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Edwardsiella tarda Serotyping Scheme for International Use

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A combination of two systems for the serotyping of *Edwardsiella tarda* developed independently at the National Institute of Health, Tokyo, Japan, and the Centers for Disease Control, Atlanta, Ga., has enabled a single serotyping scheme comprising 61 O groups and 45 H antigens to be established for international use.

Edwardsiella tarda, a group of organisms well defined by its biochemical characteristics, has been isolated not only from warm-blooded animals, including humans, but also from reptiles and amphibians. *E. tarda* has also been recognized as a pathogen of fish. Although it appears that *E. tarda* infrequently causes human diseases, a number of papers have reported the isolation of this organism from human cases of intestinal and extraintestinal infections, as reviewed by Sakazaki (3). In particular, papers from tropical countries have emphasized the etiological relationship of *E. tarda* to diarrheal disease. Van Damme and Vandepitte (4) reported that freshwater fish seem to be the most probable source of human infection in the tropics. Since most strains of *E. tarda* have uniform biochemical characters and require some vitamins and amino acids for their growth, there has been no subdividing system, such as biotyping, within the species. Serotyping is indispensable for epidemiological and ecological investigations.

Two serotyping systems have been developed for *E. tarda*. Sakazaki (2) recognized 17 O groups and 11 H antigens among 256 strains of the species; this antigenic scheme was extended later by Tamura and Sakazaki (unpublished data) to 42 O groups and 30 H antigens among 780 strains. Independently of Sakazaki, a provisional antigenic scheme with 48 O groups and 37 H antigens was described by McWhorter et al. (A. C. McWhorter, W. H. Ewing, and R. Sakazaki, Bacteriol. Proc., p. 89, 1967). For convenience in this paper, we refer to these two schemes as JNIH (National Institute of Health, Tokyo, Japan) and CDC (Centers for Disease Control, Atlanta, Ga.). However, a single typing system is clearly desirable for international use, and the present study was carried out to establish such a scheme for *E. tarda* by combining the two different schemes.

MATERIALS AND METHODS

Strains. A total of 109 strains comprising the 45 reference strains of the JNIH system and the 64 reference strains of the CDC system were used. All strains were maintained at room temperature in a semisolid medium containing 0.3% yeast extract, 1.0% Casitone (Difco Laboratories, Detroit, Mich.), 0.5% NaCl, and 0.3% agar, with the stoppers tight.

Preparation of antisera. O and H antisera were prepared as described by Sakazaki (3). For O antisera, selected smooth colonies were inoculated into 10 ml of brain heart infusion broth supplemented with 0.3% yeast extract and adjusted to pH 7.8. After overnight incubation at 35°C with continuous shaking, the broth culture was heated at 100°C for 2.5 h and then centrifuged, and the deposit was suspended in 20 ml of 0.9% saline. Rabbits were then immunized by intravenous injection of 0.5, 1.0, 2.0, and 3.0 ml at 4-day intervals, and blood was obtained 6 to 7 days after the final injection.

For H antisera, cultures in which motility was promoted by serial passage through semisolid medium were used. One loopful of culture from semisolid medium was inoculated into 10 ml of brain heart infusion broth containing 0.3% yeast extract (pH 6.6 to 6.8), which was then incubated overnight at 30°C and subsequently preserved by the addition of 0.3% Formalin. The immunization and bleeding schedule was the same as that for the preparation of O antisera.

Agglutination and agglutinin absorption. For O antigens,

TABLE 1. Relationship of O and H antigens between JNIH and CDC systems

O group		H antigen	
JNIH	CDC	JNIH	CDC
1	6490	1	30, 31
3	3888	2	10
4	1456	5	12
5	5765	6	15
6	4862	8	11
7	4830	9	9
9	1956	10	1
10	4167	11	27
13	3882	12	22
14	3854	13	19
15	3975	14	3
16	3592	15	5
17	6243	16	17
19	5343	17	2
21	5159	18	28
24	2451	19	6
25	2356	26	25
28	3143	27	4
31	3371	28	23
32	4582	29	18
33	1483	30	7
34	2012		
35	5497		
36	3889		
37	4789		
38	1958		
39	4226		
40	2521		
41	5377		
42	1540		

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TABLE 2. Reference strains for O and H antigens of *E. tarda* arranged according to O-group numbers

