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Emergence of *optrA*-Mediated Linezolid-Nonsusceptible *Enterococcus faecalis* in a Tertiary Care Hospital

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This study investigated resistance mechanisms and epidemiology of emerging linezolid-nonsusceptible *Enterococcus faecalis* (LNSEF) in a tertiary care hospital. LNSEF isolated from clinical samples were collected from November 2017 to June 2019. The isolates were investigated for linezolid resistance and the associated molecular mechanisms, including mutations of 23S rRNA domain V and acquisition of the *cfr* or *optrA* resistance gene. We used pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing for the molecular typing of the isolates. Among 4,318 *E. faecalis* isolates, 10 (0.23%) were linezolid-nonsusceptible. All LNSEF isolates were *optrA*-positive and *cfr*-negative. Of these isolates, five were sequence type (ST) 476, two ST585, one ST16, one ST16-like, and one ST480. Six LNSEF isolates obtained in the first year clustered to three types in the PFGE analysis: two ST476 isolates of type A, two ST585 isolates of type B, and two ST16 or ST16-like isolates of type C. Seven cases were of community-onset and three were hospital acquired, but total of eight were healthcare-associated including five community-onset. None of the patients had a history of linezolid treatment, and in one patient, we detected linezolid-susceptible *E. faecalis* one month before LNSEF detection. In conclusion, heterogenous clones of *optrA*-positive LNSEF emerged in the hospital mainly via community-onset.

Key Words: Linezolid resistance, Mechanism, Epidemiology, *optrA*, *Enterococcus faecalis*, Pulsed-field gel electrophoresis, Multilocus sequence typing

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Linezolid is an oxazolidinone that binds 23S rRNA and inhibits protein synthesis by preventing 70S ribosomal unit formation [1]. Since its introduction in 2000, linezolid has been the last-resort antimicrobial for vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* [1]. Linezolid-resistant *Enterococcus faecium* was first observed in patients infected with vancomycin-resistant *E. faecium* who were treated with linezolid for a prolonged period. The resistance was due to mutations in domain V of the 23S rRNA of *E. faecium* [2]. While this mechanism of linezolid resistance in *E. faecium* and *S. aureus* is predominant in Korea, resistance due to variants in ribosomal proteins L3, L4, and L22 have also been reported [3]. Acquisition of the linezolid resistance gene *cfr* or, more recently, *optrA* is more threatening because these genes are plasmid encoded and, thus, transmissible [4]. Human isolates of *optrA*-positive linezolid-nonsusceptible *Enterococcus faecalis* (LNSEF) were reported first in 2016 in China [4] and later in the USA, Sweden, France, Thailand, Taiwan, Malaysia, and Korea [3, 5]. However, *optrA* has been detected among archival clinical isolates traceable to 2012 in Korea [3]. In Asan Medical Center, a

2,715-bed, university-affiliated tertiary care hospital in Seoul, Korea, LNSEF was first detected in November 2017. There is a lack of data on LNSEF in Korea. In this study, we investigated the resistance mechanisms and epidemiology of LNSEF isolates.

Clinical isolates of *Enterococcus* species collected at the Asan Medical Center were subjected to species identification and antimicrobial susceptibility testing using the MicroScan PBC 28 panel (Beckman Coulter, Brea, CA). Linezolid susceptibility was confirmed by the E-test (bioMérieux SA, Marcy l'Etoile, France). LNSEF isolates were collected for 20 months, from November 2017 to June 2019.

The molecular mechanism for linezolid resistance was investigated by 23S rRNA gene sequencing and *cfr* or *optrA*-specific PCR. For molecular epidemiological analysis, seven housekeeping genes (*gdh, gyd, pstS, gki, aroE, xpt*, and *yiqL*) were sequenced using PCR, and sequence types (STs) were determined by comparison with an open-access database (PubMLST, https://pubmlst.org/efaecalis/). The primers were adopted from a previous report [6]. Six LNSEF strains isolated in the first year until October 2018 were subjected to pulsed-field gel electrophoresis (PFGE) typing with *Sma*I restriction (Takara, Tokyo, Japan) using the CHEF Mapper system (Bio-Rad, Hercules, CA, USA). Clonality based on PFGE analysis was interpreted according to a previous report [7].

Demographic findings and clinical history, including infection, operation, antimicrobial treatment, and microbiological data, were obtained from electronic medical records of the patients. This study was approved by the Institutional Review Board of Asan Medical Center (S2019-1169-0001).

