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LETTER TO THE EDITOR

Detection of *optrA*-positive enterococci clinical isolates in Belgium

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Dear Editor,

Linezolid, the first member of the oxazolidinone family, is one of the major antimicrobial agents used for the treatment of vancomycin-resistant enterococcus (VRE) infections. This antibiotic binds to the domain V of the 23S ribosomal RNA (rRNA) of the 50S subunit inhibiting the bacterial protein synthesis. Diverse mechanisms conferring linezolid resistance have been described in enterococci, including point mutations in genes encoding 23S rRNA, mutations in ribosomal proteins L3 and/or L4, or the presence of the plasmid-borne ribosomal methyltransferase gene *cfr* or its variant *cfr*(B) [1, 2]. The new variant *cfr*(C) has only been described in *Clostridium* [3] and *Campylobacter* [4]. Recently, new oxazolidinone resistance genes coding for an ABC transporter (*optrA*) and for an ARE ABC-F family protein (*poxtA*) have been described. Both genes have been detected in plasmids and/or transposons in enterococci and/or staphylococci of human and animal origin [5–9]. Most reports about *optrA* in enterococcus clinical samples are from China [10], while only few reports have been published from European countries, including Austria [11], Denmark [12], France [13], Germany [14], Ireland [6], Italy [15, 16], Poland [17], Spain [18], Sweden [19] and UK [20]. Therefore, data about the prevalence of this gene in nosocomial settings in Europe are limited.

In Belgium, the prevalence of *optrA* among enterococci is unknown, although an *optrA*-positive *Enterococcus faecalis* has been detected in an elderly patient attending the University Hospital of Antwerp and was reported in a recent conference communication [21]. The aim of this study was to investigate the presence of linezolid-resistant genes among linezolid non-susceptible enterococci isolated from clinical samples collected at Erasme Hospital (Brussels, Belgium) between 2014 and June 2018.

During the study period, a total of 7331 enterococci were isolated from clinical samples of 3726 patients. Antimicrobial susceptibility was determined by using Vitek2 AST P586 (bioMérieux) for the following antibiotics: ampicillin, ampicillin-sulbactam, clindamycin, erythromycin, gentamicin, imipenem, linezolid, moxifloxacin, nitrofurantoine, penicillin, streptomycin, teicoplanin, tetracycline, tigecycline and vancomycin. Linezolid minimal inhibitory concentrations (MICs) were further determined on resistant strains by E-test (bioMérieux). EUCAST breakpoints were used for interpretation [22]. Only 29 (0.8%) patients carried linezolid non-susceptible enterococci (21 *E. faecalis*, 8 *E. faecium*) including full ($n = 12$) and intermediate ($n = 17$) resistance against linezolid. Out of these 29 isolates, ten *E. faecalis* (including 2 strains from the same patient) and seven *E. faecium* (including 2 strains from the same patient) were stored and available for further characterisation. These 17 isolates were analysed for the presence of *cfr*, *cfr*(C) and *optrA* genes by using oligonucleotides previously described [4, 10, 23]. Specific detection of *cfr*(B) and *poxtA* was performed by using the primers cfr(B)-Fw1 (5′ TGCTTTAAGTCCGCGTAGG3′), cfr(B)-Rv1 (5′ CTTCTTCAAACCGCATCCG3′) poxtA-F (5′ GTACAAGCGTGTGAAGATGG3′) and poxtA-R (5′ CCACAAAGGATGGGTTATGG3′) designed for this study. The *optrA*-positive isolates were subjected to MLST [24, 25].

