

## *Campylobacter curvus*-Associated Hepatic Abscesses: a Case Report

Nicole M. Wetsch,<sup>1</sup> Karim Somani,<sup>2</sup> Gregory J. Tyrrell,<sup>3</sup> Carol Gebhart,<sup>1</sup> R. J. Bailey,<sup>2</sup>  
and Diane E. Taylor<sup>1\*</sup>

Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada<sup>1</sup>; Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada<sup>2</sup>; and Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada<sup>3</sup>

Received 30 November 2005/Returned for modification 9 January 2006/Accepted 28 February 2006

***Campylobacter curvus* was isolated from blood cultures of a patient with liver abscesses. Bacterial identification involved Gram staining, biochemical analysis, gas-liquid chromatography, and 16S rRNA sequencing. The difficulty in isolation, identification, and growth of the species confirms previous work that these organisms may be overlooked by conventional detection methods.**

### CASE REPORT

A 52-year-old carpenter with a history of smoking and diverticulitis, but otherwise generally healthy, presented with general malaise and pyrexia. A chest X-ray image revealed minor changes suggestive of pneumonia, and the patient was treated with intravenous cefuroxime. He improved and was discharged.

Three weeks later, he returned with pyrexia (temperature, 39.5°C), night sweats, anorexia, and a 30-lb weight loss. The abdominal exam was unremarkable. Investigations revealed a white blood cell count of 27,000. The chest X-ray image showed some minor changes consistent with chronic obstructive pulmonary disease. Ultrasound of the abdomen and pelvis revealed two lesions on his liver, which may have been cysts or abscesses. Empirically, he was started on ceftriaxone (2 g, given intravenously once daily) and metronidazole (500 mg, given perorally thrice daily). He improved within 48 h and went on to make an uneventful recovery.

At this time the patient was referred to our center, from a rural hospital, because of the diagnostic dilemma, as well as the two lesions on his liver detected by ultrasound. A computed tomography scan could not be performed because of the patient's allergy to the contrast media; however, a repeat ultrasound showed two complex masses within the right lobe of the liver consistent with abscesses. Aspirates were obtained and sent for analysis. Pathology showed "necro-inflammatory" material. Aerobic and anaerobic cultures of the aspirated material were negative. One anaerobic blood culture demonstrated growth of curved gram-negative rods. The organism grew on both tryptic soy and brain heart infusion agars but failed to grow on chocolate agar.

The organism was subjected to Gram staining and oil immersion phase-contrast microscopy, which indicated it to be a gram-negative curved rod. The unknown bacterial strain was grown at 37°C on brain heart infusion (Becton Dickinson, Mississauga, Ontario, Canada) agar (Invitrogen, Burlington, Ontario, Canada) plates with 5% defibrinated sheep blood

(Dalynn Biologicals, Calgary, Alberta, Canada) only under strict anaerobic conditions. Three days of uninterrupted growth was required to produce a lawn of confluent growth.

Routine biochemical tests (Table 1) were used to compare the organism with *Helicobacter pylori* strain J99 and *Campylobacter jejuni* strain NCTC 11168. Indoxyl acetate hydrolysis was done by using the method described by Popovic-Uroic et al. (19) and commercially prepared disks (American Type Culture Collection [ATCC], Rockville, MD). Nitrate reduction, catalase, and urease tests were all done by using previously described methods (2, 10, 14). Oxidase tests were performed with 1% tetramethyl-*p*-phenylenediamine (TMPD; Sigma, Oakville, Ontario, Canada) (10). The motility of the organism was assessed by using the agar method (Dalynn Biologicals) (14).

A RapID ANA II Code Compendium identified two possibilities for the unknown organism, *Wolinella succinogenes* (48.5% probability) or "*Bacillus gracilis*" (51% probability). Gas and liquid chromatographic analysis (data not shown) revealed a succinic acid peak. This peak is characteristic of *Wolinella* spp., as well as certain other  $\epsilon$ -proteobacteria that are capable of reducing fumarate (as a terminal electron acceptor) to succinate in the presence of formate or hydrogen (11, 18). *Wolinella* sp., a commensal organism related to *Campylobacter* spp. (25), is generally found in the gastrointestinal tract of cattle and had never been identified as a human pathogen. The positive motility test definitively ruled out "*B. gracilis*," since it is nonmotile. In contrast, *W. succinogenes* is motile, further corroborating the identification and placing the probability of this being the correct species at 98%. Subsequently, 16S rRNA sequencing was performed to confirm the identity of the strain.

The first 600 bp of the unknown gene sequence were compared to the GenBank data (National Center for Biotechnology Information, National Institutes of Health). The sequence was a 100% match to *C. curvus* strain LMG 11034 (GenBank accession AF550650) (5). The previously described biochemical tests were all consistent with *C. curvus*.

