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

Lalitagauri M. Deshpande,^a Deborah S. Ashcraft,^b Heather P. Kahn,^b George Pankey,^b Ronald N. Jones,^a David J. Farrell,^a Rodrigo E. Mendes^a

JMI Laboratories, North Liberty, Iowa, USA^a; Ochsner Clinic Foundation, New Orleans, Louisiana, USA^b

Two linezolid-resistant *Enterococcus faecium* isolates (MICs, 8 µg/ml) from unique patients of a medical center in New Orleans were included in this study. Isolates were initially investigated for the presence of mutations in the V domain of 23S rRNA genes and L3, L4, and L22 ribosomal proteins, as well as *cfr*. Isolates were subjected to pulsed-field gel electrophoresis (just one band difference), and one representative strain was submitted to whole-genome sequencing. Gene location was also determined by hybridization, and *cfr* genes were cloned and expressed in a *Staphylococcus aureus* background. The two isolates had one out of six 23S rRNA alleles mutated (G2576T), had wild-type L3, L4, and L22 sequences, and were positive for a *cfr*-like gene. The sequence of the protein encoded by the *cfr*-like gene was most similar (99.7%) to that found in *Peptoclostridium difficile*, which shared only 74.9% amino acid identity with the proteins encoded by genes previously identified in staphylococci and non-*faecium* enterococci and was, therefore, denominated Cfr(B). When expressed in *S. aureus*, the protein conferred a resistance profile similar to that of Cfr. Two copies of *cfr*(B) were chromosomally located and embedded in a Tn6218 similar to the *cfr*-carrying transposon described in *P. difficile*. This study reports the first detection of *cfr* genes in *E. faecium* clinical isolates in the United States and characterization of a new *cfr* variant, *cfr*(B). *cfr*(B) has been observed in mobile genetic elements in *E. faecium* and *P. difficile*, suggesting potential for dissemination. However, further analysis is necessary to access the resistance levels conferred by *cfr*(B) when expressed in enterococci.

Linezolid resistance remains rare among clinical isolates from global surveillance programs (1, 2). Among isolates exhibiting linezolid-nonsusceptible results, alterations in the 23S rRNA genes remain responsible for the majority of the resistance mechanisms observed (2). Mutations in the L3 and L4 ribosomal proteins were demonstrated to cause decreased susceptibility to linezolid (3, 4). In fact, alterations in these ribosomal proteins are currently often observed among staphylococcal isolates, especially among coagulase-negative staphylococci, demonstrating a shift in the epidemiology of linezolid-nonsusceptible isolates and associated resistance mechanisms (2). Efflux-pump mechanisms have also been implicated as a linezolid resistance mechanism (5), and a plasmid-mediated gene (transferable) was recently described (6).

A plasmid-encoded linezolid resistance mechanism, namely, *cfr*, was reported in human specimens (7). Cfr belongs to the radical S-adenosylmethionine (SAM) family of enzymes that mediate transfer of methyl residue to unactivated C-H bonds. Cfr methylates the unreactive C2- and C8-carbon atoms on the A2503 residue located in a functionally critical region of the 23S rRNA component (8). The methylation at C8 protects the Cfr-producing bacteria from the action of five major classes of antibiotics, namely, phenicols, oxazolidinones, pleuromutins, macrolides, and streptogramin A compounds (PhLOPS_A phenotype) (9). Furthermore, it has been proposed that the *cfr* gene evolved from the naturally occurring *rlmN* methyltransferase (10), which is widely distributed in several organisms (11).

The gene encoding Cfr was initially observed in *Staphylococcus sciuri* from calves (12) and other staphylococcal species from animal and human origins from the United States, Italy, China, Canada, India, and Brazil (13–17). Moreover, the *cfr* gene has been

reported in a variety of Gram-positive and Gram-negative isolates (18). However, reports of *cfr* among enterococcal isolates of human origin have remained sporadic (19–22). Recently, Marin et al. reported a *cfr*-like gene in seven *Peptoclostridium* (previously *Clostridium*) *difficile* isolates recovered from human clinical specimens (23). However, the resistance phenotype conferred by the *cfr*-like gene remained to be investigated (23, 24). Here, we report the characterization and genetic context of this *cfr* variant recently reported in *P. difficile* that was detected in two human clinical isolates of *Enterococcus faecium* from a medical center in New Orleans (1, 25). Our analysis showed that the complete encoded protein variant had approximately 75% amino acid identities with the Cfr protein commonly found in staphylococci and enterococci (12, 20, 26), and this variant was assigned the name Cfr(B).

