Intra-abdominal Infections Due to *Comamonas kerstersii*

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Abstract

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 would demonstrate the association of *C. kerstersii*, with peritonitis secondary to appendix rupture.
Cases 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 another 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 at air atmosphere, growth of a non-fermenting Gram negative bacilli was observed in all abdominal fluid cavity cultures. The colonies grew on blood agar and on nutrient agar at air atmosphere reaching a diameter of 1.5 mm. They were white in color, smooth and non-adherent, and had entire edges.

The organisms were identified using standard biochemical tests following the scheme of biochemical tests proposed by Wauters et al (1-3) as *C. kerstersii*. This scheme is centered around three enzymatic activities, oxidase, trypsin (benzyl-arginine aminopeptidase) and pyrrolidonyl aminopeptidase.

Additionally, biochemical tests as acid production from glucose, colistin and desferrioxamine susceptibility, urease, motility, nitrate reduction, growth at 42 °C and tyrosine hydrolysis were required to address the final identification (Table 2). The isolates were also analyzed on a VITEK 2 Compact System (bioMérieux) using GN Colorimetric Identification Card and by 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 (MALDITOF) mass spectrometry (MS) (Bruker Daltonik) showing a spectral score of 2.022, 2.066, 2.097 and 2.251, respectively for *C. kerstersii* (4).
Differential biochemical tests between our isolates, the different species of *Comamonas* and other non-saccharolytic related 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, using the primers described by Weisburg et al (5), were obtained with the *Taq* DNA polymerase based on the manufacturer’s specifications (Promega). Sequencing of the 1.4 kb PCR product was performed on both DNA strands at Macrogen, Inc., Seoul, Korea sequencing facility. The obtaining sequences of the 4 isolates were analyzed using the Blast V2.0 software ([http://www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). The result showed a 99% of identity with the sequences corresponding to the 16s RNA ribosomal gene of *Comamonas kerstersii* strain LMG 5323 (GenBank accession No. AJ430348.1), exhibiting a 2-base mismatch between the 4 isolates sequences and the *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 have 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 2008 (6). In all cases, sequences analysis revealed 98 % of identity with the *gyrB* sequence of the *Comamonas kerstersii* strain CIP 107987, corresponding to 8 mismatches between the compared sequences (GenBank accession No. EU024199), 92% of identity with the *gyrB* sequence of the *C. aquatica* strain CIP 107986 (GenBank accession number EU024201) and 90% of identity with the *gyrB* sequence of the *C. testosteroni* strain CNB-2 (GenBank accession number CP001220). These results confirm the species identification.

The antibiotic susceptibility test was performed using the VITEK 2 System employing the panel AST-082 (GNS susceptibility card). The MIC results were interpreted using CLSI categories (7). MIC range for different antibiotics were as follows (µg/ml): ampicillin; ≤ 2-16; ampicillin/sulbactam; ≤ 2- ≤ 2;
piperacillin/tazobactam: ≤ 4- ≤ 4; cefalotin: ≤ 2- ≤ 2; cefoxitin: ≤ 4-8; cefotaxime: ≤ 1- ≤ 1;
ceftazidime: ≤ 1- ≤ 2; ceftepime: ≤ 1- ≤ 1; imipenem: ≤ 1- ≤ 1; meropenem: ≤ 0.25- ≤ 0.25; gentamicin: ≤ 2-4; amikacin: 16-16; ciprofloxacin: ≤ 0.25- ≥ 4; colistin: ≤ 0.5-1; trimethoprim-sulfamethoxazole: ≤ 2-
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 appendix as specific anatomic defects responsible for 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 remaining four reports the infection corresponded to bacteremia (two cases), genitourinary tract infection, and central nervous system infection, respectively (10). However, it is possible that isolates described by Barbaro et al could have been identified as *C. testosteroni* because *C. kerstersii* is not found in the VITEK database which is the identification method used by these authors.

Gul M et al. also have referred to the association of *C. testosteroni* with perforated appendix. They were the first to report a case of bacteremia due to this organism in Turkey in a 22-year-old man with...
perforated acute appendicitis (11). However, again, this microorganism might have been *C. kerstersii*
since it was only identified by phenotypic methods. Our work would be 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* spp. infection, identification of isolates has been achieved only by phenotypic
methods that does 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 of 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, or liver disease (11, 13, 14, 16,
19-22) and endocarditis (23, 24), among others. There is only one case 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 as *P. alcaligenes*. Sensitivity to
deferoxamine, non-use of testosterone, the pyrrolidone arylamidase negative test, growth at 42 °C and
a positive tyrosine hydrolysis differentiates *C. kerstersii* from other *Comamonas* species; while the
sensitivity to desferrioxamine and negative trypsin activity differentiate it from *P. alcaligenes* (Table
2).