The MICs for several antibiotics were determined by using the Etest method (Epsilon meter gradient agar diffusion test strips; AB Biodisk, Solna, Sweden) according to manufacturer's directions. Antibiotic susceptibility testing was done as follows (MICs in parentheses): clarithromycin (3  $\mu$ g/ml),

\* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of Alberta, 1-63 Medical Sciences Building, Edmonton, Alberta T6G 2H7, Canada. Phone: 780-492-4777. Fax: 780-492-7521. E-mail: diane.taylor@ualberta.ca.

TABLE 1. Results of biochemical tests of patient isolate

Test	Actual result			Expected result <sup>a</sup>		
	Unknown	NCTC 11168	J99	<i>C. curvus</i> <sup>d</sup>	<i>C. jejuni</i>	<i>H. pylori</i>
Nitrate reduction	+	+	NR <sup>c</sup>	+	+	Variable
Indoxyl acetate <sup>b</sup>	+	+	-	+	+	-
Catalase	-	+	+	-	+	+
Urease	-	-	+	-	-	+
Oxidase	+	+	+	+	+	+
Motility	+	+	+	+	+	+

<sup>a</sup> Expected results, except that indoxyl acetate and *C. curvus* results were taken from *Biochemical Tests for Identification of Medical Bacteria* (14).

<sup>b</sup> The indoxyl acetate expected results were obtained from Popovic-Uroic et al. (19).

<sup>c</sup> NR, no result was recorded after several attempts since bacteria would not grow in media.

<sup>d</sup> The expected results for this species were taken from *Bergey's Manual of Determinative Bacteriology* (8). *Wolinella succinogenes* results were identical in all tests except for the indoxyl acetate test, in which the result was negative.

erythromycin (2 µg/ml), cefuroxime (>256 µg/ml), ceftriaxone (>32 µg/ml), metronidazole (0.38 µg/ml), amoxicillin-clavulanic acid (>256 µg/ml), imipenem (>32 µg/ml), quinupristin (>32 µg/ml), nalidixic acid (>256 µg/ml), and ciprofloxacin (0.047 µg/ml). Nalidixic acid resistance is characteristic of *C. curvus* (8). To confirm the differences in MIC for ciprofloxacin and nalidixic acid, these were repeated by using agar dilution testing, which gave similar results (a ciprofloxacin MIC of 0.125 µg/ml and a nalidixic acid MIC of 128 µg/ml). These MICs are similar to those obtained for the *Campylobacter fetus* subsp. *fetus* type strain ATCC 27374 (23), suggesting that the resistance profiles for quinolone antibiotics in wild-type *C. curvus* and *C. fetus* subsp. *fetus* may be similar.

Agarose-embedded chromosomal DNA preparation, restriction endonuclease digestion, and pulsed-field gel electrophoresis (PFGE) were performed essentially according to the protocol described by Ribot et al. (20). The unknown isolate was digested with 20 U each of SmaI (10 U/µl; Roche Applied Science, Laval, Quebec, Canada), SnaBI (5 U/µl; New England Biolabs, Inc. [NEB], Mississauga, Ontario, Canada), RsrII (4 U/µl; NEB), or PmeI (10 U/µl; NEB). The size reference strain, *Salmonella choleraesuis* subsp. *choleraesuis* serotype Braenderup H9812, was digested with XbaI (20 U/µl; NEB). A 50- to 1,000-kb Lambda Ladder PFG Marker (N0340S; NEB) was included on the gel as standard. DNA fragments were separated by using a CHEF-DR II PFGE system (Bio-Rad Laboratories, Hercules, Calif.). Images were captured by using a Gel Logic 200 Imaging System (Kodak, New Haven, Conn.) and were saved as 8-bit no scaling tagged image file format (tif) images for transfer to a BioNumerics database (Bionumerics Version 3.5; Applied Maths, Austin, Tex.). All lanes were normalized to the lambda ladder PFG marker prior to base pair analysis. The PFGE profile after PmeI digestion was useful for the estimation of genome size of the *C. curvus* isolate (Fig. 1). The size of *C. curvus* genome was calculated to be approximately 1.6 Mb (1,555 kb).

Several attempts were made to isolate plasmid DNA using both midi- and maxikits from QIAGEN (Mississauga, Ontario, Canada). The isolation protocol for plasmid preparation by

alkaline lysis with sodium dodecyl sulfate as outlined previously (21) was also attempted. No plasmids were found to be present.

