JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2005, p. 5091–5096 Vol. 43, No. 10
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.10.5091–5096.2005

Delineation of *Campylobacter concisus* Genomospecies by Amplified Fragment Length Polymorphism Analysis and Correlation of Results with Clinical Data

Rune Aabenhus,[1] Stephen L. W. On,[2]* Berit L. Siemer,[2] Henrik Permin,[3] and Leif P. Andersen[4]

Department of Clinical Microbiology, Rigshospitalet,[1] Danish Institute of Food and Veterinary Research,[2] Department of Internal Medicine, Hovedstatens Sygehusfaelleskab Bispebjerg Hospital,[3] and Department of Infection Control and Prevention, Rigshospitalet,[4] Copenhagen, Denmark

Received 11 February 2005/Returned for modification 10 May 2005/Accepted 7 July 2005

Campylobacter concisus **has been as frequently isolated from human diarrhea as the important enteropathogen *Campylobacter jejuni*, but it also occurs in the feces of healthy individuals. The role of *C. concisus* in human disease has been difficult to determine, since the species comprises at least two phenotypically indistinguishable but genetically distinct taxa (i.e., genomospecies) that may vary in pathogenicity. We examined 62 *C. concisus* strains by amplified fragment length polymorphism (AFLP) profiling and correlated the results with clinical data. All *C. concisus* strains gave unique AFLP profiles, and numerical analysis of these data distributed the strains among four clusters. The clustering was of taxonomic significance: two clusters contained, respectively, the type strain (of oral origin) and a reference strain (from diarrhea) of each of the known genomospecies. Genomospecies 2 strains were more frequently isolated from immunocompetent patients and/or patients without concomitant infections that presented with fever, chronic diarrhea, and gut inflammation than was genomospecies 1, clustering with the type strain of oral origin. Bloody diarrhea was recorded only with *C. concisus* genomospecies 2 infections. We identified two additional *C. concisus* genomospecies: genomospecies 3 comprised a single strain from an immunocompetent patient, and genomospecies 4 contained five isolates from severely immunodeficient patients, i.e., organ transplantation recipients or those with hematological malignancies. All genomospecies 4 strains were of the same protein profile group and failed to react with a *C. concisus* species-specific PCR assay based on 23S rRNA gene sequences: the taxonomic position of this group requires closer investigation. *Campylobacter concisus* is genetically and taxonomically diverse and contains at least four distinct genomospecies that may exhibit differences in their spectra of virulence potential.**

Campylobacter concisus is a gram-negative, curved, microaerophilic bacterium that is found in the human oral cavity and is sometimes associated with periodontal lesions (13, 19, 28). However, it has also been detected in fecal samples from both healthy (9, 10, 30) and diarrhetic patients (1, 9, 10, 15, 18, 20, 22, 29, 30). As a consequence, its primary pathogenic potential is uncertain. We have previously reported a high prevalence of *C. concisus* in diarrheal cases from a tertiary hospital setting (1), but in pediatric wards in South Africa, the isolation rate has been consistently high (15), as has also been observed in Denmark in patients at the extremes of age (10). In many diarrheal cases, *C. concisus* is isolated as the only potential intestinal pathogen, and the need to determine its role in human enteric disease is evident.

The complex taxonomy of *C. concisus* is well established. Vandamme and colleagues (29) used DNA-DNA hybridization to demonstrate that some diarrheal isolates that conformed to the phenotypic description of this species exhibited just ca. 42 to 50% DNA-DNA hybridization values with the type and reference strains of oral origin. These diarrheal and oral strains should be considered to be distinct genomic species according to current taxonomic guidelines (32); however, since no phenotypic tests are known to distinguish them, such taxa may be referred to as genomospecies (7). There are at least two well-defined *C. concisus* genomospecies according to these criteria (29), but the extent of genetic diversity displayed by use of macrorestriction profiling (21) has suggested that *C. concisus* represents a taxonomic complex potentially encompassing several genomospecies. This is an issue of some clinical relevance, since among other bacterial genera, distinct genomospecies may differ in their pathogenicities or antibiotic resistance profiles (5, 6), and previous studies of the ability of *C. concisus* to cause human diarrheal disease rarely consider the diversity of this species in their findings. A study from Belgium (30) that compared isolation rates of *C. concisus* from diarrhetic and healthy children found no significant difference between the patient groups and concluded that *C. concisus* was not a pathogen. Nonetheless, the complex genomospecific structure of *C. concisus* make it impossible to determine if the Belgian strains from the different patient groups belonged to distinct genomospecies. Clearly, the application of a powerful discriminatory tool is needed to identify possible pathogenic and/or nonpathogenic *C. concisus* genomospecies.

