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CONCISE ARTICLE

H. Adler · A. Widmer · R. Frei

Emergence of a Teicoplanin-Resistant Small Colony Variant of *Staphylococcus epidermidis* During Vancomycin Therapy

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Abstract Small colony variants of *Staphylococcus aureus* can cause persistent and recurrent infections. There are only a few reports of small colony variants of coagulase-negative staphylococci. Herein a case of infection with a teicoplanin-resistant small colony variant of *Staphylococcus epidermidis* is presented. The small colony variant was isolated from blood cultures of a patient with acute leukaemia and therapy-induced neutropenia who was treated with vancomycin for catheter-associated bloodstream infection. Despite removal of the catheter and adequate antibiotic therapy, the infection did not clear and the patient died 20 days after continuous antibiotic therapy.

Introduction

Small colony variants (SCVs) of *Staphylococcus aureus* have been found in a variety of clinical specimens and have been associated with persistent and recurrent infections [1, 2, 3]. Only a few cases of infection with SCVs of coagulase-negative staphylococci (CoNS) have been reported. SCVs of *Staphylococcus epidermidis* have been linked to prosthetic heart valve endocarditis and infection of pacemaker electrodes [4, 5]. Bloodstream infection caused by *Staphylococcus epidermidis* and *Staphylococcus capitis* following pacemaker implantation has also been reported [6]. Here we describe a teicoplanin-resistant SCV of *Staphylococcus epidermidis* which emerged during vancomycin therapy of a catheter-associated bloodstream infection in a neutropenic patient.

H. Adler (✉) · R. Frei
Bacteriology Laboratory,
University of Basel Hospitals and Clinics,
Petersgraben 4, 4031 Basel, Switzerland
e-mail: hadler@uhbs.ch
Tel.: +41-61-6254228
Fax: +41-61-2655355

A. Widmer
Division of Hospital Epidemiology,
University of Basel Hospitals and Clinics,
Petersgraben 4, 4031 Basel, Switzerland

Case Report

A 69-year-old patient was admitted to hospital for treatment of acute myeloid leukaemia. He was treated with high-dose chemotherapy and became neutropenic and febrile. Despite broad-spectrum antibiotic and amphotericin B therapy, the fever persisted. Between day 5 and day 16 of hospitalisation 15 blood cultures were taken, which yielded no growth. On day 20 of hospitalisation *Staphylococcus epidermidis* was isolated from two of two blood cultures (blood culture results from day 20–42 are listed in Table 1). The isolates were methicillin resistant and susceptible to vancomycin and teicoplanin. Treatment with vancomycin 1 g b.i.d. was started, adjusted to renal insufficiency. In addition, an antibiotic-lock (with vancomycin 1000 µg/ml in liquemin) was infused into the central venous catheter in an attempt to save it. A normal colony form and an SCV of *Staphylococcus epidermidis* were isolated from a blood culture taken 11 h after the start of vancomycin therapy. Both colony forms were methicillin resistant and vancomycin sensitive. While the normal form was teicoplanin sensitive, the SCV was teicoplanin resistant. A blood culture taken 24 h after the start of antibiotic therapy yielded no bacterial growth but *Staphylococcus epidermidis* was again isolated from a blood culture of the following day. Three days after the initiation of vancomycin therapy the central-venous catheter was replaced. Cultures of the catheter tip yielded no growth. On day 31 of hospitalisation amikacin was added to the treatment regimen. Despite adequate therapy, the patient continued to be septic and both colony forms of *Staphylococcus epidermidis* were isolated from several blood cultures. The central venous catheter was removed on day 35 of hospitalisation. No bacteria could be cultured from the catheter. On day 38, rifampicin was added to the treatment regimen. Ultrasound examination excluded the presence of septic thrombophlebitis. Transesophageal echocardiography did not show any evidence of endocarditis. Life support was suspended because the patient did not recover from neutropenia and fungal pneumonia was suspected. High-dose corticosteroid therapy was added to the regimen to control continuous febrile episodes with shivering. The patient died on day 20 after the start of antimicrobial treatment as a result of non-responding clinical sepsis. Autopsy results confirmed there was no evidence of endocarditis or septic thrombophlebitis. Histologic examination revealed multiple nodes with fungal elements compatible with aspergillosis in the lower right lobe of the lung. However, no fungi grew in cultures from lung tissue, presumably due to the antifungal therapy.

