JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 931–934 Vol. 38, No. 2
0095-1137/00/$04.00+0

Bacteremia Caused by a Strain of *Desulfovibrio* Related to the Provisionally Named *Desulfovibrio fairfieldensis*

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Received 5 August 1999/Returned for modification 22 September 1999/Accepted 19 November 1999

Eight isolates of *Desulfovibrio* spp. have been obtained over 5 years from abdominal or brain abscesses or blood. In seven patients these strains were part of a mixed flora. One strain was isolated in pure culture from the blood of a patient with peritonitis of appendicular origin. According to the 16S rRNA gene sequences, this strain was close to *Desulfovibrio fairfieldensis*. The present report describes the fourth isolate of this recently described species to be isolated in pure culture or as a predominant part of the flora and to be associated with infectious processes. Thus, *D. fairfieldensis* may possess a higher pathogenic potential than other *Desulfovibrio* species.

CASE REPORT

A 23-year-old man with gastric ulcer antecedents consulted his practitioner for an epigastric pain, which was suspected of being of gastric origin. On the following day, exacerbation of symptoms necessitated hospitalization at the hospital of Saint-Avold (Lorraine region, France). Leukocytosis and abdominal tenderness, which was accentuated in the right iliac fossa, suggested a diagnosis of perforating appendicitis. This was confirmed by surgery, which consisted of appendix removal and peritoneal lavage. Abdominal drainage and empirical intravenous antibiotic therapy with cefamandole (1.5 g per day) and metronidazole (1 g per day) permitted a full recovery. Peritoneal fluid examined by routine bacteriological methods remained sterile. An anaerobic culture of blood obtained at the time of admission was positive and was sent to the Laboratory of Bacteriology of the University Hospital of Nancy for identification of the bacterium.

Bacteriological investigations. Blood samples were inoculated in anaerobic blood culture vials (Vital; bioMérieux, Marcy l'Etoile, France). Subcultures were performed on Wilkins-Chalgren anaerobic agar at 37°C in an anaerobic chamber. Pinpoint, round, smooth, nonhemolytic, and greyish colonies were observed after 5 days of incubation. Gram staining of the colony showed curved gram-negative rods. The organism was motile and asaccharolytic and tested positive in catalase reactions but negative in oxidase, nitrate reduction, and urease reactions. It produced H_2S from sulfate. The desulfoviridin test was positive. Growth was detected only under anaerobic conditions at 25, 37, and 42°C. Thus, this bacterium possessed the phenotypic properties of *Desulfovibrio* spp. (16, 19).

Antibiotic susceptibility was determined by the agar dilution method with Wilkins-Chalgren medium. Because of the slow growth of this organism, MICs were determined after 96 h of anaerobic incubation at 37°C (13). MIC results were interpreted with reference to the guidelines of the National Committee for Clinical Laboratory Standards. They indicated that the isolate was resistant to penicillin G (32 μg/ml), amoxicillin (32 μg/ml), amoxicillin-clavulanate (16 μg/ml), ticarcillin (256 μg/ml), ticarcillin-clavulanate (256 μg/ml), piperacillin (>256 μg/ml), piperacillin-tazobactam (>256 μg/ml), cefoxitin (>256 μg/ml), cefotetan (64 μg/ml), and cefotaxime (256 μg/ml). It was sensitive to metronidazole (0.25 μg/ml), imipenem (0.5 μg/ml), and clindamycin (0.5 μg/ml).

To obtain a more precise identification of this bacterium, the complete 16S rRNA gene (16S rDNA) sequence of the isolate was determined as described previously (13, 17). The 16S rDNA sequences of the reference strains *Desulfovibrio desulfuricans* Essex 6 (type strain, ATCC 29577; isolated from soil), *D. desulfuricans* MB (ATCC 27774; isolated from sheep rumen), and *Desulfomonas pigra* (type strain, ATCC 29098; isolated from human feces) were also determined for comparison. Briefly, DNA extracts were obtained from 500 μl of bacterial suspension in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]) by the standard phenol-chloroform-isoamyl alcohol method. The 16S rDNA (about 1,500 nucleotides) was amplified by PCR with the consensus terminal primers 27f and 1525r (17). PCR products were purified with the Wizard PCR Preps DNA Purification System (Promega Corp., Madison, Wis.) according to the manufacturer's instructions. 16S rDNA sequencing was performed in an automated DNA sequencer (model 373A DNA sequencer; Applied Biosystems Inc., Foster City, Calif.) by the dye-labeled dideoxy chain-termination method (Dye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems Inc.) with terminal and internal primers specific for 16S rDNA (17). However, the 900r consensus primer was unable to amplify a DNA segment and was then modified according to the *D. desulfuricans* MB sequence (GenBank accession no. M34113). A total of 1,542 continuous nucleotides of 16S rDNA were determined, and these corresponded to *Escherichia coli* 16S rDNA sequence positions 8 to 1540 (2). The complete 16S rDNA sequence of the clinical isolate was compared to all bacterial sequences available from the GenBank database by using the Blast, version 2.0, program (National Center for Biotechnology Institute) and showed 99.9% similarity (corresponding to 2 nucleotide differences) to

