Cloacibacillus sp., a potential human pathogen associated with bacteremia in Quebec and New Brunswick

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Abstract

Bacteremia with *Cloacibacillus* species is poorly described. We present three cases involving either *Cloacibacillus evryensis* or *Cloacibacillus porcorum*. The isolates were identified by 16S rRNA gene sequencing and were susceptible to antibiotics commonly used for anaerobic infections. The clinical significance of these organisms as potential emerging pathogens is discussed.

**CASE REPORT**

**Case 1.** A 90-year-old man was admitted to the Centre hospitalier universitaire Dr Georges-L.-Dumont (Moncton, NB, Canada) for the care of a severe proctitis following rectal radiotherapy. The patient was receiving oral palliative chemotherapy with capecitabine for a rectal adenocarcinoma with lung metastasis. The patient had developed rectal bleeding and pain associated with fecal incontinence three days before his admission. He had a pacemaker and a past medical history of haemorrhoidectomy.

On admission, the patient was afebrile, with rectal pain and redness. There were no signs or symptoms of infection. The patient was managed with analgesia and local topical corticotherapy. The complete blood count on admission was within normal range, except for a hemoglobin of 107 g/L (normal: 132-170 g/L). On the 16th day of hospitalization, he developed a fever of 39.8°C, with no other symptoms except for the rectal pain. There was redness of the rectal area and pain at rectal examination. Hematological analysis revealed leukocytosis with a white cell count of 23.9 x 10^9/L (normal: 4.0-11.0 x 10^9/L), hemoglobin level of 101 g/L and a platelet count of 185 x 10^9/L (normal: 130-400 x 10^9/L).
$10^9$/L). A computerized tomography (CT)-scan of the chest, abdomen and pelvis showed a small amount of right-sided pleural effusion, two pulmonary nodules, and bladder and rectal wall thickening with prostate enlargement. Two sets of aerobic and anaerobic blood cultures were collected. Empirical antimicrobial therapy consisted of 3.375g of intravenous piperacillin-tazobactam administered every 6 h. The patient defervesced within 24 hours of the introduction of the intravenous antibiotic. On the 18th day of hospitalization, the intravenous antibiotic was discontinued. Oral ciprofloxacin and metronidazole were prescribed for 10 days. After 141 hours of incubation at 37°C in aerobic and anaerobic blood culture systems (BD BACTEC, Becton, Dickinson, Sparks, MD, USA), one anaerobic blood culture bottle was positive for rare Gram-negative bacilli. Strictly anaerobic Gram-negative bacilli were later isolated on BD Brucella blood agar (Becton, Dickinson) supplemented with hemin (5 µg/mL) and vitamin K (1 µg/mL), after 6 days of incubation at 37°C. Preliminary identification tests for anaerobes using special potency disks showed an inhibition zone diameter $>$ 10 mm for kanamycin (1000 µg), whereas there was no inhibition zone for colistin (10 µg) and vancomycin (5 µg). The isolate could not be identified by RapID ANA II system (Remel, Lenexa, KS, USA). The strictly anaerobic, slow growing Gram-negative bacillus was identified as *Cloacibacillus evryensis* based on 16S rRNA gene nucleotide sequence identities with the species type strain (158$^T$) and designated strain LSPQ-04215 (Laboratoire de santé publique du Québec, strain 04215). No other organism was isolated from blood cultures. The patient was discharged on the 22nd day of hospitalization. One year later, he was living at home, under oral palliative chemotherapy. He had no relapse of his bacteremia.
Case 2. A 94-year-old community-dwelling woman was admitted to the McGill University Health Centre (Montreal, QC, Canada) for presumed urosepsis complicated by acute delirium and hypovolemic hyponatremia. Her past medical history included atrial fibrillation requiring anticoagulation, coronary artery disease, hypertension, dyslipidemia, osteoporosis, and penicillin allergy. On arrival at hospital, the patient was delirious, with a temperature of 36.8°C. Other vital signs were within normal parameters. A urinalysis revealed pyuria and the presence of urinary nitrates. A complete blood count revealed leukocytosis (white blood cells 17.8 x 10⁹/L), hemoglobin concentration of 133 g/L and a platelet count of 313 x 10⁹/L. Chest radiography was unremarkable. Urine and one set each of aerobic and anaerobic blood cultures were performed (BD BACTEC). Empirical antimicrobial therapy with intravenous ceftriaxone 1 g every 12 hours was initiated for presumed community-acquired urosepsis. The patient’s condition improved rapidly and on the third day of admission, antibiotherapy was changed to oral trimethoprim-sulfamethoxazole (TMP-SMX). The urine culture yielded *Escherichia coli* >10⁸ CFU/mL, whereas growth was detected in the anaerobic blood culture bottle at 91 hours of incubation and yielded small Gram-negative bacilli. Slow growth of a strictly anaerobic Gram-negative bacillus was observed on 5% sheep blood agar. After five days of incubation, preliminary identification tests for anaerobes using special potency disks showed an inhibition zone diameter of 14 mm for kanamycin (1000 µg), and no inhibition zone for colistin (10 µg) or vancomycin (5 µg). Bile media did not inhibit growth of the organism. The isolate could not be identified by RapID ANA II system. The strictly anaerobic, slow growing Gram-negative bacillus was identified as *Cloacibacillus evryensis* based on 16S rRNA gene nucleotide sequence identities with
the species type strain (158T) and designated strain LSPQ-04216. No other organism was
isolated from blood cultures. The patient was discharged on the fourth day following
admission with a prescription of TMP-SMX to complete 14 days of antibiotherapy.