(Part of these data was presented at the 115th General Meeting

Received 23 June 2015 · Returned for modification 11 July 2015

Accepted 19 July 2015

Accepted manuscript posted online 27 July 2015

Citation 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. doi:10.1128/AAC.01473-15.

Address correspondence to Rodrigo E. Mendes, rodrigo-mendes@jmlabs.com.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AAC.01473-15>.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.01473-15

of the American Society for Microbiology, New Orleans, LA, May 2015 [27].)

MATERIALS AND METHODS

Identification and antimicrobial susceptibility testing. *E. faecium* clinical isolates included in this study were isolated in a medical site in New Orleans and submitted to a central monitoring laboratory (JMI Laboratories, North Liberty, IA), as part of the 2012 to 2013 SENTRY Antimicrobial Surveillance Program. Species identification and initial susceptibility testing were performed by the use of a MicroScan WalkAway system (Dade Behring, Inc. Sacramento, CA) by the submitting site. Identification was confirmed by the central laboratory using matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). Confirmation of the initial susceptibility profile and interpretation was carried out by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (28, 29). Validation of the MIC values was performed by concurrent testing of *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Detection of linezolid resistance mechanisms. Isolates that showed elevated MIC results for linezolid (i.e., an MIC of ≥ 4 $\mu\text{g/ml}$) were selected for further characterization at the central laboratory. Presence of *cfr*, mutations in 23S rRNA, and ribosomal proteins (L3, L4, and L22) were investigated by PCR and sequencing of amplicons on the two strands, as previously described (13, 30). 23S rRNA and ribosomal protein sequences obtained were compared to those from wild-type *E. faecium* ATCC 35667 using the Lasergene software package (DNASTar, Madison, Wisconsin).

Epidemiology typing. *E. faecium* isolates were epidemiologically typed by pulsed-field gel electrophoresis (PFGE) using genomic DNA prepared in agarose blocks, digested with SmaI (New England BioLabs, Beverly, MA, USA) and resolved in the CHEF-DR II apparatus (Bio-Rad, Richmond, CA, USA). Results were analyzed by GelCompar II software (Applied Math, Kortrijk, Belgium). Multilocus sequence typing (MLST) was performed according to instructions on the website efaecium.mlst.net.

Gene location. Genomic DNA was prepared in 1% agarose blocks and digested separately with S1 nuclease (partial digest), I-CeuI, SmaI, and EcoRI. DNA fragments were resolved by PFGE using CHEF-DR II and transferred to nylon membranes. Membranes were hybridized with a digoxigenin-labeled *cfr*-specific probe (Roche Diagnostics GmbH, Mannheim, Germany) (26).

Whole-genome sequencing and genetic environment of the *cfr* variant. Genomic DNA, extracted using the QIAamp genomic DNA kit (Qiagen, Hilden, Germany), was used to prepare paired-end TrueSeq library and cluster generation. Samples were sequenced using an Illumina MiSeq instrument (SeqWright, Houston, TX, USA). Sequences were aligned into multiple contigs using Lasergene NGen *de novo* assembly protocol (DNASTar, Madison, WI). Contigs were searched for *cfr*-like genes and their surrounding sequences. *In silico* analysis was also performed to investigate the presence of other antimicrobial resistance markers (i.e., *erm*, *mef*, *tet*, *van*, and aminoglycoside-modifying genes) and additional genetic analysis.

Cloning and expression of the *cfr* variant. The *cfr*-like gene investigated here and that from plasmid pSCFS3 (EMBL accession number AM086211) (9, 17, 26) were amplified using High-Fidelity PCR master mix and cloned into the PCRII-TOPO cloning vector (Invitrogen, Carlsbad, CA). Ligation reactions were transformed into *Escherichia coli* TOP10 electrocompetent cells (Gene Pulser; Bio-Rad, Hercules, CA) and selected on nutrient agar plates containing 50 $\mu\text{g/ml}$ ampicillin. The recombinant PCRII-TOPO plasmids and the *E. coli*-*S. aureus* shuttle vector pEPSA5 were extracted using a Qiagen plasmid miniprep kit. Double digests using BamHI and XbaI were performed, and *cfr* inserts were ligated into pEPSA5 using T4 DNA ligase. Ligation mixtures were transformed into *E. coli* NEB5 α electrocompetent cells (New England BioLabs, Beverly, MA). Transformants were selected in 50 $\mu\text{g/ml}$ ampicillin. Plas-

mid preparations containing the recombinant shuttle vector pEPSA5 carrying the *cfr* genes were electroporated into *S. aureus* RN4220. Transformants were selected on 30 $\mu\text{g/ml}$ chloramphenicol, and the presence of *cfr* was confirmed by PCR and sequencing.

