JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1994, p. 2598–2599
0095-1137/94/$04.00+0

Vol. 32, No. 10

Detection of *Campylobacter upsaliensis* from a Blood Culture by Using the BacT/Alert System

AMY M. CARNAHAN,[1]* JOYCE BEADLING,[1] DONNA WATSKY,[1] AND NANCY FORD[2]

Department of Microbiology, Anne Arundel Medical Center, Annapolis, Maryland 21401,[1] and Microbiology Division, Department of Pathology, Johns Hopkins Hospital, Baltimore, Maryland 21287-7093[2]

Received 4 April 1994/Returned for modification 18 May 1994/Accepted 29 June 1994

***Campylobacter upsaliensis* was isolated from the blood of a 60-year-old female with hairy cell leukemia. This spiral-shaped organism was detected in the aerobic BacT/Alert bottle (Organon Teknika, Durham, N.C.) by acridine orange staining and was recovered only on chocolate agar in a microaerophilic atmosphere at 35°C.**

The name *Campylobacter upsaliensis* was proposed and later validated for a group of thermotolerant *Campylobacter* strains, isolated from dogs, that were catalase negative or weakly positive (10, 11). Similar catalase-negative or weakly positive strains have since been reported from domestic cats (2), human fecal specimens (4, 6, 12–14), blood cultures (1, 5, 8, 14), and a breast abscess (3). This case report details the detection of *C. upsaliensis* from the blood culture of a leukemic patient and its subsequent isolation and identification.

Case report. A 60-year-old female with complaints of fever, malaise, and facial pain radiating to her anterior back and neck was seen in the emergency room of Anne Arundel Medical Center. Her past medical history included a diagnosis of hairy cell leukemia 19 years previously and a recent diagnosis of adult onset diabetes mellitus. Vital signs included a slight fever of 100.6°F (38.1°C), a pulse of 125, respiration of 22, and blood pressure of 150/90. X rays for sinusitis were negative, and complete blood count results were a leukocyte count of $12.9 \times 10^3/\mu l$, an erythrocyte count of $3.86 \times 10^6/\mu l$, a hemoglobin count of 11.1 g/dl, and a hematocrit of 38.4. The patient was started on ciprofloxacin pending blood culture results.

One of four blood culture bottles (two sets) collected in the emergency room was positive after 2.5 days of incubation with *Staphylococcus epidermidis*, which was considered a probable skin contaminant. The other aerobic bottle was detected as positive by the BacT/Alert system after 4.8 days of incubation. A Gram stain and an acridine orange stain were performed, but no microorganisms were seen on the Gram stain. However, the microbiologist reported "faint, funny-looking, seagull-shaped bacteria" on the acridine orange slide and subcultured the blood culture to the following media (Becton Dickinson Microbiology Systems [BDMS], Cockeysville, Md.): Trypticase soy agar (TSA) with 5% sheep blood (both aerobic and anaerobic), chocolate agar, and eosin-methylene blue (EMB) agar. All plates except the anaerobic blood agar plate were incubated at 35°C in 5% CO_2. After 24 h of incubation, there was only very slight growth of faintly staining, gram-negative spiral bacilli on the sheep blood agar plate. The original positive aerobic blood culture was then subcultured again to chocolate agar, Campy-BAP media, Skirrows media, and BCYE (buffered charcoal yeast extract) media, and all plates were incubated in a microaerophilic atmosphere by utilizing a Campy-Pak (BDMS) at 35°C. After 48 h of incubation, growth was visible only on the chocolate agar plate and a dark-field preparation of this growth showed very motile, spirochetal organisms. The physician was sent a preliminary report of "spiral-shaped gram-negative bacilli," and the chocolate agar and positive bottle were shipped to Johns Hopkins Hospital Microbiology Laboratory for organism identification.

The following tests were performed at Johns Hopkins Hospital Microbiology Laboratory following growth at 35°C in a microaerophilic environment (Campy-Pak): catalase, oxidase, growth in the presence of 1% glycine and 1.5% NaCl, production of hydrogen sulfide in triple sugar iron (TSI) agar, nitrate reduction, hippurate hydrolysis, urease activity, susceptibility to nalidixic acid and cephalothin, and a latex slide agglutination assay (Campyslide; BDMS). Temperature and atmospheric growth requirements were determined on TSA with 5% sheep blood and chocolate agar (BDMS). Following growth on chocolate agar at 35°C for 48 h and saponification, extraction, and derivatization of whole cells (7), cellular fatty acid analysis was performed by using an automated cellular fatty acid identification system (mis; Microbial ID, Newark, Del.).

The microorganism grew only in microaerophilic conditions at 35°C, with no growth at 25 and 42°C. It was catalase negative and weakly oxidase positive. Nitrate was reduced, hydrogen sulfide was not produced in TSI, hippurate and urea were not hydrolyzed, growth was not obtained in 1% glycine or 1.5% NaCl, and the organism was susceptible to both nalidixic acid and cephalothin. The major cellular fatty acids from this isolate were octadecenoic acid (41%) and palmitic acid (32%), with smaller amounts of tetradecanoic acid (9%), hexadecanoic acid (8%), and 3-hydroxy tetradecanoic acid (7%), which is consistent with previous reports (8). The current database for organisms recovered from the BacT/Alert system contains over 100 organisms, including gram-positive and gram-negative organisms as well as anaerobes and yeasts. Prior to this case report, only *Campylobacter jejuni* and *Campylobacter fetus* had been reported to Organon Teknika as recoverable species. A similar *C. upsaliensis* isolate was previously detected from a pediatric blood culture by the Bactec 660 system (BDMS), and this organism was visible only with acridine orange and grew only on chocolate agar in a microaerophilic environment (1).

