Case Report

Bacteremia Caused by *Dysgonomonas* spp.: a Report of Two Cases

Marisa N. Almuzara, Ph.D.,[1] Roxana Cittadini, Ph.D.,[2] Pascual J. Ladelfa, Ph.D.,[3] Liliana Olivieri, M.D.,[4] Maria Soledad Ramirez, Ph.D.,[5] Cristian Zamponi, M.D.,[2] Angela M.R. Famiglietti, Ph.D.,[1] and Carlos A.Vay, Ph.D.[1] [1]Laboratorio de Bacteriología, Departamento de Bioquímica Clínica, Instituto de Fisiopatología y Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, [2]Laboratorio de Bacteriología y Servicio de Clínica Médica, Sanatorio Mater Dei, [3]Laboratorio de Bacteriología, Complejo Médico de la Policía Federal Churruca Vista, [4]Instituto de Nefrología del Oeste, Ciudad Autónoma de Buenos Aires, [5]Facultad de Medicina, Universidad de Buenos Aires, Depto. de Microbiología, Parasitología e Inmunología, Buenos Aires, Argentina

Introduction

The genus *Dysgonomonas* was recently created by Hofstad et al. (1) to accommodate a group of fastidious gram-negative, facultative anaerobic, coccobacillus-shaped organisms. This genus constitutes a phylogenetic cluster within the *Bacteroides-Prevotella-Porphyromonas* group. Three species are included in the genus. *Dysgonomonas gadei* was first isolated from an infected human gall bladder (1). *Dysgonomonas capnocytophagoides* has been recovered from stool samples, primarily in immunocompromised patients or those with severe underlying diseases (2,3), and from blood and wound and abscess specimens (4,5). This species includes organisms formerly designated CDC group DF-3 (1). *Dysgonomonas mossii* has been described recently by Lawson et al. (6). It was isolated from abdominal drainage from a 68-year-old woman with extensive colon adenocarcinoma and from intestinal fluid from a patient with pancreatic cancer (7).

We report the first documented case of bacteremia caused by a *Dysgonomonas* species in a patient undergoing hemodialysis and a new case of bacteremia caused by *D. capnocytophagoides* in a patient with biliary tract infection.

Mailing Address: Marisa N. Almuzara, Ph.D., Laboratorio de Bacteriología, Departamento de Bioquímica Clínica, Hospital de Clinicas, Avenida Córdoba 2351, Ciudad Autónoma de Buenos Aires, Argentina 1120. Tel.: 5411-5950-8663. Fax: 5411-5950-8694. E-mail: marisanalmuzara@yahoo.com.ar

Case Reports

Case 1

The patient was a 59-year-old male with a medical history of type 2 diabetes and pancreatic cancer. He required palliative drainage of the biliary tract via a percutaneous approach with collocation of a stent because an endoscopic retrograde cholangiopancreatography could not be performed.

In April 2006, the patient was hospitalized with fever and bacteremia associated with cholestasis. Upon admission, the laboratory, parameters were as follows: alanine aminotransferase, 120 IU/L; aspartate aminotransferase, 132 IU/L; alkaline phosphatase, 352 IU/L; total bilirubin, 2.4 mg/dL; direct bilirubin, 1.5 mg/dL; and 18,300 leukocytes/μL. In addition, dilation of the intrahepatic biliary tract was diagnosed by ultrasound.

Two blood cultures were collected and processed by an automated system (BACTEC, Becton Dickinson, Sparks, MD). After 48 h of incubation, gram-negative coccobacilli to short rods were observed in a Gram-stained smear of broth. Empiric treatment was begun with ertapenem. Subsequently, a partial

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stent stenosis was observed during percutaneous drainage of the biliary tract. Balloon dilation was conducted, and an external catheter was placed.

