestone in terms of the therapeutic impasse for extensively drug-resistant (XDR) Gram-positive bacteria [7–10]. The mechanisms of resistance to vancomycin are mostly associated with the *vanA* or *vanB* genes in clinical isolates from Europe, North America, Africa and Asia [11,12]. Several mechanisms of acquired linezolid resistance have been described in enterococci, including chromosomal mutations, mostly affecting the 23S rDNA subunit (especially the G2576T substitution), and the acquisition of transferable genes encoding oxazolidinone resistance (*i.e.* *cfr*-like, *optrA* and *poxtA*) [13].

Reunion Island is a French overseas territory with 850 000 inhabitants located in the Southwest Indian Ocean Area, close to Madagascar. It provides healthcare facilities similar to those on offer in mainland France, with the University Hospital of Reunion Island (UHRI) considered the reference hospital in the Southwest Indian Ocean Area. At the crossroads of the Indian subcontinent and Southern Africa, this island is exposed to multiple and repeated importations of XDR bacteria [14,15]. The first linezolid-resistant and vancomycin-resistant *E. faecium* (LVREf) isolates in the region were described in India in 2015, and co-resistance mediated by the *optrA* gene has recently been reported at a hospital in Vellore (Tamil Nadu, Southern India) [16,17]. Vancomycin-resistant *E. faecium* has been described in Southern Africa, but no LVREf isolates have yet been reported on this continent [18,19].

This study describes the first outbreak caused by an LVREf clone in a French territory, which occurred at the UHRI. It reports the epidemiological and molecular investigation of the spread of this LVREf clone.

2. Materials and Methods

2.1. Description and investigation of the outbreak

2.1.1. Outbreak detection

The UHRI is a two-site, 1800-bed facility managing 175 000 days of hospitalisation and 315 000 outpatient consultations per year. At the end of January 2019, two VREf isolates were simultaneously detected in the general medicine department (GMD) of the UHRI, leading to suspicion of an outbreak. A multidisciplinary team was set up due to the high risk of spread and low frequency of VRE on Reunion Island. In accordance with French guidelines, the UHRI imposed isolation precautions and screening of all patients, on admission to the UHRI, from foreign countries or territories (including Mayotte Island) or recently hospitalised in another healthcare facility [20]. A 'case' was defined as a patient colonised or infected with a VRE strain. The outbreak was managed in accordance with the French High Council for Public Health (French acronym: HCSP) recommendations for preventing the spread of VRE, with contacts classified as being of high, medium or low risk [21]. Contacts underwent rectal swab screening on days 0, 5 and 10 after exposure.

2.1.2. Data collection

This study included all the isolates obtained during the outbreak, together with all the VREf isolates detected on Reunion Island between 2015 (first detection on this island) and 2019. These

isolates were included retrospectively, based on data from the regional antimicrobial resistance surveillance system of Reunion Island (French acronym: CPIAS-FELIN), which collects XDR bacteria (VRE and carbapenemase-producing Enterobacterales) from the four public hospitals and the private clinics on the island [22]. The following demographic and clinical data were collected for each patient concerned for each strain: age, gender, comorbid conditions, site at which the isolate was obtained, ward of hospitalisation, links to foreign countries, antibiotic treatment in the three months preceding VRE isolation and clinical outcome.

2.2. Microbiological analysis

2.2.1. Bacterial isolates

All rectal swabs were cultured on selective ChromID VRE agar (bioMérieux, Marcy l'Étoile, France) under ambient air at 35 ± 2°C and observed after 24 h and 48 h of incubation, according to the manufacturer's instructions. During the outbreak, contacts were also screened by performing the Xpert *vanA/vanB* assay (GeneXpert; Cepheid, Röntgenvägen, Sweden) directly on rectal swabs, in accordance with the manufacturer's recommendations. *Enterococcus faecium* strains were initially identified by MALDI-TOF mass spectrometry (Microflex; Bruker Daltonics, Bremen, Germany) and this identification was then confirmed by whole-genome sequencing (WGS).

