Journal of Global Antimicrobial Resistance 34 (2023) 234–239

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Carriage of linezolid-resistant enterococci (LRE) among humans and animals in Nigeria: coexistence of the *cfr*, *optrA*, and *poxtA* genes in *Enterococcus faecium* of animal origin

Emmanuel O. Ngbede[a,b,c,*], Issa Sy[a], Chinedu A. Akwuobu[b,d], Maurice A. Nanven[e], Alex A. Adikwu[f], Paul O. Abba[g], Mohammed I. Adah[d,h], Sören L. Becker[a,i,j,**]

[a] *Institute of Medical Microbiology and Hygiene, Saarland University, Kirrberger Straße, Gebäude 43D-66421 Homburg/Saar, Germany*
[b] *Department of Veterinary Microbiology, Federal University of Agriculture, Makurdi, Nigeria*
[c] *Leibniz-Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany*
[d] *Amadu Ali Centre for Public Health and Comparative Medicine, Federal University of Agriculture, Makurdi, Nigeria*
[e] *National Veterinary Research Institute, Vom, Plateau State, Nigeria*
[f] *Department of Veterinary Public Health and Preventive Medicine, Federal University of Agriculture, Makurdi, Nigeria*
[g] *Department of Medical Microbiology and Parasitology, Benue State University Teaching Hospital, Makurdi, Nigeria*
[h] *Department of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria*
[i] *Swiss Tropical and Public Health Institute, CH-4002 Allschwil, Switzerland*
[j] *University of Basel, CH-4003 Basel, Switzerland*

ARTICLE INFO

Article history:
Received 10 March 2023
Revised 9 July 2023
Accepted 25 July 2023
Available online 28 July 2023

Editor: Stefania Stefani

Keywords:
Antimicrobial resistance
Linezolid-resistant enterococci
cfr
oprtA
poxtA
Nigeria

ABSTRACT

Objectives: In contrast to increasing reports of the emergence of linezolid-resistant enterococci (LRE) emanating from many countries in Europe, Asia, and North America, data on its status and dissemination from the African continent remain scarce, with the information available limited to countries in North Africa. This study investigated the carriage of LRE and the genetic mechanism of resistance among *Enterococcus faecium* and *Enterococcus faecalis* strains recovered from humans and animals in Makurdi, Nigeria.
Methods: We conducted a cross-sectional study between June 2020 and July 2021 during which 630 non-duplicate human and animal faecal samples were collected and processed for the recovery of LRE. The genetic mechanisms for resistance were investigated using polymerase chain reaction (PCR) and Sanger sequencing.
Results: Linezolid-resistant enterococci were recovered from 5.87% (37/630; 95% CI: 4.17–8.00) of the samples, with the prevalence in animals and humans being 6.22% [(28/450); 95% CI: 4.17–8.87] and 5.00% [(9/180); 95% CI: 2.31–9.28], respectively. All isolates remained susceptible to vancomycin. No known point mutation mediating linezolid resistance was detected in the 23S rRNA and ribosomal protein genes; however, acquisition of one or more potentially transferable genes (*cfr, optrA*, and *poxtA*) was observed in 26 of the 37 LRE isolates. Co-existence of all three transferable genes in a single isolate was found in four *E. faecium* strains of animal origin.
Conclusion: This study provides baseline evidence for the emergence and active circulation of LRE driven majorly by the acquisition of the *optrA* gene in Nigeria. To the best of our knowledge, our study is the first to report a co-carriage of all three transferable linezolid resistance determinants in *E. faecium*. Active LRE surveillance is urgently required to understand the extent of LRE spread across sub-Saharan Africa and to develop tailored mitigation strategies.

1. Introduction

Antimicrobial resistance is a globally recognized economic and public health threat frequently associated with prolonged hospitalization, increased treatment cost, treatment failure, and mortalities [1]. Of great concern is the emergence and spread of resistance to

* Corresponding author. Mailing address: Leibniz-Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany.
** Alternative corresponding author.
E-mail addresses: emmanuel.ngbede@dsmz.de (E.O. Ngbede), soeren.becker@uks.eu (S.L. Becker).

https://doi.org/10.1016/j.jgar.2023.07.016

antimicrobials categorized as 'last line' drugs for the treatment of infections caused by multidrug-resistant (MDR) bacteria [2].

The oxazolidinones, particularly linezolid and tedizolid, are important therapeutic options against Gram-positive bacteria such as vancomycin-resistant *Enterococcus* spp. (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) [3]. Oxazolidinones act by binding to the 23S rRNA of the 50S subunit. This prevents the formation of a functional 70S initiation complex, thus inhibiting protein synthesis [4]. Yet, resistance to these agents has emerged in the last two decades, threatening their clinical efficacy for the treatment of MDR infections [4,5]. Thus far, two major mechanisms are reportedly responsible for the loss of enterococci susceptibility to oxazolidinones; (i) chromosomal mutations or alterations in the V domain of the 23S rRNA and rarely ribosomal proteins L3 and L4, and (ii) acquisition of transferable resistance genes; *cfr, optrA*, and/or *poxtA* [4].

