Evaluation of Commercial Antisera for Serotyping Heat-Labile Antigens of Campylobacter jejuni and Campylobacter coli

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Commercial antisera for serotyping 22 heat-labile antigens of Campylobacter jejuni and Campylobacter coli were evaluated by using 66 isolates from human and nonhuman sources. Test results were compared with results of tests using antisera produced at the Centers for Disease Control (CDC), Atlanta, Ga. All strains (three isolates of each of the 22 serotypes) were typeable with the CDC antisera. Of 66 test strains, 39 (59%) were typed as the same serotype with both sets of antisera. Twenty-four strains (36%), including two heat-labile serotype reference strains, were nonreactive with the commercial antisera, and three strains (4.5%) were typed as serotypes different from those obtained with CDC antisera. Five of the 22 commercial antisera correctly serotyped all homologous strains. Our study indicated that two polyvalent antisera pools, 7 unabsorbed antisera, and 16 absorbed monovalent antisera are weak and need modification to enhance their antibody titers. Further studies are necessary to explain the antigenic change to a different serotype in three strains.

Several serotyping techniques for tracing the etiologies of Campylobacter jejuni and C. coli illnesses in humans have been described (1, 9, 14, 16b, 22, 28). The techniques to detect somatic O (formerly heat-stable) and heat-labile (HL) antigens, which are similar to that for serotyping O and H antigens of Salmonella and other enteric pathogens, have proved valuable, are used more frequently than other serotyping methods, and are recommended by the International Committee on Serotyping Campylobacter (5, 20).

Penner and Hennessy and coworkers described a serotyping scheme to identify the O thermostable lipopolysaccharide antigens of C. jejuni and C. coli (17, 22, 24). The O scheme identifies 60 serotypes using 42 C. jejuni strains and 18 C. coli strains. Later, Lior et al. (16b) developed a serotyping scheme to identify HL factors of Campylobacter bacteria. Flagellar protein occasionally accounts for the serotype specificity in the HL scheme, but undetermined proteins are the primary contributing factor (2, 27). The HL scheme utilizes 63 C. jejuni, 37 C. coli, and 8 C. lari strains to identify 108 Campylobacter serotypes.

In a study of 10 phenotypic and genotypic methods to distinguish epidemic-associated Campylobacter strains, Patton et al. (21) found that both the O and the HL serotyping schemes were capable of separating Campylobacter strains obtained during outbreaks in the United States and were recommended for routine use in clinical laboratories to distinguish outbreak strains. The major hindrance to the routine use of serotyping in clinical and diagnostic laboratories is the unavailability of high-quality commercial antisera and the increased demand on time and personnel to produce and maintain antisera to the numerous serotypes.

Several measures to simplify serotyping have been introduced. These include separate serotyping schemes for C. jejuni and C. coli (23), modification of existing serotyping procedures (6, 10, 17a), making commercial antisera available (3), and testing only for the common Campylobacter serotypes (7, 11, 12, 19, 24a, 25).

In 1989, Bar et al. tested 185 Campylobacter strains from infected humans in the Federal Republic of Germany using commercial antisera to 26 HL antigens (3). They found that 48.1% of the strains were typeable and 51.9% were unidentifiable. Similar results were obtained in Tokyo with antisera from the same commercial source (3). Bar et al. suggested an expansion of the antisera to accommodate other serotypes; however, antisera from this source are no longer available.

In this study, we evaluated another source of commercial antisera for serotyping 22 HL antigens of C. jejuni and C. coli using strains isolated in sporadic cases of diarrhea and the homologous HL reference strains of Lior et al. (16b). The rationale for the selection of the 22 antisera was not indicated by the manufacturer. Ten of the 22 antisera are among the 15 most common serogroups in human isolates identified by Lior (16), and 14 of the 22 are among the 23 most common HL serotypes from humans identified in the United States (18a). Using these 22 antisera we might expect to serotype 62.4% of C. jejuni, C. coli, and C. lari strains isolated in the United States.

MATERIALS AND METHODS

Commercial antisera. The commercial Campylobacter antisera to 22 HL antigens (Eco-Bio/Diagnostics Pasteur, Genk, Belgium) included (i) four pools of unabsorbed antisera to Campylobacter antigens HL 4 to 6, 20, and 38 (pool I); HL 1, 2, 7, 9, 36, 41, and 44 (pool II); HL 8, 11, 28, 29, 53, and 60 (pool III); and HL 15, 18, 19, and 45 (pool IV); (ii) individual vials of the 22 unabsorbed Campylobacter antisera listed above; and (iii) individual vials of the 22 absorbed antisera that were monospecific for the HL antigens listed above.

Bacterial strains. The 66 Campylobacter strains (57 C. jejuni strains and 9 C. coli strains) used in the evaluation included 3 strains each of the same serotype as that of the
commercial antisera, resulting in 3 reactors and 63 nonreactors with each of the monospecific, absorbed commercial antisera.

