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Extended Scheme for Serotyping *Campylobacter jejuni*: Results Obtained in Israel from 1980 to 1981

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The serotyping scheme for *Campylobacter jejuni* previously developed in the National Center for Campylobacter, Jerusalem, was extended by the use of 20 new sera and modified by the absorption of the sera, when necessary, with homologous boiled cultures or heterologous live cultures. The extended scheme is based on slide agglutination of live suspensions and is performed in two stages: pretesting with four pooled sera and final testing with monovalent sera. So far, 34 serotypes have been recognized. Among 442 isolates of *C. jejuni*, 86.4% could be typed with this scheme. Of the 382 cultures typed, 90% reacted with one single serum, and the remainder showed a complex antigenic structure. The frequent serotypes identified were: 11 (12.9%), 12 (8.2%), 18 (6.3%), and 3 (6.1%). When epidemiological data were available, the results of the serotyping corresponded with the epidemiological evidence.

Campylobacter jejuni is now recognized as a main etiological factor of enteritis (4, 9, 17, 22, 26), especially in children. Publications of the last few years (26, 28) have already emphasized the role of certain animals and fowl as sources of infection. Outbreaks caused by *C. jejuni* have been reported, and in some of them, transmission by water (7, 18), milk (3, 23), or food (6, 19) has been observed. Since the group of microorganisms identified as *C. jejuni* is characterized by only a few biochemical reactions but has a large antigenic heterogeneity, any epidemiological investigation in this field has to be supported by serotyping the isolates. The serological study of *C. jejuni*, initiated by King (10), was further developed by Berg et al. (2) and Butzler and Skirrow (5).

At the first workshop on *Campylobacter* infections held at the University of Reading (March 1981), two methods for serotyping *C. jejuni* were presented: that of Penner et al. (20, 21) and Lauwers (12), based on a passive hemagglutination technique, and that of Lior (14) and Rogol (24), based on slide agglutination of live suspensions. Since then, all these methods were further developed (12, 14, 16, 25), and each of them covered most of the isolates from the respective country.

This paper presents the further development of the Jerusalem serotyping scheme presented at the workshop of the University of Reading and the results of its application in typing *C. jejuni* isolates from Israel.

MATERIALS AND METHODS

Bacterial strains. A total of 34 reference strains designated 1 through 31 and 35 through 37 were used for the preparation of antisera. Of these 34 strains, 30 originated from human cases of enteritis in Israel and 4 (numbers 4 through 7) were received from Sweden. Most of these cultures (24) belonged to biotype I; 4 belonged to biotype II and 5 to *Campylobacter coli*, as described by Skirrow et al. (27). A total of 442 cultures of *C. jejuni* isolated from human cases of enteritis in 1980 to 1981 were obtained from 22 hospital laboratories in Israel. The isolates were preserved at −80°C as cultures in 2 ml of fluid thioglycolate medium plus the same volume of 70% glycerol in distilled water and 2 drops of defibrinated human blood. For serotyping, the cultures were grown on blood agar slants containing blood agar base (Oxoid Ltd.) with 6% human blood. They were incubated at 42°C in an anaerobic jar (without catalyst), with an anaerobic system GasPack (BBL Microbiology Systems). Autoagglutinable cultures were passed successively on fluid thioglycolate and blood agar slants several times; only five of the cultures remained autoagglutinable.

Antisera. Agglutinating sera were prepared against the reference strains as previously described (24). White rabbits were inoculated intravenously with cultures on blood agar suspended in 0.5% formolized saline and adjusted to an optical density of 0.5 at 540 nm on a Spectronic 20 spectrophotometer. These suspensions were used for the first three inoculations (0.25, 0.5, and 1.0 ml) at 4 to 5 day intervals, followed by two inoculations (2.0 and 4.0 ml) of live suspensions of the same concentration. No adjuvant was used. Bleeding was done at 5 to 7 days after the last inoculation. The crude serum was preserved with an equal column of glycerol and kept at 4°C. Homologous

and heterologous titers were determined by slide agglutination of fresh suspensions (0.5 ml of phosphate-buffered saline for the culture on a blood agar slant) with twofold diluted sera beginning from 1/20. One drop of the diluted serum was placed on a slide, and a loopful of the bacterial suspension was stirred in it. Agglutination appearing within 30 to 60 s was noted as 3^+ or 2^+ and was considered a positive reaction. The working dilution of each serum was established as 1/4 of the homologous titer.

