Antigenic Relationships Between *Escherichia coli* O Antigens O149 to O163 and *Shigella* O Antigens

B. ROWE, R. J. GROSS, AND M. GUINEY

*Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory,
London NW9 5HT, England*

O antigens O149 to O163 are recent additions to the *Escherichia coli* antigenic scheme. Their antigenic relationships with *Shigella* serovars were examined. O antigen identity was demonstrated between *E. coli* O149 and *Shigella boydii* 1 and between *E. coli* O152 and provisional *Shigella* serovar 3341-55. A reciprocal relationship was found between *E. coli* O159 and *Shigella dysenteriae* 4 but identity was not demonstrated. The remaining *E. coli* O antigens were not significantly related to any *Shigella* serovar.

There is extensive sharing of antigenic components throughout the *Enterobacteriaceae*, and numerous examples exist between *Shigella* and *Escherichia coli* serovars. Some *Shigella* O antigens are identical to *E. coli* O antigens whereas in other instances the antigens are closely related but not identical.

In 1950 Veazie (11) described O antigen relationships between *Shigella boydii* 4 and *E. coli* O53 and between *S. boydii* 5 and *E. coli* O79. Kauffmann (4) confirmed these findings and demonstrated that the O antigens were identical in each of these pairs. In 1953 Piechaud (10) showed the O antigen identity of *S. boydii* 11 with *E. coli* O105ab.

In 1972 Edwards and Ewing (1) reported that, with only two exceptions, the O antigens of all *Shigella* serovars were related to one or more of the O antigens of *E. coli* O groups 1 to 148. Furthermore the O antigens of 13 *Shigella* serovars were identical to one or other of these *E. coli* O antigens.

Since 1968, antigens O149 to O163 have been added to the antigenic scheme for *E. coli*. This study reports their antigenic relationships to established and provisional *Shigella* serovars.

Bacterial strains. *E. coli* O antigens 154, 155, 156, 158, 159, 160, and 161 were originally characterized at the Salmonella and Shigella Reference Laboratory (SSRL) and test strains from the laboratory collection were used. Test strains for O antigens 149, 150, 151, 152, 153, 157, 162, and 163 were obtained from the Collaborative Centre for Reference and Research on Escherichia (World Health Organization). The full serovar and a reference to the descriptive literature of each of the 15 strains is shown in Table 1.

Test strains for all accepted *Shigella* serovars (1) and provisional serovars 3873-50, 2000-53, 3341-55, 3615-53, 2710-54, 2044-54, and 1621-

54 (2) were present in the SSRL culture collection.

Preparation of antisera. O antigen suspensions were prepared from all the strains by harvesting the growth from two nutrient agar slopes into 0.9% saline and heating to 100 C for 2.5 h. After centrifugation, the heated organisms were resuspended in 15 ml of saline and commercial formalin was added to a final concentration of 0.3%. Rabbits were immunized by intravenous injections of 0.5, 1.0, 2.0, 2.0, and 2.0 ml of suspension at 5-day intervals; 40 ml of blood was taken 5 and 10 days after the final injection, and the rabbits were exsanguinated 5 days later. The products of all three bleedings were pooled.

O antigen suspensions for agglutination tests. O antigen suspensions for agglutination tests were prepared by heating overnight broth cultures at 100 C for 30 min. Commercial formalin was 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 *E. coli* O149-O163 were tested against the O antigen suspensions of all the *Shigella* strains, and O antigen suspensions of *E. coli* O149-O163 were tested against O antisera for all the *Shigella* strains.

Absorption studies. Where cross-reactions were found, reciprocal absorptions were performed to determine the relationships between the strains.

Serological cross-reactions were considered significant only if the cross-reaction titer was greater than or equal to one-sixteenth of the homologous titer. Significant cross-reactions were found between certain *Shigella* serovars and *E. coli* O149, O152, and O159 (Tables 2, 3 and 4), but the remaining *E. coli* strains

TABLE 1. Antigenic relationships between *Escherichia coli* O antigens 149-163 and *Shigella* serovars

