Sutterella wadsworthensis gen. nov., sp. nov., Bile-Resistant Microaerophilic *Campylobacter gracilis*-Like Clinical Isolates

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Campylobacter gracilis (formerly *Bacteroides gracilis*) is an asaccharolytic, nitrate-positive, urease-negative organism that requires formate and fumarate or hydrogen as a growth additive and may pit agar media. Clinical isolates that were obtained primarily from appendiceal and peritoneal fluid specimens and initially were identified in our laboratory as *B. gracilis* were later found to include “unusual” strains that could be distinguished by biochemical and genetic criteria. These unusual *C. gracilis* strains were bile resistant, could not reduce tetrazolium chloride under aerobic conditions if formate and fumarate were added to the medium, and could grow in the presence of 2 or 6% oxygen if no blood was added to the medium. *C. gracilis*, other campylobacters, and the unusual strains produced distinctive dehydrogenase patterns when gels were incubated anaerobically. A cellular fatty acid analysis revealed that the cluster formed by the unusual organisms was distinct from the (separate) clusters formed by *C. gracilis*, *Bacteroides ureolyticus*, and other *Campylobacter* species. 16S rRNA sequence data indicated that these organisms are not related phylogenetically to either *C. gracilis* or other *Campylobacter* species; the most closely related taxa as determined by rRNA sequence analysis were unrelated aerobes (members of the genera *Bordetella*, *Alcaligenes*, *Rhodocyclus*, and *Comamonas*). DNA homology data confirmed that these taxa are separate groups. Our data indicate that the unusual organisms are members of a new genus and new species, for which we propose the name *Sutterella wadsworthensis*. The type strain of *S. wadsworthensis* is strain WAL 9799 (= ATCC 51579).

The bacteria originally known as *Bacteroides ureolyticus* and later as the *B. ureolyticus* group are asaccharolytic, nitrate-positive organisms that require formate or hydrogen as an electron donor and may pit agar media. *B. ureolyticus* and *Bacteroides gracilis* clearly do not belong to the genus *Bacteroides* sensu stricto (currently limited to the *Bacteroides fragilis* group [13]), and the results of 16S rRNA sequence analysis and DNA-rRNA hybridization studies support the hypothesis that there is a tight homology group that includes *B. ureolyticus*, *B. gracilis*, and the genus *Campylobacter* (including some species previously placed in the genus *Wolinella*) (10, 17). It has been proposed that both *B. ureolyticus* and *B. gracilis* are microaerophiles and not anaerobes (and therefore should be included in the group containing the true campylobacters) (5). *B. gracilis* has been reclassified as *Campylobacter gracilis*, and this name is used below. However, while *B. ureolyticus* is considered a member of the family *Campylobacteraceae*, it was not renamed along with *C. gracilis*; the recommended status of this taxon was a species incertae sedis pending further investigation (16).

C. gracilis was obtained originally from a gingival crevice (15). We have examined the role of *C. gracilis* in various non-dental infections and have found that it is more likely to be involved in serious deep-seated infections than *B. ureolyticus* is (6). During biochemical characterization and susceptibility testing of isolates obtained from clinical specimens in the Wads-

worth Anaerobe Laboratory, we observed heterogeneity among the strains previously identified as *C. gracilis*. These organisms differed in their bile resistance and their ability to reduce triphenyltetrazolium chloride (TTC). Isolates that were bile resistant and unable to reduce TTC aerobically were compared with other strains of *C. gracilis* (including the American Type Culture Collection type strain) and strains of five other *Campylobacter* species by using biochemical characteristics, cellular fatty acid profiles, levels of DNA homology, and levels of 16S rRNA sequence homology.

In this paper, we describe the results of these taxonomic and phylogenetic studies. We concluded that these organisms are members of a new genus and new species, for which we propose the name *Sutterella wadsworthensis*. The type strain of *S. wadsworthensis* is strain WAL 9799 (= ATCC 51579).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study included 37 human clinical isolates obtained from the Wadsworth Anaerobic Bacteriology Laboratory collection and one strain each of *Campylobacter* sp. and *C. gracilis* type 2 kindly provided by Anne Tanner (Forsyth Dental Center, Boston, Mass.) (Table 1). The American Type Culture Collection strains used as controls included *B. gracilis* ATCC 33236^T (T = type strain), *Campylobacter showae* ATCC 51146^T, *Campylobacter concisus* ATCC 33237^T, *Campylobacter rectus* ATCC 33238^T, *Campylobacter curvus* ATCC 35224^T, and *Campylobacter sputorum* ATCC 35980^T.

