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CASE REPORT

The expanding spectrum of human infections caused by *Kocuria* species: a case report and literature review

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Although not previously known to cause human infections, *Kocuria* species have now emerged as human pathogens, mostly in compromised hosts with severe underlying disease. Recently, there has been an increasing incidence of different types of *Kocuria* infections reported, most likely due to the adoption of better identification methods. Here, we report a case of peritonitis caused by *Kocuria rosea* in a diabetic nephropathy patient who was on continuous ambulatory peritoneal dialysis. Sepsis and peritonitis caused by *K. rosea* in our case yielded two identical *Kocuria* isolates from the peritoneal dialysate fluid within a period of three days. The infection was subsequently resolved by antibiotic treatment and catheter removal. In addition to reporting this case, we herein review the literature concerning the emergence of *Kocuria* as a significant human pathogen. The majority of cases were device-related, acquired in the hospital or endogenous, and different *Kocuria* species appear to share a common etiology of peritonitis. The overall disease burden associated with *Kocuria* appears to be high, and the treatment guidelines for diseases associated with *Kocuria* have not yet been clearly defined.

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INTRODUCTION

Organisms of the genus *Kocuria* (family Micrococcaceae, order Actinomycetales, class Actinobacteria) are gram-positive coccoid bacteria often found as tetrads and irregular clusters that are catalase-positive and coagulase-negative. These bacteria are responsible for different types of infection, mostly in immunocompromised hosts with serious underlying conditions.[1,2] However, the prevalence of human infections caused by *Kocuria* species is underestimated, as commonly used phenotypic assays are known to misidentify *Kocuria* isolates as *Staphylococci*.[2] Accordingly, a number of presumed staphylococcal pathologies might have been caused by *Kocuria* species, although it is plausible that a variety of presumed *Kocuria* infections might have actually been due to coagulase-negative *Staphylococci* (CoNS). Here, we report a case of peritonitis caused by *Kocuria rosea* that was initially assumed to be due to CoNS. The present article reviews all the cases of *Kocuria* infections reported in the English literature and discusses important issues pertaining to the diagnosis, etiology, identification, drug resistance, epidemiology, clinical presentation and management of such infections.

CASE PRESENTATION

A 57-year-old man, a previously known case of diabetic nephropathy with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis (CAPD) for the last four years, was admitted to a tertiary-care hospital in Puducherry, with complaints of abdominal pain, pedal edema and loose stool for three days. Upon initial physical examination, he was not febrile and had a normal-appearing catheter exit site. However, the peritoneal dialysate fluid was turbid. Subsequently, he became febrile, and a peripheral blood examination showed a total cell count of white blood cells 8700 cells/cu mm, neutrophils 84%, lymphocytes 12% and eosinophils 4%. The hemoglobin concentration was 9.0 g/dL, and the blood urea and creatinine levels were 161 mg/dL and 8.4 mg/dL, respectively. Random blood sugar was 170 mg/dL, total protein 5.9 mg/dL, serum albumin 1.8 mg/dL and globulin 4.1 mg/dL; the C-reactive protein level was raised to 10 mg/dL.

The peritoneal dialysis fluid (7 mL) inoculated into a BacT-alert FA bottle showed growth within 12 h (BacT-alert 240; bioMérieux, France). Direct microscopy of the sample showed gram-positive cocci in pairs and clusters. We performed all culturing procedures according to the 2005 update of the International Society for Peritoneal Dialysis recommendations and guidelines using specimens collected prior to antibiotic treatment.[3] Subcultures of the peritoneal fluid were performed using sheep blood agar, MacConkey agar and chocolate agar; the plates were incubated at 35 ℃ for 48 h. After incubation, sheep blood agar yielded the pure luxuriant growth of pale-pink non-hemolytic colonies that were 1–2 mm size (Figure 1A). Subculture on nutrient agar yielded smooth, small, pale-cream to pale-pink colonies (Figure 1B). Gram staining of the culture revealed the cells to be gram-positive cocci in pairs or clusters (Figure 2). The organism was preliminarily identified as *Kocuria* based on phenotypic test results, such

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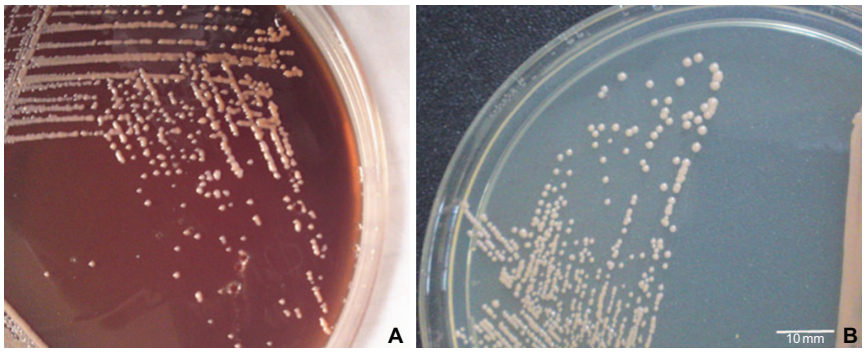

Figure 1 (**A**) Smooth, small, moist, slightly convex, pale-pink colonies of *Kocuria* observed on sheep blood agar. (**B**) Smooth, small, pale-cream to pale-pink colonies grown on nutrient agar.

as positive reactions for catalase, oxidase, nitrate reduction and growth in 5% NaCl and motility test negativity.

Subsequent additional tests, such as bacitracin susceptibility, lysozyme sensitivity at 200 μg, resistance to furazolidone and lysostaphin, helped to discriminate micrococci from *Staphylococci*.[4,5] The culture (designated as PKS1409) was later identified with a 97% probability as *Kocuria rosea* using a Vitek-2 system (bioMérieux) of 64 tests; the ID-GPC card panel tested positive only for α-glucosidase, leucine arylamidase, α-galactosidase, alanine arylamidase and tyrosine arylamidase. To verify the culture results, a second sample from the peritoneal fluid was evaluated, which also grew the same organism. The second isolate obtained from the second peritoneal dialysate fluid taken after 3 days showed a colony morphology, growth characteristics, biochemical test results and antibiogram identical to that of first isolate. However, an examination of the removed CAPD catheter and subsequent blood culture results showed no growth. Additionally, repeat blood samples collected from the patient who was already on antibiotics showed no growth.

