**Linezolid-Resistant Staphylococcus epidermidis, Portugal, 2012**

To the Editor: Linezolid is a therapeutic option for skin and soft tissue infections and pneumonia caused by multidrug-resistant gram-positive bacteria (e.g., *Staphylococcus* spp.), which occur at higher rates in Portugal than in other European countries (www.ecdc.europa.eu/en/publications/Publications/annual-epidemiological-report-2013.pdf). *Staphylococcus epidermidis* are skin and mucosal commensal bacteria; infections in humans are mostly linked to indwelling medical devices. The ability of *S. epidermidis* to acquire resistance to antimicrobial drugs and to produce biofilm can seriously compromise the success of therapy; in many institutions worldwide, rates of methicillin resistance are >70% (1).

Rates of *S. epidermidis* linezolid resistance on various continents have been low and are associated with mutations in the central loop of 23S rRNA V domain and in genes (*rplC*, *rplD* and *rplV*) encoding the L3, L4, and L22 ribosomal proteins (6–8). Clonal relatedness was determined by pulsed-field gel electrophoresis (macrorestriction with Smal) and by multilocus sequence typing (www.cdc.gov/ndc/pdfs/labs/sets/ar_mras_pfge_s_aureus.pdf and http://sepidermidis.mlst.net). *S. epidermidis* from patient 1 was searched for in vitro adherence to abiotic surfaces by using a biomass quantification assay (9) and strain ICE9 as a positive control.

All *S. epidermidis* isolates were resistant to multiple drugs, including linezolid (MIC>32 mg/L), cefoxitin, chloramphenicol, cotrimoxazole, ciprofloxacin, clindamycin, and aminoglycosides, and susceptible to only 4 drugs tested, including vancomycin (MIC = 2 mg/L) (Table). To characterize linezolid resistance, we compared the study isolates with linezolid-susceptible *S. epidermidis* RP62A/American Type Culture Collection 35984 sequence (GenBank accession no. CP000029). Study isolates contained the mutations T2530A, T2504A, and G2631T, although T2504A and G2631T were also present in linezolid-susceptible *S. epidermidis* RP62A (1,2). The most commonly reported G2576T mutation was not detected (1,2). We also compared study isolates with *S. epidermidis* RP62A and observed nucleotide mutations consistent with L94V (L101V from *S. epidermidis* American Type Culture Collection 12228, not associated with linezolid resistance) and G152D amino acid changes (2,8) and amino acid changes in the new D159E and A160P in L3 ribosomal protein. Mutations in this protein were linked to linezolid resistance, although definitive conclusions are not available (8). The cfr gene and mutations in ribosomal proteins L4 or L22 were not detected in the study isolates.

All isolates recovered had the same pulsed-field gel electrophoresis type and belonged to sequence type (ST) 2/clonal complex (CC) 5 (ST2 formerly belonged to CC2) (10) also detected among linezolid-resistant *S. epidermidis* from Europe, Brazil, and the United States (1–3). *S. epidermidis* from patient 1, considered representative of the observed clone, revealed a high ability to adhere to abiotic surfaces and grow in the biofilm form, which can facilitate infections associated with indwelling medical devices. This strain was classified as strongly adherent and had higher optical density (OD570nm = 3.33 ± 0.34) than a blank sample (culture medium: Luria Bertani broth + glucose; OD570nm = 0.2 ± 0.03). The OD570nm of the positive-control was 2.69 ± 0.44.

*S. epidermidis* ST2/CC5 is disseminated in hospital settings worldwide and is characterized by a high level of genetic diversity, an increased recombination/mutation rate, biofilm production ability, and acquisition of a high number of staphylococcal cassette chromosome mec elements (10). In Portugal, *S. epidermidis* ST2/CC5 has been observed in the community (10). We report emergence of methicillin- and linezolid-resistant *S. epidermidis* in a hospital in Portugal and its persistence for at least 7 months. Identification of the successful multidrug-resistant *S. epidermidis* ST2/CC5 clonal lineage highlights the need for strict infection control.
control procedures and revision of therapeutic strategies (e.g., linezolid use for the treatment of methicillin-resistant Staphylococcus spp. only when vancomycin is not a treatment option because of elevated MIC or clinical failures) to preserve therapeutic effectiveness of linezolid. Effective control of linezolid-resistant S. epidermidis, including among hospital-discharged patients who had received linezolid, is critical for preventing the potential for an epidemic in this hospital, and, on a larger scale, in Portugal, as has occurred for other gram-positive methicillin-resistant S. aureus and vancomycin-resistant enterococci.