2.2.2. Phenotypic and genotypic characterisation

Antimicrobial susceptibility testing was performed on each isolate by the disk diffusion method. The MICs of vancomycin, teicoplanin, linezolid, tedizolid, daptomycin and tigecycline were determined by the broth microdilution reference method (Sensititre; ThermoFisher Scientific, Courtaboeuf, France). Results were interpreted according to the 2020 EUCAST breakpoints [23].

Isolates were tested for the presence of the *vanA* and *vanB* genes by performing the Xpert *vanA/vanB* assay on colonies, in accordance with the manufacturer's recommendations. All strains were then sent to the French National Reference Centre for Enterococci (FNRC-Enc) for confirmation and additional genotypic analyses. At the FNRC-Enc, the presence of the *van* operon and of acquired linezolid resistance genes (*cfr*-like, *optrA* and *poxtA*) was investigated with specific real-time PCR assays, as previously described [24].

2.3. Whole genome sequencing and bioinformatic analysis

Genomic DNA was isolated with the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA). The DNA libraries were prepared with the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) and sequenced as paired end reads (2 × 300 bp) on an Illumina MiSeq platform with the MiSeq Reagent Kit version 3. The Illumina reads were assembled with SPAdes v3.14.0 software [25] and the assembly was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (www.ncbi.nlm.nih.gov/genome/annotation_prok/). The MLST was performed with the MLST database (<https://pubmlst.org/>), and raw reads were also submitted to the ResFinder 4.1 and LRE-Finder 1.0 servers (<https://cge.cbs.dtu.dk>) for the identification of acquired resistance genes and chromosomal resistance mutations [26,27]. A phylogenetic analysis based on single-nucleotide polymorphisms (SNPs) was performed with Snippy (<https://github.com/tseemann/snippy>) on a reference core genome MLST (<https://www.cgmlst.org/ncs/schema/991893/>). The resulting filtered SNPs were transformed into a distance matrix for tree construction with the neighbour-joining algorithm. The phylogenetic tree and a heatmap for resistance mechanisms were visualised using iTOL v5 (<https://itol.embl.de/>) [28]. The genomic sequences of the 16 VREf isolates

Table 1Description of the vancomycin-resistant *Enterococcus faecium* (*E. faecium*) isolates in colonised or infected patients identified on Reunion Island, France, 2015–2019 ($n = 16$).

Case	Strain	Species	Isolation (date, location)	Link to a foreign country	Ward	Specimen	Infection	CC17	ST
1	15-024	<i>E. faecium</i>	13/01/2015, Saint-Pierre	Yes (Madagascar)	Infectious diseases	Rectal swab	No	No	1498
2	15-196	<i>E. faecium</i>	11/05/2015, Saint-Pierre	No	ICU	Rectal swab	No	No	5
3	17-040	<i>E. faecium</i>	18/01/2017, Saint-Denis	Yes (Mauritius)	ICU	Peritoneal fluid	Yes	Yes	2086
4	17-180	<i>E. faecium</i>	10/03/2017, Saint-Pierre	Yes (India)	ICU	Rectal swab	No	Yes	80
5	18-190	<i>E. faecium</i>	04/04/2018, Sainte-Clotilde	Yes (Mauritius)	General medicine	Rectal swab	No	Yes	80
6	18-492	<i>E. faecium</i>	19/08/2018, Saint-Denis	No	ICU	Rectal swab	No	No	5
7	18-562	<i>E. faecium</i>	23/09/2018, Sainte-Clotilde	Yes (India)	Cardiology	Rectal swab	No	Yes	761
8	19-183	<i>E. faecium</i>	30/01/2019, Saint-Denis	No	General medicine	Rectal swab	No	Yes	761
9	19-184	<i>E. faecium</i>	30/01/2019, Saint-Denis	No	General medicine	Rectal swab	No	Yes	761
10	19-185	<i>E. faecium</i>	01/02/2019, Saint-Denis	No	General medicine	Rectal swab	No	Yes	761
11	19-186	<i>E. faecium</i>	01/02/2019, Saint-Denis	No	General medicine	Rectal swab	No	Yes	761
12	19-213	<i>E. faecium</i>	02/02/2019, Saint-Denis	Yes (India/Saudi Arabia)	Infectious diseases	Rectal swab	No	Yes	80
13	19-287	<i>E. faecium</i>	07/02/2019, Saint-Denis	No	Infectious diseases	Rectal swab	No	Yes	761
14	19-288	<i>E. faecium</i>	11/02/2019, Saint-Denis	No	ENT surgery	Rectal swab	No	Yes	761
15	19-289	<i>E. faecium</i>	14/02/2019, Saint-Denis	No	Cardiothoracic surgery	Rectal swab	No	Yes	80
16	19-611	<i>E. faecium</i>	15/04/2019, Saint-Denis	No	ICU	Rectal swab	No	Yes	761