We performed 16S rRNA gene sequencing, as previously described and compared the sequence results using the basic local alignment search tool with the EzTaxon database.[6,7] The 16S rRNA gene amplified using a polymerase chain reaction Mastermix (Fermentas, Thermo Scientific, MA, USA) with the conserved primers PKRF (5′-ATC CTG GCT CAG AGC GAA CG-3′) and PKRR (5′-CCC TAC GGC TAC CTT GTT ACG-3′) generated a nearly complete sequence of the 16S rRNA gene (~1365 bp) (Veriti-PCR; Applied Biosystems Inc., CA, USA). The polymerase chain reaction product was purified (Hi-Media, Mumbai, India) and sequenced using ABI technology (Eurofins, Bangalore, India). The basic local alignment search tool nucleotide sequence analysis revealed a high degree of homology (99.78%) with *K. rosea* (GenBank accession number JN084143); and the second closest match was *K. polaris*, with 99.56% homology (accession number AJ278868). This isolate was precisely identified and confirmed as *K. rosea* by a phylogenetic analysis of the 16S rRNA gene sequences by EzTaxon, a web-based tool used for the identification, using 16S rRNA gene sequences.[7] Similar results were obtained when we constructed a neighbor-joining phylogenetic tree with the 16S rRNA gene sequences of all *Kocuria* species using MEGA v5.1 program (Figure 3).

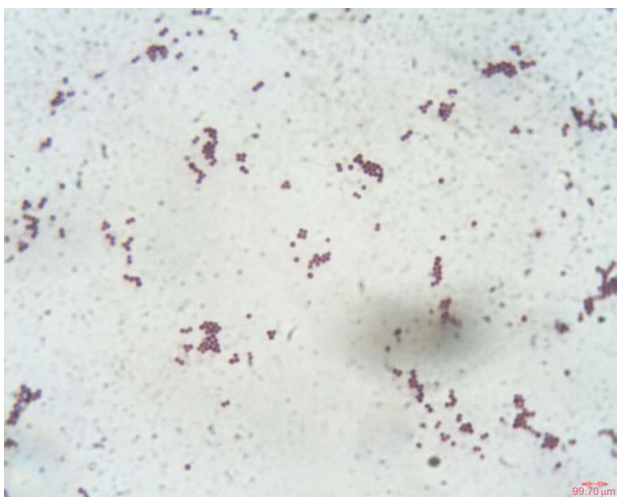

Figure 2 Gram-stain micrograph of *Kocuria rosea* PKS1409 obtained from a blood agar culture plate showing gram-positive cocci in pairs or clusters observed under oil immersion (×100).

Susceptibility testing through a modified Kirby-Bauer disc-diffusion technique and minimum inhibitory concentration (MIC) by microbroth dilution method was performed according to the Clinical and Laboratory Standards Institute guidelines for *Staphylococcus*.[8] The disc-diffusion method revealed that PKS1409 was susceptible to ampicillin, cefotaxime, ciprofloxacin, cloxacillin, gentamicin, erythromycin, amikacin, imipenem, linezolid, teicoplanin and vancomycin, but showed intermediate resistance to ceftazidime. The MIC values for co-trimoxazole (10 mg/L), tetracycline (2 mg/L) and ceftazidime (2 mg/L) were relatively high when compared to other antimicrobials, such as benzylpenicillin (0.06 mg/L), clindamycin (0.5 mg/L) and levofloxacin (0.5 mg/L). The MIC value was less than 0.5 mg/L MIC for all the other antibiotics tested, including ciprofloxacin, erythromycin, gentamicin, linezolid, rifampicin, vancomycin and quinupristin–dalfopristin. The disc-diffusion results correlated well with the MIC values for all the common antibiotics evaluated. Biofilm production was assessed by the microtitre plate assay, and PKS1409 did not produce a biofilm.[9] Biofilm production was further checked by growing the bacterium under a different set of conditions with different media, namely, brain–heart infusion, nutrient and Luria-Bertani broth, for different incubation time intervals of 6, 12 and 24 h. American Type Culture Collection reference strain *Staphylococcus epidermidis* 35984 was used as a positive control. All the experimental conditions reconfirmed that PKS1409 was not able to produce biofilm.

A chest X-ray of the patient was normal, and an abdominal ultrasound showed findings suggestive of chronic renal failure. The intraperitoneal administration of amikacin and cefazolin was started for the empirical treatment of CAPD peritonitis. In addition, intravenous injections of vancomycin (1 g) and amikacin were given for 5 days. Improvement of the patient was visible after the initiation of antibiotic treatment, with fever subsiding and a decrease in the C-reactive