Oxygen tolerance. Strains were grown for 48 to 72 h in an anaerobic chamber on brucella agar supplemented with 0.3% formate and 0.3% fumarate with and without 5% laked sheep blood. Aerolerance was determined by transferring the strains to agar plates containing the medium described above and incubating the plates in an atmosphere containing either 2 or 6% O₂. The oxygen concentration desired was obtained by using an automated evacuation-replacement technique (Anoxomat; Mart BV Laboratorium, Lichtenvoorde, Holland).

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TABLE 1. Isolates used and their sources and GenBank accession numbers

Strain ^a	Other designation ^a	GenBank nucleotide sequence accession no.	Source
<i>C. gracilis</i> (previously <i>B. gracilis</i>) strains			
WAL 6823			Pleural fluid
WAL 7701			Brain abscess
WAL 7728			Brain abscess
WAL 8030			Maxilla
WAL 8072			Neck mass
WAL 9656			Appendix
WAL 10160			Periodontal
WAL 10204			Pleural fluid
WAL 6989	ATCC 33236 ^T	L37787	Gingival sulcus
<i>C. gracilis</i> type 2 strain WAL 10733	FDC 20A1 ^b		Periodontal (oral pocket)
<i>S. wadsworthensis</i> strains			
WAL 7095			Appendiceal abscess
WAL 7115			Peritoneal fluid
WAL 7877		L37785	Appendix
WAL 8074			Unknown
WAL 8522			Peritoneal fluid
WAL 8870			Abdominal fluid
WAL 8948			Appendix
WAL 8960			Peritoneal fluid
WAL 8962			Appendix
WAL 9041			Appendix
WAL 9054		L37786	Appendix
WAL 9156			Peritoneal fluid
WAL 9244			Appendix
WAL 9317			Abdominal fluid
WAL 9631			Appendix
WAL 9689			Appendix
WAL 9799 ^T	ATCC 51579 ^T		Abdominal fluid
WAL 11060			Appendix
WAL 11078			Peritoneal fluid
WAL 11175			Peritoneal fluid
<i>C. concisus</i> strains			
WAL 9036 ^T	ATCC 33237 ^T		Gingival sulcus
WAL 10200			Transtracheal aspirate
WAL 10277			Lung aspirate
<i>C. curvus</i> strains			
ATCC 35224 ^T			Jaw abscess
WAL 8827			Peritoneal fluid
WAL 9636			
<i>C. rectus</i> strains			
ATCC 33238 ^T			Periodontal
WAL 7791			Peritoneal fluid
WAL 7943			Appendix
WAL 9675			Appendix
WAL 10143			Neck abscess
<i>C. showae</i> WAL 10729 ^T	ATCC 51146 ^T		Gingival crevice
<i>C. sputorum</i> WAL 10734 ^T	ATCC 35980 ^T		Oral cavity
<i>Campylobacter</i> sp. strains			
WAL 4864			Transtracheal aspirate
WAL 10732	FDC 286 ^b		Periodontal (oral pocket)

^a WAL, Wadsworth Anaerobe Laboratory; ATCC, American Type Culture Collection; FDC, Forsyth Dental Center.

^b Strain obtained from Anne Tanner.

Effect of bile. The effect of bile was determined by using 20% bile disks (Remel, Lenexa, Kans.) as described previously (14).

Enzyme activities. Enzyme activities were determined by using an API ZYM kit (Analytab Products, Plainview, N.Y.). Urease activity was determined with urease disks according to the directions of the manufacturer (Rosco Diagnostics, Taastrup, Denmark). Indoxyl acetate hydrolysis was determined as described previously (11). Oxidase activity was also determined as described previously (14).

Reduction of TTC. The ability to reduce TTC was determined by inoculating brucella base laked blood agar (with and without formate and fumarate) with 10⁵ CFU by using a Steers replicator, incubating the plate for 48 to 72 h anaerobically, and dropping a solution containing equal parts 0.1% TTC and 2% molten agar onto the inoculum spots. These tests were performed either in an anaerobic chamber or in the ambient atmosphere within 5 min of the time that the plates

were removed from the anaerobic chamber. Reduction of TTC was determined by the appearance of a red color, which indicated that formazan had been formed.

