JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1993, p. 708–710
0095-1137/93/030708-03$02.00/0

Vol. 31, No. 3

Three Supplementary Diagnostic Tests for *Campylobacter* Species and Related Organisms

ANDRÉ P. BURNENS* AND JACQUES NICOLET

Institute for Veterinary Bacteriology, University of Berne, Länggass-Strasse 122, CH-3012 Berne, Switzerland

Received 15 July 1992/Accepted 23 November 1992

We analyzed 307 recent clinical isolates and reference strains of *Campylobacter* spp., *Helicobacter* spp., and *Arcobacter* spp. for arylsulfatase and pyrazinamidase activities and susceptibility to polymyxin B. All isolates studied were identified by DNA dot hybridization prior to testing. *Campylobacter concisus*, *C. sputorum*, and *Helicobacter fennelliae* were positive for arylsulfatase. *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. concisus* were positive for pyrazinamidase. Only *Helicobacter* spp. were resistant to polymyxin B. *C. fetus* subsp. *fetus*, *C. hyointestinalis*, *C. mucosalis*, and *Arcobacter* spp. were negative in all three tests. The tests were simple to perform and read, and with the exception of *C. sputorum* biovars, all isolates within a species gave consistently positive or negative results in all the assays. These three phenotypic tests may help refine current methods for phenotypic identification of *Campylobacter* spp. and related organisms in a reference laboratory setting.

Campylobacter jejuni and *C. coli* have been recognized as important causes of bacterial gastroenteritis worldwide (1). However, with the increasing use of less-selective culture conditions, many other species of *Campylobacter* and related organisms have been found in fecal specimens from gastroenteritis patients (10). Because of their clinical significance, such isolates should be identified.

The present methods for identification of *Campylobacter* spp. and related organisms are few (11), and many of them are difficult to perform. The tests involving rating of growth responses are particularly difficult to use (9). On the other hand, tests for enzymatic activities usually give clear results. Therefore, we evaluated the use of arylsulfatase for *Campylobacter* identification and developed a test for measurement of pyrazinamidase activity. Furthermore, we showed that testing for susceptibility to polymyxin B by disk diffusion is useful in differentiating *Campylobacter* spp. from *Helicobacter* spp. The tests were evaluated with a collection of recent isolates from veterinary and human clinical specimens, representing species likely to be encountered in a reference laboratory setting. For species of *Campylobacter* and related organisms rarely encountered in clinical specimens, reference strains were included in the study.

(Part of the results have been presented at the 92nd General Meeting of the American Society for Microbiology [2a].)

The arylsulfatase test medium was a modification of a previously described formula (4). The medium consisted of Brucella broth (BBL 11088) with 4 g of Bacto-agar (Difco) and 650 mg of tripotassium salt of phenolphthalein disulfate (Fluka 77635) per liter. The pyrazinamidase medium was composed of Mueller-Hinton broth (BBL 11443) with 4 g of Bacto-agar and 200 mg of pyrazinamide (Merck 821050) per liter. Both media were added in 3-ml amounts to tubes (12 by 120 mm) and then autoclaved. Tubes were inoculated by stabbing the upper 0.5 cm of the agar three times with a straight wire. The tubes were then incubated for 48 h (96 h for isolates of *C. concisus*) at 37°C in an atmosphere containing 7% H_2, 7% CO_2, and 6% O_2 in N_2, created by evacuating the incubation chambers and filling them with the appropriate gas mixture. For development of the color reaction, the arylsulfatase medium was overlaid with 0.5 ml of a 1 M Na_2CO_3 solution, and the immediate appearance of a red color was scored as a positive result. The pyrazinamidase reaction was developed by overlaying the medium with 0.5 ml of freshly prepared 10% $FeSO_4$ in distilled water. The appearance of a brownish-pink band in the agar after 15 min at room temperature was scored as a positive reaction.

Susceptibility to polymyxin B was measured by swabbing the surface of a Columbia agar plate with 5% sheep blood (BioMérieux 4 304 1) with a 0.5 McFarland standard suspension of the organism. A disk containing 300 IU of polymyxin B was applied, and the plate was incubated for 24 h at 37°C in the atmosphere described above. The organism was considered susceptible if any zone of inhibition around the disk was apparent. The zones for susceptible organisms were at least 9 mm and usually larger than 12 mm.