After 48 h of aerobic incubation at 35ºC in a 5% CO_2-enriched atmosphere, small (1- to 2-mm), nonhemolytic colonies were observed on a 5% sheep blood agar plate. They were non-adherent with entire edges and a smooth surface, grey-white in color, and had a "strawberry-like" odor. The organism was identified as *D. capnocytophagoides* by using standard biochemical tests (8) and with the API ZYM system (bioMérieux, Marcy l'Etoile, France) performed according to the manufacturer's directions. To confirm the species identification, PCR amplification of the 16S rRNA was performed by using *Taq* DNA polymerase (Promega) and primers described by Weisburg et al. (9). Sequencing was performed on both DNA strands with an ABI Prism 3100 BioAnalyzer at the Utah State University sequencing facility. The sequences were analyzed by using Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/). The sequence analysis for the isolate revealed 98% identity with the AN U41355 strain, which corresponded to *D. capnocytophagoides*. The biochemical properties of the organism are listed in Table 1, and the API ZYM system results are in Table 2.

A non-standardized antimicrobial susceptibility test with 16 antimicrobial agents was performed by using Etest strips (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France). The plates were incubated for 24 h at 35ºC in an atmosphere of 5% CO_2. The MICs for the *D. capnocytophagoides* isolate were as follows: penicillin, >32 µg/ml; amoxicillin, >256 µg/ml; ampicillin-sulbactam, >256 µg/ml; piperacillin, >64 µg/ml; piperacillin-tazobactam, 48 µg/ml; ceftriaxone, >32 µg/ml; ceftazidime, >32 µg/ml; imipenem, >32 µg/ml; ertapenem, >32 µg/ml; amikacin, >256 µg/ml; gentamicin, 32 µg/ml; trimethoprim-sulfamethoxazole, 0.064 µg/ml; ciprofloxacin, >32 µg/ml; levofloxacin, >32 µg/ml; rifampin, 0.75 µg/ml; and minocycline, 0.032 µg/ml.

Despite the in vitro resistance of the isolate to the carbapenems, the patient was afebrile and recovered well. He was released on intramuscularly administered ertapenem,.

Table 1. Differences in biochemical profiles of *D. capnocytophagoides*, *D. gadei*, and our strains using conventional biochemical tests[a]

	Result[b] for			
Test	Isolate 1	*D. capnocytophagoides*	Isolate 2	*D. gadei*
Oxidase	-	-	-	-
Catalase	-	-	+	+
Nitrate reductase	-	-	-	-
Esculin hydrolysis	+	V	+	+
Urease	-	-	-	-
Indole	-	V	+	+
Gelatinase	-	-	-	-
Acid from:				
L-Arabinose	+	+	+	+
Cellobiose	+	ND	+	+
Fructose	+	ND	+	+
Lactose	+	+	+	+
D-Mannose	+	+	+	+
Melezitose	+	ND	+	+
Melibiose	+	+	+	+
Raffinose	+	+	+	+
L-Rhamnose	+	ND	+	+
Salicin	+	ND	+	+
Sucrose	+	+	+	+
Trehalose	-	-	+	+
Xylose	+	+	+	+
Adonitol	-	-	-	-
Dulcitol	-	-	-	-
Erythritol	-	ND	-	-
Inositol	-	-	-	-
D-Mannitol	-	-	-	-
D-Sorbitol	-	-	-	-
Maltose	+	+	-	ND

[a] Data for *D. capnocytophagoides* and *D. gadei* are from reference 1.
[b] -, negative; +, positive; V, variable; ND, not done.

Case 2

The patient was a 24-year-old male with hepatitis C virus infection who had been undergoing chronic hemodialysis since 1988 because of renal hypoplasia. In that same year, a renal transplant was performed, resulting in a graft loss 3 years later. From then, the patient was on peritoneal dialysis alternating with hemodialysis. From 2003 to May 2006, he presented with multiple episodes of bacteremia and vascular access infections caused by *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae,* and *Citrobacter freundii.* He received multiple antimicrobial treatment regimens for the various septic episodes, including Intravenous (i.v.) vancomycin plus imipenem, i.v. ceftazidime plus amikacin, and oral ciprofloxacin and amoxicillin-clavulanic acid.

