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Two Cases of Peritonitis Caused by *Kocuria marina* in Patients Undergoing Continuous Ambulatory Peritoneal Dialysis▽

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***Kocuria* spp. are members of the *Micrococcaceae* family that are frequently found in the environment and on human skin. Few human infections have been reported. We describe what appear to be the first two cases of *Kocuria marina* peritonitis in patients undergoing continuous ambulatory peritoneal dialysis.**

CASE REPORTS

Case 1. A 57-year-old man was admitted to the emergency department because of turbid dialysis effluent for 1 day. He had end-stage renal disease as a result of diabetic nephropathy and had been undergoing continuous ambulatory peritoneal dialysis (CAPD) for 6 years. Upon physical examination, he was afebrile, with a normal-appearing catheter exit site. However, the peritoneal dialysate fluid was straw colored and cloudy, with a total leukocyte count of 0.78×10^9 leukocytes/liter and a neutrophil count of 90%. No microorganisms were seen on a Gram stain. In the peripheral blood, the hemoglobin concentration was 10.1 g/dl, the white blood cell (WBC) count was 7.40×10^9 cells/liter, and the platelet count was 171×10^9 platelets/liter. The C-reactive protein concentration was 2.76 mg/dl (reference concentration, <0.5 mg/dl), and the serum urea and creatinine concentrations were 53 mg/dl and 11.2 mg/dl, respectively. Intraperitoneal administration of netilmicin and narrow-spectrum cephalosporin (ceftezole) was started for empirical treatment of CAPD peritonitis, which was changed to intraperitoneal ceftazidime and clindamycin when there was no response.

Culture of the dialysate yielded a pure culture of gram-positive cocci in pairs or clusters (strain M07-0128). After 48 h of incubation at 35°C in 5% CO_2 on sheep blood agar, the 1- to 2-mm colonies were nonhemolytic and yellow. The isolate was identified as *Kocuria varians*/*Kocuria kristinae* with a 50.28%/49.72% probability, respectively, by a Vitek 2 system (bioMérieux, St. Louis, MO) and as *K. kristinae* (code number 6714014) with a 99.3% probability by an API Staph system (bioMérieux, Marcy l'Etoile, France). We performed 16S rRNA gene sequencing as previously described (5) and compared the obtained sequence with sequences similar to those of the type strains using BLAST and EzTaxon (4). The result showed 99.86% homology with *Kocuria marina*; the second closest match was *Kocuria carniphila*, with 98.30% homology. This isolate was finally identified as *K. marina* by 16S rRNA gene sequence analysis. In spite of the start of administration of intravenous vancomycin on day 10, the response remained unsatisfactory. The Tenckhoff catheter in his abdomen was removed on day 17, and he was switched to hemodialysis with the placement of an arteriovenous shunt. The patient improved with antibiotic therapy for 7 days after catheter removal and was discharged.

We performed antimicrobial susceptibility testing on the isolate using the agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for *Staphylococcus* (4a). The isolate was susceptible to penicillin, ampicillin, ampicillin-sulbactam, gentamicin, cephalothin (cefalotin), ciprofloxacin, moxifloxacin, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, vancomycin, chloramphenicol, tetracycline, and rifampin (rifampicin) (Table 1).

Case 2. A 73-year-old man visited the section for peritoneal dialysis of our hospital because of turbid dialysis effluent for 3 days. He had end-stage renal disease as a result of diabetic nephropathy and had been undergoing CAPD for 4 years. The peritoneal fluid was cloudy, with a total leukocyte count of 2.58×10^9 leukocytes/liter and a neutrophil count of 90%. No microorganisms were seen on a Gram stain. In the peripheral blood, the hemoglobin concentration was 11.7 g/dl, the WBC count was 6.49×10^9 cells/liter, and the platelet count was 314×10^9 platelets/liter. The C-reactive protein concentration was 4.03 mg/dl, and the serum urea and creatinine concentrations were 34 mg/dl and 7.5 mg/dl, respectively. Intraperitoneal administration of netilmicin and narrow-spectrum cephalosporin (ceftezole) was started.