Whole-genome fingerprinting by amplified fragment length polymorphisms (AFLP) is a powerful tool for concurrent taxonomic and subtyping analyses of various bacterial species, and it has been shown to accurately identify interstrain relationships at the subspecies, species, and strain levels (8, 14, 23–26). It has been suggested (23) that the use of AFLP profiling could help resolve the complex taxonomic structure of *C. concisus* and assess the prevalence of diverse genomospecies isolated

* Corresponding author. Present address: Institute of Environmental Science & Research, Christchurch Science Center, 27 Creyke Rd., Ilam, P.O. Box 29 181, Christchurch 8004, New Zealand. Phone: 643 351 6019, Fax: 643 351 0010. E-mail: stephen.on@esr.cri.nz.

from clinical samples, thus elucidating more clearly their potential roles in gastrointestinal illness. The present study applies AFLP profiling to 60 clinical *C. concisus* strains from, predominantly, diarrhetic patients of known immunological status, along with type and reference strains of the two known genomospecies, in order to clarify the taxonomic structure of this organism and identify genomospecies that may differ in their pathogenic potentials.

MATERIALS AND METHODS

Bacterial strains. A total of 62 strains were analyzed in the study (Table 1). Type and reference strains of the known *C. concisus* genomospecies, i.e., the oral isolate CCUG 13144^T and the intestinal isolate CCUG 19995, were included, as well as 56 stool isolates from diarrhetic patients and 4 oral *C. concisus* strains. Isolation of *C. concisus* from fresh clinical samples was achieved using the filter method as previously described (1). The 60 clinical strains analyzed were selected from Danish patients of defined immunological status (see below) to represent an approximately equal distribution between immunocompetent (IC) and immunodeficient (ID) patient origins. The isolates were obtained between August 1997 and November 2002 and were epidemiologically unrelated. Strains were identified as *C. concisus* by biochemical and protein profile criteria described previously (1). In addition, a species-specific PCR assay derived from the 23S rRNA gene (3) was applied to some strains.

The strains were grown under appropriate microaerobic conditions on 5% blood agar plates containing 1% yeast extract as previously described (1).

Patient data. The medical records of *C. concisus*-positive patients were examined retrospectively, and details of underlying diseases, recent travel abroad, bloody and chronic (≥3 weeks duration) diarrhea, fever, lactoferrin, and medication were obtained when possible. Stool cultures were also evaluated for other *Campylobacter* spp., gram-negative enteric pathogens (including *Salmonella*, *Yersinia*, *Vibrio*, *Shigella*, *Aeromonas*, *Erwinia*, and *Plesiomonas* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Hafnia alvei*), and *Clostridium difficile* by use of appropriate selective media (4, 10, 11). The presence of *Clostridium perfringens*, *Staphylococcus aureus*, and *Listeria* and *Candida* spp. in stools was investigated by incubation of inoculated 10% blood agar media anaerobically (*C. perfringens* only) and 5% blood agar with 1.0% yeast extract aerobically (*S. aureus* and *Listeria* and *Candida* spp.) at 37°C for 2 days. Suspect colonies of any of the above-mentioned bacteria were identified by conventional methods (2). All isolation procedures were performed to international standards ratified by the Danish accreditation body (DANAK), and coisolations with *C. concisus* were noted. Patients with cancer (solid tumors and hematological malignancies) or human immunodeficiency virus (HIV) infection or who were receiving immunosuppressant medication were characterized as ID patients. Patients with no underlying diseases or who did not receive immunosuppressant medication were characterized as IC patients.

Chromosomal DNA extraction. Freshly grown cells were harvested and washed once in phosphate-buffered saline. Extraction of chromosomal DNA was performed using protocol 3 of the EasyDNA kit (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. The DNA content was measured with a spectrophotometer and standardized by dilution in sterile deionized (Milli-Q) water to a final concentration of 250 ng/μl.