Nomenclature of the *cfr* variant. The *cfr*-like gene and deduced protein sequences were submitted to Marilyn Roberts (Washington State University, WA, USA) and the new variant designated *cfr*(B) (24).

Nucleotide sequence accession number. *cfr*(B) and its adjacent regions were submitted to the EMBL database and deposited under the accession number [KR610408](https://www.ebi.ac.uk/ena/record/KR610408).

RESULTS

Patient histories. A 56-year-old male from south Louisiana received a kidney-pancreas transplant 2 years earlier (2010) with associated immunosuppression. Other pertinent past medical history includes a splenectomy and partial liver resection following a motor vehicle accident. In September 2011, he received linezolid therapy (600 mg orally [p.o.] twice a day [b.i.d.], length of time unknown) to treat right-sided mastoiditis. In February 2012, he developed septic shock secondary to Fournier's gangrene and was treated with vancomycin, piperacillin-tazobactam, and surgical debridement. In March 2012, he was hospitalized for diarrhea, abdominal pain, nausea, and vomiting. An exploratory laparotomy resulted in small bowel resection for perforation. Initial treatment included vancomycin, piperacillin-tazobactam, and fluconazole. Cultures of the peritoneal fluid grew *Peptostreptococcus* spp., *Klebsiella* spp., vancomycin- and linezolid-resistant *E. faecium* (18203R), and *Candida glabrata*. The patient was treated with micafungin, metronidazole, and ciprofloxacin until discharged. The patient was also diagnosed with orchitis and upon discharge, doxycycline at 100 mg p.o. b.i.d. was prescribed for 1 month. As of August 2014, the patient was alive and doing well.

A 61-year-old male from south Louisiana being treated for neurosarcoidosis and disseminated *Nocardia* with a trimethoprim-sulfamethoxazole regimen and chronic steroids developed an intra-abdominal abscess in October 2012 that required drainage. Culture of the abscess yielded vancomycin-resistant *E. faecium* that was susceptible to linezolid. He was treated with linezolid, piperacillin-tazobactam, and ciprofloxacin. He remained on all antibiotics through mid-December. In January 2013, the patient developed fever and was hospitalized. Blood cultures were positive for linezolid-susceptible, vancomycin-susceptible *E. faecium*. The patient received doripenem and micafungin but continued to have intra-abdominal abscesses requiring drain placement. In March 2013, he remained on doripenem, linezolid, ciprofloxacin, and micafungin (total length of time unknown). In April 2013, blood cultures revealed vancomycin- and linezolid-resistant *E. faecium* (18961R). He was started on daptomycin and metronidazole but expired in May 2013.

The two patients lived in different towns, but they were inpatients in the same two hospitals and long-term acute care center in the New Orleans area. However, the patients were not hospitalized at the same time.

Susceptibility profile, epidemiology, and linezolid resistance mechanisms. The two *E. faecium* isolates exhibited elevated MIC results to linezolid, vancomycin, teicoplanin (VanA phenotype), gentamicin, ciprofloxacin, and tetracycline (Table 1). The two isolates were susceptible to doxycycline (MIC, 4 $\mu\text{g/ml}$) (Table 1). The isolate recovered during the 2012 sampling year displayed resistance phenotypes to erythromycin and clindamycin, while

TABLE 1 Antimicrobial susceptibility profile of Cfr-producing *E. faecium* isolated in a New Orleans hospital and *S. aureus* RN4220 carrying *cfr* and *cfr*(B) recombinant plasmids

Antimicrobial agent	MIC (μg/ml) for:					
	<i>E. faecium</i>		<i>S. aureus</i>			
	18203R	18961R	RN4220	RN4220(pEPSA5)	RN4220(pEPSA5- <i>cfr</i>) ^a	RN4220(pEPSA5- <i>cfr</i> (B)) ^b
Linezolid	8	8	0.5	0.5	2	2
Clindamycin	>64	0.25	0.06	0.06	16	16
Erythromycin	>64	2	0.25	0.25	0.25	0.25
Chloramphenicol	8	4	4	64 ^c	128	128
Retapamulin	0.5	0.5	0.06	0.06	4	4
Quinupristin-dalfopristin	1	1	0.5	0.25	1	1
Virginiamycin	0.5	0.25	0.25	0.12	1	1
Ampicillin	>8	>8	ND ^d	ND	ND	ND
Tetracycline	32	32	ND	ND	ND	ND
Tigecycline	0.06	0.06	ND	ND	ND	ND
Doxycycline	4	4	ND	ND	ND	ND
Vancomycin	>16	>16	ND	ND	ND	ND
Teicoplanin	>16	>16	ND	ND	ND	ND
Daptomycin	2	4	ND	ND	ND	ND
Gentamicin	>8	>8	ND	ND	ND	ND
Ciprofloxacin	>4	>4	ND	ND	ND	ND