In May 2006, the patient experienced fever and chills during hemodialysis. Two conventional blood culture sets were collected, and empirical treatment with 2 g of i.v. ceftazidime was begun. After 24 h of incubation, Gram stain and subculture of broth were performed. Gram-negative coccobacilli were observed in the Gram-stained smear. After 48 h of incubation, the colonies of the organism grew on blood agar in a CO_2-enriched atmosphere. They were gray-white in color, smooth and non-adherent, and had entire edges and a slightly aromatic odor. The organism was identified by using the same methods as described for case 1, and identification was confirmed by 16S rRNA gene sequencing. The sequence analysis revealed 97% identity with AN Y18530, corresponding to a *D. gadei* strain. The biochemical properties of this organism, determined by conventional tests are listed in Table 1, and the API ZYM system results are shown in Table 2.

The phenotypic profiles and the 16S rRNA gene sequence analysis strongly

suggest that this strain is almost certainly *D. gadei.* Susceptibility to 16 antimicrobial agents was determined by Etest strips in the same manner as described for the isolate in case 1. The MICs for the *D. gadei* isolate were as follows: penicillin, >32 μg/ml; amoxicillin, 12 μg/ml; piperacillin, 12 μg/ml; piperacillin-tazobactam, 2 μg/ml; ampicillin-sulbactam, 24 μg/ml; ceftriaxone, >32 μg/ml; ceftazidime, >32 μg/ml; imipenem, 0.5 μg/ml; ertapenem, 1 μg/ml; amikacin, >256 μg/ml; gentamicin, >256 μg/ml; trimethoprim-sulfamethoxazole, 0.004 μg/ml; ciprofloxacin, ≥32 μg/ml; levofloxacin, 3 μg/ml; rifampin, 0.25 μg/ml; and minocycline, 0.016 μg/ml.

In spite of the susceptibility test results, the patient completed 3 weeks of treatment with ceftazidime. Blood cultures collected during treatment were negative.

Table 2. Reactivities of *Dysgonomonas* spp. in the API ZYM system

Enzyme	Result[b] for Isolate 1	*D. capnocytophagoides*	Isolate 2	*D. gadei*
Alkaline phosphatase	+	+	+	+
Esterase (C4)	-	V	+	-
Esterase lipase(C8)	-	-	+	+ (w)
Lipase (C14)	-	-	-	-
Leucine arylamidase	-	-	-	-
Valine arylamidase	-	-	-	-
Cystine arylamidase	-	-	-	-
Trypsin	-	-	+	+
Chymotrypsin	-	-	-	+
Acid phosphatase	+	+	+	+
Phosphoamidase	+	+	+	+
α-Galactosidase	+	+	+	+
β-Galactosidase	+	+	+	+
β-Glucuronidase	-	-	-	-[b]
α-Glucosidase	+	+	+	+
β-Glucosidase	+	+	+	+
N-Acetyl-β-glucosaminidase	-	-	+	+
α-Mannosidase	-	-	-	+(w)
α-Fucosidase	-	-	+	+

[a] +, positive; -, negative; V, variable; +(w), weakly positive. Data for *D. capnocytophagoides* and *D. gadei* are from reference 1.

[b] Results for the production of β-glucuronidase differed among the different API systems employed. In the API ID 32A and rapid ID32E systems, β-glucuronidase was detected, but in the API ZYM kit, this enzyme was not produced.

Discussion

Until 1988, except for the description of DF-3 isolates from multiple stool samples from a patient with common variable hypogammaglobulinemia, this fastidious gram-negative bacterium has not been described in association with human disease. The Special Bacteriology Reference Laboratory of the CDC had a collection of only 53 DF-3 isolates recovered from a variety of sources, principally blood and wounds (10). To date, of more than 28 DF-3 isolates described in the literature, 22 have been isolated from the stool of immunocompromised patients, but there has been an association with diarrhea in only 50% of the cases (11).

