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CASE REPORT

Intra-Abdominal Infections Due to *Comamonas kerstersii*

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Herein, we report four cases of *Comamonas kerstersii* intra-abdominal infections representing the first report of human infections caused by this *Comamonas* species. In addition, our work demonstrates the association of *C. kerstersii* with peritonitis secondary to appendix rupture.

CASE REPORT

Herein we describe four cases of intra-abdominal infections due to *Comamonas kerstersii*. In all of them, *C. kerstersii* was isolated from free fluid in the abdominal cavity. In three cases, a perforated appendix was the source of intra-abdominal infection; in the other case, it was a sigmoid colon perforation. *C. kerstersii* was always isolated in conjunction with other pathogens. Only one patient had an underlying disease. In all cases, the clinical evolution was favorable. The main clinical features of each case are presented in Table 1.

After 48 h of incubation at 35°C and in ambient air, growth of a nonfermenting Gram-negative bacillus was observed in all abdominal fluid cavity cultures. The colonies grew to a diameter of 1.5 mm on blood agar and on nutrient agar in ambient air. They were white, smooth, and nonadherent, and they had entire edges.

The organisms were identified as *C. kerstersii* by using standard biochemical tests (1) and according to the scheme proposed by Wauters et al. (2, 3). This scheme is centered around three enzymatic activities, oxidase, trypsin (benzyl-arginine aminopeptidase), and pyrrolidonyl aminopeptidase. Additionally, biochemical tests, such as determination of acid production from glucose, colistin and desferrioxamine susceptibility, urease production, motility, nitrate reduction, growth at 42°C, and tyrosine hydrolysis, were required to make the final identification (Table 2). The isolates were also analyzed on a Vitek 2 compact system (bioMérieux) using a GN colorimetric identification card and with API 20NE version 6.0 (numerical profiles were interpreted using the APILAB software, version 3.3.3 [bioMérieux]). The Vitek 2 and API 20NE results are summarized in Table 3. Identification was also carried out by using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik), which resulted in spectral scores of 2.022, 2.066, 2.097, and 2.251 for the four *C. kerstersii* isolates (4).

The results of differential biochemical tests on our isolates,

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TABLE 1 Clinical and microbiological characteristics of patients with infections due to *Comamonas kerstersii*

Case	Age (yr), sex[a]	Clinical presentation	Underlying disease(s)	Predisposing condition(s)	Identified pathogens	Antibiotic treatment
1	43, F	Febrile syndrome, abdominal pain	Ovarian tumor with peritoneal metastases	Sigmoid perforation by foreign body (biliary stent), rectovaginal fistula, and colostomy	*Escherichia coli*, *Bacteroides fragilis*, *Comamonas kerstersii*	Ampicillin-sulbactam followed by piperacillin-tazobactam and then ertapenem
2	48, M	Febrile syndrome, abdominal pain for 3 days	No underlying disease	Perforated appendix	*Streptococcus anginosus* group, *Aeromonas hydrophila* group, *Escherichia coli*, *Comamonas kerstersii*	Ampicillin-sulbactam, ciprofloxacin, and then amoxicillin-clavulanic acid
3	10, F	Abdominal pain for 3 days, bilious vomiting, and febrile events	No underlying disease	Perforated gangrenous appendix	*Streptococcus anginosus* group, *Escherichia coli*, *Comamonas kerstersii*	Ampicillin + metronidazole + gentamicin and then amoxicillin-clavulanic acid
4	21, F	Abdominal pain for 3 days associated with vomiting	No underlying disease	Perforated gangrenous appendix	*Citrobacter amalonaticus*, *Comamonas kerstersii*	Ampicillin + metronidazole + gentamicin

[a] F, female; M, male.

TABLE 2 Biochemical identification of *Comamonas kerstersii* isolates

Test	Result[a] for:					
	Our isolates	*Comamonas kerstersii*	*Comamonas terrigena*	*Comamonas testosteroni*	*Comamonas aquatica*	*Pseudomonas alcaligenes*
Oxidase activity	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+
Trypsin	−	−	−	−	−	+
Susceptibility to desferrioxamine	S	S	S	R	S	R
Susceptibility to colistin	S	S	S	S	S	S
Pyrrolidonyl aminopeptidase	−	−	+	+	−	−
Acid from glucose	−	−	−	−	−	−
Urease	−	−	−	−	−	−
Growth at 42°C	+	+	−	−	−	+
Tyrosine hydrolysis	+	+	+	+	−	ND

[a] +, positive; −, negative; S, susceptible; R, resistant; ND, not done. Data are from references 1, 2, and 3.

different species of *Comamonas*, and other nonsaccharolytic microorganisms are shown in Table 2.

Because of the rarity of this pathogen, PCR amplification of the 16S rRNA was performed in order to confirm the species. PCR products of the 16S rRNA gene were obtained with the primers described by Weisburg et al. (5) and by using the *Taq* DNA polymerase according to the manufacturer's specifications (Promega). Sequencing of the 1.4-kb PCR product was performed on both DNA strands at the Macrogen, Inc., Seoul, South Korea, sequencing facility. The obtained sequences of the 4 isolates were analyzed using the BLAST V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/). The result showed a 99% identity with the sequences corresponding to the 16S RNA ribosomal gene of *Comamonas kerstersii* strain LMG 5323 (GenBank accession no. AJ430348.1); there was a 2-base mismatch between the 4 isolate sequences and *Comamonas kerstersii* strain LMG 5323. In order to obtain a more discriminatory sequence and also confirm the obtained result, we amplified the *gyrB* gene (coding for subunit beta of DNA gyrase), which has been shown to resolve phylogenetic relationships in various bacterial groups (6). A PCR product of around 420 bp was obtained using the primers described by Tayeb et al. (6). In all cases, sequence analysis revealed 98% identity with the *gyrB* sequence of *Comamonas kerstersii* strain CIP 107987, which corresponds to 8 mismatches between the compared sequences (GenBank accession no. EU024199), 92% identity with the *gyrB* sequence of *Comamonas aquatica* strain CIP 107986 (GenBank accession no. EU024201), and 90% identity with the *gyrB* sequence of *Comamonas testosteroni* strain CNB-2 (GenBank accession no. CP001220). These results confirm the species identification.