Twenty-two of the 66 strains were Campylobacter reference strains used in the Lior serotyping scheme and were kindly contributed by H. Lior, Laboratory Centre for Disease Control, Ottawa, Canada. The remaining 44 strains included 38 human isolates from patients with sporadic cases of diarrhea in the United States, one pig isolate from Vermont, and one chicken and four human isolates from Bangladesh. All strains were identified phenotypically in the Campylobacter Reference Laboratory and the Analytical Chemistry Laboratory of the Centers for Disease Control (CDC) or at the laboratories of state departments of public health. Strains referred to CDC were confirmed by hippurate hydrolysis (both ninhydrin and gas-liquid chromatography methods) and cellular fatty acid profiles (4, 13, 15). The test strains were previously serotyped with Campylobacter HL antisera that was prepared and standardized at CDC by using the reference strains and method of Lior et al. (19).

Strain preparation. Each of the 66 test strains was given a code number and tested as an unknown. To obtain cultures having 70% or greater bacterial motility by dark-field microscopy, all strains were transferred five or more times to fresh (<1-week-old) Mueller-Hinton agar plates prepared with Mueller-Hinton broth (Oxoid Ltd., Basingstoke, England), 1% plain agar (Difco Laboratories, Detroit, Mich.), and 5% sheep blood. The inoculated plates were incubated, according to the package insert, for 24 h at 42°C in a microaerobic atmosphere of approximately 5% O2, 7.5% CO2, 7.5% H2, and 80% N2 generated by an evacuation-replacement system using a gas mixture of 10% CO2, 10% H2, and 80% N2 (18).

Strains determined to be actively motile by dark-field microscopy were added to 0.5 ml of 0.02% DNase (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M phosphate-buffered saline, pH 7.2, to make a turbid suspension. Each strain was tested for reactivity by and confirming 1 drop of bacterial suspension in 1 drop of 2% saline on a glass slide and reading for absence of autoagglutination after 1 min. Strains showing autoagglutination were transferred three to five additional times to fresh Mueller-Hinton agar, and the above procedure was repeated until a smooth test culture was obtained.

Slide agglutination test. Each commercial antisera was used prior to expiration and according to the manufacturer’s instructions. These instructions followed the serotyping scheme of Lior et al. (16b) and consisted of three steps: (i) screening strains with polyvalent antisera (pools I to IV), (ii) presumptive identification of serotype using individual unabsorbed antisera, and (iii) confirmation of serotype using the monovalent absorbed antisera.

The test was performed by placing 1 drop of the turbid emulsion of test bacteria in DNase on a slide along with 1 drop of the appropriate commercial antisera. The drops were mixed, the slide was tilted back and forth for 45 s, and the suspension was read for agglutination by using an indirect light source. Reactions were scored from negative to 4+, with readings of 1+ considered to reflect weak reactions and readings of 2+ or greater considered positive.

RESULTS

Of the 66 strains, 39 (59%) were typed as the same serotype with both the commercial and the CDC antisera (Table 1). Included in this number are clinical strain HL 18,23 that was serotyped as HL 18,19 with the commercial antisera, one HL 41,60 clinical strain that was serotyped as HL 60, two HL 19 clinical strains that were serotyped as HL 19,9, and HL 18,19, and one HL 36 reference strain that was serotyped as HL 36,53.

Three clinical strains (4.5%) were typed as serotypes different from those obtained with CDC antisera. Two HL 20 strains and one HL 53 strain were serotyped as HL 1, HL 6, and HL 8, respectively, with the commercial antisera (Table 1).

Twenty-four strains (36.4%) were nontypeable with the commercial antisera. To identify the antisera that yielded the nontypeable results, we decoded the strain numbers and retested all nonreactive strains with their corresponding commercial antisera.

All commercial polyvalent pools (except pool I, which was nontypeable with one of three HL 20 test strains, and pool IV, which was reactive with all three HL 15 test strains) were reactive with their respective positive strains (Table 2). The unabsorbed antisera HL 1, 7, 8, and 53 were reactive with one of three homologous strains, HL 20 and 29 antisera were nonreactive with two strains, and the HL 15 antisera was nonreactive with all three strains. A total of 16 absorbed antisera were nonreactive with one or more homologous strains (Table 2). Ten absorbed antisera (HL 2 to 9, 11, 41, and 44) were nonreactive with one of three homologous strains, four absorbed antisera (HL 20, 29, 38, and 53) were nonreactive with two of three homologous strains, and two absorbed antisera (HL 15 and 45) did not serotype any homologous strains.

