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Serological Comparison of Two Collections of *Vibrio cholerae* Non O1

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Two large, independently obtained collections of *Vibrio cholerae* non O1, containing 59 and 67 reference strains, respectively, were compared serologically in four laboratories. Twenty strains in each collection were considered identical, and an additional 16 strains in each collection were probably identical. Twenty-eight unique strains were identified, and inconclusive results were obtained with 25 strains. Nine strains were not considered *V. cholerae* in at least one testing laboratory. Of these, five showed insufficient correspondence to *V. cholerae* O1 by DNA hybridization to be considered *V. cholerae*.

Vibrio cholerae strains produce a single type of H antigen but a large variety of O antigens. Classification of *V. cholerae* on the basis of O antigen type, determined serologically, was begun by Gardner and Venkatraman (2) and has continued in several laboratories (4–8). Although there is agreement that serotype O1 should be used to designate the type of *V. cholerae* that causes epidemic and pandemic cholera, no common system for the designation of other serotypes has been developed, nor have the strains included in different collections been compared.

This report describes a collaborative serological comparison of two large, independently obtained collections of *V. cholerae* serotypes. Each collection includes strains from clinical and nonclinical sources which were isolated throughout the world, and each has been reported in detail elsewhere (4–8); typing sera derived from each collection have also been used in different regions of the world to classify *V. cholerae* isolates. It is hoped that his comparison will aid interpretation of studies based on these collections and will advance efforts to develop a uniform serotyping system for *V. cholerae*. Such a system would be of obvious value in describing and comparing the growing number of *V. cholerae* isolated from human, animal, and environmental sources.

MATERIALS AND METHODS

Strains. Two previously described collections of reference strains were studied. These included *V. cholerae* serotypes O2 to O60 (5, 6) provided by R. Sakazaki, National Institute of Health, Tokyo (NIHT), Japan, and 67 reference strains (7) from H. Smith, Jr., Jefferson Medical College, Philadelphia, Pa. All strains possessed the minimal characteristics for identification of *V. cholerae* as proposed by Hugh and Sakazaki (3), and all failed to agglutinate in antiserum to *V. cholerae* type O1. Freeze-dried strains were provided to each collaborating laboratory.

Antisera. Antisera for slide agglutination testing were prepared at three laboratories according to published techniques. Antisera preparation at NIHT and Tokyo Metropolitan Research Laboratory of Public Health involved hyperimmunization of rabbits with heat-killed bacteria as previously described (5). Sera prepared by NIHT were routinely absorbed with an R strain of *V. cholerae* O1 to remove anti-R antibodies; this absorption step was not used with sera prepared at Tokyo Metropolitan Research Laboratory. Antisera preparation at Jefferson Medical College involved the single inoculation of rabbits with live bacteria (7). In instances of cross-reactions, individual antisera in all sets were absorbed with live or killed bacteria of the cross-reacting serotype.

Agglutination testing. Slide agglutination testing was done with bacteria suspended in the test serum; at NIHT heat-killed bacteria were used, whereas live bacteria were used in all other laboratories. At NIHT the results of slide agglutination testing were confirmed by a tube agglutination test with heat-killed bacteria. Sera were supplied and tested at dilutions previously shown to be just sufficient to agglutinate the homologous strain promptly.

Agglutination studies performed at the four individual laboratories are summarized in Table 1. Each laboratory did not test each antiserum against each strain; however, every strain was studied in at least three laboratories, usually with two or more sets of antisera.

DNA relatedness to *V. cholerae*. For some strains, DNA relatedness was determined at the Centers for Disease Control by reassociating labeled DNA from *V.*

TABLE 1. Summary of antisera and strains tested at individual laboratories

Strains tested	Antisera used[a]			
	TML	NIHT	CDC	JMC
Sakazaki				
2–39	1, 2, 3	1, 2, 3	2, 3	2, 3
40–60	1, 3	1, 3		3
Smith				
67 types	1, 2, 3	1	2, 3	2, 3

[a] TML, Tokyo Metropolitan Research Laboratory of Public Health; NIHT, National Institute of Health, Tokyo; CDC, Centers for Disease Control; JMC, Jefferson Medical College; 1, antisera prepared at NIHT; 2, antisera prepared at TML; 3, antisera prepared at JMC.

cholerae 1845-73 with unlabeled DNA from each test strain and by using hydroxyapatite to separate related from unrelated DNA (1). *V. cholerae* 1845-73 is biochemically typical and agglutinates in Smith type 12 and Sakazaki type 5 antisera.