Absorptions of sera. All the sera were tested, at working dilution, by slide agglutination against a boiled (1 h at 100°C) suspension of the homologous reference strain. Most of the sera did not react at all or reacted after 2 to 3 min with a fine granular agglutination, clearly different from the rapid reaction of live suspensions. Only nine sera (numbers 3, 5, 8, 11, 12, 17, 18, 23, and 24) reacted stronger, and these sera were absorbed with the homologous boiled (2 h at 100°C) strain as described by Lior (14). The cross-reactions of the sera observed in the testing with heterologous live antigens were eliminated by absorptions with live bacterial suspensions. The whole culture of five blood agar slants was harvested in 10 ml of phosphate-buffered saline and centrifuged (10 min at 11,180 × *g*). A 2-ml amount of serum (diluted 1/2) was added to the packed cells, and the mixture was incubated for 2 h at 37°C and then centrifuged. The supernatant was collected, preserved with merthiolate, and tested in successive dilutions against the homologous and the cross-reacting strains. The following absorptions were performed: antiserum 1 absorbed with reference strain 18 (noted as 1/18; 4/24, 10/11, 11/1 and 11/10, 12/14, 14/6 and 14/12, 18/10, 20/26, 21/20, 23/8, 24/4 and 24/23, and 26/25. The absorbed sera were diluted so as to react only with the homologous reference strain.

Testing of strains isolated in Israel. Slide agglutination was first performed with pooled sera. Four pools were used: pool I comprised the sera 1 through 9; pool II the sera 10 through 15; pool III the sera 16 through 25, and pool IV the sera 26 through 31 and 35 through 37. The final dilution of each serum in a pool was identical to the working dilution of the respective serum. Further tests were performed with the single sera of the reacting pool and, when necessary, with antisera absorbed for heterologous cross-reacting antibodies. Only reactions appearing within 30 to 60 s were considered.

RESULTS

Antisera. All 34 sera (among them 9 absorbed with the homologous boiled culture) were tested in slide agglutination against the homologous and heterologous reference strains. Twofold dilutions of the sera were used beginning from 1/20. The results are presented in Table 1. The homologous titers varied between 1/80 and 1/320. Among the 34 sera tested, 20 reacted with the homologous strain only, 9 sera cross-reacted with one heterologous strain, and 5 cross-reacted with two to four heterologous strains. Cross-reactions of two of the sera were eliminated by using the working dilution at 1/4 of the homologous titer. For the remaining 12 sera, absorptions with cross-reacting strains led to monovalent sera.

TABLE 1. Homologous and heterologous slide agglutination titers of *C. jejuni* antisera

Reference serum no.	Homologous titer	Heterologous reaction [reference strain (titer)]
1	80	18 (40)
2	160	
3[a]	160	4 (20)
4	160	24 (80), 3 (20), 8 (20), 27 (20)
5[a]	320	
6	160	
7	320	
8[a]	80	
9	160	
10	80	11 (40)
11[a]	160	10 (40)
12[a]	80	14 (20)
13	80	
14	80	6 (40), 12 (40)
15	160	
16	160	
17[a]	80	
18[a]	80	10 (40)
19	80	18 (20)
20	80	26 (40)
21	80	20 (20)
22	80	
23[a]	160	8 (40), 16 (20), 24 (20)
24[a]	80	4 (40), 23 (40), 7 (20)
25	160	
26	80	25 (40)
27	80	
28	80	
29	160	
30	80	
31	80	
35	80	
36	80	
37	80	

[a] Sera absorbed with the homologous boiled strain.

