NOTES

Antigenic Scheme for the *Hafniae*

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The role of opportunistic pathogens in infectious pathological conditions of humans is increasingly being appreciated. Some organisms that have been considered nonpathogenic occasionally cause severe hospital infections and outbreaks of acute enteric diseases.

Although considered to be a commensal inhabitant of the intestinal tract (6), *Hafnia alvei* (syn. *Enterobacter hafniae*) has been reported to cause acute enteric diseases, urogenital inflammatory conditions, septic poisoning, and other diseases (1, 3, 4, 8, 9).

During the past several years, a study of biochemical and antigenic properties of 303 *H. alvei* strains was carried out in the Laboratory of Opportunistic Enteric Pathogens at the Mechnikov Research Institute for Vaccines and Sera, Moscow. One hundred and sixty-seven of the *Hafnia* strains used in this study were isolated from enteric patients or were obtained from other sources in the U.S.S.R.; other strains were kindly supplied by L. Le Minor (Paris, France), P. Carpenter (England), J. Kiese-walter (G.D.R.), and K. Lachowicz (Warsaw, Poland).

All of these strains had similar enzymatic properties and could be identified as members of *H. alvei* by their phenotypic characteristics. For the serological grouping of these strains by antigen type, 92 O antisera and 37 OH antisera were prepared from rabbits.

For O antisera, bacterial suspensions prepared in physiological saline and heated at 100°C for 2.5 h were used as antigens. The rabbits were immunized with four increasingly larger doses of the suspension containing 10^9 cells per ml as follows: 0.5, 1, 2, and 3 ml injected intravenously at 4-day intervals.

OH antisera were obtained by immunizing rabbits with 6-h-old broth cultures killed with 0.3% Formalin. To obtain H antigens, the strains were subcultured several times (up to 10 passages) in 0.3% agar medium at 25°C.

The serological grouping of the strains by O antigen was done in the usual way by agglutination and absorption tests (7). If the cross-absorption of agglutinins did not cause a decrease in the titer of homologous antibodies in the antisera, the strains were considered to belong to the corresponding O groups.

A total of 39 serological O groups was estab-

lished by the results of the agglutination and agglutinin absorption tests. In some strains, O antigens consisted of several partial factors. Thus, the existence of single and double intrageneric antigenic relationships, more or less pronounced, was established. The heterologous antibody titers varied between 1:100 and 1:800.

The grouping of the strains by H antigen was carried out as described for O antigens. From the results of the agglutination and agglutinin adsorption tests, 36 varieties of H antigen were identified. H-antigenic relationships were established, and these were found to be more pronounced than the O-antigenic relationships.

In accordance with the various combinations of H and O antigens discovered, the strains tested were found to belong to 57 different serovars. To our regret, the strains used by R. Sakazaki (7) and H. Matsumoto (5), in working out the provisional scheme for the differentiation of the *hafniae*, were not available and thus could not be directly compared with the strains studied here.

From the results obtained from this study, a provisional antigenic and diagnostic scheme for the differentiation of the *hafniae* was developed. It includes 39 serological O groups with 36 varieties of H antigens, which occur in various combinations. Thus, 57 serovars of the *hafniae* were recognized. For each specific H or O antigen, a corresponding reference strain has been determined. These strains are available from the Laboratory of Opportunistic Enteric Pathogens at the Mechnikov Research Institute for Vaccines and Sera, Moscow. The proposed antigenic scheme (Table 1) provides basic guidelines for the preparation of diagnostic agglutinating antisera, which can be used in the routine work of bacteriology laboratories. We have rather frequently isolated from patients serovars O4:H6,7, O4:H-, O6:H12,13, and O37:H-.

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TABLE 1. *Antigenic scheme for the hafniae*

<i>H. alvei</i> strain	O-group	O-antigen	H-antigen	<i>H. alvei</i> strain	O-group	O-antigen	H-antigen
<i>H. alvei</i>	1	1	1	121"K"	20	20	26,13
PC-32011	2	2	2	133"K"	21	21	
1-65	3	3a3b	3,5	174"K"	22	22	27,19
469/64		3a3c	4,5	176	23	23	28,7
12-66	4	4a4b	6,7	183	24	24	29,21
166		4a4c		184"K"	25	25	
206	5	5a5b	8,9	186	26	26	17,7
196g/s		5a5c	11	111"K"	27	27	30
21/7	6	6	12,13	118	28	28	31
203	7	7		178	29	29	28,7
755/64	8	8,16c	14,13	17-66	30	30	20,21
180/66	9	9	15	19f	31	31	20,21
2-66	10	10a10b	16	773/66	32	32	33
181"K"	11	11a11b	17,7	139-4	33	33	34
47/65	12	12a12b		20-2	34	34,14a	1,35
624		12a12c	18,19	WF-3	35	35a	18,19
388	13	13,10b	20,21	23-65		35a35b	
15/2	14	14a14b	22	76	36	36a36b	9,10
81/64	15	15,12b	15	240-5	37	37	
602	16	16a16b	23	528	38	38	
18-65		16a16c		120"K"	39	39	36
2965	17	17,36b	1,24				
262	18	18,11b	25,13				
8-66	19	19a19b	17,7				
177"K"		19a19c	16				

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