JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2304–2306 Vol. 34, No. 9
0095-1137/96/$04.00+0

Isolation of *Campylobacter concisus* from Feces of Children with and without Diarrhea

R. VAN ETTERIJCK,[1] J. BREYNAERT,[1] H. REVETS,[1]† T. DEVREKER,[2] Y. VANDENPLAS,[2] P. VANDAMME,[3] AND S. LAUWERS[1]*

Departments of Microbiology[1] and Pediatrics,[2] Akademisch Ziekenhuis, Vrije Universiteit Brussel, B-1090 Brussels, and Department of Microbiology, Universiteit Gent, B-9000 Gent,[3] Belgium

Received 16 February 1996/Returned for modification 19 April 1996/Accepted 12 June 1996

A prospective study compared fecal isolation rates of *Campylobacter concisus* for children with diarrhea and without diarrhea by a filter technique in which media were incubated for 4 days in a microaerobic atmosphere. No statistically significant difference in isolation rates was found (13.2% in patients with diarrhea and 9% in controls). Moreover, 35 of 37 children attending the same day care center harbored different *C. concisus* strains, as was demonstrated by arbitrary primer PCR DNA fingerprinting. These data suggest a lack of a pathogenic role for *C. concisus* in enteritis.

Campylobacter concisus, a fastidious and rather slowly growing gram-negative rod of the human oral cavity (14), has been associated with periodontal disease in humans. *C. concisus* strains were recovered from the gingival crevices of patients who had gingivitis and periodontitis with advancing bone loss (3, 6, 13, 14), but their role in the pathogenesis of periodontal disease has not yet been firmly established. Hitherto, reports on the isolation of *C. concisus* from other human sources have been scant. Johnson and Finegold (2) and Vandamme et al. (18) identified *C. concisus* strains that were isolated mainly from the feces of patients with gastrointestinal disorders. More recently, two prospective studies (4, 5) indicated that *C. concisus* is commonly found in the feces of children with diarrhea, but no real pathogenic role for *C. concisus* was established.

These reports prompted us to investigate the pathogenic role of *C. concisus* in gastrointestinal disease in children by comparing fecal isolation rates for patients with enteritis and a control population. In addition, the epidemiological relationships of *C. concisus* strains isolated from children attending the same day care center were studied by PCR-mediated fingerprinting.

For a period from September 1991 to March 1992, the isolation rate of *C. concisus* in 174 children with enteritis was determined. One hundred and thirty-four children (1 month to 3 years old) were admitted to the hospital for enteritis, 14 children (1 month to 3 years old) developed symptoms of enteritis while in the hospital, and 26 children (1 to 8 months old) suffered from diarrhea at the time they were admitted to the sleep unit for sudden infant death syndrome screening. The control group consisted of 958 children: 272 children (1 month to 3 years old) who were admitted to the hospital for a variety of disorders but who did not have enteritis and 686 healthy siblings (1 to 8 months old) who were admitted to the hospital for sudden infant death syndrome screening and who did not have diarrhea at the time of admission. The chi-square test was used to compare the isolation rates for patients and controls.

All stool specimens were cultured for *C. concisus* on a selective Mueller-Hinton agar supplemented with 5% (vol/vol) sheep blood, 10 μg of nalidixic acid per ml, and 10 μg of vancomycin per ml and on two nonselective Columbia blood agars (with either a 0.45- or a 0.65-μm-pore-size filter). Both selective and nonselective media were incubated at 37°C for up to 4 days in an atmosphere of 7% CO_2, 4.5% O_2, 81.5% N_2, and 7% H_2. A Gram stain was performed on all suspected colonies. For species identification, the following cultural and biochemical characteristics were determined: oxidase activity; catalase activity; growth at 25, 37, and 42°C; microaerophilic growth with and without H_2; anaerobic growth in the presence of fumarate and fumarate plus formate; hydrolysis of hippurate; hydrolysis of indoxyl acetate; H_2S production in triple sugar iron agar; and finally, susceptibility to nalidixic acid and cephalothin. Identification of *C. concisus* strains was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins (9, 17). Arbitrary primer PCR DNA fingerprinting was performed on 42 *C. concisus* strains isolated from 34 children with diarrhea who attended the day care center of our medical campus during overlapping time periods and on 9 unrelated *C. concisus* strains. Boiled suspensions of *C. concisus* were used as the target in the arbitrary primer PCR. A standard protocol was followed (20), with a combination of arbitrary primers 1247 (5′-AAGAGCCCGT-3′) and 1253 (5′-GTTTCCGCCC-3′) being used. The reproducibility of the results was examined by repeating the arbitrary primer PCR procedure at least twice for each isolate. Isolates were considered to represent the same strain if their DNA profiles were indistinguishable, whereas isolates were categorized as unrelated if their DNA profiles differed by more than two DNA band shifts.