Culture of the dialysate yielded pure growth of gram-positive cocci in pairs or tetrads (strain M07-1336). After a 48-hour incubation at 35°C in 5% CO_2 on sheep blood agar, the organism grew as nonhemolytic orange colonies that were 1 to 2

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TABLE 1. Antimicrobial susceptibility profiles of two *K. marina* strains

Strain	Susceptibility (MIC[s] [μg/ml])[a]													
	PC	AM	SAM	GM	CF	CIP	MXF	SXT	E	CC	VA	CM	TET	RIF
M07-0128	0.06	≤0.03	0.06/0.03	0.06	0.06	0.5	0.25	2/38	0.06	0.06	0.5	4	0.06	≤0.03
M07-1336	0.06	≤0.03	0.06/0.03	0.06	0.06	0.5	0.25	4/76	0.06	0.06	0.5	2	0.06	≤0.03

[a] Abbreviations: PC, penicillin; AM, ampicillin; SAM, ampicillin-sulbactam; GM, gentamicin; CF, cephalothin; CIP, ciprofloxacin; MXF, moxifloxacin; SXT, trimethoprim-sulfamethoxazole; E, erythromycin; CC, clindamycin; VA, vancomycin; CM, chloramphenicol; TET, tetracycline; RIF, rifampin.

mm in diameter. We initially reported the isolate as *Staphylococcus hominis* according to the Vitek 2 system. However, upon reexamination, the isolate was identified as *K. kristinae* with a 94.32% probability by a Vitek 2 system and as *Staphylococcus chromogenes* (code number 6716016) with a 94.1% probability by an API system. Sequencing of the 16S rRNA genes showed 99.72% homology with *K. marina* (GenBank accession no. AY211385) and 98.17% homology with *K. carniphila* (GenBank accession no. AJ622907). By day 5 of empirical treatment, the CAPD effluent had cleared, and the WBC count decreased. He was discharged with complete resolution of the peritonitis. This isolate was likewise susceptible to penicillin, ampicillin, ampicillin-sulbactam, gentamicin, cephalothin, ciprofloxacin, moxifloxacin, erythromycin, clindamycin, vancomycin, chloramphenicol, tetracycline, and rifampin but not to trimethoprim-sulfamethoxazole (Table 1).

Kocuria spp. are aerobic, gram-positive cocci occurring in tetrads. They are members of the *Micrococcaceae* family. More than 11 species of *Kocuria* are recognized. The organism is widespread in nature, frequently being found as normal skin flora in humans and other mammals (7, 13). However, we do not know the source of the organisms in these two infections. There are only a few reports of human infection caused by *Kocuria*. Specifically, we found a single case of acute cholecystitis caused by *K. kristinae* (9) and three cases of catheter-related bacteremia, attributing one each to *Kocuria rosea*, *K. kristinae*, and *Kocuria rhizophila* (1–3). Most patients with catheter-related bacteremia were immunocompromised by malignancy or a metabolic disorder. Strain KMM 3905^T, isolated from a high-salt environment, was recently given the name *Kocuria marina* sp. nov. (6). There have been no reports of *K. marina* being isolated from clinical specimens.

Peritonitis is one of the common complications of CAPD. The most common causative organisms are gram-positive bacteria, particularly *Staphylococcus aureus* and coagulase-negative staphylococci, which are part of the normal skin flora (10, 12). The peritonitis is usually diagnosed on the basis of cloudy effluent and the effluent cell count. There have been no published reports of CAPD peritonitis caused by *K. marina*, but we are confident that it was the causative organism in our patients. We performed all culture procedures according to the 2005 update of the International Society for Peritoneal Dialysis guidelines/recommendations (11), using specimens collected prior to antibiotic treatment. We employed a standard blood culture system with inoculation of the sediment obtained by centrifuging 50 ml of effluent, as recommended by the International Society for Peritoneal Dialysis guidelines. Pure cultures were obtained in both cases.

To confirm the identification of these two isolates, we performed 16S rRNA gene sequencing with an ABI Prism 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) and a BigDye Terminator cycle sequencing kit (Applied Biosystems) (5). We finally identified these two strains as *K. marina*. *Kocuria* spp. are distinguished from the genus *Micrococcus* and related gram-positive cocci on the basis of phylogenetic analysis using 16S rRNA gene sequences (13). *K. marina* is most closely related to *K. rhizophila* DSM 11926, *K. varians* DSM 20033, and *K. carniphila* CCM 132. However, *K. marina* forms an independent phylogenetic lineage within *Kocuria* (6, 14). We constructed a phylogenetic tree using the neighbor-joining method with MEGA version 4, and the same results were acquired (Fig. 1).

