

Four Cases of Bacteremia Caused by *Oscillibacter ruminantium*, a Newly Described Species

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The genus *Oscillibacter* has been known since 2007, but no association to human infection has been reported. Here, we present four cases of *Oscillibacter ruminantium* bacteremia from hospitals across Denmark from 2001 to 2010. Correct identification is now possible, as the 16S rRNA gene sequence was recently made publicly available.

CASE REPORTS

Case 1 (year 2001) is a 53-year-old man who was admitted to the medical department with nausea and diarrhea, with a previous medical history of insulin-dependent diabetes mellitus, alcohol dependency, and chronic pancreatitis. On admittance, his temperature was 35.6°C and his blood pressure was 70/50 mm Hg. The total leukocyte count was 32.3×10^9 cells/liter (normal value range, 3.5×10^9 to 8.8×10^9), and the C-reactive protein level was 169 mg/liter (normal value, <10 mg/liter). Blood cultures were drawn and antibiotic treatment was initiated with intravenous benzylpenicillin, gentamicin, and metronidazole. After 1 day of incubation, blood cultures (Bactec Plus aerobic/F vial and Bactec 9240 automated instrument; Becton, Dickinson Diagnostic Instrument Systems, Franklin Lakes, NJ, USA) were positive with coagulase-negative staphylococci, and the following day, after 45 h of incubation, the anaerobic vial (Bactec Plus anaerobic/F) showed growth of anaerobic Gram-negative rods. Benzylpenicillin was discontinued in favor of intravenous cefuroxime.

The patient became afebrile but developed diffuse abdominal pains. A computerized tomography scan of his abdomen showed pancreatic calcifications and small quantities of peritoneal ascites. New blood cultures drawn were negative, and antibiotics were discontinued after a total of 10 days of treatment. The patient subsequently developed a large pleural effusion, but successful pleuracentesis could not be performed and the patient succumbed.

The Gram-negative rods isolated from the first set of blood cultures were sent to an anaerobe reference laboratory and identified using conventional anaerobic identification methods (1) as a possible *Clostridium* species.

Case 2 (year 2002) is a 78-year-old man who was admitted to the department of medicine with fatigue and epistaxis and with a previous medical record of ulcerative colitis, a mechanical aortic valve due to severe aortic stenosis, and hemicolectomy, including sigmoidectomy due to colon adenocarcinoma. The C-reactive protein level was 121 mg/liter, and the erythrocyte sedimentation rate was elevated to 110 (normal value, 0). The total blood leukocyte count was not elevated, and he was afebrile. Blood cultures drawn on admittance were without growth. The patient was diagnosed with a relapse of the colonic adenocarcinoma with metastasis to the liver and discharged 20 days later. Blood cultures (Bactec Plus anaerobic/F vial) drawn 1 day prior to discharge showed growth of anaerobic Gram-negative rods. The isolate was sent to

an anaerobe reference laboratory and identified as a possible *Clostridium* sp. by using conventional anaerobic identification methods (1). To our knowledge, the patient did not receive antibiotic treatment. In the following months, the patient was frequently readmitted for drainage of copious amounts of ascites. Ascites cultures were without growth. The patient was treated with chemotherapy and prednisolone and died 6 months after the initial admittance.

Case 3 (year 2009) is a 61-year-old man with known alcohol dependency who was admitted to the emergency medical ward due to a fall at home and confusion. On admittance, his temperature was 37.1°C. On suspicion of urinary tract infection, treatment with oral pivmecillinam was initiated. Pivmecillinam is an oral agent used for treating lower urinary tract infections. The patient showed signs of alcohol withdrawal syndrome, and 4 days after admission he developed stage four hepatic encephalopathy and was transferred to the intensive care unit. Due to suspected aspiration pneumonia, blood cultures were drawn and intravenous treatment with cefuroxime and metronidazole was initiated. Cefuroxime was subsequently changed to piperacillin-tazobactam due to a rise in C-reactive protein levels to 83 mg/liter from 61 mg/liter. He received a total of 10 days of intravenous antibiotic treatment. The patient recovered from the hepatic encephalopathy and was discharged to a nursing home 5 weeks after admittance.

