Title	**Gordonia Species as Emerging Causes of Continuous-Ambulatory-Peritoneal-Dialysis-Related Peritonitis Identified by 16S rRNA and secA1 Gene Sequencing and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)**
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Gordonia Species as Emerging Causes of Continuous-Ambulatory-Peritoneal-Dialysis-Related Peritonitis Identified by 16S rRNA and *secA1* Gene Sequencing and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)

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We report here four cases of continuous ambulatory peritoneal dialysis-related peritonitis caused by three different species of *Gordonia*. The portal of entry was likely through Tenckhoff catheters. 16S rRNA and *secA1* gene sequencing are so far the most reliable methods for the accurate identification of *Gordonia* species.

Gordonia species are Gram-positive weakly acid-fast coryneform bacteria. Although *Gordonia* species have been implicated in a variety of infections, only seven cases of continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis caused by *Gordonia* species have been described (1–4). Furthermore, the methods used for identifying these seven *Gordonia* isolates were not mentioned in five cases (2, 4), and the accuracy of the identifications could not be ascertained. In this article, we described four cases of CAPD-related peritonitis caused by three different species of *Gordonia* confirmed by 16S rRNA and *secA1* gene sequencing.

Clinical specimens were collected and handled according to standard protocols, and the clinical data were collected by analyzing the patients' hospital records. Phenotypic identification was performed using standard conventional biochemical methods and the API Coryne system (bioMérieux, France). All tests were performed in triplicate. The MICs were determined using Etests (bioMérieux), according to the standards of the Clinical and Laboratory Standards Institute (CLSI) (5), with *Escherichia coli* strain ATCC 25922 and *Pseudomonas aeruginosa* strain ATCC 27853 as controls; the results were compared with the CLSI MIC interpretive standards for *Staphylococcus* spp. and *Streptococcus pneumoniae* (6) for evaluation. Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA and *secA1* genes were performed according to our previous publication (7), except for the primer pairs used (G268F/G1096R [8] for the 16S rRNA gene and *SecA1*-f/*SecA1*-r [9] for the *secA1* gene). Comparative sequence identity analysis and phylogenetic analysis using the maximum likelihood method were performed according to our previous publication (10), except that MEGA 6.06 (11) was used instead. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was performed according to our previous publication (12), and the protein profiles obtained were processed and analyzed using MALDI Biotyper 3.1 (Bruker Daltonics, Germany) for the generation of the hierarchical cluster analysis (HCA) dendrogram and for identification.

Case 1. A 65-year-old Chinese man was admitted for cloudy dialysis effluent for 1 day. He had end-stage renal failure (ESRF) of unknown etiology and had been undergoing CAPD for 7 years. He was afebrile but had turbid dialysis effluent. The total leukocyte count of the dialysis fluid was 930/mm^3, with neutrophil predominance. A Gram smear of the dialysis effluent after centrifugation revealed numerous leukocytes without any organisms. Empirical intraperitoneal (i.p.) cefazolin and gentamicin therapies were started. As the clinical condition of the patient did not improve, intravenous (i.v.) imipenem-cilastatin and amikacin were commenced and continued for a total of 6 weeks.

Case 2. A 64-year-old Chinese woman was admitted for abdominal pain and cloudy dialysis effluent for 1 day. She had ESRF due to hypertensive nephropathy and had been undergoing CAPD for 4 years. She was afebrile but had generalized abdominal tenderness and turbid dialysis effluent. The total leukocyte count of the dialysis fluid was >1,000/mm^3, with neutrophil predominance. A Gram smear of the dialysis effluent after centrifugation revealed numerous leukocytes and Gram-positive bacilli. Empirical i.p. cefazolin and gentamicin therapies were started. The patient did not respond to i.p. cefazolin and gentamicin, which were stopped after 1 week, and i.v. meropenem and amikacin therapies were commenced. As the response was still unsatisfactory after another 2 weeks, the Tenckhoff catheter was removed, and temporary hemodialysis was commenced. She received three more weeks of i.v. meropenem, followed by 4 weeks of oral levofloxacin.

