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Case report

Linezolid-resistant *Staphylococcus epidermidis* associated with long-term, repeated linezolid use in a pediatric patient

Naruhiko Ishiwada [a, *], Akiko Takaya [b], Asahi Kimura [b], Masaharu Watanabe [c], Moeko Hino [d], Hidemasa Ochiai [d], Mari Matsui [e], Keigo Shibayama [e], Tomoko Yamamoto [a, b]

[a] Department of Infectious Diseases, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan
[b] Department of Microbiology and Molecular Genetics, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan
[c] Division of Laboratory Medicine, Chiba University Hospital, Chiba 260-8677, Japan
[d] Department of Pediatrics, Chiba University Hospital, Chiba 260-8677, Japan
[e] Department of Bacteriology II, National Institute of Infectious Diseases, Musashimurayama, Tokyo 208-0011, Japan

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ABSTRACT

We report an 8-year-old patient with catheter-related bacteremia caused by linezolid-resistant *Staphylococcus epidermidis* that was isolated after the long-term, repeated use of linezolid. Three *S. epidermidis* strains isolated from this patient were bacteriologically analyzed. While the strain isolated prior to linezolid initiation was susceptible to linezolid, two strains after linezolid therapy displayed low-level linezolid susceptibility (MIC, 4 mg/L) and linezolid resistance (MIC, 16 mg/L). T2500A mutation in two copies and G2575T mutations in three copies of 23S rRNA were detected in the low-susceptible strain and the resistant strain, respectively. Linezolid-resistant *S. epidermidis* infection is rare, but may occur with the long-term administration of linezolid.

1. Introduction

Linezolid is a therapeutic agent used to treat infections caused by multidrug-resistant Gram positive bacteria such as *Staphylococcus* species. *Staphylococcus epidermidis* are skin and mucosal commensal bacteria and infection in humans are mostly linked to immune-suppression therapy and indwelling medical devices. The rate of methicillin resistance in *S. epidermidis* is >70%, however, rates of *S. epidermidis* linezolid resistance have been low [1]. Recently, cases of linezolid-resistant *S. epidermidis* infection were reported in several countries [2,3]. One of the risk factors for linezolid resistance in *Staphylococcus* species is prolonged linezolid therapy [4].

In the present report, we analyze clinical *S. epidermidis* isolates from pediatric patient that became resistant to linezolid after long-term, repeated use.

The 23S rRNA domain mutations and the PFGE profiles of two isolated strains after linezolid therapy were different, suggesting that linezolid resistance developed independently during therapy. To our knowledge, neither of these findings was described previously in clinical isolates of *S. epidermidis*.

2. Case report

The patient was an 8-year-old girl with acute myeloid leukemia (AML-M2). She was admitted to our hospital for the second time for the treatment of bone-marrow relapsed AML. She received cord blood transplantation but failed to engraft. After a second relapse, the patient was given supportive therapy including pain control. During the course of supportive therapy, *S. epidermidis* I-1557 was isolated from a blood sample that was collected from the lumen of her central venous catheter (CVC). Blood culture was carefully done by the experienced pediatric oncologist. Two sets of blood cultures

* Corresponding author. Department of Infectious Diseases, Medical Mycology Research Center, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8673, Japan. Tel.: +81 43 226 2799; fax: +81 43 226 2786.
E-mail address: ishiwada@faculty.chiba-u.jp (N. Ishiwada).

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with a set collected simultaneously from each lumen of the CVC were taken and all blood samples were positive for *S. epidermidis*. Clinicians diagnosed catheter-related bacteremia rather than contamination. Based on antimicrobial susceptibility test results for the isolated strain, the patient received intravenous linezolid (10 mg/kg dose three times daily) for 14 days with a good clinical response. Another reason why the clinician chose linezolid was that the agent could be used without regard to renal function and did not require measurement of blood drug concentration. She was not given linezolid since her initial admission to our hospital. The blood culture was sterile during linezolid therapy. However, the patient developed a fever 2 weeks after completing linezolid therapy and *S. epidermidis* was re-isolated from her blood sample. The patient received the same linezolid scheme once again, but, *S. epidermidis* was cultured from blood taken from the CVC lumen after 6 (I-0202) and 8 (I-0237) weeks of linezolid therapy. The CVC was difficult to remove because her general condition had worsened and her all nutrition was given through CVC. Eight weeks after the second linezolid therapy, daptomycin was initiated. However, the patient died of a cerebral hemorrhage associated with AML 10 days after initiating daptomycin therapy.

The three isolates analyzed in the present study were obtained before (I-1557), 2.5 months after (I-0202) and 3 months after (I-0237) initiating linezolid therapy. *S. epidermidis* was identified using a matrix assisted laser desorption/ionization system. All isolates were tested for antimicrobial susceptibility by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) method. Minimal inhibitory concentration (MIC) results were interpreted on the basis of published CLSI criteria. To test for mutations in individual copies of 23S rRNA by polymerase chain reaction (PCR) amplification, six specific primer sets were used to individually amplify copies of 23S rRNA from all isolates. The resulting DNA fragments were sequenced using the same primers [5]. A specific primer set to amplify for L3 and L4 riboprotein genes was used to analyze ribosomal protein genes by PCR amplification, followed by DNA sequencing [5]. The presence of *cfr* was investigated by PCR amplification [6]. The clonal relatedness of the strains was determined by PFGE using the restriction enzyme *Sma*I [7]. The DNA fragments were separated in a 1% agarose slab gel using a CHEF-Mapper system with a DNA size standard lambda ladder used as a reference marker.