Abbreviations: ICU = intensive care unit; ENT = Ear nose and throat.

The highlighted rows in the table correspond to the patients and isolates involved in the outbreak.

identified in this study were deposited in GenBank as bioproject PRJNA791620.

3. Results

3.1. Description and investigation of the outbreak

3.1.1. Data collection

Sixteen VRE_f isolates were obtained between January 2015 and December 2019, seven of which were involved in the outbreak. These isolates came from the UHRI North site ($n = 11$), the UHRI South site ($n = 3$) and a private clinic ($n = 2$). One patient was infected (peritonitis), the other 15 were colonised. Twelve of the 16 VRE_f-positive patients were male (SR = 0.75), and the median age of the patients was 69 years (range 35–90). A link to a foreign country, mostly India or Mauritius, was identified in six of the 16 cases (Table 1). Five of the cases detected at the UHRI ($n = 14$) were obtained from the intensive care unit (ICU) (35.7%), four were obtained from the GMD (28.6%), three from the infectious diseases department (IDD) (21.4%), and one case each was obtained from the cardiothoracic surgery and ENT departments (Table 1). Four of the 16 patients died (25%); one of these deaths was considered directly attributable to VRE infection. Eleven patients (69%) received antibiotics before VRE detection, including vancomycin in one patient. None of the patients received linezolid, phenicols or daptomycin.

3.1.2. Outbreak investigation

In the context of the outbreak, 505 patients were screened for VRE colonisation (97% at the UHRI) and 219 contact patients were monitored (i.e. marked as a potential VRE carrier and to be screened at a possible future admission). The outbreak investigation (coordinated by a multidisciplinary team) involved the repeated rectal swab screening of contact patients. Efforts were also made to raise the awareness of nursing staff concerning measures for preventing cross-transmission through a dedicated hygienist nurse. All VRE-infected or VRE-colonised patients were isolated, with enhanced contact precautions, as recommended by the HSCP. When two additional patients were detected on 31 January 2019, two days after the first two cases, the transfer of cases and contacts to other units of the UHRI was halted until the third rectal swab screening result was obtained for all high-risk contacts (Figure S1a). Epidemiological investigations revealed that seven patients colonised with LVRE_f (Cases 8–11, 13, 14 and 16) were probably involved in the outbreak; none of these patients had any links

to a foreign country. After the detection of Case 16, no further LVRE_f isolates were obtained at the UHRI, despite active surveillance in the GMD/IDD for a further three weeks and the routine weekly screening of patients in the ICU.

The seven suspected cases were all hospitalised at the North site of the UHRI, mostly (five of seven) in the GMD (Figures S1a and S1b). The other two cases (Cases 13 and 16) were detected in the IDD and ICU. Case 14 was hospitalised in the same room of the GMD directly after Case 11, and Case 16 was hospitalised in the same room of the ICU three days after Case 11 (Figure S1a). No epidemiological link was found between Case 13 and the six other suspected outbreak cases.

3.2. Microbiological analysis

All 16 isolates displayed high levels of resistance to vancomycin (MIC ≥ 128 mg/L) and teicoplanin (MIC ≥ 16 mg/L) (Table 2). Eight isolates (50%), including the seven outbreak isolates of the outbreak, were also resistant to linezolid (MIC = 8–256 mg/L) (Table 2). One isolate (Case 16) was simultaneously resistant to vancomycin, teicoplanin, linezolid and daptomycin (MIC = 8 mg/L), even though the patient had not received any of these antibiotics. Antimicrobial susceptibility testing was performed for 11 other antimicrobial agents: all seven suspected outbreak isolates were resistant to amoxicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, levofloxacin, rifampicin, tetracycline and trimethoprim-sulfamethoxazole (Table 2). The UHRI laboratory detected the *vanA* gene in all 16 isolates, and the presence of this gene was subsequently confirmed by the FNRC-Enc.