Original reference no.	Original source	Reference to literature	Serovar	Relationship
CS1483	Enteric disease in piglets, Ireland	9	O149.K91.K88ac.H10	<i>S. boydii</i> 1 (identical) <i>S. dysenteriae</i> 2 (a,b-a,c) <i>S. boydii</i> 4 (one way)
1935	Diseased chickens, England	3	O150.K93.H6	None
880-67	Gastroenteritis in adults and children, Japan	5	O151.K?H50	None
1184-68	Dysentery-like disease in adults and children, Japan	5	O152.K?H-	Provisional serovar 3341-55 (identical)
14097	Infantile diarrhea, Germany	5	O153.K?H7	None
E1541/68	Human feces and food, Arabian Gulf	7	O154.K94.H4	None
E1529/68	Human feces and food, Arabian Gulf	7	O155.K?H9	None
E1585/68	Human feces and food, Arabian Gulf	7	O156.K?H47	None
A2	Enteric disease in piglets, Ireland	3	O157.K88ac.H19	None
E1020/72	Infantile diarrhea, England	8	O158.K?H20	None
E2476/72	Infantile diarrhea, Scotland	8	O159.K?H23	<i>S. dysenteriae</i> 4 (a,b-a,c)
E110/69	Human feces and food, Arabian Gulf	8	O160.K?H34	None
E223/69	Human feces, Arabian Gulf	8	O161.K?H54	None
10B1/1	Human feces, England	6	O162.K?H10	None
SN3B/1	Human feces, England	6	O163.K?H19	None

TABLE 2. Antigenic relationships between *Escherichia coli* O149 and *Shigella* serovars

Serum	Absorbed with	Agglutination titer of O antigen suspension			
		O149	<i>S. dysenteriae</i> 2	<i>S. boydii</i> 1	<i>S. boydii</i> 4
O149	Nil	25,600	200	12,800	3,200
	<i>S. dysenteriae</i> 2	25,600	<100	12,800	1,600
	<i>S. boydii</i> 1	<100	<100	<100	<100
	<i>S. boydii</i> 4	25,600	<100	6,400	<100
<i>S. dysenteriae</i> 2	Nil	1,600	25,600	6,400	<100
	O149	<100	25,600	<100	<100
<i>S. boydii</i> 1	Nil	1,600	<100	3,200	<100
	O149	<100	<100	<100	<100
<i>S. boydii</i> 4	Nil	<100	<100	<100	3,200
	O149	<100	<100	<100	25,600

TABLE 3. Antigenic relationships between *Escherichia coli* O152 and *Shigella* serovars

Serum	Absorbed with	Agglutination titer of O antigen suspension	
		O152	3341.55
O152	Nil	12,800	25,600
	3341-55	<100	<100
3341-55	Nil	12,800	25,600
	O152	<100	<100

showed no significant cross-reactions with any *Shigella* serovars.

Reciprocal absorptions showed that the O antigen of *E. coli* O149 was identical to that of *S. boydii* 1. The test strain of *E. coli* O149 also

TABLE 4. Antigenic relationships between *Escherichia coli* O159 and *Shigella* serovars

Serum	Absorbed with	Agglutination titer of O antigen suspension	
		O159	<i>S. dysenteriae</i> 4
O159	Nil	1,600	400
	<i>S. dysenteriae</i> 4	800	<100
<i>S. dysenteriae</i> 4	Nil	200	25,600
	O159	<100	25,600

showed a reciprocal relationship of the a,b-a,c variety with the test strain of *S. dysenteriae* 2. The test strain of *S. boydii* 4 was agglutinated

by *E. coli* O149 antiserum, but the reciprocal reaction was not found.

The O antigen of *E. coli* O152 was identical to that of provisional *Shigella* serovar 3341-55, and *E. coli* O159 showed a small reciprocal relationship of the a,b-a,c variety with *S. dysenteriae* 4.

The antigenic relationships found are summarized in Table 1.

The first stage in the identification of *Enterobacteriaceae* is the determination of genus or species by means of biochemical tests. Within these biochemically defined taxa, serological analysis provides more precise identification.

Biochemically atypical strains occur in several genera, and these strains can resemble members of other genera so closely that errors in identification may arise. There is no problem in differentiating biochemically typical *E. coli* from shigellas. However, if only a limited range of biochemical tests is used, nonmotile, anaerogenic, and lactose-negative strains of *E. coli* may be suspected of being shigellas. This is particularly likely to occur if the strain is strongly agglutinated by antiserum for *Shigella*. The problem can be resolved by the use of tests for lysine-decarboxylase, β -galactosidase, and Christensen citrate and by remembering that *Shigella* species are much less active against sugars than is *E. coli*.

The systematic study of biochemical characters is essential in the differentiation of genera, but it is useful to be aware of the intergeneric relations of antigens within the *Enterobacteriaceae*.

REPRINT REQUESTS

Address reprint requests to: Dr. B. Rowe, Salmonella

and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale Ave., London NW9 5HT, England.

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