After 72 h of incubation, blood cultures (BacT/Alert using standard anaerobic culture bottles; bioMérieux, Marcy l'Etoile, France) showed growth of anaerobic Gram-negative rods. Growth on solid chocolate agar with added vitamin K and cysteine (Statens Serum Institut Diagnostica, Hillerød, Denmark) was observed after 44 h. Partial 16S rRNA gene sequencing (MicroSeq 500 system; Perkin-Elmer, Applied Biosystems Division, Foster City, CA) was performed, and submission of the consensus sequence to the EzTaxon server (2) resulted in a pairwise similarity score of

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94.363% to *Oscillibacter valericigenes* (GenBank accession no. AB238598.1).

Case 4 (year 2010) is an 80-year-old woman who was admitted to the department of abdominal surgery with a distended abdomen and with a previous medical history of Alzheimer's dementia and hypothyroidism. Oral benzylpenicillin prescribed 12 h prior to admittance on suspicion of pneumonia was discontinued. An X ray of the abdomen showed volvulus, and a sigmoidoscopy with successful desufflation was performed. Shortly thereafter, the patient developed fever with a temperature of 38.2°C and septic shock and died. As part of the diagnostic workup, two sets of blood cultures (BacT/Alert standard blood culture bottles) were drawn. After 2 days of incubation, both anaerobe blood culture bottles were positive with anaerobic Gram-negative rods. Growth on solid chocolate agar with added vitamin K and cysteine was observed after 24 h. Submitting the partially sequenced 16S rRNA consensus sequence to the EzTaxon server resulted in a pairwise similarity score of 94.316% to *O. valericigenes* (GenBank accession no. AB238598.1).

In 2009, Justesen et al. undertook a project to identify anaerobic Gram-positive rods from an anaerobe isolate collection using partial 16S rRNA gene sequencing (19). Isolates from cases 1 and 2 had been identified as possible *Clostridium* species and were included, as some *Clostridium* species are known to appear Gram negative or Gram variable (1). It was noted that attempts to identify these isolates by querying sequences to NCBI BLAST and EzTaxon yielded low sequence similarity scores to known species, the closest being *O. valericigenes* (just below 95% similarity). This was also later noted for case 3 and case 4. Recently, final identification was made possible with the submission of the 16S rRNA gene sequence for *Oscillibacter ruminantium* by Lee and colleagues (4). Consensus sequences from partial 16S rRNA sequencing of isolates from the four described cases were compared to the EzTaxon-e database (5). The highest sequence similarity (99.78%) for the four strains was obtained with the *O. ruminantium* strain GH1 (GenBank accession no. JF750939.1).

The organisms were handled similarly to other clinical anaerobes. Anaerobe blood culture bottles were plated on a solid chocolate agar with added vitamin K and cysteine (Statens Serum Institut Diagnostica, Hillerød, Denmark) or similar in-house-produced agar plates and incubated at 37°C in an atmosphere of 10% CO₂, 10% H₂, and 80% N₂ in an anaerobic chamber. After 24 h of growth, colonies were smooth textured and transparent, punctuated, raised, and varied in shape from circular to irregular with entire edges.

Antimicrobial susceptibility was determined by the Etest gradient method (bioMérieux, Craponne, France) on brucella blood agar supplemented with hemin and vitamin K (Becton, Dickinson GmbH, BD Diagnostics, Heidelberg, Germany) according to the manufacturer's instructions. Clindamycin results were confirmed after 48 h of incubation. *Bacteroides fragilis* ATCC 25285 was used as a quality control.