Antigenic formula[a]		Reference strain	Antigenic formula		Reference strain
O group	H antigen		O group	H antigen	
1	**1**	223-61 (JCM 7154)[b]	34	**34**	5091-63 (JCM 7191)
2	**2**	404-62 (JCM 7155)	**35**	1	223-60 (JCM 7192)
3	**3**	1058-60 (JCM 7156)	**36**	**19**	2010-67 (JCM 7193)
4	**4**	1068-60 (JCM 7157)	**37**	**15**	1001-65 (JCM 7194)
5	**5**	387-60 (JCM 7158)	**38**	**29**	1034-64 (JCM 7195)
6	**8**	389-63 (JCM 7159)	**39**	**17**	389-68 (JCM 7196)
7	**6**	408-68 (JCM 7160)	**40**	1	394-68 (JCM 7197)
7	**42**	4282-67 (JCM 7161)	**41**	**28**	5377-62 (JCM 7198)
8	3	1079-60(JCM 7162)	41	**35**	4462-64 (JCM 7199)
9	**9**	444-61 (JCM 7163)	**42**	13	1540-65 (JCM 7200)
10	**20**	86-61 (JCM 7164)	**43**	14	3979-60 (JCM 7201)
10	**33**	4167-62 (JCM 7165)	**44**	8	434-62 (JCM 7202)
11	**7**	471-62 (JCM 7166)	**45**	**32**	3610-62 (JCM 7203)
12	1	219-63 (JCM 7167)	**46**	**37**	3075-63 (JCM 7204)
13	1	72-73 (JCM 7168)	**47**	**38**	3339-64 (JCM 7205)
14	**21**	490-72 (JCM 7169)	**48**	10	3344-64 (JCM 7206)
15	**12**	1113-65 (JCM 7170)	**49**	19	3893-64 (JCM 7207)
16	**1**[c]	1004-65 (JCM 7171)	**50**	6	3152-65 (JCM 7208)
17	**11**	1058-70 (JCM 7172)	**51**	18	3852-65 (JCM 7209)
18	3	689-65 (JCM 7173)	**52**	1	3853-65 (JCM 7210)
19	**14**	1053-77 (JCM 7174)	**53**	**39**	4199-65 (JCM 7211)
20	**16**	1001-68 (JCM 7175)	**54**	17	4321-65 (JCM 7212)
21	**27**	24-73 (JCM 7176)	**55**	10	4646-65 (JCM 7213)
22	8	237-73 (JCM 7177)	**56**	10	5258-66 (JCM 7214)
23	**18**	489-72 (JCM 7178)	**57**	15	2528-66 (JCM 7215)
24	**13**	334-62 (JCM 7179)	**58**	11	4257-66 (JCM 7216)
25	1	486-72 (JCM 7180)	**59**	17	4751-66 (JCM 7217)
26	**24**	1049-70 (JCM 7181)	**60**	10	5159-63 (JCM 7218)
27	**22**	29-73 (JCM 7182)	**61**	11	2451-65 (JCM 7219)
28	8	40-71 (JCM 7183)	—[d]	**12**	3345-64 (JCM 7220)
29	1	1059-70 (JCM 7184)	—	**30**	217-61 (JCM 7221)
30	**25**	31-73 (JCM 7185)	—	**31**	1806-61 (JCM 7222)
31	**23**	395-68 (JCM 7186)	—	**36**	3343-64 (JCM 7223)
32	**27**	4582-60 (JCM 7187)	—	**40**	4899-66 (JCM 7224)
32	**44**	942-68 (JCM 7188)	—	**41**	542-67 (JCM 7225)
33	**10**	223-71 (JCM 7189)	—	**43**	3844-67 (JCM 7226)
34	**26**	465-72 (JCM 7190)	—	**45**	472-67 (JCM 7227)

[a] Numbers in boldface type are reference antigens.
[b] JCM, Japan Collection of Microorganisms.
[c] For the determination of H-1 antigen, a pooled serum containing equal amounts of two H antisera prepared from strains 223-71 and 1004-65 was used.
[d] —, R antigen.