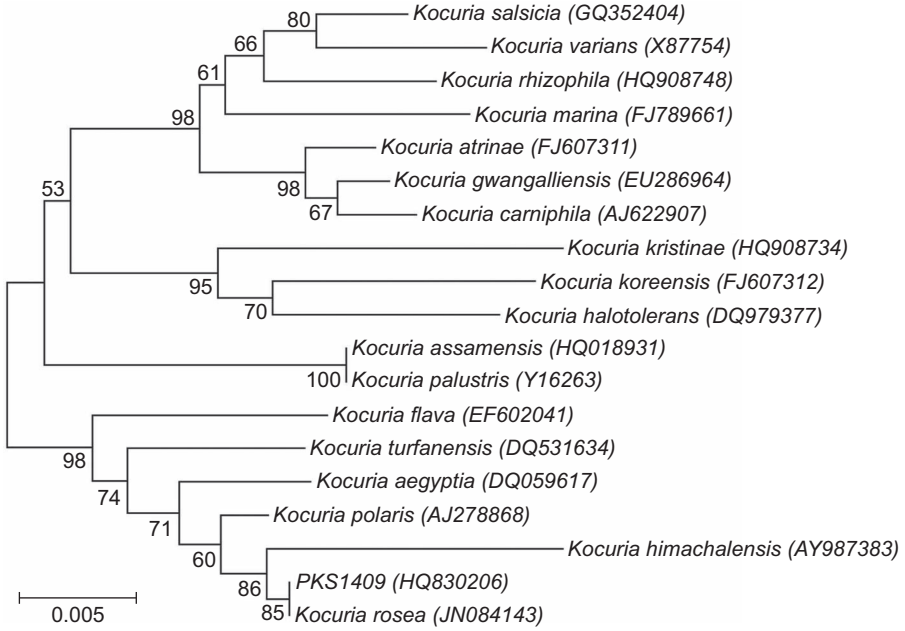

Figure 3 Phylogenetic analysis using MEGA V-5.1 based on the 16S rRNA gene sequences of all the representative *Kocuria* species, along with strain *K. rosea* PKS1409 obtained in this study. Bar=0.005 nucleotide substitutions per position.

protein level. Two methods of susceptibility testing showed that PKS1409 was susceptible to vancomycin. The patient responded well to the vancomycin treatment and did not develop any other complications. The patient was symptomless after 14 days of antibiotic therapy and catheter removal and was discharged.

Nucleotide sequence accession number

The nearly complete sequence of the 16S rRNA gene of the *Kocuria rosea* isolate from this study has been deposited in GenBank under accession number HQ830206.

DISCUSSION AND LITERATURE REVIEW

Kocuria is a member of the Micrococcaceae family and presently comprises 18 species.[1,2,10] This group was previously classified into the *Micrococcus* genus, but was later removed based on the phylogenetic and chemotaxonomic analyses of Stackebrant *et al.*[1,10] *Kocuria* is ubiquitous in nature and is frequently found as normal skin flora in humans and other mammals. Only five of the 18 species in this genus are known to be opportunistic pathogens.[2] Indeed, the documented cases of *Kocuria* infections in humans are very limited; however, many cases might have been missed owing to their misidentification as *Staphylococci* due to the limited biochemical tests and also due to automated identification systems.[2,11–14] A search of the English-language research literature revealed nineteen other reports on *Kocuria* infections (Table 1).

HUMAN INFECTIONS CAUSED BY *Kocuria* SPP.

The number of cases reported has increased over the past five years, most likely due to the improved identification systems for the diagnosis of this infective agent. Recovery in many of the cases of *Kocuria* infection required catheter removal, which resolved the infection. Among the members of this genus, *K. kristinae*, first described in 1974 (previously *Micrococcus kristinae*), is known to cause catheter-related bacteremia and infective endocarditis. These infections are associated with different cancers, acute cholecystitis and other metabolic disorders.[15,17,18,27] The recently introduced Vitek-2 GP-card supplemented with an additional database allows for the identification of *K. kristinae*.[28] Bacteremia caused by *K. kristinae* in a patient suffering from ovarian cancer who had multiple febrile episodes for a prolonged period of >six months has been described[15] (Table 1). The strains yielded from all the blood and central venous catheter (CVC) cultures of this patient were indistinguishable and clonal in nature, strongly suggesting recurrent infection and the possibility of a CVC line as a port of entry.

On one occasion, a three-year screening of blood cultures in the National Taiwan University hospital led to the isolation of 21 *Kocuria* isolates from five patients, with 20 being *K. kristinae* and responsible for either catheter-related bacteremia or infective endocarditis.[18] Subsequent random amplification of polymorphic DNA genotyping of these isolates revealed that there was no nosocomial transmission. Furthermore, multiple isolates from individual patients with persistent blood-stream infection (BSI) revealed identical random amplification of polymorphic DNA profiles, implying that there was either incomplete treatment of an occult focus or persistent carriage of the same bacterium. *K. kristinae* as the cause of acute peritonitis in a patient with end-stage renal failure undergoing CAPD for two years has also been reported, and bacterial entry into the peritoneal cavity through touch contamination of the catheter was suspected.[20,21] The continued monitoring of bacterial growth for up to 72 h without any clinical indication after the onset of acute peritonitis was proven to be beneficial, as this led to an interruption and change in the antibiotic treatment, leading to improvement in the patient's condition.

Surprisingly, *K. kristinae* was also reported to cause severe intravascular infections in an immunocompetent host, an infection that was complicated by septic pulmonary emboli that were secondary to suppurative thrombosis. A problematic catheter-related BSI leading to such complications was striking in this case.[22] A recent report of a female infant with a history of prolonged diarrhea showed the blood culture growth of *K. kristinae*, and this organism was considered responsible for bacteremia with black hairy tongue symptoms.[25] Unlike the initial empirical ceftriaxone therapy, the infant showed a

Table 1 Details of all the significant cases of *Kocuria* infections reported to date