Dehydrogenase analysis. Cells were grown for 48 to 72 h on brucella blood agar plates supplemented with 0.3% formate and 0.3% fumarate in an anaerobic chamber and were then suspended in 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.5). The cells were disrupted by three passes through a French pressure cell (SLM Instruments, Inc., Urbana, Ill.), and a cell-free supernatant was obtained by centrifuging each preparation at 37,000 × *g* for 15 min. Undenatured samples containing 20 µg of protein were subjected to polyacrylamide gel electrophoresis on a 4% stacking gel and a 10% running gel at 25 mA and 200 V (7). The gels were stained overnight in a solution containing 0.08 M Tris (pH 7.5), malate (2 mg/ml), phenazine methosulfate (0.04 mg/ml), thiazolyl blue tetrazolium (0.2 mg/ml), and NAD (0.1 mg/ml). For

staining, the gels were incubated anaerobically with and without substrate (to distinguish substrate-specific bands from nonspecific bands) and under ambient conditions.

Cellular fatty acid analysis. Organisms were initially identified by using the Microbial Identification System, which analyzes cell wall fatty acids. The fatty acid methyl esters of whole-cell extracts were identified by the method of Moss and Nunez-Montiel (9). Briefly, cells were grown for 48 to 72 h in peptone-yeast extract-glucose medium (Carr Scarborough, Stone Mountain, Ga.) supplemented with formate and fumarate. Growth was harvested by centrifugation at $5,000 \times g$ for 20 min, and the resulting pellets were kept frozen at -20°C until they were processed. The cells were saponified in aqueous methanol, methylated in hydrochloric acid in aqueous methanol, extracted in hexane and methyl-*tert*-butyl ether, washed in dilute NaOH, and analyzed with a model 5890A gas-liquid chromatograph (Hewlett-Packard) equipped with a silicone-lined capillary column by using the Microbial ID software package (MIDI; Microbial ID, Inc., Newark, Del.).

16S rRNA sequencing. Nucleic acids were purified as described previously. RNA was purified, and the sequence of the 16S rRNA fragment was determined by using reverse transcriptase as described previously (18). DNA was amplified by using primers directed at sequences specific for eubacterial 16S ribosomal DNA (4). The PCR product was reamplified by using the method of Zintz and Beebe (19). The resulting amplicons were sequenced by using a DyeDeoxy terminator sequencing kit (Applied Biosystems, Foster City, Calif.) in conjunction with a model 373A automated sequencer (Applied Biosystems). A phylogenetic analysis was performed by using the maximum-likelihood method (3) and the entire 16S rRNA sequence. The GenBank and Ribosomal Database Project databases were searched for similar sequences in PCGENE (Intelligenetics, Mountain View, Calif.) by using the program FSTNSCAN (a subroutine of PCGENE), and the 20 most similar sequences were identified. The phylogenetic tree available from the Ribosomal Database Project indicated that these sequences could be located in two (separate) clusters, and representative sequences from these two regions of the tree were included in our tree.

DNA extraction and purification. Strains were grown as described above. The growth from three or four plates was scraped into tubes containing 0.5 ml of Tris-EDTA (10 mM Tris [pH 7.6], 1 mM EDTA), centrifuged for 15 to 20 min at 8,000 rpm ($8,000 \times g$), and resuspended in Tris-EDTA. The suspensions were treated with lysozyme (1 mg/ml) for 30 min at 37°C . Sodium dodecyl sulfate (final concentration, 0.5%) and proteinase K (final concentration, 0.1 mg/ml) were then added, and the suspensions were incubated for 60 min at 37°C . Hexadecyltrimethylammonium bromide (final concentration, 1%) was added (to solubilize the lipopolysaccharide), and the suspensions were incubated at 65°C for 20 min. Then the DNA was extracted by using an equal volume of chloroform-isoamyl alcohol (24:1). The aqueous phase was removed and extracted by using an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). The DNA was then precipitated from the aqueous supernatant with NaCl (final concentration, 1 M) and 0.6 volume of isopropanol. The DNA pellets were then redissolved in 300 μl of Tris-EDTA and treated with 4 μl of RNase (10 mg/ml) for 60 min at 50°C . The final extraction was performed by using an equal volume of chloroform-isoamyl alcohol. The preparations were then precipitated with sodium acetate (final concentration, 0.3 M) and 2 volumes of 95% ethanol, washed twice with 70% ethanol, and resuspended in 200 μl of 0.42 M NaCl. DNA concentration and purity were determined spectrophotometrically.