Currently, only four cases of DF-3-associated bacteremia have been reported: one of them was a patient with acute lymphocytic leukemia on broad-spectrum antibiotic therapy (4), the second was a febrile patient with acute myelocytic leukemia during aplasia (11), the third was a neutropenic patient treated for acute myeloid leukemia (12), and the fourth case was a 78-year-old male with pancreatic cancer, ischemic heart disease, and diabetes mellitus (13). In the last case, a portal of entry into the patient's vascular system existed through multiple perianal defects (a large perianal decubitus ulcer contiguous with the rectal mucosa and two anal fissures). In the second case, reported by Grob et al. (11), DF-3 was isolated from a stool specimen of normal consistency. This patient did not have diarrhea at the time or during the period of bacteremia with fever. The lack of isolation of DF-3 outside the human body and its predominant isolation from stool specimens suggest that the gastrointestinal tract is its natural habitat (11).

The second case described in this article represents a new case of *D. capnocytophagoides* bacteremia, with a biliary focus in a patient with a solid neoplasm (pancreatic cancer). Although the origin of the bacteremia could not be determined, microbial colonization of the biliary tract via the intestinal tract is likely.

D. gadei was isolated originally from a 68-year-old male with non-insulin-dependent diabetes mellitus and essential hypertension. The patient was admitted to the hospital because he fractured his tibia and metatarsal bones during a car accident. Nine days later, he suddenly became ill with fever, chills, and vascular collapse. *E. coli* was the only pathogen isolated from the patient's blood. An ultrasound scan of the abdomen showed gallstones and a distended gall bladder. Aerobic and anaerobic culture of pus aspirated from the gall bladder yielded growth of *E. coli*, *Klebsiella* species, and enterococci. In addition, *D. gadei* was recovered by anaerobic cultivation on kanamycin/vancomycin laked blood agar. The patient was treated with cefuroxime and metronidazole for 2 weeks and recovered uneventfully (1).

Our case represents the first time a *Dysgonomonas* species has been isolated from the blood of a hemodialysis patient. Although the origin of the bacteremia could not established, we believe that it was due to multiple infected vascular accesses, including at the femoral site, because of its proximity to the perianal region. Also, the patient's treatment with multiple antibiotics could have led to the selection of this multidrug-resistant microorganism.

Dysgonomonas spp. resemble *Capnocytophaga* spp. in their requirement for enriched media, facultative anaerobiosis, lack of flagellae, and slow growth (8). *D. capnocytophagoides* must be differentiated from the *Capnocytophaga* species of human origin because they are both oxidase and catalase negative, esculin hydrolysis variable, and ONPG positive. Acid production from xylose and melibiose differentiates *D. capnocytophagoides* from *Capnocytophaga* spp. Conversely, *D. gadei* is catalase positive, produces indole and ferments

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melibiose and xylose, all test reactions that differ from those of *Capnocytophaga* spp.

Both species grow on nutritive agar around X and XV factor disks, suggesting that they depend on hemin for growth, a characteristic shared with *Haemophilus ducreyi* and sometimes with *Aggregatibacter aphrophilus,* with which they may also be misidentified. Catalase production is an important differentiating characteristic between the two *Dysgonomonas* spp. described here. *D. gadei* is catalase positive, and *D. capnocytophagoides* is catalase negative.

Dysgonomonas spp. are usually susceptible to tetracyclines, rifampin, and co-trimoxazole (SXT). Susceptibility to imipenem is variable. Some reports have described the resistance of strains to imipenem (11,13). Resistance to carbapenems has also been observed in the *D. capnocytophagoides* strain described in this report.

In the two cases presented, the patients had favorable outcomes, even though their isolates tested resistant to the antimicrobial agents used to treat them. Although these organisms are rarely recovered from clinical specimens, their pathogenic potential is unknown. In this report, our objective is to alert microbiologists to the possible isolation of *Dysgonomonas* spp. from patient blood samples and their possible role in human disease.

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