Case Report

Linezolid-resistant *S. epidermidis* clone ST-2 isolated from a patient who did not receive any course of oxazolidinone therapy: a case report

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Introduction: Linezolid has excellent activity against multidrug-resistant *Staphylococcus* spp. and linezolid resistance has emerged mainly in isolates recovered from patients who are receiving courses of oxazolidinone therapy.

Case presentation: We report a clinical case of bacteraemia caused by a linezolid-resistant *Staphylococcus epidermidis* clone ST-2 with the G2576T mutation in five copies of the 23S rRNA gene (MIC, 32 µg ml⁻¹) isolated from a patient who did not receive any course of oxazolidinone therapy. The *S. epidermidis* HSPE24-SP strain was WT for L3, L4 and L22 ribosomal proteins, *cfr* negative and indistinguishable by PFGE from the clone that circulated at the same time in another tertiary care hospital located in the same city.

Conclusion: This report suggests the inter-hospital spread of *S. epidermidis* clone ST-2 and calls attention to the possibility of isolation of a linezolid-resistant strain with multiple 23S rRNA mutated copies in an environment under no oxazolidinone selective pressure.

Keywords: drug resistance; linezolid; *S. epidermidis*.

Received 15 January 2014

Accepted 30 July 2014

Introduction

Linezolid, a synthetic antibiotic of the oxazolidinone class, has excellent activity against Gram-positive cocci. Mutations in the central loop of domain V of the 23S rRNA gene or, less often, an adenosine modification catalysed by Cfr at position 2503 of the 23S rRNA and mutations in the ribosomal proteins L3 and L4 are mechanisms emerging in linezolid-resistant isolates. Linezolid resistance has emerged mainly in isolates recovered from patients who are receiving courses of oxazolidinone therapy. However, several reports of linezolid-resistant strains from patients with no prior drug exposure have already been reported (Jones *et al.*, 2002;

Ikeda-Dantsuji *et al.*, 2011). Since 2008, we have documented the spread of the linezolid-resistant *Staphylococcus epidermidis* clone ST-2 in patients who were treated with linezolid in a tertiary care hospital (A) of the city of São Paulo, Brazil. In this study, we report a clinical case of bacteraemia caused by a linezolid-resistant *S. epidermidis* clone ST-2 from a patient without prior exposure to linezolid in another tertiary care hospital (B) located in the same city and we investigated the resistance mechanisms associated with this phenotype. This case report illustrates the inter-hospital spread of *S. epidermidis* clone ST-2 with a G2576T mutation in the 23S rRNA and calls attention to the possibility of isolation of a linezolid-resistant strain in an environment under no oxazolidinone selective pressure.

Abbreviations: CC, clonal complex; ICU, intensive care unit

Case report

A 75-year-old male was hospitalized for 26 days for treatment of a diabetic foot, acute renal failure and hyperkalemia. During this period, the patient was treated with teicoplanin, ciprofloxacin and metronidazole for 14 days. He was discharged, and 8 days later he was admitted again due to worsening of the injury. A linezolid-susceptible *Staphylococcus hominis* strain was isolated from a blood culture drawn on day 4 of his second hospitalization, but it was considered to be a contaminant. Vancomycin, cefepime and piperacillin/tazobactam were prescribed for 20 days for the infection of the diabetic foot. The patient was submitted to debridement, but the cultures collected during this procedure were negative. Despite the use of antimicrobials and the debridement, the clinical condition of the patient deteriorated and he was transferred to the intensive care unit (ICU) where sepsis secondary to diabetic foot infection was diagnosed. The antimicrobial regimen was modified to teicoplanin (due to renal insufficiency) and meropenem. The patient showed improvement of his clinical conditions and was discharged from the ICU 12 days later. A blood culture drawn on the last day of ICU admission, because the patient presented an episode of fever, revealed the presence of a linezolid-resistant *S. epidermidis* strain. Soon after, the patient was transferred to the internal medicine ward, and fluconazole was prescribed for 7 days due to a positive urine culture for *Candida* sp. After the result of this blood culture, an oesophageal echocardiography was performed with no endocarditis signals, but an abdominal computed tomography scan performed to investigate a splenomegaly revealed multiple splenic abscesses. Because of a diagnostic hypothesis of haematogenous dissemination, the patient received teicoplanin and meropenem for 52 days, and the splenic abscess was drained by a guided puncture during this period with negative culture. At the end of the treatment, he remained for 11 days without antibiotics. However, due to an appearance of nosocomial pneumonia, piperacillin/tazobactam was again introduced; however, he died 4 days later with a diagnosis of severe sepsis and nosocomial pneumonia. This patient did not receive linezolid.