The four polyvalent antisera pools serotyped 62 of 66

<table>
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<tr>
<th>Reference strain</th>
<th>Clinical strain:</th>
<th>Commercial antisera</th>
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strains (94%), the unabsorbed antisera serotyped 55 of 66 strains (83%), and the absorbed antisera serotyped 42 of 66 strains (63%). Six of the 22 absorbed commercial antisera (HL I, 18, 28, 36, and 60) were reactive with all three of their homologous strains (Table 2), although one HL I clinical strain did not react with its homologous unabsorbed antisera (Table 1).

Of the 22 HL reference strains, 20 were correctly identified by both the CDC antisera and the commercial antisera (Table 1). Reference strains HL 15 and HL 45 along with their homologous clinical test strains were nontypeable with the commercial antisera (Table 1).

DISCUSSION

Campylobacters are a common cause of food-borne human gastroenteritis. Because epidemiological data are important in the control of campylobacteriosis, the commercial availability of high-quality Campylobacter antisera would enable more diagnostic laboratories to become involved with the epidemiology of this illness.

At the present time, commercial serotyping antisera for identifying 22 HL antigens of C. jejuni and C. coli are available. Our study showed that these antisera followed Lior’s simple slide agglutination technique with live cultures and absorbed antisera and were convenient to use. However, with these antisera, a significant number of strains (36.4%) were nontypeable and three were misidentified compared with the results of tests using the CDC antisera to HL antigens. The discrepancy in serotypes of these three strains may represent antigenic variation of the flagella. Certain strains of C. coli and C. jejuni have been shown to undergo antigenic variation (8). Further studies are necessary to determine whether this phenomenon occurred.

Several factors may cause strains to be nonreactive or nontypeable in serotyping schemes. Nontypeable strains are often caused by low levels of antibody in the antiserum. The unabsorbed antiserum, resulting from rabbit immunization with formalized whole cells of a known HL serotype, are polyvalent and may react with homologous and heterologous O and HL antigens. To prepare HL specific antisera, the O and heterologous HL antibodies are removed by absorption (20). These absorptions may weaken the titers of HL specific antisera.

In this study, 11 absorbed antisera were unable to serotype one or more homologous strains that were typeable with the analogous unabsorbed antisera (Table 1). These results suggest that the absorption process reduced specific-antibody titers to a level such that antigenically weak strains could not be typed. Likewise, nontypeable results for unabsorbed antisera with one or both homologous clinical strains (Table 2) indicate that these antisera have borderline levels of antibody. The nonreactivity of a single strain of serotype HL 20 with pool I (Table 2) is also most likely related to low antibody titer.

The observation that none of the three HL 15 strains, including the reference strain, reacted with any of the pooled, unabsorbed or absorbed antisera (Table 2) suggests extremely low antibody levels or inadvertent error in preparing the antisera. Reference strain HL 45 was also nontypeable with its absorbed antisera. Historically, reference strains give strong, reproducible reactions with their homologous antisera. With the HL scheme, the immunizing vaccines must be prepared from actively motile bacteria, i.e., over 70% of cells with flagellar movement, to obtain high-titer antisera to HL antigens (16a).

Nontypeable results can also be due to instability of antibody titer in the antiserum, which would necessitate an earlier expiration date. For the commercial antisera used in this study, 4½ months remained in the shelf life. In our experience, weakly positive, low-titer HL antiserum deteriorates to a nonreacting antiserum in approximately 6 months and high-titer, strongly reacting antisera are stable for several years when stored at −20°C.

Another contributing factor in the identification of nontypeable strains, particularly in the HL serotyping scheme, is the absence of flagella. Flagella appear to carry the serotype-specific antigens of certain serotypes in the Lior HL serotyping scheme, and nonflagellated mutants can become nontypeable (26, 27). Lack of flagella was not a characteristic of the test strains in this study, as each showed at least 70% motility before being serotyped.

The method of production of Campylobacter HL serotyping antisera conforms to the principles outlined for the production of Salmonella antisera (5). With Salmonella antisera, prolonged rabbit immunization beyond the recommended vaccine injections may result in antiserum with heterologous cross-reacting antibodies. Cross-reacting antibodies did not present a problem with these commercial Campylobacter antisera.

Our study indicated that 25 of the 48 antisera included in the commercial kit (four antiserum pools, 22 unabsorbed antisera, and 22 absorbed antisera) failed to serotype one or more of three homologous Campylobacter strains. Therefore, we conclude that further work is needed before these antisera gain widespread use.

### Table 2. Reactivities of Campylobacter strains with polyvalent, unabsorbed, and absorbed commercial antisera

<table>
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<tr>
<th>HL serotype</th>
<th>No. of reactive strains&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup> Sixty-six decoded test strains and nontypeable strains tested with their homologous antisera.

<sup>b</sup> Of three strains total.
ANTISERA FOR HL ANTIGENS OF C. JEUNI AND C. COLI

REFERENCES