^a *cfr* gene from *S. aureus* carrying pSCFS3 (17, 26).
^b *cfr*(B) gene from this study.
^c Plasmid pEPSA5 carried the chloramphenicol resistance marker (*cat*) for selection of transformants in *S. aureus*.
^d ND, not determined.

the *E. faecium* isolate collected during 2013 was susceptible to the two compounds (Table 1). The *E. faecium* isolates shared highly similar PFGE profiles (one band difference) (Fig. 1A) and were assigned ST794. The two isolates were *cfr* positive, and one out of six 23S rRNA alleles had a G2576T alteration. Modifications in the L3, L4, and L22 proteins were not observed.

Characterization of the *cfr* gene variant, *cfr*(B), and encoded protein. The *cfr*(B) nucleotide sequence was most similar (99.7%)

to those found in *P. difficile* (GenBank accession numbers KM359438, KM359439, CP003939, HG002389, and HG002396). The Cfr(B) amino acid sequences from the two *E. faecium* isolates were identical and had only 74.9% amino acid or 72.0% nucleotide identities compared with those of the Cfr proteins and *cfr* genes carried in plasmids pSCFS1 or pSCFS3 (Fig. 2; see also Fig. S1 in the supplemental material). However, the Cfr(B)-encoded protein showed complete identity with 23S rRNA methyltrans-

FIG 1 PFGE of *E. faecium* genomic DNA digests and Southern blot hybridization with a *cfr*-specific digoxigenin-labeled probe. (A) SmaI digests; (B) EcoRI digests; (C) ICEul digests. Lanes λ, 1, and 2 represent lambda DNA molecular marker, *E. faecium* 18203R, and *E. faecium* 18961R, respectively.

FIG 2 Evolutionary relationships predicted from the Cfr, Cfr(B), and RlmN sequence alignment. The length of each branch represents the distance between sequences, while the units at the bottom of the tree indicate the number of substitution events. Reference information and accession numbers are given in parentheses here for the following proteins: Cfr(B) ([KR610408](#)) from *E. faecium* strains 18203R and 18961R (this study; USA), Cfr(B) (GenBank accession number [EFQ16578](#)) from *E. faecalis* TX0635/WH245 (USA) (37), Cfr(B) (GenBank accession number [EOI25076](#)) from *E. faecalis* WH571 (USA) (37), Cfr(B) (GenBank accession number [EOI92877](#)) from *E. faecalis* TX0635/WH245 (USA) (37), Cfr(B) (GenBank accession number [EJX57584](#)) from *E. faecium* R497 (USA) (38), Cfr(B) (GenBank accession number [AIX48091](#)) from *P. difficile* 11107643 (Spain) (23), Cfr(B) (GenBank accession number [CDF47262](#)) from *P. difficile* Ox2167 (England) (32), Cfr(B) (GenBank accession number [EQF87020](#)) from *P. difficile* 824 (USA; not available), Cfr (GenBank accession number [NP_899167](#)) from *S. sciuri* (Germany) (39), Cfr (GenBank accession number [ACC77590](#)) from *S. aureus* 737X (USA) (13), RlmN (GenBank accession number [AIL04523](#)) from *E. faecalis* ATCC 29212 (USA; not available), RlmN (GenBank accession number [EFS10488](#)) from *E. faecium* TX0082 (USA) (40), and RlmN (GenBank accession number [ZP_06924569](#)) from *S. aureus* ATCC 51811 (USA) (41).

ferase or SAM-dependent methyltransferase described in *E. faecium* (GenBank accession number [EJX74584](#)) or 99.7% with those from *E. faecalis* (GenBank accession numbers [EFQ16578](#), [EOI25076](#), and [EOI92877](#)) and *P. difficile* (GenBank accession numbers [AIX48091](#), [CDF47262](#), and [EQF87020](#)) (Fig. 2; see also Fig. S1 in the supplemental material). In addition, Cfr(B) showed the conserved amino acid positions that are associated with Cfr and that differentiate it from the intrinsic RlmN (Fig. 2; see also Fig. S1 in the supplemental material) (11). Compared with examples of the indigenous RlmN, those of Cfr(B) showed approximately 35.0% identity (Fig. 2; see also Fig. S1 in the supplemental material). Moreover, the *cfr* and *cfr*(B) expressed in the *S. aureus* RN4220 background demonstrated that the two proteins similarly increased the MIC results for linezolid, chloramphenicol, clindamycin, retapamulin, quinupristin-dalfopristin, and virginiamycin (Table 1).