Comparison of fecal isolation rates for children with and without enteritis. Twenty-three of 174 children with diarrhea were culture positive for *C. concisus*, giving an isolation rate of 13.2%. In the control group, 86 of 958 children were positive for *C. concisus*, giving an isolation rate of 9.0%. This difference in isolation rates is statistically not significant ($P = 0.15$). As can be seen from Table 1, the sensitivities of the individual culture methods compared with that of the three methods combined are rather low: 44% for culturing on selective medium with vancomycin and nalidixic acid, 50.9% for the filtration technique using a 0.45-μm-pore-size filter, and 53.4% for

* Corresponding author. Mailing address: Department of Microbiology, Akademisch Ziekenhuis, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium. Phone: 32-2-4775000. Fax: 32-2-4775015.

† Present address: Laboratory for Cellular Immunology, Vlaams Interuniversitair Instituut voor Biotechnologie, Vrije Universiteit Brussel, B-1640 Sint-Genesius-Rode, Belgium.

TABLE 1. Sensitivities of different culture methods for *C. concisus*

Culture method	No. of positive cultures	Sensitivity (%)[a]
Filter (0.45-μm pore size)	59	50.9
Filter (0.65-μm pore size)	62	53.4
Selective medium	51	44.0
Filter (0.45- and 0.65-μm pore sizes)	81	69.8
Filter (0.45-μm pore size) and selective medium	97	83.6
Filter (0.65-μm pore size) and selective medium	99	85.3

[a] Sensitivity was calculated on the basis of the total number of positive cultures (116) obtained by the use of the three methods combined.

the filtration technique using a 0.65-μm-pore-size filter. A combination of culturing on selective medium with either one of the filtration techniques improved sensitivities to more than 80%.

Phenotypic characteristics of *C. concisus*. In comparison with those of other enteric *Campylobacter* species, *C. concisus* cells are much less curved. Growth is rather slow; after 3 to 4 days of incubation, small (0.5- to 1-mm diameter), flat to convex, translucent to brownish nonhemolytic colonies appeared on the Columbia blood agar. The major features distinguishing *C. concisus* from other catalase-negative (or weakly positive) *Campylobacter* species are microaerophilic growth with and without H_2, anaerobic growth with fumarate and fumarate plus formate, hydrolysis of indoxyl acetate, and susceptibility to cephalothin. *C. concisus* grows only in the presence of H_2 in a microaerobic atmosphere; anaerobic growth is observed only in the presence of fumarate plus formate. In contrast to reports in the literature (10, 16, 19) in which *C. concisus* is reported to be negative for indoxyl acetate hydrolysis, 6% of our *C. concisus* strains were positive for indoxyl acetate hydrolysis. In these cases, routine phenotypic tests do not allow discrimination of *C. concisus* from the indoxyl acetate hydrolysis-positive *Campylobacter curvus* (15), another campylobacter that is found in the human mouth and that has also been isolated from feces (4). Results of SDS-PAGE of whole-cell proteins of *C. concisus* strains correlate with results of DNA-DNA binding (18), and SDS-PAGE can differentiate *C. concisus* from *C. curvus* (12), a result which was corroborated in our study (data not shown). In addition, *C. concisus* has been reported to be resistant to cephalothin and nalidixic acid (10, 16, 19). However, in these studies, only a limited number of strains were tested. In our study, all *C. concisus* clinical isolates were sensitive to cephalothin and 49% were sensitive to nalidixic acid.