3.3. Whole genome sequencing and bioinformatic analysis

Most of the *E. faecium* isolates (13 of 16, 81.3%), including the seven suspected outbreak isolates, belonged to clonal complex (CC) 17 (Table 1). Genotyping analysis revealed that the 16 isolates belonged to five different sequence types (STs), the three main STs identified being ST761 ($n = 8$), ST80 ($n = 4$) and ST5 ($n = 2$) (Table 1 and Figure 1). All seven outbreak isolates belonged to ST761, as did an isolate obtained in September 2018 (Case 7) from a patient hospitalised in a Reunionese private clinic reporting recent prior hospitalisation in India (Figures 1, S1a and S1b); this patient was also found to be colonised with NDM-5 carbapenemase-producing *E. coli*.

Phylogenomic analysis revealed the presence of a main cluster comprising the seven outbreak LVRE_f isolates, which were extremely closely related (difference of 0–2 SNPs, using the *E. faecium*

Table 2

Description of the antimicrobial susceptibility profiles of vancomycin-resistant *Enterococcus faecium* (*E. faecium*) isolates obtained on Reunion Island, France, 2015–2019 (*n* = 16).

Case	Strain	Species	Phenotype of resistance	MIC (mg/L)					
				LZD	TZD	VAN	TEC	DAP	TGC
1	15-024	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, TET, SXT, RIF	2	0.25	> 256	16	4	0.06
2	15-196	<i>E. faecium</i>	AMP, LVX, ERY, CLI, VAN, TEC, CHL, TET	2	0.5	> 256	16	1	0.12
3	17-040	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, SXT, RIF	1	0.25	256	256	2	0.03
4	17-180	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, RIF	> 256	> 8	> 256	64	4	0.06
5	18-190	<i>E. faecium</i>	AMP, LVX, ERY, CLI, VAN, TEC	4	1	> 256	64	4	0.06
6	18-492	<i>E. faecium</i>	AMP, ERY, CLI, VAN, TEC, TET	2	0.5	> 256	64	1	0.25
7	18-562	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, TET, RIF	2	0.25	> 256	64	2	0.03
8	19-183	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	16	2	> 256	64	2	0.5
9	19-184	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	8	2	128	64	2	0.25
10	19-185	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	16	2	> 256	64	4	0.25
11	19-186	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	16	2	128	64	4	0.5
12	19-213	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, TET, RIF	2	0.25	128	128	4	0.12
13	19-287	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	8	2	> 256	64	2	0.25
14	19-288	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF	8	2	128	32	2	0.25
15	19-289	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, TET, SXT, RIF	2	0.25	> 256	32	4	0.12
16	19-611	<i>E. faecium</i>	AMP, GEN, LVX, ERY, CLI, VAN, TEC, LZD, CHL, TET, SXT, RIF, DAP	32	4	> 256	64	8	0.25

Abbreviations : AMP = ampicillin; CHL = chloramphenicol; CLI = clindamycin; DAP = daptomycin; ERY = erythromycin; GEN = gentamicin; LZD = linezolid; LVX = levofloxacin; RIF = rifampicin ; SXT = trimethoprim-sulfamethoxazole; TEC = teicoplanin; TET = tetracycline ; TGC = tigecycline; TZD = tedizolid; VAN = vancomycin. The highlighted rows in the table correspond to the patients and isolates involved in the outbreak.

