Kocuria rhizophila Adds to the Emerging Spectrum of Micrococcal Species Involved in Human Infections[▽]

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We describe the first case of a *Kocuria rhizophila* infection in a boy with methylmalonic aciduria. A single clone was isolated from blood samples drawn through a port system and from peripheral veins during septic episodes within a 2-year period. *K. rhizophila* expands the emerging number of “micrococci” considered to be etiologically relevant.

CASE REPORT

The patient was an 8-year-old boy with methylmalonic aciduria due to a noncobalamin-responsive deficiency of methylmalonyl coenzyme A mutase that had been diagnosed during his neonatal period based on fibroblast studies. Although he was treated with a protein-restricted diet as well as a carnitine- and leucine-free amino acid supplement, the clinical course was complicated by frequent episodes of vomiting and abdominal pain. Following a metabolic crisis with lactic acidosis and severe pancreatitis complicated by the formation of a pancreatic pseudocyst, a subcutaneous implantable vascular-access port (Port-A-Cath; Vital-Port) was placed in his left internal jugular vein at the age of 6 years.

Two years later, the patient's first septic episode due to *Kocuria rhizophila* was documented with the repeated recovery of this species by culturing blood samples drawn through the Port-A-Cath and from a peripheral vein (Fig. 1). While the fever resolved promptly after the initiation of antimicrobial therapy with cefuroxime (90 mg/kg body weight per day for 10 days), the patient was readmitted with signs of acute pancreatitis. After the initiation of total parenteral nutrition, the patient developed a fever and *K. rhizophila* again was repeatedly cultivated from blood samples taken through the Port-A-Cath. Again, the patient recovered following therapy with cefuroxime (90 mg/kg body weight per day for 18 days). Since it was assumed that the Port-A-Cath might be the focus of the infection, this device was additionally “locked” with 5 mg vancomycin over a period of 7 days. After further febrile episodes in the following months without the recovery of *K. rhizophila* from blood cultures, a third septic episode due to this pathogen was noted 2 years after the first isolation of *K. rhizophila* from this patient. Again, *K. rhizophila* was detected following the initiation of high-calorie parenteral-infusion therapy via the Port-A-Cath. Since *Candida parapsilosis* was also recovered from the Port-A-Cath after 1 week of treatment with vancomycin (33 mg/kg body weight per day) and cefotaxime (100

mg/kg body weight per day), the port system was explanted and a central venous catheter was placed through the patient's right femoral vein. Finally, after successful antimycotic treatment with amphotericin B, a new Port-A-Cath was placed in his left internal jugular vein without further complications.

A total of 10 cultures done using the Bactec 9240 system (Becton Dickinson, Cockeysville, MD) with blood sampled from the port and peripheral veins yielded gram-positive cocci occurring in pairs, tetrads, and packets that were preliminarily identified by basic characteristics as *Micrococcus* species. The only colonies that grew under strictly aerobic conditions were smooth and circular with a yellow tinge and appeared dull and creamy on Mueller-Hinton blood agar. The isolates were catalase positive and oxidase negative. Only a few positive reactions were found when the Vitek 2 automated system (bioMérieux Vitek, Hazelwood, MO) and the ID 32 Staph ATB gallery (bioMérieux Vitek) were used. The Vitek 2 ID-GPC card ambiguously identified several isolates as *Kocuria varians* or *Kocuria rosea* or as *Dermaococcus nishinomiyaensis* or *Micrococcus luteus*, with probabilities of 50.53% to 98.23%. One isolate was identified as *K. rosea* (probability of 97.95%). By use of the ID-GPC card panel, the isolates tested positive for only the alanine arylamidase reaction and the alkalization of L-lactate. The ID 32 Staph system identified four isolates as staphylococcal species (*Staphylococcus auricularis* and *Staphylococcus capitis*) but could not validate these results (profiles 060000000 and 060000200, respectively). Further isolates were identified as *Kocuria kristinae* (probabilities of 44.3% to 99.4%). DNA extraction and 16S rRNA gene sequence analyses of selected isolates were done as previously described (8). 16S rRNA gene sequences of two isolates recovered in 2005 (K1373-05, accession number FM177895) and in 2007 (K1458-07, accession number FM177896) showed complete identity to sequences of *K. rhizophila* (accession number Y16264) deposited in the GenBank nucleotide database. When arbitrarily primed PCR with prolonged ramp times was used (10), all isolates were shown to be clonal, representing one strain (data not shown). If the operator of the Vitek 2 system declared the isolates as coagulase-negative staphylococci, all isolates tested were determined to be susceptible in vitro to a wide range of antibiotics, including all β -lactams, macrolides, glycopeptides, and quinolones tested, with the exception of norfloxacin, to

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FIG. 1. Schematic representation showing the time course of the port-associated *Kocuria rhizophila* infection. i.v., intravenous. Dates are given in month/year.

which the isolate was reported as resistant. These findings were confirmed by an agar disk diffusion assay on Mueller-Hinton agar.