All isolates used in the study had been previously identified by DNA hybridization as described before (2). Strains from clinical veterinary specimens had been isolated in our laboratory. Strains from humans were isolated in clinical microbiology laboratories in Switzerland and sent to us for confirmation. For every species analyzed, the type strain was included. Some of the type strains used have been described before (2). Campylobacter-like strain CLO3 was from C. Fennell (Seattle, Wash.), *Helicobacter fennelliae* NCTC 11612 was obtained from the National Collection of Type Cultures (London, United Kingdom), and the remaining type strains were obtained from the American Type Culture Collection (Rockville, Md.). For species of *Campylobacter* and related organisms rarely encountered in clinical specimens, the following reference strains from the Culture Collection of the University of Göteborg (CCUG) were included in the study: *C. lari* CCUG strains 15031, 15035, 19512, 19519, 19528, 20575, 20581, 20593, 29405, and 29406; *C. mucosalis* CCUG strains 10771, 21559, 21560, 23203, 23204, and 24190; *C. sputorum* biovars CCUG 886, 11290, 12014, 12016, 20703, 22572, 22574, 22579, and 24262; and *H. cinaedi* CCUG strains 15432, 17733, 18819, 19218, 19503, 19504, and 20698.

The results of our study are summarized in Table 1. We

* Corresponding author.

TABLE 1. Arylsulfatase and pyrazinamidase activity and polymyxin B susceptibility of *Campylobacter* spp. and related organisms

Species[a]	No. of strains tested	% of strains		
		Positive for arylsulfatase activity	Positive for pyrazinamidase activity	Resistant to polymyxin B
C. jejuni	54	2	100	0
C. coli	19	0	100	0
C. lari	12	0	100	0
C. upsaliensis	115	0	100	0
C. fetus subsp. *fetus*	24	0	0	0
C. hyointestinalis	16	0	0	0
C. mucosalis	7	0	0	0
C. concisus	10	100	100	0
C. sputorum[b]	13	100	62	0
H. fennelliae	2	100	0	100
Helicobacter spp.[c]	21	0	0	86
Arcobacter spp.[d]	14	0	0	0

[a] The type strain was included for each species tested.
[b] Includes one isolate of biovar sputorum, five of biovar bubulus, and seven of biovar faecalis.
[c] Includes eight isolates of *H. cinaedi*, isolate CLO3, six of *H. fennelliae*-like organisms, and six of *H. pylori*.
[d] Includes five isolates of *A. butzleri* and nine of *A. cryaerophilus*.

tested some of the strains with different medium formulations and incubation conditions; the conditions and media described above gave the best results. Only the results for strains tested with the above-mentioned procedures are included in the table.

Testing of *Campylobacter* spp. for arylsulfatase activity has been described before (4, 16). However, in one study, only five *Campylobacter* species were included in the analysis (4). The other report considered arylsulfatase results only for the *Campylobacter* spp. present in the oral flora (16). The simplified formula for and preparation of the medium that we propose give results comparable to those described for both the original medium (4) and the semisynthetic plating medium with an indolyl chromogenic substrate (16).

In the present study, members of 11 species and groups of recently described campylobacters and related organisms have been tested for arylsulfatase activity for the first time. *C. mucosalis* has been recognized as a species separate from *C. sputorum* since the first description of the test (14). Therefore, *C. mucosalis* can now be differentiated from *C. sputorum* by a negative test for arylsulfatase activity. Furthermore, arylsulfatase activity is very helpful for differentiation of *C. upsaliensis* from *C. concisus*, both of which are catalase-negative species found in feces (13).

Some isolates of *C. upsaliensis* were reported to be positive for arylsulfatase in a previous report (8). We found no strain of *C. upsaliensis* that was positive for arylsulfatase in the present study. Because all our strains were identified by DNA hybridization, we are confident about our results. The *C. upsaliensis* strains that were reportedly arylsulfatase positive (8) were not available for study. These differences may be explained by the fact that arylsulfatase activity is medium dependent. Evidence for this may be that no strain of any *Campylobacter* species tested showed arylsulfatase activity when tested in semisolid Mueller-Hinton agar. Further evidence for the medium dependence of arylsulfatase activity is that *C. concisus* tended to give weaker or negative reactions in batches of medium that had been refrigerated for more than 3 weeks.

