Persistent Bloodstream Infection with *Kocuria rhizophila* Related to a Damaged Central Catheter

Didier Moissenet, Karsten Becker, Audrey Mérens, Agnès Ferroni, Béatrice Dubern, and Hoang Vu-Thien

Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Service de Microbiologie, Faculté de Médecine, Université Pierre et Marie Curie, Paris, France; University Hospital of Münster, Institute of Medical Microbiology, Münster, Germany; Hôpital d'Instruction des Armées Bégin, Service de Biologie Médicale, Saint Mandé, France; Assistance Publique-Hôpitaux de Paris, Hôpital Necker, Service de Microbiologie, Faculté de Médecine, Université René Descartes, Paris, France; and Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Service de Gastro-Entérologie-Nutrition, Faculté de Médecine, Université Pierre et Marie Curie, Paris, France

A case of persistent bloodstream infection with *Kocuria rhizophila* related to a damaged central venous catheter in a 3-year-old girl with Hirschsprung’s disease is reported. The strain was identified as *K. rhizophila* by 16S rRNA gene sequencing and matrix-assisted laser desorption ionization–time of flight mass spectrometry. Arbitrarily primed PCR analysis showed a clonal strain. The repeated septic episodes were resolved with the catheter repair.

**CASE REPORT**

The patient was a 3-year-old girl with total colonic form of Hirschsprung’s disease. On day 2 of life (27 April 2006), an emergency surgery for small-intestine occlusion due to atresia was performed, with removal of 31 cm of small intestine, followed by terminal ileostomy and colostomy. A total parenteral nutrition was then needed. On 25 August 2006, a subcutaneous implantable vascular-access port (Cook Spectrum Central Venous Catheter, Cook Ireland Ltd.) was placed for home parenteral nutrition after an accidental removal of the Nutricath (Vygon, France) catheter.

Four months later (December 2006), the first septic episode was observed, with seven positive blood samples drawn through the catheter and from a peripheral vein. The fever resolved promptly after the initiation of antimicrobial therapy combining vancomycin (40 mg/kg body weight/day, 10 days) and gentamicin (3 mg/kg/day, 2 days). Blood isolates were first identified as *Micrococcus* spp. by routine biochemical galleries and subsequently as *Kocuria rhizophila* by using molecular tools. Afterward, seven other septic episodes with *K. rhizophila* (two in 2007, four in 2008, and one in 2009) were observed and resolved with the same antimicrobial therapy. Since it was assumed that colonization of the catheter was the cause of sepsis, ethanol locks (70% ethanol was instilled into the catheter lumen for 12 h and then withdrawn from the catheter lumen; then, an isotonic sodium chloride flush was performed) were made in the catheter during the last four episodes associated with systemic antibiotics (17). At the time of the last septic event in 2009, a hole in the catheter was detected and repaired using a specific repairing kit. After April 2009, no novel septic episode was observed.

