AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Nationwide Surveillance of Novel Oxazolidinone Resistance Gene *optrA* in *Enterococcus* Isolates in China from 2004 to 2014

Lanqing Cui,[a] Yang Wang,[b] Yuan Lv,[a] Shan Wang,[a] Yunjia Song,[a] Yun Li,[a] Jian Liu,[a] Feng Xue,[a] Weiwei Yang,[a] Jia Zhang[a]

Institute of Clinical Pharmacology, Peking University First Hospital, Beijing, China[a]; Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China[b]

A total of 2,201 nonduplicate enterococcal isolates collected from 29 hospitals in 23 cities in China between 2004 and 2014 were screened for the oxazolidinone resistance gene *optrA*; 45 isolates (2.0%) were *optrA* positive with 11 OptrA variants identified. The positive rate of *optrA* increased from 0.4% to 3.9% during the 10-year surveillance period. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence type (MLST) analysis revealed that 37 *optrA*-positive *Enterococcus faecalis* isolates clustered into 25 PFGE patterns and 21 sequence types, while 6 *Enterococcus faecium* isolates represented 6 PFGE patterns and 6 sequence types. The present study underscores the importance of routine and persistent monitoring of oxazolidinone resistance and *optrA* gene.

Linezolid is an important oxazolidinone antibiotic, approved for clinical use since 2000, that has shown great potency against most Gram-positive organisms, especially vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) (1). According to the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) and Linezolid Experience and Accurate Determination of Resistance (LEADER) program for 2014, linezolid demonstrated excellent activity against Gram-positive pathogens *in vitro* with overall susceptibility rates of 99.88% and 99.78%, respectively (2, 3). However, increasing numbers of linezolid-resistant isolates have been detected worldwide. Mutations in domain V of the 23S rRNA are the main resistance mechanisms, and G2576T is the most frequently seen alteration (4). Furthermore, linezolid resistance has also been associated with mutations in ribosomal proteins L3 and L4 (4). Besides these mutations, the *cfr* gene, the first reported transferable oxazolidinone resistance gene, encodes a methyltransferase that modifies the 23S rRNA at position A2503 that mediates the resistance not only to linezolid but also to phenicols, lincosamides, pleuromutilins, and streptogramin A. Moreover, a *cfr* variant gene, designated *cfr*(B) (5, 6), and the second transferable oxazolidinone resistance gene *optrA* have been identified (7).

The *optrA* gene (oxazolidinone phenicol transferable resistance) encodes an ATP-binding cassette transporter, resulting in resistance or elevated MICs for oxazolidinones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol), and the plasmid-borne *optrA* can be transferred among *Enterococcus* spp. (7). *optrA* was initially identified in *Enterococcus faecalis* isolate E349 recovered from a Chinese patient and to date has been mainly detected in *Enterococcus* spp. and very recently in one *cfr*-positive *Staphylococcus sciuri* isolate from a pig in China (8, 9). Although the *optrA* gene was originally detected in human isolates, it was also identified in food-producing animals and is more frequently seen in *Enterococcus* spp. from food-producing animals than from humans (15.9% and 2% to 2.9%, respectively) and in *E. faecalis* than in *Enterococcus faecium* (7, 9). However, the previous surveillance data on *optrA* from hospitals of China were territorial; thus, to understand the comprehensive epidemiology of *optrA*, we screened enterococci isolated from hospitalized patients across China during 10 years.