Pyrazinamidase activity has been widely used for the differentiation of diverse genera of bacteria (3, 5, 7, 12). To our knowledge, *Campylobacter* spp. have never been tested for pyrazinamidase before. We found that a concentration of 200 mg of pyrazinamide per liter was optimal for reading of results. The group of thermophilic enteropathogenic *Campylobacter* spp. consistently gave strongly positive results in the test, whereas *C. concisus* was always weakly positive. The test was also very useful for differentiation of *Arcobacter butzleri* (6, 14), which is pyrazinamidase negative, from the occasional aerotolerant strains of *C. coli* (positive). Furthermore, it differentiates *C. lari* (positive) from thermotolerant *C. fetus* subsp. *fetus* and *C. hyointestinalis* (negative).

Resistance to polymyxin B has been widely used for the differentiation of bacteria (15). We observed that the level of resistance to polymyxin B was species specific for *Campylobacter* spp. and related organisms. *H. fennelliae*, a group of *H. fennelliae*-like organisms isolated from dog feces, *H. cinaedi*, strain CLO3, and 50% of the *H. pylori* isolates grew with a disk containing 300 IU of polymyxin B, whereas for all 284 isolates of *Campylobacter* spp. and *Arcobacter* spp. tested, the zone of inhibition was at least 9 mm and usually greater than 12 mm. Therefore, we conclude that only *Helicobacter* spp. are able to grow with polymyxin B, even if some *H. pylori* fail to do so.

Only six enzymatic tests for the differentiation of campylobacters and related organisms have been described in standard texts so far (see references in reference 10). The arylsulfatase and pyrazinamidase tests thus add significantly to the reactions available for the phenotypic identification of clinically important *Campylobacter* spp. and related organisms. The enzymatic tests were particularly useful because they were simple to read and interpret. However, the results obtained with a few strains of rare organisms will need further confirmation. Polymyxin B resistance, on the other hand, gave very useful information for differentiating between *Helicobacter* spp. and *Campylobacter* spp.

REFERENCES

1. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. **5**:157–176.
2. **Burnens, A. P., and J. Nicolet.** 1992. Detection of *Campylobacter upsaliensis* in diarrheic dogs and cats, using a selective medium with cefoperazone. Am. J. Vet. Res. **53**:48–51.
2a. **Burnens, A. P., and J. Nicolet.** 1992. Abstr. 92nd Gen. Meet.

Am. Soc. Microbiol. 1992, abstr. C-103, p. 437.
3. **Carnahan, A., L. Hammontree, L. Bourgeois, and S. W. Joseph.** 1990. Pyrazinamidase activity as a phenotypic marker for several *Aeromonas* spp. isolated from clinical specimens. J. Clin. Microbiol. **28:**391–392.
4. **Hebert, G. A., D. G. Hollis, R. E. Weaver, M. A. Lambert, M. J. Blaser, and C. W. Moss.** 1982. Thirty years of campylobacters: biochemical characteristics and a biotyping proposal for *Campylobacter jejuni*. J. Clin. Microbiol. **15:**1065–1073.
5. **Kandolo, K., and G. Wauters.** 1985. Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. J. Clin. Microbiol. **21:**980–982.
6. **Kiehlbauch, J. A., D. J. Brenner, M. A. Nicholson, C. N. Baker, C. M. Patton, A. G. Steigerwalt, and I. K. Wachsmuth.** 1991. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. J. Clin. Microbiol. **29:**376–385.
7. **Konno, K., F. M. Feldman, and W. McDermott.** 1967. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am. Rev. Respir. Dis. **95:**461–469.
8. **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.
9. **On, S. L. W., and B. Holmes.** 1991. Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. J. Clin. Microbiol. **29:**923–926.
10. **Penner, J. L.** 1988. The genus *Campylobacter*: a decade of progress. Clin. Microbiol. Rev. **1:**157–172.
11. **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.
12. **Sulea, I. T., M. C. Pollice, and L. Barksdale.** 1980. Pyrazine carboxylamidase activity in *Corynebacterium*. Int. J. Syst. Bacteriol. **30:**466–472.
13. **Vandamme, P., E. Falsen, B. Pot, B. Hoste, K. Kersters, and J. de Ley.** 1989. Identification of EF group 22 campylobacters from gastroenteritis cases as *Campylobacter concisus*. J. Clin. Microbiol. **27:**1775–1781.
14. **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.
15. **von Graevenitz, A.** 1986. The use of antimicrobial agents as tools in epidemiology, identification, and selection of microorganisms, p. 810–824. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
16. **Wyss, C.** 1989. *Campylobacter-Wolinella* group organisms are the only oral bacteria that form arylsulfatase-active colonies on a synthetic indicator medium. Infect. Immun. **57:**1380–1383.