Enterococcus spp. (particularly *Enterococcus faecalis* and *Enterococcus faecium*) listed among the global priority resistant bacteria by the World Health Organization (WHO) are among the most common causes of nosocomial, life-threatening infections, especially in the elderly, immunocompromised, and children [6,7]. Linezolid-resistant enterococci (LRE) are reportedly increasing and rapidly spreading across the world and have been recovered from humans, animals, and food products [8–10]. Of particular concern is the reported detection of similar strains between humans and animals (although from different continents), suggesting the potential for bidirectional exchange [9,11,12]. However, despite the increasing prevalence and dissemination of LRE in Europe, North America, and Asia, little has been reported from sub-Saharan Africa on its status and spread. The available data from the African continent are limited to countries in North Africa, with reports of an occurrence rate of ca. 5% from wastewater, humans, and animals [12–15].

The high burden and negative effect of antimicrobial resistance (AMR) in sub-Saharan Africa is a source of concern [16]. It is therefore imperative that the efficacy of the last resort antimicrobials in the region be preserved; we recommend active surveillance to promptly detect and curtail the evolution and spread of bacteria resistant to these drugs and to study the factors promoting their emergence. Similar to many African countries, studies on linezolid resistance in Nigeria are scarce. The weak or nonexistent surveillance network for linezolid resistance and rare use of the agent in human and veterinary medicine in Nigeria may fuel a perception of the absence of LRE in this region. Widespread, unrestricted access to antimicrobials and increasingly close human-animal interactions in Nigeria create a potential 'hotspot' for the emergence and dissemination of multidrug-resistant bacteria, including LRE. Therefore, the need to investigate the status of this pathogen in animal and human populations persists. In the present study, we investigated the occurrence of LRE among humans and animals and identified the potential mechanism(s) mediating observed resistance. This information is important for understanding the threat of LRE locally in addition to its global spread.

2. Materials and methods

2.1. Study design and location

This prospective cross-sectional study was conducted between June 2020 and July 2021. During the study period, a total of 630 non-duplicate faecal samples from humans (n = 180), cattle (n = 85), pigs (n = 125), dogs (n = 50), shrews (n = 40), and poultry (n = 150) were collected and processed for the recovery of LRE. The human faecal samples originated from patients visiting two [2] major health facilities (Federal Medical Center Makurdi and Benue State University Teaching Hospital) in Makurdi, Benue State, Nigeria. The samples included faeces submitted to the clinical microbiology laboratories for investigation of enteric pathogens. Similarly, all animals, with the exception of shrews, were sampled from farms, slaughter facilities, and households in Makurdi, Benue State, North central Nigeria. The periurban shrews were captured and sampled in Surulere, Lagos State, southwest Nigeria during a Nationwide surveillance for Emerging Infectious Diseases in wildlife across Nigeria.

2.2. Recovery of enterococci

The faecal samples collected were processed for the recovery of florfenicol-resistant enterococci [presumptive linezolid-resistant enterococci (LRE)] and vancomycin-resistant enterococci (VRE) within 24 h of collection. Briefly, 0.5 g of each faecal sample was inoculated into 5 mL of tryptone soya broth (Oxoid, UK) and enriched overnight at 37°C. Following overnight enrichment, 500 µL of the broth was inoculated into two different tubes of 4.5 mL bile esculin azide broth (Lioflichem, Italy), one supplemented with florfenicol (10µg/mL) and the other with vancomycin (6µg/mL) for the selective enrichment of LRE and VRE, respectively. The broths were then incubated for 48 h at 37°C. Following selective enrichment, a loopful of the broths positive for enterococci (evidenced by browning/blackening of the medium) was streaked on florfenicol- and vancomycin-supplemented Slanetz Bartley (SB) plates and incubated for 48 h at 37°C. A single colony with typical enterococci morphology on SB medium was picked from each supplemented plate (for each sample) and sub-cultured to purity on tryptone soya agar. Isolates were confirmed as enterococci based on Gram staining reaction and results of catalase and pyrrolidonyl peptidase (PYRase) tests (Hardy Diagnostics, Santa Maria, CA). Isolates were further speciated based on carbohydrate [mannitol, xylose, arabinose, pyruvate, and Methyl-alpha-D-glucopyranoside (MDG)] fermentation reactions as described by Manero and Blanch [17] and Schwaiger et al. [18]. All isolates were stored on tryptone soya agar slants and transferred to the Institute of Medical Microbiology and Hygiene (IMMH), Saarland University Homburg/Saar, Germany for further analysis.

2.3. Species confirmation

The species identification of the isolates was further confirmed at IMMH using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).

2.4. Phenotypic screening for linezolid and vancomycin resistance

The linezolid and vancomycin minimum inhibitory concentrations (MICs) for the isolates were determined by the gradient diffusion test using E-test strips (Liofilchem, Roseto degli Abruzzi, Italy). Briefly, linezolid and vancomycin strips (Liofilchem, Italy) were placed on Mueller Hinton agar plates pre-inoculated with a suspension (equivalent of 0.5 McFarland Standard) of the isolates and then incubated for 18 to 24 h at 37°C. Subsequently, the MICs of the respective antibiotics were then read according to the manufacturer's instructions. The isolates were categorized as LRE or VRE using interpretative breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19] and the Clinical and Laboratory Standards Institute (CLSI) [20] i.e., LRE = linezolid MIC >4µg/mL (EUCAST) or ≥8µg/mL (CLSI); VRE = vancomycin MIC >4µg/mL (CLSI and EUCAST).

