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Evaluation of the Indoxyl Acetate Hydrolysis Test for the Differentiation of Campylobacters

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Indoxyl acetate hydrolysis is a rapid, inexpensive differential test which can be performed easily to help identify campylobacters. A total of 571 *Campylobacter* cultures, including atypical variants, representing 10 species was tested.

During the past decade, the number of recognized and/or proposed species and subspecies in the genus *Campylobacter* has been expanded to 17 (5, 12). Definitive identification of campylobacters requires a variety of physiological and biochemical tests which are, for the most part, too laborious and time-consuming to be of practical value in routine diagnostic laboratories. Unlike other common clinical isolates, these organisms do not characteristically ferment or oxidize carbohydrate substrates; hence, the choices of practical tests for their identification and differentiation are relatively few (12, 17, 18).

Recently, Mills and Gherna (9) reported that only certain *Campylobacter* species have the ability to hydrolyze indoxyl acetate and proposed that this substrate be used in the rapid identification of *Campylobacter* species. The aim of the present study was to evaluate the usefulness of the indoxyl acetate hydrolysis (IAH) test for differentiation of campylobacters.

Most of the *Campylobacter* cultures used in this study were single fecal isolates submitted to our reference laboratory by various clinical laboratories in the province of Ontario, Canada, for identification or confirmation. Control cultures for this study included stock strains derived from our *Campylobacter* culture collection, as well as those generously provided by H. Lior, National Laboratory of Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada, and M. Karmali, Hospital for Sick Children, Toronto, Ontario, Canada. Altogether, 571 cultures were examined (see Table 1). Cultures were propagated on tryptic soy agar and/or Mueller-Hinton agar containing 5% sheep blood and incubated at 36°C for 48 h in a microaerophilic atmosphere. Stock cultures were maintained in Wilkins-Chalgren holding medium at −20°C or in glycerol (12 to 15% [vol/vol])-nutrient broth at −70°C.

Phenotypic characterization of strains was performed by using methods previously described (6, 7, 10, 13). The following tests were carried out: Gram staining; growth in aerobic (ambient) and microaerophilic atmospheres; growth at 25, 36, and 42°C; production of catalase and oxidase; hydrolysis of hippurate and urea; production of H_2S in TSI agar, iron metabisulfite medium, and lead acetate strips; tolerance to 1% glycine, 1% bile, and 3.5% NaCl; susceptibility to nalidixic acid and cephalothin (30-μg disks); reduction of nitrate; and anaerobic growth in trimethylamine-*N*-oxide. The differential reactions and characteristics used for species identification were those reviewed and tabulated by Penner (12).

Indoxyl acetate differential disks were prepared by placing 100 sterile, blank (0.25-in. [1 in. = 2.54 cm]-diameter) concentration disks (Difco Laboratories, Detroit, Mich.) into a dark bottle containing 0.25 g of indoxyl acetate (Sigma Chemical Co., St. Louis, Mo.) dissolved in 2.5 ml of acetone. Saturated disks were spread on a glass petri dish and allowed to air dry away from direct light. Once dried, the disks were stored in a tightly capped opaque bottle at 4°C. Freshly prepared disks are white; however, on prolonged storage (up to a year) they gradually become discolored (pale purple). A shelf life of 6 to 8 months and discard of discolored disks are recommended. The hydrolysis test was performed by using a slight modification of the method of Mills and Gherna (9). A dried disk was placed on a glass microscope slide and moistened with 1 to 2 drops of sterile distilled water. With an inoculating wire or wooden applicator stick, a heavy growth of the test culture was smeared onto the disk, which was then observed for up to 20 min at room temperature for signs of color change. IAH was indicated by the development, usually within a few minutes, of blueness, which intensified on prolonged standing. The test was considered negative if no color change occurred within 20 min.

Table 1 shows the IAH reactions of 571 cultures representing 10 different *Campylobacter* species. All cultures of *C. jejuni*, *C. coli*, *C. cryaerophila*, "*C. upsaliensis*," and *C. fennelliae* hydrolyzed indoxyl acetate. Cultures of strains belonging to the remaining five species failed to hydrolyze this substrate.

Our results agree with those reported by Mills and Gherna (9) for the nine species which were common to both studies. In the latter study, one of four cultures of *C. cinaedi* strains yielded a weakly positive reaction. In both studies, all other species were definitively positive or negative. No firm conclusions could be made for *C. cinaedi* or *C. fennelliae*, since only one strain of each species was available for our study. Conclusive evidence for the IAH characteristics of less common species in Table 1 requires that more cultures be tested; however, the virtual absence of variable reactions in our studies suggests that the ability to hydrolyze indoxyl acetate may be a valuable phenotypic marker for these organisms.

Tests for catalase activity, hippurate hydrolysis, and susceptibility to cephalothin and nalidixic acid are now routinely performed in many laboratories. However, their use-

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TABLE 1. IAH properties of 571 *Campylobacter* cultures

Organism (phenotype)[a]	No. of cultures	No. of cultures positive for IAH
C. jejuni (Nal^s)	473	473
C. jejuni (Nal^r)	18	18
C. coli (Nal^s)	35	35
C. coli (Nal^r)	2	2
C. cryaerophila	6	6
"*C. upsaliensis*"	6	6
C. fennelliae	1	1
C. fetus subsp. *fetus*	13	0
C. laridis	7	0
C. hyointestinalis	5	0
C. cinaedi	1	0
C. pylori	4	0

[a] Nal^s, Nalidixic acid susceptible; Nal^r, nalidixic acid resistant.

fulness for differentiation of campylobacters has been limited to some degree by the emergence of such atypical strains as catalase- and hippurate-negative *C. jejuni* (2, 15, 18), Nal^r *C. jejuni* (1, 16), cephalothin-susceptible ($Ceph^s$) *C. coli* (3), and cephalothin-resistant ($Ceph^r$) *C. fetus* subsp. *fetus* (4). Other atypical campylobacters which have been reported in the literature recently include urease-positive variants of *C. laridis* (8) and nitrate-negative and $Ceph^s$ *C. jejuni* (*C. jejuni* subsp. *doylei*) (14). The only atypical strains encountered in the present study were Nal^r *C. jejuni* and Nal^r *C. coli*. Unless additional tests are done, for example, the trimethylamine-*N*-oxide test, such strains can easily be confused with *C. laridis*. The IAH test is particularly useful for differentiation of Nal^r *C. coli* from the latter species. Also, its rapidity and ease of performance make it a practical alternative to the trimethylamine-*N*-oxide test, which requires up to 7 days of incubation.

The IAH properties of "*C. upsaliensis*" have not been described previously (9, 12). Our data suggest that the IAH test may also be useful in distinguishing *C. cinaedi* from "*C. upsaliensis*," especially "*C. upsaliensis*" variants which fail to grow at 42°C (11). The only remaining *Campylobacter* species for which IAH characteristics have not been described is *C. concisus* (9, 12). In a recent study of *C. concisus*-like organisms (E. Falsen group 22), Vandamme and colleagues (19) reported that most of their strains were originally misidentified, emphasizing again the shortcomings of traditional methods used in *Campylobacter* identification.

In summary, we confirm the findings of Mills and Gherna (9) and conclude that the IAH test can be used reliably to aid in differentiation of *Campylobacter* species. In addition, we found the test very useful for identification of strains that exhibit aberrant phenotypic properties. The rapidity, ease of performance, and low cost of the IAH test make it suitable for routine use.

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