AFLP fingerprinting. AFLP fingerprinting was performed according to the method of Siemer et al. (26). In brief, MfeII and BspDI (1 U) were used to digest 625 ng chromosomal DNA for 1 hour in NEBuffer 4 (New England BioLabs) at 37°C, and restriction half-site-specific adapters were subsequently ligated to the fragments for 3 hours at 37°C. Five microliters of a 10-fold-diluted ligation mixture was used as the PCR template. The PCR was carried out using the adapter-specific primers MfeI-F (carboxyfluorescein labeled) and BspDI in 25 PCR cycles. The final products were separated on a 6% denaturing polyacrylamide sequencing gel using an ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA). Data collection and preprocessing were performed using GeneScan v. 3.1 fragment analysis software (Applied Biosystems).

Numerical analysis of AFLP profiles. AFLP strain profiles comprising 6-carboxyfluorescein-labeled fragments ranging from 50 to 500 bp were imported and compared using the software BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium) as described previously (26), except that no fragments were excluded from the comparisons. Interstrain relationships were inferred by use of the Dice coefficient and clustered by the unweighted pair group with mathematical average (UPGMA) method.

Fecal lactoferrin titers. Fecal lactoferrin is an indirect measurement of inflammation in the gut caused by neutrophil degranulation. Lactoferrin was measured by enzyme-linked immunosorbent assay using polystyrene microtiter plates (Maxiplate P96; NUNC, Denmark) incubated with rabbit antibodies against human lactoferrin (A 186; DAKO-Cytometrica A/S), and uncoupled antibodies were removed by washing the plates. Fecal samples diluted in phosphate-buffered saline (pH 7.2) and biotinylated antibody against lactoferrin were mixed in the plates and incubated for 2 hours at 37°C. Uncoupled lactoferrin was removed, and the results were measured using a spectrophotometer. A purified (90%) human lactoferrin (MO 63178; Sigma Chemical Co.) served as a standard. Values were defined as high (>1,000 picograms/ml), medium (100 to 1,000 picograms/ml), or low (<100 picograms/ml). Intersample variation was less than 10%, and day-to day variation was less than 5%.

Statistical analysis. The correlation between AFLP cluster composition and clinical data was determined using the chi-square test. P values of <0.05 were regarded as significant.

RESULTS

Patient data. A total of 60 patients were included in the study, 31 female and 29 male, of whom 6 were children. The median age was 38 years (range, 1 to 89 years). Fifty-five percent of the patients were classified as ID (Table 1). A large proportion of patients (30%) suffered from an underlying malignancy (solid tumors or hematological malignancies), 13% were diagnosed with HIV infection, and 10% of the patients had undergone transplantation (bone marrow or organ transplants). Inflammatory bowel disease (IBD) (Crohn's disease or ulcerative colitis) was recorded in 12%, and travel abroad immediately prior to onset of symptoms and subsequent isolation was noted in 14% of the *C. concisus*-positive patients (Table 1). No pathogen other than *C. concisus* was isolated in 65% of the cases. Concomitant isolation rates among AFLP cluster 2 (i.e., genomospecies 2) strains were considerably lower (25%) than those in cluster 1 (i.e., genomospecies 1) (55%; $P < 0.05$) (Table 2).

General features and reproducibility of AFLP profiles. The AFLP patterns of the *C. concisus* strains examined contained between 2 and ca. 70 bands in the 50- to 500-bp detection range (Fig. 1). Each strain yielded a unique profile.

Reproducibility was calculated by examining DNA samples of 29 strains on two different occasions. The mean reproducibility was 91.27% between duplicate analyses, a value similar to those in previous studies (23, 24, 26).

Delineation of *C. concisus* genomospecies. The number and distribution of bands among *C. concisus* AFLP profiles were broadly reflected in the cluster analysis (examples are shown in Fig. 1). The 62 strain profiles were distributed among four distinct clusters that were defined at the 21% similarity level (data not shown), with the type and reference strains of known genomospecies (see below) clearly separated. The cluster designation by the UPGMA method was statistically robust, as determined by the algorithm described by Schouls et al. (25).

