

Case Report of an Unclassified Microaerophilic Bacterium Associated with Gastroenteritis

SANDRA ROMERO,^{1,2} JOHN R. ARCHER,^{1*} MARJORIE E. HAMACHER,¹ STEPHEN M. BOLOGNA,³
AND RONALD F. SCHELL^{1,2}

State Laboratory of Hygiene¹ and Departments of Medical Microbiology² and Pathology,³ University of Wisconsin, Madison, Wisconsin 53706

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An unusual microaerophilic gram-negative bacterium was isolated from the stools of two individuals presenting with chronic diarrhea. This bacterium resembled *Campylobacter* species by colonial morphology and biochemical reactions. However, microscopic examination revealed a fusiform rod with a corrugated surface, rather than a spiral rod. This is the first reported isolation of this bacterium from humans.

Since the first successful isolation of *Campylobacter* species utilizing selective plating media and optimal growth conditions (17), the number of clinical isolates has steadily increased (11). These human isolates include *Campylobacter jejuni*, which causes enteritis (21), whereas other *Campylobacter* species have been associated with diarrhea, enterocolitis, enteritis, and gastritis (6, 14, 16, 19, 20). This study is the first reported isolation of an unclassified bacterium from two humans with mild chronic gastroenteritis; this bacterium shares some cultural and biochemical characteristics with *Campylobacter* species.

Case 1. A 47-year-old male with symptoms of gastroenteritis was seen in May 1985 by a physician in Madison, Wis. The patient had a history of recent travel to the Dominican Republic in January 1985 but appeared healthy until April, when he developed symptoms. He demonstrated a chronic diarrhea with two to three loose stools per day which were mushy but not bloody or watery. Other symptoms were fever, headache, and lower abdominal pain with no nausea or vomiting. Stool cultures yielded a gram-negative bacterium which resembled *Campylobacter* species on initial inspection. This isolate was subsequently lost during biochemical testing. A second specimen was requested, and the unusual bacterium was reisolated. Cultures for enteric pathogens, as well as microscopic examinations for ova and parasites, were negative. The patient was treated with erythromycin and became asymptomatic. Follow-up cultures were negative, and no relapse occurred. The family members of patient 1 were cultured for enteric pathogens, and a second isolate of the unusual bacterium was recovered from the 16-year-old daughter, who was asymptomatic. Other family members were negative. The bacterium was also isolated from an asymptomatic young dog (5-month-old female) owned by the family, but an older dog (16 years old) was negative.

Case 2. A 40-year-old male with symptoms of chronic gastroenteritis was seen in October 1985 by a physician in Janesville, Wis. The patient had a 2-month history of six to eight soft-to-watery bowel movements a day. His stools were not bloody and did not contain mucus. He showed no weight loss or fever. No inflammatory changes were evident in the bowel by fiberoptic sigmoidoscopy. When stool cultures were performed for enteric pathogens, a bacterium resembling the bacterium in case 1 was isolated. No other

enteric pathogens were found, and microscopic examinations for ova and parasites were negative. The patient had no history of foreign travel or known association with animals. The patient was administered 250 mg of erythromycin 4 times a day for 10 days, and the symptoms resolved with no relapse. Follow-up specimens for bacterial pathogens were not available.

Stool specimens were cultured for enteric pathogens with conventional plating media (5). Specimens were cultured for *Campylobacter* species with Skirrow plating medium (17) under microaerophilic conditions at 42°C for 72 h. After incubation, the same microaerophilic gram-negative bacterium was isolated from three human and one canine stool specimen. The colonies resembled *Campylobacter* species. They were transparent, confluent or watery, flat, and spreading along the streak lines. Older cultures appeared wavelike or rippled. The bacterium grew microaerophilically but not aerobically or anaerobically. Attempts at growth under a CO₂ atmosphere were negative. Subcultures were maintained by passage on blood agar plates (7% sheep blood; Difco Laboratories). Microscopic examination was performed by phase-contrast microscopy and transmission electron microscopy (1). Microscopically, the bacterium was fusiform shaped and approximately 6.5 by 0.5 μm. The bacterium possessed multiple bipolar flagella that allowed for random and, occasionally, polar oscillating movement. The number of flagella was approximately seven; the flagella were sheathed. The bacterium also had a corrugated surface formed by periplasmic fibers (1). The fusiform-shaped rods began to develop coccoid forms after 48 h of culture.

Biochemical characterizations were determined by the methods of Benjamin et al. (2). The bacterium was oxidase positive, catalase negative, and nonfermentative. In addition, tests for 1% glycine, 3.5% NaCl, and hippurate hydrolysis (9) were negative. The bacterium, however, demonstrated a very rapid hydrolysis of urea (4). Trace amounts of H₂S production were also detected only on lead acetate strips. Tests for tolerance to nalidixic acid and cephalothin were performed using 30-μg disks (Difco) placed on blood agar plates previously inoculated with a suspension of 10⁸ bacteria per ml. Zones of inhibition were determined as previously described by Karmali et al. (10). The bacterium was resistant to nalidixic acid and cephalothin.

The microaerophilic bacterium shared characteristics with *Campylobacter* species. Similarities were established on the basis of colony morphology, development of coccoid forms

* Corresponding author.

after 48 to 72 h of incubation, growth and isolation under microaerophilic conditions, and biochemical reactions. Although the unidentified bacterium resembled *Campylobacter* species, its microscopic morphology was fusiform, rather than spiral, and the surface was corrugated due to the presence of periplasmic fibers. The number and arrangement of flagella was different from *Campylobacter* species since the unidentified bacterium had bipolar tufts of sheathed flagella, instead of single bipolar flagella. Additional studies to determine the relationship between this bacterium and the genus *Campylobacter* are presented in the accompanying paper (1).