*C. curvus* was discovered in 1984 and originally classified as *Wolinella curva* (22). The organism was reclassified as *Campylobacter curvus* in 1991 based on DNA-DNA hybridization and immunotyping analysis (24). Recently, Han et al. found isolates of *C. curvus* causing liver abscesses, in conjunction with *Streptococcus* species, in a lung cancer patient with a postobstructive bronchial abscess, as well as in an ovarian cancer patient with a liver abscess (6).

*C. curvus* has been found in a wide variety of clinical presentations (1-3), but culture and identification difficulties may be the cause of its under-recognition (16, 17). The species has been isolated from the blood (6) and the oral cavity, although it is noninvasive orally and is not believed to be a causative agent of periodontal disease (7, 15). Although not well documented, the gastrointestinal tract has also been shown previously to be a reservoir for this species (1, 9) and is a far more likely source in this case than the well-documented oral cavity (7, 15, 18).

The genome size for *C. curvus* has not been published; however, PFGE revealed the genome size for the patient's isolate to be approximately 1.6 Mb. *C. jejuni* is the only completely sequenced strain of this genus; however, *C. coli*, *C. lari*, and *C. upsaliensis* have all been partially sequenced. Their genome sizes were estimated to be approximately 1.68, 1.5, and 1.66 Mb, respectively (4). Thus, the *C. curvus* genome is in a size range similar to that of other *Campylobacter* species.

These findings support other evidence that *C. curvus* may be

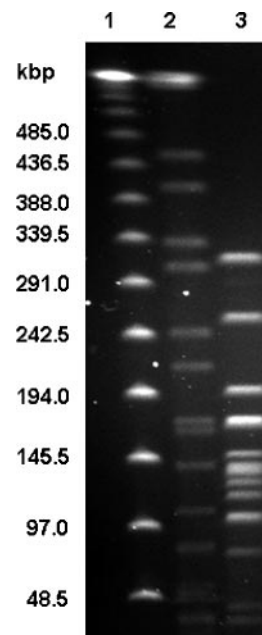


FIG. 1. PFGE of the *C. curvus* sample DNA. Lanes: 1, lambda DNA ladder run as size marker; 2, *S. choleraesuis* subsp. *choleraesuis* serotype Braenderup H9812 DNA digested with XbaI (size marker); 3, unknown *C. curvus* DNA digested with PmeI.

more pathogenic than originally believed (1, 6). Selective media for *Campylobacter* usually contains antibiotics to which species other than *C. jejuni*, *C. coli*, and *C. lari* are susceptible (13). This may explain the difficulties found in culturing and maintaining *C. curvus* in a laboratory setting. Several species, including *C. curvus*, do not respond well to conventional isolation methods and, therefore, may remain undetected, particularly if present in conjunction with another *Campylobacter* species that may be cultured under microaerobic conditions unsuitable for *C. curvus* (1, 9, 16, 17). Based on our results, this organism is likely being under-reported due to both selective media and unfavorable growth conditions (13). In a few selected cases, higher than usual numbers of *C. curvus* isolates have been isolated by using filtration methods (1, 12), lending support to the addition of filtration to standard isolation methods. To increase the isolation rate of *C. curvus*, we recommend a longer incubation period, filtration, and the use of brain heart infusion–5% sheep blood plates (without selection) with incubation under anaerobic conditions. This should be carried out in conjunction with the selective plates that are currently used.

We thank Robert White, the family practice physician who collected blood cultures, as well as Kathy Pon and the laboratory staff of the Westlock Healthcare Centre for the isolation of the bacterium. Joanne Simala-Grant and Kelly Baptista are acknowledged for critical reading of the manuscript.

D.E.T. is a scientist with Alberta Heritage Foundation for Medical Research.