Preparation of labeled DNA. DNA was sheared by sonication with a Microson XL 2500 apparatus (Heat Systems, Farmington, N.Y.) for 4 min at a power setting of 4 by using a microcup horn to give a fragment size of approximately 500 bp. Each reference DNA was labeled with [$1',2',5',3'\text{-}^3\text{H}$]dCTP by nick translation by using a commercial nick translation kit (Amersham Corp., Arlington Heights, Ill.) according to manufacturer's instructions. The labeled DNA was separated from unincorporated nucleotides by gel exclusion chromatography by using Sephadex G-50.

DNA-DNA hybridization. Unlabeled DNA preparations were sheared as described above. DNA-DNA reassociation experiments and S1 nuclease treatments were performed as described previously (12). Briefly, 0.02 μg of labeled reference DNA and 20 μg of unlabeled DNA in 200 μl of 0.42 M NaCl were denatured at 100°C and hybridized at 65 or 70°C (as indicated in Table 2) for 90 h in screw-cap microcentrifuge tubes. Fifty-four microliters of S1 nuclease (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 95 U/ml, and the tubes were then incubated for 20 min at 50°C . Precipitates were collected on nitrocellulose filters (pore size, 0.45 μm ; type HAWP; Millipore Corp., Bedford, Mass.), dried, and counted with a model LS 6000 TA liquid scintillation counter (Beckman Instruments, Fullerton, Calif.). All hybridization experiments were performed in triplicate. Levels of hybridization were corrected for self-reassociation by using calf thymus DNA as a control. Levels of homology were determined by comparison with the corrected absolute value obtained for hybridized homologous DNA.

Antimicrobial agent susceptibility tests. The MICs of antimicrobial agents for selected strains were determined as described previously (14) with formate and fumarate added to the media.

Nucleotide sequence accession numbers. The 16S rRNA sequences of *S. wadsworthensis* WAL 7877 and WAL 9054 and *B. gracilis* (*C. gracilis*) WAL 6989^T (= ATCC 33236^T) have been deposited in the GenBank database under accession numbers L37785, L37786, and L37787, respectively.

TABLE 2. Levels of DNA-DNA homology for 20 *Sutterella* strains, 10 *C. gracilis* strains, and 11 strains belonging to other *Campylobacter* species

Source of unlabeled DNA	% Homology with labeled DNA from:		
	<i>S. wadsworthensis</i> WAL 9799 ^{Ta}	<i>C. gracilis</i> ATCC 33236 ^{Tb}	<i>C. gracilis</i> WAL 8030 ^b
<i>Sutterella</i> strains			
WAL 9799	100	0	0
WAL 7095	97	3	
WAL 7115	81		
WAL 7877	67	0	
WAL 8074	100		
WAL 8522	71	0	
WAL 8870	78		
WAL 8948	100	0	
WAL 8960	102	0	
WAL 8962	100		
WAL 9041	96	5	
WAL 9156	100	0	
WAL 9244	100		
WAL 9317	100		
WAL 9631	98	12	
WAL 9689	85	0	
WAL 9899 ^T	104	0	
WAL 11060	100		
WAL 11078	100	0	0
WAL 11175	83	0	
<i>C. gracilis</i> strains			
ATCC 33236 ^T	0	100	
WAL 8030	0	43	100
WAL 6823	0	22	
WAL 7701	0	30	48
WAL 7728	2	30	
WAL 8072	0	42	74
WAL 9656	0	33	58
WAL 10160	0	75	43
WAL 10204	0	79	
WAL 10733 ^c	42		58
<i>Campylobacter</i> sp. strain	0		
WAL 4864			
<i>C. rectus</i> WAL 7791	0		
<i>C. rectus</i> WAL 7943		15	
<i>C. curvus</i> WAL 9636	0	0	
<i>C. rectus</i> WAL 9675	0	1	
<i>C. rectus</i> WAL 10143	0	0	
<i>Campylobacter</i> sp. strain	0		
WAL 10732 ^d			
<i>C. concisus</i> ATCC 33237 ^T	0	0	
<i>C. rectus</i> ATCC 33238 ^T	2	15	23
<i>C. curvus</i> ATCC 35224 ^T	0	0	
<i>C. sputorum</i> ATCC 35980 ^T		0	
<i>C. showae</i> ATCC 51146 ^T	1	0	

^a Levels of hybridization at 70°C .