Location of *cfr*(B) and genetic context. PFGE followed by Southern blotting hybridization of I-CeuI digests revealed two signals from the *E. faecium* isolates (Fig. 1). This was confirmed by additional Southern blotting hybridizations of SmaI and EcoRI digests (Fig. 1), each generating two hybridization signals. S1 nuclease digest blotting did not yield hybridization signals, suggesting the absence of extrachromosomal locations for *cfr*(B).

The genetic context of *cfr*(B) included a Tn6218 structure similar to the *cfr*-carrying transposon described in *P. difficile* (GenBank accession number [HG002396](#)) (Fig. 3) including integrase, excisionase, regulatory proteins, sigma 70 region, and other open reading frames flanked by chromosomal DNA. Only 94 single nucleotide polymorphisms (SNPs) were observed in the se-

quence spanning 8,363 nucleotides surrounding the *cfr*(B) gene compared to that under GenBank accession number [HG002396](#). Among these SNPs, 91 were within the transposase/integrase (*int*) gene, which translated in 86% identity between the two *int* genes. In addition, the *int* gene from *E. faecium* was identical to that encoding a transposase described in *E. faecium* under GenBank accession number [WP_002350216](#). The *cfr*(B)-containing genetic element was inserted in the chromosome in between 16S rRNA pseudouridylylase synthase and a bacteriophage integrase. Further *in silico* sequence analysis demonstrated that *E. faecium* isolates harbored *tet*(L), *tet*(M), *aac*(6')-aph(2''), *aph*(3')-III, *vanA*, and *erm*(B) genes; however, the *erm*(B) gene in 18961R was disrupted by *sasK*.

DISCUSSION

Cfr belongs to the S-adenosylmethionine (SAM) enzyme superfamily of proteins, which are widely distributed in nature (11). These proteins have similarities to the Cfr(B) protein described here, and many sequences belonging to this class observed in several bacterial species (e.g., *E. faecium*, *P. difficile*, *Bacillus amyloliquefaciens*, and *Bacillus methylotrophicus*) have been deposited in GenBank as putative chloramphenicol-florphenicol-resistance proteins. Similar to Cfr and Cfr(B), three Cfr-like proteins from *B. amyloliquefaciens*, *Bacillus clausii*, and *Brevibacillus brevis* were shown to confer decreased susceptibility to five classes of antibiotics when expressed in *E. coli* or *S. aureus* (31).

With rare exceptions, *cfr* has been plasmid located and surrounded by mobile elements (18, 20, 26). Likewise, *cfr*(B) was located on a Tn6218 element, the structure of which was published

FIG 3 Schematic representation of DNA sequences (8.4 kb) surrounding *cfr*(B).

in GenBank (accession number [HG002396](#)) and obtained from a *P. difficile* isolate (Ox2167) collected from a human specimen in 2009 in Oxford, England (32). These results suggest that *cfr*(B) was incorporated by Tn6218, and this genetic structure was disseminated among *E. faecium* (this study) and *P. difficile* (England) isolates and likely among the *P. difficile* isolates from Spain (23) and other bacterial species yet to be detected. Tn6218 has been detected in other *P. difficile* clinical isolates (Ox42 [GenBank accession number [HG002387](#)] and Ox1485 [GenBank accession number [HG002395](#)]); however, these genetic structures lack the *cfr* or *cfr*(B) gene. Moreover, the presence of multiple copies of *cfr*(B) in the *E. faecium* chromosome (this study) emphasizes the capability of this transposon to mobilize.

It was demonstrated here that Cfr(B) confers a similar phenotype compared to Cfr expressed in an *S. aureus* background. However, it is tempting to speculate that the same may not be true when Cfr(B) is expressed in an enterococcal background since *E. faecium* 18961 displayed a low MIC value for clindamycin, and the two isolates showed low MIC results for chloramphenicol. As isolate 18961 had a nonfunctional Erm(B), it would be expected that the clindamycin MIC value would remain elevated due to the production of Cfr(B). Thus, it appears that, at least in isolate 18961, Cfr(B) does not confer the usual PhLOPS_A phenotype, which has been previously reported in *E. faecalis* (33). In addition, the two *E. faecium* isolates exhibited a linezolid MIC value of 8 µg/ml, which would be consistent with a low number of alleles mutated in 23S rRNA (34). Therefore, further investigations are needed to access the resistance levels conferred by *cfr*(B) when expressed in enterococci and its possible effect when associated with 23S rRNA mutations.

