

# *Cloacibacillus* sp., a Potential Human Pathogen Associated with Bacteremia in Quebec and New Brunswick

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**Bacteremia due to *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 was that of a 90-year-old man who was admitted to the Centre Hospitalier Universitaire Dr. Georges-L.-Dumont (Moncton, NB, Canada) for 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 3 days before his admission. He had a pacemaker and a medical history of hemorrhoidectomy.

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 the normal range, except for a hemoglobin level of 107 (normal, 132 to 170) g/liter. On day 16 of hospitalization, he developed a fever of 39.8°C with no other symptoms except for rectal pain. There was redness of the rectal area and pain upon rectal examination. Hematological analysis revealed leukocytosis with a white cell count of  $23.9 \times 10^9$  (normal,  $4.0 \times 10^9$  to  $11.0 \times 10^9$ )/liter, a hemoglobin level of 101 g/liter, and a platelet count of  $185 \times 10^9$  (normal,  $130 \times 10^9$  to  $400 \times 10^9$ )/liter. A computerized tomography (CT) scan of the chest, abdomen, and pelvis showed a small amount of right-side 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 3.375 g of intravenous piperacillin-tazobactam administered every 6 h. The patient defervesced within 24 h of the introduction of the intravenous antibiotic. On day 18 of hospitalization, the intravenous antibiotic was discontinued. Oral ciprofloxacin and metronidazole were prescribed for 10 days. After 141 h 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 with special-potency disks showed an inhibition zone diameter of >10 mm for kanamycin (1,000 µg), whereas there was no inhibition zone for colistin (10 µg) or vancomycin (5 µg). The isolate could not be identified by the RapID ANA II system (Remel, Lenexa, KS, USA). The strictly anaerobic, slowly

growing, Gram-negative bacillus was identified as *Cloacibacillus evryensis* on the basis of 16S rRNA gene nucleotide sequence identity with the species type strain (158<sup>T</sup>) 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 day 22 of hospitalization. One year later, he was living at home under oral palliative chemotherapy. He had no relapse of his bacteremia.

Case 2 was that of a 94-year-old community-dwelling woman who was admitted to the McGill University Health Centre (Montreal, QC, Canada) for presumed urosepsis complicated by acute delirium and hypovolemic hyponatremia. Her medical history included atrial fibrillation requiring anticoagulation, coronary artery disease, hypertension, dyslipidemia, osteoporosis, and penicillin allergy. On arrival at the hospital, the patient was delirious with a temperature of 36.8°C. Other vital signs were within normal parameters. Urinalysis revealed pyuria and the presence of urinary nitrates. A complete blood count revealed leukocytosis (white blood cell count,  $17.8 \times 10^9$ /liter), a hemoglobin concentration of 133 g/liter, and a platelet count of  $313 \times 10^9$ /liter. Chest radiography was unremarkable. A urine culture and one set each of aerobic and anaerobic blood cultures were performed (BD Bactec). Empirical intravenous antimicrobial therapy with 1 g of ceftriaxone every 12 h was initiated for presumed community-acquired urosepsis. The patient's condition improved rapidly, and on day 3 after admission, her antibiotherapy was changed to oral trimethoprim-sulfamethoxazole (TMP-SMX). The urine culture yielded *Escherichia coli* at  $>10^8$  CFU/ml, whereas growth was detected in the anaerobic blood culture bottle at 91 h of incubation

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and yielded small Gram-negative bacilli. Slow growth of a strictly anaerobic Gram-negative bacillus was observed on 5% sheep blood agar. After 5 days of incubation, preliminary identification tests for anaerobes using special-potency disks showed an inhibition zone diameter of 14 mm for kanamycin (1,000 µg) and no inhibition zone for colistin (10 µg) or vancomycin (5 µg). Bile medium did not inhibit the growth of the organism. The isolate could not be identified by the RapID ANA II system. The strictly anaerobic, slowly growing, Gram-negative bacillus was identified as *C. evryensis* on the basis of 16S rRNA gene nucleotide sequence identity with the species type strain (158<sup>T</sup>) and was designated strain LSPQ-04216. No other organism was isolated from blood cultures. The patient was discharged on day 4 following admission with a prescription for TMP-SMX to complete 14 days of antibiotic therapy.

