BRIEF REPORT

Multicity Outbreak of Linezolid-Resistant *Staphylococcus epidermidis* Associated with Clonal Spread of a *cfr*-Containing Strain

Hector Bonilla,[1] Michael D. Huband,[3] Joan Seidel,[2] Helen Schmidt,[2] MaryKay Lescoe,[3] Sandra P. McCurdy,[3] M. Megan Lemmon,[3] Lori A. Brennan,[3] A. Tait-Kamradt,[3] Laura Puzniak,[3] and John P. Quinn[3]

[1]Summa Health System, Akron, and [2]Robinson Memorial Hospital, Ravenna, Ohio; and [3]Pfizer Global Research and Development, Groton, Connecticut

We report a multicity outbreak of *cfr*-containing linezolid-resistant *Staphylococcus epidermidis* in Ohio. Thirty-nine isolates were obtained from 2 hospitals. Two clones with different mechanisms of linezolid resistance were circulating in hospital A. One of these contained the *cfr* gene, and the other a ribosomal mutation. The clone containing *cfr* was identical in both hospitals.

Linezolid is an oxazolidinone antibacterial approved for treatment of infections caused by several gram-positive organisms, including *Staphylococcus aureus* and *Staphylococcus epidermidis* [1]. Since its clinical introduction in the United States in 2000, the emergence of resistant strains has remained relatively rare [2–4]. Several multicenter and multinational surveillance studies have shown that >99% of clinical strains of coagulase negative staphylococci and *S. aureus* still remain susceptible to linezolid [2–7]. When resistance does occur, it is seen most commonly in coagulase-negative staphylococci and enterococci [4, 7–9]. Resistance may arise during therapy, especially in deep-seated infections treated over prolonged courses [8, 9]. Most isolates of this type in both enterococci and staphylococci have mutations at the site of action, in the central loop of domain V of the 23S rRNA; the most common of these mutations is G2576T [10]. Recently, linezolid resistance due to acquisition of a gene known as *cfr* (chloramphenicol and florfenicol resistance) has been reported [11–17]. The product of this gene is a methyltransferase that modifies adenosine at position 2503 in the 23S rRNA, which is located in the drug-binding site.

This methylation affects the binding of and susceptibility to 4 other antimicrobial classes (phenicols, lincosamides, pleuromutilins, and streptogramin A), leading to a multidrug-resistant phenotype. The *cfr* gene has been found primarily on plasmids, and in the laboratory, it can transfer between staphylococci [13]. Although the first human isolate was found to have a chromosomal location, flanking regions suggested a plasmidic origin [14]. The *cfr* gene was first seen in veterinary isolates of *Staphylococcus warneri, Staphylococcus sciuri, Staphylococcus hyicus,* and *S. aureus* perhaps associated with veterinary use of phenicols [18], but it has also been reported in several isolates of *S. aureus* from humans, including an outbreak in Spain [19]. The epidemiology of linezolid-resistant coagulase-negative staphylococci has not been well characterized. However, 2 nosocomial outbreaks of such organisms have occurred that have been associated with clonal spread [20, 21]. We describe here the clinical and molecular epidemiology of an outbreak of linezolid-resistant *S. epidermidis* containing *cfr* in 2 hospitals located 39 miles apart in northeastern Ohio. Susceptibility testing, molecular typing, and mechanistic studies were performed in a central laboratory.

Methods. This study was conducted in a 280-bed, rural, county hospital with an on-site–affiliated, long-term acute care (LTAC) facility, located in northeast Ohio (hospital A). The patients in this LTAC facility often have multiple comorbidities, with a mean hospital stay of 25 days. An index isolate of linezolid-resistant *S. epidermidis* was isolated from a patient's blood in April 2008. From then until May 2009, isolates of linezolid-resistant *S. epidermidis* from blood cultures were stored in a −70°F freezer. Demographic data, current and prior hospitalizations over the prior 12 months, comorbidities, and antibacterial use during the prior 6 months were recorded by reviewing patient medical and pharmacy records. The clinical events were classified as nosocomial acquired (>48 h after admission) or community acquired (<48 h after admission). Bacteremic episodes were classified as true infection on the basis of the following criteria: multiple positive blood culture results, a positive blood culture result along with a positive culture result with the same organism from an infected device or line, and bacteremia associated with systemic symptoms (fever [temperature >8.5°C], hypotension [systolic blood pressure <90 mm/Hg], and leukocytosis [>13,000 cells/mL]) that could not be attributed to other processes [22].