In total, 4,318 *E. faecalis* isolates were obtained from 2,639 patients. Ten isolates (0.23%) were found to be linezolid-nonsusceptible and included five linezolid-intermediate (0.115%) and five linezolid-resistant (0.115%) isolates. Minimum inhibitory concentrations of linezolid as determined by E-tests were 6–12 μg/mL, which were resistant. The isolates were obtained from urine (N=6), Jackson Pratt drainage (N=2), and open pus (N=2) samples. The antimicrobial susceptibility and resistance genes of the LNSEF isolates are listed in Table 1. All LNSEF isolates were susceptible to penicillin, ampicillin, daptomycin, nitrofurantoin, teicoplanin, and vancomycin, but resistant to fluoroquinolone and quinupristin/dalfopristin.

All 10 LNSEF isolates were positive for *optrA*, but none were positive for *cfr*. The domain V of the 23S rRNA genes of all six isolates in the first year were wild-type. Multilocus sequence typing (MLST) revealed that five isolates were ST476, two ST585, one ST16, one ST16-like, and one ST480. The pulsotypes of the six isolates in the first year were clustered into three types (Fig. 1). The ST16-like isolate harbored a thymine at the 27th position of *yiqL*, while ST16 had cytosine. Clinical and epidemiological features of the 10 patients with LNSEF are presented in Table 2. Seven of these LNSEF isolates were obtained from urine samples and open pus samples in an outpatient setting, whereas the remaining three were hospital-acquired. In one patient, linezolid-susceptible enterococci were recovered from a urine sample one month before LNSEF isolation. None of the patients carrying LNSEF had been treated with linezolid. Eight

Table 1. Antibiogram, resistance genes, and molecular epidemiology of LNSEF

Isolate	MIC (mg/L)									*optrA*	*cfr*	Mutation of 23SrRNA domain V	PFGE	MLST
	LNZ*	AMP	CIP	DAP	RIF	TC	VAN	Syn-SM	Syn-GM					
1	4/8	4	>2	2	≤1	>8	2	>1,000	>500	(+)	(–)	(–)	A	ST476
2	4/6	4	>2	2	≤1	>8	2	>1,000	>500	(+)	(–)	(–)	B	ST585
3	4/6	4	>2	≤1	≤1	>8	1	>1,000	>500	(+)	(–)	(–)	B	ST585
4	>4/12	4	>2	2	>2	>8	2	>1,000	>500	(+)	(–)	(–)	C	ST16
5	>4/12	4	>2	≤1	≤1	≤4	2	>1,000	>500	(+)	(–)	(–)	A	ST476
6	>4/12	4	>2	2	>2	>8	2	≤1,000	>500	(+)	(–)	(–)	C	ST16-like
7	>4/12	4	>2	≤1	≤1	≤4	2	≤1,000	>500	(+)	(–)	ND	ND	ST476
8	4/6	4	>2	2	≤1	≤4	2	≤1,000	≤500	(+)	(–)	ND	ND	ST476
9	>4/12	4	>2	4	≤1	>8	2	≤1,000	≤500	(+)	(–)	ND	ND	ST476
10	4/8	4	>2	≤1	≤1	>8	2	≤1,000	>500	(+)	(–)	ND	ND	ST480

*Linezolid MICs are denoted as the MicroScan MIC/E-test MIC.
Abbreviations: AMP, ampicillin; CIP, ciprofloxacin; DAP, daptomycin; MIC, minimal inhibitory concentration; LNZ, linezolid; LNSEF, linezolid-nonsusceptible *Enterococcus faecalis*; MLST, multilocus sequence typing; ND, not determined; PFGE, pulsed-field gel electrophoresis; RIF, rifampin; Syn-GM: high-dose gentamicin; TC, tetracycline; VAN, vancomycin; Syn-SM, high-dose streptomycin.