^b Levels of hybridization at 65°C .

^c *C. gracilis* type 2 strain obtained from Anne Tanner.

^d *Campylobacter* sp. strain most closely related to *C. showae* and obtained from Anne Tanner.

RESULTS

Resistance to bile. Strains belonging to the typical *C. gracilis* group were sensitive to bile, while the *Sutterella* strains were bile resistant. The strains that grouped with *Campylobacter* species were variable in their resistance to bile.

Reduction of TTC. TTC reduction by *C. gracilis* was affected by the basal growth medium and atmosphere used. Both the typical *C. gracilis* strains and *Sutterella* strains reduced TTC in an anaerobic atmosphere on blood agar plates when no formate and fumarate were added, but did not reduce TTC aer-

FIG. 1. Malate dehydrogenase patterns of *C. gracilis*, *Sutterella*, and *Campylobacter* strains. Gels were incubated anaerobically. Lane 1, *C. rectus* ATCC 33238^T; lane 2, *C. showae* WAL 10729^T; lane 3, *C. gracilis* ATCC 33236^T; lane 4, *C. gracilis* WAL 8030; lane 5, *S. wadsworthensis* WAL 9799^T (= ATCC 51579^T); lane 6, *S. wadsworthensis* WAL 8960. Lanes 5 and 6 contained non-substrate-specific dehydrogenase activity with anaerobic incubation if the gel was incubated anaerobically. Substrate-specific *Campylobacter* bands were present under both aerobic and anaerobic incubation conditions.

obically. However, if formate and fumarate were added to the medium, the typical *C. gracilis* strains reduced TTC in an aerobic atmosphere (as did *Campylobacter* and *B. ureolyticus* strains), while *Sutterella* strains did not.

Dehydrogenase profiles. Three general patterns of dehydrogenase activity were observed with the typical *C. gracilis*, *S. wadsworthensis*, and *Campylobacter* strains which we studied (Fig. 1). The members of the group that included the type strain of *C. gracilis* (as well as *C. gracilis* type 2 strain WAL 10733 [FDC 20A1]) produced no bands or very faintly reactive malate-specific bands (which appeared only when the gels were very heavily loaded), whereas the members of the *Sutterella* group produced strongly reactive single or multiple nonspecific dehydrogenase bands (but only when they were incubated anaerobically). The *Campylobacter* strains (including strain WAL 10732 [FDC 286]) produced different malate dehydrogenase-specific bands that migrated various distances under both aerobic and anaerobic incubation conditions. As the different *Campylobacter* species cannot be differentiated by using routine biochemical tests, malate dehydrogenase profiles may provide a useful tool for identifying species belonging to this genus.

Cellular fatty acid analysis. The fatty acid methyl esters found in the different groups of organisms are shown in Table 3. A cluster analysis of the cellular fatty acid data revealed the following three major groups: (i) typical *C. gracilis* (including

FIG. 2. Dendrogram based on the results of a cellular fatty acid cluster analysis showing the positions of *C. gracilis*, other *Campylobacter* species, and *S. wadsworthensis*. Numbers in parentheses are the numbers of strains.

strain ATCC 33236^T), (ii) the *Sutterella* group, and (iii) *Campylobacter* species (Fig. 2). Among other things, the absence of the 12:0 fatty acid methyl ester distinguished *Sutterella* strains from typical *C. gracilis* strains and strains of other *Campylobacter* species. The typical *C. gracilis* strains, *Campylobacter* strains, and *B. ureolyticus* strains also clustered quite independently of each other. *S. wadsworthensis* WAL 9799 was selected as the type strain because it occurred in the center of the *Sutterella* cluster. *C. gracilis* ATCC 33236^T occurred at the edge of the *C. gracilis* cellular fatty acid cluster.

Biochemical characteristics and oxygen tolerance. Like *B. ureolyticus*, *C. gracilis*, and other *Campylobacter* species, members of the genus *Sutterella* were asaccharolytic and reduced nitrate to nitrite. Both *Campylobacter* and *Sutterella* strains grew on solid media in the presence of 2 and 6% oxygen. *C. gracilis* grew in the presence of 6% oxygen only on plates containing brucella agar supplemented with formate and fumarate with no blood added; if blood was added, *C. gracilis* did not grow.