Table 1 Blood culture results from days 20–42 of hospitalization

Day of hospitalisation	Blood culture no.	Isolation of *S. epidermidis*		Remarks
		Large colonies	SCVs	
20	16	+, teicoplanin S	–	
	17	+, teicoplanin S	–	
21	18	+, teicoplanin S	–	
22	19	+, teicoplanin S	–	start of vancomycin therapy
	20	+, teicoplanin S	+, teicoplanin R	
23	21	–	–	
24	22	+, teicoplanin S	–	
25	23	–	–	replacement of catheter
	24	+, teicoplanin S	–	
26	25	+, teicoplanin S	+, teicoplanin S	
27	26	+, teicoplanin S	+, teicoplanin R	
28	27	+, teicoplanin S	+, teicoplanin R	
29	28	+, teicoplanin S	–	
30	29	+, teicoplanin S	–	
31	30	+, teicoplanin S	+, teicoplanin R	addition of amikacin to therapy
35	31	+, teicoplanin S	+, teicoplanin R	replacement of catheter
	32	+, teicoplanin S	–	
	33	+, teicoplanin S	–	
37	34	+, teicoplanin S	–	
	35	+, teicoplanin S	–	
38				addtion of rifampicin to therapy
39	36	–	–	
40	37	+, teicoplanin S	–	
41	38	+, teicoplanin S	–	
	39	–	–	
42	40	–	–	patient died

+, growth; –, no growth; *S*, susceptible; *R*, resistant

Materials and Methods

Blood cultures were processed with an automated system (BacT/ALERT; bioMérieux, USA). Aliquots of the broth were subcultured on Columbia agar with 5% sheep blood and incubated at 37°C in 5% CO_2 and under anaerobic conditions for 48 h. Intravenous catheter tips were cultured as described by Maki et al. [7]. The clinical isolates of *Staphylococcus epidermidis* were identified based on their biochemical profile with the API Staph Kit (bioMérieux, France). Identification was confirmed using the MicroSeq 16S rRNA gene kit (Applied Biosystems, USA). The amplified DNA was sequenced on an automated DNA sequencer (ABI PRISM 310 Sequencer, Applied Biosystems). Molecular typing was performed by pulsed-field gel electrophoresis (PFGE) as previously described [8], genomic DNA being digested by the restriction endonuclease *Sma*I. Susceptibility testing was performed according to NCCLS guidelines [9] by broth microdilution using commercially manufactured plates (Micronaut-S; Merlin Diagnostics, Germany). Resistance to methicillin was confirmed by detection of penicillin-binding protein 2' (MRSA-Screen; Denka Seiken, Japan) after induction with oxacillin [10]. MICs of teicoplanin and vancomycin were confirmed by the E test (AB-Biodisk, Sweden), applying an inoculum of 0.5 McFarland on Mueller Hinton agar with 5% sheep blood. In addition, isolates were screened for hetero-resistance to vancomycin using the vancomycin E test with an inoculum of 2.0 McFarland on brain heart infusion agar [11].

Results and Discussion

The normal colonies of *Staphylococcus epidermidis* were found after overnight incubation of subculture plates. SCVs were usually detected after 24–72 h of incubation. Their colony diameter was around 1 mm. SCVs showed a tendency to revert to the normal form upon further subculturing and we were not able to conserve the small colony phenotype over prolonged periods of time. Both colony forms were identified as *Staphylococcus epidermidis.* Sequence analysis of a 500-bp fragment of the rRNA gene showed 100% homology with the type strain. PFGE restriction fragment patterns of normal colony form and of the SCVs were indistinguishable. All isolates of *Staphylococcus epidermidis* (normal form and SCVs) were resistant to methicillin, tobramycin, cotrimoxazole, ciprofloxacin and levofloxacin and sensitive to netilmicin. They were uniformly susceptible to vancomycin (MIC 3 mg/l) and there was no evidence of vancomycin hetero-resistance. While the normal colony forms were susceptible to teicoplanin (MIC 1 mg/l), all but one of the isolates of SCVs were teicoplanin resistant (MIC 24 mg/l).