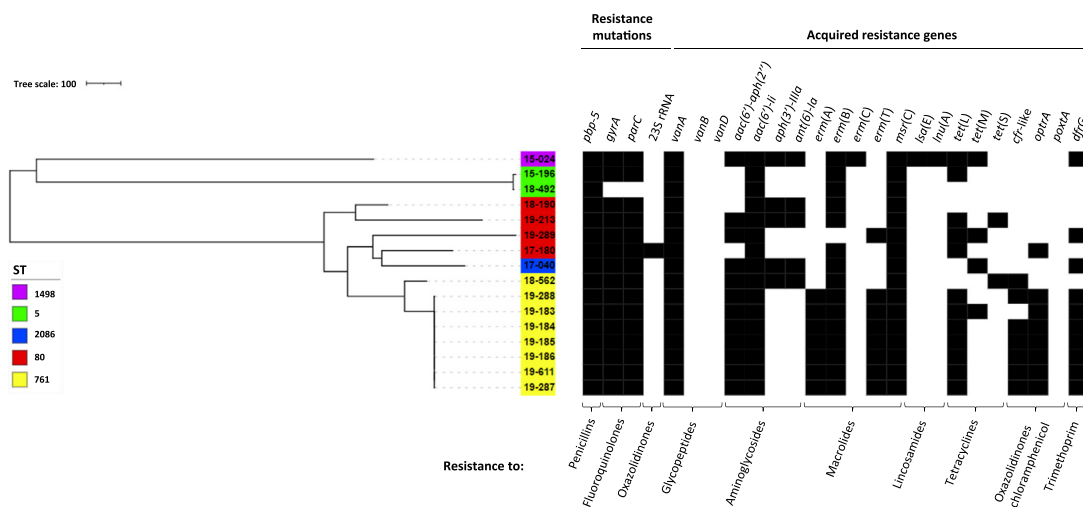


Figure 1. Dendrogram representing the phylogenetic analysis of the VREf strains (Reunion Island, France, 2015–2019), together with a heatmap for resistance mechanisms. A phylogenetic analysis based on SNPs in the core genome was performed with Snippy. The dendrogram, together with ST affiliation and a heatmap for resistance mechanisms (resistance mutations and acquired resistance genes) were generated with iTOL. The scale bar represents 100 SNPs. The presence of mutations and acquired genes is indicated by black boxes. All isolates exhibited many diverse (> 10) *pbp-5* mutations. All isolates (except 18–492) harboured mutations in both *GyrA* (S83Y [*n* = 12] or S83I [*n* = 3]) and *ParC* (S80I [*n* = 15]). The 17-180 isolate presented the G2576T mutation in the 6/6 23S rRNA alleles.

core genome as a reference) and all of which harboured the *optrA* gene (Figure 1). These seven LVREf outbreak isolates displayed genetic proximity to the ST761 VREf isolate (Case 7, strain number 18–562) imported from India in 2018 (151–153 SNPs) but were not considered as clonally related. A second cluster, including two ST5 isolates (Cases 2 and 6), was also detected. This cluster contained linezolid-susceptible *E. faecium* strains with no obvious epidemiological links, detected three years apart at the two sites of the UHRI.

4. Discussion

Linezolid-resistant and vancomycin-resistant *E. faecium* is a matter of growing concern in hospitals, due to the ability of such bacteria generating large-scale outbreaks and becoming resistant to all available antibiotics [29,30].

The current analysis found that Case 9 was the index patient of the outbreak at the North site of the UHRI. The mode of LVREf acquisition by Case 9 remains unknown (no link to a foreign coun-

try), but it should be noted that Cases 7 and 9 had recently been hospitalised in the same private clinic (Figure S1b). Case 7 probably imported this ST761 VREf clone into Reunion Island from India in 2018, but it is difficult to establish whether there was a relationship between this case and the index case of the outbreak (Case 9) or whether these two cases correspond to independent contaminations. Furthermore, it remains unclear how Case 13 was contaminated at the UHRI. This outbreak confirms the known exposure of Reunion Island to very high risk of importation of XDR bacteria, particularly from the Indian subcontinent (5 of 6 VRE-positive patients linked to foreign countries had travelled to either India or Mauritius) [14,31]. Reunion Island has historic links to India, as a proportion of its population originates from this country and regularly travels there for family holidays [32]. Interestingly, *optrA*-mediated linezolid resistance in India was first described at a hospital in the Tamil Nadu region, the region of origin of most of the Hindu inhabitants of Reunion Island [17]. However, ST761 vancomycin-susceptible *E. faecium* was only described in 2016 in China, where the emergence of *optrA*-mediated linezolid resistance

has also been reported [33,34]. This outbreak highlights the need for the systematic screening of all patients entering the UHRI (and all other healthcare facilities on Reunion Island) who have travelled abroad, particularly if they report having been hospitalised in a high-risk area such as India or China [35].

