Case Report

Recovery of linezolid-resistant, methicillin-susceptible *Staphylococcus aureus* in a case of implanted pacemaker-associated infection

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**Introduction:** Linezolid resistance among *Staphylococcus aureus* has emerged almost exclusively in those organisms with methicillin resistance (MRSA). To our knowledge, recovery of linezolid-resistant (LR) methicillin-susceptible *S. aureus* (MSSA) from humans has been described in only a single case.

**Case presentation:** A 63-year-old Japanese man was referred for removal of an infected cardiac resynchronization device. He had received a 2-week administration of linezolid approximately 6 months previously. LR MSSA (linezolid MIC = 8 µg ml⁻¹) was recovered from purulent materials around the exposed generator and removed electrode leads, concurrently with linezolid-susceptible (LS) MRSA and LS MSSA. The LR MSSA and LS MRSA demonstrated close genetic relatedness in a macrorestriction analysis and were categorized into the same molecular types (multilocus sequence type 239 and spa type t137), suggesting that they originated from an identical ancestor. The LR MSSA had a G2576T mutation in the 23S rRNA genes in two of five *rrn* operons.

**Conclusion:** Linezolid resistance may occur not only among multidrug-resistant Gram-positive organisms but also in those for which a number of antibiotics are still effective.

**Keywords:** 23S ribosomal RNA; linezolid; methicillin-susceptible *Staphylococcus aureus*; pacemaker-associated infection.

**Introduction**

Linezolid, the first oxazolidinone approved for clinical use, has often been used for treatment of infections caused by multidrug-resistant Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci and vancomycin-resistant enterococci (Diekema & Jones, 2001). Since the first report appeared in 2001 (Tsiodras et al., 2001), linezolid-resistant (LR) *S. aureus* has been detected in separate cases and outbreaks worldwide (Gu et al., 2013). Well-characterized mechanisms conferring linezolid resistance include mutations in the domain V region of the 23S rRNA gene, acquisition of the ribosomal methyltransferase gene (*cfr*) and mutations in the 50S ribosomal proteins L3 and L4 (encoded by the *rplC* and *rplD* genes, respectively) (Shaw & Barbachyn, 2011; Long & Vester, 2012). Interestingly, nearly all LR *S. aureus* strains have co-exhibited methicillin resistance (Gu et al., 2013; Flamm et al., 2013a, b) and, to our knowledge, only a report from Spain has described the recovery of methicillin-susceptible *S. aureus* (MSSA) with linezolid resistance from humans (Quiles-Melero et al., 2012). In the present report, we describe the recovery of LR MSSA, concurrent with linezolid-susceptible (LS) MRSA and LS MSSA, in a case of implanted pacemaker-associated infection. In addition, the recovered organisms were characterized genetically.

**Case report**

A 63-year-old Japanese man was referred for removal of an infected cardiac resynchronization device (CRD). He had been receiving haemodialysis caused by diabetic
nephropathy for 6 years. An artificial pacemaker was first implanted to treat a complete atrioventricular block 5 years earlier and replaced with the CRD 8 months before referral. One month after the replacement, dehiscence of the skin and partial exposure of the implanted generator, accompanied by fever, chills and shivering, occurred. MRSA was recovered from purulent materials discharged from the site. Initially, the patient did not consent to removal of the CRD. Thus, oral linezolid was administered but was terminated after 2 weeks because thrombocytopenia progressed. Thereafter, the patient was treated with intravenous vancomycin, together with cleansing of the wound, until referral. At the operation for removal of the infected CRD, massive purulent matter was present around the generator. No vegetation was visible on any of the electrode leads. After removal of the infected CRD, the patient’s general condition rapidly improved. He was transferred to the referring hospital 7 days after the operation whilst administration of vancomycin was continued.