The demonstration of this unidentified bacterium in humans is interesting, although its association with gastroenteritis may be coincidental. It is recognized that more patients would be needed to confirm the relationship between the presence of the bacterium and development of disease. One would also expect rare or no isolates from healthy subjects, disappearance of the bacterium after treatment, absence of other pathogens in stools, demonstration of virulence factor, and production of disease in animals. Several of these factors have been addressed in this case report. Both cases of gastroenteritis resolved immediately after treatment with erythromycin, and no relapses were reported. The rapidity of resolution after treatment suggests a nonviral etiology. No other common enteric pathogens or ova and parasites were cultured or detected from the stools of the two patients. The fact that the bacterium was not associated with gastroenteritis in the daughter of one of the patients is difficult to explain. Neither patient has an underlying immune deficiency to account for the symptoms of the disease. The same bacterium was reisolated from the dog owned by patient 1. It is known that animals can be reservoirs for *Campylobacter* species (3, 8, 18). There have been reports of bacteria resembling our isolate found in lower animals (7, 12, 13, 15), although association with disease in humans has not been reported. Patient 2 of our study had gastroenteritis with no known association with animals. Additional studies need to be performed to determine if dogs or other animals can transmit or acquire this bacterium.

This study represents the first reported isolation of an unclassified microaerophilic bacterium associated with mild chronic gastroenteritis in humans.

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LITERATURE CITED

1. Archer, J. R., S. Romero, A. E. Ritchie, M. E. Hamacher, B. M. Steiner, J. H. Bryner, and R. F. Schell. 1988. Characterization of an unclassified microaerophilic bacterium associated with gastroenteritis. *J. Clin. Microbiol.* **26**:101-105.
2. Benjamin, J., S. Leaper, R. J. Owen, and M. B. Skirrow. 1983. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic campylobacter (NARTC) group. *Curr. Microbiol.* **8**:231-238.
3. Blaser, M. J., J. Cravens, and B. W. Towers. 1978. *Campylobacter* enteritis associated with canine infection. *Lancet* **ii**: 979-987.
4. Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bacteriol.* **52**: 461-466.
5. Ewing, W. H. (ed.). 1986. Isolation and preliminary identification, p. 27-45. In Edwards and Ewings' identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing, Co., New York.
6. Fennell, C. L., P. A. Totten, T. C. Quinn, D. L. Patton, K. K. Holmes, and W. E. Stamm. 1984. Characterization of *Campylobacter*-like organisms isolated from homosexual men. *J. Infect. Dis.* **149**:58-66.
7. Foster, J. R. 1984. Bacterial infection of the common bile duct in chronic fascioliasis in the rat. *J. Comp. Pathol.* **94**:175-181.
8. F6x, J. G., R. Moore, and J. I. Ackerman. 1983. Canine and feline campylobacteriosis: epizootiology and clinical and public health features. *J. Am. Vet. Med. Assoc.* **183**:1420-1424.
9. Hwang, M.-N., and G. M. Ederer. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *J. Clin. Microbiol.* **1**:114-115.
10. Karmali, M. A., S. De Grandis, and P. C. Fleming. 1980. Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus* to eight cephalosporins with special reference to species differentiation. *Antimicrob. Agents Chemother.* **18**:948-951.
11. Klein, B. S., J. M. Vergeront, M. J. Blaser, P. Edmonds, D. J. Brenner, D. Janssen, and J. P. Davis. 1986. *Campylobacter* infection associated with raw milk. *J. Am. Med. Assoc.* **255**: 361-364.
12. Lockard, V. G., and R. K. Boler. 1970. Ultrastructure of a spiraled microorganism in the gastric mucosa of dogs. *Am. J. Vet. Res.* **31**:1453-1462.
13. Phillips, M. W., and A. Lee. 1983. Isolation and characterization of a spiral bacterium from the crypts of rodent gastrointestinal tracts. *Appl. Environ. Microbiol.* **45**:675-683.
14. Rathbone, B. J., J. I. Wyatt, and R. V. Heatley. 1986. *C. pyloridis*—a new factor in peptic ulcer disease? *Gut* **27**:635-641.
15. Salomon, H. 1896. Ueber das Spirillum des Saugtiermagens und sein Verhalten zu den Belgzellen. *Centralbl. Bakteriol.* **19**:433-442.
16. Simor, A. E., and L. Wilcox. 1987. Enteritis associated with *Campylobacter laridis*. *J. Clin. Microbiol.* **25**:10-12.
17. Skirrow, M. B. 1977. *Campylobacter* enteritis, a "new disease." *Br. Med. J.* **2**:9-11.
18. Skirrow, M. B., G. L. Turnbull, R. E. Walker, and S. E. J. Young. 1980. *Campylobacter jejuni* enteritis transmitted from cat to man. *Lancet* **i**:1188.
19. Totten, P. A., C. L. Fennell, F. C. Tenover, J. M. Wezenberg, P. L. Perine, W. E. Stamm, and K. K. Holmes. 1985. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennelliae* (sp. nov.): two new *Campylobacter* sp. associated with enteric disease in homosexual men. *J. Infect. Dis.* **151**:131-139.
20. Vesterby, A., U. Baandrup, N. O. Jacobsen, and K. Albertsen. 1983. *Campylobacter* enterocolitis. *Acta Pathol. Microbiol. Immunol. Scand.* **91**:31-33.
21. Walker, R. I., M. B. Caldwell, E. C. Lee, P. Guerry, T. J. Trust, and G. M. Ruiz-Palacios. 1986. Pathophysiology of *Campylobacter* enteritis. *Microbiol. Rev.* **50**:81-94.