Case 3. A 54-year-old woman was admitted to the Centre Hospitalier de l’Université de
Montréal-Hôpital Saint-Luc (Montreal, QC, Canada) for vomiting, right iliac fossa pain
and tenderness and temperature of 39.5°C for the last 24 hours. The patient was known
for obesity (118 kg) and a previous medical history of cholecystectomy. The medical
exam revealed a positive McBurney point. Hematological investigations revealed
leukocytosis with a white cell count of 17.5 x 10⁹/L with 90% neutrophils, hemoglobin
level of 155 g/L, platelet count of 230 x 10⁹/L, creatinine at 62 µmol/L, and total bilirubin
at 29.6 µmol/L (normal 7-23). The aspartate aminotransferase, alanine transaminase and
alkaline phosphatase were normal. The urine biochemical analysis and bacterial culture
were normal and negative, respectively. Two sets of blood cultures were done (BD
BACTEC). The abdomino-pelvic CT scan showed a 9 cm uterine fibroma, hepatic
steatosis, inflamed fat with gas bubbles around the appendix, compatible with acute
appendicitis. Empirical antimicrobial therapy consisted of 3.375 grams of intravenous
piperacillin-tazobactam every 6 hours. The patient improved progressively, became
afebrile with a decrease in abdominal pain. One anaerobic bottle out of the two blood
cultures was positive for a slowly growing anaerobic Gram-negative rod and later
isolated on fastidious anaerobe agar (Oxoid, Basingstoke, United Kingdom). No other
organism was isolated. The strictly anaerobic, slow growing Gram-negative bacillus was
identified as *Cloacibacillus porcorum* based on 16S rRNA gene nucleotide sequence identities with the species type strain (CL-84T) and designated strain LSPQ-04226. On the 4th day, the white cell count was $8.6 \times 10^9$/L with 73% neutrophils, the hemoglobin level at 115 g/L, the platelet count at $269 \times 10^9$/L and the creatinine at 62 µmol/L. The intravenous antimicrobial agent was changed for oral amoxicillin-clavulanic acid at 875 mg every 12 h for 10 days. The patient continued to improve and was followed at the outpatient clinic by the digestive surgeon. Three months later, a complete colonoscopy showed only slight sigmoid diverticular disease.

