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Suggestion To Supplement *Shigella flexneri* Classification Scheme with the Subserovar *Shigella flexneri* 4c: Phenotypic Characteristics of Strains

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A new serovar of *Shigella flexneri* has been isolated with increasing frequency in the USSR. It has the antigenic formula IV:7,8. We suggest that it be incorporated into the classification scheme as *S. flexneri* subserovar 4c. The 212 strains of this proposed subserovar examined to date display biochemical and serological properties typical of the species, are sensitive to the genus-specific bacteriophage, and cause keratoconjunctivitis in the Sereny test.

Recently, changes in the classification scheme of *Shigella* spp., in particular *Shigella flexneri* (4), indicate that *S. flexneri* serovar 4 is subdivided into two subserovars, 4a and 4b, with the abbreviated formulae IV:3,4 and IV:6, respectively.

However, an uncommon subserovar of *S. flexneri* 4 having the antigenic formula IV:7,8 has been found in a number of republics of the USSR since the early 1980s (12, 15). Several strains of *S. flexneri* 4 with antigenic group complex 7,8 have been isolated in other countries (6, 7), but the occurrence of such a subserovar in the USSR had not been reported earlier. No cultures reacting with the antiserum against group-specific antigens 7,8 have been found among 323 strains of *S. flexneri* 4 isolated in different regions of the USSR (8). This information has been confirmed by the All-Union Shigellosis Center, where bacteria from 19 base regions have been examined during the period 1973 to 1979 (12).

Recently, *S. flexneri* 4 (IV:7,8) has been involved in moderately severe or severe dysentery in children and adults sporadically or in clustered epidemics, leading to a detailed investigation of the properties of this new dysentery agent. This report summarizes the investigation of the phenotypic characteristics of the *S. flexneri* 4 subserovar IV:7,8.

MATERIALS AND METHODS

Bacteria. The 212 strains of *S. flexneri* 4 (IV:7,8) examined included 210 isolates from patients and carriers, 1 strain from water, and 1 strain from a monkey. In addition, two strains of *S. flexneri* 4 (IV:7,8) obtained from England and Czechoslovakia, 66 strains of *S. flexneri* 4a, and four strains of *S. flexneri* 4b were included for comparison. Thus, a total of 284 strains of *S. flexneri* 4 were examined.

Biochemical and miscellaneous tests. The biochemical reactions of the strains were determined by standard methods (5, 11) (see Table 1). The sensitivity of the organisms to the genus-specific bacteriophage was assessed by a conventional technique (11). Colonial morphology was analyzed with slanting-light-beam microscopy by the method of Landy (10). Serological identification of all cultures was performed with adsorbed *Shigella* antiserum by slide agglutination. The invasiveness of 47 *S. flexneri* 4 (IV:7,8) isolates was evaluated in the keratoconjunctivitis (Sereny) test (14).

RESULTS

The growth of freshly isolated *S. flexneri* 4 (IV:7,8) cultures on strictly selective media (HE-agar from England [Oxoid], DDC-agar from the German Democratic Republic [Germed], Ploskirev agar from the USSR) was diminished (about twofold) as compared with that of other *S. flexneri* strains. This was not the case with differential media (Endo agar and eosin-methylene blue agar from the USSR).

Colony morphology examined by slanting-light-beam microscopy revealed that most of the strains were in the S form. The colonies had even edges, homogeneous surface structure, and bright bluish-pink coloring. The surfaces of colonies of some strains were slightly lined.

All of the cultures examined possessed the biochemical characteristics of the genus *Shigella* and of the species *S. flexneri* (Table 1). It was of interest that the cultures isolated in different regions of the USSR from patients, from a monkey, and from water and those obtained from abroad had identical enzymatic properties. Also, it is notable that the strains of the *S. flexneri* 4 subserovar IV:7,8 belonged to a single biovar, 6, in terms of the scheme used; the following characteristics were recognized: maltose positive, arabinose positive or strongly positive, sorbitol negative, rhamnose negative (11). In contrast, the cultures of the subserovars 4a and 4b belonged to several biovars (9, 13). The strains of *S. flexneri* 4 (IV:7,8) which we studied can be distinguished from the other subserovars on the basis of their inability to ferment rhamnose or sorbitol as well as their inability to produce indol.