None of the 17 isolates screened carried *cfr*, *cfr*(B), *cfr*(C) or *poxtA* genes, but five (29.4%) carried the recently described *optrA* gene: four *E. faecalis* and one *E. faecium* (Table 1). The two *E. faecium* isolates recovered from the same patient were *optrA*-negative. Interestingly, for the other patient with two isolates, the first *E. faecalis* isolate was *optrA*-negative (urine sample, April 2017), while the second (urine sample,

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Table 1 Characteristics of the enterococci carrying *optrA*

Strain	Month and year of samples	Source	Patient (sex and birth date)	Species	CMI linezolid (mg/L)	MLST
1	November, 2014	Urine	F, 14-05-1952	*E. faecalis*	8	ST474
2	August, 2016	Urine	F, 12-10-1943	*E. faecalis*	16	ST480
3	July, 2017	Rectal smear	M, 01-04-1961	*E. faecalis*	16	ST16
4[a]	December, 2017	Urine	F, 11-03-1973	*E. faecalis*	32	ST480
5	May, 2018	Blood	F, 27-01-1955	*E. faecium*	24	ST202

F feminine, *M* masculine

[a] The patient with this *optrA*-positive *E. faecalis* ST480 isolate, carried 6 months before a linezolid-resistant *optrA*-negative *E. faecalis* ST480 strain

December 2017) was *optrA*-positive. Both strains revealed to belong to the same ST (ST480) (Table 1, isolate no. 4).

Our first *optrA*-positive *E. faecalis* isolate (Table 1, no. 1) was isolated in December 2014 from a urine sample, a year before the first description of this gene [5]. The patient had been previously treated with nitrofurantoin and with ciprofloxacin by her general practitioner within 10 days before the sample was taken. The isolate belonged to ST474, a clone that was first reported in an animal from China in 2011 [26]. But recently, ST474 *optrA*-positive clinical isolates have been detected in a Spanish teaching hospital [18].

The *optrA*-positive *E. faecalis* isolates no. 2 and no. 4 were recovered from patients presenting asymptomatic bacteriuria, one patient was suffering of kidney stones (no. 2) and the other (no. 4) was a kidney transplant recipient. Both isolates belonged to the clone ST480. ST480 clinical isolates have been detected first in Spain and in France in 2011 [26]. Recently, ST480 *optrA*-positive isolates have been detected in Germany [14]; and the unique Belgian *E. faecalis optrA*-positive previously described also belongs to ST480 clone [21]. ST480 *E. faecalis* isolates were also reported in wastewater and surface water samples from Tunisia [27].

The *optrA*-positive *E. faecalis* isolate no. 3 was obtained from a rectal screening sample of a patient with metastatic rectal adenocarcinoma. This isolate belonged to ST16. ST16 *optrA*-positive isolates were previously reported in China [28] and Denmark [12].

The unique *optrA*-positive *E. faecium* isolate (no. 5) was recovered from blood cultures drawn from a patient suffering from an acute myeloid leukaemia under chemotherapy. The bacteraemia was probably due to bacterial translocation from the digestive tract. This patient was treated with vancomycin, which was switched to linezolid then to tigecycline because of the emergence of a renal dysfunction. The treatment with tigecycline was maintained for 14 days. This isolate belonged to ST202, which is considered as a major hospital-associated lineage [29].

In conclusion, our study shows that *optrA* gene is detected in linezolid non-susceptible enterococci isolated from clinical samples collected in our Belgian hospital. We were able to demonstrate that the *optrA* gene was already present in our institution in 2014, prior to its description in 2015 [5]. Although, the number of enterococcus isolates investigated in this study was limited, the *optrA* gene was found in enterococci belonging to different clones. Its presence in different lineages is concordant with the fact that this gene has been described on mobile genetic elements (mainly plasmids, but also transposons). The *optrA* gene has been found at a higher frequency in isolates from animal origin than from human origin and its prevalence is higher in *E. faecalis* than in *E. faecium* [1]. Nevertheless, *optrA* clinical *E. faecium* isolates have been described. Furthermore, an outbreak of vancomycin-resistant *cfr*- and *optrA*-positive *E. faecium* has recently been reported in an Irish hospital [6]. Further studies are needed to determine the exact prevalence of this gene among enterococci in nosocomial settings, to evaluate its potential transmission to vancomycin-resistant *E. faecium* and its impact on the treatment strategies of patients infected with isolates resistant to both glycopeptides and linezolid. Meanwhile, vigilance is required to avoid linezolid-resistant VRE to become a major nosocomial pathogen.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study formal consent is not required.

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