**Kocuria rhizophila** adds to the emerging spectrum of micrococcal species involved in human infections

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Short title

*Kocuria rhizophila* infection

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Abstract

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

The patient was an 8-year-old boy with methyl-malonic aciduria due to a non-cobalamin responsive deficiency of methylmalonyl CoA mutase diagnosed in his neonatal period based on fibroblast studies. Although he was treated with a protein-restricted diet as well as with a carnitine and a 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 a severe pancreatitis complicated by the formation of a pancreas pseudocyst, a subcutaneous implantable vascular access port (Port-a-Cath, Vital-Port®) was placed in the left internal jugular vein at the age of six years.

Two years later, the first septic episode due to Kocuria rhizophila was documented with repeated recovery of this species from blood cultures drawn through the Port-a-Cath and from a peripheral vein. While the fever resolved promptly after initiation of antimicrobial therapy with cefuroxime (90 mg/kg body weight per day for 10 days), the patient was readmitted with signs of an acute pancreatitis. After initiation of total parenteral nutrition, the patient developed fever and K. rhizophila was again repeatedly cultivated from blood 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 recovery of K. rhizophila from blood cultures, a third septic episode due to this pathogen was noted - two years after the first isolation of K. rhizophila from this patient. Again, K. rhizophila was detected following initiation of a high caloric parenteral infusion therapy via the Port-a-Cath. Since after one week of treatment with vancomycin (33 mg/kg body weight per day) and cefotaxim (100 mg/kg body weight per day) also Candida parapsilosis was recovered from the Port-a-Cath, the port system was explanted and a central venous catheter was placed.
through the right femoral vein. Finally, after successful antifungal treatment with amphotericin B, a new Port-a-Cath was placed without further complications.

A total of ten blood cultures using the BACTEC™ 9240 system (Becton Dickinson, Cockeysville, MD) sampled from the port and peripheral veins yielded gram-positive cocci occurring in pairs, tetrads and packets preliminary identified by basic characteristics as *Micrococcus* species. The only under strictly aerobic conditions growing smooth colonies were circular with 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 automated system VITEK® 2 (bioMérieux Vitek, Hazelwood, MO) and the ID 32 STAPH ATB gallery (bioMérieux Vitek) were applied. The VITEK® 2 ID-GPC card identified several isolates ambiguously as *Kocuria varians*/*Kocuria rosea* or as *Dermacoccus nishinomiyaensis*/*Micrococcus luteus* with probabilities of 50.53% - 98.23%. One isolate was identified as *K. rosea* (probability of 97.95%). Of the ID-GPC card panel, only the alanin arylamidase reaction and the alkalization of L-lactat were tested positive. The ID 32 STAPH identified four isolates as staphylococcal species (*S. auricularis* and *S. capitis*, respectively) but could not validate these results (profiles 060 000 000 and 060 000 200, respectively).

Further isolates were identified as *Kocuria kristinae* (probabilities, 44.3% - 99.4%). DNA extraction and 16S rRNA gene sequence analyses of selected isolates were done as previously described (8). 16S rRNA 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-polymerase chain reaction with prolonged ramp times was used (10), all isolates were shown to be clonal, representing one strain (data not shown). Using VITEK® 2 by declaring the isolates as coagulase-negative staphylococci by the operator, all isolates were tested *in vitro* susceptible to a wide range of antibiotics, including all β-lactams, macrolides, glycopeptides, and quinolones tested with the exception of
norfloxacin reported as resistant. These findings were confirmed by agar disk diffusion 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 those bacteria, which were formerly classified into the genus *Micrococcus* (triv. “micrococci”). This genus was dissected into the genera *Kocuria, Micrococcus, Nesterenkonia, Kytococcus*, and *Dermacoccus* followed by rearrangement into two families (*Micrococcaceae, Dermacoccaceae*) both belonging to the suborder *Micrococcineae* (23). In this report, the authors used the trivial terms “micrococci” as well as “micrococcal” in quotes to indicate members of these genera. Many novel species of these genera, established in the last decade, are known as part of the microbial biocenosis of water, sediments, soils, sludges, and fermented foods forming complex biofilms together with various 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 as emerging pathogens not only in immunocompromized patients (1,19,20). However, these species were also found to cause (i) infections such as endocarditis, pneumonia, and sepsis, predominantly in immunocompromized 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 was first described in 1999 isolated from the rhizoplane of the narrowleaved cattail (*Typha angustifolia*) inhabiting a floating mat on a creek of the Hungarian part of the Danube river (12). Since its first description, only few reports on this actinobacterial species have been published so far. *K. rhizophila* was found in co-culture with other species of this genus
by El-Baradei studying the bacterial biodiversity occurring in the traditional Egyptian soft Domiat cheese (9). Furthermore, the widely used quality-control strain for sterility testing and assaying a variety of antibiotics and fungicide residues ATCC 9341 - originally deposited as *Sarcina lutea* and later re-designated as *Micrococcus luteus* – was recently re-classified as *K. rhizophila* (25). Recently, this microorganism was the predominant bacterium isolated from chicken treated with oxalic acid for reducing populations of naturally occurring microorganisms on raw chicken (2). However, infections in humans or animals were not described so far. 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 is due to a contact with an environmental source, e.g. freshwater, dust or contaminated food.

The susceptibility to bacitracin and lysozyme and the resistance to lysostaphin and nitrofurantoin are major criteria for the conventional preliminarily 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, do not cover the 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 as described here and elsewhere have to be considered if micrococci are involved (7).

In case reported here, a Port-a-Cath device provided a niche for the recurrence of this pathogen for a period of more than two years, temporarily in co-existence with *C. parapsilosis* colonizing this long-lasting implanted foreign body. To what extent, a pre-colonization of foreign bodies by “micrococcal” species might facilitate the colonization by other microorganisms due to generation of a biofilm and, thus, establishing a pre-formed, bacterial growth enhancing microenvironment should be object of further studies. Based on studies on 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 general accepted therapeutic regime for severe infections with micrococcal species has not yet been defined. A combination of rifampin with 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 the suborder *Micrococcineae* that are able to cause infections in humans. If “micrococcal” species are considered as etiologically relevant, failures in databases and outdated nomenclature of identification systems should be considered.

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References


Figure legends

Figure 1. Schematic representation showing the time course of the *Kocuria rhizophila* port-associated infection.
Clinical signs

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<td>Port</td>
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<td>Antibiotic therapy (cefuroxim i.v., vancomycin i.v., vancomycin lock)</td>
<td>Antibiotic therapy (cefuroxim i.v., vancomycin i.v., amphotericin B i.v.) Port explantation</td>
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Recovery of:
- *Kocuria rhizophila*  
- *Candida parapsilosis*  

Recovery from:
- Blood (peripheral vein)  
- Port