16S rRNA sequencing. We determined the 16S rRNA sequences of *C. gracilis* ATCC 33236^T, *C. rectus* WAL 7943, and two *Sutterella* strains (WAL 9054 and WAL 7877). *C. rectus* WAL 7943 was most similar to *C. rectus* ATCC 33238^T; these two strains differed at only 2 of 1,420 base pairs (level of similarity, 99.9%). The type strain of *C. gracilis* was also very

TABLE 3. Cell wall fatty acid analysis of *C. gracilis* strains, *Sutterella* strains, and strains of other *Campylobacter* species

Taxon	No. of strains	% of total cell wall fatty acids						Summed feature 10 ^a
		12:0 Fatty acid methyl ester	14:0 Fatty acid methyl ester	16:1 <i>cis</i> 9 Fatty acid methyl ester	16:0 Fatty acid methyl ester	16:0 Dimethyl acetal	18:1 <i>cis</i> 11 Di-methyl acetal	
<i>C. gracilis</i> (formerly <i>B. gracilis</i>)	10	10–20	8–13	<5	6–12	15–23	10–12	10–20
<i>Sutterella</i>	30	<1	3–8.5	17–24	26–44	0.2–6	0–1	11–30
<i>Campylobacter</i> ^b	16	4–13	8.5–13	9–18	22–34	2–5 ^c	0	16–30

^a 18:1 C11/t9/t6 fatty acid methyl ester and/or unknown peak at 17.834.

^b The strains examined included eight *C. rectus* strains, three *C. curvus* strains, three *C. concisus* strains, and two *Campylobacter* sp. strains.

^c The *C. curvus* strains contained 2 to 5% 16:0 dimethyl acetal, and the other *Campylobacter* strains contained none of this compound.

FIG. 3. Phylogenetic tree showing the positions of *S. wadsworthensis*, *C. gracilis*, other *Campylobacter* species, and related organisms based on 16S rRNA sequence similarity data.

similar to *C. rectus*, but the level of sequence similarity was lower (96%) (1,432 base pairs were compared). A phylogenetic tree constructed by using the maximum-likelihood method is shown in Fig. 3. *C. gracilis* ATCC 33236^T branches at the same point as *C. rectus* and belongs to a cluster that contains other *Campylobacter* species. The 16S rRNA sequences of the two *Sutterella* strains (WAL 9054 and WAL 7877 [1,454 and 1,419 base pairs were sequenced, respectively]) were identical to each other but were not similar to the sequences of the *C. gracilis* and *Campylobacter* strains analyzed or the sequences obtained from the GenBank, Ribosomal Database Project, and PCgene databases. A total of 15 sequences (the sequences of 13 named and 2 unnamed *Campylobacter* species) were included in the comparison. The taxa that were most closely related to the two *Sutterella* strains as determined by 16S rRNA sequences were unrelated aerobes (members of the genera *Bordetella*, *Alcaligenes*, *Rhodocyclus*, and *Comamonas*), as shown by the phylogenetic tree in Fig. 3.

DNA hybridization. The levels of DNA-DNA homology between members of the groups examined are shown in Table 2. ³H-labeled DNA from *C. gracilis* ATCC 33236^T hybridized moderately with the DNAs of other typical *C. gracilis* strains (the levels of homology were 70 to 80% for two of nine strains, 40 to 50% for three of nine strains, 30 to 50% for three of nine strains, and 22% for one of nine strains). The DNAs of 10 *Sutterella* strains did not hybridize at all with the DNA of the *C. gracilis* type strain, and the DNAs of three *Sutterella* strains hybridized very slightly (levels of homology, 3.5 and 12%). The DNAs of seven *Campylobacter* strains did not hybridize at all with *C. gracilis* ATCC 33236^T DNA, and the DNAs of two strains of *C. rectus* hybridized slightly (level of homology, 15%). ³H-labeled DNA from another *C. gracilis* strain (WAL 8030) hybridized more strongly with the DNAs of other *C. gracilis* strains, did not hybridize at all with the DNAs of *Sutterella* strains, and hybridized slightly with the DNA of the single *C. rectus* strain tested (level of homology, 23%).

³H-labeled DNA from strain WAL 9799 (the *S. wadsworthensis* type strain) did not hybridize with the DNAs of 10 *C. gracilis* strains or the DNAs of nine strains belonging to other

Campylobacter species, but hybridized strongly (levels of homology, $\geq 96\%$) with the DNAs of 68% of the *Sutterella* strains (19 strains) and exhibited levels of homology of $\geq 67\%$ with all of the *Sutterella* strains tested, indicating that these strains belong to the same species (1).