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TABLE 1. Characteristics of patients from whom *Desulfovibrio* strains have been isolated

Patient	Age (yr)	Sex	Antecedent(s)	Diagnosis	Surgical treatment
1	39	Male	Maxillary sinusitis, alcoholism	Brain abscess	Yes
2	3	Female	None	Appendicitis	Yes
3	61	Female	Alcoholic cirrhosis, sigmoid resection	Abdominal wall abscess, peritonitis	Yes
4	23	Male	Gastric ulcer	Appendicitis, peritonitis	Yes
5	59	Female	Hemicolectomy, cholecystectomy	Intra-abdominal abscesses	Yes
6	85	Male	Rectorrhagia, chronic heart and renal failure	Alteration of clinical status	No
7	65	Male	Splenopancreatectomy	Abdominal wall abscess with fistulization	Yes

that of *Desulfovibrio fairfieldensis* (GenBank accession no. U42221). The sequence of reference strain *D. desulfuricans* MB had 97.2% similarity (corresponding to 43 nucleotide differences) to the sequence of the same strain deposited in the GenBank database (accession no. M34113). This previously deposited sequence was identical to that determined for *D. desulfuricans* Essex 6 except for one nucleotide.

Seven other *Desulfovibrio* clinical strains have been isolated in Nancy, France, over a 5-year period. These seven strains and the strain described above were recovered from seven patients, designated patients 1 to 7 (Table 1). The strains from these patients were labeled D1 to D7, according to patient number, except for those from patient 3, from whom two strains were isolated; the two strains from patient 3 were labeled D31 and D32, respectively (Table 2). Five strains (strains D1, D2, D31, D5, and D7) were isolated from abscesses, and three strains (strains D32, D4, and D6) were isolated from blood. These eight clinical isolates differed according to their catalase, nitrate reduction, and urease reactions. Only one isolate was in pure culture (strain D4), and this strain could be isolated from the patient described above. The 16S rDNA sequences of these seven clinical isolates were also determined.

Nucleotide sequence accession numbers. The sequences of reference strains *Desulfomonas pigra*, *D. desulfuricans* Essex 6, and *D. desulfuricans* MB and of *Desulfovibrio* clinical isolate D4 can be recovered from the GenBank database under accession nos. AF192152, AF192153, AF192154, and AF192155, respectively.

The sulfate-reducing eubacteria represent a class of anaerobic microorganisms that conduct dissimilatory sulfate reduction. In this process, sulfate acts as an oxidizing agent for the dissimilation of organic matter, and the reduced sulfur is released into the external environment as sulfide, a toxic and corrosive compound. Thus, these bacteria have detrimental industrial and environmental impacts, such as anaerobic corrosion of steel. They have mostly been isolated from environmental sources (16), but they are also present in the intestinal tracts (6, 16) and mouths (18) of humans and animals. The predominant species in humans belong to the genus *Desulfovibrio* (5, 20). *Desulfovibrio* spp. have been associated with proliferative bowel disease in animals (3, 4, 10, 11), and they have been implicated in ulcerative colitis in humans because of the toxic effects of sulfide, which inhibits butyrate oxidation by colonic epithelial cells (5, 7, 14, 20). However, their role in

TABLE 2. Characteristics of *Desulfovibrio* strains isolated from clinical samples