**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 a temperature of 39.5°C for the last 24 h. The patient was known for obesity (118 kg) and a medical history of cholecystectomy. The medical exam revealed a positive McBurney point. Hematological investigations revealed leukocytosis with a white cell count of  $17.5 \times 10^9$ /liter with 90% neutrophils, a hemoglobin level of 155 g/liter, a platelet count of  $230 \times 10^9$ /liter, a creatinine level of 62 µmol/liter, and a total bilirubin level of 29.6 (normal, 7 to 23) µmol/liter. The aspartate aminotransferase, alanine transaminase, and alkaline phosphatase levels were normal. The urine biochemical analysis and bacterial culture were normal and negative, respectively. Two sets of blood cultures were done (BD Bactec). An abdomino-pelvic CT scan showed a 9-cm uterine fibroma, hepatic steatosis, and inflamed fat with gas bubbles around the appendix, compatible with acute appendicitis. Empirical antimicrobial therapy consisted of 3.375 g of intravenous piperacillin-tazobactam every 6 h. The patient improved progressively and 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 that was later isolated on fastidious anaerobe agar (Oxoid, Basingstoke, United Kingdom). No other organism was isolated. The strictly anaerobic, slowly growing, Gram-negative bacillus was identified as *Cloacibacillus porcorum* on the basis of 16S rRNA gene nucleotide sequence identity with the species type strain (CL-84<sup>T</sup>) and was designated strain LSPQ-04226. On day 4, the white cell count was  $8.6 \times 10^9$ /liter with 73% neutrophils, the hemoglobin level at 115 g/liter, the platelet count was  $269 \times 10^9$ /liter, and the creatinine level was 62 µmol/liter. The intravenous antimicrobial agent was changed to oral amoxicillin-clavulanic acid at 875 mg every 12 h for 10 days. The patient continued to improve and was followed up at the outpatient clinic by the digestive surgeon. Three months later, a complete colonoscopy showed only slight sigmoid diverticular disease.

The genus *Cloacibacillus* was first described by Ganesan et al. (1) and comprises two validly described species, *C. evryensis* and *C. porcorum* (2). Cells of the genus *Cloacibacillus* are strictly anaerobic, Gram-negative bacilli that are nonmotile and were originally isolated from environmental sources. *C. evryensis* was isolated from an anaerobic digester of a wastewater treatment plant

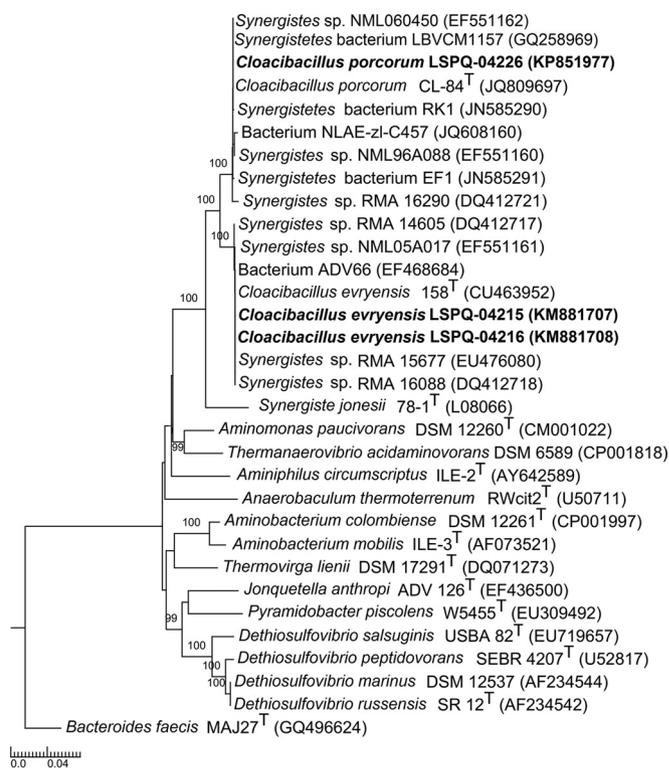


FIG 1 Neighbor-joining phylogenetic tree based on a comparison of partial 16S rRNA gene sequences (1,136 nucleotides) showing the relationships among strains LSPQ-04215, LSPQ-04216, and LSPQ-04226 and related taxa within the *Cloacibacillus* genus and other members of the *Synergistetes* phylum. *Bacteroides faecis* MAJ27<sup>T</sup> was used as an outgroup. Bootstrap percentages (based on 1,000 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 16S rRNA gene sequences are shown in parentheses. The scale bar indicates the estimated number of substitutions per site. A superscript T indicates a type strain.

