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Antigenic Relationships Among Type Strains of *Yersinia enterocolitica* and Those of *Escherichia coli, Salmonella* spp., and *Shigella* spp.

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The antigenic type strains for *Yersinia enterocolitica* antigens O:1 to O:34 were examined and their antigenic relationships with the type strains of *Escherichia coli, Shigella* spp., and *Salmonella* spp. were determined. *Y. enterocolitica* O:5,27 was antigenically identical to *E. coli* O97 and *Y. enterocolitica* O:11 was antigenically identical to *E. coli* O98.

Yersinia enterocolitica has been intensively studied in recent years both in Europe and in the United States. Improved methods of isolation and identification have been developed and have led to a greater appreciation of the clinical importance of this organism. In particular, the role of *Y. enterocolitica* in enteritis and its pathogenic mechanism in such infections have been increasingly well documented. Biotyping and serotyping schemes have been developed and have led to an increased understanding of the epidemiology of *Y. enterocolitica* enteritis. For example, there is now evidence for an animal reservoir as well as for direct or indirect spread of infection from person to person. Serotyping has also shown that enteric infections are usually due to serovars O:3, O:8, and O:9 (1).

It has been shown that there is widespread sharing of antigens among the *Enterobacteriaceae*, and antigenic cross-reactions may cause difficulties in the identification of some species. For example, many *Shigella* antigens are identical or closely related to *Escherichia coli* antigens (2, 4). Since *Y. enterocolitica* is now regarded as a member of the family *Enterobacteriaceae*, we examined type strains for *Y. enterocolitica* antigens O:1 to O:34 and determined their antigenic relationships among *E. coli, Shigella* spp., and *Salmonella* spp.

MATERIALS AND METHODS

Bacterial strains. Type strains for *Y. enterocolitica* serovars O:1,2a,3 to O:34 were used (6). Strains O:7 and O:8 were obtained from S. Winblad, University of Lund, Malmö, Sweden, and the remaining strains were from N. S. Mair, University of Leicester, Leicester, England. During the course of this investigation it was discovered that the type strain for serovar O:7,13 (strain number 553) no longer possessed antigen O:13. This was kindly confirmed by D. G. Wauters, Université Catholique de Louvain, Louvain, Belgium. Since no suitable alternative strain possessing antigen O:13 was available, this serovar was omitted from the study. Type strains for all *E. coli* O antigens O1 to O164, all recognized and provisional *Shigella* serovars (3), and all *Salmonella* O antigens 1 to 67 were present in the culture collection of the Division of Enteric Pathogens.

Preparation of antisera and antigen suspensions. O antisera were prepared by standard methods, using all of the *Y. enterocolitica, E. coli, Shigella* spp., and *Salmonella* spp. type strains. *Y. enterocolitica* cultures for the preparation of vaccines and antigen suspensions were grown at room temperature (ca. 22°C). All other cultures were grown at 37°C. Cultures for the preparation of vaccines were each grown on two nutrient agar slopes and harvested in 0.9% saline before heating at 100°C for 2.5 h. After centrifugation, the heated organisms were suspended in 15 ml of saline, and commercial Formalin was added to a final concentration of 0.3%. Rabbits were immunized by intravenous injection.

For the preparation of O antigen suspensions for agglutination tests, cultures were grown in nutrient broth overnight and then heated at 100°C for 30 min. Commercial Formalin was then added to a final concentration of 0.3%.

Agglutination tests. Agglutination tests were performed in plastic agglutination trays and were incubated at 50°C for 16 h. O antisera for all of the *Y. enterocolitica* strains were tested against O antigen suspensions of all of the *E. coli, Shigella*, and *Salmonella* strains. O antisera for all of the *E. coli, Shigella*, and *Salmonella* strains were tested against O antigen suspensions of all the *Y. enterocolitica* strains.

Absorption studies. Cross-reacting titers of 1:16 or greater to the homologous titer were considered significant. Where significant cross-reactions were found among the test strains, reciprocal absorptions were performed.

RESULTS

Significant cross-reactions of *Y. enterocolitica* antisera are shown in Table 1. Significant cross-

TABLE 1. Significant cross-reactions of *Y. enterocolitica* antisera

Serovar	Strain no.	Homologous titer	Cross-reactions with other antigens (titer)
O:1,2a,3	64	12,800	None
O:2a,2b,3	178	12,800	None
O:3	134	12,800	None
O:4,32	96	25,600	None
O:4,33	1476	6,400	*E. coli* O10 (800), O25 (400)
O:5	123	6,400	*E. coli* O97 (800)
O:5,27	885	12,800	*E. coli* O97 (12,800)
O:6,30	102	6,400	None
O:6,31	1477	25,600	None
O:7	605	3,200	*E. coli* O86 (200), O87 (200), O127 (800), O136 (6,400)
O:8	636	1,600	*E. coli* O136 (400)
O:9	382	6,400	None
O:10,K1	551	6,400	*E. coli* O164 (400)
O:11,23	105	3,200	*E. coli* O98 (1,600)
O:11,24	841	25,600	*E. coli* O98 (3,200)
O:12,25	490	12,800	*E. coli* O12 (800), provisional *Shigella* 3873.50 (1,600)
O:12,26	103	25,600	*E. coli* O12 (1,600), provisional *Shigella* 3873.50 (1,600)
O:14	480	3,200	*E. coli* O55 (200), O136 (200), *Shigella boydii* 1 (200)
O:15	614	25,600	*Shigella boydii* 6 (3,200)
O:16	1475	12,800	None
O:16,29	867	6,400	*E. coli* O46 (400)
O:17	39621	25,600	None
O:18	846	25,600	*E. coli* O11 (6,400)
O:19,8	842	25,600	*E. coli* O39 (1,600), O87 (1,600), O136 (800)
O:20	845	25,600	None
O:21	1110	25,600	None
O:22		25,600	None
O:28	1474	25,600	*E. coli* O102 (1,600), O139 (3,200)
O:34	1501	12,800	None