Clinical sources. The strains belonging to the *Sutterella* group were isolated mainly from infections of gastrointestinal tracts (Table 1). *C. gracilis* strains were found virtually only in infections that occurred above the diaphragm (brain, pleural fluid, etc.); only one strain of *C. gracilis* was isolated from an appendiceal specimen. The resistance of the *Sutterella* strains to both human bile (unpublished data) and oxgall is notable; this may account for the ability of these organisms to survive in biliary tracts and bowels.

Metronidazole resistance of *C. gracilis*. The typically poor growth of *C. gracilis* strains (even on control plates) makes endpoint determinations particularly difficult. We found that adding the growth supplements formate and fumarate made determining MICs easier and minimized the "haze" observed even at high antimicrobial agent concentrations. The MICs for *C. gracilis* were considerably lower when formate and fumarate were added to the medium. We used TTC to facilitate endpoint determinations and established a new antibiogram for this organism (8). We subsequently reanalyzed the susceptibility profiles of these organisms in light of our revised classification and found that the metronidazole-resistant strains all belonged to the oxygen-tolerant, bile-resistant *Sutterella* group. Antibiograms for the new taxa belonging to this group should be determined.

DISCUSSION

Strains of *C. gracilis* (formerly *B. gracilis*) are associated with severe infections and high levels of resistance to several antimicrobial agents, although recently we have found that incorporating formate and fumarate into media (which results in better growth of these organisms) results in clearer and considerably lower MICs. Previous studies performed in our laboratory revealed that *C. gracilis* was more likely than *B. ureolyticus* to cause serious and deep-seated infections (6). In this study, we found that all of the *C. gracilis* strains except one were isolated from infections that occurred above the diaphragm, while most of the *Sutterella* strains were isolated from infections that occurred below the diaphragm, and that the metronidazole-resistant strains all belonged to the genus *Sutterella*. The relative importance of these two taxa in infections of various types and the differences in their antibiograms should be evaluated in light of the revised taxonomic status of these organisms. Workers in a clinical laboratory can easily distinguish *Sutterella* strains, *C. gracilis* strains, and strains of other *Campylobacter* species by using two criteria, resistance to 20% bile disks and cell wall fatty acid patterns. One of the differences is the absence of 12:0 fatty acid methyl ester in *Sutterella* strains. The fatty acid patterns of *C. gracilis* strains and other *Campylobacter* species include 12:0 fatty acid methyl ester, as described previously (2).

The results of previous 16S rRNA sequence studies have suggested that *C. gracilis* belongs phylogenetically to the group that contains the true campylobacters (10). Our results for the 16S rRNA sequence of *C. gracilis* were virtually identical to the previously published sequence (the same strain was used) (2); these sequences differed at only 1 of 1,381 bases after resolution of some ambiguous positions. A phylogenetic analysis revealed that the strains of *C. gracilis* tested were very closely related to *Campylobacter* species. Vandamme et al. (16) found that *C. gracilis* differed from some campylobacters only in the

TABLE 4. Characteristics of *B. ureolyticus*, the genus *Campylobacter*, *C. gracilis*, and the genus *Sutterella*

Taxon	Oxidase activity	Urease activity	Indoxyl acetate	Growth in the presence of:		Resistance to 20% bile	TTC reduction ^a
				2% O ₂	6% O ₂		
<i>B. ureolyticus</i>	+	+	NT	+	+ or w	S	NT
<i>Campylobacter</i>	+	—	V	+	+	V ^c	+
<i>C. gracilis</i> (formerly <i>B. gracilis</i>)	—	—	+	+	— ^d	S	+
<i>Sutterella</i>	—	—	—	+	+ (—)	R	—

^a Ability to reduce TTC in an aerobic atmosphere when formate and fumarate are added to the medium.

^b +, positive; —, negative; NT, not tested; V, variable within the same species; w, weakly positive; S, sensitive; R, resistant; + (—), occasional strains negative.

^c The strains tested included two *C. curvus* strains (which were resistant), four *C. rectus* strains (which were sensitive), one *C. concisus* strain (which was sensitive), and *Campylobacter* sp. strain WAL 4864 (which exhibited an intermediate level of sensitivity).