Reference(s)	Age (years)/ sex	Site of infection	Type of infection and associated comorbidities	Susceptibility pattern[a]			Treatment (antibiotics prescribed)[c]	Change in antibiotics after inappropriate therapy	Species	Removal of catheter/ clinical prognosis
				S	R	I[b]				
Basaglia *et al.*[15]	51/F	Bloodstream	Catheter-related bacteremia, ovarian cancer	CD, E, CF, P, OX, CN, VA	—	—	Meropenem, ciprofloxacin and clindamycin	Yes	*K. kristinae*	Yes/recovered
Ben-Ami *et al.*[11]	47/F	Kidney	Ventriculo-peritoneal (VP) shunt infection	OX, VA, R, CD, TR, C	—	—	Vancomycin	No	*K. varians*	NM[d]/edema resolved
Altuntas *et al.*[16]	39/M	Bloodstream	Catheter-related bacteremia	AS, E, VA	—	—	Imipenem/cilastatin, amikacin, vancomycin	Yes	*K. rosea*	No/recovered
Ma *et al.*[17]	56/M	Gall bladder	Acute cholecystitis	P, CLX, E, CD, LZ, LE, TS, VA	—	—	Levofloxacin	No	*K. kristinae*	NM/recovered
Becker *et al.*[13]	8/M	Bloodstream	Sepsis		NX	—	Cefuroxime, vancomycin and amphotericin B	Yes	*K. rhizophila*	NM/multiple febrile episodes; subsequent recovery
Lee *et al.*[14]	57/M	Peritoneum	CAPD-related peritonitis	P, A, G, CN, MO, TR, E, CD, VA, C, T, R	—	—	Ceftazidime and clindamycin	Yes	*K. marina*	Yes/recovered
Lee *et al.*[14]	73/M	Peritoneum	CAPD-related peritonitis	P, A, G, CN, MO, E, CD, VA, C, T, R	TR	—	Netilmicin	No	*K. marina*	NM/recovered
Lai *et al.*[18]	89/F	Bloodstream	Infective endocarditis/ ischemic bowel disease	P, A, E, CD, VA, AC, TE, CF, LE, F, QD, LZ, R	OX, CZ	CE	Vancomycin, teicoplanin, oxacillin	No	*K. kristinae*	Yes/improved
	37/F	Bloodstream	Catheter-related bacteremia, gastric cancer	P, A, E, CD, VA, AC, TE, CF, LE, F, QD, LZ, R	OX, CZ	CE	Piperacillin-tazobactam, ciprofloxacin	No	*K. kristinae*	Yes/improved
	2/M	Bloodstream	Catheter-related bacteremia, congenital short bowel syndrome, hypogammaglobulemia	P, A, E, CD, VA, AC, TE, CF, LE, F, QD, LZ, R	OX, CZ	CE	Oxacillin, vancomycin	Yes	*K. kristinae*	Yes/improved
	68/F	Bloodstream	Catheter-related bacteremia, gastric cancer	P, A, E, CD, VA, AC, TE, CF, LE, F, QD, LZ, R	OX, CZ	CE	Oxacillin	Yes	*K. kristinae*	Yes/improved
Tsai *et al.*[19]	52/M	Brain	Brain abscess	P, CLX, CF, CD, MR, AS, PT	—	—	Ceftazidime and ceftibuten	No	*K. varians*	NM/recovered
Carlini *et al.*[20]	78/M	Peritoneum	CAPD-related peritonitis	—	—	—	Cefotaxime, tobramycin, tazobactam, ciprofloxacin, teicoplanin, amoxicillin/ clavulanic acid	Yes	*K. kristinae*	No/recovered
Cheung *et al.*[21]	69/M	Peritoneum	CAPD-related peritonitis	CLX, G, E, CD, F	—	—	Cefazolin and cefepime	No	*K. kristinae*	No/recovered
Dunn *et al.*[22]	29/F		Catheter-related bacteremia with pulmonary septic emboli	OX, VA, CZ, CD, R, TS	—	—	Ceftriaxone, azithromycin, vancomycin, clindamycin, oxacillin	Yes	*K. kristinae*	Yes/improved
Moissenet *et al.*[23]	3/F	Bloodstream	Catheter-related bacteremia, hirschsprung's disease	P, G, AK, TOB, T, VA, TE	CF	E	Vancomycin, gentamicin	No	*K. rhizophila*	NM/septic episodes resolved
Meletis *et al.*[24]	70/M	Peritoneum	CAPD-related peritonitis	G, E, CD, T, LZ, glycopeptides	LE	—	Vancomycin, aztreonam	No	*K. varians*	Yes/improved

Table 1 Continue

Reference(s)	Age (years)/ sex	Site of infection	Type of infection and associated comorbidities	Susceptibility pattern[a]			Treatment (antibiotics prescribed)[c]	Change in antibiotics after inappropriate therapy	Species	Removal of catheter/ clinical prognosis
				S	R	I[b]				
Oncel *et al.*[25]	4 months/F	Bloodstream	Black hairy tongue	-	—	—	Ceftriaxone, vancomycin	No	*K. kristinae*	NM/recovered
Citro *et al.*[26]	74/M	Bloodstream	Endocarditis, sepsis				Sulbactam/ampicillin, gentamicin	No	*K. kristinae*	NM/severe sepsis, MOF[e], expired
Our study	57/M	Peritoneum	Peritonitis, diabetic nephropathy	AC, CE, CF, CLX, G, E, MR, AK, LZ, TE, VA		CAZ	Vancomycin, cefazolin, amikacin	No	*K. rosea*	Yes/recovered

[a] According to Clinical and Laboratory Standards Institute guidelines for *Staphylococcus*.
[b] A, ampicillin; AC, amoxycillin/clavulanic acid; AK, amikacin; AS, ampicillin/sulbactam; C, chloramphenicol; CE, cephotaxime; CD, clindamycin; CLX, cloxacillin; CF, ciprofloxacin; CMZ, cefmetazole; CN, cefalothin; CZ, cefozolin; E, erythromycin; F, fusidic acid; G, gentamicin; LE, levofloxacin; LZ, lenezolid; MR, meropenem; MO, moxifloxacin; NX, norfloxacin; OX, oxacillin; P, penicillin; PT, piperacillin/tazobactam; QD, quinupristin/dalfopristin; R, rifampin; S, sulbactum; TE, teicoplanin; T, tetracycline; TOB, tobramycin; TR, trimethoprim; TS, trimethoprim/sulphamethoxazole; VA, vancomycin.
[c] 'and' in between two antibiotics indicates simultaneous administration.
[d] NM, not mentioned in the report.
[e] MOF, multiple organ failure.

dramatic response to vancomycin treatment, which completely resolved the infection and symptoms within a week. A case of *K. kristinae* infection was recently documented in an elderly patient affected by endocarditis who was suffering from diabetes mellitus and hypertension who additionally had minor amputation for a left forefoot ulcer.[26] This was a rare and unusual case, whereby a central venous catheter was not involved, but the disease was associated with a diabetic foot infection. Catheter removal prior to the clearance of infection was noted in many investigations, implying that the removal of the device should be considered and recommended for the treatment of *K. kristinae* infection. It is also evident that any repeat isolation of *K. kristinae* from blood cultures can no longer be ignored by either the microbiologist or clinician.