In total, 2,201 enterococcal isolates, including 987 *E. faecalis* isolates, 1,115 *E. faecium* isolates, and 99 other *Enterococcus* species isolates, were collected from 29 hospitals in 23 different cities from 21 provinces and municipalities between 2004 and 2014 through the Center Net of Mohnarin program (Ministry of Health National Antimicrobial Resistance Investigation Net, established by the China Ministry of Health in 2004) and the CARST program (the China Antimicrobial Resistance Surveillance Trial; the Center Net of Mohnarin was renamed CARST in 2013). Species were initially identified by the participating hospital using Vitek and API systems, and identifications of *optrA*-positive isolates were confirmed through 16S rRNA sequence and *Enterococcus* genus-specific primers (10, 11). Screened isolates were collected from urine (37.2%), drainage (23.3%), blood (17.3%), body secretions (9.8%), sputum (6.5%), and other specimens (3%, including cerebrospinal fluid, feces, etc.); 18.6% of strains were collected from intensive care units (ICU), and 78.6% of strains were not from ICUs. In addition, there were 2.9% and 2.8% of the isolates with unknown specimen origin and ward information (ICU or not ICU), respectively.

The *optrA* gene was also detected in linezolid-susceptible *Enterococcus* spp. in a previous study (9); therefore, all 2,201 isolates were investigated for the *optrA* gene using an internal primer pair ($optrA_{internal}$ F, CAGGTGGTCAGCGAACTAAG; $optrA_{internal}$ R, GCCACACCACCCATAAGTGTT). For *optrA*-positive isolates, the complete *optrA* gene was amplified by another primer pair ($optrA_{complete}$ F, TAGGAGGTAGAAGCAAATGA; $optrA_{complete}$ R, CGGCAAACTCAAAAGGTC) and sequenced. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

Received 12 June 2016 **Returned for modification** 2 July 2016
Accepted 2 September 2016

Accepted manuscript posted online 19 September 2016

Citation Cui L, Wang Y, Lv Y, Wang S, Song Y, Li Y, Liu J, Xue F, Yang W, Zhang J. 2016. Nationwide surveillance of novel oxazolidinone resistance gene *optrA* in *Enterococcus* isolates in China from 2004 to 2014. Antimicrob Agents Chemother 60:7490–7493. doi:10.1128/AAC.01256-16.

Address correspondence to Yuan Lv, lvyuan0901@sina.com.

TABLE 1 Numbers of *Enterococcus* species isolates screened for the *optrA* gene and the positive rate from 2004 to 2014 in China[a]

Yr and species	No. of isolates	LZD[b] MIC_{50} (mg/liter)	LZD MIC_{90} (mg/liter)	No. *optrA* positive	Percentage (%)
2004-2005					
E. faecalis	125	ND[c]	ND	0	0
E. faecium	115	ND	ND	1	0.9
Other *Enterococcus* spp.	22	ND	ND	0	0
Overall	262			1	0.4
2007-2008					
E. faecalis	158	ND	ND	1	0.6
E. faecium	149	ND	ND	0	0
Other *Enterococcus* spp.	39	ND	ND	0	0
Overall	346			1	0.3
2009-2010					
E. faecalis	113	2	2	6	5.3
E. faecium	195	1	2	1	0.5
Other *Enterococcus* spp.	36	1	2	0	0
Overall	344			7	2.0
2011-2012					
E. faecalis	276	1	2	8	2.9
E. faecium	304	1	2	1	0.3
Other *Enterococcus* spp.	1	—[d]	—	1	100
Overall	581			10	1.7
2013-2014					
E. faecalis	315	2	2	22	7.0
E. faecium	352	2	2	3	0.9
Other *Enterococcus* spp.	1	—	—	1	100
Overall	668			26	3.9
2004-2014					
E. faecalis	987	2[e]	2[e]	37	3.7
E. faecium	1,115	1[e]	2[e]	6	0.5
Other *Enterococcus* spp.	99	1[e]	2[e]	2	2.0
Overall	2,201			45	2.0

[a] The Center Net of Mohnarin program and CARST program collected the isolates from the middle of one year to the middle of the following year.
[b] LZD, linezolid.
[c] ND, not detected.
[d] —, linezolid MIC_{50} and MIC_{90} were not calculated.
[e] MIC_{50} and MIC_{90} were calculated according to the MICs of isolates screened from 2009 to 2014.

were performed to analyze the genotypes of the *optrA*-positive strains. MICs were determined by the 2-fold agar dilution method according to the recommendations of the CLSI, and the reference strain *E. faecalis* ATCC 29212 served as a quality control (12). Moreover, all of the *optrA*-positive isolates were screened for *cfr* and *cfr*(B), and isolates with linezolid resistance (MIC of ≥8 mg/liter) were also screened for the presence of the 23S rRNA mutation and ribosomal protein (L3/L4) mutation as previously described (5, 7, 13).