Cluster 1 contained the type strain (CCUG 13144) of oral origin that represents the archetypal genomospecies 1 reference and a further 22 Danish clinical isolates. Cluster 2 contained CCUG 19995, the reference strain for genomospecies 2, originally isolated from human diarrhea (29), and also 32 Danish clinical isolates. Cluster 3 comprised a single Danish diarrheal isolate from an immunocompetant patient. Cluster 4 contained five strains, all from severe-ID patients (three patients with transplants and two with hematological malignancies). These strains did not yield an amplicon in the 23S rRNA

TABLE 1. Details of *C. concisus* strains examined

Strain no.	Underlying disease[a]	Status[b]	AFLP cluster	Age (yr)	Remarks
RH 13996.02	CML	ID	1	54	
RH 1048.98	Obs IBD	IC	1	39	Travel
RH 5627.98	Heart disease	IC	1	52	
RH 7766.98	NHL	ID	1	58	
RH 7311.98	None	IC	1	63	
RH 9185.98	Medulloblastoma	ID	1	4	
CCUG13144			1		Oral type strain
RH 13867.98	Leukemia	ID	1	74	
RH 15691.98	Wilm's tumor	ID	1	3	
RH 10284.98	HIV	ID	1	41	
RH 5977.99	HIV	ID	1	42	
RH 4482.98	IBD, CLL	ID	1	49	
RH 13950.02	CVID	ID	1	30	Chronic diarrhea
RH 15688.98	IBD	IC	1	21	
RH 5684.98	HIV	ID	1	46	
RH 9309.98	Cancer cervicis; alcoholism	ID	1	53	Chronic diarrhea
RH 13210.98	CF/transplanted lungs	ID	1	18	No diarrhea
RH 9966.98	HIV	ID	1	36	Chronic diarrhea
RH 4766.98	CLL	ID	1	54	
RH 16731.98	HIV	ID	1	24	
RH 4441.98	None	IC	1	36	Travel
RH 11184.98	CML	ID	1	39	
RH 4900.99	None	IC	1	50	Sputum sample
RH 14331.97	None	IC	2	28	Chronic diarrhea
RH 7891.99	HIV	ID	2	42	Sputum sample
RH 3157.99	HIV	ID	2	38	
RH 16290.98	Breast cancer (treated)	IC	2	66	
RH 7309.98	None	IC	2	22	Travel, chronic diarrhea
RH 5097.98	ALL	ID	2	18	Bloody stool
RH 13545.99	None	IC	2	1	
RH 12106.97	Renal cancer	ID	2	53	
RH 2183.99	None	IC	2	28	
RH 12753.98	Neurodermatitis	IC	2	34	
RH 11909.98	Myelomatosis	ID	2	65	
RH 13826.98	Asthma	IC	2	27	Bloody stool
RH 1374.99	IBD	IC	2	44	Chronic diarrhea
RH 14973.98	None	IC	2	58	Travel, bloody stool
RH 11857.98	None	IC	2	21	Travel
RH 10001.98	HIV	ID	2	49	Chronic diarrhea
RH 14381.02	Renal transplant	ID	2	55	
RH 14533.02	NHL	ID	2	2	
RH 6189.99	Polymyalgia rheumatica	ID	2	66	Chronic diarrhea
RH 13956.02	Renal transplant	ID	2	46	
RH 4647.98	COLD	IC	2	80	
RH 9118.99	IDDM, psoriasis	IC	2	45	Sputum sample
RH 4350.99	Heart disease	IC	2	80	Chronic diarrhea
RH 4966.99	Dementia	IC	2	77	Sputum sample
Anders	IBD	IC	2	10	Hemorrhagic colitis
RH 13922.98	ALL	ID	2	9	
RH 15808.98	IBD	IC	2	56	Chronic diarrhea
RH 11560.98	IBD	IC	2	49	
RH 4204.98	IBD	IC	2	67	Chronic diarrhea
CCUG19995			2		Intestinal type strain
RH 8101.98	None	IC	2	28	Travel
RH 11070.97	Breast cancer (treated)	ID	2	89	Chronic diarrhea
RH 6213.98	NIDDM	IC	2	56	
RH 7656.99	IDDM, psoriasis	IC	3	45	
RH 15500.97	Transplanted kidney	ID	4	65	
RH 13153.02	CF/Transpl. TJEK	ID	4	23	
RH 13570.97	Transplanted liver	ID	4	46	Liver abscess
RH 17503.98	CML	ID	4	46	Chronic diarrhea
RH 5288.98	AML	ID	4	49	

