Enterococcal isolates carrying the novel oxazolidinone resistance gene *optrA* from hospitals in Zhejiang, Guangdong, and Henan, China, 2010–2014

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Abstract

A collection of 1159 enterococcal isolates from five Chinese hospitals were screened for the presence of the novel oxazolidinone resistance gene *optrA*, which was found in 34 (2.9%) isolates. Pulsed-field gel electrophoresis (PFGE) typing of 29 *optrA*-carrying *Enterococcus faecalis* isolates revealed 25 PFGE patterns, and multilocus sequence typing yielded 20 sequence types. Routine surveillance of *optrA*-positive enterococci in hospitals should be conducted to monitor and counteract their further dissemination. The data of this study may be used as a baseline from which to judge future decreases or increases in *optrA*-positive enterococci.

Keywords: Antimicrobial resistance, enterococci, linezolid, molecular epidemiology, nosocomial infection, phenicols, tedizolid
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As last-resort antimicrobial agents for the control of clinical infections caused by vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*, oxazolidinones have shown potent activity against most Gram-positive pathogens [1,2]. Mutations or modifications of the target site within the peptidyl transferase centre of the 50S ribosomal subunit can result in resistance to oxazolidinones. Alterations in domain V of the 23S rRNA, most frequently seen as a G2576T mutation, constitute the main oxazolidinone resistance mechanism in enterococci [3,4]. In addition, acquisition of the *cfr* gene, which encodes a methyltransferase that modifies the adenine at position 2503 in the peptidyl transferase centre and thereby confers combined resistance to oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A antibiotics, has been detected in enterococci and other Gram-positive and Gram-negative bacteria [5,6].

Recently, the new oxazolidinone resistance gene *optrA* has been identified in *Enterococcus faecalis* and *Enterococcus faecium* isolates of human and animal origin [7]. This gene codes for an ATP-binding cassette transporter that results in resistance or elevated MICs for oxazolidinones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol), and was found in 2.0% (12/595) of the tested clinical *E. faecalis* and *E. faecium* isolates collected from the Second Affiliated Hospital of Zhejiang University, during 1998–2014 in Hangzhou, China [7]. However, the exact mechanism by which this ATP-binding cassette transporter confers resistance to oxazolidinones and phenicols is not fully understood, as it lacks a membrane component. The *optrA* gene is the first identified transferable gene that results in an elevated MIC for tedizolid, a recently approved novel oxazolidinone with improved activity against linezolid-resistant isolates carrying *cfr* [8,9]. The emergence of *optrA* in clinical enterococci is of great concern, as the spread of this gene could significantly limit the treatment options for vancomycin-resistant enterococci. To obtain a better understanding of the presence of *optrA* in enterococci of human clinical origin, we screened enterococci derived from hospitalized patients in China for the presence of *optrA*, and also investigated the molecular epidemiology of *optrA*-carrying isolates.

A total of 1159 non-duplicate enterococcal isolates were randomly collected from five hospitals in five different cities of China from 2010 to 2014 (Table S1). These isolates are different from the enterococcal isolates previously reported [7]. Species identification was performed with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonik, Bremen, Germany). Isolates were obtained from urine (62.9%), body fluid (20.5%, including hydrothorax, ascites, bile, cerebrospinal fluid, and prostate fluid), secretions

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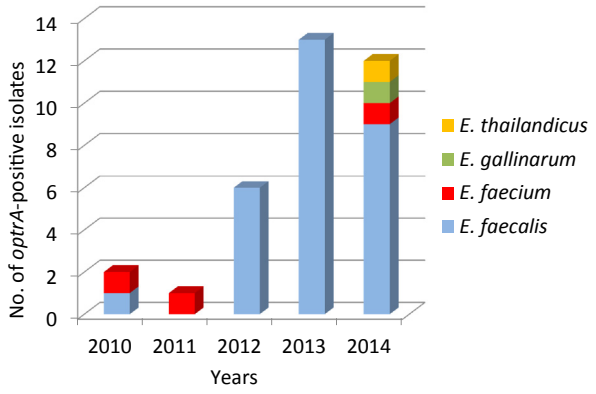

FIG. 1. Year-by-year comparison of the presence of *optrA*-positive enterococcal isolates.

(11.1%, including pus, wound secretions, and secretions or drainage of operation incision), and blood (5.5%). Urology wards were the most common sources of samples (29.4%), followed by hepatopancreatobiliary surgery wards (15.4%) and intensive-care units (8.2%). Colonizing enterococcal isolates from faecal or anal/rectal samples were not included in this study.