A total of 45 isolates (2.0%, 45/2201), including 37 *E. faecalis* isolates, 6 *E. faecium* isolates, and 1 isolate each of *Enterococcus thailandicus* and *Enterococcus gallinarum*, were positive for the *optrA* gene. The *optrA*-positive incidence increased over time from 0.4% (1/262) in 2004 to 2005 to 3.9% (26/668) in 2013 to 2014 (Table 1). Interestingly, the *optrA* gene was initially detected in an *E. faecium* isolate, 05K173, collected in the year 2005, 2 years before linezolid was approved for clinical use in China (2007). Similar to the results of the aforementioned study, the *optrA* gene was more frequently seen in *E. faecalis* (3.7%, 37/987) than in *E. faecium* (0.5%, 6/1115). The 37 *optrA*-positive *E. faecalis* isolates clustered into 25 PFGE patterns using the criteria of Tenover et al. (14). Further, MLST analysis revealed that 37 *E. faecalis* isolates belonged to 21 sequence types (ST), and ST476 was the most common type (6/37), followed by ST116 (5/37). Overall, the clones of 37 *E. faecalis* isolated in different hospitals and different time periods were diverse. However, occasional clonal dissemination was observed in the same hospital; for example, three isolates (09E349, 09E363, and 09E399) from one hospital were clustered into one clone and one sequence type (ST116), and isolates 13F074 and 13F075 with identical PFGE and ST grouping (ST714) were observed from another hospital. Moreover, 6 *optrA*-positive *E. faecium* isolates represented 6 PFGE patterns and 6 different sequence types.

Antimicrobial susceptibility testing of 45 *optrA*-positive isolates revealed that 34 *E. faecalis* isolates were not susceptible to linezolid (MIC of ≥4 mg/liter), and the remaining 11 isolates were susceptible to linezolid (MIC of ≤2 mg/liter). With the exception of one vancomycin-intermediate *Enterococcus gallinarum* isolate,

TABLE 2 Polymorphisms of the OptrA protein detected in 45 *optrA*-positive *Enterococcus* species isolates

OptrA variant	Mutations compared with first-reported $OptrA_{E349}$ from *E. faecalis* E349[a]	No. of isolates
$OptrA_{E349}$	No mutations	8
DD	Tyr176Asp, Gly393Asp	2
DK	Tyr176Asp, Glu256Lys	1
DP	Tyr176Asp, Thr481Pro	7
ED	Lys3Glu, Tyr176Asp	3
EDD	Lys3Glu, Tyr176Asp, Gly393Asp	6
EDM	Lys3Glu, Tyr176Asp, Ile622Met	6
EYDNDM	Lys3Glu, Asn12Tyr, Tyr176Asp, Asp247Asn, Gly393Asp, Ile622Met	1
KD	Thr112Lys, Tyr176Asp	3
KDP	Thr112Lys, Tyr176Asp, Thr481Pro	4
RDK	Ile104Arg, Tyr176Asp, Glu256Lys	4

[a] D, Asp; E, Glu; K, Lys; M, Met; N, Asn; P, Pro; R, Arg; Y, Tyr.