In summary, this study reports the first detection of *cfr* genes in *E. faecium* clinical isolates in the United States and the characterization of a new variant, *cfr*(B), which was shown to confer a resistance phenotype to several antimicrobial agents similar to that of *cfr* when expressed in an *S. aureus* background. The origin of *cfr*-like genes and their dissemination within several distinct genetic structures remains to be elucidated. Just like the *cfr* gene detected in several bacterial species from human and animal specimens, *cfr*(B) has been observed in mobile genetic elements and among at least three bacterial species (i.e., *E. faecium*, *E. faecalis*, and *P. difficile*). Overall, linezolid resistance rates remain low (1, 2, 25, 35, 36); however, sporadic outbreaks and clonal spread of linezolid-resistant Gram-positive isolates remain a threat, highlighting the importance of active surveillance and screening for mobile genes, such as those described here.

ACKNOWLEDGMENTS

We thank the following staff members at JMI Laboratories (North Liberty, IA, USA) for technical support and manuscript assistance: J. Oberholser, P. Rhomberg, J. Ross, J. Schuchert, J. Streit, and L. Woosley. We are also grateful to Alex Horswill, University of Iowa, for providing the *E. coli*-*S. aureus* shuttle vector pEPSA5.

JMI Laboratories, Inc., received research and educational grants from 2012 to 2014 from Achaogen, Actelion, Affinium, American Proficiency Institute (API), AmpliPhi Biosciences, Anacor, Astellas, AstraZeneca, Basilea, BioVersys, Cardeas, Cempira, Cerexa, Cubist, Daiichi, Dipexium, Durata, Exela, Fedora, Forest Research Institute, Furiex, Genentech, GlaxoSmithKline, Janssen, Johnson & Johnson, Medpace, Meiji Seika Kaisha, Melinta, Merck, MethylGene, Nabriva, Nanosphere, Novartis, Pfizer, Polypor, Rempex, Roche, Seachaid, Shionogi, Synthes, The Med-

icines Co., Theravance, Thermo Fisher, VenatoRx, Vertex, Waterloo, Wockhardt, and some other corporations.

Some JMI employees are advisors/consultants for Astellas, Cubist, Pfizer, Cempira, Cerexa-Forest, and Theravance.

With regard to speakers bureaus and stock options, we have no conflicts of interest to declare. The Ochsner Clinic Foundation authors declare no conflicts of interest.

REFERENCES

- Flamm RK, Mendes RE, Hogan PA, Ross JE, Farrell DJ, Jones RN. 2015. *In vitro* activity of linezolid as assessed through the 2013 LEADER surveillance program. *Diagn Microbiol Infect Dis* 81:283–289. <http://dx.doi.org/10.1016/j.diagmicrobio.2014.12.009>.
- 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.drug.2014.04.002>.
- Locke JB, Hilgers M, Shaw KJ. 2009. Mutations in ribosomal protein L3 are associated with oxazolidinone resistance in staphylococci of clinical origin. *Antimicrob Agents Chemother* 53:5275–5278. <http://dx.doi.org/10.1128/AAC.01032-09>.
- Locke JB, Hilgers M, Shaw KJ. 2009. Novel ribosomal mutations in *Staphylococcus aureus* strains identified through selection with the oxazolidinones linezolid and torezolid (TR-700). *Antimicrob Agents Chemother* 53:5265–5274. <http://dx.doi.org/10.1128/AAC.00871-09>.
- Floyd JL, Smith KP, Kumar SH, Floyd JT, Varela MF. 2010. LmrS is a multidrug efflux pump of the major facilitator superfamily from *Staphylococcus aureus*. *Antimicrob Agents Chemother* 54:5406–5412. <http://dx.doi.org/10.1128/AAC.00580-10>.
- 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>.
- Toh SM, Xiong L, Arias CA, Villegas MV, Lolans K, Quinn J, Mankin AS. 2007. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. *Mol Microbiol* 64:1506–1514. <http://dx.doi.org/10.1111/j.1365-2958.2007.05744.x>.
- Challand MR, Salvadori E, Driesener RC, Kay CW, Roach PL, Spencer J. 2013. Cysteine methylation controls radical generation in the Cfr radical AdoMet rRNA methyltransferase. *PLoS One* 8:e67979. <http://dx.doi.org/10.1371/journal.pone.0067979>.
- Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. 2006. The *cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* 50:2500–2505. <http://dx.doi.org/10.1128/AAC.00131-06>.
- Kaminska KH, Purta E, Hansen LH, Bujnicki JM, Vester B, Long KS. 2010. Insights into the structure, function and evolution of the radical-SAM 23S rRNA methyltransferase Cfr that confers antibiotic resistance in bacteria. *Nucleic Acids Res* 38:1652–1663. <http://dx.doi.org/10.1093/nar/gkp1142>.
- Atkinson GC, Hansen LH, Tenson T, Rasmussen A, Kirpekar F, Vester B. 2013. Distinction between the Cfr methyltransferase conferring antibiotic resistance and the housekeeping RlmN methyltransferase. *Antimicrob Agents Chemother* 57:4019–4026. <http://dx.doi.org/10.1128/AAC.00448-13>.
- Schwarz S, Werckenthin C, Kehrenberg C. 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrob Agents Chemother* 44:2530–2533. <http://dx.doi.org/10.1128/AAC.44.9.2530-2533.2000>.
- Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN. 2008. First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob Agents Chemother* 52:2244–2246. <http://dx.doi.org/10.1128/AAC.00231-08>.
- Bongiorno D, Campanile F, Mongelli G, Baldi MT, Provenzano R, Reali S, Lo Russo C, Santagati M, Stefani S. 2010. DNA methylase modifications and other linezolid resistance mutations in coagulase-negative

