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Confirmation of Human *Campylobacter concisus* Isolates Misidentified as *Campylobacter mucosalis* and Suggestions for Improved Differentiation between the Two Species

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A strain from human diarrhea originally identified as *Campylobacter mucosalis* (NCTC 12408) was examined by using 64 phenotypic characters. The similarity of this strain to 297 isolates of *Campylobacter*, *Helicobacter*, *Arcobacter*, and related taxa was then determined with a computer-supported data analysis program, MVSP. NCTC 12408 showed closest similarity to 20 type, reference, and field isolates of *Campylobacter concisus*. These strains were clearly separated from those of *C. mucosalis* in the numerical analysis of phenotypic tests; a table was constructed from the data used to aid in differentiating these two species in the clinical laboratory. The identity of NCTC 12408 was confirmed as *C. concisus* by visual comparison of its sodium dodecyl sulfate-polyacrylamide gel whole-cell protein electrophoregram with those of type strains of *C. concisus* and *C. mucosalis*. These data suggest that genuine human infection with *C. mucosalis* has not yet been reported.

The first isolations of strains considered to be *Campylobacter mucosalis* from human clinical material have been reported (3). It was subsequently suggested that these strains may have been misidentified isolates of *Campylobacter concisus* (6), although this claim was refuted (2). This debate has highlighted the principal problems faced by clinical laboratories in accurately identifying campylobacteria, namely, taxonomic complexity and biochemical inertness. These problems are compounded by the absence of standardized methods for the useful discriminatory tests, since differing protocols may lead to significant differences in test outcome; examples of discrepant results have been noted previously (7–9). The value of most identification schemes for this group is therefore compromised since they have been compiled from various sources and do not allow for methodological differences which may occur.

Ideally, phenotypic identification schemes for microorganisms should comprise data which are directly comparable. Three such schemes for campylobacters and related organisms have been described. Barrett et al. (1) published a database comprising 25 biochemical tests recorded for 16 taxa, and a commercial system (API Campy) which comprises 21 tests and 18 taxa has been described. However, no objective data on the accuracy and integrity of these schemes are available.

An evaluation of a prototype probabilistic identification database for campylobacters and related organisms has been described elsewhere (4). Continuing research has resulted in the accumulation of extensive phenotypic data (for 64 test characters) for 297 strains representing 35 *Campylobacter*, *Arcobacter*, *Helicobacter*, and related taxa (unpublished data). These data are used here to confirm the identity of one of the clinical isolates (NCTC 12408; strain NCTC 12407 is no longer extant) originally described as *C. mucosalis* (3) and also to determine whether *C. mucosalis* and *C. concisus* can be differentiated by conventional methods.

Sixty-four phenotypic characters were determined for strain NCTC 12408 by standardized methods established in this laboratory, of which 44 have been described previously (4, 7–9). However, hydrogen sulfide production in triple sugar iron medium was recorded as copious (blackening of >70% of the agar butt and slope) and/or trace (blackening of part of the slope only). The remaining tests were as follows: cell morphology (recorded as gram-negative spirals, vibrioid [small curved rods], and/or straight rods); aerobic growth on blood agar (BA) at 25°C; growth on lecithin and tyrosine media; pitting on BA; alpha-hemolytic activity; and tolerance to 1.5 and 2.0% bile (BA base), 4.0% NaCl (BA base), 0.05% safranin (nutrient agar [NA] base), basic fuchsin (0.005%; NA base), crystal violet (0.0005%; NA base), janus green (0.01%; NA base), methyl orange (0.032%; NA base), sodium fluoride (0.05%; BA base), sodium deoxycholate (0.1%; NA base), and pyronin (0.02%; BA base). Inoculation (7) and incubation (8) procedures for tests requiring bacterial growth were as described previously. The results were compared, by numerical analysis, with similar data previously obtained for each of the 297 strains mentioned above. Similarities between strains were calculated by using the simple matching coefficient and clustered with the unweighted pair group with mathematical averaging algorithm. Calculations were performed with a Multi-Variate Statistical Package (MVSP version 2.1; Kovach Computing Services, Anglesey, Wales, United Kingdom) supported on a personal-computer-compatible microcomputer.

NCTC 12408 clustered at the 73.4% similarity level with 20 reference strains (including the type strain) and field isolates of *C. concisus*. These 21 strains formed a distinct cluster within which five subphenons were recognized at the 87.3% similarity level. All 10 *C. mucosalis* strains examined formed a discrete cluster at the 82.8% similarity level and were clearly separated from the *C. concisus* cluster, with which they exhibited only 62.8% similarity. These results were augmented by comparing the whole-cell electrophoretic protein profile of NCTC 12408 with those of the type strains of the aforementioned species. This confirmed the identity of NCTC 12408 as *C. concisus*; its diarrheal source is consistent with previous data (10). These findings suggest that genuine cases of *C. mucosalis* infection in humans have not yet been reported, since both of the isolates

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TABLE 1. Percentages of strains of *C. concisus* and *C. mucosalis* able to grow on media containing various inhibitory agents (4, 7, 8)[a]

Test	% of strains	
	C. concisus (n = 21)	*C. mucosalis* (n = 10)
1% Bile	5	100
0.02% Safranin	29	100
Metronidazole, BA base	19	90
MacConkey agar	0	80
0.05% Sodium fluoride, BA base	76	0

[a] References cite basal media and test protocols used in this study.

described by Figura et al. (3) were identical in all but one biochemical test (hydrogen sulfide production in triple sugar iron medium), in which only a quantitative difference was noted (3). Furthermore, *C. concisus* may also give a positive reaction in this test (1).

C. concisus as presently defined is both genomically (10) and phenotypically diverse and can be regarded as a complex. Differences between the overall phenotype of the *C. concisus* complex and those of *C. mucosalis* were apparent from the numerical analysis. Although no single test used in this study was able to unequivocally separate these species, the combined results of certain tests used in this study demonstrated significant differential potential (Table 1). Although it has been suggested that only molecular techniques can positively identify rare campylobacteria (6), the data presented here indicate that phenotypic characterization is still a valid and accurate tool for this purpose, provided that comparable data are used. However, caution should be exercised when employing the API Campy identification system, since misidentifications of *C. concisus* as *C. mucosalis* (as recorded here) and of *Arcobacter butzleri* as *Arcobacter cryaerophilus* or *Helicobacter cinaedi* (5) have been reported.

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