Received 24 March 2010; accepted 22 June 2010; electronically published 20 August 2010.

Reprints or correspondence: Michael D. Huband, Pfizer Global Research and Development, Antibacterial Biology, Eastern Point Rd, MS 8220-2443, Groton, CT 06340 (michael.huband@pfizer.com).

Clinical Infectious Diseases 2010; 51(7):796–800

1058-4838/2010/5107-0006$15.00
DOI: 10.1086/656281

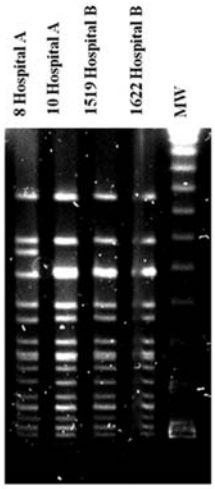

Figure 1. Pulsed-field gel electrophoresis of *Staphylococcus epidermidis* (SE) from hospitals A and B. MW, molecular weight.

The linezolid-resistant bacterial isolates recovered were referred to the antibacterial department at Pfizer Global Research and Development in Groton, Connecticut (the central laboratory), for confirmation of species identification, minimum inhibitory concentrations (MICs), clonal analysis, and characterization of the mechanisms underlying linezolid resistance. Because our central laboratory had been sent previous linezolid-resistant *S. epidermidis* isolates from this part of Ohio, we were aware of similar cases occurring in another Ohio hospital 39 miles away (hospital B). The first such case actually occurred there in 2006, 2 years prior to our index case. Isolates that had been recovered during the period from October 2006 to July 2007 were obtained from hospital B. Hospital B is a larger medical facility; with 476 bed, it serves 8 outpatient medical centers and has an on-site, 30-bed LTAC facility. Although we could obtain isolates from hospital B, clinical information was not available. To the best of our knowledge, no patients from hospital B have been admitted to hospital A or its LTAC facility in the previous 5 years. Antimicrobial susceptibility testing of bacterial isolates was performed according to the Clinical and Laboratory Standards Institute's approved methods [23, 24]. Pulsed-field gel electrophoresis (PFGE) was performed using a modification of the method of McDougal et al [25]. The criteria for clonality were those used by Tenover et al [26]. Presence of the *cfr*-methylase gene (ie, G2576T) and L4 mutations of the 50S ribosomal subunit were confirmed by polymerase chain reaction analysis. Less common ribosomal mutations, such as T2500A, were not tested for.

Results and discussion. PFGE typing of 15 isolates from hospital A revealed an interesting gel pattern (Figure 1). First, 2 major clone types were circulating in hospital A. Type 1 was generally *cfr* positive and had no G2576T mutation. Type 2 was generally *cfr* negative but had G2576T mutations. In addition, linezolid-resistant isolates from hospital B also belonged to the same pulsotype as type 1 from hospital A. These isolates were mostly, but not universally, *cfr* positive. Exceptions at hospital A included a type 1 isolate that was both *cfr* negative and

Table 1. Hospital A: Linezolid-Resistant *Staphylococcus epidermidis* (April 2008–May 2009)

S. epidermidis strain	PFGE type	Minimum inhibitory concentration, μg/mL					
		Linezolid[a]	Clindamycin	Chloramphenicol	G2576T	*cfr*	L4
1634-09	1	>256	>64	>64	Neg	Pos	Pos
1635-09	1	>256	0.25	16	Neg	Neg	Pos
1519-08	1	>256	>64	>64	Neg	Pos	Pos
1622-09	1	>256	>64	>64	Neg	Pos	Pos
1625-09	1	>256	>64	>64	Neg	Pos	Neg
1628-09	1	>256	>64	>64	Neg	Pos	Pos
1631-09	1	>256	>64	>64	Neg	Pos	Pos
1516-08	2	16	>64	64	Pos	Neg	Neg
1517-08	2	>256	0.5	32	Pos	Neg	Pos
1518-08	2	>256	2	ND	Pos	Neg	Pos
1520-08	2	>256	2	ND	Pos	Neg	Pos
1626-09	2	>256	>64	>64	Neg	Pos	Pos
1627-09	2	>256	1	32	Pos	Neg	Pos
1629-09	2	>256	0.5	32	Pos	Neg	Pos
1630-09	2	>256	16	64	Pos	Neg	Pos
1633-09	ND	>256	1	32	Pos	Neg	Pos

NOTE. ND, no data; Neg, negative; PFGE, pulsed-field gel electrophoresis; Pos, positive.

