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Desulfovibrio fairfieldensis Bacteremia Associated with Choledocholithiasis and Endoscopic Retrograde Cholangiopancreatography▽

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Desulfovibrio fairfieldensis **is a gram-negative, curved, motile, anaerobic bacillus.** ***D. fairfieldensis*** **has been isolated only from human specimens and is considered a normal resident of the human gastrointestinal tract. We report the second case of** ***Desulfovibrio*** **bacteremia associated with choledocholithiasis and review the other reported cases of** ***D. fairfieldensis*** **bacteremia.**

CASE REPORT

In January 2007, a 77-year-old man with a history of choledocholithiasis was electively admitted to the day-procedure unit of the Royal Prince Alfred Hospital, Camperdown, NSW, Australia, for repeat endoscopic retrograde cholangiopancreatography (ERCP) with common bile duct stone extraction and lithotripsy. On admission to the unit, he was clinically stable and his laboratory values were as follows: white cell count, 11.8×10^9/liter; hemoglobin, 154 g/liter; platelets, 203×10^9/liter; red cell count, 5.31×10^{12}/liter; neutrophils, 10.1×10^9/liter; bilirubin (total), 37 μmol/liter; alkaline phosphatase, 440 U/liter; gamma-glutamyl transpeptidase, 562 U/liter; alanine transaminase, 243 U/liter; and aspartate transaminase, 384 U/liter.

Upon ERCP, there was extensive stone disease, including involvement of the hepatic ducts. The stones were trawled and cleared with a lithotripter, basket, and balloon, followed by stent insertion. After the procedure, the patient was admitted to the ward overnight for observation. That evening, the patient had rigors and a low-grade fever (37.4°C) but denied abdominal pain, nausea, or vomiting and remained hemodynamically stable. Blood cultures were taken, and the patient was commenced on ticarcillin-clavulanate intravenously (i.v.) at 3.1 g every 6 h. The patient's fever resolved the next day. After 4 days of i.v. ticarcillin-clavulanate, he was discharged home on oral ciprofloxacin at 500 mg twice a day (b.i.d.). At the time of discharge, blood cultures remained negative. The patient returned 1 month later for a further ERCP and remains well on follow-up.

Microbiology. Blood for culture was collected into BacT/ALERT FA and SN blood culture bottles (bioMérieux, Durham, NC) and incubated in a BacT/ALERT automated incubator (bioMérieux). Four days after the initial set of blood cultures were drawn, growth was detected in the anaerobic bottle. A strong sulfur smell was detected from the medium aspirated from the bottle. The aerobic bottle remained negative. Gram staining revealed curved, gram-negative bacilli, which were motile on the wet preparation. The initial impression was that of a *Campylobacter* species. Broth from the anaerobic bottle was subcultured onto horse blood agar plates (Oxoid, Thebarton, SA, Australia) and incubated in both 5% CO_2 and an Oxoid anaerobic jar with an AnaeroGen gas pack (Oxoid, Basingstoke, Hampshire, United Kingdom) at 37°C. After 48 h, no growth was detected on aerobic or anaerobic subcultures. However, after 4 days, inspection of the anaerobic subculture plate with a hand loupe revealed tiny colonies. Again, microscopy revealed curved, gram-negative bacilli. At 7 days, the colonies on the anaerobic plate were clear and pinpoint in size. The organism was catalase positive but oxidase, indole, and urease negative. Antibiotic susceptibilities were determined using the Epsilometer test (Etest; AB Biodisk, Solna, Sweden) as described by McDougall et al. (11), and the results were read at 96 h. The organism was found to be resistant to ticarcillin-clavulanate (MIC, >256 mg/liter) but sensitive to metronidazole (MIC, 0.002 mg/liter) and ciprofloxacin (MIC, 0.5 mg/liter).

The isolate was referred to the Identification Reference Laboratory at the Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead, NSW, Australia, for further analysis. Additional phenotypic features reported were a negative reaction for glucose fermentation, positive reactions for nitrate reduction and H_2S production (in sulfur-indole-motility medium), and a positive desulfoviridin test. Identification by long-chain-fatty-acid analysis was performed using a Hewlett-Packard 5890 series II gas chromatograph unit (Hewlett Packard Corporation, Palo Alto, CA), with analysis by the automated MIDI microbial identification system (MIDI, Newark, DE). The MIDI identification was consistent with *Desulfovibrio pigra* (*Desulfovibrio fairfieldensis* is not in the MIDI database). However, cluster analysis using MIS software clustered this isolate with previously identified *D. fairfieldensis* strains (M. Yuen, personal communication).