RESULTS

Agreement on identification of strains as *V. cholerae*. Some strains submitted for testing were not considered *V. cholerae* in one or more laboratories, based on additional tests done in those laboratories. These strains and the reasons given for questioning their identification are summarized in Table 2. All questioned strains were tested for DNA hybridization at the Centers for Disease Control; these results are also shown in Table 2. Strains showing greater than 70% relatedness with typical *V. cholerae* in 75°C DNA hybridization reactions were considered *V. cholerae*; all strains that showed less than 50% relatedness were considered not to be *V. cholerea*.

Corresponding serotypes. Twenty strains from each collection produced identical O antigens (Table 3). In each instance, antisera to a single strain in the Smith collection consistently agglutinated only a single strain in the Sakazaki collection, and antisera to that Sakazaki strain, made at either NIHT or Tokyo Metropolitan Research Laboratory, consistently agglutinated only the single strain in Smith's collection.

TABLE 3. Serotypes with identical counterparts in the opposite collection (corresponding serotypes)

Sakazaki strain no.	Smith strain no.	Sakazaki strain no.	Smith strain no.
3	62	35	15
6	14	40	11
7	13	41	106
12	33	48	60
14	31	49	69
17	57	51	40
18	25	52	21
28	75	53	59
30	110	54	308
34	42	57	32

Apparently corresponding serotypes. An additional 17 strains from the Sakazaki collection and 16 from that of Smith were considered to be probably identical based upon agglutination tests which showed nearly perfect agreement in the different testing laboratories (Table 4); these numbers are unequal because one strain in the Smith collection (no. 29) appeared to correspond with two in the Sakazaki collection (no. 13 and 29). Several patterns of minimal disagreement were observed, as follows.

In 11 instances, a single cross-reaction with an additional antiserum was observed in one or more laboratories. Thus, for example, Smith strain no. 20 was agglutinated in all laboratories by antisera to Sakazaki strain no. 4, but it was also agglutinated in one laboratory by antiserum to Sakazaki strain no. 10.

In five instances, the agreement was imperfect because strains failed to agglutinate in some

TABLE 2. Strains considered not *V. cholerae* in one or more laboratories[a]

Relation to *V. cholerae* 1845-73[b]	Smith strain no.	Corresponding Sakazaki strain no.	Basis of failure[c]		% Relatedness[d]
			NIH	CDC	
Highly related	16	None	1, 2		85
	201	None	2		83
	148	None	1, 2		77
	14	6	1, 2		74
Weakly related	50	None	1, 2	3	36
	160	13? or none	1, 2	3, 4	26
	113	20		3, 4	21
	107	26	1, 2	3, 4	19

[a] Questioned strains are underlined.
[b] By DNA hybridization.
[c] Not *V. cholerae*, on the basis of: 1, numerical analysis; 2, failure to agglutinate in *V. cholerae* H antiserum; 3, less than 40% relatedness to *V. cholerae* 1845-73 in DNA hybridization tests; 4, failure to ferment sucrose.
[d] Relatedness to *V. cholerae* 1845-73 at 75°C.

TABLE 4. Apparently corresponding serotypes

Deviation	Sakazaki strain no.	Smith strain no.	Cross-reaction (serum:strain)[a]	Failure(s) to agglutinate (serum:strain)[b]
Single cross-reaction	4	20	Sa 10:Sm 20	
	8	22	Sm 176:Sa 8	
	13	29	Sa 29:Sa 13	
	20	113	Sa 22:Sa 20	
	21	44	Sa 8:Sm 44	
	23	19	Sm 115:Sa 23	
	27	312	Sm 37:Sa 27	
	29	29	Sa 13:Sa 29	
	31	320	Sa 36:Sa 31	
	44	61	Sa 25:Sm 61	
	47	175	Sa 7:Sm 175	
One-way failure to agglutinate	10	27		Sa:Sm
	11	102		Sa:Sm
Two-way failure to agglutinate	26	107		Sa:Sm; Sm:Sa[c]
	37	23		Sa:Sm; Sa:Sa[d]
	5	12		Sa:Sm; Sm:Sa
Single cross-reaction and one-way failure to agglutinate	39	68	Sm 44:Sa 39	Sa:Sm

[a] Sa, Sakazaki; Sm, Smith. In this category, a single cross-reaction with an additional antiserum was seen in one or more laboratories. Thus, Smith strain no. 20 was agglutinated in all laboratories by antisera to Sakazaki strain no. 4, but it was also agglutinated in one laboratory by antiserum to Sakazaki strain no. 10.

[b] Antiserum to the indicated strain in one collection failed to agglutinate the indicated strain in the opposite collection. Thus, antiserum to Sakazaki strain no. 10 failed to agglutinate Smith strain no. 27 in some tests.

[c] Failures to agglutinate, as defined in footnote *b*, were observed in both directions.

