Caroline Theunissen, Peter Janssens, Anne Demulder, Denis Nouboussié, Marjan Van Esbroeck, Alfons Van Gompel, and Jef Van den Ende

Author affiliations: Brugmann University Hospital, Brussels, Belgium (C. Theunissen, P. Janssens, A. Demulder, D. Nouboussie); and Prince Leopold Insitute of Tropical Medicine, Antwerp, Belgium (M. Van Esbroeck, A. Van Gompel, J. Van den Ende)

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Address for correspondence: Caroline Theunissen, Department of Internal Medicine, Brugmann University Hospital, Van Gehuchtenplein 4, Brussels 1020, Belgium; email: caroline.theunissen@chu-brugmann.be

Linezolid-Resistant *Staphylococcus cohnii*, Greece

To the Editor: Since 2003, linezolid has typically been used to treat infections caused by multidrug-resistant gram-positive cocci such as vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* (*1*). In Greece, a major problem is nosocomial dissemination of vancomycin-resistant enterococci. Use of linezolid for the treatment of such infections led to the emergence of linezolid–vancomycin resistant *E. faecium*; however, linezolid resistance of staphylococci is still relatively low in this country (*2*). We describe an outbreak caused by a linezolid-resistant *S. cohnii* in an intensive care unit (ICU) in Greece.

From July through October 2007, nonrepetitive coagulase-negative staphylococci that exhibited resistance to linezolid, were isolated from blood cultures from 4 separate patients hospitalized in the ICU at Sismanoglion General Hospital of Athens, a 450-bed tertiary care hospital. The ICU is a 10-bed, level II unit, comprising 2 rooms with 1 bed each and 2 rooms with 4 beds each. Each isolate was recovered in 2 of 2 blood culture sets per patient, indicating true bacteremia. The demographic and clinical information for the patients is described in the Table. The mean duration of time preceding linezolid therapy was 22 days.

Isolates were first identified to the species level by using an API Staph system (bioMérieux, la Balme les Grottes, France) and a molecular method based on the *tuf* gene followed by sequencing analysis (*3*). Susceptibility testing for various antimicrobial agents was performed by disk diffusion and using Clinical Laboratory Standards Institute criteria; susceptibilities were interpreted according to Institute guidelines (*4*). In addition, MICs to oxacillin, vancomycin, teicoplanin, quinupristin-dalfopristin, linezolid, daptomycin, and tigecycline were determined by Etest (AB Biodisk, Solna, Sweden) according to manufacturer's instructions. Resistance genes *mecA*, *vat*, *vga*, *erm*, *aac(6′)-Ie+aph(2″)*, *ant(4′)-Ia*, and *aph(3′)-IIIa*, as markers for resistance to β-lactams, dalfopristin, macrolides, and aminoglycosides, were identified by PCR as previously reported (*5*,*6*). The presence of G2576T in domain V of the 23S rRNA, which is mainly associated with linezolid resistance in clinical isolates, was detected by using PCR and digestion of the product with *NheI* (*2*). The number of mutated versus nonmutated alleles was determined as described by Pillai et al. (*7*). In addition, isolates were examined for the presence of the *cfr* gene, which was found to be correlated with linezolid resistance in some coagulase-negative staphylococci and for mutations of ribosomal protein L4, L22 genes (*8*,*9*). Clonality of isolates was assessed by pulsed-field gel electrophoresis (PFGE) after digestion of chromosomal DNA with *SmaI* (*2*).

The molecular method identified the isolates as *S. cohnii* subsp. *ureolyticus*. The API Staph system has correctly identified 2 of them; the re-

Table. Clinical characteristics of 4 patients from whom linezolid-resistant *Staphylococcus cohnii* was isolated, Greece, 2007*