A brain abscess caused by *Kocuria varians* has been described, affecting the right occipital region, and was successfully treated with surgical excision and antimicrobial therapy.[19] The potential source of the organism was suspected to be the hematogenous spread to brain parenchyma. The *K. varians* identification was based on Vitek-2, which showed a relatively reduced probability level (93%); however, the main drawback in this case was the lack of a 16S rRNA gene sequence analysis for species confirmation. CVC placement was suspected to be a possible risk factor for *K. varians* peritonitis relapse in patients undergoing dialysis, and the treatment of such infections might be a complicated task, as one study demonstrated that *K. varians* isolated from the environment was capable of producing biofilms.[24,29] Interestingly, one investigation used Vitek-2 for the detection of a ventriculoatrial shunt infection by phenotypically variable *Staphylococcus epidermidis* that was masquerading as polymicrobial bacteremia caused by CoNS and *K. varians*.[11] Therefore, the erroneous identification of CoNS as *Kocuria* or vice-versa is possible and should be excluded with certainty only through a genome-based analysis. Clearly, it is now more important than earlier to confirm whether *K. varians* indeed can cause different types of infection or not.

K. marina and *K. rhizophila* have also joined the emerging spectrum of *Kocuria* species causing human infections.[13,14] Cases of *K. marina* peritonitis in patients undergoing CAPD revealed that dialysis fluid from these patients becoming turbid and straw colored and an increase in white blood cell and platelet counts was noticed;[14] empirical therapy failed in these cases, and only catheter removal resolved the infection.[14] The first case of *K. rhizophila* infection was reported in a boy with methylmalonic aciduria where the boy suffered multiple episodes of sepsis for more than two years, and the Port-A-Cath was shown to be the focus of infection. Indeed, the Port-A-Cath device might have provided a favorable niche for prolonged survival of the organism and subsequent recurrence.[13] *K. rhizophila* has been widely used as a quality-control strain (American Type Culture Collection-9341) for sterility testing,[30] and a recent case of *K. rhizophila* infection presented as persistent BSI associated with a damaged CVC in a girl with Hirschsprung's disease; advanced molecular methods were used for the identification in this case.[23]

K. rosea is yet another species capable of causing human infections, and the first report of *K. rosea* infection describes multiple episodes of febrile neutropenia in a patient with relapsed Hodgkin disease undergoing peripheral blood stem cell transplantation.[31] Although the isolate was susceptible to vancomycin, its administration did not alter the clinical picture of the infection until the catheter was removed. It was previously reported that *K. rosea* can cause catheter-related bacteremia and peritonitis; however, no genotypic identification methods were employed to confirm this, thus the etiology is uncertain.[16,31,32] Only an abstract was available as the reference in two of three cases because

the original publication was not in English; thus, precise information could not be obtained. Slightly more information was available for the third case: the bacteremic patient was human immunodeficiency virus positive and was successfully treated with vancomycin and catheter removal.[32] Such scanty information forced us to rely on a single existing report.[16] However, the present study conclusively confirms *K. rosea* as an opportunistic pathogen that is able to cause peritonitis and BSI.

Catheter removal was one of the treatment options followed, and the possibility of a CVC line as the portal of entry was suspected. The possibility of biofilm formation on the catheter was attributed to treatment failure in one of the cases, although it was not investigated.[16] Conversely, the *K. rosea* isolated in our case did not produce any biofilm, and hence the hypothesis of biofilm formation appears to be premature. Biofilm assays involving a large number of isolates may provide definite answers with regard to the biofilm-forming capability of *K. rosea*. Furthermore, *K. rosea* strain PKS1409 investigated in our study grew well and faster with enhanced pigment production when maintained at 4 ℃. Interestingly, contrary to the earlier observations, our isolate is very close to *K. polaris*, a known psychrophile isolated from Antarctica, as revealed by 16S rRNA gene analysis.[2,33] To sum up, human infections caused by only a few species of *Kocuria* affirm the presence of certain species-specific virulence characteristics that enable these bacteria to cause infections. Moreover, several new cases have expanded the clinical spectrum of disease caused by this unusual pathogen.

IDENTIFICATION OF *Kocuria* SPECIES

Phenotypic identification may fail to recognize all the members of *Kocuria* species because the results of biochemical and carbon assimilation tests are shown to be heterogeneous in different species of *Kocuria*.[2] In reality, these organisms are often not correctly identified and are many times hastily discarded as contaminants. Furthermore, accurate identification is difficult because commercially available databases do not include all the classified *Kocuria* species. Even cellular fatty acid profile analyses were not much of use in identification.[1,34] However, *Kocuria* classification is now typically confirmed by 16S rRNA gene sequencing, and one study even used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for species identification, which appears to be an efficient method.[2,26]

A series of 12 *Kocuria* isolates obtained from a patient (subsequently identified as *K. rhizophila* by 16S rRNA gene sequencing) in a study were ambiguously identified as either belonging to any one of the four different bacterial species, namely, *K. varians*, *K. rosea*, *Dermacoccus nishinomiyaensis* and *Micrococcus luteus*, by the Vitek 2 ID-GPC-card phenotypic system.[14] The ID-32 Staph system also misidentified approximately four isolates in this collection as either *Staphylococcus auricularis* or *S. capitis*. Another study employing the Vitek-2 system initially identified two *K. marina* isolates as *K. varians* and *Staphylococcus hominis*; upon re-examination, they were once again misidentified as *K. kristinae* and *Staphylococcus chromogenes*,[14] whereas subsequent 16S rRNA gene sequencing confirmed these two isolates as *K. marina*. When subjected to the Vitek ID-GPC-card panel test, *Kocuria* isolates are positive for only the alanine arylamidase reaction and alkalinization of *L*-lactate;[13] strain PKS1409 from our case was positive for only five tests in the Vitek array. Such instances of only a few positive reactions observed in an array of 64 biochemical tests (Vitek-2-Compact; bioMérieux) with an automated system questions the validity of this identification process.