During the 3-year period (2007 to 2009), using the BacT/Alert three-dimensional (3D) system (bioMérieux, Marcy l’Etoile, France), a total of 22 positive blood cultures were obtained, with 21 samples drawn from the catheter and one from a peripheral vein. All the cultures yielded Gram-positive cocci occurring in pairs, tetrads, and clusters that were preliminarily identified as *Micrococcus* species with basic characteristics. The colonies grew under aerobic conditions and appeared smooth and circular with a lemon-yellow tinge or cream color on blood agar. The isolates were catalase positive, oxidase negative, susceptible to bacitracin, and resistant to furazolidone. Only a few positive reactions were found with the ID 32 Staph gallery (bioMérieux), yielding *Staphylococcus auricularis* with weak probability of 57%. In 2006 and 2007, the clinical strain was considered *Micrococcus* without other investigation in the identification process. In 2008, we used the Vitek 2 ID-GPC card (bioMérieux) that yielded *Kocuria varians* with an apparent “excellent” confidence score (98%). It showed only a single discordant test (urea) since it appeared negative for this species, whereas it should be positive in 87% of *K. varians* isolates. In order to clarify this discordant test and confirm *K. varians*, we used 16S rRNA gene analysis. DNA extraction and 16S rRNA gene sequence analyses were performed as previously described (16). The procedure was performed on 11 clinical isolates: four recovered in 2006, six in 2008, and one in 2009. Surprisingly, all the sequences obtained showed a complete identity to those of *K. rhizophila* deposited in the GenBank nucleotide database. The 16S rRNA gene sequence of the *K. rhizophila* isolate showed a similarity of 98% (457/466) with that of the 16S rRNA gene of the *K. rhizophila* DC22201 strain (GenBank accession no. NC010617) and showed similarity with the sequences of 10 other *K. rhizophila* strains, including Kovacs’ historic type strain *K. rhizophila* TA68T (GenBank accession no. NR_026452; similarity of 99%) and *Micrococcus luteus* NCTC 2665 (GenBank accession no. NC012803; similarity of 93%). Other *Kocuria* species showed a similarity of 451/462 for *Kocuria carniphila* and 452/467 for *Kocuria marina*. Finally and recently, we have used matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as previously described (6), confirming the strain as *K. rhizophila*. The spectral profiles of four clinical isolates were identical to the spectral profile of the *K. rhizophila* type strain (Fig. 1) and quite distinct from the profiles of *Kocuria kristinae*, *Kocuria palustris*, *Kocuria rosea*, and *Kocuria varians*, all present in the Andromas database. With the disk diffusion method, all the isolates were
resistant to ciprofloxacin, intermediate to erythromycin, and susceptible to penicillin, gentamicin, amikacin, tobramycin, tetracycline, vancomycin, and teicoplanin, according to EUCAST breakpoints determined for Staphylococcus spp. since there are not breakpoints available for Kocuria spp. With broth microdilution, MICs were 0.03 mg/liter for penicillin, 0.5 mg/liter for gentamicin, 1 mg/liter for amikacin, 2 mg/liter for tobramycin, 8 mg/liter for ciprofloxacin, 4 mg/liter for erythromycin, 0.125 mg/liter for tetracycline, 0.5 mg/liter for vancomycin, and 1 mg/liter for teicoplanin.

Clinical isolates genotyping with the arbitrarily primed PCR technique needed prolonged ramp times, as reported by Ellinghaus et al. (8). It was performed on 9 representative isolates of the eight septic episodes and showed the same pattern, representing a clonal K. rhizophila strain recovered for 3 years (Fig. 2).

In 1995, the genus Micrococcus was dissected into five genera (Kocuria, Nesterenkonia, Kytococcus, Dermacoccus, and Micrococcus) (23) with description of three Kocuria species (K. rosea, K. varians, and K. kristinae). Here, the trivial terms “micrococci” and “micrococcals” are used to indicate members of these genera. The genus Kocuria belongs to the family of Micrococcaceae, which is part of the order of Actinomycetales, class Actinobacteria. In 1999, K. rhizophila was described with K. palustris by Kovacs et al. (10). It was isolated from the rhizoplane of the narrow-leaved cattail (Typha angustifolia) inhabiting a floating mat on a creek of the Hungarian part of the Danube River. In 2003, the strain ATCC...
9341, originally deposited as *Sarcina lutea* and later redesignated *Micrococcus luteus*, was reclassified as *K. rhizophila* (25). From 2004, 11 other *Kocuria* species were described (*K. marina* sp. nov. [9], *K. carniphila* sp. nov. [28], *K. aegyptia* sp. nov. [13], *K. himachalensis* sp. nov. [15], *K. flava* sp. nov. and *K. turfanensis* sp. nov. [32], *K. halotolerans* sp. nov. [26], *K. gwangalliensis* sp. nov. [22], *K. koreensis* sp. nov. [20], *K. atrinae* sp. nov. [19], and *K. salsicia* sp. nov. [31]). *K. rhizophila* is also important in industrial applications and is commonly used as a standard quality control strain for antimicrobial susceptibility testing. In 2008, the complete genome sequence of *K. rhizophila* was determined by Takarada et al. (24).