All enterococcal isolates were inoculated on Columbia agar plates (containing 5% v/v sheep blood) supplemented with 10 mg/L florfenicol, as the two transferable oxazolidinone resistance genes known to date, *optrA* and *cfr* [6,7], also confer resistance to chloramphenicol and florfenicol. Isolates growing on the selective medium were screened for the presence of 23S rRNA mutations, *optrA* and *cfr*, and the phenicol exporter genes *fexA* and *fexB*, with specific PCR assays and sequence analysis of the corresponding amplicons [7]. For *optrA*-positive isolates, MICs were determined by broth microdilution according to the recommendations of the CLSI [10,11]. Molecular typing was performed by analysis of pulsed-field gel electrophoresis (PFGE) profiles of *Sma*I-digested chromosomal DNA [12] and multilocus sequence typing, as described previously [13,14].

Among the 1159 enterococcal isolates tested, 38 (3.3%) grew on florfenicol-supplemented medium, and 34 of 38 (89.5%) were positive for *optrA* (Table S1). Thirty-two of the 34 *optrA*-positive isolates also carried the phenicol resistance genes *fexA* and/or *fexB*. The remaining four florfenicol-resistant but

TABLE 1. Clinical characteristics and molecular typing of 34 *optrA*-positive enterococcal isolates

Isolate	Hospital	Year	Sample	Sex	Age (years)	Ward	*optrA* variant[a]	LZD MIC (mg/L)	PFGE	MLST[b]	CC[c]
Enterococcus faecalis											
SR12140	A	2012	Prostate fluid	M	53	Urology	RDK	8	A	ST207	CC28
SR12155	A	2012	Urine	M	41	Urology	DP	4	B	ST632	CC96
SR12179	A	2012	Urine	M	86	Vasculocardiology	DP	4	C1	ST476	CC476
SR12188	A	2012	Urine	F	29	Urology	DP	4	D	ST49	CC49
SR12205	A	2012	Urine	F	51	Urology	EDM	8	E	ST59	CC59
SR12219	A	2012	Wound secretion	M	29	Hand surgery	W	8	C2	ST476	CC476
SR136	A	2013	Blood	F	71	Haematology	W	4	F	ST655	None
SR139	A	2013	Urine	M	75	Urology	W	4	F	ST655	None
SR1311	A	2013	Urine	M	81	Urology	W	4	G	ST619	CC81
SR1341	A	2013	Urine	M	79	ICU	W	8	H	ST81	CC81
SR1382	A	2013	Ascites	M	71	Anorectal	EDD	4	I	ST192	None
SR13118	A	2013	Urine	M	84	Vasculocardiology	KD	8	J1	ST16	CC16
SR13158	A	2013	Urine	M	83	Urology	W	8	L	ST585	CC4
SR13179	A	2013	Urine	F	66	Urology	KD	8	J2	ST16	CC16
SR13213	A	2013	Urine	F	39	Urology	DP	4	K	ST16	CC16
SR13217	A	2013	Urine	M	86	Vasculocardiology	DP	8	M	ST480	None
SR13293	A	2013	Urine	F	24	Urology	DP	8	N	ST480	None
TZ32	B	2014	Bile	M	73	Gastroenterology	EYDNDM	2	O	ST368	None
TZ145	B	2014	Urine	F	54	Gynaecology	KD	8	P	ST16	CC16
TZ147	B	2014	Urine	F	20	Urology	W	4	Q	ST656	None
TZ207	B	2014	Wound secretion	M	93	Burn	RDK	8	R	ST314	CC314
CX81	C	2013	Blood	M	58	Hepatopancreatobiliary	EDM	2	S	ST657	CC93
CX84	C	2013	Wound secretion	F	63	Orthopaedics	EDM	2	S	ST657	CC93
GZ6	D	2010	Urine	M	56	Urology	EYDNDM	2	T	ST593	None
ZZ12	E	2014	Urine	M	79	Urology	W	8	U	ST658	CC476
ZZ31	E	2014	Urine	M	56	Urology	DP	4	V	ST659	None
ZZ36	E	2014	Wound secretion	F	22	Gynaecology	EDP	4	W	ST480	None
ZZ129	E	2014	Ascites	F	59	Hepatopancreatobiliary	EDM	2	X	ST591	CC16
ZZ133	E	2014	Wound secretion	F	50	Gynaecology	EYDNDM	2	Y	ST593	None
Enterococcus faecium											
TZ199	B	2014	Wound secretion	M	66	Orthopaedics	EDM	4	—	ST97	CC17
GZ124	D	2010	Urine	F	48	Urology	DD	4	—	ST882	CC17
GZ129	D	2011	Wound secretion	F	45	Gastrointestinal surgery	DD	4	—	ST885	CC17
Enterococcus thailandicus											
TZ198	B	2014	Bile	M	74	Hepatopancreatobiliary	EDM	2	—	—	—
Enterococcus gallinarum											
TZ208	B	2014	Ascites	M	40	Hepatopancreatobiliary	EDD	2	—	—	—