Previously, Ben-Ami *et al*.[11] reported on the misidentification of CoNS as *Kocuria* species by the Vitek-2 assay and warned that a clinical specimen yielding *Kocuria* could easily raise suspicion of CoNS infection. However, recent studies, including ours, correctly identified *Kocuria* using the Vitek-2 ID-GPC gram-positive identification card, perhaps due to the recently introduced larger database that allows the identification of additional taxa.[12] However, it is not clear whether the Vitek-2 GP-system will reduce the number of CoNS falsely identified as *Kocuria* spp., although there are claims of improvement in specificity after the addendum of new database.[12] However, misidentification among members of the *Kocuria* genus cannot be ruled out altogether, as recent studies have reported such occurrences.[13,14,17] Nonetheless, in a recent screening of 21 *Kocuria* isolates, only one was misidentified, suggesting that the newer Vitek-2 has improved efficiency.[18]

Furthermore, both *Micrococci* and *Staphylococci* are known to show phenotypic variability (PV) that complicates their identification.[11] PV among isolates might be misidentified by phenotypic assays, as biochemical activities may change during growth to allow the microbes to adapt to changing environmental conditions.[5,11,14,35] Moreover, PV becomes more common under stress conditions and likely contributes to virulence.[36,37] It has been shown that the pathogenic clinical isolates cultured from blood are more phenotypically variable than the saprophytic strains cultured from skin or mucosal surfaces.[38] Because PV is common in *S. epidermidis* isolates, misidentification of these isolates as *Kocuria* by commercial systems may be frequent and widespread. More troubling is the fact that such errors are likely to occur more frequently with the highly virulent strains of *Staphylococcus* that are known to show high PV.[11] Thus, the biochemical analysis-based misidentification of CoNS as *Kocuria* and vice-versa due to PV appears to be a major problem faced by diagnostic laboratories. A summary of the reports on the misidentification of *Kocuria* by phenotypic systems is given in Table 2.

Despite its limitations, simple tests, such as susceptibility to bacitracin and lysozyme and resistance to nitrofurantoin/furazolidone and lysostaphin, appear to be very useful in differentiating *Kocuria* from *Staphylococci*. In addition, the typical pigmentation of *Kocuria* colonies generally becomes more distinct with age.[2] Hence, the prolonged incubation of a culture for >48 h is recommended to appreciate the pigmentation better, which can also discriminate the organism from *Staphylococci*. Importantly, in most of the previous reports of misidentification, there was no mention of incubation time, and it can be assumed that the plates were kept only up to 24 h, thereby missing the characteristic pigmentation, which may appear some time later. We suggest that clinical laboratories worldwide prolong the incubation time to >48 h to avoid any chance of misidentification. In particular, our observations revealed massive growth and pigment enhancement of *K. rosea* at refrigeration temperatures. Finally, the identification of *Kocuria* spp. by any number of phenotypic tests should be always placed under suspicion and should be confirmed explicitly by performing molecular assays.

PATHOPHYSIOLOGY, VIRULENCE FACTORS AND EPIDEMIOLOGY

Multiple episodes of sepsis, febrile neutropenia, and increased platelets, white blood cell counts and C-reactive protein levels are the common manifestations of *Kocuria* infection.[2,22] Although very little is known about the epidemiology and virulence properties of *Kocuria*, the involvement of biofilm in adhesion, colonization and subsequent infection has been suggested.[24] Surprisingly, *K. rosea* PKS1409 characterized by us did not produce any biofilm. Furthermore, there are no reports of biofilm production by *Kocuria* in the literature, yet it has

Table 2 Misidentification of *Kocuria* species and members of the *Kocuria* genus by different automated phenotypic identification systems

Study	Vitek2	Bactec/BactAlert	Phoenix	API-STAPH/ATB	16S rRNA gene sequencing (a genome-based assay)
Ma *et al.*[17]	*K. kristinae*	—	*K. varians*	*K. kristinae.*	*K. marina*
Becker *et al.*[13]	*K. varians/K. rosea/Dermacoccus nishinomiyaensis/Micrococcus luteus/K. rhizophila*	*Micrococcus* sp.	—	*K. varians/K. rosea/Dermacoccus nishinomiyaensis/Micrococcus luteus/ K. rhizophila*	*K. rhizophila*
Lee *et al.*[14]	*Kocuria varians/Kocuria kristinae*	—	—	*K. kristinae*	*K. marina*
	Staphylococcus hominis/K. kristinae	—	—	*Staphylococcus Chromogenes*	*K. marina*
Tsai *et al.*[19]	*K. varians*	—	—	—	—
Cheung *et al.*[21]	*K. kristinae*			*Staphylococcus lugdunensis/ Staphylococcus hominis*	—
Moissenet *et al.*[23]	*K. rhizophila*	—	—	*Staphylococcus auricularis*	*K. rhizophila*

been implicated in device-related bacteremia, implying that the colonization of *Kocuria* on a device will likely result in chronic recurrent bacteremia in a compromised host.[15,16,18] Hence, clinicians should not overlook and underestimate the isolation of *Kocuria* from implanted devices.

K. kristinae is shown to be a component of the skin and oral cavity flora and has been implicated as a common source of contamination in clinical specimens.[5,17,39] Interestingly, *K. rhizophila* is a predominant bacterium isolated from chicken meat treated with oxalic acid.[13,40] The most likely suspected source of *K. rhizophila* is patient contact with contaminated meat and dust from the environment, which may result in colonization. The possibility of a CVC line as a portal of entry should also be evaluated. A recent study emphasized the adherence of bacteria to the silastic tube as a possibility for the failure of antibiotic treatment.[16] Biofilm production in any pathogen makes them more resistant and difficult to eradicate; nonetheless, it remains unclear whether *Kocuria* species produce biofilm. The lack of genotypic studies has greatly impacted epidemiological investigations and the understanding of the basic differences between the saprophytic and pathogenic traits of *Kocuria* strains. Indeed, an understanding of these aspects may avoid the hasty classification of *Kocuria* as contaminating micrococci in a clinical setup.

