Proposal of Minimal Standards for Describing New Species of the Family Campylobacteraceae

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The International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Campylobacter and Related Bacteria has agreed in principle on minimum requirements for the description of new species of the family Campylobacteraceae. These requirements, as well as methods for determining specific characteristics, are proposed as minimal standards for the description of new species. In addition to specified phenotypic characteristics, molecular data are required. The placement of a new species should be consistent with the current view on classification usually based on methods such as nucleic acid sequencing, hybridization, or protein fingerprinting.

Members of the family Campylobacteraceae are curved or spiral bacteria mainly encountered as commensals or parasites in humans and other warm-blooded animals. The genus Campylobacter was created in 1963 (27), and as a consequence of the taxonomic revisions proposed by Vandamme et al. (37), the emended genus now contains (March 1993) 13 species and four subspecies. The genus Arcobacter was suggested for the former species Campylobacter cryaerophilus and Campylobacter nitrofugalis (37); recently, two additional species, Arcobacter butleri (12) and Arcobacter skirrowii (38), have been described. The inclusion of the genera Campylobacter and Arcobacter in a separate family, Campylobacteraceae, was suggested by Vandamme et al. (36). Helicobacter pylori, Helicobacter mustelae, Helicobacter cinaedi, and Helicobacter fennelliae, which were originally described as campylobacters (7, 37), will not be considered here.

General features of Campylobacteraceae. Curved or occasionally straight rods, 0.2 to 0.9 µm wide and 0.5 to 5 µm long. The rods may appear as spiral, S-, V-, or comma-shaped forms and may also occur in short or occasionally long chains. Cells may become spherical or coccoid, especially in old cultures. Nonspore-forming. Gram negative. Motile by means of single or occasionally multiple unsheathed flagella at one or both ends. Optimum temperatures of growth range from 30 to 42°C. They are usually microaerophilic and have a respiratory type of metabolism. Some species may be aerotolerant. Oxidase producing. Carbohydrates are neither fermented nor oxidized. Respiratory quinones are menaquinones. The G+C content of the DNA ranges from 28 to 46 mol%. The type genus is Campylobacter; at present, the only other genus is Arcobacter.

The following key features of the genus Arcobacter can be used to distinguish species from those of the genus Campylobacter: growth at 15°C but not at 42°C, aerobic growth at 30°C, G+C contents of 27 to 30 mol%, and methyl-substituted menaquinone-6 not present as a major isoprenoid quinone (36, 37). Detailed morphological, physiological, and biochemical test results for the various species have been reported previously (8, 22, 25, 26, 29) (see also the references given in Table 1).

General comments. The description of a new species or subspecies of Campylobacter or Arcobacter should be based on characteristics necessary for assigning the new taxon to the genus and on characteristics serving to differentiate the new taxon from existing taxa of the genus. The description should be based on as many strains as possible, preferably not fewer than 10. For critical comparisons with other species, controls consisting of type or reference strains of other species should be tested. For all test procedures, the inoculum size, composition of the gaseous atmosphere, and composition and pH of the medium should be stated. When possible, standardized, well-described tests and methods should be followed (1, 15, 16, 23, 24, 29).

Cell morphology. The shape, size, and spiral wavelength (where appropriate) of bacteria should be reported. The tendency to undergo rapid transformation to coccoid forms on exposure to air should also be indicated. Indicate whether stained preparations or wet mounts are used for microscopic observations. The number and arrangement of flagella should preferably be determined by electron microscopy; in which case, the presence or absence of flagellar sheaths should be reported.

Staining behavior. The behavior of the cells in the Gram stain must be stated.

Motility. Active, darting motility should be observed by microscopic examination of wet mounts or hanging drop preparations of young cultures in buffered saline or broth.

Colony morphology. The shape, size, and color of colonies should be described. The presence of.swarming on solid media should be noted.

Growth conditions. Factors affecting growth should be tested under conditions that are near optimal unless stated otherwise.

(i) Culture medium. Growth on unsupplemented nutrient agar should be tested. When using blood-supplemented nutrient agar, the type and the percentage of blood used should be reported as well as any hemolytic activity observed.

(ii) Temperature range. The ability to grow in specified broth or agar media from standardized inocula at various temperatures should be reported with the time of incubation. The following temperatures should be used: 15, 25, 36, or 37°C and 42 or 43°C (preferably the latter).

(iii) Gaseous requirements. The ability of the strains to grow under aerobic conditions and under microaerobic conditions should be reported; the oxygen content should be specified in the latter instance and if formate or hydrogen is needed.

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Anaerobic growth should be tested, and the requirement for fumarate alone, hydrogen and fumarate, or fumarate and formate should be investigated if considered appropriate (25, 30).

Biochemical properties. The tests for the following are required: (i) oxidase activity, by using any conventional method such as that described by Kovacs (14); (ii) catalase activity, with percentage of reagent solution and time of observation given; (iii) acid production from glucose by oxidation and fermentation by the method of Hugh and Leifson (10); (iv) nitrate reduction in a broth or a semisolid medium or by the method of Cook (4); (v) indoxyl acetate hydrolysis, preferably by the method of Hwang and Ederer (11) or an appropriate modification (9) (it is recommended that negative or equivocal results are confirmed by chromatographic assay for benzoic acid [13]).

Other tests. Tests for the following are desirable: (i) tolerance to 1.5 and 3.5% NaCl in brucella alibi broth or agar medium, (ii) anaerobic growth in the presence of 0.1% (wt/vol) trimethylamine N-oxide hydrochloride (TMAO) (2), (iii) rapid H$_2$S production in iron-bisulfitie-pyruvate (FBP) agar (1, 29), (iv) growth in the presence of triphenyltetrazolium chloride (0.04%) (2), and (v) tolerance to 1% glycine in brucella alibi broth medium.

Resistance to antimicrobial agents. Susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg) should be determined by diffusion tests with Mueller-Hinton agar. The absence of a clear zone of inhibition should be recorded as resistance; for susceptible strains, the zone sizes should be stated.

Molecular data. Standard methods for the determination of the guanine-plus-cytosine content of DNA (melting temperature or chemical) may be used (20). Reference DNA preferably of Escherichia coli NCTC 9001 (G+C, 51 mol%) should be analyzed at the same time. The strain used and its estimated G+C content (moles percent) expressed relative to the reference DNA should be reported.

Determination of major cellular fatty acids and respiratory quinone content is strongly recommended (3, 41), particularly for the presence of methyl-substituted menaquinone-6.

The suggested new species and its nearest neighbors should be investigated by a method giving a broader view of genotypic relationships. Thus, relationships to other species of Campylobacteraceae should be determined by DNA-DNA hybridization (20), and percentage of relatedness to the type strains of the other species should be stated. It should be noted that computer-assisted analysis of one-dimensional electrophoretic protein patterns provides taxonomic discrimination comparable to that of DNA-DNA hybridization (18) and could be considered a possible alternative. If the new species is not significantly related by DNA-DNA hybridization to any species of Campylobacteraceae, its relationship to the two genera of the family and to other allied genera, particularly Helicobacter and Wolinella, should be established by 16S rRNA sequence similarities (35) or by rRNA-DNA hybridization and thermal stability studies (37).

Ecology. The natural habitat(s) of the proposed species should be detailed as much as possible, and its pathogenicity and host range, if known, should be reported.

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