**Discussion.**

The genus *Cloacibacillus* was first described by Ganesan *et al* (1) and comprises 2 validly described species, *C. evryensis* and *C. porcorum* (2). Cells of the genus *Cloacibacillus* are strictly anaerobic Gram-negative bacilli, non-motile and originally isolated from environmental sources. *C. evryensis* was isolated from an anaerobic digester of a wastewater treatment plant (1); *C. porcorum* was isolated from the mucosal lining of a pig caecum (2). Members of this genus can be identified by 16S rRNA gene sequencing, cellular fatty acid profiles, DNA G+C content and metabolic end products analysis (1). The genus *Cloacibacillus* is a member of the family *Synergistaceae* in the phylum *Synergistetes* described in 2009 (3). The phylum contains 12 genera: *Aminiphilus, Aminobacterium, Aminomonas, Anaerobaculum, Cloacibacillus, Dethiosulfovibrio, Fretibacterium, Jonquetella, Pyramidobacter, Synergistes, Thermanaerovibrio, and Thermovirga* (4). Previously, most 16S rRNA gene sequences
available from this phylum were obtained from culture-independent studies (3, 5-11). A small number of isolates were shown to be present in human infections, including soft tissue infections, abscesses, blood, peritoneal fluid, and dental infections (5-11).