Strain KMM 3905^T was isolated from a marine sediment sample taken from Troitsa Bay and was named a novel *Kocuria* species, *K. marina*, by Kim et al. (6). The G+C content of the genomic DNA is 60 mol%, the lowest reported G+C content among species of *Kocuria*. Those investigators described the biochemical characteristics of *K. marina* as growth at both 4°C and 43°C and in the presence of as much as 15% NaCl, although salt is not required. The organism showed some biochemical characteristics different from those of other *Kocuria* spp., as follows: it was positive for urease and nitrate reduction, negative for oxidase and alkaline phosphatase, and negative for acid production from glucose, lactose, or sucrose (6). However, it was doubtful that we could identify *K. marina* to the species level with these biochemical tests, as our two *K. marina* strains differed from those reported by Kim et al. (6). Moreover, the two isolates showed different pigmentation on sheep blood agar; one strain was orange and the other was yellow. At present, correct identification of *Kocuria* spp. by commercial systems is problematic because systems such as Vitek 2 GP card and API Staph do not include all *Kocuria* spp. in their database. To describe the common phenotypic properties of *K. marina*, further investigation should be conducted on many strains.

Empirical therapy for CAPD peritonitis is an intraperitoneal narrow-spectrum cephalosporin such as cefazolin for gram-positive cocci and a fluoroquinolone or an expanded-spectrum cephalosporin for gram-negative bacilli (8). Our patients were treated empirically with a narrow-spectrum cephalosporin and netilmicin by intraperitoneal infusion before culture results were available, and one was cured. However, the other patient did not respond in spite of the addition of intravenous vancomycin, and catheter removal was necessary. This is similar to

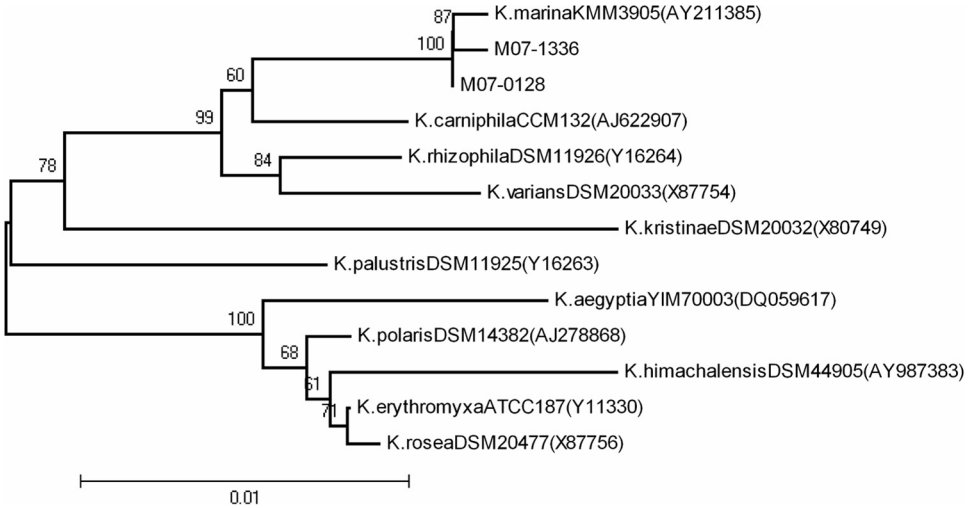

FIG. 1. Phylogenetic analysis using the neighbor-joining method based on the 16S rRNA gene sequences of representative *Kocuria* species and strains M07-0128 and M07-1336. Bar, 0.01 nucleotide substitutions per position.

the situation with catheter-related bacteremia. In those cases, therapy with a glycopeptide failed, but the patients were cured after removal of the catheter (1–3). Our case 1 patient likewise recovered with the removal of the catheter and continuous antibiotic therapy.

Our two strains were isolated with a 9-month interval between them, and there was no relationship between these two patients. The source could not be identified. Because there are no interpretative guidelines for antimicrobial susceptibility testing for *Kocuria* spp., we did not test any antimicrobial agents at the time the organisms were isolated. Later, however, we performed susceptibility testing using an agar dilution method according to the CLSI recommendations for *Staphylococcus*, because this is the first report of *Kocuria marina* as a pathogen, and there are no data on MICs for *Kocuria* spp. Notably, these two strains were susceptible to most antimicrobial agents (Table 1).

We describe two cases of *K. marina* peritonitis in patients undergoing CAPD; this is, to our knowledge, the first report of human infection caused by this organism.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rRNA gene sequences of the two strains of *K. marina* identified in the present study are FJ789660 and FJ789661.

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