ANTIBIOTIC RESISTANCE AND TREATMENT

The lack of proper guidelines and breakpoints for *Kocuria* has forced many studies, including ours, to follow the susceptibility breakpoints used for *Staphylococcus*. As a result, the numerous reports referring to the interpretive values of *Staphylococcus* may cause the misdiagnosis of either sensitivity or resistance. We therefore emphasize the urgent need for the formulation of specific criteria for interpreting susceptibility in *Kocuria* species. A recent review analyzed the antibiotic resistance patterns of various *Kocuria* species and their clinical impact.[2] Most *Kocuria* isolates were reported to be susceptible to many of the first- and second-line drugs, with the exception of ampicillin and norfloxacin.[2,13,41]

Decreased cell wall permeability and the presence of efflux pumps are implicated in the resistance of *Kocuria* species.[2] The recently sequenced genome of *K. rhizophila* has revealed the presence of many proteins that are predicted to be involved in multidrug efflux mechanisms, although their role in imparting resistance is not clear.[42] Pathogenic *Kocuria* species are highly susceptible to broad-spectrum antibiotics. Szczerba proposed amoxicillin/clavulanate along with such drugs as ceftriaxone, cefuroxime, doxycycline and amikacin as a first-line therapy against micrococcal pathologies.[41] In addition, it has been suggested that the treatment duration should depend on the type of infection.[2,13–15] The infusion of antibiotics in a catheter lock or catheter removal appears to be the best option for the treatment of implant-related sepsis.[2,17,18,41] As there are no evidence-based guidelines for the management of *Kocuria* infections, previously reported successfully treated cases can only become the guiding principle for treating *Kocuria* infections.

Unfortunately, the skill required and high cost involved in molecular assays may limit their use in routine practice, and consequently, *Kocuria* species misidentification in many laboratories is likely to continue for some time. However, it is probable that the prevalence of *Kocuria* infections in coming years may increase as genome-based identification becomes routinely available. The first indication of such a scenario becoming true has been observed through the numerous new cases of *Kocuria* infections being reported in a short span of time.[20,22,23,32] Owing to misidentification over the years, the diseases caused by *Kocuria* species are erroneously believed to be rare. Hence, it is suggested that physicians should not underestimate the importance of *Kocuria* spp. when isolated from clinical samples, particularly from blood and inert medical implants. Furthermore, cause for concern remains, as therapy guidelines for illnesses involving *Kocuria* are lacking, mainly due to the absence of established criteria for evaluating the killing and/or growth inhibition of *Kocuria* in the presence of antibiotics. Although a comprehensive view of *Kocuria* infections will have to await the documentation of many more cases, several recent reports of *Kocuria* species as the cause of different diseases clearly reveal the expanding clinical spectrum of infections caused by *Kocuria*.

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1 Stackebrandt E, Koch C, Gvozdiak O, Schumann P. Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* 1995; **45**: 682–692.
2 Savini V, Catavitello C, Masciarelli G *et al.* Drug sensitivity and clinical impact of members of the genus *Kocuria*. *J Med Microbiol* 2010; **59**: 1395–402.
3 Piraino B, Bailie G, Bernardini J *et al.* for the ISPD Ad Hoc Advisory Committee. Peritoneal dialysis-related infections recommendations2005 update. *Perit Dial Int* 2005; **25**: 107–131.
4 Baker JS. Comparison of various methods for differentiation of *Staphylococci* and *Micrococci*. *J Clin Microbiol* 1984; **19**: 875–879.