SCVs of *Staphylococcus aureus* have been reported to cause persistent and recurrent infections [1, 2, 3]. Compared with their normal phenotypes, they often show reduced susceptibilities to several antimicrobial agents, namely aminoglycosides and cotrimoxazole [3, 12]. In the few reported cases of infection with SCVs of CoNS [4, 5, 6] such differences between the two colony forms were not observed. Here we describe the first case of an infection with a teicoplanin-resistant staphylococcal SCV.

The patient was treated with vancomycin including vancomycin-lock for suspected bloodstream infection after methicillin-resistant *Staphylococcus epidermidis* had been isolated from two blood cultures. A normal colony form and an SCV of *Staphylococcus epidermidis* were recovered from a blood culture performed 11 h after the start of vancomycin therapy. In contrast to the normal phenotype, the SCV was resistant to teicoplanin. Both

phenotypes were susceptible to vancomycin. Identical PFGE patterns showed both forms to be clonal.

Two scenarios exist for the occurrence of the teicoplanin-resistant SCV. Firstly, resistance to teicoplanin developed during exposure to the high concentration of vancomycin associated with vancomycin-lock. Similarly, SCVs isolated from patients after local treatment for osteomyelitis with gentamicin beads had up to 32-fold higher MICs of gentamicin than the parent strain (1 μg/ml vs. <0.031 μg/ml) [2]. It is remarkable that resistance to teicoplanin, but not to vancomycin, may have evolved under vancomycin therapy. Even though development of vancomycin-resistant SCVs of *Staphylococcus haemolyticus* after exposure to vancomycin has been reported [13], resistance to teicoplanin might still be easier to obtain under selective pressure from vancomycin than resistance to vancomycin itself. Cercenado et al. [14] found CoNS with reduced susceptibilities to teicoplanin but full susceptibility to vancomycin in 32 patients, eight of whom had been treated with vancomycin. Secondly, taking into account the tendency of SCVs to revert to the normal form, we cannot exclude the possibility that they developed spontaneously before vancomycin therapy but were missed in the corresponding blood cultures. Emergence of a teicoplanin-resistant SCV of *Staphylococcus aureus* during experimental foreign body infection in a rat without antibiotic therapy has been reported [15]. However, this is the first report of the emergence of a teicoplanin-resistant SCV of a *Staphylococcus* species in a patient.

Based on the following evidence, it is conceivable that persistence of the bloodstream infection was caused by the development of the SCV. (i) Antibiotic therapy was performed according to established treatment guidelines and susceptibility testing. (ii) The central-venous line was promptly removed once persistent bacteremia was observed, whereas bacteria could not be cultured from the catheter. A new catheter was inserted at a different location. (iii) Neither doppler ultrasound of the subclavian vein and transesophageal echocardiography nor the autopsy showed any evidence of septic thrombophlebitis or endocarditis. Treatment failure despite adequate antibiotic therapy has been reported in four patients with osteomyelitis due to SCVs of *Staphylococcus aureus* [2] and in a patient with pacemaker-associated vegetations due to SCVs of *Staphylococcus capitis* [6]. SCVs of *Staphylococcus epidermidis* were also isolated in a fatal case of prosthetic heart valve endocarditis [4].

Between the start of vancomycin therapy and the death of the patient, both colony forms were isolated from six blood cultures while ten blood cultures yielded solely the normal form. Taking into account its tendency to revert, we believe that *Staphylococcus epidermidis* survived as SCV and reverted to the normal form upon cultivation. Because of both their tendency to revert and their slow growth, SCVs of staphylococci may easily go undetected. Thus, the number of cultures should be increased and incubation of subcultures should be extended if infection with SCVs of staphylococci is suspected.

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