2.5. DNA extraction, quantification and purity control

Extraction of genomic DNA (gDNA) from overnight blood agar plates of the LRE was carried out using the Maxwell® 16 MDx

Table 1
Distribution of linezolid resistance and associated mechanisms among enterococci recovered from humans and animals in Nigeria.

S/No.	Strain ID	Species	Host	Minimum Inhibitory Concentration				Acquired Resistance Genes		
				VAN	LZD	TZD	FFC	*optrA*	*potxA*	*cfr*
1	EH2	*E. faecalis*	Human	1.5	24	2	≥15	+	-	-
2	EH3	*Enterococcus faecalis*	Human	1.5	16	4	≥15	+	-	-
3	EH6	*E. faecalis*	Human	2	32	4	≥15	+	-	-
4	EH11	*E. faecalis*	Human	1.5	6	ND	≥15	-	-	-
5	EH13	*E. faecalis*	Human	1.5	6	ND	≥15	-	-	-
6	EH17	*E. faecium*	Human	0.5	24	1.5	≥15	+	-	-
7	EH18	*E. faecium*	Human	3	12	ND	≥15	+	-	-
8	EH21	*E. faecalis*	Human	1.5	6	ND	≥15	-	-	-
9	EH23	*E. faecium*	Human	0.38	6	ND	≥15	-	-	-
10	E2	*E. faecalis*	Cattle	0.75	8	ND	≥15	+	-	-
11	E7	*E. faecalis*	Cattle	1	6	ND	≥15	-	-	-
12	E8	*E. faecalis*	Cattle	0.75	6	ND	≥15	-	-	-
13	E10	*E. faecalis*	Dog	2	12	3	≥15	+	-	-
14	E11	*E. faecalis*	Cattle	0.5	6	ND	≥15	-	-	-
15	E12	*E. faecium*	Pigs	0.50	8	1.5	≥15	+	+	+
16	E13	*E. faecium*	Pigs	0.25	8	2	≥15	+	+	+
17	E14	*E. faecium*	Pigs	0.38	8	ND	≥15	+	+	-
18	E15	*E. faecium*	Pig	0.38	6	ND	≥15	-	-	-
19	E16	*E. faecium*	Pig	0.125	6	ND	≥15	-	-	-
20	E17	*E. faecalis*	Cattle	1	8	ND	≥15	+	-	-
21	E19	*E. faecium*	Cattle	0.25	16	3	≥15	+	-	-
22	E20	*E. faecium*	Cattle	0.5	6	ND	≥15	-	-	-
23	E21	*E. faecalis*	Cattle	0.5	12	2	≥15	+	-	-
24	E22	*E. faecium*	Cattle	0.5	8	3	≥15	+	+	+
25	E24	*E. faecium*	Cattle	1	≥256	1.5	≥15	+	-	+
26	E26	*E. faecium*	Cattle	0.25	8	3	≥15	+	+	+
27	E27	*E. faecalis*	Pig	0.75	24	2	≥15	+	-	-
28	E28	*E. faecalis*	Dog	1.5	24	1.5	≥15	+	-	-
29	E29	*E. faecalis*	Dog	2	16	3	≥15	+	-	-
30	E30	*E. faecalis*	Dog	1.5	6	ND	≥15	-	-	-
31	E31	*E. faecalis*	Dog	1.5	16	ND	≥15	+	-	-
32	6EW	*E. faecalis*	Shrew	1.5	8	ND	≥15	+	-	-
33	13EW	*E. faecalis*	Shrew	0.38	16	ND	≥15	+	-	-
34	15EW	*E. faecalis*	Shrew	0.75	12	ND	≥15	+	-	-
35	21EW	*E. faecalis*	Shrew	0.75	8	ND	≥15	+	-	-
36	23EW	*E. faecalis*	Shrew	0.5	16	3	≥15	+	-	-
37	31EW	*E. faecalis*	Shrew	2	8	ND	≥15	+	-	-

A plus sign (+) indicates the presence of the gene, a minus sign (−) the absence of the gene, determined by PCR.
FFC, florfenicol; TZD, tedizolid; VAN, vancomycin.

version 1.60 instrument system (Promega, Madison, WI) according to the manufacturer's instructions. The concentration of the extracted DNA was measured at an absorbance of 260 nm and the purity was assessed by calculating the A260/280 and A260/230 ratios using the NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

2.6. Resistance mechanism

The genetic basis of linezolid resistance among the LRE isolates was investigated using polymerase chain reaction (PCR) and subsequent sequencing of the obtained amplicons. The PCR targeted previously reported mechanisms, including mutation in domain V of the 23S rRNA [21] and/or ribosomal proteins L3 (*rplC*), L4 (*rplD*), [22] and L22 (*rplV*) [23], and presence of *cfr* [24], *optrA* [25] and *poxtA* [26] using respective previously reported primers and conditions. Electrophoresis of all PCR products was carried out on 2% agarose gel stained with ethidium bromide and visualized using a UV transilluminator. The positive amplicons were further purified and sequenced on both strands using the GenomeLab GeXP sequencer and kit (Beckman Coulter, Germany). The chromatograms obtained were visualized and edited using BioEdit Sequence Alignment Editor version 7.2.5 [27].