The first 500 bp of the 16S rRNA gene of this isolate were also sequenced after PCR amplification with both the blood culture medium and colonies from the anaerobic subculture plate. DNA extraction from colonies was performed using the QIAamp DNA Mini kit spin column method (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Formal DNA extraction was not performed on the blood culture medium. Instead, 2 drops of the medium was added to 1 ml of H_2O. This mixture was vortexed and added directly to the master mix.

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TABLE 1. Characteristics of six patients with *Desulfovibrio fairfieldensis* bacteremia[a]

Patient no.	Yr of report	Age (yr)	Sex	Past medical history	Source	Procedures	Antimicrobials	Recovery from other sites	Outcome	Reference
1	1997	75	M	SGNP, COAD, HTN, DVT, NOCL	BBAP	CLNSC	i.v. CIP, p.o. CIP	No	Recovery	11
2	2000	23	M	GU	P. Apdx	Apdct	i.v. FAM, i.v. MTZ	No	Recovery	9
3	2000	85	M	CCF, RF	RCTRG	No	NR	No	Recovery	9
4	2005	NR	NR	NR	NR	NR	NR	NR	NR	15
5	2007	77	M	DM2, CVA, HTN, CAD, CDL	BT	ERCP	i.v. TIM, p.o. CIP	No	Recovery	This study
6	1977	67	M	CP, SYN	BT	CHCYST	Nil	No	Recovery	13

[a] Apdct, appendectomy; BBAP, bleeding benign adenomatous polyps; BT, biliary tract; CAD, coronary artery disease; CCF, chronic cardiac failure; CDL, choledocholithiasis; CHCYST, cholecystectomy; CIP, ciprofloxacin; CLNSC, colonoscopy; COAD, chronic obstructive airway disease; CP, chest pain; CVA, cerebrovascular accident; DM2, type 2 diabetes mellitus; DVT, deep venous thrombosis; ERCP, endoscopic retrograde cholangiopancreatography; FAM, cefamandole; GU, gastric ulcer disease; HTN, hypertension; M, male; MTZ, metronidazole; NOCL, nonobstructive cholelithiasis; NR, not reported; P. Apdx, perforated appendix; p.o., oral administration; RCTRG, rectorrhagia; RF, renal failure; SGNP, sclerosing glomerulonephritis; SYN, syncope; TIM, ticarcillin-clavulanate.

Amplification of the partial 16S rRNA gene was performed using a monochrome, real-time format. The reaction mixture consisted of SensiMix (Quantace, London, United Kingdom) (25 μl), SYBR green I (1 μl), the universal 16S rRNA gene primers Fd1 (5′-CAG AGT TTG ATC CTG GCT CAG-3′) (2 μl; 10 pmol) and R2 (5′-GTA TTA CCG CGG CTG CTG-3′) (2 μl; 10 pmol) (10), DNA template (10 μl), and H_2O (10 μl). Thermocycling proceeded in a Corbett Rotor-Gene RG-3000 instrument (Corbett Research, Sydney, Australia). The parameters were 1 cycle at 95°C for 10 min, followed by 30 cycles at 95°C for 20 seconds, 58°C for 30 seconds, and 72°C for 30 seconds.

The entire 50-μl real-time reaction mixture containing the PCR products was purified using a Marligen Rapid DNA purification kit (Marligen Biosciences, Ijamsville, MD). Sequencing was carried out on-site at SUPAMAC, the Sydney University Prince Alfred Macromolecular Analysis Centre, using ABI Prism BigDye Terminator 3.1 chemistry (Applied Biosystems, Foster City, CA) and a GeneAmp 9700 thermocycler (Applied Biosystems). The sequence cycling products were analyzed by capillary electrophoresis and fluorescence detection in a 3730*xl* DNA analyzer (Applied Biosystems). The fluorescence data were analyzed with Applied Biosystems DNA sequencing analysis software (version 5.2; Applied Biosystems).

The sequence data derived from the isolates from the blood culture medium and the anaerobic subculture colonies were identical. A BLAST (1) search using the partial 16S rRNA gene sequence for this isolate showed it to be 100% similar (485 of 485 bases) to AJ251630 and AF192155, both of which were described as *D. fairfieldensis* by the citing publications (5, 9). The sequence was also 99% similar (483 of 485 bases) to *D. fairfieldensis* U42221, the first described isolate of this species (14).