[a] Determined by use of the Etest (AB Biodisk).

Table 2. Hospital B: Linezolid-Resistant *Staphylococcus epidermidis* (October 2006–July 2007)

S. epidermidis strain	PFGE type	MIC of linezolid,[a] μg/mL	G2576T	*cfr*	L4
1243-07	1	>256	Neg	Pos	Pos
1246-07	1	>256	Neg	Pos	Pos
1247-07	1	>256	Neg	Pos	Pos
1251-07	1	>256	Neg	Pos	Pos
1252-07	1	>256	Neg	Pos	Pos
1253-07	1	>256	Neg	Pos	Pos
1255-07	1	>256	Neg	Pos	Pos
1256-07	1	>256	Neg	Pos	Pos
1261-07	1	>256	Neg	Pos	Pos
1262-07	1	>256	Neg	Pos	Pos
1263-07	1	>256	Neg	Pos	Pos
1275-07	1	>256	Neg	Pos	Pos
1250-07	1 A	128	Neg	Neg	Pos
1259-07	1 A	>256	Neg	Neg	Pos
1260-07	1	>256	Neg	Neg	Pos
1248-07	ND	>256	Pos	Neg	Neg
1249-07	ND	>256	Pos	Neg	Neg
1254-07	ND	>256	Pos	Neg	Neg
1257-07	ND	>256	Neg	Neg	Pos
1258-07	ND	>256	Neg	Pos	Pos
1264-07	ND	>256	Neg	Neg	Pos

NOTE. MIC, minimum inhibitory concentration; ND, no data; Neg, negative; PFGE, pulsed-field gel electrophoresis; Pos, positive.

[a] Determined by use of the Etest (AB Biodisk).

G2576T negative, and a type 2 isolate that was positive for *cfr*. We did not find any isolates carrying both the *cfr* gene and the G2576T ribosomal mutation. The majority of the isolates from both clonal types were found to also possess a concomitant glycine insertion in a highly conserved region of the L4 ribosomal gene: $_{71}GR_{72}$ to $_{71}GGR_{72}$. This glycine insertion has been described once before in *S. epidermidis,* where it was the only mutation thought to explain linezolid resistance [27]. The exact contribution of this insertion to linezolid resistance is difficult to discern in strains with underlying *cfr* or G2576T mutations. It is interesting to note that a few strains from both medical centers demonstrated only L4 mutations and had high MICs of linezolid (>256 μg/mL). However, in these strains, it should be noted that other, less common linezolid-resistant mutations (L3, L22, and T2500A) were not tested for.

Bacterial isolates were recovered from both medical centers (18 isolates from hospital A and 21 isolates from hospital B). In hospital A, 16 strains were identified as *S. epidermidis,* and 2 strains were identified as *Staphylococcus haemolyticus.* These strains were isolated from blood cultures from 11 different patients. Because *S. epidermidis* cases predominated, we focused our attention on those isolates. Medical records from these patients were reviewed. Six were females with a mean age of 64 years (range, 34–83 years). All had comorbidities. The most common of these comorbidities were diabetes mellitus, hypertension, cardiovascular disease, and malignancies. Eight of the 11 isolates were nosocomially acquired, and the remaining 3 were acquired in the LTAC facility. Bacteremia was considered a true infection on the basis of the criteria outlined above in 5 of 11 patients. Of these 5 cases of bacteremia, 3 were associated with central line use. All patients had received antibacterials 5–13 days prior to the bacteremic episode, and 5 had received linezolid. None had received other agents that might select *cfr,* such as clindamycin or chloramphenicol. Pharmacy records showed that all patients with a linezolid-resistant isolate had received linezolid treatment within the previous 6 months.

Bacterial identification, antibiograms, molecular typing data, and molecular mechanistic data from isolates in hospital A are summarized in Table 1, and those from hospital B in Table 2. It is noteworthy that >94% of isolates in both medical centers, independent of the resistance mechanism, had linezolid MICs >256 μg/mL. As expected, the *cfr*-positive isolates in both medical centers also displayed a multidrug-resistant phenotype, including resistance to clindamycin and chloramphenicol.