This episode is the first outbreak of *vanA* linezolid-resistant and vancomycin-resistant enterococci to be described on a French territory. Before that, four LVREf isolates harbouring the *vanA* and *optrA* genes (three of which were also linked to India, but were not ST761), detected in four different French hospitals, had been identified in mainland France [24]. This outbreak was controlled by the implementation of early, reactive hygiene measures, which limited the number of secondary cases to six, if the separate case reported in 2018 (Case 7) was excluded. Frequent multidisciplinary crisis cell meetings (with medical doctors, hygienists, microbiologists and senior nurses) and a fast molecular assay (Xpert *vanA/vanB*) enabled the investigations to be optimised. Contact patients should be screened at least three times before release from isolation, in line with recommendations, as confirmed by the current observations that some cases, such as Case 14, were not detected until the third rectal swab, which is consistent with previous findings [36].

A small number of patients were infected (1 of 16; 6.3%), but the emergence of this XDR *E. faecium* clone in this geographical area is a matter of concern, due to the very limited number of alternative treatments available in case of infection. In the UHRI, the use of linezolid is controlled and only reserved for cases of infection (especially osteoarticular) with methicillin-resistant *Staphylococcus* or in very rare cases with enterococci (VRE or co-infection with another multidrug-resistant germ). For the last isolate detected (Case 16), tigecycline was the only remaining effective treatment.

The acquisition of transferable genes encoding oxazolidinone resistance (in this case, *optrA*) in a linezolid-susceptible VREf clone is also a matter of concern. The *optrA* gene is frequently carried by plasmids also bearing macrolide-resistance or aminoglycoside-resistance genes [37]. Therefore, it is important to understand that the use of other classes of antibiotics can favour the transfer of such plasmids, resulting in an XDR clone. Antibiotic sparing, for all these therapeutic classes, is therefore essential in patients potentially colonised with VRE.

5. Conclusions

This study of the emergence and possible inter-hospital circulation of an XDR linezolid- and vancomycin-resistant ST761 *E. faecium* clone on Reunion Island emphasises the importance of the strict application of prevention and infection control measures against highly epidemic strains in this highly exposed territory with links to the Indian subcontinent. It also documents the spread of the *optrA* gene in VREf in the Southwest Indian Ocean area, in which the French territory of Reunion Island could serve as a relay point for the transmission of these high-risk clones to Southern Africa or Europe, due to its bi-weekly or daily air connections to South Africa and mainland France, respectively.

Acknowledgements

The authors wish to thank the members of the infection control team for their help in the investigation and control of this outbreak.

Funding

This work was partly supported by the Reunion Regional Health Agency (ARS Réunion) and the ERDF European program (RESISTO-RUN study, 20201126-0022906). The FNRC-Enc is funded by the

French Ministry of Health, through the *Santé Publique France* agency.

Competing Interests

None.

Ethical Approval

Not required.

Sequence Information

GenBank: bioproject PRJNA791620.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2022.106686.