Mutations/alterations in domain V of the 23S rRNA and/or ribosomal proteins gene sequences were detected by alignment with appropriate reference linezolid-susceptible (wild type sequence) *E. faecalis* ATCC 29212 (CP008816.1) or *E. faecium* DO (CP003583.1) with the aid of the BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in the GenBank database. Similarly, the OptrA amino acid sequences obtained were aligned to the originally described *optrA* gene sequence from *E. faecalis* E349 (KP399637.1) [28].

2.7. Accession numbers

The nucleotide sequences from the *optrA*, *poxtA*, *cfr*, *rplD* (L4), *rplC* (L3) and *rplV* (L22) genes of the isolates in this study have been deposited in the NCBI GenBank database under accession numbers OQ122146-OQ122167, OQ117757-OQ117759, OQ117760-OQ117761, OQ289005-OQ289025, OQ289026-OQ289054, and OQ289055-OQ289074, respectively.

3. Results

During the study period, enterococci were recovered only on florfenicol-supplemented plates from 67 of the 630 collected faecal samples. MALDI-TOF analyses identified 64 of the 67 isolates as enterococci distributed into four different species, of which *E. faecalis* (41/64) and *E. faecium* (20/64) dominated in both animals (23/37 vs. 13/37) and humans (18/27 vs. 7/27) (Supplementary Table S1).

The linezolid MICs of the 64 isolates ranged from 1 to ≥256 µg/mL (MIC_{50} = ≤ 6 µg/mL; MIC_{90} = ≤16 µg/mL) (Supplementary

Table 2
Amino acid sequence alterations detected in the *optr*A protein sequences of the linezolid-resistant enterococci recovered from humans and animals in Nigeria.

Strain	% Similarity[a]	Observed mutations
NGEH2	98.8	G405, T481P
NGEH6	98.5	G349S, T481P
NGEH17	98.3	G375E, F457N
NGEH18	99.5	T481P
NGE2	96.5	G393D
NGE13	99.2	R340K, K569I
NGE14	99.6	G393D
NGE17	99.6	G393D
NGE19	98.2	Q338H, T481P
NGE21	99.0	L374T, G393D
NGE29	98.6	L361R, A362S, T481P
NGE31	97.7	L361P, T481P, E564S, R565Q
NGE6EW	99.5	G393D
NGE21EW	99.1	L348F, K373R
NGE31EW	98.8	R370K, G393D

[a] % identity with reference gene, *optrA E. faecalis* E349 (KP399637.1).

Table S1). Of the 64 isolates recovered, 37 (11.6%; 95% CI: 8.6 – 15.6) were linezolid resistant with MICs >4 µg/mL (range: 6–≥256 µg/mL) (Table 1). However, no VRE-resistant strain was recovered (i.e., none of the samples were positive for vancomycin-resistant enterococci).

The distribution of LRE comprised cattle (11/85), dogs (5/50), pigs (6/125), shrews (6/40), and humans (9/180). No LRE was recovered from poultry sources. Similarly, all LRE isolates tested expressed co-resistance to tedizolid (MIC >0.5 µg/mL; range: 1.5–4 µg/mL) (Table 1, Supplementary Table S1). Of note, none of the isolates were resistant to vancomycin (MIC_{range}: 0.38–4 µg/mL; MIC_{50} = ≤0.75 µg/mL; MIC_{90} = ≤ 2 µg/mL) (Table 1, Supplementary Table S2).

Acquisition of potentially transferable linezolid resistance genes *cfr, optrA*, and *potxA* was detected in most of the LRE isolates, with the *optr*A gene being the predominantly occurring resistance determinant (26/37). The *optr*A gene occurred alone (n = 21) or in coexistence with *cfr* (n = 1), *poxt*A (n = 1), or in combination with *cfr* and *poxt*A (n = 4) genes. Coexistence of more than one resistance determinant in a single isolate was observed only among *E. faecium* isolates originating from pigs (n = 3) and cattle (n = 3) (Table 1).

A 98 to 99% sequence homology between the *optr*A genes of isolates in this study and the first reported *optr*A gene sequence (*E. faecalis:* GenBank AKA86814.1) was observed. The amino acid substitutions, T481P and G393D, were the most frequently detected alterations in the *optr*A gene sequence (Table 2).

No known point mutation(s) associated with linezolid resistance were detected in the V domain of the 23S rRNA. However, multiple mutations were detected in the ribosomal protein (L3, L4, and L22) genes. (Table 3).

4. Discussion

Emergence of resistance to last resort drugs is a serious therapeutic concern, especially in countries of sub–Saharan Africa, including Nigeria, faced with the high burden and negative effect of antimicrobial resistance [1]. In this study we observed an LRE carriage rate of ca. 5% and 6% for humans and animals, respectively, providing evidence for the emergence of LRE in spite of the rare consumption or usage of linezolid in Nigeria. The carriage rate is comparable to that reported from other parts of the globe [9,13,29,30]. Interestingly, all the isolates were susceptible to vancomycin. This observation may be attributed to the rare use of vancomycin or its analogues in veterinary and human medicine in the country and provides an alternative for treatment in the event of MDR enterococci infection.