TABLE 3. O-antigen relationships of *E. tarda*[a]

O antiserum	Homologous titer	Titer with other O groups (O group:titer)
1	1,600	2:100, **29:100**, 46:800
3	800	1:200, 57:100
4	1,600	12:200, 40:400
5	1,600	1:100, 11:100, 18:100, 21:200, 27:100, 29:200, 48:400, 58:200
8	1,600	9:100, 39:100
9	1,600	1:200, 7:200, 19:200, 48:200
10	1,600	4:400, 14:200
12	1,600	1:100, 4:100
14	1,600	**23:400**, 28:200, 59:100
15	1,600	8:100, 10:100
16	1,600	19:200, 41:100
17	1,600	**44:200**
18	1,600	19:100, 29:100
19	3,200	1:200, 21:100, 27:100, 29:100, 33:400
20	1,600	56:400, 59:200
21	1,600	58:100
23	800	**14:100**, 38:200
29	1,600	**1:100**
33	1,600	12:200, 44:200
34	1,600	21:100, 40:200
39	800	23:100
40	1,600	10:100
44	1,600	10:100, **17:200**, 45:200
52	1,600	28:100
53	800	45:200
54	1,600	52:200

[a] Minor reactions with titers <1:50 are not recorded. Boldface type indicates reciprocal relationships. No significant cross-reactions were recognized among antisera for O groups 2, 6, 7, 11, 13, 22, 24 to 28, 30 to 32, 35 to 38, 41 to 43, 45 to 51, and 55 to 61.

reciprocal agglutination tests were carried out in tubes with all of the O antisera against all of the O reference strains, using overnight cultures of brain heart infusion broth containing 0.3% yeast extract (pH 7.8) heated at 100°C for 1 h. Agglutinin absorption tests were performed with the reference strains of the various O groups in every instance in which antigenic relationships were indicated by cross-agglutination at a dilution of 1:50 or more. The absorptions were done with centrifuged packed cells from saline suspensions which had been heated at 100°C for 1 h. The packed cells were added to antiserum diluted 1:5, and the mixture was incubated at 50°C for 2 h.

Antigens used for H agglutination tests were prepared by culturing the strains in the same way as for the H antisera and preserved by adding an equal volume of saline containing 0.2% thimerosal. All H-antigen reference strains were titrated in tube tests against all H antisera. The tests were read after incubation for 3 h in a water bath at 50°C. If agglutination occurred at a dilution of 1:100 or more with heterologous antisera, agglutinin absorption tests were performed with centrifuged packed cells from saline suspensions of actively motile cultures grown on soft agar plates.

Details of the methods for identifing O and H antigens have been described by Sakazaki (3).

RESULTS AND DISCUSSION

By reciprocal agglutination and agglutinin absorption tests it was shown that 30 of the O groups and 22 of the H antigens of the two systems were either identical or very closely related (Table 1). In view of this, new numbers were assigned to the remaining 18 O groups and 23 H antigens of the CDC system which did not react with any antisera in the JNIH system to make a single serotyping scheme. As a result, the new scheme comprises 61 O groups and 45 H antigens; the reference strains for these are shown in Table 2. These strains were deposited in the Japan Collection of Microorganisms.

Although the reference strains of 34 of the 60 O groups were specific, some reciprocal and many unilateral relationships were observed among strains of the remaining 26 O groups. Similarly, 23 of the 45 H reference strains showed no significant reactions with other H antisera, but there were many relationships among the remaining 22 reference strains. These O and H interactions are shown in Tables 3 and 4. In general, the relationships among the H reference strains were more complex than those among the O reference strains. Because many H reference strains were agglutinated to a high titer by other H antisera, reciprocally absorbed antisera were required for the determination of H antigens.

H antigen 1 was assigned to a complex group in which the H antigens were closely related to each other. Fifteen strains which were agglutinated strongly by H1 antisera prepared from strains 223-71 and 1004-65 were selected at random and used to produce H antisera. By reciprocal agglutination and absorption tests the H1 antigen group was divided into five subgroups. The results obtained with representative strains of the five subgroups shown in Table 5 suggest that the H1 antigen consists of several antigenic components and that the H antigens of each subgroup share more than one component. In some instances the relationships among subgroups were minor when judged by agglutination, but the homologous titers were reduced markedly by absorption of the antisera with the heterologous strains. For example, the H antiserum of strain 49-61, which gave a homologous titer of 1:16,000, agglutinated the H antigen of strain 72-73 to only

TABLE 4. H-antigen relationships of *E. tarda*[a]