44 isolates were susceptible to vancomycin, teicoplanin, and tigecycline but resistant to erythromycin. Among 37 *optrA*-positive *E. faecalis* isolates, 2 isolates (5.4%) were resistant to ampicillin, while 30 (81.1%), 34 (91.9%), and 35 (94.6%) isolates were resistant to levofloxacin, minocycline, and chloramphenicol, respectively. Furthermore, the analysis of mutations in 23S rRNA and L3/L4 protein in 13 linezolid-resistant strains (MIC of 8 mg/liter) revealed that only the mutation F101L in L4 protein was detected; this mutation has been considered to be associated with linezolid resistance (15). However, this mutation has also been observed in two linezolid-susceptible isolates, 09E317 (MIC of 0.5 mg/liter) and 09K619 (MIC of 1 mg/liter), which were randomly selected in isolates our laboratory preserved, implying that this mutation may be not strongly associated with linezolid resistance. Moreover, 45 *optrA*-positive isolates were all negative for the *cfr* or *cfr*(B) gene.

It was previously reported that 655 amino acid sequences of OptrA protein varied in the *optrA*-positive isolates, and, to date, 10 variants have been identified compared with those for the original OptrA from *E. faecalis* E349 (designated $OptrA_{E349}$) (9). In this study, 11 OptrA variants were observed, and 3 novel variants, the DK variant, ED variant, and KDP variant, were identified (Table 2). Among 11 OptrA variants, $OptrA_{E349}$was the most observed (8/45), followed by the DP variant (7/45), EDD variant (6/45), and EDM variant (6/45). Five OptrA variants, including the EDD variant (4/11), EDM variant (3/11), DP variant (2/11), ED variant (1/11), and EYDNDM variant (1/11), were identified in 11 linezolid-susceptible isolates, which included 9 *E. faecalis* isolates, 1 *E. gallinarum* isolate, and 1 *E. faecium* isolate. The amino acid alterations of the 5 OptrA variants occurred at positions 3 (Lys→Glu), 12 (Asn→Tyr), 176 (Tyr→Asp), 247 (Asp→Asn), 393 (Gly→Asp), 481 (Thr→Pro), and 622 (Ile→Met). However, the EYDD variant with positions at 3 (Lys→Glu), 12 (Asn→Tyr), 176 (Tyr→Asp), and 393 (Gly→Asp) from *S. sciuri* has been shown to cause 2- to 8-fold increased MICs for oxazolidinones in *S. aureus* and *E. faecalis* (8). It remains unclear whether any of these amino acid substitutions or the low expression of the *optrA* gene or some other mechanisms resulted in the lower linezolid MICs.

To the best of our knowledge, this is the first study of *optrA* nationwide surveillance on a large scale that involved isolates collected from 29 hospitals in 23 cities in China from 2004 to 2014. This study revealed that the presence of the oxazolidinone resistance gene *optrA* increased slightly in China and occurred prior to the approval of linezolid in the Chinese market. Owing to the important role of linezolid against Gram-positive pathogens in clinical practice and the potential risks of the transfer of plasmid-borne *optrA* between *Enterococcus* spp., it is necessary to routinely and persistently screen for oxazolidinone resistance and *optrA* gene.

Accession number(s). The sequences of the *optrA* gene encoding different OptrA variants have been deposited in GenBank under the following accession numbers: KX620932 ($OptrA_{E349}$), KX620933 (DD), KX620934 (DK), KX620935 (DP), KX620936 (ED), KX620937 (EDD), KX620938 (EDM), KX620939 (EYDNDM), KX620940 (KD), KX620941 (KDP), and KX620942 (RDK).

ACKNOWLEDGMENTS

This work was funded by grants from the National Natural Science Foundation of China (grants 81572033 and 31422055).

We are truly grateful to all of the hospitals participating in the Center Net of Mohnarin program and CARST program.

FUNDING INFORMATION

This work, including the efforts of Yuan Lv, was funded by National Natural Science Foundation of China (NSFC) (81572033). This work, including the efforts of Yang Wang, was funded by National Natural Science Foundation of China (NSFC) (31422055).