Investigations

The identification and antimicrobial susceptibility testing were initially performed using the Vitek-2 system (bioMérieux). Subsequently, confirmation of species identification was performed as described previously (Hirota et al., 2011). Susceptibility to ciprofloxacin, teicoplanin, trimethoprim-sulfamethoxazole, amikacin, gentamicin, erythromycin, clindamycin, tetracycline and chloramphenicol was evaluated by a disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). The susceptibility to linezolid, oxacillin and vancomycin was determined by the CLSI broth microdilution method. *Staphylococcus aureus* ATCC

25923 and *Enterococcus faecalis* ATCC 29212 were tested as quality-control strains. Susceptibility testing results were interpreted based on the CLSI (2013) criteria. The domain V region of the 23S rRNA gene was amplified by PCR (Almeida et al., 2012) and sequenced. In order to distinguish a homozygous from a heterozygous profile, the domain V fragment was digested with *NheI* restriction enzyme. Determination of the number of mutated 23S rRNA alleles was assessed as described by Liakopoulos et al. (2009a). The presence of the *cfr* gene and mutations in the *rplC*, *rplD* and *rplV* genes were investigated as described previously (Kehrenberg & Schwarz, 2006; Miller et al., 2008; Mendes et al., 2010). PFGE of *SmaI* digests and multilocus sequence typing were performed to determine genetic relatedness.

Results and discussion

S. epidermidis HSPE24-SP strain was shown to be susceptible to vancomycin (MIC, 1 µg ml⁻¹) and teicoplanin (MIC, 4 µg ml⁻¹) but resistant to oxacillin (MIC, >256 µg ml⁻¹), linezolid (MIC, 32 µg ml⁻¹) and the other antimicrobial agents tested, as shown in Table 1. Incomplete digestion of domain V with *NheI* suggested the presence of fragments with both G2576T mutant and WT sequences. Through DNA sequencing, the G2576T mutation was identified in five copies of the 23S rRNA gene of the HSPE24-SP strain. It was not possible to amplify the allele *rrlD*. The presence of the *cfr* gene was not detected in HSPE24-SP. This strain was also shown to WT for the L3, L4 and L22 ribosomal proteins.

The *S. epidermidis* HSPE24-SP strain belongs to clone ST2, which is frequently isolated from hospital A, where linezolid has usually been prescribed for the treatment of vancomycin-resistant *Enterococcus* spp. infections. *S. epidermidis* strains resistant to linezolid isolated from hospital A usually exhibit linezolid MICs of 16–64 µg ml⁻¹, and the G2576T mutation in domain V of the 23S rRNA gene has been the main determinant of linezolid resistance found among these strains. *S. epidermidis* grouped under clone ST2 isolated from hospital A usually shows a substitution of Leu101Val in the L3 protein, which was not present in the HSPE24-SP strain. This mutation is probably a clonal marker of this population, as it is not involved in linezolid resistance. PFGE analysis showed that the *S. epidermidis* HSPE24-SP strain was indistinguishable from the clone circulating in the hospital A from 2010 to 2011 and was clonally related to the clone that was identified from 2008 to 2009 (Almeida et al., 2012). All these strains belonged to sequence type 2 [clonal complex (CC) 2] by MLST.

