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Evaluation of API Campy in Comparison with Conventional Methods for Identification of Thermophilic Campylobacters

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API Campy was compared with conventional biochemical methods for its ability to identify 100 thermophilic campylobacter isolates. When the results were read according to the manufacturer's instructions, API Campy showed 92% agreement with conventional methods. Extended incubation of the assimilation strip resulted in the correct identification of an additional two isolates. Discrepant results occurred for six isolates. Overall, API Campy offered no advantages over conventional methods.

Campylobacter jejuni, *Campylobacter coli*, and *Campylobacter lari* are established etiological agents of bacterial gastroenteritis (12) and have been associated with bacteremias (13) and continuous ambulatory peritoneal dialysis-associated peritonitis (17). For many diagnostic laboratories, conventional identification schemes for thermophilic campylobacters usually consist of a few discriminatory tests: growth at 42°C, oxidase and catalase tests, cephalothin and nalidixic acid susceptibility, and hippurate hydrolysis. Such identification schemes are not always precise, because reproducibility in some key tests may be influenced by both inoculum size and basal medium (11). Furthermore, the use of nalidixic acid resistance as a means of identifying *C. lari* is becoming less reliable because of the emergence of resistance to quinolones among other campylobacters (3). In addition to biochemical tests, methods incorporating latex particle agglutination and DNA probe technology have been used for identification. However, latex agglutination has exhibited a low level of sensitivity for *C. lari* (8), while the use of DNA probes for campylobacter identification (5) requires specialization beyond the means of many diagnostic laboratories. API Campy (API-bioMérieux SA, Marcy l'Etoile, France) is a miniaturized identification system that uses 11 enzymatic and conventional tests plus 9 assimilation and inhibition tests. To date, API Campy has been used for biotyping (6), but there is no published information regarding the performance of this system for the routine determination of the species of campylobacters. Therefore, we performed a study to evaluate API Campy and to compare this system with the conventional identification methods cited above.

Bacteria. A total of 100 thermophilic campylobacter isolates, consisting of 97 clinical isolates (93 from feces, 2 from blood culture, and 2 from peritoneal dialysates) and 3 reference strains (*C. jejuni* NCTC 11351, *C. coli* NCTC 11366, and *C. lari* NCTC 11352), were tested. The isolates were maintained at −70°C in glycerol broth and were subcultured twice onto Columbia agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% defibrinated horse blood prior to testing. The subcultures were then incubated at 35°C in a microaerophilic atmosphere of 5% O_2, 10% CO_2, 10% H_2, and the balance N_2 for 24 to 48 h. The same inoculum was used for both the conventional tests and API Campy.

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Conventional identification. The following criteria were used to identify isolates of thermophilic campylobacters: curved or spiral gram-negative rods, distinctive colony morphology, positive catalase and oxidase reactions (16), and growth at 42°C. Isolates were further identified as *C. jejuni* or *C. coli* if they were resistant to cephalothin (30-μg disk) and susceptible to nalidixic acid (30-μg disk). *C. jejuni* was distinguished from *C. coli* on the basis of positive hippurate hydrolysis (16). Isolates resistant to nalidixic acid and negative for hippurate hydrolysis were presumptively identified as *C. lari.*

API Campy strip system. API Campy is a two-part system developed from the work of Elharrif, Megraud, and colleagues (1, 2, 7) for the identification of thermophilic *Campylobacter* species. The first strip consists of 10 enzymatic tests, and the second strip consists of 1 enzymatic, 6 assimilation, and 3 susceptibility tests. Preparation of the two strips and the inoculum was in accordance with the manufacturer's instructions. Results for all enzymatic tests were obtained with the addition of appropriate reagents after 24 h of incubation at 35°C under aerobic conditions. Results for the assimilation and inhibition tests were recorded after 24 h at 35°C under microaerophilic conditions. Incubation was extended to 48 h if the succinate assimilation test was negative, in accordance with the manufacturer's instructions.

After 24 h of incubation, API Campy had correctly identified 60 of 65 (92%) succinate-positive isolates. The assimilation and inhibition test strips for these isolates were incubated for an additional 24 h, despite the manufacturer's instructions. This extended incubation changed the profile codes for 21 of 65 isolates and resulted in an additional 2 isolates being correctly identified. The identifications of the remaining 19 of 21 isolates were unaltered. The increase was not statistically significant. Therefore, after 48 h of incubation, 62 of 65 (95%) succinate-positive isolates were correctly identified. Of the succinate-negative isolates, 32 of 35 (91%) were correctly identified. Overall, there was 94% agreement with the conventional tests after 48 h of incubation.

Seventy-eight isolates were identified by both systems as *C. jejuni*, 14 were identified as *C. coli*, and 2 were identified as *C. lari*. All reference strains were correctly identified by both systems. Three isolates had discrepant identifications, and three isolates yielded profile codes not present in the API Campy database. The correct identification of these six isolates was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4) and DNA-DNA dot blot hybridization (15) to obtain protein banding profiles. Their identities were found to be in agreement with those obtained by the conventional

tests. Of the three isolates incorrectly identified by API Campy, two were *C. coli* (misidentified as *Arcobacter cryaerophilus* and *C. jejuni*) and one was *C. lari* (misidentified as *A. cryaerophilus*). The three isolates for which no profile code existed were all *C. coli.*

On and Homes (11), in a study on useful enzyme detection tests for campylobacters, commented on the difficulty of establishing a clearly defined cutoff point for the alkaline phosphatase test. In the present study we also found it difficult to interpret results for alkaline phosphatase, as well as γ-glutamyl transferase, pyrrolidonyl arylamidase, L-arginine arylamidase, and L-aspartate arylamidase. We observed frequent differences in the intensities of the colors for these tests after the addition of the appropriate reagents. Very faint (but not colorless) reactions were difficult to interpret.

All isolates were cephalothin resistant (MICs, ≥32 μg/ml) when tested by agar dilution by the methodology of the National Committee for Clinical Laboratory Standards (9) using Mueller-Hinton agar containing 5% lysed horse blood. API Campy, which uses cefazolin, a compound similar to cephalothin, determined only 16 of 78 (20%) of *C. jejuni* and 14 of 19 (74%) of *C. coli* isolates as resistant. This was a disappointing feature of API Campy, although susceptibility to cephalothin has been observed with *C. jejuni* subsp. *doylei* (14) and *C. coli* (10).

Our results indicate 100% correlation between API Campy and the conventional tests for the identification of *C. jejuni*. Since only 14 of 19 (74%) *C. coli* isolates and 2 of 3 *C. lari* isolates were correctly identified, the API Campy system may not be reliable for the routine identification of these species. However, additional isolates would need to be tested. This may also be true for other *Campylobacter* species, as reported by Jacob et al. (6), who found strains of *Arcobacter butzleri* identified as either *Campylobacter cinaedi* or *A. cryaerophilus* by API Campy. Conventional tests alone appear to be satisfactory for the routine identification of thermophilic campylobacters. API Campy offered no advantages over conventional methods.

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