Mutations, particularly the G2576 substitution in V domain of the 23S rRNA, have been identified as major mechanisms responsible for linezolid resistance and are associated with prior linezolid or tedizolid exposure [31]. Similar to reports from North African countries and other parts of the world, none of the isolates in this study harboured the relevant mutations in V domain of the 23S rRNA gene mediating linezolid resistance [32]. To our knowledge, none of the patients or animals in this study had prior linezolid exposure, implying that the linezolid resistance detected may have emerged independently of oxazolidinone consumption and is likely a result of co-selection (selective pressure) from the widespread use of other antimicrobials, including phenicols, tiamulin, and tetracyclines [33].

Multiple substitutions not previously reported were observed in ribosomal proteins L3, L4, and L22 genes in this study. However, the role of these substitutions in linezolid resistance remains unknown and requires further expression-level analysis, including transcriptomics and complementation assays, to clarify their role in the observed linezolid resistance phenotype.

All three currently known transferable linezolid resistance genes, *cfr, optrA* and *poxtA*, were detected in this study. Consistent with recently increasing reports across Europe, Asia, and North America, the *optr*A resistance gene was the predominantly detected mechanism for the observed linezolid resistance [8–12,34]. This observation, in addition to depicting the success of this resistant genotype, suggests the likelihood of a change or shift in the epidemiology of LRE from the previously known predominance of mutational mechanisms to transferable resistance genes, particularly the *optrA* gene. We observed similarities between our findings and those from North Africa, particularly in the detection of the *optrA* and *poxtA* genes and absence of mutations in the 23S rRNA and ribosomal protein genes [12–15]. However, in contrast to our findings and to the best of our knowledge, none of the studies from the North African countries have yet reported the detection of the *cfr*- gene.

Since its first detection, the *optrA* gene has spread to almost all continents, conferring resistance to linezolid and tedizolid, in agreement with our findings. Several genetic variants of the *optr*A gene have been reported; however, similar to our findings, the relationships between these variants and increased or reduced linezolid MICs remains controversial [34]. Coincidently, previously reported mutations in the *optr*A variants from international collections, particularly the T481P and G393D substitutions, were detected among our isolates. This further supports evidence for the expansion and successful spread of these mutations/variants globally. The observed 45 to 100% similarity between our *optr*A sequence and the original reported by Wang et al. [28] may suggest the existence of multiple variant(s) among our collection. Unfortunately, because of the short sequence length (partial sequence) of our *optr*A gene, we could not, with certainty, assign them to any defined *optr*A variant groups as described by Cai et al. [34] and Bender et al. [35].

Co-occurrence of the *optrA* and other genes, including *cfr* [36], *poxtA* gene, [37] and simultaneous co-existence of all three genes, in *E. faecalis* and *E. gallinarum* of human and animal origin have been described [38]. However, our study describes, for the first time, the co-existence of all three genes in *E. faecium* of animal origin and the emergence of *cfr-, optrA-*, and *poxt*A-carrying enterococci in humans and animals in Nigeria. These findings further highlight the potential role that animal production settings may play in the emergence and dissemination of linezolid-resistant enterococci.

Noteworthy in this study is the detection of linezolid resistance among enterococci originating from shrews captured around hu-

Table 3
Amino acid sequence alterations detected in the ribosomal protein gene sequences (in comparison with reference gene sequences) of the linezolid-resistant enterococci recovered from humans and animals in Nigeria.

Strains	% Similarity[a]	Observed mutations in the respective genes
rplC		
NGEH2	99.5	I23F
NGEH6	99.0	E29D
NGEH17	96.4	L22V, I23F, P24G, V25R, V27A, E29Q, A30V
NGEH21	99.5	T17H
NGEH23	99.4	I23F
NGE2	99.0	T17N, L22F
NGE7	99.5	K196I
NGE11	99.5	T18K
NGE12	97.9	Q14G, L22F, V149I, A150P
NGE13	99.0	V149I, A150P
NGE14	99.5	I23F
NGE15	99.0	V149I, A150P
NGE19	99.5	L22F
NGE20	98.5	T2I, V149I, A150P
NGE26	98.5	T31P, V149I, A150P
NGE27	98.4	I23V, T26N, A30D
NGE28	98.5	T40N, E42G
NGE6EW	96.0	Q14H, V27E, E29Q, P32T, V35I, L36I, E182G
NGE23EW	99.0	T17N
NGE31EW	99.0	G11A, Q14R
rplD		
NGEH11	94.4	K8I, T18P, F24I, L129V, R168S, N169Y, S178P, D185Y, A189T, K191S, Q201R
NGEH17	94.4	I26L, E138K
NGEH23	99.5	Q9P
NGE8	98.5	E22D, L156V
NGE11	99.5	K8N
NGE15	99.0	K8N, E27A
NGE19	92.9	S31T, V38I, E120S, I126V, N130S, S147T, K158T, G159E, N172S, S178A, N189E, T190I, A194V
NGE21	99.5	V186D
NGE26	99.5	E27A
NGE21EW	99.5	P28T
NGE23EW	99.0	Q9P, I23C
rplV		
NG6EW	99.1	T113S
NGE15EW	85.9	A10G, T14K, R16S, S18P, P19H, A22E, L28I, S33R, A35E, I38F, I40F, A49T, S58L, A61D, S73R
NGE31EW	91.5	R16G, S73R, S77F, E78G, F80I, P92R, K103R