H antiserum	Homologous titer	Titer with other H antigens (H antigen:titer)
1	3,2000	3:100, **4:1,000**, **11:500**, 14:100
2	16,000	1:100, 5:500
3	16,000	2:100, **14:100**
4	16,000	**1:200**, **17:1,000**
5	16,000	7:200, 17:200, 19:100
6	8,000	5:100, 21:100
7	16,000	**5:2,000**
8	8,000	**13:200**, **19:200**, **20:500**
11	16,000	**1:500**, 30:1,000
12	16,000	20:500, 24:200, 36:500
13	32,000	**8:2,000**, **19:1,000**, 20:500
14	32,000	**3:2,000**, 9:200, 10:2,000, 13:200, 33:2,000, 38:2,000, 42:8,000
17	16,000	**4:1,000**
18	8,000	6:200, 17:100
19	16,000	**13:100**, **20:1,000**
20	32,000	**8:2,000**, **19:2,000**, 21:2,000
21	16,000	12:200, 16:200, 24:200
24	8,000	39:1,000
25	16,000	16:100, 21:1,000, 33:1,000
32	16,000	15:4,000
35	8,000	**37:2,000**
37	16,000	**35:2,000**

[a] Minor reactions with titers of <1:50 are not recorded. Boldface type indicates reciprocal reactions. No significant cross-reactions were recognized among antisera for H antigens 9, 10, 15, 16, 22, 23, 26 to 31, 33, 34, 36, and 38 to 45.

TABLE 5. Relationships among the H-1 antigen complexes

Antiserum	H antigen				
	223-71	72-63	1004-55	49-61	486-72
223-61					
Unabsorbed	32,000	8,000	8,000	16,000	8,000
Absorbed with:					
72-63	2,000	—[a]	1,000	—	1,000
1004-55	8,000	8,000	—	1,000	—
49-61	8,000	2,000	—	—	1,000
486-72	8,000	1,000	—	1,000	—
72-63 + 49-61 + 486-72	2,000	—	—	—	—
72-63					
Unabsorbed	16,000	16,000	4,000	2,000	4,000
Absorbed with:					
223-61	—	2,000	—	1,000	500
49-61	2,000	2,000	1,000	—	—
223-61 + 49-61	—	1,000	—	—	—
1004-55					
Unabsorbed	500	4,000	16,000	2,000	—
Absorbed with:					
223-61	—	—	8,000	1,000	1,000
72-63	—	—	4,000	4,000	—
49-61	—	—	1,000	—	—
223-61 + 72-63 + 49-61	—	—	1,000	—	—
49-61					
Unabsorbed	8,000	500	8,000	16,000	8,000
Absorbed with:					
223-61	—	500	2,000	4,000	—
72-63	—	—	2,000	500	1,000
1004-55	500	—	—	1,000	100
486-72	—	2,000	1,000	2,000	—
223-61 + 72-63 + 1004-55 + 486-72	—	—	—	500	—
486-72					
Unabsorbed	4,000	4,000	4,000	8,000	8,000
Absorbed with:					
72-63	—	—	—	2,000	2,000
49-61	1,000	1,000	—	—	2,000
72-63 + 49-61	—	—	—	—	1,000

[a] —, No agglutinination at a dilution of 1:50 or more.

1:500, but absorption of the 49-61 H antiserum by strain 72-63 reduced the homologous titer of the 49-61 H antiserum to 1:500. During the examination of additional strains with the same H subgroups as that of strain 72-73, some cultures were found to be agglutinated to a high titer by the H antiserum of strain 49-61. From these results it can be reasoned that the various H-antigen components in some strains are either more sensitive to agglutination or present in larger amounts than in other strains. In addition, although five subgroups were distinguished within the H1 antigen complex of 15 strains, many other strains also reacted to more than one of the five "subgroup-specific" antisera, thus suggesting that further subdivision of H-antigen complexes is possible. However, such subdivision of H-antigen complexes may not be practicable as it would complicate serotyping of the organisms. Because of this, the single symbol H-1 was given to this antigen complex. A pooled serum containing equal amounts of two antisera prepared from H reference strains 223-71 and 1004-65 is sufficient to identify the H-1 antigen complex.

Serotyping of *E. tarda* is no longer done at CDC, so request for this work should be directed to K. Tamura at NIH, Tokyo.

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