[d] Sa:Sa indicates that antiserum to Sakazaki strain no. 37, made at Tokyo Metropolitan Research Laboratory, failed to agglutinate Sakazaki strain no. 37 in some tests, although antiserum to that strain made at NIHT gave satisfactory results.

antisera. In two cases, this failure showed a "one-way" pattern. Thus, antisera to Sakazaki strain no. 10 usually, but not always, agglutinated Smith strain no. 27, whereas antiserum to Smith strain no. 27 consistently agglutinated Sakazaki strain no. 10. In three other instances, a two-way pattern of failure to agglutinate was observed, i.e., failures with two antisera were involved.

In one instance, the imperfect agreement involved both a single cross-reaction and a one-way failure to agglutinate.

Serotypes unique to each collection. Fifteen strains from the Sakazaki collection and 13 strains from that of Smith were unique, i.e., a corresponding serotype was not found in the opposite collection (Table 5). In these instances, strains from one collection were not agglutinated by antisera to other strains in the same collection or to any strains in the opposite collection. The only exceptions allowed were those in which one or two antisera to other serotypes agglutinated the test strain, but only in one testing laboratory.

Strains requiring further study. With 25 strains (7 of Sakazaki, 18 of Smith), results did not show consistent correspondence or lack of correspondence (Table 6). In some instances, results suggested specific correspondence, but the patterns of disagreement were more complex than those in Table 4. Nevertheless, it seemed worthwhile to report these "possible" correspondence patterns.

DISCUSSION

All strains tested in this study met the minimal criteria of Hugh and Sakazaki (3) for classification as *V. cholerae*. Nevertheless, eight of the Smith strains and one of Sakazaki were not considered *V. cholerae* in one or more laboratories, based mostly on additional criteria used in individual laboratories. Of these, four Smith strains and one Sakazaki strain did not show sufficient correspondence to a representative strain of *V. cholerae* to be considered *V. cholerae* by DNA hybridization. It is possible that some of these strains may eventually be excluded from *V. cholerae* when uniform criteria for use of numerical taxonomy, DNA hybridization assays, or other tests are applied. However, because these tests were not used in all laboratories and were not included in the published minimal criteria for *V. cholerae*, it was decided to include these strains in this report.

Recognizing that this study involved four laboratories, antisera made by different methods, and different methods for performing the slide agglutination test, it appeared that a high level of agreement between laboratories was obtained. Of the 126 strains tested (59 of Sakazaki and 67 of Smith), 20 pairs were identical, an additional 16 pairs were probably identical, and 28 were unique to one or the other of the collections (15 of Sakazaki and 13 of Smith). Thus, only 25 of the 126 strains remained in question and require further study.

It is likely that some of the observed cross-reactions or failures of agglutination reflect different methods of serum preparation or performance of slide agglutination. For example, Sakazaki and Shimada have described weak cross-reactions with some antisera which were not absorbed with an R strain of *V. cholerae* O1 (4). The same authors have also reported that some live strains that fail to agglutinate in homologous antisera will agglutinate after being boiled, washed, and suspended in saline (4). It is also possible that some strains produce O antigens that are considerably interrelated. This may be true of Sakazaki strains no. 13 and 29.

TABLE 5. Serotypes unique to each collection

Collection	Strain no.	Cross-reacting antisera[a]
Sakazaki	15	Sa 25
	19	
	22	
	25	Sa 15, Sm 59
	32	
	33	
	38	
	42	
	43	
	45	Sm 79
	50	
	55	Sm 320
	56	
	58	
	59	
Smith	16	Sa 39
	18	
	38	
	43	
	50	
	77	
	94	Sm 38
	111	
	148	
	309	
	332	
	340	
	342	

[a] Cross-reactions were observed only in a single testing laboratory. Sa, Antiserum to indicated Sakazaki strain; Sm, antiserum to indicated Smith strain.

TABLE 6. Strains requiring further study

Strain	Possible corresponding strain[a]	
	Smith	Sakazaki
Sakazaki		
2	17	
9	343	
16	48, none	
24	74, none	
36	30	
46	76	
60	28, none	
Smith		
17		2
24		None
28		60
30		36, 33
37		27, none
45		2, 5, 10, 8, none
48		None
56		24, none
64		12, none
74		None
76		46
79		46
83		None
115		23
160		13, none
176		8, none
201		None
343		2, 9

[a] See text for definition of this category.

An interrelation of the O antigens of these strains has been noted previously (6) and may account for the apparent correspondence of Smith type 29 with each of these serotypes in the Sakazaki collection. An interrelation between the O antigens of Sakazaki serotypes 15 and 25 has also been noted previously and was observed in this study (6).

The results of this study should improve the ability to compare results of studies which have used serotyping systems based on these two collections. An even more valuable outcome would be the development of a single scheme for serotyping *V. cholerae*. It is hoped that this comparison will facilitate the development of such a system.

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