Case 3. A 67-year-old Chinese man was admitted for abdominal pain and cloudy dialysis effluent for 1 day. He had ESRF due to

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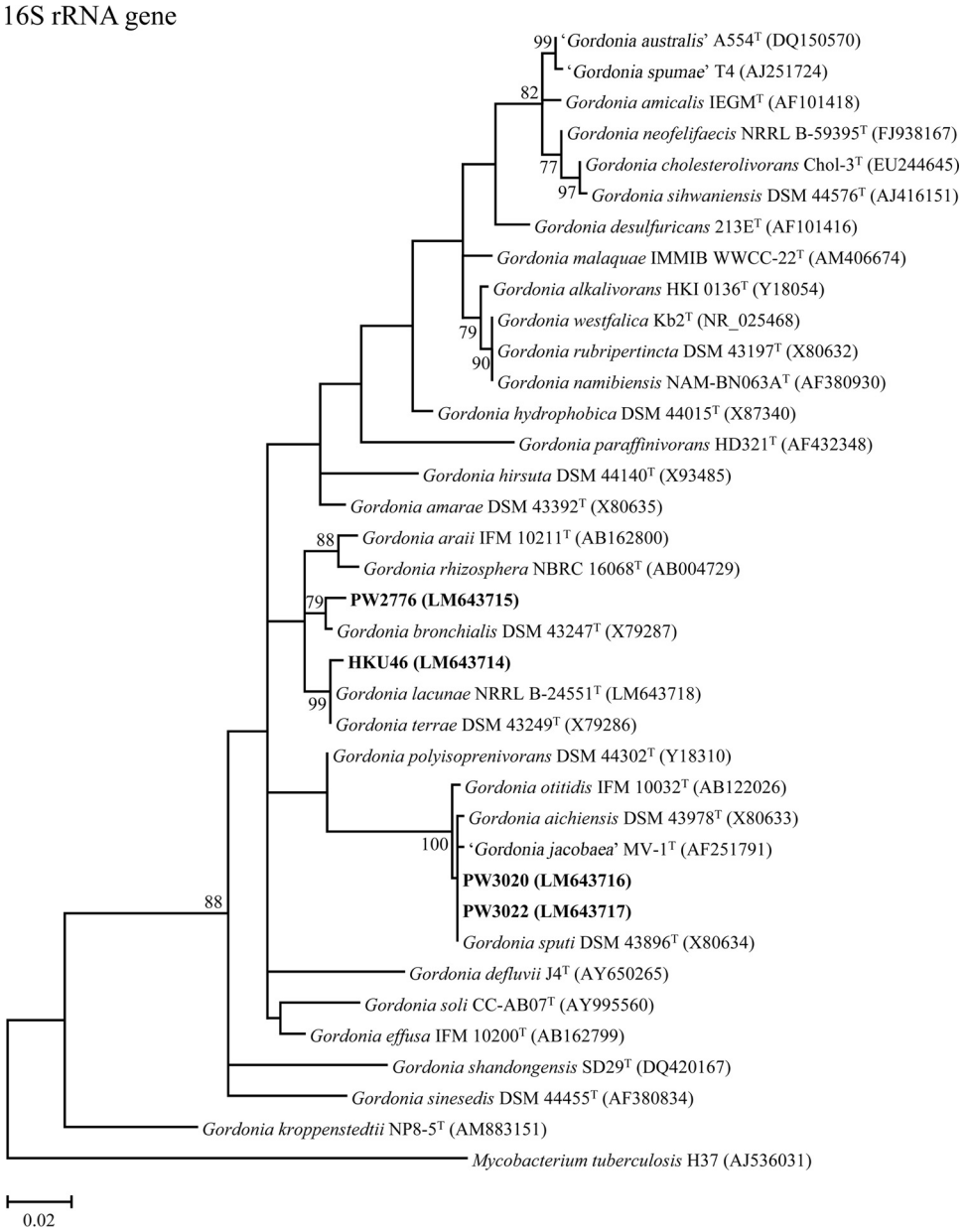