- staphylococci in Italy. *J Antimicrob Chemother* 65:2336–2340. <http://dx.doi.org/10.1093/jac/dkq344>.
15. Rajan V, Kumar VG, Gopal S. 2014. A *cfr*-positive clinical staphylococcal isolate from India with multiple mechanisms of linezolid-resistance. *Indian J Med Res* 139:463–467.
 16. Gales AC, Deshpande LM, de Souza AG, Pignatari AC, Mendes RE. 2015. MSSA ST398/t034 carrying a plasmid-mediated *cfr* and *erm(B)* in Brazil. *J Antimicrob Chemother* 70:303–305. <http://dx.doi.org/10.1093/jac/dku366>.
 17. Kehrenberg C, Schwarz S. 2006. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrob Agents Chemother* 50:1156–1163. <http://dx.doi.org/10.1128/AAC.50.4.1156-1163.2006>.
 18. Shen J, Wang Y, Schwarz S. 2013. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* 68:1697–1706. <http://dx.doi.org/10.1093/jac/dkt092>.
 19. Liu Y, Wang Y, Schwarz S, Li Y, Shen Z, Zhang Q, Wu C, Shen J. 2013. Transferable multiresistance plasmids carrying *cfr* in *Enterococcus* spp. from swine and farm environment. *Antimicrob Agents Chemother* 57:42–48. <http://dx.doi.org/10.1128/AAC.01605-12>.
 20. Diaz L, Kiratisin P, Mendes R, Panesso D, Singh KV, Arias CA. 2012. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother* 56:3917–3922. <http://dx.doi.org/10.1128/AAC.00419-12>.
 21. Liu Y, Wang Y, Wu C, Shen Z, Schwarz S, Du XD, Dai L, Zhang W, Zhang Q, Shen J. 2012. First report of the multidrug resistance gene *cfr* in *Enterococcus faecalis* of animal origin. *Antimicrob Agents Chemother* 56:1650–1654. <http://dx.doi.org/10.1128/AAC.06091-11>.
 22. Patel SN, Memari N, Shahinas D, Toye B, Jamieson FB, Farrell DJ. 2013. Linezolid resistance in *Enterococcus faecium* isolated in Ontario, Canada. *Diagn Microbiol Infect Dis* 77:350–353. <http://dx.doi.org/10.1016/j.diagmicrobio.2013.08.012>.
 23. Marin M, Martin A, Alcalá L, Cercenado E, Iglesias C, Reigadas E, Bouza E. 2015. *Clostridium difficile* isolates with high linezolid MICs harbor the multiresistance gene *cfr*. *Antimicrob Agents Chemother* 59:586–589. <http://dx.doi.org/10.1128/AAC.04082-14>.
 24. Schwarz S, Wang Y. 2015. Nomenclature and functionality of the so-called *cfr* gene from *Clostridium difficile*. *Antimicrob Agents Chemother* 59:2476–2477. <http://dx.doi.org/10.1128/AAC.04893-14>.
 25. Mendes RE, Flamm RK, Hogan PA, Ross JE, Jones RN. 2014. Summary of linezolid activity and resistance mechanisms detected during the 2012 LEADER surveillance program for the United States. *Antimicrob Agents Chemother* 58:1243–1247. <http://dx.doi.org/10.1128/AAC.02112-13>.
 26. Mendes RE, Deshpande LM, Bonilla HF, Schwarz S, Huband MD, Jones RN, Quinn JP. 2013. Dissemination of a pSCFS3-like *cfr*-carrying plasmid in *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical isolates recovered from hospitals in Ohio. *Antimicrob Agents Chemother* 57:2923–2928. <http://dx.doi.org/10.1128/AAC.00071-13>.
 27. Kahn HP, Deshpande LM, Mendes RE, Ashcraft DS, Pankey G. 2015. Abstr 115th Gen Meet Am Soc Microbiol, abstr 103.
 28. Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—10th ed. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
 29. Clinical and Laboratory Standards Institute. 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