Patient no.	Gender/ age, y	Medical history	Reason for hospitalization	Previous treatment	Date of hospital admission	Date of sample collection	Outcome	Date of death or discharge
1	F/82	No relevant history	Acute cholecystitis, necrotizing pancreatitis	CIP, CAZ, IMP, TZP, LIN	May 10	Jul 20	Death	Aug 21
2	M/38	Alcoholism	Lung abscess	CIP, CAZ, LIN	Jul 14	Aug 21	Recovery	Oct 20
3	M/52	COPD, melitensus diabetes	Necrotizing pneumonia	TZP, CLI, IMP, LIN	Aug 21	Sep 25	Recovery	Oct 30
4	M/65	No relevant history	Septic shock	IMP, CIP, TZP, LIN	Sep 17	Oct 28	Recovery	Dec 10

*CIP, ciprofloxacin; CAZ, ceftazidime; IMP, imipenem; TZP, piperacillin-tazobactam; LIN, linezolid; CLI, clindamycin; COPD, chronic obstructive pulmonary disease.

maining 2 isolates were falsely characterized as *S. xylosus*. According to disc diffusion test results, the isolates were resistant to cefoxitin, oxacillin, penicillin, rifampin, quinupristin-dalfopristin, erythromycin, clindamycin, fusidic acid, tobramycin, gentamicin, and linezolid. MICs were linezolid 32, oxacillin 256, quinupristin-dalfopristin 8, vancomycin 2, teicoplanin 2, tigecycline 0.125, and daptomycin 0.5 mg/L. Molecular methods detected the following resistance genes: *mecA, ermA, aac(6´)-Ie+aph(2″),* and *aph(3´)-IIIa.* The isolates, despite their resistance to streptogramins, were negative for *vat* and *vgaA* genes. In addition, all isolates carried the G2576T mutation and had 4 of 5 mutated alleles. No isolate carried the *cfr* gene or any mutation on ribosomal protein L4 and L22 genes. PFGE results indicated that all isolates were clonally related, belonging to the same clone.

Outbreaks caused by linezolid-resistant staphylococci are rare worldwide (*10*); in Sismanoglion Hospital, before the outbreak period, no linezolid-resistant staphylococci and enterococci had been isolated. However, in the ICU, a statistically significant increase in the use of linezolid was observed in 2004 and in 2007 (0.58 vs. 1.34 defined daily doses/100 patient-days, respectively); heavy use of linezolid may have created substantial selection pressure in favor of the linezolid-resistant isolates.

The 4 patients were treated in the same room by the same personnel; thus, a potential explanation for this outbreak is patient-to-patient transmission of linezolid-resistant strains on the hands of healthcare personnel. However, cultures of ICU personnel (nasal cavity and hands) grew only methicillin-resistant *S. aureus* and methicilllin-resistant *S. epidermidis*. In addition, environmental samples taken from the beds and the equipment of these patients were negative for *S. cohnii*. Strict control measures were taken (e.g., isolation of infected patients, increased environmental cleaning, and reinforcement of proper glove and gown use and hand disinfection with alcohol gel), and the outbreak strain was not recovered from other patients in the ICU or in other departments of the hospital after the initial outbreak. In conclusion, to avoid spread of staphylococcal resistance in ICUs, measures such as hand hygiene and adequate central venous catheter handling should be taken, and policies regarding antimicrobial drug use must be applied.

Efi Petinaki, Maria Kanellopoulou, Anthi Damani, Antigoni Foka, Iris Spiliopoulou, Nikoletta Skalmoutsou, Bogdan Raitsiou, Konstantinos Valakis, and Evangelos Papafragas

Author affiliations: University of Thessalia, Larissa, Greece (E. Petinaki); Sismanoglion General Hospital of Athens, Athens, Greece (M. Kanellopoulou, N. Skalmoutsou, B. Raitsiou, K.Valakis, E. Papafragas); Institute of BioMedical Research and Technology, Larissa (E. Petinaki, A. Damani); and University of Patras, Patras, Greece (A. Foka, I. Spiliopoulou)

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Address for correspondence: Efi Petinaki, Department of Microbiology, University of Thessalia, Medical School Mezourlo, Larissa, Greece; e-mail: petinaki@med.uth.gr

Buruli Ulcer in Long-Term Traveler to Senegal

To the Editor: Buruli ulcer (BU) is caused by infection of subcutaneous fat with the environmental pathogen *Mycobacterium ulcerans*. BU has been reported or suspected in more than 30 countries. It has never been reported in Senegal and Guinea-Bissau (*1*). We report a case of travel-associated BU in a French traveler to Senegal.