DNA fingerprinting of *C. concisus* strains. All strains examined showed stable profiles on repeated testing. Figure 1 shows DNA fingerprints from a selection of strains. A total of 49 different PCR types could be distinguished. On two occasions, the same DNA pattern was observed in two different children attending the day care center. All other children harbored unique strains. Six children after a mean follow-up period of 4 to 12 months harbored two or three unrelated strains that were distinguished by arbitrary primer PCR patterns with at least three fragment mismatches.

The routine use of a filter paper technique (11) for the isolation of campylobacters from feces has resulted in the isolation of *Campylobacter* species other than *Campylobacter jejuni* and *Campylobacter coli* (4, 5). One of the species that can frequently be recovered if the media are incubated in an H_2-enriched atmosphere is *C. concisus*, a campylobacter mainly associated with the oral cavity. Until recently, only 13 fecal isolates of *C. concisus* from 10 Swedish and 3 British patients

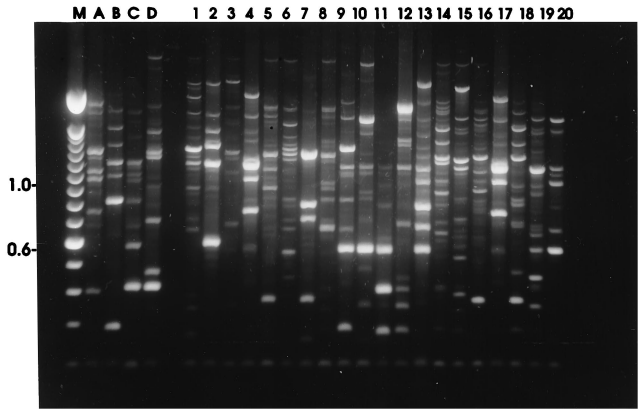

FIG. 1. DNA fingerprints of a selection of *C. concisus* strains isolated from children attending the same day care center (lanes 1 to 20) compared with those of *C. concisus* control strains isolated from patients who were unrelated to the day care center (lanes A to D). Lane M contains a 100-bp DNA ladder as a molecular weight marker.

with symptoms of enteritis have been described in the literature (2, 5, 18). In 1991, we reported the isolation of *C. concisus* from 19 adults and 75 children, most of whom had diarrhea (4). Figura et al. (1) recently reported the isolation of *Campylobacter mucosalis* from two children with enteritis by using a filtration technique and incubation in an H_2-enriched microaerobic atmosphere. However, the characteristics described for these *C. mucosalis* strains conformed to the description of *C. concisus* (8, 19). Using 64 phenotypic characteristics and SDS-PAGE whole-cell protein electropherograms, On demonstrated that Figura's strains were *C. concisus* that had been misidentified as *C. mucosalis* (7). That study and our own study show that adequate discrimination of *C. concisus* from other catalase-negative (or weakly positive) *Campylobacter* species is very difficult when a limited number of phenotypic tests are used.

Although *C. concisus* can frequently be isolated from patients with diarrhea, a comparison of the isolation rates of *C. concisus* for children with and without diarrhea in this study showed that isolates are found in normal subjects as often as they are in patients with diarrhea. Moreover, *C. concisus* strains isolated from children attending the same day care center during overlapping periods of time did not show any epidemiological relationships. Consequently, *C. concisus* should not be considered a primary pathogen associated with gastrointestinal disease.

We thank Anita Wijns and Ann De Backer for technical assistance.

P.V. is indebted to the National Fund for Scientific Research (Belgium) for a position as a postdoctoral research fellow.