However, the lack of identification of *Synergistetes* isolates at the genus level led to the generic grouping under the *Synergistes* group of organisms (SGOs) (3, 7, 11-13). With the initial description of the genus *Cloacibacillus* in 2008, 16S rRNA gene sequencing and phylogenetic analysis are now available for the proper identification of *Cloacibacillus* species (1, 2). We report here the first clinical description of *Cloacibacillus* bacteremia in three patients from whom either *C. evryensis* or *C. porcorum* was isolated. The three isolates, LSPQ-04215, LSPQ-04216 and LSPQ-04226, were identified by 16S rRNA gene sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit's on an ABI3130 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) (14). To identify the taxonomic neighbours of the three clinical isolates, the 16S rRNA gene sequences (GenBank accession number for isolates LSPQ-04215, LSPQ-04216 and LSPQ-04226 are KM881707, KM881708 and KP851977, respectively) were used for an initial BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) against GenBank. Phylogenetic and molecular evolutionary analyses with genera of the phylum *Synergistetes* were performed on 1136 nucleotides using MEGA version 5 (15). Isolates LSPQ-04215 and LSPQ-04216 showed 100% nucleotide sequence identities with *C. evryensis* strain 158T (GenBank accession CU463952). Likewise, LSPQ-04226 showed 100% nucleotide sequence identities with *C. porcorum* strain CL-84T (GenBank accession JQ809697). The two *C. evryensis* isolates, LSPQ-04215 and LSPQ-04216, shared 97% nucleotide sequence identities with the 16S rRNA gene sequences of both *C.
porcorum CL-84T and LSPQ-04226. A Neighbour-joining phylogenetic tree showed that isolates LSPQ-04215 and LSPQ-04216 clustered with C. evryensis 158T while LSPQ-04226 clustered with C. porcorum CL-84T (Figure 1). Interestingly, we found that several isolates previously named Synergistes sp., Synergistes bacterium, or simply ‘Bacterium’ in the features contained in their annotation sequences submitted to GenBank, clearly belonged to the species C. evryensis or C. porcorum (Figure 1). Several Cloacibacillus-like strains were isolated from human infections, but the use of older taxonomic classification positioned them on various branches of the SGO phylogeny (Figure 1). In the three University Hospitals from which isolates LSPQ-04215, LSPQ-04216 and LSPQ-04226 originated, conventional anaerobic characterization was performed according to the Wadsworth Anaerobic Bacteriology Manual (16) and was based on growth under anaerobic conditions, Gram stain, commercially available anaerobe identification substrates, susceptibility testing to kanamycin, colistin, vancomycin and growth on bile media. Cloacibacillus species are obligate anaerobes that grow slowly (1, 2). The calculated doubling time previously reported, varies from 8 hours for C. porcorum to 15 hours for C. evryensis (1, 2), which could explain the rare Gram negative bacilli observed from blood culture after more than 3 days of incubation in our case reports. All three isolates, LSPQ-04215, LSPQ-04216 and LSPQ-04226, are susceptible to kanamycin and resistant to colistin and vancomycin, like C. evryensis strain 158T and C. porcorum CL-84T (1, 2). The conventional phenotypic based-methods for anaerobes failed to identify Cloacibacillus species. The three isolates could not be identified using Vitek MS MALDI-TOF (bioMérieux Canada, Saint-Laurent, QC). A single isolate, C. evryensis LSP-04215, was tested on Bruker MALDI Biotyper (Bruker, Madison, WI,
USA). It could not be identified. The genus *Cloacibacillus* was not included in the Vitek MS IVD version 2.0 (bioMérieux Canada), a clinically relevant species database, and SARAMIS version 4.1 (Spectral ARchive And Microbial Identification System) (bioMérieux Canada) or in the Bruker MALDI Biotyper databases. New spectra entries were added to the SARAMIS database using cultures from three different times of incubation (3, 7 and 10 days) for each of the three isolates. Based on this newly updated library, the SARAMIS database was capable of identifying the three isolates and assign them to either *C. evryensis* or *C. porcorum*, respectively. We are planning to add both type strains (*C. evryensis* 158\textsuperscript{T} and *C. porcorum* CL-84\textsuperscript{T}) in the SARAMIS database in the near future. Antimicrobial susceptibility of the three isolates was determined by dilution agar method, after 48 h of anaerobic incubation on prerduced sheep laked blood agar supplemented with hemin (5 µg/mL) and vitamin K (1 µg/mL), as recommended by the Clinical and Laboratory Standards Institute (17). The minimal inhibitory concentration (MIC) of each isolate is reported in Table 1. As human infections with both *Cloacibacillus* species have remained largely uncharacterized, we reviewed the patients' charts for symptoms of infection, inflammation parameters such as fever, leukocyte counts, monomicrobial bacteremia, and clinical diagnosis. We also compared isolation sites and clinical diagnosis of our three cases to the previous cases of *Cloacibacillus*-like strains using the features contained in the annotation of the sequence data submitted to GenBank (Table 2). The latter were isolated from the environment and from human blood, peritoneal fluid and digestive tract (Table 2). The patients in cases 1 and 3 had proctitis and appendicitis, respectively, indicating a probable intestinal origin of the bacteremia. The probable intestinal origin of the *Cloacibacillus* bacteremia in our three
case reports is in accordance with the isolation sites of several *Cloacibacillus*-like strains reviewed in Table 2. The antimicrobial susceptibility testing of the three *Cloacibacillus* isolates showed susceptibility to antibiotics commonly used for anaerobic infections. The patients all improved clinically after initiation of antibiotherapy. The three cases presented suggest that *Cloacibacillus* sp are low-virulence, opportunistic pathogens associated with elderly or otherwise debilitated hosts. It is noteworthy that the person described in case 2 made a full clinical recovery despite receiving only ceftriaxone and trimethoprim-sulfamethoxazole, two antibiotics with limited uses against anaerobes. None of the patients described relapsed with *Cloacibacillus* bacteremia, and none of the patients died within 30 days.

Physicians should be aware that *Cloacibacillus* species can be opportunistic human pathogens associated with intestinal infections.
Table 1. Minimal inhibitory concentration (MIC) of selected antibiotics on *C. evryensis* LSPQ-04215 and LSPQ-04216 and *C. porcorum* LSPQ-04226

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>C. evryensis</em> LSPQ-04215</th>
<th><em>C. evryensis</em> LSPQ-04216</th>
<th><em>C. porcorum</em> LSPQ-04226</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.25</td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≤ 2</td>
<td>≤ 2</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.125</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.5</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>2/4</td>
<td>1/4</td>
<td>2/4</td>
</tr>
</tbody>
</table>
TABLE 2: Species identifications of *Cloacibacillus* and *Cloacibacillus*-like 16S rRNA sequences deposited in GenBank, their isolation sites and relevant clinical data.