Access to patient records was approved by The Danish Health and Medicines Authority (case no. 3-3013-382/1) and the Danish Data Protection Agency (case no. 13/17994).

Oscillibacter ruminantium is a strictly anaerobic, Gram-negative, non-spore-forming bacterium recently identified from the

rumen of Korean native cattle, showing a 97.3% 16S rRNA sequence similarity to *Oscillibacter valericigenes* (4). *O. valericigenes* was isolated from the alimentary canal of a Japanese *Corbicula* clam (6).

O. ruminantium belongs to the family *Ruminococcaceae* of the order *Clostridiales* and is the second identified species belonging to the genus *Oscillibacter* (4). Apart from *O. valericigenes*, *O. ruminantium* shows close phylogenetic association with *Oscillospira guilliermondii*, *Pseudoflavonifractor capillosus*, and *Flavonifractor plautii* based on 16S rRNA gene sequence similarity. The family *Ruminococcaceae* contains both Gram-negative and Gram-positive organisms (7), and it is known that some anaerobes, including some *Clostridium* species, stain variably or consistently stain Gram-negative despite belonging to a Gram-positive genera (8, 9). This was taken into account when identifying anaerobes using conventional methods at the anaerobe reference laboratory, explaining why the isolates from the cases in 2001 and 2002 could be identified as possible *Clostridium* species, despite staining Gram negative.

The four cases presented above are summarized in Table 1. Regrettably, we were unable to access the full patient record for one patient, and laboratory data for two of the cases are incomplete. All patients were over 50 years of age, and two suffered from alcohol dependency. In case 1, the patient presented with severe sepsis with a suspected intra-abdominal source; in case 3, the patient developed sepsis due to a suspected aspiration pneumonia; and in case 4, the obvious diagnosis was perforation and sepsis as complications to sigmoidoscopy and volvulus desufflation. As the isolation of coagulase-negative staphylococci was interpreted as contamination in case 1, the only possible pathogenic agent isolated in these three cases was *O. ruminantium*. To the best of our knowledge, the patient in case 2 did not receive antibiotic treatment for the anaerobe bacteria isolated from blood cultures drawn prior to discharge. The patient's death 6 months later was attributed to his underlying condition of metastatic colon adenocarcinoma, not infection, so the significance of the isolation of *O. ruminantium* in this case is uncertain. With this in mind, it seems plausible that *O. ruminantium* was the causative agent of the patients' septic conditions in cases 1, 3, and 4 and a possible pathogen in case 2.

All four patients exhibited risk factors for bacteremia with anaerobic bacteria to some degree; age in all cases, malignancy in one case, and in the cases of the two patients with excessive alcohol consumption, it is plausible that they suffered from some degree of chronic liver disease, which is a known risk factor for anaerobic bacteremia (10).

In one case, the suspected source of infection was the respiratory tract, but in the majority of cases, the likely source of infection was intra-abdominal. Indeed, in recent studies of the human colonic microbiota, *Oscillibacter* has been consistently detected at the genus level, and *O. valericigenes* has been detected at the species level in one study, using 16S rRNA-based analysis (11–14). *Oscillibacter* species have now been isolated from the rumen of cattle in Korea and from the alimentary canal of clams in Japan and have been identified in 16S rRNA gene clone libraries from broilers in China (15). With the identification in blood cultures from patients with a suspected abdominal source of infection in Denmark, it is reasonable to speculate that the species could be part of the microbiome of several other species. Indeed, environ-