 30. Miller K, Dunsmore CJ, Fishwick CW, Chopra I. 2008. Linezolid and tiamulin cross-resistance in *Staphylococcus aureus* mediated by point mutations in the peptidyl transferase center. *Antimicrob Agents Chemother* 52:1737–1742. <http://dx.doi.org/10.1128/AAC.01015-07>.
 31. Hansen LH, Planellas MH, Long KS, Vester B. 2012. The order Bacillales hosts functional homologs of the worrisome *cfr* antibiotic resistance gene. *Antimicrob Agents Chemother* 56:3563–3567. <http://dx.doi.org/10.1128/AAC.00673-12>.
 32. Dingle KE, Elliott B, Robinson E, Griffiths D, Eyre DW, Stoesser N, Vaughan A, Golubchik T, Fawley WN, Wilcox MH, Peto TE, Walker AS, Riley TV, Crook DW, Didelot X. 2014. Evolutionary history of the *Clostridium difficile* pathogenicity locus. *Genome Biol Evol* 6:36–52. <http://dx.doi.org/10.1093/gbe/evt204>.
 33. Liu Y, Wang Y, Schwarz S, Wang S, Chen L, Wu C, Shen J. 2014. Investigation of a multiresistance gene *cfr* that fails to mediate resistance to phenicols and oxazolidinones in *Enterococcus faecalis*. *J Antimicrob Chemother* 69:892–898. <http://dx.doi.org/10.1093/jac/dkt459>.
 34. Lobritz M, Hutton-Thomas R, Marshall S, Rice LB. 2003. Recombination proficiency influences frequency and locus of mutational resistance to linezolid in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 47:3318–3320. <http://dx.doi.org/10.1128/AAC.47.10.3318-3320.2003>.
 35. Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK. 2015. Update on the linezolid *in vitro* activity through the Zyvox Annual Appraisal of Potency and Spectrum program, 2013. *Antimicrob Agents Chemother* 59:2454–2457. <http://dx.doi.org/10.1128/AAC.04784-14>.
 36. Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK. 2014. Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) program: report of linezolid activity over nine years (2004–12). *J Antimicrob Chemother* 69:1582–1588. <http://dx.doi.org/10.1093/jac/dkt541>.
 37. McBride SM, Fischetti VA, Leblanc DJ, Moellering RC, Jr, Gilmore MS. 2007. Genetic diversity among *Enterococcus faecalis*. *PLoS One* 2:e582. <http://dx.doi.org/10.1371/journal.pone.0000582>.
 38. Arias CA, Panesso D, McGrath DM, Qin X, Mojica MF, Miller C, Diaz L, Tran TT, Rincon S, Barbu EM, Reyes J, Roh JH, Lobos E, Sodergren E, Pasqualini R, Arap W, Quinn JP, Shamoo Y, Murray BE, Weinstock GM. 2011. Genetic basis for *in vivo* daptomycin resistance in enterococci. *N Engl J Med* 365:892–900. <http://dx.doi.org/10.1056/NEJMoa1011138>.
 39. Kehrenberg C, Ojo KK, Schwarz S. 2004. Nucleotide sequence and organization of the multiresistance plasmid pSCFS1 from *Staphylococcus sciuri*. *J Antimicrob Chemother* 54:936–939. <http://dx.doi.org/10.1093/jac/dkh457>.
 40. Nallapareddy SR, Singh KV, Murray BE. 2006. Construction of improved temperature-sensitive and mobilizable vectors and their use for constructing mutations in the adhesin-encoding *acm* gene of poorly transformable clinical *Enterococcus faecium* strains. *Appl Environ Microbiol* 72:334–345. <http://dx.doi.org/10.1128/AEM.72.1.334-345.2006>.
 41. Su YC, Wong AC. 1995. Identification and purification of a new staphylococcal enterotoxin, H. *Appl Environ Microbiol* 61:1438–1443.