FIG 1 Phylogenetic trees showing the relationship of the four case isolates to *Gordonia* species. The trees were inferred from partial 16S rRNA and partial *secA1* gene sequence data by the maximum likelihood method, with the substitution models T92 (Tamura 3-parameter model) + G (gamma-distributed rate variation) + I (estimated proportion of invariable sites) and GTR (general time-reversible model) + G + I, respectively. Totals of 789 and 469 nucleotide positions of the 16S rRNA and *secA1* genes, respectively, were included in the analyses. The scale bars indicate the estimated numbers of substitutions per base. The numbers at the nodes, expressed in percentages, indicate the levels of bootstrap support calculated from 1,000 trees, and bootstrap values of <70 are not shown. All accession numbers (in parentheses) are given as cited in the ENA/GenBank/DDBJ databases, and the names "*G. australis*," "*G. jacobaea*," and "*G. spumae*" are not validly published and have no standing in bacterial nomenclature. The case isolates reported in this study are highlighted in bold type.

diabetic nephropathy and had been undergoing CAPD for 1 year. His Tenckhoff catheter broke 1 week prior to symptom onset. He was afebrile but had generalized abdominal tenderness and turbid dialysis effluent. The total leukocyte count of the dialysis fluid was 1,060/mm^3, with neutrophil predominance. A Gram smear of the dialysis effluent after centrifugation revealed numerous leukocytes without any organisms. Empirical i.p. cefazolin and ceftazidime therapies were started. The patient did not respond to i.p. cefazolin and ceftazidime, which were stopped after 1 week, and i.v. imipenem-cilastatin and amikacin therapies were commenced. As the response was still unsatisfactory after another 3 weeks, the Tenckhoff catheter was removed, and temporary hemodialysis was commenced.

Case 4. A 52-year-old Chinese man was admitted for abdominal pain and cloudy dialysis effluent for 1 day. He had ESRF due to immunoglobulin A nephropathy and had been undergoing CAPD for 1 year. His Tenckhoff catheter broke 2 weeks prior to symptom onset. He was febrile (38.4°C) and had generalized abdominal