Discussion. *Desulfovibrio fairfieldensis* is a gram-negative, curved, motile, non-spore-forming, asaccharolytic, anaerobic bacillus. It is catalase positive, oxidase negative, indole negative, and urease negative and produces H_2S (4). Excellent photomicrographs and electron micrographs of this organism have been published previously (11, 14). While most *Desulfovibrio* species are found in the environment, *D. fairfieldensis* has been isolated only from human specimens and is thought to be a normal resident of the human intestinal tract (4). The first report of clinical infection with *Desulfovibrio fairfieldensis* was due to a liver abscess in Australia over 10 years ago (14). Since that time, however, this organism has not been reported frequently. We report the second case of *D. fairfieldensis* bacteremia reported from Australia; it is also the second case of *Desulfovibrio* infection associated with choledocholithiasis.

Our review of the literature identified four other cases of confirmed *D. fairfieldensis* bacteremia (Table 1). McDougall et al. identified the organism from the first case in a patient with a history of nonobstructive cholelithiasis and benign, bleeding adenomatous polyps (11). The second case, reported by Loubinoux et al., was a patient who presented with a perforated appendix (9). The same case report discusses a third patient with a history of rectorrhagia at the same center (9). The fourth case is mentioned by Warren et al. in a case series from California (15). No demographic details of this patient were provided. The investigators from the same center note that the very first case report of *Desulfovibrio* infection (13) may actually have been due to *D. fairfieldensis*. In this first report, also from California, a patient with choledocholithiasis (similar to the present case) was found to be bacteremic with an organism identified at the time as *D. desulfuricans*. However, Warren et al. comment that this organism may have instead been *D. fairfieldensis*. This proposal was made on the basis that the isolate was reported to be catalase positive and urease negative, which are two key reactions that differentiate *D. fairfieldensis* from *D. desulfuricans*. We have included this case in our review.

Most patients (including the one from the present case) were >65 years of age and male. In three patients, compromise of the gastrointestinal tract, from bleeding polyps (11), a perforated appendix (9), and rectorrhagia (9), was the source of infection; in another two patients, the likely source was the biliary tree (13; the present case). Surgical or invasive intervention was reported in four of the cases, including colonoscopy (11), appendectomy (9), ERCP (present case), and cholecystectomy (13). The antimicrobial regimens employed were varied. The outcomes, where reported, indicated recovery in every case.

The organisms (Table 2) were relatively slow growing (4 to 6 days until positive), even in automated blood culture systems. The only biochemical variation was the presence or absence of

a nitrate reaction. Antimicrobial susceptibility varied for the beta-lactams, but there was consistent susceptibility to metronidazole. Methods used to determine antimicrobial susceptibility included disk diffusion (11), Etest (11), and CLSI agar dilution (9, 15) with results read at 96 h.

In addition to blood, *D. fairfieldensis* has been isolated from various other sites of infection. The most frequent sites of isolation have been peritoneal fluid ($n = 8$) (8, 15), periodontal pockets ($n = 6$) (5, 7), and abdominal collections ($n = 5$) (8, 9, 15). This organism has also been isolated from a pelvic and a colorectal collection (15), a liver abscess (14), and urine (6). The majority of the 26 clinical isolates have been isolated in France ($n = 10$) (6, 7, 9), the United States ($n = 10$) (15), and Australia ($n = 5$) (8, 11, 14). The true incidence of disease is likely to be underestimated because of the difficulties associated with the identification of anaerobic organisms (2, 9). Still, it appears that *D. fairfieldensis* is found most frequently by those who seek it. Indeed, the majority of the reported isolates are from the following three groups: J. Loubinoux and coworkers in France (7, 8, 9), Y. Warren and coworkers in the United States (15), and W. Tee and coworkers in Australia (8, 11, 14). We noted that molecular identification has played a major role in the identification of this organism, as all of the isolates reported were definitively identified by either 16S rRNA gene sequencing ($n = 17$) (5, 6, 9, 11, 14, 15) or amplification with specific PCR primers ($n = 9$) (7, 8).