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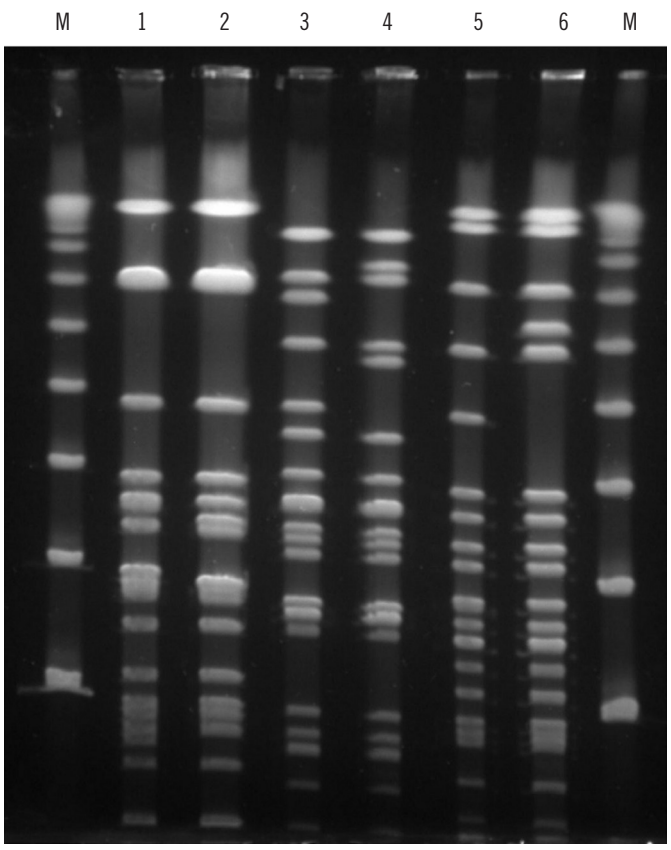

Fig. 1. Pulsed-field gel electrophoresis (PFGE) analysis of linezolid-nonsusceptible *Enterococcus faecalis* isolates after *Sma*I-digestion. M denotes Lambda Ladder PFG Marker (New England Biolabs Inc., N0341S, Beverly, MA). Each of lanes from 1 to 6 denoted the pulsotype of isolate number 1, 5, 2, 3, 4, and 6 in order.

patients had a history of hospitalization. Two patients had an overlap in admission period, but they had been treated in different wards, ruling out contact transmission. The five ST476 isolates were from patients who lived in three different cities.

In this study, the prevalence of *optrA*-positive LNSEF of 0.23% was comparable to those of a linezolid resistance surveillance program (2011 to 2015) in the United States, in which 0.3%–0.7% of enterococci were linezolid-nonsusceptible [8]. In line with this finding, a recent study in a tertiary care hospital in Korea revealed that 1.65% of enterococci were nonsusceptible to linezolid, and 20.8% of LNSEF isolates carried *optrA*, accounting for approximately 0.3% of the total *E. faecalis* isolates [3]. This prevalence of *optrA*-mediated linezolid resistance is similar to that observed in the present study. We identified *optrA* as the primary mechanism of linezolid resistance in *E. faecalis*, whereas during the same period, 23S rRNA mutations was identified as the primary mechanism underlying linezolid resistance in *E. faecium* [9]. Considering the ability of bacteria to

Table 2. Clinical and epidemiological features of 10 patients from whom LNSEF was isolated

Patient	Sex/age (year)	Sample date, type	Admission date	Admission history (≤1 yr)	Underlying disease	Surgical history (≤1 yr)	Previous antimicrobial treatment (≤30 days)	Type of infection	Location (city)	Clinical outcome
1	M/24	17.11.01, urine	Outpatient	Yes	Retrocaval ureter	Yes	Cefixime, ceftriaxone	Colonization	Wonju, Kangwon	
2	F/46	17.11.02, urine	Outpatient	Yes	Cervical cancer	No	Levofloxacin, piperacillin/tazobactam, ceftizoxime, ceftazidime, ampicillin, cotrimoxazole, ceftriaxone	Acute pyelonephritis	Guri, Gyeonggi	Resolved
3	M/77	18.06.07, JP drainage	18.05.20	Yes	Hepatocellular carcinoma	Yes	Ciprofloxacin, meropenem	Liver abscess	Buk, Busan	Resolved
4	F/64	18.06.07, urine	18.05.31	Yes	Thrombotic microangiopathy	No	Cephradine	Colonization	Hanam, Gyeonggi	Not applicable
5	F/59	18.09.27, urine	Outpatient	Yes	Endometrial cancer	Yes	No	Cystitis	Seocho, Seoul	Resolved
6	M/70	18.11.06, urine	Outpatient	Yes	Benign prostate hyperplasia	Yes	Ciprofloxacin	Colonization	Gangdong, Seoul	Not applicable
7	F/25	19.01.01 open pus	Outpatient	Yes	Ischemic cardiomyopathy	No	Piperacillin/tazobactam, cefpodoxime, cefazedone	Chronic wound	Wanju, Chonbuk	Resolved
8	F/84	19.02.20 urine	Outpatient	No	Atrial fibrillation	No	No	Cystitis	Gangdong, Seoul	Resolved
9	F/74	19.05.31 JP drainage	19.05.25	Yes	Rectal cancer	Yes	Ciprofloxacin, metronidazole, cefoxitin	Colonization	Wonju, Kangwon	
10	M/70	19.06.07 open pus	Outpatient	No	Acute myeloid leukemia	No	Ciprofloxacin, metronidazole	Perianal abscess	Gwangjin, Seoul	Resolved