The antibiotic susceptibility test was performed using the Vitek 2 system employing panel AST-082 (GNS susceptibility card). The MIC results were interpreted using CLSI categories (7). MIC ranges for different antibiotics were as follows (μg/ml): ampicillin, ≤2 to 16; ampicillin-sulbactam, ≤2; piperacillin-tazobactam, ≤4; cephalothin, ≤2; cefoxitin, ≤4 to 8; cefotaxime, ≤1; ceftazidime, ≤1 to 2; cefepime, ≤1; imipenem, ≤1; meropenem, ≤0.25; gentamicin, ≤2 to 4; amikacin, 16; ciprofloxacin, ≤0.25 to ≥4; colistin, ≤0.5 to 1; and trimethoprim-sulfamethoxazole, ≤2 to 4. *C. kerstersii* was highly susceptible to antibiotics, except for one of the isolates, which showed resistance to ciprofloxacin.

The genus *Comamonas* was originally created in 1985, and it included a single species, *Comamonas terrigena* (8). In 1987, *Pseudomonas acidovorans* and *Pseudomonas testosteroni* were reclassified as members of the genus *Comamonas*. *Comamonas acidovorans* was subsequently reclassified as *Delftia acidovorans* (9). *Comamonas terrigena* actually comprises three genotypically separate groups: *Comamonas terrigena*, *Comamonas aquatica*, and *Comamonas kerstersii* (2).

Barbaro et al. have reported the tendency of *C. testosteroni* to cause peritoneal cavity infections and perforated appendixes as specific anatomic defects resulting from the infection (10). They identified 10 cases of infections due to this microorganism in patients hospitalized at a single metropolitan hospital during a 3-year period. In 6 of them, *C. testosteroni* was isolated from the peritoneal cavity; in 5 cases, a perforated appendix was the source of intra-abdominal infection. In the four remaining cases, the infection corresponded to bacteremia (two cases), genitourinary tract infection, and central nervous system infection (10). However, it is possible that the isolates described by Barbaro et al. were identified as *C. testosteroni* because *C. kerstersii* is not found in the

TABLE 3 Phenotypic identification results of the Vitek 2 and API 20NE systems

Case(s)	Vitek 2 system identification			API 20NE system identification		
	Biocode	Identification	Level of confidence	Biocode	Identification	Level of confidence
1	0000000000500042	*Acinetobacter junii/ C. testosteroni/Acinetobacter ursingii*	Low discrimination	1000044	*C. testosteroni/P. alcaligenes*	Low discrimination (58.5%)
2	0000000100500041	*C. testosteroni*	Excellent identification (99%)	1000074	*C. testosteroni/P. alcaligenes*	Good identification (95.9%)
3 and 4	0000000100500042	*A. junii/C. testosteroni*	Low discrimination	1000074	*C. testosteroni/P. alcaligenes*	Good identification (95.9%)

Vitek database, which is the identification source used by these authors.

Gul et al. also have referred to the association of *C. testosteroni* with a perforated appendix. They were the first to report an acute case of bacteremia due to this organism in Turkey in a 22-year-old man with a perforated appendix (11). However, again, this microorganism might have been *C. kerstersii*, since it was identified only by phenotypic methods. Our work is the first to demonstrate the association of *C. kerstersii* with peritonitis secondary to appendix rupture.

In the literature, infections due to *C. kerstersii* may be underestimated because in previously published cases of *Comamonas* infection, identification of isolates has been achieved only by phenotypic methods, which do not allow differentiation among species of the genus (10–16).

Very few members of the *Comamonadaceae* family have been reported to cause infections in humans. However, most reported cases are due to *Delftia acidovorans* or to *C. testosteroni*. Both organisms are known to produce ocular infections (15, 17, 18), bacteremia, and central-line-associated bloodstream infections in patients with any underlying disease, such as malignancy, liver disease (11, 13, 14, 16, 19–22), and endocarditis (23, 24), among others. There is only one report of human infection due to *C. terrigena* in the literature. It was a case of acute bacterial endocarditis which responded appropriately to antibiotic treatment (25).

C. kerstersii should be differentiated from other *Comamonas* species and from other related organisms that also reduce nitrates and do not assimilate or acidify sugars, such as *Pseudomonas alcaligenes*. Sensitivity to deferoxamine, nonuse of testosterone, a negative pyrrolidone arylamidase test, growth at 42°C, and a positive tyrosine hydrolysis test differentiate *C. kerstersii* from other *Comamonas* species, while its sensitivity to desferrioxamine and lack of trypsin activity differentiate it from *P. alcaligenes* (Table 2).

We emphasize that the isolation of *C. kerstersii* from free fluid in the abdominal cavity and a perforated appendix are indications of intra-abdominal infection. Also, we highlight the need to request polyphasic identification to obtain definitive identification.

Nucleotide sequence accession numbers. The obtained sequences for the *C. kerstersii* rRNA and *gyrB* genes have been submitted to GenBank under accession no. KC714046 and KC714047, respectively.

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