Serotyping of local isolates. This serotyping scheme was used with 442 cultures. Most of the strains (382 = 86.4%) reacted with one or some of the pooled sera; 60 cultures did not react with any of them and served for preparation of new sera and further development of the scheme. Among the typed cultures, 240 (62.8%) reacted with one pool and one single serum; 103 (27.0%) reacted with two sera and 39 (10.2%) with three or more sera. All the cultures reacting with more than one serum were tested with the respective sera which had been absorbed for heterologous cross-reactions. The results are presented in Table 2. After testing with absorbed sera, 244 cultures (90%) reacted with one single serum and 38 (10%) showed a more complex antigenic structure.

The most frequent serotypes so far identified in Israel are: 11 (12.9%), 12 (8.2%), 18 (6.3%), 3

(6.1%), 24 (4.8%), and 15 (3.4%). These six serotypes represent together more than 40% of the isolates. During the study of 6 family outbreaks, 3 hospital events and one community outbreak, good correlation was found between the results of serotyping the isolates and the epidemiological evidence (Table 3).

TABLE 3. Serotypes of *C. jejuni* isolated from related cases

No. of foci (case)	No. of cases	Serotype
1 (Family)	2	3
2 (Family)	7	12
1 (Family)	2	2
1 (Family)	2	8
1 (Hospital)	4	10
2 (Hospital)	4	11
1 (Community)	8	17

DISCUSSION

The serotyping systems for *C. jejuni* described by Penner and Hennessy (20, 21) and by Lauwers (12, 13), based on passive hemagglutination with heat-stable soluble antigens, are elegant and very sensitive but may show some difficulties when used in various hospital laboratories. On the other hand, the slide agglutination methods, developed by Lior (12, 13, 15) and by us, based on the use of sera absorbed for homologous heat-stable and heterologous heat-labile antigens, are suitable for national centers and not for hospital laboratories. Other authors (8, 11) have compared several methods of serotyping and expressed their preference for the slide agglutination method. We propose here a serotyping system working in two stages: pooled crude sera, to be used for diagnosis and a first serogrouping at the level of hospital and other laboratories, and a second stage of confirmation and complete serotyping at the level of the respective National Center for Campylobacter. The complete antigenic structure of the isolates is a problem to be solved in future studies. In our experience, about 10% of the isolates seem to have a complex antigenic structure. Similar observations have also been done by other authors (1, 16, 17).

TABLE 2. Serotypes of *C. jejuni* isolated in Israel from human sources[a]

Serotype[b]	No. of strains	%	Serotype[c]	No. of strains
11	57	12.9	1, 10	1
12	36	8.2	1, 12	1
18	28	6.3	3, 5	1
3	27	6.1	3, 11	2
24	21	4.8	4, 12	6
15	15	3.4	4, 29	1
1	13	2.9	5, 6	2
5	13	2.9	6, 24	1
8	13	2.9	7, 11	1
23	13	2.9	8, 11	1
9	12	2.7	8, 12	1
4	11	2.5	11, 12	2
20	10	2.3	11, 13	1
17	9	2.1	11, 14	7
6	8	1.8	11, 18	3
13	8	1.8	11, 24	1
10	7	1.6	11, 28	1
2	6	1.4	12, 15	1
14	6	1.4	14, 15	2
7	5	1.1	14, 20	1
16	5	1.1	16, 25	1
22	5	1.1		
19, 21, 25–31, 35–37	16	3.6		
Total	344	77.8		38 (8.6%)

[a] Of the total number of strains (442), 382 (86.4%) were typable and 60 (13.6%), comprising five autoagglutinable cultures, were nontypable.

[b] Strains reacting with one serum (crude or absorbed).

[c] Strains reacting with 2 absorbed sera, comprising 5 autoagglutinable cultures.

The actual methods used in serotyping *C. jejuni*, based mostly on sera prepared against respective local isolates, are efficient for about 90% of the strains isolated in the respective country. Only a large cooperation between the national centers involved in the isolation and serotyping of *Campylobacter* spp. may lead to an international scheme, which has to cover isolates from various countries. Such a scheme will be useful for discovering sources of infection and ways of transmission on a local and an international level.

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