[a] COLD, chronic obstructive lung disease; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; CF, cystic fibrosis; CLL, chronic lymphatic leukemia; CML, chronic myeloid leukemia; CVID, common variable immunodeficiency. NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia; ALL, acute lymphatic leukemia; Obs, suspected diagnosis for investigation; treated, 10 years control completed without relapse.

[b] Immune status of patients with nonimmunosuppressive diseases is related to medical treatment with immunosuppressant drugs at time of isolation of *C. concisus* from stools (glucorticoids and/or cytostatic agents, etc.).

TABLE 2. Summary of clinical presentations encompassed by each *C. concisus* AFLP cluster

AFLP cluster	No. of strains	No. (%)[a] of patients with:					
		Other pathogens[b]	Bloody stools	Chronic diarrhea	Fever >38°C	Lactoferrin >1,000	IC
1	22	12 (55)	0	3 (14)	5 (31)[c]	9 (53)[c]	6 (27)
2	32	8 (25)[d]	4 (13)	9 (28)	10 (50)[c]	13 (65)[c]	19 (59)[d]
3	1	0	0	0	1 (50)	1 (50)	1 (50)
4	5	1 (20)	0	1 (20)	3 (60)	1 (20)	0

[a] Type strains not included.
[b] Other pathogens include *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and *Clostridium* spp. (see the text for details).
[c] Smaller sample sizes.
[d] $P < 0.05$ compared to AFLP cluster 1.

species-specific PCR as described by Bastyns and colleagues (3), although the 16S rRNA sequence of a representative strain was 97% similar to that of the type strain in a BLAST comparison (data not shown).

Correlation of clinical data with *C. concisus* genomospecies designation. Table 2 summarizes the clinical data associated with each of the four *C. concisus* genomospecies and the statistically significant differences between the clinical associations of the predominant genomospecies, i.e., 1 and 2. Danish *C. concisus* genomospecies 2 strains were statistically ($P < 0.05$) more likely to occur in immunocompetent patients and less likely to be isolated alongside other gastrointestinal pathogens than *C. concisus* genomospecies 1 strains. There was a tendency for *C. concisus* genomospecies 2 strains to occur more frequently than *C. concisus* genomospecies 1 strains in chronic diarrhea cases or in patients with a fever at admission or during diarrheal episodes (>38°C) and/or with high lactoferrin levels, although these differences were not statistically significant at a P value of <0.05 level. Patients with bloody diarrhea ($n = 4$) were observed only with *C. concisus* genomospecies 2 infections; conversely, one patient from whom *C. concisus* genomospecies 1 was recovered had no diarrhea.

Genomospecies 3 consisted of a single isolate from an acute diarrheal episode reported in an immunocompetant patient from whom no other pathogen was isolated. In contrast, all five *C. concisus* genomospecies 4 strains were recovered from immunodeficient patients who either were recovering from organ transplants or suffered from hematological malignancies.

No patterns of *C. concisus* infection in regard to patient wards and isolation dates were seen among the different AFLP clusters.

DISCUSSION

The efficacy of AFLP profiling for revealing genetic relationships among *Campylobacter* and related species has been well