Abbreviations: F, female; JP, Jackson Pratt; LNSEF, linezolid-nonsusceptible *Enterococcus faecalis*; M, male.

transfer *optrA* via plasmids and the marked increase in *optrA*-positive *E. faecalis* cases in China [4], the spread of *optrA*-mediated linezolid resistance could be an imminent threat in Korea.

In this study, all LNSEF isolates except one were multidrug-resistant. The susceptibility rates of LNSEF isolates to ciprofloxacin, high-level gentamicin, and high-level streptomycin were 0.0%, 20.0%, and 50.0%, respectively, whereas the average susceptibility rates of the total *E. faecalis* isolates to these antimicrobials were 59.9%, 51.4%, and 86.1%, respectively (unpublished data). Except two, all patients underwent antimicrobial treatment within 30 days before LNSEF isolation. Therefore, most cases were healthcare-associated, although they were isolated from an outpatient setting. Antibiotic pressure seemed to trigger the spread of LNSEF, but none of the patients had a history of linezolid treatment. Therefore, linezolid resistance is likely to spread via clonal expansion or horizontal transfer of resistance genes in a community setting, but in a healthcare-associated manner. Increased spread of LNSEF would complicate the antimicrobial treatment of urogenital and wound infections.

ST476 was the most prevalent strain in this study. Previously *optrA*-positive ST476 strains were reported only in China, where it has been frequently found in humans and pigs [10-12]. The epidemiological interpretation of these findings needs further investigation. Among other STs, ST16 is an *optrA*-carrying clone with a worldwide distribution [12]. ST585 strains harboring *optrA* have been reported in the USA, China, and Germany [5, 10, 13]. ST480 strains harboring *optrA* have been reported in China, France, Germany, and Belgium [12-15]. Studies in China have revealed that *optrA* is more prevalent in animal enterococcal isolates (15.9%) than human isolates (2.0%) [10] and that 3.5% of healthy individuals carry *optrA*-carrying *E. faecalis*, regardless of age [16]. The *optrA* gene has been found from the enterococcal isolates of food animals since 2008 in Korea, and *optrA*-positive LNSEF from food animals belong to several STs, of which only ST16 was detected in the present study [17, 18]. Therefore, ST16 may be responsible for the spread of *optrA*-positive LNSEF in both food animals and humans. All STs in this study have been previously detected in China. Geographical vicinity along with substantial food exchange and extensive human traveling may explain the epidemiological linkage of *optrA*-positive *E. faecalis* between these two countries.

In this study, none of the patients with LNSEF were epidemiologically related to one another. Most LNSEF infections were of the community-onset and healthcare-associated type. This may herald an era of linezolid resistance by plasmid-mediated resistance gene acquisition. A Chinese hospital reported that as high as 3.93% of *E. faecalis* isolates were linezolid-resistant, and all those isolates were positive for *optrA, cfr*, or both [11]. Therefore, the rapid spread of LNSEF via clonal expansion or horizontal transfer of resistance genes is a possible scenario once it is introduced [3, 4, 19].

There were some limitations in this study. First, the number of LNSEF isolates studied was relatively small, because linezolid resistance in *E. faecalis* is still rare. Second, this was a single-center study; further multi-center studies are needed to confirm the prevalence and epidemiology of *optrA*-harboring *E. faecalis*. In conclusion, the emergence of *optrA*-mediated LNSEF in a community setting is alarming. Therefore, adequate infection control measures and surveillance of linezolid resistance are necessary in Korea.

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AUTHOR CONTRIBUTIONS

MNK directed the study. KP and YSJ conducted the experiments. HS and JC analyzed the data. KP wrote the paper.

CONFLICTS OF INTEREST

The authors report no potential conflicts of interest relevant to this article.

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