5 Ben-Ami R, Navon-Venezia S, Schwartz D, Schlezinger Y, Mekuzas Y, Carmeli Y. Erroneous reporting of coagulase-negative *Staphylococci* as *Kocuria* spp. by the Vitek 2 system. *J Clin Microbiol* 2005; **43**: 1448–1450.
6 Wauters G, Charlier J, Janssens M, Delme'e M. *Brevibacterium paucivorans* sp. nov., from human clinical specimens. *Int J Syst Evol Microbiol* 2001; **51**: 1703–1707.
7 Chun J, Lee JH, Jung Y *et al*. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 2007; **57**: 2259–2261.
8 Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; 19th informational supplement*. CLSI document M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute, 2009.
9 Duggirala A, Kenchappa P, Sharma S, Peeters JK, Das T, Hasnain SE. High-resolution genome profiling differentiated *Staphylococcus epidermidis* strains isolated from patients with ocular infections and normal individuals. *Invest Ophthalmol Vis Sci* 2007; **48**: 3239–3245.
10 Stackebrandt E, Frederiksen W, Garrity GM *et al*. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 2002; **52**: 1043–1047.
11 Ben-Ami R, Navon-Venezia S, Schwartz D, Carmeli Y. Infection of a ventriculoatrial shunt with phenotypically variable *Staphylococcus epidermidis* masquerading as polymicrobial bacteremia due to various coagulase-negative *Staphylococci* and *Kocuria varians*. *J Clin Microbiol* 2003; **41**: 2444–2447.
12 Boudewijns M, Vandeven J, Verhaegen J, Ben-Ami R, Carmeli Y. Vitek 2 automated identification system and *Kocuria kristinae*. *J Clin Microbiol* 2005; **43**: 5832.
13 Becker K, Rutsch F, Uekötter A *et al*. *Kocuria rhizophila* adds to the emerging spectrum of micrococcal species involved in human infections. *J Clin Microbiol* 2008; **46**: 3537–3539.
14 Lee JY, Kim SH, Jeong HS *et al*. Two cases of peritonitis caused by *Kocuria marina* in patients undergoing continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 2009; **47**: 3376–3378.
15 Basaglia G, Carretto E, Barbarini D *et al*. Catheter-related bacteremia due to *Kocuria kristinae* in a patient with ovarian cancer. *J Clin Microbiol* 2002; **40**: 311–313.
16 Altuntas F, Yildiz O, Eser B, Gündogan K, Sumerkan B, Cetin M. Catheter-related bacteremia due to *Kocuria rosea* in a patient undergoing peripheral blood stem cell transplantation. *BMC Infect Dis* 2004; **4**: 62.
17 Ma ESK, Wong CL, Lai KT, Chan EC, Yam WC, Chan AC. *Kocuria kristinae* infection associated with acute cholecystitis. *BMC Infect Dis* 2005; **5**: 60.
18 Lai CC, Wang JY, Lin SH *et al*. Catheter-related bacteraemia and infective endocarditis caused by *Kocuria* species. *Clin Microbiol Infect* 2011; **17**: 190–192.
19 Tsai CY, Su SH, Cheng YH, Chou YL, Tsai TH, Lieu AS. *Kocuria varians* infection associated with brain abscess: a case report. *BMC Infect Dis* 2010; **10**: 102.
20 Carlini A, Mattei R, Lucarotti I, Bartelloni A, Rosati A. *Kocuria kristinae*: an unusual cause of acute peritoneal dialysis-related infection. *Perit Dial Int* 2011; **31**: 105–107.
21 Cheung CY, Cheng NH, Chau KF, Li CS. An unusual organism for CAPD-related peritonitis: *Kocuria kristinae*. *Perit Dial Int* 2011; **31**: 107–108.
22 Dunn R, Bares S, David MZ. Central venous catheter-related bacteremia caused by *Kocuria kristinae*: case report and review of the literature. *Ann Clin Microbiol Antimicrob* 2011; **10**: 31.
23 Moissenet D, Becker K, Mérens A, Ferroni A, Dubern B, Vu-Thien H. Persistent bloodstream infection with *Kocuria rhizophila* related to a damaged central catheter. *J Clin Microbiol* 2012; **50**: 1495–1498.
24 Meletis G, Gogou V, Palamouti M *et al*. Catheter-related relapsing peritonitis due to *Kocuria varians* in a patient undergoing continuous ambulatory peritoneal dialysis. *Nefrologia* 2012; **32**: 541–542.
25 Karadag Oncel E, Boyraz MS, Kara A. Black tongue associated with *Kocuria* (Micrococcus) *kristinae* bacteremia in a 4-month-old infant. *Eur J Pediatr* 2012; **171**: 593.
26 Citro R, Prota C, Greco L *et al*. *Kocuria kristinae* endocarditis related to diabetic foot infection. *J Med Microbiol* 2013; **62**: 932–934.
27 Martinaud C, Gisserot O, Gaillard T, Brisou P, de Jaureguiberry JP. Bacteremia caused by *Kocuria kristinae* in a patient with acute leukaemia. *Med Mal Infect* 2008; **38**: 334–335.
28 Funke G, Funke-Kissling P. Performance of the new Vitek 2 GP card for identification of medically relevant gram-positive cocci in a routine clinical laboratory. *J Clin Microbiol* 2005; **43**: 84–88.
29 Midelet G, Kobilinsky A, Carpentier B. Construction and analysis of fractional multifactorial designs to study attachment strength and transfer of *Listeria monocytogenes* from pure or mixed biofilms after contact with a solid model food. *Appl Environ Microbiol* 2006; **72**: 2313–2321.
30 Tang JS, Gillevet PM. Reclassification of ATCC 9341 from *Micrococcus luteus* to *Kocuria rhizophila*. *Int J Syst Evol Microbiol* 2003; **53**: 995–997.
31 Kaya KE, Kurtoğlu Y, Cesur S *et al*. [Peritonitis due to *Kocuria rosea* in a continuous ambulatory peritoneal dialysis case.] *Mikrobiyol Bul* 2009; **43**: 335- 337. Turkish.
32 Corti M, Villafañe MF, Soto I, Palmieri O, Callejo R. [Bacteremia by *Kocuria rosea* in an AIDS patient.] *Rev Chilena Infectol* 2012; **29**: 355–356. Spanish.
33 Reddy GS, Prakash JS, Prabahar V, Matsumoto GI, Stackebrandt E, Shivaji S. *Kocuria polaris* sp.nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *Int J Syst Evol Microbiol* 2003; **53**: 183–187.
34 Stead DE, Sellwood JE, Wilson J, Viney I. Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* 1992; **72**: 315–321.
35 Savini V, Catavitello C, Bianco A, Balbinot A, D'Antonio D. Epidemiology, pathogenicity and emerging resistances in *Staphylococcus pasteuri*: from mammals and lampreys, to man. *Recent Pat Anti infect Drug Discov* 2009; **4**: 123–129.
36 Christensen GD, Baddour LM, Madison BM *et al*. Colonial morphology of *Staphylococci* on Memphis agar: phase variation of slime production, resistance to β-lactam antibiotics, and virulence. *J Infect Dis* 1990; **161**: 1153–1169.
37 Deighton M, Pearson S, Capstick J, Spelman D, Borland R. Phenotypic variation of *Staphylococcus epidermidis* isolated from a patient with native valve endocarditis. *J Clin Microbiol* 1992; **30**: 2385–2390.
38 Ziebuhr W, Heilmann C, Gotz F *et al*. Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infect Immun* 1997; **65**: 890–896.
39 Kloos WE, Tornabene TG, Schleifer KH. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *Int J Syst Bacteriol* 1974; **24**: 79–101.
40 Anang DM, Rusul G, Radu S, Bakar J, Beuchat LR. Inhibitory effect of oxalic acid on bacterial spoilage of raw chilled chicken. *J Food Prot* 2006; **69**: 1913–1919.
41 Szczerba I. [Susceptibility to antibiotics of bacteria from genera *Micrococcus*, *Kocuria*, *Nesternkonia*, *Kytococcus* and *Dermacoccus*.] *Med Dosw Mikrobiol* 2003; **55**: 75–80. Polish.
42 Takarada H, Semine M, Kosugi H *et al*. Complete genome sequence of the soil actinomycete *Kocuria rhizophila*. *J Bacteriol* 2008; **190**: 4139–4146.