There are both infection control and mechanistic implications to our observations. LTAC facilities have been implicated as reservoirs in regional outbreaks of multidrug-resistant organisms [28]. The majority of our patients had multiple risk factors for colonization and/or infection with multidrug-resistant organisms, including multiple comorbidities, presence of intravascular catheter, and prior antibacterial exposure [21, 29, 30]. All patients received linezolid at some point during the prior 6 months, which may have contributed to the emergence of resistant strains [8, 31, 32]. Two major clones were circulating in hospital A, and the one containing *cfr* was common to both hospitals. We believe that the *cfr* strain may have originated in hospital B. Hospital B is a larger facility, and resistant strains were originally identified there 2 years earlier. Although the 2 hospitals are not affiliated, they are close enough to one another that it is possible that some patients may have received care in both. Unfortunately, because of the absence of clinical information from hospital B, we can neither confirm nor refute this hypothesis. The demonstration that the clone in both hospitals contained *cfr* is disturbing, because this gene is usually plasmid mediated, raising the concern about spread between strains in addition to clonal spread. Plasmid studies as well as attempts to elucidate the exact role of the L4 insertion and other less common resistance mutations in mediating linezolid resistance in these strains are the subject of ongoing work in our laboratories. Strains with L4 mutations may be more common than previously thought, because they are not always tested for. Recent reports have shown L4 mutations in laboratory strains of linezolid-resistant *S. aureus* [33] as well as in pneumococci from humans [34] and *Clostridium* species from animals [35].

Acknowledgments

We thank Jafri Khatib, MD, at Mercy Medical Center in Canton, Ohio, for providing bacterial strains. Pfizer provided no financial support to Mercy Medical Center, Summa Health System, or Robinson Memorial Hospital for this study and data analysis.

Potential conflicts of interest. M.D.H., M.L., S.P.M., M.M.L., L.P., and J.P.Q. report that they are currently employees of Pfizer Global Research and Development in Groton, Connecticut, and may also hold Pfizer stock. All other authors: no conflicts.