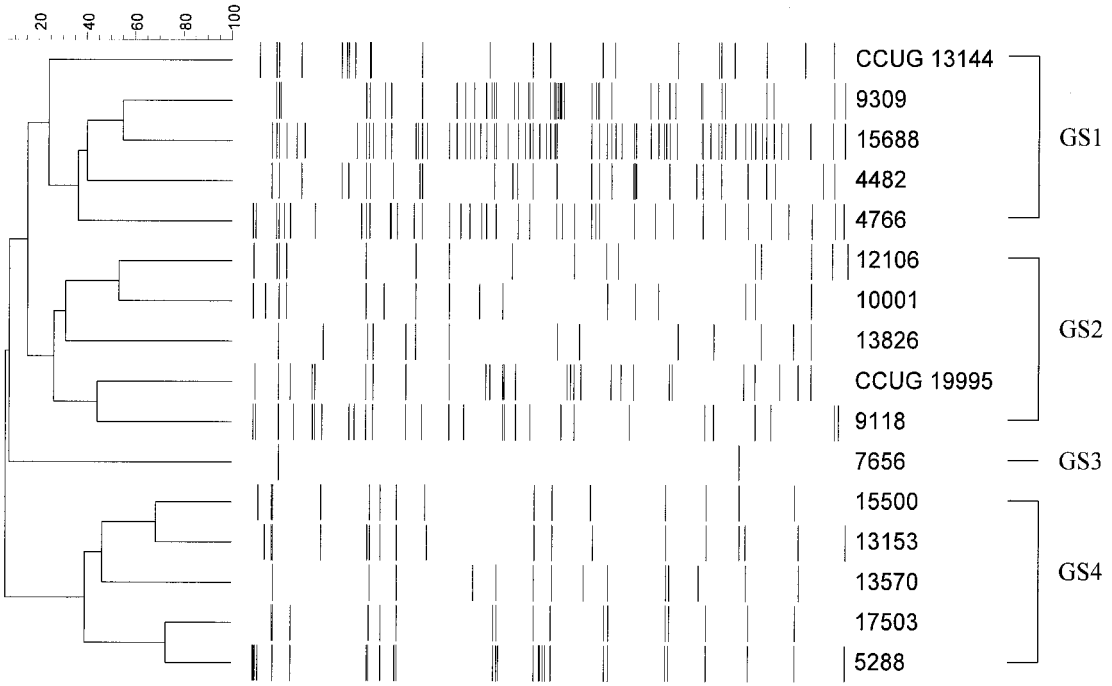

FIG. 1. Representative AFLP profiles of selected *C. concisus* strains. The horizontal axis indicates percent similarity as determined by the Dice coefficient and UPGMA clustering. The genomospecies (GS) designation is listed to the right of the strain number. CCUG, Culture Collection of the University of Göteborg, Sweden.

documented (8, 23–26), and this makes it an ideal tool to investigate and resolve problems in taxonomically complex groups. The results of the current study suggest that *C. concisus* as currently defined represents a complex of at least four genetically distinct taxa, i.e., genomospecies. Correlations with clinical data may indicate that the pathogenic potentials of these genomospecies differ.

The high level of genetic diversity in *C. concisus* as revealed by AFLP profiling correlates with previous findings based on macrorestriction profiling, another whole-genome fingerprinting tool, whereby 52 macrorestriction profiles were found among 53 strains (21). In the present study, no two strains were alike: however, the dendrogram constructed by numerical analysis of the AFLP profiles revealed relationships at the taxonomic level. Two main clusters were defined that contained, respectively, the type and reference strains of oral and diarrheal origin (CCUG 13144 and CCUG 19995) that corresponded to the genomospecies previously delineated (29). Since DNA-DNA hybridization values between these strains are just 46% (29), they clearly represent genetically distinct bacterial species according to current rules of nomenclature (32). Their distinction in the AFLP analysis confirms the taxonomic resolution of the approach.

Two additional clusters that we consider to represent additional genomospecies of *C. concisus* were identified. Genomospecies 3 comprises a single strain (7656), albeit one with a highly unusual AFLP profile containing only two 6-carboxyfluorescein-labeled fragments in the detection range of the sequencer. Five strains were assigned to *C. concisus* genomospecies 4; interestingly, these strains did not yield an amplicon with the species-specific PCR based on the 23S rRNA gene (3). However, BLAST comparison of a near-complete 16S rRNA gene sequence from a representative strain revealed it to be 97% similar to GenBank sequences of *C. concisus* (data not shown). Furthermore, all *C. concisus* genomospecies 4 strains were assigned to protein profile group 1 as previously defined (1); in contrast, the remaining Danish *C. concisus* strains were assigned to protein profile group 2. Since *C. concisus* genomospecies 4 strains also appear to be somewhat more distantly related to the other strains examined by AFLP (Fig. 1), their taxonomic position warrants closer investigation.