References

- [1] Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill* 2008;13:19046.
- [2] Ayobami O, Willrich N, Reuss A, Eckmanns T, Markwart R. The ongoing challenge of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* in Europe: an epidemiological analysis of bloodstream infections. *Emerg Microbes Infect* 2020;9:1180–93.
- [3] Lode HM. Clinical impact of antibiotic-resistant Gram-positive pathogens. *Clin Microbiol Infect* 2009;15:212–17.
- [4] MacDougall C, Johnstone J, Prematunge C, Adomako K, Nadolny E, Truong E, et al. Economic evaluation of vancomycin-resistant enterococci (VRE) control practices: a systematic review. *J Hosp Infect* 2020;105:53–63.
- [5] Arias CA, Murray BE. The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol* 2012;10:266–78.
- [6] Pfaller MA, Cormican M, Flamm RK, Mendes RE, Jones RN. Temporal and geographic variation in antimicrobial susceptibility and resistance patterns of enterococci: results from the SENTRY antimicrobial surveillance program, 1997–2016. *Open Forum Infect Dis* 2019;6:S54–62.
- [7] Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–81.
- [8] Krull M, Klare I, Ross B, Trenschele R, Beelen DW, Todt D, et al. Emergence of linezolid- and vancomycin-resistant *Enterococcus faecium* in a department for hematologic stem cell transplantation. *Antimicrob Resist Infect Control* 2016;5:31.
- [9] Egan SA, Corcoran S, McDermott H, Fitzpatrick M, Hoyne A, McCormack O, et al. Hospital outbreak of linezolid-resistant and vancomycin-resistant ST80 *Enterococcus faecium* harbouring an *optrA*-encoding conjugative plasmid investigated by whole-genome sequencing. *J Hosp Infect* 2020;105:726–35.
- [10] Fang H, Fröding I, Ullberg M, Giske CG. Genomic analysis revealed distinct transmission clusters of vancomycin-resistant *Enterococcus faecium* ST80 in Stockholm, Sweden. *J Hosp Infect* 2021;107:12–15.
- [11] Cattoir V, Giard JC. Antibiotic resistance in *Enterococcus faecium* clinical isolates. *Expert Rev Anti Infect Ther* 2014;12:239–48.
- [12] Miller WR, Murray BE, Rice LB, Arias CA. Resistance in vancomycin-resistant enterococci. *Infect Dis Clin North Am* 2020;34:751–71.
- [13] Dejoies L, Sassi M, Schutz S, Moreaux J, Zouari A, Potrel S, et al. Genetic features of the *poxA* linezolid resistance gene in human enterococci from France. *J Antimicrob Chemother* 2021;76:1978–85.
- [14] Miltgen G, Cholley P, Martak D, Thouverez M, Seraphin P, Leclaire A, et al. Carbenemase-producing *Enterobacteriaceae* circulating in the Reunion Island, a French territory in the Southwest Indian Ocean. *Antimicrob Resist Infect Control* 2020;9:36.
- [15] Gay N, Lugagne N, Miltgen G, Belmonte O, Cardinale E. Reunion Island, a sentinel territory for antimicrobial-resistant bacteria surveillance in the South-Western Indian Ocean: a retrospective survey using hospitalized patient screening, 2015–2017. *BMC Public Health* 2020;20:1488.
- [16] Rai S, Niranjana DK, Kaur T, Singh NP, Hada V, Kaur IR. Detection of the classical G2576U mutation in linezolid resistant *Staphylococcus aureus* along with isolation of linezolid resistant *Enterococcus faecium* from a patient on short-term linezolid therapy: first report from India. *Indian J Med Microbiol* 2015;33:21–4.
- [17] Bakthavatchalam YD, Vasudevan K, Babu P, Neeravi AR, Narasiman V, Veer-araghavan B. Genomic insights of *optrA*-carrying linezolid-resistant *Enterococcus faecium* using hybrid assembly: first report from India. *J Glob Antimicrob Resist* 2021;25:331–6.