Patient	Strain	Site of isolation	Reactions for variable characteristics			Associated flora
			Catalase reaction	NO_3^- reduction	Urease reaction	
1	D1	Brain abscess	−	−	+	*Capnocytophaga ochracea*, *Eubacterium exiguum*, and *Streptococcus constellatus*
2	D2	Appendicular abscess	−	−	+	*Bacteroides merdae*, *Eubacterium lentum*, *Escherichia coli*, and *Enterococcus* sp.
3	D31	Abdominal wall abscess	+	+	−	*Bacteroides fragilis*, *Eubacterium lentum*, *Clostridium* sp., *Escherichia coli*, *Enterobacter cloacae*, and *Enterococcus* sp.
3	D32	Blood	+	+	−	*Escherichia coli*, and *Enterobacter cloacae*
4	D4	Blood	+	−	−	None
5	D5	Abdominal abscess	+	−	−	*Bacteroides vulgatus*, *Eubacterium lentum*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus intermedius*
6	D6	Blood	+	−	−	*Bacteroides fragilis*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Clostridium innocuum*, *Clostridium* sp., and *Enterococcus avium*
7	D7	Abdominal wall abscess	+	−	−	*Bacteroides thetaiotaomicron*, *Eubacterium lentum*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus* sp., and *Streptococcus intermedius*

TABLE 3. 16S rDNA sequence similarities of reference and clinical *Desulfovibrio* strains[a]

Species or strain	% Nucleotide sequence similarity							
	D. desulfuricans		*D. fairfieldensis*[b]	Clinical strains				
	Essex 6 (ATCC 29577)	MB (ATCC 27774)		D1 and D2[c]	D31 and D32[c]	D4	D5	D6 and D7[c]
D. pigra ATCC 29098	95.9	94.5	95.8	96.1	94.6	95.7	95.7	95.6
D. desulfuricans Essex 6		97.3	97.1	99.4	97.3	97.0	97.0	96.9
D. desulfuricans MB			95.8	97.1	99.9	95.7	95.7	95.6
D. fairfieldensis[b]				97.1	95.7	99.9	99.9	99.1
D1 and D2[c]					97.2	97.1	97.2	97.1
D31 and D32[c]						95.6	95.6	95.6
D4							99.8	99.1
D5								99.2

[a] The complete 1,542-nucleotide sequences of the strains were compared.
[b] The sequence has been recovered from GenBank (accession no. U42221).
[c] Strains with identical sequences.

human disease remains speculative, and their isolation from clinical samples is seldom described. To date, only eight cases of human infection with *Desulfovibrio* spp. have been documented. *D. desulfuricans* has been isolated from blood (15), and *Desulfovibrio vulgaris* has been isolated from an intra-abdominal abscess (8). *Desulfovibrio* spp. have been recovered from appendiceal tissue and peritoneal fluid (1) and from a brain abscess (12), and three *Desulfovibrio* strains have recently been isolated from a pyogenic liver abscess (17), blood (13), and a urine specimen (9). The latter three strains have provisionally been named *Desulfovibrio fairfieldensis* (17) because of their unique 16S rDNA sequences.

According to phenotypic characters and 16S rDNA sequences, the eight *Desulfovibrio* clinical strains isolated in Nancy can be distributed into three groups: strains D1 and D2 are related to *D. desulfuricans* Essex 6 (99.4% 16S rDNA similarity), strains D31 and D32 are related to *D. desulfuricans* MB (99.9% 16S rDNA similarity), and strains D4, D5, D6, and D7 are related to *D. fairfieldensis* (99.9% 16S rDNA similarity for D4 and D5 and 99.1% 16S rDNA similarity for D6 and D7). The strains in each group show less than 97.3% 16S rDNA similarity with strains of another group (Table 3). It has been suggested that a 16S rDNA sequence similarity of <98% is evidence that strains belong to separate species (17). However, it remains to be determined by more complete phenotypic and genotypic analyses whether these three groups of strains represent different species of *Desulfovibrio*.

The isolation of *Desulfovibrio* spp. from human clinical samples might seldom occur because of an absence of systematic searches for the bacteria, the slow and fastidious growth of these bacteria, and the presence of mixed flora. Thus, their prevalence in human diseases may be underestimated. In our series, the outcomes for the seven patients were always favorable once appropriate therapy was undertaken. As is often the case in anaerobic infections, a mixed flora was generally involved. *Desulfovibrio* spp. may be present in abdominal abscesses as part of the anaerobic intestinal flora. These abscesses are associated with factors that favor infection, such as cirrhosis and previous intestinal surgery, suggesting that *Desulfovibrio* strains are opportunistic agents with somewhat limited pathogenicities.

Desulfovibrio strains have seldom been isolated in pure culture from clinical samples. *D. desulfuricans* has been reported in blood (15). The four additional isolates were from an hepatic abscess, blood, and urine (9, 13, 17) and from blood, as reported above. These four strains have provisionally been labeled *D. fairfieldensis* and have almost identical 16S rDNA sequences, despite a few phenotypic differences, such as esculin hydrolysis, growth at 25°C, and antibiotic susceptibility profile (differences in resistance or susceptibility to amoxicillin-clavulanate, imipenem, ciprofloxacin, and chloramphenicol). They were sensitive to clindamycin and metronidazole, two drugs commonly used for the treatment of anaerobic infections. These four isolates may represent different strains of the same species which remains to be ascertained by further studies. Compared to other *Desulfovibrio* spp., *D. fairfieldensis* may have unique properties that confer some invasive power and make this newly described bacterium a potential human pathogen.