The role of *C. concisus* in human diarrhea has been a controversial issue. Several studies indicate its pathogenic potential; the organism is as frequently recovered from diarrhea as the common enteric pathogens *Campylobacter jejuni* and *Campylobacter coli* (1, 10, 15), and in many cases no other diarrheagenic pathogen is detected. Moreover, colonization of the intestines of mice by *C. concisus* is transiently achieved (R. Aabenhus, unpublished data), whereby infected mice exhibited a significant loss of body weight compared with uninfected mice. Istivan and colleagues (12) have identified a membrane-bound hemolytic phospholipase in *C. concisus* strains, and many *C. concisus* strains show cytotoxic activity (9). However, the prevalence of *C. concisus* in 4 to 15% of asymptomatic patients (9, 10, 30) has been used by some to suggest that *C. concisus* should be regarded as at best an opportunistic pathogen, most relevant to immunocompromised patients and those at the extremes of age. These studies were not able to account for the fact that *C. concisus* is a heterogeneous species complex comprising several genomospecies that may vary in their pathogenic potentials.

We noted a difference in clinical associations of *C. concisus* strains according to their genomospecies designation as determined by AFLP profile analysis. Although only comprising a single strain, the genotype of 7656 (genomospecies 3) was highly distinctive, and the strain was recovered from an immunocompetent patient. In contrast, all *C. concisus* genomospecies 4 strains were isolated from immunodeficient patients, suggesting that these strains may be less invasive; this observation must be confirmed by the study of additional isolates. However, our observation resembles that of Engberg et al. (9), in which cluster analysis of randomly amplified polymorphisms in DNA profiles identified two strains from the feces of healthy human carriers that were distinct from the majority of strains of diarrheal origin. Considered together, these data support the existence of avirulent or at least comparatively less pathogenic genomospecies of *C. concisus*.

Bias in the potential of *C. concisus* strains to infect immunocompetent patients between the major genomospecies (1 and 2) was also evident. Fifty-nine percent of strains from immunocompetent patients were identified as *C. concisus* genomospecies 2, which was the only taxon to be associated with bloody stools. In addition, patients infected with *C. concisus* genomospecies 2 more frequently presented with fever, chronic diarrhea, and gut inflammation in the absence of other established pathogens, although these results were not significant at a P value of <0.05 level. Also of interest is the fact that five of seven IBD patients were colonized with genomospecies 2, also consistent with the former findings, although the numbers are too small for statistical analysis. Taken together, these findings suggest that a pathogenic role exists for at least certain subtypes of *C. concisus* strains. The need to establish whether *C. concisus* genomospecies 2 is an emerging human pathogen is evident in light of its high isolation rates in selected hospital settings (1, 10, 15, 18, 30).

Bacterial virulence encompasses a wide range of traits (31), and infection with a less toxigenic or hemolytic strain does not necessarily obviate a role in disease. The different genomospecies of *C. concisus* may be preferentially adapted for colonization of either oral (where some strains occur as normal flora [28]) or intestinal tissue (as with the closely related commensal species *Campylobacter hominis* [16]), with certain genomospecies possibly causing gastrointestinal illness only when the host immune system is compromised. The high incidence of *C. concisus* in pediatric patients is especially noteworthy in this respect (1, 15, 18), and clearly the increasing burden of infection with HIV worldwide (17) is also relevant. A study to elucidate the distribution of *C. concisus* genomospecies in South African patients could prove enlightening, in light of the high incidence of HIV in that region (27). Determining the distribution of *C. concisus* genomospecies between healthy and diarrhetic patients could also provide new insight into *Campylobacter* gastrointestinal pathology.

In conclusion, our study demonstrates that at least four distinct *C. concisus* genomospecies can be recognized. Furthermore, there are indications that these four genomospecies exhibit differences in their spectra of virulence potential. The taxonomic position of *C. concisus* genomospecies 4 needs clarification. More work is required to develop simple diagnostic

tests to accurately differentiate *C. concisus* genomospecies, so that their prevalence and significance can be properly ascertained. Given the high prevalence of this species in human diarrhea (1, 10, 15, 18, 30), such studies are certainly justified.

ACKNOWLEDGMENTS

R.A. and L.P.A. thank Direktør Henrik Ibsens Fond for financial support.

We thank Anne Skov and Penny Jordan for excellent technical assistance.

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