#### REFERENCES

- Abbott, S. L., M. Waddington, D. Lindquist, J. Ware, W. Cheung, J. Ely, and J. M. Janda. 2005. Description of *Campylobacter curvus* and *C. curvus*-like strains associated with sporadic episodes of bloody gastroenteritis and Brainerd's diarrhea. *J. Clin. Microbiol.* **43**:585–588.
- Atlas, R. M. 2004. Handbook of microbiological media. CRC Press, Boca Raton, Fla.
- Baar, C., M. Eppinger, G. Raddatz, J. Simon, C. Lanz, O. Klimmek, R. Nandakumar, R. Gross, A. Rosinus, H. Keller, P. Jagtap, B. Linke, F. Meyer, H. Lederer, and S. C. Schuster. 2003. Complete genome sequence and analysis of *Wolinella succinogenes*. *Proc. Natl. Acad. Sci. USA* **100**:11690–11695.
- Fouts, D. E., E. F. Mongodin, R. E. Mandrell, W. G. Miller, D. A. Rasko, J. Ravel, L. M. Brinkac, R. T. DeBoy, C. T. Parker, S. C. Daugherty, R. J. Dodson, A. S. Durkin, R. Madupu, S. A. Sullivan, J. U. Shetty, M. A. Ayodeji, A. Shvartsbeyn, M. C. Schatz, J. H. Badger, C. M. Fraser, and K. E. Nelson. 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. *PLoS Biol.* **3**:e15.
- GenBank. 2003. GenBank: BX571656. National Center for Biotechnology Information, Bethesda, Md. [Online.] <http://www.ncbi.nlm.nih.gov/genomes/framick.cgi?db=genome&gi=323>.
- Han, X. Y., J. J. Tarrand, and D. C. Rice. 2005. Oral *Campylobacter* species involved in extraoral abscess: a report of three cases. *J. Clin. Microbiol.* **43**:2513–2515.
- Han, Y. W., W. Shi, G. T. Huang, S. Kinder Haake, N. H. Park, H. Kuramitsu, and R. J. Genco. 2000. Interactions between periodontal bacteria and human oral epithelial cells: *Fusobacterium nucleatum* adheres to and invades epithelial cells. *Infect. Immun.* **68**:3140–3146.
- Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. 1994. Bergey's manual of determinative bacteriology, 9th ed. The Williams & Wilkins Co., Baltimore, Md.
- Koga, M., N. Yuki, M. Takahashi, K. Saito, and K. Hirata. 1999. Are *Campylobacter curvus* and *Campylobacter upsaliensis* antecedent infectious agents in Guillain-Barre and Fisher syndromes? *J. Neurol. Sci.* **163**:53–57.
- Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn. 1997. Color atlas and textbook of diagnostic microbiology. Lippincott-Raven Publishers, Philadelphia, Pa.
- Lancaster, C. R., and J. Simon. 2002. Succinate:quinone oxidoreductases from epsilon-proteobacteria. *Biochim. Biophys. Acta* **1553**:84–101.
- Lastovica, A. J., and E. le Roux. 2000. Efficient isolation of campylobacteria from stools. *J. Clin. Microbiol.* **38**:2798–2799.
- Loades, C. J., L. E. Reiman, and C. W. Keevil. 2005. Abstr. 105th Gen. Meet. Am. Soc. Microbiol. 2005, abstr. D-198, p. 234–235.
- MacFaddin, J. F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott/The Williams & Wilkins Co., Philadelphia, Pa.
- Macuch, P. J., and A. C. Tanner. 2000. *Campylobacter* species in health, gingivitis, and periodontitis. *J. Dent. Res.* **79**:785–792.
- Maher, M., C. Finnegan, E. Collins, B. Ward, C. Carroll, and M. Cormican. 2003. Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of feces. *J. Clin. Microbiol.* **41**:2980–2986.
- Ng, L. K., D. E. Taylor, and M. E. Stiles. 1988. Characterization of freshly isolated *Campylobacter coli* strains and suitability of selective media for their growth. *J. Clin. Microbiol.* **26**:518–523.
- Paster, B. J., and R. J. Gibbons. 1986. Chemotactic response to formate by *Campylobacter concisus* and its potential role in gingival colonization. *Infect. Immun.* **52**:378–383.
- Popovic-Uroic, T., C. M. Patton, M. A. Nicholson, and J. A. Kiehlbauch. 1990. Evaluation of the indoxyl acetate hydrolysis test for rapid differentiation of *Campylobacter*, *Helicobacter*, and *Wolinella* species. *J. Clin. Microbiol.* **28**:2335–2339.
- Ribot, E. M., C. Fitzgerald, K. Kubota, B. Swaminathan, and T. J. Barrett. 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. *J. Clin. Microbiol.* **39**:1889–1894.
- Sambrook, J., and D. W. Russell. 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Tanner, A. C. R., M. A. Listgarten, and J. L. Ebersole. 1984. *Wolinella curva* sp. nov.: “*Vibrio succinogenes*” of human origin. *Int. J. Syst. Bacteriol.* **34**:275–282.
- Taylor, D. E., and A. S. Chau. 1997. Cloning and nucleotide sequence of the *gyrA* gene from *Campylobacter fetus* subsp. *fetus* ATCC 27374 and characterization of ciprofloxacin-resistant laboratory and clinical isolates. *Antimicrob. Agents Chemother.* **41**:665–671.
- Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley. 1991. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. *Int. J. Syst. Bacteriol.* **41**:88–103.
- Wolin, M. J., Wolin, E. A., and M. J. Jacobs. 1961. Cytochrome-producing anaerobic vibrio, *Vibrio succinogenes* sp. n. *J. Bacteriol.* **81**:911–917.