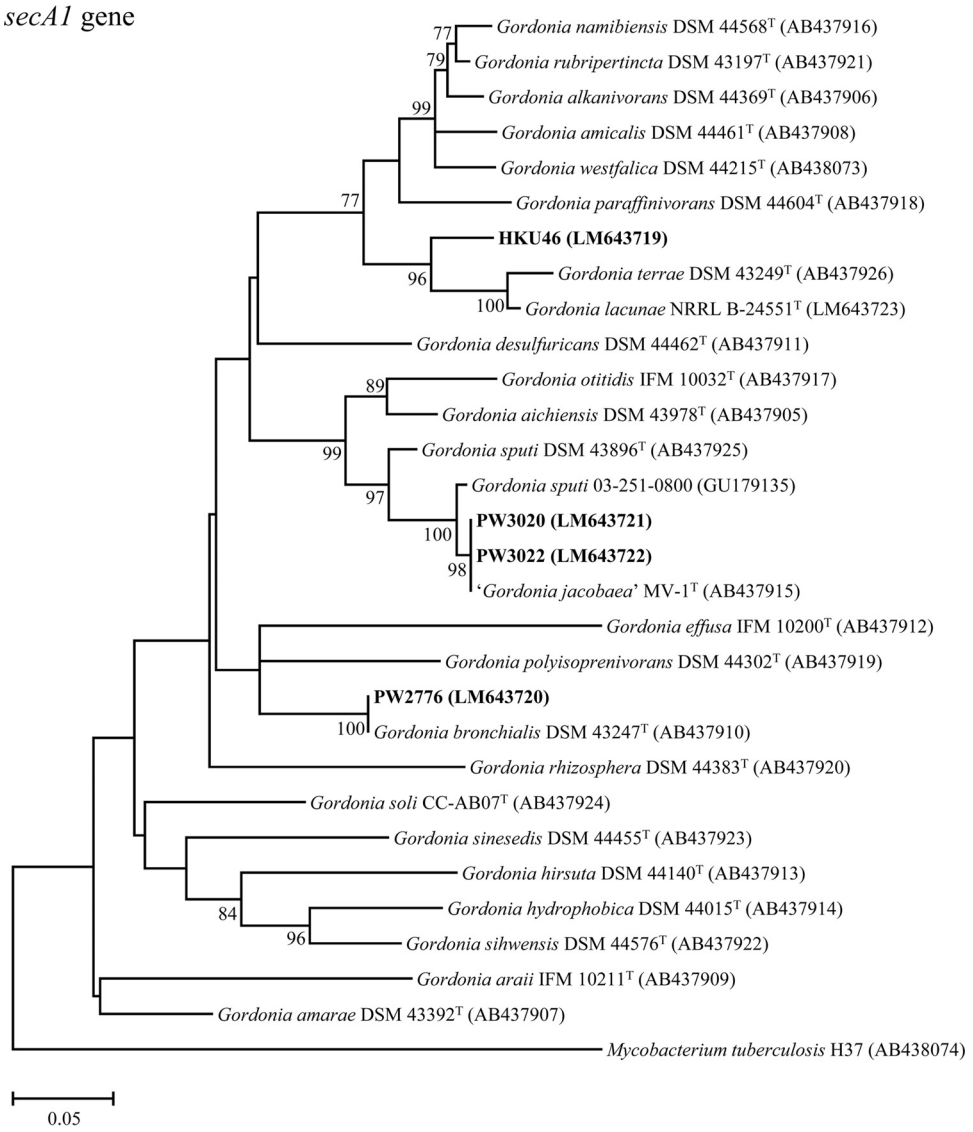

FIG 1 continued

tenderness and turbid dialysis effluent. The total leukocyte count of the dialysis fluid was 1,030/mm^3, with neutrophil predominance. A Gram smear of the dialysis effluent after centrifugation revealed numerous leukocytes without any organisms. Empirical i.p. cefazolin and ceftazidime therapies were started. The patient did not respond to i.p. cefazolin and ceftazidime, which were stopped after 1 week, and the therapy was changed to i.p. vancomycin and amikacin, which were continued for a total of 3 weeks.

The culture of the dialysis effluents of all four patients obtained on admission yielded Gram-positive bacilli (strains PW3022, PW2776, PW3020, and HKU46, respectively). All four isolates grew on horse blood agar as small, light pink, dry, rough, irregular, and nonhemolytic colonies after 5 days of incubation at 37°C in an aerobic environment with 5% CO_2. They did not grow under anaerobic conditions. The Gram smears of the colonies showed nonsporulating beaded Gram-positive bacilli, which were acid-fast by modified acid-fast stain. The bacteria were catalase positive but nonmotile. The API Coryne system showed that the four isolates were *Rhodococcus* species (code: 1110004, 3110004, or 7151104). All four isolates were susceptible to vancomycin, amikacin, imipenem, and ciprofloxacin, with MICs of 0.75 to 1.5 μg/ml, 0.125 to 1 μg/ml, 0.008 to 0.094 μg/ml, and 0.094 to 0.125 μg/ml, respectively.