Taxonomically, the *Campylobacter* species have been arranged into groups, with subgroup IA containing those species considered to be thermophilic, enteropathogenic species: *C. jejuni* subsp. *jejuni*, *C. jejuni* subsp. *doylei*, *C. coli*, *C. lari* (formerly *C. laridis*), and *C. upsaliensis* (9). Phenotypically, *C. upsaliensis* is most closely related to *C. jejuni* subsp. *doylei*.

* Corresponding author. Present address: Department of Medical and Research Technology, University of Maryland School of Medicine, 100 Penn St., Baltimore, MD 21201-1082. Phone: (410) 706-1830. Fax: (410) 706-5229.

Both species are catalase negative or weakly positive, but *C. jejuni* subsp. *doylei* differs from *C. upsaliensis* in that the former species is variable in its ability to hydrolyze hippurate and cannot reduce nitrate. *C. upsaliensis* has been reported as positive for growth at 42°C, but even though our isolate did not grow at 42°C, other such strains have been reported (8). This isolate gave a positive agglutination result (Campyslide) with latex-bound antibodies for *C. jejuni*, *C. coli*, *C. laridis*, and *C. fetus*. It may be that *C. upsaliensis* shares cell wall antigens with these four species.

Previous studies have suggested a pathogenic role for *C. upsaliensis* in cases of diarrhea, bacteremia, and respiratory ailments (5, 8, 13). Most patients have been very young or immunocompromised or debilitated in some way, suggesting that *C. upsaliensis* may be an opportunistic pathogen. Future isolations of *C. upsaliensis* must take into account this fastidious requirement for a microaerophilic environment, lack of growth on most commercial media, and atypical reactions (catalase negative and hippurate hydrolysis negative). *C. upsaliensis* could easily be overlooked in a positive blood culture if acridine orange and Gram stains aren't both performed and unless an enriched medium like chocolate agar is inoculated and incubated in a microaerophilic atmosphere. Increased awareness of this organism may result in more frequent isolations of this species and a better understanding of its clinical and epidemiological significance as a human pathogen.

We acknowledge Charlotte Patton, Susan Schilling, and Enser Cole III for valuable discussions during the preparation of the manuscript.

REFERENCES

1. **Chusid, M. J., D. W. Wortmann, and W. M. Dunne.** 1990. "*Campylobacter upsaliensis*" sepsis in a boy with acquired hypogammaglobulinemia. Diagn. Microbiol. Infect. Dis. **13:**367–369.
2. **Fox, J. G., K. O. Maxwell, N. S. Taylor, C. D. Runsick, P. Edmonds, and D. J. Brenner.** 1989. "*Campylobacter upsaliensis*" isolated from cats as identified by DNA relatedness and biochemical features. J. Clin. Microbiol. **27:**2376–2378.
3. **Gaudreau, C., and F. Lamothe.** 1992. *Campylobacter upsaliensis* isolated from a breast abscess. J. Clin. Microbiol. **30:**1354–1356.
4. **Goosens, H., B. Pot, L. Vlaes, C. van den Boore, R. van den Abbeele, C. van Naelten, J. Levy, H. Cogniau, P. Marbehant, J. Verhoef, K. Kersters, J.-P. Butzler, and P. Vandamme.** 1990. Characterization and description of "*Campylobacter upsaliensis*" isolated from human feces. J. Clin. Microbiol. **28:**1039–1046.
5. **Lastovica, A. J., E. le Roux, and J. L. Penner.** 1989. "*Campylobacter upsaliensis*" isolated from blood cultures of pediatric patients. J. Clin. Microbiol. **27:**657–659.
6. **Megraud, F., and F. Bonnet.** 1986. Unusual *Campylobacters* in human feces. J. Infect. **12:**275–276.
7. **Osterhous, G. J., V. H. Shull, and J. D. Dick.** 1991. Identification of clinical isolates of gram-negative nonfermentative bacteria by an automated cellular fatty acid identification system. J. Clin. Microbiol. **29:**1822–1830.
8. **Patton, C. M., N. Shaffer, P. Edmonds, T. J. Barrett, M. A. Lambert, C. Baker, D. M. Pearlman, and D. J. Brenner.** 1989. Human disease associated with "*Campylobacter upsaliensis*" (catalase-negative or weakly positive *Campylobacter* species) in the United States. J. Clin. Microbiol. **27:**66–73.
9. **Penner, J. L.** 1991. *Campylobacter*, *Helicobacter*, and related spiral bacteria, p. 402–409. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.) Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
10. **Sandstedt, K., and J. Ursing.** 1991. Description of *Campylobacter upsaliensis* sp. nov. previously known as the CNW group. Syst. Appl. Microbiol. **14:**39–45.
11. **Sandstedt, K., J. Ursing, and M. Walder.** 1983. Thermotolerant *Campylobacter* with no or weak catalase activity isolated from dogs. Curr. Microbiol. **8:**209–213.
12. **Steele, T. W., N. Sangster, and J. A. Lanser.** 1985. DNA relatedness and biochemical features of *Campylobacter* spp. isolated in Central and South Australia. J. Clin. Microbiol. **22:**71–74.
13. **Taylor, D. E., K. Hiratsuka, and L. Mueller.** 1989. Isolation and characterization of catalase-negative and catalase-weak strains of *Campylobacter* species, including "*Campylobacter upsaliensis*," from humans with gastroenteritis. J. Clin. Microbiol. **27:**2042–2045.
14. **Walmsley, S. L., and M. A. Karmali.** 1989. Direct isolation of atypical thermophilic *Campylobacter* species from human feces on selective agar medium. J. Clin. Microbiol. **27:**668–670.