[a] % identity with reference gene, *rplC*, *rplD*, or *rplV* (*E. faecium* DO CP003583.1 or *E. faecalis* ATCC29212 CP008816.1, depending on the species of the isolate).

man habitations, raising questions and concerns on the extent of dispersal of this resistance phenotype.

Congruent to a report from Germany by Bender et al. [34], only among isolates with linezolid MICs ≥8 µg/mL were linezolid resistance determinants detected. Eleven of the isolates in our study with linezolid MICs of 6µg/mL categorized as resistant based on the EUCAST guidelines, but susceptible based on the CLSI guidelines, completely lacked any currently known linezolid resistance genes. This contrasts to reports from Asia, particularly China, where the *optr*A or *poxt*A genes were detected among linezolid-susceptible isolates, including isolates with linezolid MICs as low as 2 µg/mL [14,28,35]. This discrepancy and low-range MIC phenomenon have been reported to create inconsistencies that make the identification and reporting of LRE isolates difficult and suggest the presence and role of other yet to be known mechanisms in low level linezolid resistance [39]. It is also plausible that this observed phenotype may be as a result of other linezolid resistance mechanisms, including presence of variant *cfr* genes *cfr*(B) and *cfr*(D) [40,41], biofilm formation and cell wall thickening [42], and over-expression of efflux-pump systems [43], which were not investigated in this study.

A limitation of this study was the lack of sufficient genomic data to investigate the genetic backbone/environment of the resistance genes detected and the relationship between the detected isolates for evidence of transmission between humans and animals or an ongoing outbreak/intra hospital spread (in the case of human isolates). Other limitations included our inability to investigate the isolates for the presence of other linezolid resistance mechanisms [including variants *cfr*(B) and *cfr*(D) genes and or cell wall thickening], which may have resulted in underestimation of the occurrence of resistant isolates. Moreover, we did not employ PCR for detection of *vanA/B/C* genes that might confer vancomycin resistance, as not all VRE strains can be detected using E-tests and samples were only collected from one area in Nigeria.

In conclusion, our work underscores that linezolid resistance in enterococci driven majorly by the acquisition of potentially transferable genes has emerged in Nigeria independently of linezolid consumption. Our findings raise awareness of and concerns on the circulation of LRE, particularly as it pertains to the treatment of MDR enterococci or staphylococci. It further echoes the need to prioritize antimicrobial stewardship and active surveillance to understand the extent of LRE spread and how the current antimicrobial use practices in humans and animals in the country influence the emergence and dissemination of these strains.

Funding: E.O.N. was supported by the Cooperation Visits Programme of The World Academy of Sciences and Deutsche Forschungsgemeinschaft (TWAS-DFG) (BE 6419/1–1).

Competing interests: None declared

Ethical approval: Approval was obtained from the Institutional Ethics and Research Committee (FMH/FMC/HRE/VOL/I) and the Animal Research Ethics Committee (AEC/03/80/20). Only permission from the farmers and owners (which was obtained) was required for sampling of domestic animals and at the slaughter facilities.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.07.016.

References

[1] Antimicrobial Resistance CollaboratorsGlobal burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022;399:629–55. doi:10.1016/S0140-6736(21)02724-0.

[2] Knight GM, Glover RE, McQuaid CF, Olaru LD, Gallandat K, Leclerc QJ, et al. Antimicrobial resistance and COVID-19: intersections and implications. eLife 2021;10:e64139. doi:10.7554/eLife.64139.

[3] Ament PW, Jamshed N, Horne JP. Linezolid: its role in the treatment of gram-positive, drug-resistant bacterial infections. Am Fam Physician 2002;65:663–70.

[4] Mendes RE, Deshpande LM, Jones RN. Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat 2014;17:1–12. doi:10.1016/j.drup.2014.04.002.

[5] Eliopoulos GM, Meka VG, Gold HS. Antimicrobial resistance to linezolid. Clin Infect Dis 2004;39:1010–15. doi:10.1086/423841.

[6] Brinkwirth S, Ayobami O, Eckmanns T, Markwart R. Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. Euro Surveill 2021;26:2001628. doi:10.2807/1560-7917.ES.2021.26.45.2001628.

[7] World Health Organization Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis, Geneva: World Health Organization; 2017. WHO/EMP/IAU/2017.12.

[8] Freitas AR, Finisterra L, Tedim AP, Duarte B, Novais C, Peixe Lfrom the ESCMID Study Group on Food- and Water-borne Infections (EFWISG). Linezolid- and multidrug-resistant enterococci in raw commercial dog food, Europe, 2019-2020. Emerg Infect Dis 2021;27:2221–4. doi:10.3201/eid2708.204933.