Micrococcal species were found in dust, soil, water, and food and on skin and mucosa of humans and animals. Members of these species groups have also been found to cause infections such as meningitis, endocarditis, and pneumonia, particularly in immunocompromised patients, and infections related to implanted or inserted devices (18, 21, 30). Among the recently established genus *Kocuria*, documented human cases of infections are limited. The type species *K. rosea* has been reported to cause catheter-related bacteremia (2). Another member of the genus, *K. kristinae*, has also been reported to cause a catheter-related bacteremia in patients with ovarian cancer (3) or acute cholecystitis (14). In 2009, two cases of peritonitis caused by *K. marina* were reported by Lee et al. (12). More recently, in 2010, Lai et al. (11) reported catheter-related bacteremia and infective endocarditis caused by *Kocuria sp.*, whereas Tsai et al. (27) reported a *K. varians* infection associated with brain abscess.

While *K. rhizophila* has been isolated from food such as cheese (7) and chicken meat (1), to our knowledge, our case is the second report of *K. rhizophila* human infection after the first one described by Becker et al. in 2008 (4). While the source of our 3-year-persistent *K. rhizophila* strain was unclear, it is possible that it was part of resident skin flora of the patient, colonizing the intravascular device on several occasions. The long-term intravascular device (3 years) probably provided a niche for the persistent *K. rhizophila* strain, recurring through the hole in the device. Repair of the damaged device seemed to prevent the occurrence of any novel septic episode until now. The whole ward staff is now frequently reminded of the strict application of aseptic protocol concerning central venous catheter management. Currently, the child is in good health and always has liquid stools. Parenteral nutrition could be reduced to 5 days per week.

The ID 32 Staph gallery did not allow a reliable identification of *K. rhizophila* since the database of this commercially available diagnostic kit includes only a limited number of micrococcaceae species, not covering the recently described species and not reflecting the taxonomy established by Stackebrandt et al. (23). The Vitek 2 ID-GP card ambiguously identified several *Kocuria* species (*K. varians, K. kristinae, and K. rosea*) but not *K. rhizophila*. Moreover, misidentification of coagulase-negative staphylococci as *Kocuria* using standard biochemical analysis by the Vitek 2 system is not uncommon, due to phenotypic variability (5). In contrast, the use of 16S rRNA gene analysis or MALDI-TOF MS was adequate to obtain an accurate identification of *K. rhizophila*.

Few data are currently available about antimicrobial susceptibility of *Kocuria* spp. or other micrococcaceae and, moreover, no generally accepted therapeutic regimen for severe infections has yet been defined. In 1995, von Eiff et al. (29) determined MICs of several drugs on 188 micrococcaceae strains: MICS \_{90\%} (mg/liter) of rifampin, penicillin, imipenem, ampicillin, clindamycin, cefotaxime, vancomycin/teicoplanin, and gentamicin were, respectively, \(\leq 0.031\), 0.125, 0.125, 0.25, 0.25, 1, 1/1, and 1, whereas MICs \_{90\%} of amikacin, erythromycin, fosfomycin, and fusidic acid were >2 mg/liter. In our case, the strain was susceptible to vancomycin and gentamicin, and the combination of these two drugs always allowed sterilization of blood cultures.

In conclusion, if an organism resembling micrococcaceae is repeatedly isolated from blood cultures, it is important to use means other than the routine biochemical systems, such as 16S rRNA gene sequencing or MALDI-TOF MS, to obtain an accurate species identification. It is also recommended to verify carefully the integrity of long-term intravascular devices and repair damage to salvage central lines from having to be removed.

### Nucleotide sequence accession number

The 16S rRNA gene sequence of the *K. rhizophila* isolate has been submitted to GenBank under accession number JQ272742.

### REFERENCES