PCR amplification and sequencing of the 16S rRNA and *secA1* genes of the four patient isolates and comparative sequence identity analyses showed that strains PW3020 and PW3022 were *Gordonia sputi*, and strain PW2776 was *Gordonia bronchialis* (Fig. 1). For strain HKU46, its 16S rRNA gene was found to possess 99.7% sequence identity to that of *Gordonia terrae* strain DSM 43249^T, but its *secA1* gene possessed only 94.0% sequence identity to that of *Gordonia lacunae* strain NRRL B-24551^T (Fig. 1), indicating that it was a potentially novel *Gordonia* species.

MALDI-TOF MS and HCA showed that PW3020 and PW3022 were clustered with other strains of *G. sputi* in the database (Fig. 2) and were correctly identified as *G. sputi*, with top match scores of 2.039 and 2.026, respectively (scores of ≥1.7 and <1.7 to ≥1.5

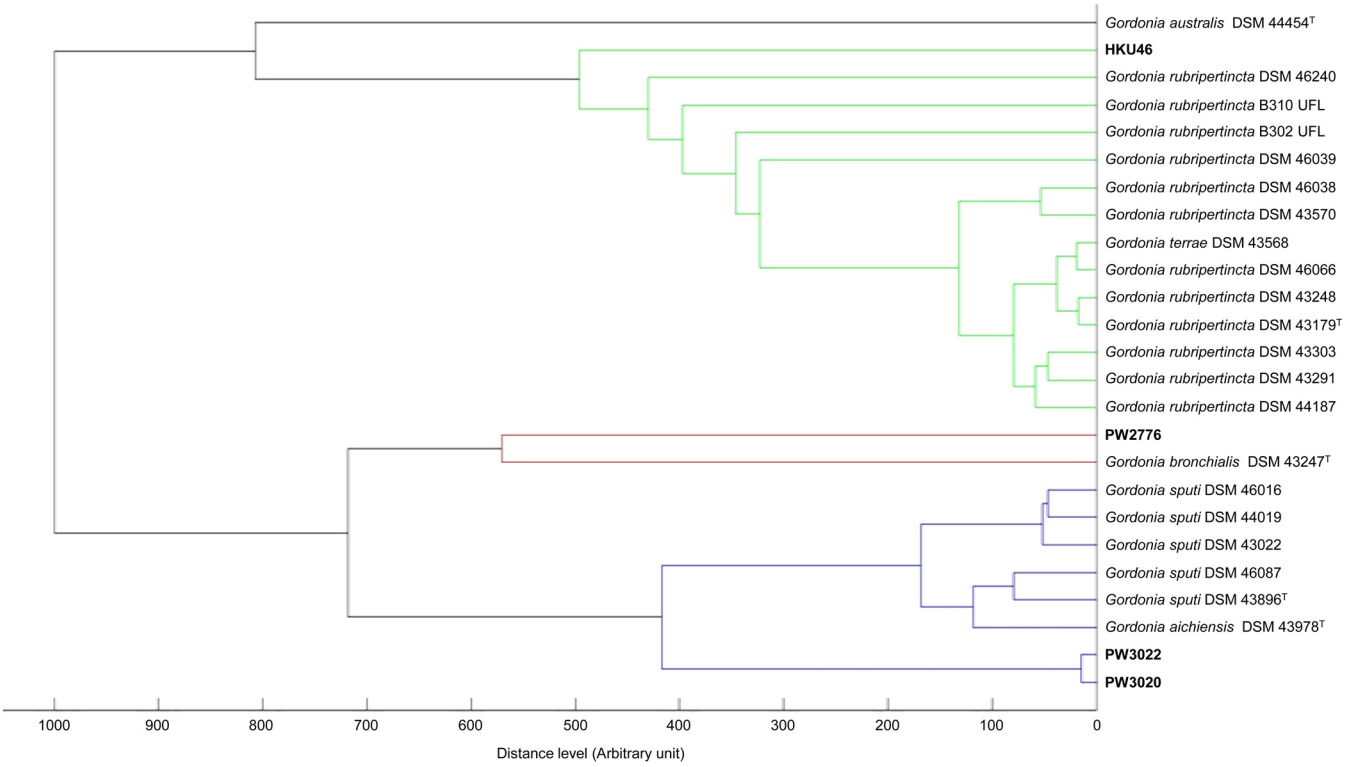

FIG 2 Dendrogram generated from HCA of MALDI-TOF mass spectra of the case isolates and strains of *Gordonia* species in the Bruker database.