[9] Timmermans M, Bogaerts B, Vanneste K, De Keersmaecker SCJ, Roosens NHC, Kowalewicz C, et al. Large diversity of linezolid-resistant isolates discovered in food-producing animals through linezolid selective monitoring in Belgium in 2019. J Antimicrob Chemother 2021;77:49–57. doi:10.1093/jac/dkab376.

[10] Dadashi M, Sharifian P, Bostanshirin N, Hajikhani B, Bostanghadiri N, Khosravi-Dehaghi N, et al. The global prevalence of daptomycin, tigecycline, and linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains from human clinical samples: a systematic review and meta-analysis. Front Med (Lausanne) 2021;8:720647. doi:10.3389/fmed.2021.720647.

[11] Huang J, Wang M, Gao Y, Chen L, Wang L. Emergence of plasmid-mediated oxazolidinone resistance gene *poxt*A from CC17 *Enterococcus faecium* of pig origin. J Antimicrob Chemother 2019;74:2524–30. doi:10.1093/jac/dkz250.

[12] Freitas AR, Tedim AP, Duarte B, Elghaieb H, Abbassi MS, Hassen A, et al. Linezolid-resistant (Tn6246::fexB-poxtA) *Enterococcus faecium* strains colonizing humans and bovines on different continents: similarity without epidemiological link. J Antimicrob Chemother 2020;75:2416–23. doi:10.1093/jac/dkaa227.

[13] Elghaieb H, Freitas AR, Abbassi MS, Novais C, Zouari M, Hassen A, et al. Dispersal of linezolid-resistant enterococci carrying *poxt*A or *optr*A in retail meat and food-producing animals from Tunisia. J Antimicrob Chemother 2019;74:2865–9. doi:10.1093/jac/dkz263.

[14] Freitas AR, Elghaieb H, León-Sampedro R, Abbassi MS, Novais C, Coque TM, et al. Detection of *optrA* in the African continent (Tunisia) within a mosaic *Enterococcus faecalis* plasmid from urban wastewaters. J Antimicrob Chemother 2017;72:3245–51. doi:10.1093/jac/dkx321.

[15] Raddaoui A, Chebbi Y, Tanfous FB, Mabrouk A, Achour W. First description of clinical linezolid resistant Enterococcus sp. in North Africa. J Glob Antimicrob Resist 2020;21:169–70. doi:10.1016/j.jgar.2020.04.012.

[16] Ikhimiukor OO, Odih EE, Donado-Godoy P, Okeke IN. A bottom-up view of antimicrobial resistance transmission in developing countries. Nat Microbiol 2022;7:757–65. doi:10.1038/s41564-022-01124-w.

[17] Manero A, Blanch AR. Identification of *Enterococcus* spp. with a biochemical key. Appl Environ Microbiol 1999;65:4425–30. doi:10.1128/AEM.65.10.4425-4430.1999.

[18] Schwaiger K, Schmied EM, Bauer J. Comparative analysis on antibiotic resistance characteristics of *Listeria* spp. and *Enterococcus* spp. isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. Zoonoses Public Health 2010;57:171–80. doi:10.1111/j.1863-2378.2008.01229.x.

[19] The European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters; 2023. Version 13.0 http://www.eucast.org .

[20] . Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2021. p. 2021. *CLSI Document M100-ED31*.

[21] Bourgeois-Nicolaos N, Massias L, Couson B, Butel MJ, Andremont A, Doucet-Populaire F. Dose dependence of emergence of resistance to linezolid in *Enterococcus faecalis* in vivo. J Infect Dis 2007;195:1480–8. doi:10.1086/513876.

[22] Diaz L, Kiratisin P, Mendes RE, Panesso D, Singh KV, Arias CA. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. Antimicrob Agents Chemother 2012;56:3917–22. doi:10.1128/AAC.00419-12.

[23] Lee SM, Huh HJ, Song DJ, Shim HJ, Park KS, Kang CI, Ki CS, Lee NY. Resistance mechanisms of linezolid-nonsusceptible enterococci in Korea: low rate of 23S rRNA mutations in *Enterococcus faecium*. J Med Microbiol 2017;66:1730–5. doi:10.1099/jmm.0.000637.

[24] Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. Antimicrob Agents Chemother 2006;50:1156–63. doi:10.1128/AAC.50.4.1156-1163.2006.

[25] Shang Y, Li D, Shan X, Schwarz S, Zhang SM, Chen YX, et al. Analysis of two pheromone-responsive conjugative multiresistance plasmids carrying the novel mobile *optrA* locus from *Enterococcus faecalis*. Infect Drug Resist 2019;12:2355–62. doi:10.2147/IDR.S206295.

[26] Lei CW, Kang ZZ, Wu SK, Chen YP, Kong LH, Wang HN. Detection of the phenicol-oxazolidinone-tetracycline resistance gene *poxt*A in *Enterococcus faecium* and *Enterococcus faecalis* of food-producing animal origin in China. J Antimicrob Chemother 2019;74:2459–61. doi:10.1093/jac/dkz198.

[27] Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT. Nucleic Acids Symposium Series 1999;41:95–8.

[28] Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, et al. A novel gene, *optr*A, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. J Antimicrob Chemother 2015;70:2182–90. doi:10.1093/jac/dkv116.