reactions of *Y. enterocolitica* antigen suspensions are shown in Table 2. Significant reciprocal cross-reactions occurred as follows: *Y. enterocolitica* O:5 and O:5,27 with *E. coli* O97; *Y. enterocolitica* O:11,23 and O:11,24 with *E. coli* O98; *Y. enterocolitica* O:12,25 and O:12,26 with provisional *Shigella* 3873-50; and *Y. enterocolitica* O:18 with *E. coli* O11. *Y. enterocolitica* O:21 was agglutinated to titer by *Salmonella* O4 antiserum, but the reciprocal reaction gave a titer of less than 1:16 of the homologous titer.

In most cases reciprocal absorptions failed to show any close antigenic relationship between cross-reacting strains. However, it was shown that *Y. enterocolitica* O:5,27 was antigenically identical to *E. coli* O97 (Table 3) and *Y. enterocolitica* O:11 was identical to *E. coli* O98 (Table 4).

TABLE 2. Significant cross-reactions of *Y. enterocolitica* O antigen suspensions

Serovar	Homologous titer	Cross-reactions with *Y. enterocolitica* O antigens (titer)
E. coli O5	3,200	O:8 (200)
E. coli O11	6,400	O:18 (800
E. coli O29	400	O:12,25 (400)
E. coli O55	25,600	O:20 (1,600)
E. coli O56	1,600	O:8 (400)
E. coli O65	800	O:7 (200)
E. coli O69	3,200	O:4,33 (400)
E. coli O97	25,600	O:5 (6,400), O:5,27 (6,400)
E. coli O98	3,200	O:11,23 (800), O:11:24 (800)
E. coli O105ac	800	O:7 (400)
E. coli O123	3,200	O:4,33 (200)
E. coli O127	3,200	O:7 (400) O:8 (200)
E. coli O139	6,400	O:28 (800)
Provisional *Shigella* 3873.50	3,200	O:12,25 (400), O:12,26 (200)
Salmonella O4	800	O:21 (800)

TABLE 3. Antigenic relationship of *Y. enterocolitica* O:5 and O:5,27 with *E. coli* O97

Antiserum to:	Absorbing suspension	Antigen suspension titer		
		Y. enterocolitica O:5	*Y. enterocolitica* O:5,27	*E. coli* O97
Y. enterocolitica O:5	None	6,400	1,600	800
	E. coli O97	<100	<100	<100
Y. enterocolitica O:5,27	None	3,200	12,800	12,800
	E. coli O97	<100	<100	<100
E. coli O97	None	6,400	6,400	25,600
	Y. enterocolitica O:5	<100	3,200	25,600
	Y. enterocolitica O:5,27	<100	<100	<100

TABLE 4. Antigenic relationship of *Y. enterocolitica* O:11,23 and O:11,24 with *E. coli* O98

Antiserum to:	Absorbing suspension	Antigen suspension titer		
		Y. enterocolitica O:11,23	*Y. enterocolitica* O:11,24	*E. coli* O98
Y. enterocolitica O:11,23	None	3,200	1,600	1,600
	E. coli O98	1,600	<100	<100
Y. enterocolitica O:11,24	None	12,800	25,600	3,200
	E. coli O98	<100	1,600	<100
E. coli O98	None	800	800	3,200
	Y. enterocolitica O:11,23	<100	<100	<100
	Y. enterocolitica O:11,24	<100	<100	<100

DISCUSSION

The antigenic cross-reactions of *Y. enterocolitica* with *Salmonella* spp., *Shigella* spp., and *E. coli* have been determined. Although few cross-reactions with *Salmonella* strains were regarded as significant according to the criteria adopted, a reciprocal cross-reaction occurred at low titer between *Y. enterocolitica* O:12 and *Salmonella* O47. This was in agreement with the findings of Wauters and his colleagues (5). In addition, there was some cross-reaction between *Salmonella* O4 and *Y. enterocolitica* O:21. No cross-reactions were found with the accepted serovars of *Shigella* spp., but *Y. enterocolitica* O:12,25 and O:12,26 had a reciprocal cross-reaction with a provisional serovar of *S. dysenteriae*. Most importantly, two *Y. enterocolitica* serovars were found to share identical O antigens with two *E. coli* serovars. These findings emphasize the importance of using biochemical tests in conjunction with serotyping methods in the identification of clinical isolates.

The development of effective serotyping schemes has been an essential prerequisite for epidemiological studies of the clinically important *Enterobacteriaceae*. Detailed, internationally recognized schemes for *Salmonella* spp., *Shigella* spp., and *E. coli* exist and are widely used. The emergence of *Y. enterocolitica* as an important enteric pathogen suggests that the serotyping scheme for this organism may need to become more generally available. An understanding of the antigenic relationships which occur between members of different genera is necessary as an aid to accurate identification.

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