- [18] McCarthy KM, Van Nierop W, Duse A, Von Gottberg A, Kassel M, Perovic O, et al. Control of an outbreak of vancomycin-resistant *Enterococcus faecium* in an oncology ward in South Africa: effective use of limited resources. *J Hosp Infect* 2000;44:294–300.
- [19] Alemayehu T, Hailemariam M. Prevalence of vancomycin-resistant *enterococcus* in Africa in one health approach: a systematic review and meta-analysis. *Sci Rep* 2020;10:20542.
- [20] Prévention de la transmission des Bactéries Hautement Résistantes émergentes (BHRe). Rapport du Haut Conseil de Santé Publique. 2013. Available at: https://www.hcsp.fr/Explore.cgi/Telecharger?NomFichier=hcspr20130710_recoprevtransxbhre.pdf [accessed 23 March 2022].
- [21] Actualisation des recommandations relatives aux BHRe. Rapport du Haut Conseil de Santé Publique. 2019. Available at: <https://www.hcsp.fr/explore.cgi/avisrapportsdomaine?clefr=758> [accessed 23 March 2022].
- [22] Réseau de surveillance régional des résistances aux antibiotiques, La Réunion. CPIAS Réunion-Mayotte. Fédération de lutte contre les infections nosocomiales (FELIN). Available at: http://www.cpias-oi.fr/images/cpias/articles/Antibioresistance/Rapport_-_rsistances-2019.pdf [accessed 23 March 2022].
- [23] Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM). Recommandations version 1.2. 2020. Available at: https://www.sfm-microbiologie.org/wp-content/uploads/2020/10/CASFM2020_Octobre2020_V1.2.pdf [accessed 14 April 2022].
- [24] Sassi M, Guérin F, Zouari A, Beyrouthy R, Auzou M, Fines-Guyon M, et al. Emergence of *optrA*-mediated linezolid resistance in enterococci from France, 2006–16. *J Antimicrob Chemother* 2019;74:1469–72.
- [25] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–77.
- [26] Hasman H, Clausen PTL, Kaya H, Hansen F, Knudsen JD, Wang M, et al. LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the *optrA*, *cfi*, *cfi(B)* and *poxA* genes encoding linezolid resistance in enterococci from whole-genome sequences. *J Antimicrob Chemother* 2019;74:1473–6.
- [27] Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020;75:3491–500.
- [28] Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 2021;49:W293–6.
- [29] Satilmis L, Vanhems P, Bénet T. Outbreaks of vancomycin-resistant enterococci in hospital settings: a systematic review and calculation of the basic reproductive number. *Infect Control Hosp Epidemiol* 2016;37:289–94.
- [30] Miller WR, Murray BE, Rice LB, Rice LB, Arias CA. Vancomycin-resistant enterococci: therapeutic challenges in the 21st century. *Infect Dis Clin North Am* 2016;30:415–39.
- [31] Miltgen G, Bour M, Allyn J, Allou N, Vedani T, Vuilleminot J-B, et al. Molecular and epidemiological investigation of a colistin-resistant OXA-23-/NDM-1-producing *Acinetobacter baumannii* outbreak in the Southwest Indian Ocean Area. *Int J Antimicrob Agents* 2021;58:106402.
- [32] Trouillet PY. Les populations d'origine indienne hors de l'Inde : fabrique et enjeux d'une « diaspora ». *Géconfluences*. 2015. Available at: <http://geoconfluences.ens-lyon.fr/informations-scientifiques/dossiers-regionaux/le-monde-indien-populations-et-espaces/articles-scientifiques/diaspora-indienne> [accessed 7 June 2022].
- [33] Wang S, Guo Y, Lv J, Qi X, Li D, Chen Z, et al. Characteristic of *Enterococcus faecium* clinical isolates with quinupristin/dalfopristin resistance in China. *BMC Microbiol* 2016;16:246.
- [34] Cui L, Wang Y, Lv Y, Wang S, Song Y, Li Y, et al. Nationwide surveillance of novel oxazolidinone resistance gene *optrA* in *enterococcus* isolates in China from 2004 to 2014. *Antimicrob Agents Chemother* 2016;60:7490–3.
- [35] Angue M, Allou N, Belmonte O, Lefort Y, Lugagne N, Vandroux D, et al. Risk factors for colonization with multidrug-resistant bacteria among patients admitted to the intensive care unit after returning from abroad. *J Travel Med* 2015;22:300–5.
- [36] Allyn J, Angue M, Belmonte O, Lugagne N, Traversier N, Vandroux D, et al. Delayed diagnosis of high drug-resistant microorganisms carriage in repatriated patients: three cases in a French intensive care unit. *J Travel Med* 2015;22:215–17.
- [37] Schwarz S, Zhang W, Du X-D, Krüger H, Feßler AT, Ma S, et al. Mobile oxazolidinone resistance genes in Gram-positive and Gram-negative bacteria. *Clin Microbiol Rev* 2021;34:e0018820.