represent confident identification to the species and genus levels for Gram-positive rods, respectively [13, 14]), whereas PW2776 was clustered with a strain of *G. bronchialis* in the database (Fig. 2) and was correctly identified as *G. bronchialis*, with a top match score of 1.743. As for HKU46, MALDI-TOF MS failed to confidently identify this isolate to the species level. However, it was identified as a *Gordonia* species, with a top match score of 1.550 (as *Gordonia rubripertincta*), suggesting it did not resemble any known *Gordonia* species in the database.

Gordonia species are emerging causes of CAPD-related peritonitis. With the increasing use of 16S rRNA and other housekeeping gene sequencing techniques, bacteria previously not known to be associated with particular clinical syndromes have been reported (15, 16). Since 2012 (i.e., in <3 years), seven cases of CAPD-related peritonitis caused by *Gordonia* species have been reported (1–4) (Table 1). In the present series, none of the four patients responded to i.p. cefazolin and ceftazidime-gentamicin treatment. When the treatment regimens were switched to i.v. imipenem-cilastatin or meropenem plus amikacin-levofloxacin or i.v./i.p. vancomycin plus amikacin with or without Tenckhoff catheter removal, all four patients responded promptly. This is in line with the treatment response of the seven cases reported in the literature, for which most patients required either i.v. vancomycin or a carbapenem with or without Tenckhoff catheter removal. Notably, CAPD was resumed in seven of the 11 (63.6%) patients afterwards.

The portal of entry in these patients with *Gordonia* CAPD-related peritonitis is likely the Tenckhoff catheter. Pathogens causing CAPD-related peritonitis originate from two major sources, either through the Tenckhoff catheter or by translocating through the intestinal wall. Since *Gordonia* species are ubiquitous bacteria that have been isolated from environmental samples, such as those from soil and water, it is likely that these bacteria reach the peritoneal cavities of patients undergoing CAPD through their Tenckhoff catheters. In fact, two of the four patients in this study had broken Tenckhoff catheter tips 1 to 2 weeks prior to the development of peritonitis, which likely predisposed those patients to the entry of the *Gordonia* bacteria into the peritoneal cavities. Notably, *Gordonia* species have been most commonly reported to be causes of indwelling device-associated infections (17–30), in line with these cases of *Gordonia* CAPD-related peritonitis.

16S rRNA and *secA1* gene sequencing are so far the most reliable ways to accurately identify *Gordonia* species, which is crucial for understanding the epidemiology, clinical features, treatment, and outcome of infections caused by this group of bacteria. *Gordonia* are diphtheroids, which are particularly difficult to identify to both the genus and species levels by conventional phenotypic tests. In this study, all four isolates were misidentified as *Rhodococcus* species by the API Coryne system. 16S rRNA and *secA1* gene sequencing accurately identified two isolates as *G. sputi* and a third one as *G. bronchialis*. As the *secA1* gene of the fourth isolate (HKU46) showed a ≥6% nucleotide difference with the most closely related *Gordonia* species, *G. lacunae* and *G. terrae*, it was likely that it represents a novel *Gordonia* species, which warranted further characterization. As for MALDI-TOF MS, all three strains of *G. sputi* and *G. bronchialis* were confidently identified to the species level, indicating that this technology is potentially also useful for identifying *Gordonia* species.