Recently, it was noted that a complete picture of infections related to *Kocuria* spp. will have to await the documentation of more clinical cases (17). The first clinical presentation of *K. rhizophila* reported here underlines the emergent role of these bacteria, which were formerly classified into the genus *Micrococcus* (trivial term “micrococci”). This genus was dissected into the genera *Kocuria*, *Micrococcus*, *Nesterenkonia*, *Kytococcus*, and *Dermacoccus*, which were then rearranged into two families (*Micrococcaceae* and *Dermacoccaceae*), both belonging to the suborder *Micrococcineae* (23). In this report, we use the trivial terms “micrococci” as well as “micrococcal” in quotation marks to indicate the members of these genera. Many novel species of these genera, established in the last decade, are known to be part of the microbial biocenosis of water, sediments, soils, sludges, and fermented foods forming complex biofilms together with a variety of other microorganisms (11, 15, 16).

Even though isolates belonging to the former genus *Micrococcus* are usually regarded as contaminants from skin and mucous membranes, “micrococci” have been reported not just as emerging pathogens in immunocompromised patients (1, 19, 20). These species have also been found to cause (i) infections such as endocarditis, pneumonia, and sepsis, predominantly in immunocompromised patients (14, 18, 22, 27), and/or (ii) infections related to implanted or inserted foreign bodies (6, 20, 21). A novel “micrococcal” species, *Kytococcus schroeteri*, involved in human infections, was described recently (5).

Here, we describe what is to our knowledge the first case of a *K. rhizophila* infection. This bacterium, 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, was first described in 1999 (12). Since that description, only a few reports on this actinobacterial species have been published so far. *K. rhizophila* was found in coculture with other species of this genus by El-Baradei et al. while studying the bacterial biodiversity occurring in traditional

Egyptian soft Domiati cheese (9). Furthermore, the widely used quality control strain for sterility testing and assaying a variety of antibiotics and fungicide residues, ATCC 9341, which was originally deposited as *Sarcina lutea* and later redesignated *Micrococcus luteus*, was recently reclassified as *K. rhizophila* (25). Recently, this microorganism was the predominant bacterium isolated from chicken meat treated with oxalic acid for reducing the populations of naturally occurring microorganisms on raw chicken (2). However, infections in humans or animals have not yet been described. While the genuine source of the *K. rhizophila* isolates reported here remains unclear, it is most likely that the colonization of the port following its implantation was due to contact with an environmental source, e.g., freshwater, dust, or contaminated food.

Susceptibility to bacitracin and lysozyme and resistance to lysostaphin and nitrofurantoin are major criteria for the conventional preliminary differentiation of “micrococci” from staphylococci, which display the opposite pattern (3). The databases of the commercially available diagnostic kits include “micrococcal” species only in a very limited manner and do not cover recently described “micrococcal” species and/or do not reflect the new taxonomy of the *Micrococcineae* order as established by Stackebrandt et al. (4, 23, 24). Thus, misidentifications between “micrococci” and staphylococci described here and elsewhere have to be considered if “micrococci” are involved (7).

In the case reported here, a Port-A-Cath device provided a niche for a period of more than 2 years for the recurrence of this pathogen, which was temporarily in coexistence with *Candida parapsilosis* colonizing this long-lasting implanted foreign body. Determining the extent that precolonization of foreign bodies by “micrococcal” species might facilitate colonization by other microorganisms due to the generation of a biofilm and, thus, the establishment of a preformed, bacterial growth-enhancing microenvironment should be the object of further studies. Based on studies of the ecology of mixed biofilms, Leriche et al. reported that *Staphylococcus sciuri* cells daily subjected to a chlorinated alkaline solution are protected by *Kocuria* microcolonies (13).

A generally accepted therapeutic regimen for severe infections with micrococcal species has not yet been defined. A

combination of rifampin and ampicillin has been shown to be effective for *M. luteus* (27). Also, successful treatment was performed with other β -lactams, vancomycin, clindamycin, gentamicin, or a combination of these agents. Overall, rifampin showed the highest in vitro activity against “micrococcal” species (26).

In conclusion, *K. rhizophila* adds to the other members of the suborder *Micrococcineae* that are able to cause infections in humans. If “micrococcal” species are considered to be etiologically relevant, failures in databases and outdated nomenclature of identification systems should be considered.

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