Five segments of the removed CRD, three tips of electrode leads, the generator and a Gore-Tex film covering the generator were immersed separately in thioglycollate broth, which was incubated overnight at 35°C and subcultured on sheep blood agar for a further 24 h. Several colonies grown on the agar were examined with Gram staining, catalase and coagulase production tests, and WalkAway 96SI (Siemens Healthcare Diagnostics), and consequently were identified as LR MSSA, LS MSSA or LS MRSA. Some of the recovered LR MSSA and LS MRSA strains were suspended in 10% skimmed milk and preserved at −85°C until further examination. Macrorestriction analysis, conducted according to a previous description (Hitomi et al., 2000), demonstrated that the LR MSSA strains were genetically identical and were closely related to the LS MRSA strains (Fig. 1). Thus, one LR MSSA (strain 178-2) and one LS MRSA (179-4) were further analysed. Molecular examinations showed that both strains were categorized into multilocus sequence type (ST) 239 (Enright et al., 2000) and spa type t137 (Harmsen et al., 2003). Strain 179-4 carried staphylococcal cassette chromosome mec (SCCmec) type III (Oliveira & de Lencastre, 2002; Zhang et al., 2005). MIC values for linezolid against strains 178-2 and 179-4, confirmed with the broth microdilution method (CLSI, 2012) using Dry Plate (Eiken Kagaku) and an Etest (Sysmex bioMérieux), were 8 and 1 μg ml⁻¹, respectively. Analysis of domain V of the 23S ribosomal RNA demonstrated that strain 178-2 possessed five rrr operons, of which rrr3 and rrr5 had a mutation of G2576T (Pillai et al., 2002). Deduced amino acid sequences of proteins L3 and L4 (Locke et al., 2009) were identical to those of S. aureus MRSA252 (Long & Vester, 2012). The cfr gene was not detected (Kehrenberg & Schwarz, 2006).

Discussion

In the present case, LR MSSA, LS MSSA and LS MRSA were recovered from nearly all of the examined samples. We speculate that organisms recovered from the tips of electrode leads originated in the purulent materials around the generator because no visible vegetation was present on the electrode leads and the dehiscent site was so purulent that the electrode leads may easily have been contaminated during removal of the CRD. Rapid improvement of the patient’s symptoms after the operation also suggested that infection was confined to the subcutaneous tissues and minimally, if at all, extended to the vascular lumens.

The growth of strain 178-2, an LR MSSA detected in the present case, was inhibited by linezolid at a concentration of 8 μg ml⁻¹, the lower limit of the ‘resistance’ category. Among the three mechanisms conferring linezolid resistance, the strain demonstrated only mutation of the 23S rRNA gene, with a G2576T substitution in two of the five rrr operons. Previous reports have shown that accumulation of mutations in the 23S rRNA gene causes stepwise increase in linezolid resistance (Besier et al., 2008; Ikeda-Dantsuiji et al., 2011). Thus, the low-level linezolid resistance of strain 178-2 may have been attributed to the partial mutation among the five rrr operons. We consider that a 2-week administration of linezolid approximately 6 months previously, the only confirmed episode of the patient’s exposure to the drug, may have caused the genetic mutation in S. aureus colonizing in the purulent material at the dehiscent site. Horizontal transmission of LR S. aureus was unlikely to have occurred in either the referring or referred hospital, because LR S. aureus has rarely been isolated in Japan to date (Ikeda-Dantsuiji et al., 2011).

Genetic analyses with PFGE and molecular typing indicated that strain 178-2 was closely related to strain 179-4, an LS MRSA recovered concurrently in the present case, suggesting that they originated from a common

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**Fig. 1.** Macrorestriction analysis of recovered LR MSSA (lanes A, B and G) and LS MRSA (lanes C–F) strains. Strains 178-2 and 179-4 are shown in lanes A and C, respectively; lane M, molecular size markers.
ancestor. Interestingly, strain 179-4 belonged to a Hungarian clonal type (ST239–SCCmec III), which has rarely been isolated in Japan (Oliveira et al., 2002). We could not confirm the origin of these strains because precise medical and microbiological records in the referring hospital were unavailable and not all of the organisms recovered from the removed CRD were preserved for genetic analyses. One possibility is that some of the LS MRSA, having colonized on or around the exposed CRD, acquired mutations in the 23S rRNA gene during exposure to linezolid and progeny that had lost the mecA gene subsequently emerged.

Strain 178-2 demonstrated resistance to linezolid through chromosomal mutation but showed susceptibility to other anti-Staphylococcus agents. Thus, it is unclear whether recovery of the strain is becoming a threat to public health. However, physicians should be aware that linezolid resistance may occur not only among multidrug-resistant Gram-positive organisms but also in those for which a number of other antibiotics are still effective. Prudent use of linezolid should be emphasized under all circumstances.

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**References**