(1); *C. porcorum* was isolated from the mucosal lining of a pig cecum (2). Members of this genus can be identified by 16S rRNA gene sequencing, cellular fatty acid profiles, DNA G+C content, and metabolic end product analysis (1). The genus *Cloacibacillus* is a member of the family *Synergistaceae* in the phylum *Synergistetes*, which was 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 of the 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 (SGO) (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 iden-

TABLE 1 MICs of selected antibiotics for *C. evryensis* LSPQ-04215 and LSPQ-04216 and *C. porcorum* LSPQ-04226

Antibiotic	MIC (μg/ml)		
	<i>C. evryensis</i> LSPQ-04215	<i>C. evryensis</i> LSPQ-04216	<i>C. porcorum</i> LSPQ-04226
Clindamycin	0.25	0.5	0.5
Penicillin	0.25	≤0.125	≤0.125
Cefoxitin	≤2	≤2	≤2
Meropenem	0.125	0.03	0.03
Metronidazole	0.5	0.06	0.5
Piperacillin-tazobactam	2/4	1/4	2/4

tified by 16S rRNA gene sequencing with the BigDye Terminator v3.1 Cycle Sequencing kit on an ABI3130 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) (14). To identify the taxonomic neighbors of the three clinical isolates, the 16S rRNA gene sequences 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 1,136 nucleotides with MEGA version 5 (15). Isolates LSPQ-04215 and LSPQ-04216 showed 100% nucleotide sequence identity with *C. evryensis* strain 158<sup>T</sup> (GenBank accession no. CU463952). Likewise, LSPQ-04226 showed 100% nucleotide sequence identity with *C. porcorum* strain CL-84<sup>T</sup> (GenBank accession no. JQ809697). The two *C. evryensis* isolates, LSPQ-04215 and LSPQ-04216, showed 97% nucleotide sequence identity with the 16S rRNA gene sequences of both *C. porcorum* CL-84<sup>T</sup> and LSPQ-04226. A neighbor-joining phylogenetic tree showed that isolates LSPQ-04215 and LSPQ-04216 clustered with *C. evryensis* 158<sup>T</sup> while LSPQ-04226 clustered with *C. porcorum* CL-84<sup>T</sup> (Fig. 1). Interestingly, we found that several isolates pre-

viously 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* (Fig. 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 (Fig. 1). In the three university hospitals where isolates LSPQ-04215, LSPQ-04216, and LSPQ-04226 originated, conventional anaerobic characterization was performed according to reference 16 and was based on growth under anaerobic conditions; Gram staining; commercially available anaerobe identification substrates; testing for susceptibility to kanamycin, colistin, and vancomycin; and growth on bile medium. *Cloacibacillus* species are obligate anaerobes that grow slowly (1, 2). The calculated doubling time previously reported varies from 8 h for *C. porcorum* to 15 h for *C. evryensis* (1, 2), which could explain the rare Gram-negative bacilli observed in blood cultures after more than 3 days of incubation in the cases reported here. All three isolates, LSPQ-04215, LSPQ-04216, and LSPQ-04226, are susceptible to kanamycin and resistant to colistin and vancomycin, like *C. evryensis* 158<sup>T</sup> and *C. porcorum* CL-84<sup>T</sup> (1, 2). The conventional phenotype-based methods for anaerobes failed to identify *Cloacibacillus* species. The three isolates could not be identified by Vitek MS matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (bioMérieux Canada, Saint-Laurent, QC, Canada). A single isolate, *C. evryensis* LSP-04215, was tested on a Bruker MALDI Biotyper (Bruker, Madison, WI, USA). It could not be identified. The genus *Cloacibacillus* was not included in Vitek MS IVD version 2.0 (bioMérieux Canada), a clinically relevant species database; SARAMIS (spectral archive and microbial identification system) version 4.1 (bioMérieux Canada); or in the Bruker MALDI Biotyper databases. New spec-

TABLE 2 Identification of *Cloacibacillus* and *Cloacibacillus*-like 16S rRNA sequences deposited in GenBank to the species level, their isolation sites, and relevant clinical data