Nucleotide sequence accession numbers. The 16S rRNA and

TABLE 1 Summary of reported cases of CAPD peritonitis due to *Gordonia* species[a]

Case	Study	Sex/age (yr)[b]	Underlying condition or risk[c]	Identity of bacterial isolate	Identification method	Treatment[d]	Treatment duration	PD catheter removal[e]	Outcome
1	Imran et al. (1)	F/78		*Gordonia* sp.	16S rRNA gene sequencing	i.v. teicoplanin and i.p. gentamicin	NA[f]	Yes	NA
2	Gardenier et al. (2)	F/50	Chronic kidney disease due to reflux, breast cancer	*Gordonia* sp.	Not specified	Amoxicillin-clavulanate	3 weeks	Yes	Cured and CAPD resumed
3	Ou et al. (3)	M/69	ESRD due to diabetic nephropathy	*Gordonia sputi*	16S rRNA gene sequencing	1st and 2nd episodes: i.p. cefazolin plus gentamicin; 3rd episode: i.v. vancomycin plus ceftazidime, and then p.o. ciprofloxacin	1st and 2nd episodes: 2 weeks; 3rd episode: NA	Yes	Cured, with 2 episodes of recurrence; switched to hemodialysis
4	Ma et al. (4)	M/45	ESRD due to immunoglobulin A nephropathy	*Gordonia terrae*	Not specified	i.p. vancomycin	3 weeks	No	Cured; cadaveric renal transplant 2 wk after antibiotic was completed
5	Ma et al. (4)	M/70	ESRD due to diabetic nephropathy, hypertension, peripheral vascular disease, ischemic heart disease	*Gordonia bronchialis*	Not specified	i.p. imipenem-cilastatin and amikacin	2 weeks	No	Cured
6	Ma et al. (4)	M/61	Alport syndrome	*Gordonia terrae*	Not specified	1st and 2nd episodes: i.p. vancomycin; 3rd episode: i.v. meropenem	1st and 2nd episodes: 2 weeks; 3rd episode: 2 weeks	Yes	Cured, with 2 episodes of recurrence; CAPD resumed
7	Ma et al. (4)	M/60	ESRD of unknown cause	*Gordonia terrae*	Not specified	i.p. meropenem	NA	Yes	Cured and CAPD resumed
8	Present case 1	M/65	ESRD of unknown cause, hypertension, gout	*Gordonia sputi*	16S rRNA and *secA1* gene sequencing	i.v. imipenem-cilastatin and amikacin	6 weeks	No	Cured and CAPD resumed, with no recurrence, succumbed 6 mo later because of *Corynebacterium jeikeium* and *Candida famata* CAPD peritonitis
9	Present case 2	F/64	ESRD due to hypertensive nephropathy	*Gordonia bronchialis*	16S rRNA and *secA1* gene sequencing	i.v. meropenem, and then p.o. levofloxacin	3 weeks/4 weeks	Yes	Cured and CAPD resumed, with no recurrence
10	Present case 3	M/67	ESRD due to diabetic nephropathy, hypertension, gout, chronic obstructive pulmonary disease	*Gordonia sputi*	16S rRNA and *secA1* gene sequencing	i.v. imipenem-cilastatin and amikacin	3 weeks	Yes	Cured and CAPD resumed with no recurrence, succumbed 11 mo later because of perforated sigmoid ulcer with secondary peritonitis
11	Present case 4	M/52	ESRD due to immunoglobulin A nephropathy	Potential novel *Gordonia* sp.	16S rRNA and *secA1* gene sequencing	i.p. vancomycin and amikacin	3 weeks	No	Cured and CAPD resumed, with no recurrence

[a] CAPD, continuous ambulatory peritoneal dialysis.
[b] F, female; M, male.
[c] ESRD, end-stage renal disease.
[d] i.p., intraperitoneal; i.v., intravenous; p.o., oral.
[e] PD, peritoneal dialysis CAPD.
[f] NA, not available.

secA1 gene sequences have been deposited in the European Nucleotide Archive (ENA), European Molecular Biology Laboratory (EMBL), under accession numbers LM643714 to LM643723.

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We declare no conflicts of interest.

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