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. **Leach KL, Brickner SJ, Noe MC, Miller PF.** 2011. Linezolid, the first oxazolidinone antibacterial agent. Ann N Y Acad Sci **1222:**49–54. http://dx.doi.org/10.1111/j.1749-6632.2011.05962.x.
2. **Flamm RK, Mendes RE, Hogan PA, Streit JM, Ross JE, Jones RN.** 2016. Linezolid surveillance results for the United States (LEADER Surveillance Program 2014). Antimicrob Agents Chemother **60:**2273–2280. http://dx.doi.org/10.1128/AAC.02803-15.
3. **Mendes RE, Hogan PA, Jones RN, Sader HS, Flamm RK.** 2016. Surveillance for linezolid resistance via the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) programme (2014): evolving resistance mechanisms with stable susceptibility rates. J Antimicrob Chemother **71:** 1860–1865. http://dx.doi.org/10.1093/jac/dkw052.
4. **Mendes RE, Deshpande LM, Jones RN.** 2014. Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat **17:**1–12. http://dx.doi.org/10.1016/j.drup.2014.04.002.
5. **Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, Farrell DJ, Mendes RE.** 2015. Detection of a new cfr-like gene, cfr(B), in *Enterococcus faecium* isolates recovered from human specimens in the United States as part of the SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother **59:**6256–6261. http://dx.doi.org/10.1128/AAC.01473-15.
6. **Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B.** 2006. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob Agents Chemother **50:**2500–2505. http://dx.doi.org/10.1128/AAC.00131-06.
7. **Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Fessler AT, Wu C, Yu H, Deng X, Xia X, Shen J.** 2015. A novel gene, *optrA*, 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 **70:**2182–2190. http://dx.doi.org/10.1093/jac/dkv116.
8. **Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Fessler AT, Zhang R, Wu C, Shen J.** 2016. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. J Antimicrob Chemother **71:**1474–1478. http://dx.doi.org/10.1093/jac/dkw040.
9. **Cai J, Wang Y, Schwarz S, Lv H, Li Y, Liao K, Yu S, Zhao K, Gu D, Wang X, Zhang R, Shen J.** 2015. Enterococcal isolates carrying the novel

oxazolidinone resistance gene *optrA* from hospitals in Zhejiang, Guangdong, and Henan, China, 2010−2014. Clin Microbiol Infect **21:**1095.e1–1095.e4. http://dx.doi.org/10.1016/j.cmi.2015.08.007.

10. **Depardieu F, Perichon B, Courvalin P.** 2004. Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. J Clin Microbiol **42:**5857–5860. http://dx.doi.org/10.1128/JCM.42.12.5857-5860.2004.
11. **Jackson CR, Fedorka-Cray PJ, Barrett JB.** 2004. Use of a genus- and species-specific multiplex PCR for identification of enterococci. J Clin Microbiol **42:**3558–3565. http://dx.doi.org/10.1128/JCM.42.8.3558-3565.2004.
12. **Clinical and Laboratory Standards Institute.** 2016. Performance standards for antimicrobial susceptibility testing; 26th ed. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA.
13. **Ntokou E, Stathopoulos C, Kristo I, Dimitroulia E, Labrou M, Vasdeki A, Makris D, Zakynthinos E, Tsakris A, Pournaras S.** 2012. Intensive care unit dissemination of multiple clones of linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Antimicrob Chemother **67:**1819–1823. http://dx.doi.org/10.1093/jac/dks146.
14. **Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **33:**2233–2239.
15. **Biedenbach DJ, Farrell DJ, Mendes RE, Ross JE, Jones RN.** 2010. Stability of linezolid activity in an era of mobile oxazolidinone resistance determinants: results from the 2009 Zyvox Annual Appraisal of Potency and Spectrum program. Diagn Microbiol Infect Dis **68:**459–467. http://dx.doi.org/10.1016/j.diagmicrobio.2010.09.018.