References

1. Diekema DJ, Jones RN. Oxazolidione antibiotics. Lancet **2001**; 358: 1975–1982.
2. Ross JE, Anderegg TR, Sader HS, Fritsche TR, Jones RN. Trends in linezolid susceptibility patterns in 2002: report from the worldwide Zyvox Annual Appraisal of Potency and Spectrum Program. Diagn Microbiol Infect Dis **2005**;52:53–58.
3. Adderegg TR, Sader HS, Fritsche, Ross JE, Jones RN. Trends in linezolid susceptibility patterns: report from the 2002–2003 worldwide Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program. Int J Antimicrob Agents **2005**; 26:13–21.
4. Jones RN, Fritsche TR, Sader HS, Ross JE. LEADER surveillance program results for 2005: an activity and spectrum analysis of linezolid using clinical isolates from the United States (50 medical centers). Diagn Microbiol Infect Dis **2007**; 59:309–317.
5. Jones RN, Ross JE, Fritsche TR, Sader HS. Oxazolidinone susceptibility patterns in 2004: report from the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program assessing isolates from 16 nations. J Antimicrob Chemother **2006**; 57:279–287.
6. Ross JE, Fritsche TR, Sader HS, Jones RN. Oxazolidinone susceptibility patterns for 2005: International Report from the Zyvox Annual Appraisal of Potency and Spectrum Study. Int J Antimicrob Agents **2007**; 29:295–301.
7. Jones RN, Kohno S, Ono Y, Ross JE, Yanagihara K. ZAAPS International Surveillance Program (2007) for linezolid resistance: results from 5591 gram-positive clinical isolates in 23 countries. Diagn Microbiol Infect Dis **2009**; 64:191–201.
8. Meka VG, Pillai SK, Sakoulas G, et al. Linezolid resistance in sequential *Staphylococcus aureus* isolates associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. J Infect Dis **2004**; 190:311–317.
9. Gómez-Gil R, Romero-Gómez MP, García-Arias A, et al. Nosocomial outbreak of linezolid-resistant *Enterococcus faecalis* infection in a tertiary care hospital. Diagn Microbiol Infect Dis **2009**; 65:175–179.
10. Marshall SH, Donskey CJ, Hutton-Thomas R, Salata RA, Rice LB. Gene dosage and linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis.* Antimicrob Agents Chemother **2002**; 46:3334–3336.
11. Kehrenberg C, Cuny C, Strommenger B, Schwarts S, White W. Methicillin-resistant and susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr.* Antimicrob Agents Chemother **2009**; 53:779–781.
12. Kehrenberg C, Schwarz S, Jacobsen L, Hansen LH, Vester B. A new mechanism for chloranphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. Mol Microbiol **2005**; 57: 1064–1073.
13. Kehrenberg C, Aarestrup FM, Schawarz S. IS21–558 insertion sequences are involved in the mobility of the multiresistance gene *cfr.* Antimicrob Agents Chemother **2007**; 51:483–487.
14. Toh SM, Xiong L, Arias CA, et al. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotics linezolid. Mol Microbiol **2007**; 64:1506–1514.
15. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob Agents Chemother **2006**; 50:2500–2505.
16. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN. First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. Antimicrob Agents Chemother **2008**; 52:2244–2246.
17. Arias CA, Vallejo M, Reyes J, et al. Clinical and microbiological aspects of linezolid resistance mediated by the *cfr* gene enconding a 23 rRNA methyltransferase. J Clin Microbiol **2008**; 46:892–896.
18. Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes fexA and *cfr* among chloramphenicol-resistant *Staphylococcus.* Antimicrob Agents Chemother **2006**; 50:1156–1163.
19. Morales G, Picazo JJ, Baos E, et al. Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus.* Clin Infect Dis **2010**; 50:821–825.
20. Rodríguez-Aranda A, Daskalaki M, Villar J, Sanz F, Otero JR, Chaves F. Nosocomial spread of linezolid-resistant *Staphylococcus haemolyticus* infection in an intensive care unit. Diagn Microbiol Infect Dis **2009**; 63:398–402.
21. Trevino M, Martinez-Lamas L, Romero-Jung PA, Giraldez JM, Alvarez-Escudero J, Regueiro BJ. Endemic linezolid-resistant *Staphylococcus epidermis* in a critical care unit. Eur J Clin Microbiol Infect Dis **2008**; 28:527–533.
22. Beekmann SE, Diekema DJ, Doern GV. Determining the clinical significance of coagulase-negative staphylococci isolated from blood cultures. Infect Control Hosp Epidemiol **2005**; 26:559–566.
23. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. CLSI document M7-A8. 8th ed. Wayne, PA: Clinical and Laboratory Standards Institute; **2009**.
24. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 19th informational supplement. CLSI document M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute; **2009**.
25. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol **2003**; 41:5113–5120.
26. Tenover FC, Arbeit R, Archer G, et al. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus.* J Clin Microbiol **1994**; 32:407–415.
27. Wong A, Reddy SP, Smyth DS, Aguero-Rosenfeld ME, Sakoulas G, Robinson DA. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. Antimicrob Agents Chemother **2010**; 54: 742–748.
28. Munoz-Price, LS. Long-term acute care hospitals. Clin Infect Dis **2009**; 49:438–443.
29. Herwaldt LA, Hollis RJ, Boyken LD, Pfaller MA. Molecular epidemiology of coagulase-negative staphylococci isolated from immunocompromised patients. Infect Control Hosp Epidemiol **1992**; 13:86–92.
30. Kirchoff LV, Sheagren JN. Epidemiology and clinical significance of blood cultures positive for coagulase-negative *Staphylococcus.* Infect Control **1985**; 6:479–486.
31. Ba BB, Arpin C, Bikie B, et al. Activity of linezolid in an in vitro pharmacokinetic-pharmocodynamic model against *Staphylococcus aureus* and *Enterococcus faecalis* strains with either a hypermutator phenotype or not. In: Programs and abstracts of the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC: American Society for Microbiology, **2009**. Abstract A1-549.
32. Lasala PR, Mccurdy S, Lemmon M, Lescoe M, Mulanovich VE. Outbreak of linezolid-resistant (LNZ-R) coagulase-negative *Staphylococcus* (CNS) in cancer patients linked to LNZ use. In: Programs and abstracts of the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC: American Society for Microbiology, **2009**. Abstract C2-132.

33. Locke JB, Hilgers M, Shaw KJ. Novel ribosomal mutations in *Staphylococcus aureus* identified through selection with the oxazolidinones linezolid and torezolid (TR-700). Antimicrob Agents Chemother **2009**; 53:5265–5274.
34. Wolter N, Smith AM, Farrell DJ, et al. Novel mechanism of resistance to oxazolidinones, macrolides, and chloranphenicol in ribosomal protein L4 of the pneumococcus. Antimicrob Agents Chemother **2005**; 49:3554–3557.
35. Hölzel CS, Harms KS, Schwaiger K, Bauer J. Resistance to linezolid in a porcine *Clostridium perfringens* strain carrying a mutation in the *rplD* gene encoding the ribosome protein L4. Antimicrob Agents Chemother **2010**; 54:1351–1353.