TABLE 1 Summary of four cases of bacteremia with *O. ruminantium*

Characteristic	Result			
	Case 1 (2001)	Case 2 (2002)	Case 3 (2009)	Case 4 (2010)
Gender	Male	Male	Male	Female
Age (yrs)	53	78	61	80
Comorbidity	Alcohol dependency, chronic pancreatitis	Ulcerative colitis, colonic adenocarcinoma	Alcohol dependency	Alzheimer's dementia, hypothyroidism
Admittance diagnosis	Septic shock	Relapse and liver metastasis of adenocarcinoma	Stage four hepatic encephalopathy, secondary aspiration pneumonia	Colonic volvulus, secondary septic shock
Temp (°C) when blood cultures were drawn	35.6	Data not available ^a	Not registered in the patient's charts	38.2
C-reactive protein (mg/liter) level when blood cultures were drawn (reference, <10 mg/liter)	169	Data not available ^a	61	<10
No. of hours of incubation for blood cultures to become positive	24 (CoNS ^b) and 45 (<i>O. ruminantium</i>)	Data not available ^a	72	48
Isolated bacteria from blood cultures	<i>O. ruminantium</i> and CoNS	<i>O. ruminantium</i>	<i>O. ruminantium</i>	<i>O. ruminantium</i>
Plausible source of infection	Intra-abdominal	Intra-abdominal	Pulmonary	Intra-abdominal
Antibiotic treatment	Gentamicin, metronidazole, cefuroxime	None	Piperacillin-tazobactam	None
Length of antibiotic treatment in days	10	None	10	None

^a One patient record was not complete.

^b CoNS, coagulase-negative staphylococci.

mental sequences similar to that of *Oscillibacter* have been found in cow and goat fecal samples (16).

The *O. ruminantium* isolates identified were susceptible to all antibiotics tested: benzylpenicillin, piperacillin-tazobactam, meropenem, metronidazole, clindamycin, tigecycline, moxifloxacin, and vancomycin (Table 2). The apparent vancomycin susceptibility is unusual for Gram-negative rods but is also found in other anaerobic rods, such as *Clostridium clostridioforme* and *Clostridium symbiosum*, that persistently stain Gram negative (17).

It is notable that we have been able to retrospectively identify four cases of bacteremia with *O. ruminantium* occurring over a 9-year period by using partial 16S rRNA gene sequencing. At the Department of Clinical Microbiology, Odense University Hospital, we use primarily an anaerobic agar, a chocolate agar containing hemin and supplemented with vitamin K and cysteine as the reducing agent, and utilize partial 16S rRNA gene sequencing as our secondary diagnostic method for species identification of anaerobic bacteria from blood and other sterile body sites if identification cannot be achieved using matrix-assisted laser desorption ionization–time of flight mass spectrometry (18, 19). This approach allows for the discovery and correct identification of anaerobic bacteria, as previously described (3, 19, 20).

Incorrect bacteriological diagnosis undoubtedly has clinical implications (21), and correct identification of isolated bacteria can aid the identification of the focus of infection. Therefore, an effort to reach the correct species identification is warranted. Misidentification of anaerobic bacteria from blood cultures by conventional biochemical methods compared to sequenced-based identification demonstrates the value of applying molecular diagnostics (19, 22).

In summary, we present the first case reports of bacteremia with *O. ruminantium*, a potential new opportunistic pathogen that is likely part of the human gut microbiota. In all four cases, growth of *O. ruminantium* was achieved using standard methods: Bactec or Bact/Alert blood culture systems, anaerobe incubation chambers, and anaerobe agar. The clinical isolates appeared susceptible to all antibiotics tested. As the species has just recently been identified and the four cases presented here have been collected over a period of 9 years, it is probable that infection with *O. ruminantium* is rare but underreported.

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TABLE 2 Antimicrobial susceptibility of the four strains of *O. ruminantium* isolated from blood cultures

Antimicrobial agent	MIC (mg/liter) range ^a
Benzylpenicillin	0.250–0.380
Piperacillin-tazobactam	0.125–0.250
Meropenem	0.047–0.094
Metronidazole	<0.016–0.064
Clindamycin	0.032–0.064
Tigecycline	<0.016
Moxifloxacin	0.380–1.000
Vancomycin	0.380–0.500

^a MIC was determined by the Etest gradient method (bioMérieux, Craponne, France).

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