CC, clonal complex; F, female; ICU, intensive-care unit; LZD, linezolid; M, male; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; ST, sequence type.
[a]DD, Tyr176Asp/Gly393Asp; EDD, Lys3Glu/Tyr176Asp/Gly393Asp; EDM, Lys3Glu/Tyr176Asp/Ile622Met; EDP, Lys3Glu/Tyr176Asp/Thr481Pro; EYDNDM, Lys3Glu/Asn12Tyr/Tyr176Asp/Asp247Asn/Gly393Asp/Ile622Met; KD, Thr112Lys/Tyr176Asp; DP, Tyr176Asp/Thr481Pro; RDK, Ile104Arg/Tyr176Asp/Glu256Lys; W, wild type.
[b]ST655, ST656, ST657, ST658 and ST659 were novel STs identified in this study.
[c]None means that the ST could not be attributed to one of the recognized CCs.

optrA-negative isolates carried only *fexB* (three *E. faecium*) or *fexA* (one *E. faecalis*). The 34 *optrA*-positive isolates comprised 29 *E. faecalis*, three *E. faecium*, and, for the first time, single isolates of *Enterococcus gallinarum* and *Enterococcus thailandicus*. Neither 23S rRNA mutations nor *cfr* were detected in any of these 34 isolates. In addition, no *optrA* was detected in 458 randomly chosen enterococcal isolates that failed to grow on florfenicol-supplemented plates. Half of the patients from whom *optrA*-positive isolates had been obtained had a history of treatment with cephalosporins, and only two patients had received previous linezolid treatment. A year-by-year comparison of *optrA*-positive isolates revealed a slight increase from two (2.2%) and one (1.0%) isolates in 2010 and 2011, respectively to 13 (2.9%) and 12 (4.1%) isolates in 2013 and 2014, respectively (Fig. 1).

All *optrA*-positive isolates were susceptible to vancomycin and daptomycin. Moreover, all 29 *optrA*-positive *E. faecalis* isolates were susceptible to penicillin and ampicillin, but 17 (58.6%) of them were ciprofloxacin-resistant. Twenty-six *optrA*-positive isolates were resistant or intermediately resistant to linezolid, with MICs of 4–8 mg/L, whereas the remaining eight isolates were borderline susceptible to linezolid (MIC of 2 mg/L). The corresponding elevated tedizolid MICs for 28 of 34 (82.4%) of isolates ranged from 1 mg/L to 2 mg/L (Table S2), as compared with the previously tested clinical isolates, with MIC_{50} and MIC_{90} values of $\leq$0.5 mg/L [15]. Comparison of the deduced OptrA amino acid sequences of the 34 isolates with that of the original OptrA from *E. faecalis* E349 (designated as the wild type) revealed nine variants. Among the eight linezolid borderline-susceptible isolates, alterations at positions 3 (Lys3Glu), 12 (Asn12Tyr), 176 (Tyr176Asp), 247 (Asp247Asn), 393 (Gly393Asp), and 622 (Ile622Met) (EYDNDM variant) were identified in four isolates, and alterations at positions 3 (Lys3Glu), 176 (Tyr176Asp) and 622 (Ile622Met) (EDM variant) were identified in three isolates (Table 1). Whether any of these amino acid substitutions—and, if so, which—account for the lower linezolid MICs is the subject of a separate study.

PFGE and multilocus sequence typing analysis revealed that the 29 *optrA*-positive *E. faecalis* isolates represented 25 PFGE patterns and 20 sequence types (Table 1). However, occasional clonal dissemination could be confirmed in the same hospital; for eaxmple, isolates SR136 and CX81 were indistinguishable from isolates SR139 and CX84, respectively. Isolates with similar PFGE patterns (difference of two to three bands) and identical multilocus STs were observed, e.g. isolates SR12179 and SR12219, and isolates SR13118 and SR13179, respectively (Fig. S1).

Owing to the important role of oxazolidinones as therapeutics in life-threatening infections caused by Gram-positive bacteria, it is essential to screen enterococci and staphylococci for the presence of oxazolidinone resistance. The occurrence of the novel transferable linezolid resistance gene *optrA*, which also results in elevated MICs for tedizolid, is alarming. Because of their low linezolid MICs of 4–8 mg/L—and taking into account that, occasionally, isolates with an MIC of 2 mg/L are also *optrA*-positive—such enterococci might be easily overlooked in the clinical microbiology laboratory. As *optrA* also confers phenicol resistance to enterococci and staphylococci, florfenicol, which is exclusively approved for food-producing animals, can be used in the initial screening, followed by PCR analysis with *optrA*-specific primers [7]. Routine surveillance of *optrA*-carrying enterococcal clinical isolates—supported by the molecular analysis of such isolates—should be conducted to monitor, trace back and counteract the further dissemination of *optrA*.

Transparency declaration

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.08.007.

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