We would like to emphasize the isolation of *C. kerstersii* from free fluid in the abdominal cavity and
perforated appendix as source of intra-abdominal infection. Also, we would like to highlight the need
to request polyphasic identification to address definitive identification.
Nucleotide sequence accession number. The obtained sequences for the *C. kerstersii* rRNA and gyrB genes have been submitted to GenBank under accession numbers KC714046 and KC714047, respectively.

Acknowledgments

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Legends to Tables

Legend to Table 2

a +, positive; -, negative; ND, not done. Data are from references 1, 2 and 3.
References


TABLE 1. Clinical and Microbiological Characteristics of Patients with Infections Due to *Comamonas kerstersii*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), sex</th>
<th>Clinical presentation</th>
<th>Underlying disease</th>
<th>Predisposing conditions</th>
<th>Identified pathogens</th>
<th>Antibiotic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43, F</td>
<td>febrile syndrome, abdominal pain</td>
<td>Ovarian tumor with peritoneal metastases</td>
<td>sigmoid perforation by foreign body (biliary stent), rectovaginal fistula and colostomy</td>
<td><em>Escherichia coli</em>, <em>Bacteroides fragilis</em>, <em>Comamonas kerstersii</em></td>
<td>ampicillin-sulbactam followed by Piperacillin-tazobactam and, then, ertapenem</td>
</tr>
<tr>
<td>2</td>
<td>48, M</td>
<td>febrile syndrome, abdominal pain for 3 days</td>
<td>No underlying disease</td>
<td>Perforated appendix</td>
<td><em>Streptococcus anginosus</em> group, <em>Aeromonas hydrophila</em> group <em>Escherichia coli</em>, <em>Comamonas kerstersii</em></td>
<td>ampicillin sulbactam, ciprofloxacin and then, amoxicillin/clavulanic acid</td>
</tr>
<tr>
<td>3</td>
<td>10, F</td>
<td>abdominal pain for 3 days, bilious vomiting and febrile events</td>
<td>No underlying disease</td>
<td>Perforated gangrenous appendix</td>
<td><em>Streptococcus anginosus</em> group, <em>Escherichia coli</em>, <em>Comamonas kerstersii</em></td>
<td>Ampicillin + metronidazole + gentamicin and then, amoxicillin/clavulanic acid</td>
</tr>
<tr>
<td>4</td>
<td>21, F</td>
<td>abdominal pain for 3 days associated with vomiting</td>
<td>No underlying disease</td>
<td>Perforated gangrenous appendix</td>
<td><em>Citrobacter amalonaticus</em>, <em>Comamonas kerstersii</em></td>
<td>Ampicillin + metronidazole + gentamicin</td>
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<table>
<thead>
<tr>
<th>Test</th>
<th>Ours isolates</th>
<th>Comamonas kerstersii</th>
<th>Comamonas terrigena</th>
<th>Comamonas testosteroni</th>
<th>Comamonas aquatica</th>
<th>Pseudomonas alcaligenes</th>
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<tr>
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<tr>
<td>Susceptibility to</td>
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<td>Colistin</td>
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<td>Acid from glucose</td>
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<td>Growth at 42 °C</td>
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<td>+</td>
<td>+</td>
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<td>-</td>
<td>ND</td>
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TABLE 2. Biochemical Identification of *Comamonas kerstersii* Isolates
TABLE 3. Phenotypic identification results of VITEK 2 and API20NE System

<table>
<thead>
<tr>
<th>Case</th>
<th>Biocode</th>
<th>VITEK2 System identification</th>
<th>API 20NE identification</th>
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<td></td>
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<td>Identification</td>
<td>Level confidence</td>
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<td>Acinetobacter junii/ C. testosteroni/ Acinetobacter ursingii</td>
<td>Low discrimination</td>
</tr>
<tr>
<td>2</td>
<td>0000000100500041</td>
<td>C. testosteroni</td>
<td>Excellent identification (99 %)</td>
</tr>
<tr>
<td>3 and 4</td>
<td>0000000100500042</td>
<td>A. junii/C. testosteroni</td>
<td>Low discrimination</td>
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