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| Characteristic                  | 1                  | 2                  | 3                  | 4                  | 5                  |
|---------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Epidemiologic features          |                    |                    |                    |                    |                    |
| Date of isolation               | 2012 May 8         | 2012 Aug 7         | 2012 Oct 23        | 2012 Nov 7         | 2012 Nov 11        |
| Hospital ward                   | Men’s surgery      | Unknown            | Medicine I         | Emergency unit†    | Emergency unit†    |
| Pathology                       | Gastric neoplasia† | Unknown            | Multiple§          | Acute lung edema  | Multiple           |
| Clinical sample                 | Catheter           | Blood              | Catheter           | Blood              | Blood              |
| Patient sex/age, y              | M/75               | Unknown            | F/87               | M/78               | M/87               |
| Previous linezolid              | Yes                | Unknown            | Yes#               | Yes‡               | Yes‡               |
| PFGE type                       | A                  | A                  | A                  | A                  | A                  |
| Sequence type                   | 2*                 |                     |                    |                    |                    |
| Biofilm production (OD570nm)    | Strong (2.33 ± 0.34)††|                |                    |                    |                    |
| Drug resistance                 |                    |                    |                    |                    |                    |
| Linezolid (MIC, mg/L)           | R (32)             | R (32)             | R (32)             | R (32)             | R (32)             |
| Vancomycin (MIC, mg/L)          | S (2)              | S (2)              | S (2)              | S (2)              | S (2)              |
| Cefoxitin                       | R                  | R                  | R                  | R                  | R                  |
| Gentamicin                      | R                  | R                  | R                  | R                  | R                  |
| Tobramycin                      | R                  | R                  | R                  | R                  | R                  |
| Ciprofloxacin                   | R                  | R                  | R                  | R                  | R                  |
| Clindamycin                     | R                  | R                  | R                  | R                  | R                  |
| Erythromycin                    | S                  | S                  | I                  | S                  | S                  |
| Quinupristin–dalfopristin       | S                  | S                  | S                  | S                  | S                  |
| Chloramphenicol                 | R                  | R                  | R                  | R                  | R                  |
| Tetracycline                    | S                  | S                  | S                  | S                  | S                  |
| Cotrimoxazole                   | R                  | R                  | R                  | R                  | R                  |
| Molecular features              |                    |                    |                    |                    |                    |
| cfr gene                        | –                  | –                  | –                  | –                  | –                  |
| 23S rRNA mutations              |                    |                    |                    |                    |                    |
| T2504A                          | +                  | +                  | +                  | +                  | +                  |
| G2631T                          | +                  | +                  | +                  | +                  | +                  |
| T2530A                          | +                  | +                  | +                  | +                  | +                  |
| L3 ribosomal protein mutations  |                    |                    |                    |                    |                    |
| Leu94Val                        | +**                |                    |                    |                    |                    |
| Gly152Asp                       | +**                |                    |                    |                    |                    |
| Asp159Glu                       | +**                |                    |                    |                    |                    |
| Ala160Pro                       | +**                |                    |                    |                    |                    |
| L4 or L22 ribosomal protein mutations | None            |                    |                    |                    |                    |

*PFGE, pulsed-field gel electrophoresis; OD, optical density; R, resistant; S, susceptible; I, Intermediate resistance; –, negative; +, positive. Blank cells indicate not tested.
†A sixth linezolid-resistant S. epidermidis isolate was detected in December 2012; however, access to this isolate was not possible during this study.
‡Patients 4 and 5 were hospitalized in Medicine II a month before linezolid-resistant S. epidermidis was isolated. Therapy with linezolid was started during this first hospitalization. For patient 4, duration of linezolid therapy was at least 12 d. For patient 5, duration of therapy is unknown.
§Long-stay hospitalization.
¶Followed up in oncology ward since 2011.
#Patient 3 received linezolid for 11 d before linezolid-resistant S. epidermidis was detected.
**Studied in S. epidermidis from patient 1 only, representative isolate of the PFGE type A.
††For the interpretation of the results, the cutoff optical density (ODc) was defined as 3 SDs above the mean OD of the negative control (culture medium). Strains were classified as nonadherent (ODc<ODc), weakly adherent (ODc<ODc<2×ODc), moderately adherent (2×ODc<ODc<4×ODc), or strongly adherent (4×ODc<ODc).
Composite SCCmec Element in Single-locus Variant (ST217) of Epidemic MRSA-15 Clone

To the Editor: Since early epidemiologic studies of methicillin-resistant Staphylococcus aureus (MRSA) were published, it has been clear that the majority of nosocomial MRSA infections worldwide are caused by isolates derived from a few highly epidemic MRSA (EMRSA) clones. These are thought to have emerged through acquisition of the staphylococcal cassette chromosome mec (SCCmec) element by successful methicillin-susceptible S. aureus strains, within 5 major lineages or clonal complexes (CCs) including CC22 (1). Although epidemic clones are found worldwide, shifts of the predominant clones over time in which the emerging and usually more antibiotic drug–susceptible clones replace the older ones have been noted in countries, in small regions within countries, and in single hospitals (2). The reasons and mechanisms of such replacement as well as the epidemiologic dynamics leading to the success of a particular epidemic clone are largely unknown.

In Italy, isolations of classical EMRSA clones such as ST8-MRSA-I, ST247-MRSA-I, and ST239-MRSA-III decreased from the 1990s to the 2000s; during the same period ST228-MRSA-I increased, became established, and turned into the predominant clone in Italy (3). The genesis of other clones, such as ST8-MRSA-IV and ST22-MRSA-IV, which were associated with a tendency towards decreased multidrug resistance, was documented during 2000–2007 (3). Similar to occurrences in other European countries, the gentamicin-susceptible Panton-Valentine leukocidin–negative ST22-MRSA-IV clone, also known as EMRSA-15 (1), is now becoming predominant in Italy, replacing ST228-MRSA-I in hospital settings (4).

As part of another investigation, we recently isolated a MRSA strain from the nasal swab samples of a 5-year-old boy and his parents. The 3 isolates shared the same antibacterial drug resistance pattern (oxacillin and ciprofloxacin resistance) and proved to be identical by pulsed-field gel electrophoresis, SCCmec typing, and agr typing. Remarkably, ≈2 months earlier, the child had been admitted to a pediatric hospital for 10 days to be evaluated and treated for behavioral problems. A MRSA isolate, which was identified in a nasal sample obtained and analyzed just before discharge in the absence of clinical symptoms and was not further investigated, showed the same antibacterial drug–susceptible clones replace the older ones have been