[29] Egan SA, Shore AC, O'Connell B, Brennan GI, Coleman DC. Linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* from hospitalized patients in Ireland: high prevalence of the MDR genes *optr*A and *poxt*A in isolates with diverse genetic backgrounds. J Antimicrob Chemother 2020;75:1704–11. doi:10.1093/jac/dkaa075.

[30] Zhang Y, Dong G, Li J, Chen L, Liu H, Bi W, Lu H, Zhou T. A high incidence and coexistence of multiresistance genes *cfr* and *optr*A among linezolid-resistant enterococci isolated from a teaching hospital in Wenzhou, China. Eur J Clin Microbiol Infect Dis 2018;37:1441–8. doi:10.1007/s10096-018-3269-8.

[31] Olearo F, Both A, Belmar Campos C, Hilgarth H, Klupp EM, Hansen JL, et al. Emergence of linezolid-resistance in vancomycin-resistant *Enterococcus faecium* ST117 associated with increased linezolid-consumption. Int J Med Microbiol 2021;311:151477. doi:10.1016/j.ijmm.2021.151477.

[32] Tamang MD, Moon DC, Kim SR, Kang HY, Lee K, Nam HM, et al. Detection of novel oxazolidinone and phenicol resistance gene *optrA* in enterococcal isolates from food animals and animal carcasses. Vet Microbiol 2017;201:252–256. doi:10.1016/j.vetmic.2017.01.035.

[33] Fioriti S, Coccitto SN, Cedraro N, Simoni S, Morroni G, Brenciani A, et al. Linezolid resistance genes in enterococci isolated from sediment and zooplankton in two Italian coastal areas. Appl Environ Microbiol 2021;87:e02958 20. doi:10.1128/AEM.02958-20.

[34] Cai J, Wang Y, Schwarz S, Lv H, Li Y, Liao K, et al. Enterococcal isolates carrying the novel oxazolidinone resistance gene *optrA* from hospitals in Zhejiang, Guangdong, and Henan, China, 2010-2014. Clin Microbiol Infect 2015;21:1095 e1-4. doi:10.1016/j.cmi.2015.08.007.

[35] Bender JK, Fleige C, Lange D, Klare I, Werner G. Rapid emergence of highly variable and transferable oxazolidinone and phenicol resistance gene *optr*A in German *Enterococcus* spp. clinical isolates. Int J Antimicrob Agents 2018;52:819–27. doi:10.1016/j.cmi.2015.08.007.

[36] Chen L, Han D, Tang Z, Hao J, Xiong W, Zeng Z. Co-existence of the oxazolidinone resistance genes *cfr* and *optrA* on two transferable multi-resistance plasmids in one *Enterococcus faecalis* isolate from swine. Int J Antimicrob Agents 2020;56:105993. doi:10.1016/j.ijantimicag.2020.105993.

[37] Ruiz-Ripa L, Feßler AT, Hanke D, Sanz S, Olarte C, Eichhorn I, et al. Detection of *poxtA*- and *optrA*-carrying *E. faecium* isolates in air samples of a Spanish swine farm. J Glob Antimicrob Resist 2020;22:28–31. doi:10.1016/j.jgar.2019.12.012.

[38] Coccitto SN, Cinthi M, Fioriti S, Morroni G, Simoni S, Vignaroli C, et al. Linezolid-resistant *Enterococcus gallinarum* isolate of swine origin carrying *cfr, optrA* and *poxtA* genes. J Antimicrob Chemother 2022;77:331–7. doi:10.1093/jac/dkab408.

[39] Mališová L, Jakubů V, Pomorská K, Musílek M, Žemličková H. Spread of linezolid-resistant *Enterococcus* spp. in human clinical isolates in the Czech Republic. Antibiotics (Basel) 2021;10:219. doi:10.3390/antibiotics10020219.

[40] Bender JK, Fleige C, Klare I, Fiedler S, Mischnik A, Mutters NT, et al. Detection of a *cfr*(B) variant in German *Enterococcus faecium* clinical isolates and the impact on linezolid resistance in *Enterococcus* spp. PLoS One 2016;11:e0167042. doi:10.1371/journal.pone.0167042.

[41] Ruiz-Ripa L, Feßler AT, Hanke D, Eichhorn I, Azcona-Gutiérrez JM, Pérez-Moreno MO, et al. Mechanisms of linezolid resistance among enterococci of clinical origin in Spain-Detection of *optrA*- and *cfr*(D)-carrying *E. faecalis*. Microorganisms 2020;8:1155. doi:10.3390/microorganisms8081155.

[42] Tian Y, Li T, Zhu Y, Wang B, Zou X, Li M. Mechanisms of linezolid resistance in staphylococci and enterococci isolated from two teaching hospitals in Shanghai, China. BMC Microbiol 2014;14:292. doi:10.1186/s12866-014-0292-5.

[43] Hua R, Xia Y, Wu W, Yan J, Yang M. Whole transcriptome analysis reveals potential novel mechanisms of low-level linezolid resistance in *Enterococcus faecalis*. Gene 2018;647:143–9. doi:10.1016/j.gene.2018.01.008.