An understanding of the molecular epidemiology of coagulase-negative staphylococci resistance to linezolid is still very limited, but some studies have shown that *S. epidermidis* ST-2 clones widely disseminated in hospitals from different countries may accumulate different mutations

Table 1. Antimicrobial susceptibility profile and molecular findings for the linezolid-resistant *S. epidermidis* strains evaluated in this study

Isolate	Hospital	ICU	Culture date	Clinical sample	PFGE	MLST
<i>S. ep</i> 25/228	A	Yes	Nov-09	Blood	I	ST2
<i>S. ep</i> 30/315	A	Yes	Mai-10	Catheter	I	ST2
<i>S. ep</i> 31/321	A	No	Jun-10	Catheter	I	ST2
<i>S. ep</i> 32/330	A	Yes	Jun-10	Blood	I	ST2
<i>S. ep</i> 36/391	A	Yes	Set-10	Blood	I	ST2
<i>S. ep</i> HSPE24	B	Yes	Nov-10	Blood	I	ST2
<i>S. ep</i> 39/476	A	Yes	Jan-11	Catheter	I	ST2
<i>S. ep</i> 47/647	A	Yes	Jul-11	Catheter	I	ST2
<i>S. ep</i> 48/753	A	Yes	Jul-11	Catheter	I	ST2
<i>S. ep</i> 45/583	A	No	Set-11	Blood	I	ST2

Table 1. Continued.

<i>cfr</i> gene	Mutations			
	23S rRNA	L3	L4	L22
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	WT	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT
–	G2576T	Leu101Val	WT	WT

Table 1. Continued.

Antimicrobial susceptibility profile [MIC ($\mu\text{g ml}^{-1}$)]											
LZD	VAN	OXA	CIP	TEC	STX	AMK	GEN	ERY	CLI	TET	CHL
64	1	>256	R	S	R	R	R	R	R	S	R
32	1	>256	R	S	R	R	R	R	R	S	R
64	1	>256	R	S	R	R	R	S	R	S	R
64	1	>256	R	S	R	R	R	S	I	S	R
64	1	48	I	S	R	R	R	R	R	S	R
32	1	>256	R	S	R	R	R	R	R	I	R
64	1	6	I	S	R	R	R	S	R	S	R
64	1	0.25	I	S	R	R	R	R	R	S	R
64	1	>256	I	S	R	R	R	R	R	S	R
32	1	>256	R	S	R	R	R	R	R	S	R

LZD, linezolid; VAN, vancomycin; OXA, oxacillin; CIP, ciprofloxacin (5 μg); TEC, teicoplanin (30 mg); STX, trimethoprim-sulfamethoxazole (1.25/23.75 mg); AMK, amikacin (30 mg); GEN, gentamicin (10 mg); ERY, erythromycin (15 mg); CLI, clindamycin (2 mg); TET, tetracycline (30 mg); CHL, chloramphenicol (30 mg); R, resistant; S, susceptible; I, intermediate; *S. ep*, *S. epidermidis*.

in domain V of the 23S rRNA gene (Liakopoulos *et al.*, 2010b; Wong *et al.*, 2010). According to these studies, we found that linezolid resistance in *S. epidermidis* from the two hospitals included in this work was also limited to CC2.

The use of linezolid in hospital B, in contrast to hospital A, is highly restricted to patients with infections associated with vancomycin-resistant *Enterococcus* strains. The limited use of this antimicrobial, however, did not prevent spread of the *S. epidermidis* HSPE24-SP strain, which could

have occurred via patient-to-patient contact at the same institution or even through a common member of staff at the two hospitals. Only one case of linezolid-resistant *E. faecalis* has been reported previously in hospital B since the introduction of this antimicrobial agent in 2005. At hospital A, the *S. epidermidis* ST-2 clone with G2576T has been established for some time, probably due to the selective pressure of antimicrobials, but this isolated case in hospital B suggests nosocomial transmission, indicating increased ability of this clone to spread and adapt to different hospital conditions.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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