REFERENCES

1. **Figura, N., P. Guglielmetti, A. Zanchi, N. Partini, D. Armellini, P. F. Bayeli, M. Bugnoli, and S. Verdiani.** 1993. Two cases of *Campylobacter mucosalis* enteritis in children. J. Clin. Microbiol. **31:**727–728.
2. **Johnson, C. C., and S. M. Finegold.** 1987. Uncommonly encountered, motile, anaerobic Gram-negative bacilli associated with infection. Rev. Infect. Dis. **9:**1150–1162.
3. **Kamma, J. J., M. Nakou, and F. A. Manti.** 1994. Microbiota of rapidly progressive periodontitis lesions in association with clinical parameters. J. Periodontol. **65:**1073–1078.
4. **Lauwers, S., T. Devreker, R. Van Etterijck, J. Breynaert, A. Van Zeebroeck, L. Smekens, K. Kersters, and P. Vandamme.** 1991. Isolation of *Campylobacter concisus* from human faeces. Microb. Ecol. Health Dis. **4**(Suppl.)**:** S91.
5. **Lindblom, G.-B., E. Sjögren, J. Hansson-Westerberg, and B. Kaijser.** 1995. *Campylobacter upsaliensis*, *C. sputorum sputorum* and *C. concisus* as common

causes of diarrhoea in Swedish children. Scand. J. Infect. Dis. **27:**187–188.
6. **Moore, W. E. C., L. V. Holdeman, E. P. Cato, R. M. Smibert, J. A. Burmeister, K. G. Palcanis, and R. R. Ranney.** 1985. Comparative bacteriology of juvenile periodontitis. Infect. Immun. **48:**507–519.
7. **On, S. L. W.** 1994. Confirmation of human *Campylobacter concisus* isolates misidentified as *Campylobacter mucosalis* and suggestions for improved differentiation between the two species. J. Clin. Microbiol. **32:**2305–2306.
8. **Penner, J. L.** 1988. The genus *Campylobacter*: a decade of progress. Clin. Microbiol. Rev. **1:**157–172.
9. **Pot, B., P. Vandamme, and K. Kersters.** 1994. Analysis of electrophoretic whole-organism protein fingerprints, p. 493–521. *In* M. Goodfellow and A. G. O'Donnell (ed.), Modern microbial methods: chemical methods in prokaryotic systematics. J. Wiley and Sons Ltd., Chichester, United Kingdom.
10. **Skirrow, M. B.** 1990. *Campylobacter*, *Helicobacter* and other motile curved Gram-negative rods, p. 531–549. *In* M. T. Parker and L. H. Collier (ed.), Topley and Wilson's principles of bacteriology, virology and immunity, vol 2. Systematic bacteriology. E. Arnold, London.
11. **Steele, T. W., and S. N. McDermott.** 1984. Technical note: the use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from faeces. Pathology **16:**263–265.
12. **Tanner, A. C. R.** 1986. Characterization of *Wolinella* spp., *Campylobacter concisus*, *Bacteroides gracilis*, and *Eikenella corrodens* by polyacrylamide gel electrophoresis. J. Clin. Microbiol. **24:**562–565.
13. **Tanner, A. C. R., S. Badger, C.-H. Lai, M. A. Listgarten, R. A. Visconti, and S. S. Socransky.** 1981. *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., and description of *Bacteriodes gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. Int. J. Syst. Bacteriol. **31:**432–445.
14. **Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontol. **6:**278–307.
15. **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.
16. **Vandamme, P., and J. De Ley.** 1991. Proposal for a new family, *Campylobacteriaceae*. Int. J. Syst. Bacteriol. **41:**451–455.
17. **Vandamme, P., D. Dewettinck, and K. Kersters.** 1992. Application of numerical analysis of electrophoretic protein profiles for the identification of thermophilic campylobacters. Syst. Appl. Microbiol. **15:**402–408.
18. **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.
19. **Vandamme, P., and H. Goossens.** 1992. Taxonomy of *Campylobacter*, *Arcobacter*, and *Helicobacter*: a review. Zentralbl. Bakteriol. **276:**447–472.
20. **Wang, G., T. S. Whittam, C. M. Berg, and D. E. Berg.** 1993. RAPD (arbitrary primer) PCR is more sensitive than multilocus enzyme electrophoresis for distinguishing related bacterial strains. Nucleic Acids Res. **21:**5930–5933.