Due to the association with infections involving the gastrointestinal tract, it has been suggested that *D. fairfieldensis* possesses more invasive pathogenic potential than other *Desulfovibrio* species (9). In addition, *D. fairfieldensis* also possesses the most antimicrobial resistance of the genus (15). In spite of these two features, there have been no deaths directly attributed to *D. fairfieldensis* infection. We also note that our review found that at least half of the bacteremic patients had gallstone disease, which may signify that this organism may be associated with the biliary tree as well. Consequently, manipulation of the biliary tree may predispose patients to *D. fairfieldensis* bacteremia.

We found the use of 16S rRNA gene sequencing to be a very useful method to identify this organism and provide a faster turnaround time than conventional methods. The time required to obtain a sequence-based identification directly from the positive blood culture medium was about 30 h. The anaerobic subculture alone took 4 days until colonies were visible with a loupe. Further investigations then required additional subcultures. However, we note that phenotypic characters still remained important, as they assisted in correlating the sequence-based data.

The use of DNA sequencing for bacterial identification has become more practical with the advent of automated genetic analyzers and alignment software, and sequence-based identification is now beginning to play a larger role in the identification of infectious organisms (3). The role of 16S rRNA gene sequencing for bacterial identification has been demonstrated for various organisms, including mycobacteria, *Nocardia* species, and noncultured bacteria (3). Since anaerobes may often be regarded as difficult to identify, there appears to be a role for sequenced-based identification of these organisms as well (4, 12). Indeed, wider use of this utility can assist in the accurate and consistent identification of *D. fairfieldensis* and other

TABLE 2. Characteristics of six isolates of *Desulfovibrio fairfieldensis* from cases of bacteremia[a]

Patient	Blood culture system	Time to positivity (days)	Test result								Antimicrobial susceptibility test	Antimicrobials		Molecular identification test	Reference
			Catalase	Oxidase	Indole	Nitrate	Urease	H_2S production	H_2S odor	Motile	Pure culture		Susceptible	Resistant		
1	BacT/ALERT	6	+	−	−	−	−	+	Yes	+	Yes	DD, Etest	MTZ, CHL, CIP, IPM, AMC, TIM, ATH, CLI	PEN, AMP, CEF, CEC, TIC, SXT, GEN, CTX, VAN	16S	11
2	Vital	NR	+	NR	−	−	−	+	NR	+	Yes	Agar dilution	MTZ, IPM, CLI	PEN, AMP, AMC, TIM, TZP, FOX, CTT, CTX	16S	9
3	NR	NR	+	NR	−	−	−	+	NR	+	No	NR	NR	NR	16S	9
4	NR	NR	+	NR	−	+	−	+	NR	+	NR	Agar dilution	MTZ, CHL, KAN	CRO, TZP, VAN, COL	16S	15
5	BacT/ALERT	4	+	−	−	+	−	+	Yes	+	Yes	Etest	MTZ, CIP	TIM	16S	This study
6	E-vac	4	+	−	−	w	−	+	Yes	+	Yes	NR	PEN, CLI, CHL, TET, ERY	NR	NP	13

[a] 16S, 16S rRNA gene sequence; AMC; amoxicillin-clavulanic acid; AMP, ampicillin; ATH, azithromycin; CEC, cefaclor; CEF, cefalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; COL, colistin; CRO, ceftriaxone; CTT, cefotetan; CTX, cefotaxime; DD, disc diffusion; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; KAN, kanamycin; MTZ, metronidazole; NP, not performed; NR, not reported; PEN, penicillin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TIC, ticarcillin; TIM, ticarcillin-clavulanic acid; TZP, piperacillin-tazobactam; VAN, vancomycin.

anaerobes, thereby further delineating their true epidemiology and importance in human infection. However, we acknowledge that culture methods, even if carried out by a reference laboratory, remain necessary in order to determine antimicrobial susceptibility.

In conclusion, 30 years on, we would like to reiterate the admonition of Porschen and Chan that the clinical microbiologist be aware of and able to identify this uncommon anaerobe (13). Suspicion should be raised when curved, motile, anaerobic bacilli are isolated after 4 to 6 days from blood cultures from patients who are known to have gastrointestinal compromise or recent biliary manipulation. If the resources for anaerobic culture and identification are not readily available, identification by 16S rRNA gene sequencing may offer a relatively rapid method of identification. When the diagnosis is suspected or confirmed, effective treatment appears to be metronidazole accompanied by surgical intervention, where appropriate, to remove the focus of infection.

Nucleotide sequence accession number. The partial 16S rRNA gene sequence of the case isolate was deposited in GenBank under accession number EF532788.

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