The susceptibility profiles of the isolates are presented in Table 1. Strain I-1557 was susceptible to linezolid. Strain I-0202 and I-0237 showed low-level linezolid susceptibility (MIC, 4 mg/L) and linezolid resistance (MIC, 16 mg/L), respectively. All *S. epidermidis* isolates were resistant to multiple drugs. The isolates were first tested for the most common mechanism, mutations in the domain V region of their 23S rRNA. Since *S. epidermidis* strains are known to have six 23S rRNA operons [5], we used PCR primers specific for each copy to sequenced the copies. No mutations were found in the linezolid-susceptible strain, I-1557 (Table 1). A T2500A mutation was identified in two copies in strain I-0202, while a G2576T mutation was detected in three copies in strain I-0237. Linezolid resistance has also been associated with mutations in the ribosomal proteins L3 and L4. All isolates in the present study had the mutation Leu-101 → Val in the L3 protein, which was not present in the L3 of the reference *S. epidermidis* RP62A (GenBank accession no. NC_002976). No mutation was identified in the L4 protein in all isolates. PCR demonstrated that no *cfr* was harbored by the linezolid-resistant *S. epidermidis* isolates characterized in the present study. The clonal relatedness of the isolates was examined by PFGE of *Sma*I-digested genomic DNA (Fig. 1). The linezolid-susceptible strain, I-1557 and strain I-0202 were indistinguishable from each other, suggesting that I-0202 was derived from I-1557. The PFGE profile revealed that strain I-0237 was not related to the susceptible strain I-1557. Taken together, these results suggest that the resistance to linezolid in *S. epidermidis* may probably be due to the selection of an intra-treatment mutant.

Table 1
MICs and resistant determinants of *S. epidermidis* clinical isolates I-1557, I-0202 and I-0237.

Parameter	Result for isolate (sample date)		
	I-1557 (Dec. 2012)	I-0202 (Feb.2013)	I-0237 (Mar. 2013)
MICs(mg/L)			
Linezolid	<1	4	16
Oxacillin	>4	>4	>4
Cefazolin	>16	>16	>16
Imipenem	>8	>8	>8
Gentamycin	>8	>8	>8
Arbekacin	4	2	>8
Erythromycin	>4	>4	>4
Clindamycin	>2	>2	>2
Levofloxacin	>4	>4	>4
Minocycline	<2	<2	>8
Vancomycin	2	2	2
Teicoplanin	4	8	4
Fosfomycin	>128	>128	>128
TMS[a]	>38/2	>38/2	>38/2
Mutations in 23S rRNA domain V allele sequence[b]			
rrlA	–	–	–
rrlB	–	–	–
rrlC	–	–	–
rrlD	–	T2500A	G2576T
rrlE	–	T2500A	G2576T
rrlF	–	–	G2576T
Mutations in ribosomal proteins[b]			
L3	Leu101Val	Leu101Val	Leu101Val
L4	–	–	–
Cfr[c]	–	–	–

[a] Trimethoprim-sulphametoxazole.
[b] A long dash indicates no mutation.
[c] A long dash indicates that *cfr* was not detected.

3. Discussion

Linezolid, approved in 2000, is the only oxazolidinone agent that is marketed for human use in the United States. In the 2011 surveillance program report on the antimicrobial susceptibility of this agent in the United States, the linezolid resistance rates of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) were 0.1% and 1.2%, respectively [8]. In Japan, linezolid was approved for the treating of MRSA infection in 2006. Only 11 linezolid-resistant MRSA strains were identified in clinical isolates collected at six hospitals during 2006–8 [9]. Linezolid has been useful and safe agent for the treatment of severe pediatric infectious diseases caused by *Staphylococcus* species and was approved for pediatric patients in 2012 [10]. In 2015, five linezolid resistant *Staphylococcus capitis* strains from clinical specimens were reported [11].

In our case, linezolid was administered for more than 2 months. During linezolid therapy, *S. epidermidis* developed linezolid resistance. Interestingly, two isolated strains after linezolid therapy had different phenotype and genotype of linezolid resistance. A G2576T mutation was identified in domain V of the 23S rRNA of I-0237, for which the MIC of linezolid was 16 mg/L. G2576T is the most frequently reported mutation in staphylococci [12], although other mutations such as T2500A, T2504A and G2447T have also been identified in 23S rRNA of clinical isolates. The T2500A mutation was identified in I-0202, for which the MIC of linezolid was 4 mg/L. This is also a reported mutation of staphylococci [13]. The linezolid-susceptible strain, I-1557 and strain I-0202 were same profile by PFGE analysis, suggesting that I-0202 was derived from I-1557 by