The patient was a 24-year-old Caucasian man who came to the University Hospital of Bordeaux, France, with a nonhealing lesion on the anterior left leg that had been present for ≈12 weeks. The patient had traveled in Senegal to the border of Guinea-Bissau from September 2006 through August 2007. His trip had begun in Dakar and proceeded south to the districts of Kaolack, Toubacouta, and Casamance. The patient stayed in Casamance during the rainy season from June 2007 through August 2007. He had been working on construction of wood dugouts, had been bare-legged regularly, and had been in contact with stagnant water.

He first noticed a lesion during June 2007, which had gradually increased to a small, centrally crusted ulcer. By the end of August 2007 (week 8 of the lesion), skin examination showed a 3 × 6-cm necrotizing ulcer with central crusting and an erythematous border (Figure). The lesion was not warm or tender but generated a seropurulent discharge. Concurrently, palpable left inguinal lymph nodes were observed.

Bacteriologic swabs identified *Staphylococcus aureus* and group A *Streptoccocus pyogenes*. Two punch-biopsy specimens were taken from the border of the lesion. Histologic analysis showed nonspecific acute and chronic dermal inflammation with necrotizing granulomas that extended into the subcutaneous tissues, suggestive of infection with atypical *Mycobacterium* spp. Bacteriologic examination did not identify acid-fast bacilli (negative direct smear result after Ziehl-Neelsen staining) or other specific microorganisms (negative direct smear results after periodic acid–Schiff, Giemsa, and Gram staining). Tissue specimens were placed into BACTEC 12B broth (Becton Dickinson, Franklin Lakes, NJ, USA) (incubated at 35°C) and onto Löwenstein-Jensen slants (incubated at 30°C). No growth was detected after 42 days. On the basis of clinical findings, we suspected a diagnosis of BU.

Taq-Man real-time quantitative PCR that used primers for 2 *M. ulcerans*–specific genes (insertion sequence *2404* and ketoreductase B gene) (*2*,*3*) and negative controls showed positive results for DNA from both biopsy specimens. A normalized standard curve was constructed, which indicated a bacterial load of ≈6×10^3 organisms/g of tissue.

Laboratory investigations indicated a total leukocyte count of 16,400 cells/μL (reference range 3,600–10,000 cells/μL) and a C-reactive protein level of 0.59 mg/mL (reference value <0.01 mg/mL). Results of radiologic investigations were normal. The

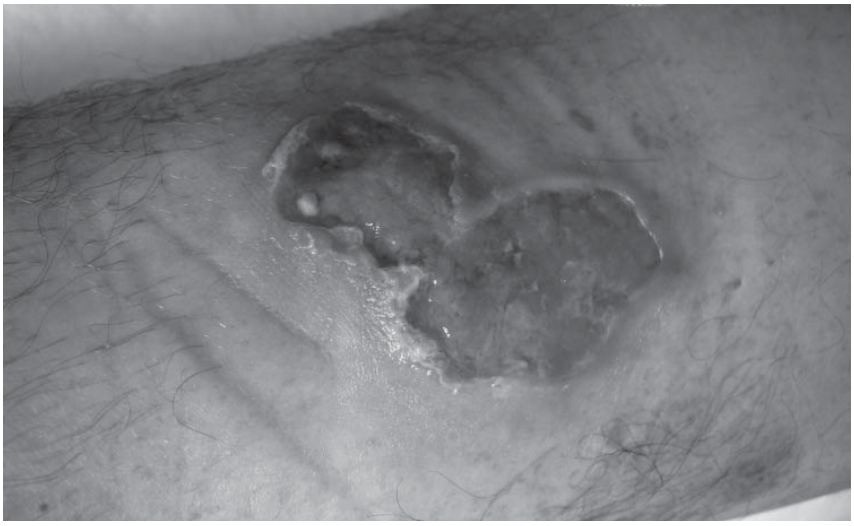

Figure. Ulcer (3 × 6 cm) on anterior side of the left leg of the patient, showing an erythematous border.