^d In the presence of 6% O₂ *C. gracilis* grew only on plates containing brucella agar supplemented with formate and fumarate but no blood; if blood was added, *C. gracilis* did not grow.

absence of flagella and the absence of oxidase activity; these authors commented that the cytochrome pattern of *C. gracilis* resembles the pattern reported for the genus *Campylobacter* and that the lack of measurable oxidase activity is not the result of a fundamental metabolic difference between *C. gracilis* and other *Campylobacter* species. In our study we found other evidence which indicates that these taxa can be distinguished. Strains belonging to *C. gracilis* did not exhibit malate dehydrogenase activity, in contrast to other campylobacters. Also, the cell wall fatty acid patterns of *C. gracilis* were distinct from those of other *Campylobacter* species. Consistent with the results of Vandamme et al. (16), we found that *C. gracilis* contained 16:0 dimethylaldehyde (*C. curvus* contained small amounts of 16:0 dimethylaldehyde) and large amounts of 18:1 *cis*9 dimethylaldehyde (other *Campylobacter* species contained no 18:1 *cis*9 dimethylaldehyde). In contrast to Vandamme et al. (16), we did not find a 16-C aldehyde in *C. gracilis*. Finally, a DNA-DNA hybridization analysis revealed that the level of homology between *C. gracilis* DNA and *C. rectus* DNA was low.

The type strain of *C. gracilis*, strain ATCC 33236^T, was at the edge of the *C. gracilis* cluster defined by the cell wall fatty acid analysis and hybridized less strongly than WAL 8030 to the other *C. gracilis* strains. *C. gracilis* WAL 8030 (a strain that occurred at the center of the cellular fatty acid cluster) exhibited high levels of DNA homology with other *C. gracilis* strains and a level of DNA homology with *C. rectus* of 23%. These data suggest that strain ATCC 33236 may not be a good type strain for *C. gracilis*. However, the *C. gracilis* strains that we used were not specifically oral isolates but were (with one exception) obtained from infections that occurred above the diaphragm (in contrast to the *Sutterella* strains, which were obtained for the most part from gastrointestinally related infections); thus, these strains may represent a different subgroup of *C. gracilis* than the subgroup used when strain ATCC 33236 was chosen as the type strain. Two of the isolates, strains WAL 10160 and WAL 10204 (a periodontal isolate and a pleural fluid isolate, respectively), exhibited higher levels of homology with the type strain (~75%) than the other isolates did (~30 to 40%). Our data (particularly our DNA homology data) indicated that the heterogeneity of *C. gracilis* strains merits further study.

The strains that originally were identified as *C. gracilis* but differed in genetic and biochemical characteristics belong to a new genus and a new species, for which we propose the name *Sutterella wadsworthensis*. The *Sutterella* strains differed from the *C. gracilis* type strain and other typical *C. gracilis* strains and from other *Campylobacter* strains in a number of characteristics (Table 4).

Description of *Sutterella* gen. nov. *Sutterella* (Sut.ter.el'la. M.L. dim. fem. n. *Sutterella*, named in memory of Vera Sutter, respected colleague and director of the Wadsworth Anaerobe Laboratory for 20 years). Gram-negative straight rods. Grows in a microaerophilic atmosphere (an atmosphere containing 2 or 6% oxygen) or under anaerobic conditions. Resistant to 20% bile disks. Asaccharolytic. Cannot reduce tetrazolium tetrahydrochloride under aerobic conditions if formate and fumarate are added to the medium. Can be differentiated from *C. gracilis* and *Campylobacter* species by the characteristics shown in Table 4. 16S rRNA sequence and DNA hybridization data indicate that the genus *Sutterella* is not related to *C. gracilis* or other *Campylobacter* species.

The genus *Sutterella* contains one species, *Sutterella wadsworthensis*, which is the type species.

Description of *Sutterella wadsworthensis* sp. nov. *Sutterella wadsworthensis* (wadsworth'en.sis. M.L. adj. *wadsworthensis*, from Wadsworth, referring to the Wadsworth Anaerobe Laboratories, VAMC West Los Angeles, where the strains were identified). Gram-negative straight rods that are 0.5 to 1 µm wide and 1 to 3 µm long. Urease negative. Oxidase negative. Indoxyl acetate negative. Reduces nitrate. Isolated mainly from infections of gastrointestinal tracts. May be resistant to metronidazole. The type strain is strain WAL 9799 (= ATCC 51579).

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