16S rRNA gene sequence identification in GenBank <sup>a</sup>	Strain name (GenBank accession no.)	Identification based on ≥99% homology with type strain	Isolation site	Clinical diagnosis	Human clinical relevance
<i>C. evryensis</i>	158 <sup>T</sup> (CU463952)	<i>C. evryensis</i>	Environment	NA <sup>b</sup>	No
<i>C. evryensis</i>	LSPQ-04215 (KM881707)	<i>C. evryensis</i>	Blood	Bacteremia	Yes
<i>C. evryensis</i>	LSPQ-04216 (KM881708)	<i>C. evryensis</i>	Blood	Bacteremia	Yes
<i>Synergistes</i> sp.	RMA16088 (DQ412718)	<i>C. evryensis</i>	Peritoneal fluid	NA	Yes
<i>Synergistes</i> sp.	NML05A017 (EF551161)	<i>C. evryensis</i>	Blood	NA	Yes
<i>Synergistes</i> sp.	RMA 14605 (DQ412717)	<i>C. evryensis</i>	Peritoneal fluid	NA	Yes
<i>Synergistes</i> sp.	ADV66 (EF468684)	<i>C. evryensis</i>	NA	NA	NA
<i>Synergistes</i> sp.	RMA 15677 (EU476080)	<i>C. evryensis</i>	Peritoneal fluid	NA	Yes
<i>C. porcorum</i>	CL-84 <sup>T</sup> (JQ809697)	<i>C. porcorum</i>	Environment	NA	No
<i>C. porcorum</i>	LSPQ-04226 (KP851977)	<i>C. porcorum</i>	Blood	Bacteremia	Yes
<i>Synergistes</i> sp.	RMA 16290 (DQ412721)	<i>C. porcorum</i>	Peritoneal fluid	NA	Yes
<i>Synergistes</i> sp.	NML96A088 (EF551160)	<i>C. porcorum</i>	Blood	NA	Yes
<i>Synergistes</i> sp.	NML060450 (EF551162)	<i>C. porcorum</i>	Blood	NA	Yes
<i>Synergistetes</i> bacterium	RK1 (JN585290)	<i>C. porcorum</i>	Digestive tract of red Kangaroo ( <i>Macropus rufus</i> )	NA	No
<i>Synergistetes</i> bacterium	EF1 (JN585291)	<i>C. porcorum</i>	Emu ( <i>Dromaius novaehollandiae</i> ) feces	NA	No
Bacterium	NLAE-zl-C457 (JQ608160)	<i>C. porcorum</i>	Cow feces	NA	No
<i>Synergistetes</i> sp.	LBVCM1157 (GQ258969)	<i>C. porcorum</i>	Blood	NA	Yes
<i>Synergistes jonesii</i>	78-1 <sup>T</sup> (L08066)	<i>C. porcorum</i>	Rumen tract	NA	No

<sup>a</sup> The two *C. evryensis* strains and the *C. porcorum* strain isolated in this study are underlined.

<sup>b</sup> NA, not available.

trum entries were added to the SARAMIS database by using cultures from three different times of incubation (3, 7, and 10 days) for each of the three isolates. On the basis of this newly updated library, the SARAMIS database was capable of identifying the three isolates and assigned them to either *C. evryensis* or *C. porcorum*, respectively. We are planning to add both type strains (*C. evryensis* 158<sup>T</sup> and *C. porcorum* CL-84<sup>T</sup>) to the SARAMIS database in the near future. The antimicrobial susceptibilities of the three isolates were determined by the agar dilution method after 48 h of anaerobic incubation on prereduced laked sheep 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 MICs for each isolate are 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 the isolation sites and clinical diagnoses of our three cases to the previous cases of *Cloacibacillus*-like strains by 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 the digestive tract (Table 2). The patients in cases 1 and 3 had proctitis and appendicitis, respectively, indicating a probable intestinal origin of their 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* species are low-virulence, opportunistic pathogens associated with elderly or otherwise debilitated hosts. It is noteworthy that the patient described in case 2 made a full clinical recovery despite receiving only ceftriaxone and TMP-SMX, 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.

**Nucleotide sequence accession numbers.** The GenBank accession numbers for isolates LSPQ-04215, LSPQ-04216, and LSPQ-04226 are [KM881707](#), [KM881708](#), and [KP851977](#), respectively.

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