We are indebted to J. Le Gall for comments and support throughout this study and thank A. Lozniewski for a critical review of the manuscript.

REFERENCES

1. **Baron, E. J., R. Bennion, J. Thompson, C. Strong, P. Summanen, M. McTeague, and S. M. Finegold.** 1992. A microbiological comparison between acute and complicated appendicitis. Clin. Infect. Dis. **14:**227–231.
2. **Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller.** 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **75:**4801–4805.
3. **Fox, J. G., F. E. Dewhirst, G. J. Fraser, B. J. Paster, B. Shames, and J. C. Murphy.** 1994. Intracellular *Campylobacter*-like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp. J. Clin. Microbiol. **32:**1229–1237.
4. **Gebhart, C. J., S. M. Barns, S. McOrist, G. F. Lin, and G. H. K. Lawson.** 1993. Ileal symbiont intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. Int. J. Syst. Bacteriol. **43:**533–538.
5. **Gibson, G. R., G. T. McFarlane, and J. H. Cummings.** 1988. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J. Appl. Bacteriol. **65:**103–111.
6. **Gibson, G. R.** 1990. Physiology and ecology of sulphate-reducing bacteria. J. Appl. Bacteriol. **69:**769–797.
7. **Gibson, G. R., J. H. Cummings, and G. T. McFarlane.** 1991. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. **86:**103–112.
8. **Johnson, C. C., and S. M. Finegold.** 1987. Uncommonly encountered, motile, anaerobic gram-negative bacilli associated with infection. Rev. Infect. Dis. **9:**1150–1162.
9. **La Scola, B., and D. Raoult.** 1999. Third human isolate of a *Desulfovibrio* sp. identical to the provisionally named *Desulfovibrio fairfieldensis*. J. Clin. Microbiol. **37:**3076–3077.
10. **Lawson, G. H. K., S. McOrist, S. Jasni, and R. A. Mackie.** 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance in vitro. J. Clin. Microbiol. **31:**1136–1142.
11. **Li, X., J. Pang, and J. G. Fox.** 1996. Coinfection with intracellular *Desulfovibrio* species and coccidia in ferrets with proliferative bowel disease. Lab. Anim. Sci. **46:**569–571.

12. **Lozniewski, A., P. Maurer, H. Schuhmacher, J. P. Carlier, and F. Mory.** 1999. First isolation of *Desulfovibrio* sp. as part of a polymicrobial infection from a brain abscess. Eur. J. Clin. Microbiol. Infect. Dis. **18:**602–603.
13. **McDougall, R., J. Robson, D. Paterson, and W. Tee.** 1997. Bacteremia caused by a recently described novel *Desulfovibrio* species. J. Clin. Microbiol. **35:**1805–1808.
14. **Pitcher, M. C. L., and J. H. Cummings.** 1996. Hydrogen sulphide: a bacterial toxin in ulcerative colitis? Gut **39:**1–4.
15. **Porschen, R. K., and P. Chan.** 1977. Anaerobic vibrio-like organisms cultured from blood: *Desulfovibrio desulfuricans* and *Succinivibrio* species. J. Clin. Microbiol. **5:**444–447.
16. **Postgate, J. R.** 1984. The sulphate-reducing bacteria, 2nd ed. Cambridge University Press, Cambridge, United Kingdom.
17. **Tee, W., M. Dyall-Smith, W. Woods, and D. Eisen.** 1996. Probable new species of *Desulfovibrio* isolated from a pyogenic liver abscess. J. Clin. Microbiol. **34:**1760–1764.
18. **Van der Hoeven, J. S., C. W. A. van den Kieboom, and M. J. M. Schaeken.** 1995. Sulfate-reducing bacteria in the periodontal pocket. Oral Microbiol. Immunol. **10:**288–290.
19. **Widdel, F., and N. Pfennig.** 1984. Dissimilatory sulfate- or sulfur-reducing bacteria, p. 663–679. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore, Md.
20. **Willis, C. L., J. H. Cummings, G. Neale, and G. R. Gibson.** 1997. Nutritional aspects of dissimilatory sulfate reduction in the human large intestine. Curr. Microbiol. **35:**294–298.