<table>
<thead>
<tr>
<th>16S rRNA gene sequence identification in GenBank</th>
<th>Strain name (GenBank accession no)</th>
<th>Identification based on ≥ 99% homology with type strain</th>
<th>Isolation site</th>
<th>Clinical diagnosis</th>
<th>Human clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. evryensis</em></td>
<td>158^T (CU463952)</td>
<td><em>C. evryensis</em></td>
<td>Environment</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td><em>C. evryensis</em></td>
<td>LSPQ-04215 (KM881707)</td>
<td><em>C. evryensis</em></td>
<td>Blood</td>
<td>Bacteremia</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>RMA16088 (DQ412717)</td>
<td><em>C. evryensis</em></td>
<td>Peritoneal fluid</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>NML05A017 (EF551161)</td>
<td><em>C. evryensis</em></td>
<td>Blood</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>RMA 14605 (DQ412717)</td>
<td><em>C. evryensis</em></td>
<td>Peritoneal fluid</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>ADV66 (EF468684)</td>
<td><em>C. evryensis</em></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>RMA 15677 (EU476080)</td>
<td><em>C. evryensis</em></td>
<td>Peritoneal fluid</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td><em>C. porcorum</em></td>
<td>CL-84^T (IQ809697)</td>
<td><em>C. porcorum</em></td>
<td>Environment</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td><em>C. porcorum</em></td>
<td>LSPQ-04226 (KF851977)</td>
<td><em>C. porcorum</em></td>
<td>Blood</td>
<td>Bacteremia</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>RMA 16290 (DQ412721)</td>
<td><em>C. porcorum</em></td>
<td>Peritoneal fluid</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>Synergistes sp.</td>
<td>NML06A088 (EF551160)</td>
<td><em>C. porcorum</em></td>
<td>Blood</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistes sp.</td>
<td>NML060450 (EF551162)</td>
<td><em>C. porcorum</em></td>
<td>Blood</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Synergistetes bacterium</td>
<td>RK1 (JN585290)</td>
<td><em>C. porcorum</em></td>
<td>Digestive tract of red Kangaroos (<em>Macropus rufus</em>)</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Synergistetes bacterium</td>
<td>EF1 (JN585291)</td>
<td><em>C. porcorum</em></td>
<td>feces of emu (<em>Dromaius novaehollandiae</em>)</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Bacterium</td>
<td>NLAE--to--C457 (JQ608160)</td>
<td><em>C. porcorum</em></td>
<td>feces of cow</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Synergistetes sp</td>
<td>LBVCM1157 (GQ258969)</td>
<td><em>C. porcorum</em></td>
<td>Blood</td>
<td>NA</td>
<td>Yes</td>
</tr>
</tbody>
</table>
The two *C. evryensis* and the *C. porcorum* strains isolated in this study are in bold characters.
Figure 1: Neighbour-joining phylogenetic tree based on a comparison of partial (1136 nt) 16S rRNA gene sequences, showing the relationships between strains LSPQ-04215, LSPQ-04216, LSPQ-04226 and related taxa within the *Cloacibacillus* genus and other members of the *Synergistetes* phylum. *Bacteroides faecis MAJ27* was used as an outgroup. Bootstrap percentages (based on 1000 replications) higher than 90% are shown at branching points. Names of strains and accession numbers of 16S rRNA genes are as cited in GenBank. GenBank accession numbers for each 16S rRNA gene sequence are given between parentheses. The scale bar indicates the estimated number of substitutions per site. T: Type strain.
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