Table 2 compares the serological reactivities of *S. flexneri* 4 subserovars. All 214 strains (212 strains from the USSR, 2 strains from England and Czechoslovakia) of *S. flexneri* 4 (IV:7,8) reacted only with antiserum to the type-specific antigen IV and group-specific antigens 7,8. Strains 4a and 4b reacted with their respective antisera; two of the four 4b strains tested were distinctly agglutinated by antiserum 3,4. Repeated serological examination of individual *S. flexneri* 4 (IV:7,8) colonies (5 to 10) removed from the nutrient plating

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TABLE 1. Biochemical reactions of 212 *S. flexneri* 4 (IV:7,8) strains isolated in the USSR

Test or substrate	Result[a]	% Positive	% Positive in ≥3 days	Test or substrate	Result[a]	% Positive	% Positive in ≥3 days
Hydrogen sulfide	−	0		Dulcitol	−	0	
Urease	−	0		Salicin	−	0	
Indole	−	0		Adonitol	−	0	
Methyl red (37°C)	+	100		Inositol	−	0	
Voges-Proskauer (37°C)	−	0		Sorbitol	−	0	
Simmons citrate	−	0		Arabinose	+ or (+)	80.6	19.6
Motility	−	0		Raffinose	(+) or +	40.5	59.5
Gelatin liquefaction (22°C)	−	0		Rhamnose	−	0	
Lysine decarboxylase	−	0		Malonate	−	0	
Arginine dihydrolase	−	0		Christensens citrate	−	0	
Ornithine decarboxylase	−	0		Sodium acetate	−	0	
Phenylalanine deaminase	−	0		Maltose	+	97.7	1.8
Acid from glucose	+	100		Xylose	−	0	
Gas from glucose	−	0		Glycerol	−	0	
Lactose	−	0		Esculin	−	0	
Sucrose	−	0		Bacteriophage	+	100	
Mannitol	+	100					

[a] +, ≥90% positive within 1 or 2 days of incubation; −, ≥90% negative within 30 days (carbohydrate), 14 days (sodium acetate), 7 days (Simmons citrate), or 4 days (Christensens citrate, decarboxylase, dihydrolase).

medium demonstrated no qualitative variations and a small number of quantitative variations (27 strains tested).

The 47 strains (from patients) tested for invasiveness caused purulent keratoconjuctivitis in the Sereny test with guinea pigs in 48 h.

DISCUSSION

Isolation of *S. flexneri* 4 (IV:7,8) strains has been reported earlier by Rumanian investigators (7). In addition, *S. flexneri* 4 strains that did not react with antiserum to *S. flexneri* 4a or 4b were found in Japan (1). Ewing and Carpenter (6) suggested that the number of *S. flexneri* subserovars not be expanded but that the antigenic formula of those strains which deviated in their antigenic structure be specified.

Since we report a considerable number of *S. flexneri* 4 (IV:7,8) strains, there is a need to expand the antigenic scheme of these bacteria. *S. flexneri* 4 (IV:7,8) accounted for as much as 17.2% of all *S. flexneri* cultures isolated in various regions of the USSR (3). Their unquestioned role as etiological agents of diarrhea indicates that they are not rarely encountered, exotic strains.

Detailed study of the phenotypic characteristics of the *S. flexneri* 4 subserovar IV:7,8 has revealed the identity of strains isolated in different years as well as in different republics of the USSR and abroad. The subserovar IV:7,8 was observed to differ biochemically from the 4a and 4b representatives. Therefore, we suggest that the *S. flexneri* 4 subserovar with the abbreviated formula IV:7,8 be incorporated into the classification scheme of *Shigella* spp. as a separate nomenclatural unit and that it be designated 4c. This suggestion was approved at the meeting of the USSR Committee on Taxonomy and Nomenclature of Pathogenic Bacteria, Protozoa, and Fungi, Moscow, 22 February 1984.

TABLE 2. Serological identification of *S. flexneri* 4 (IV:7,8)

Subserovar	Adsorption of antisera to *S. flexneri* 4 (IV:7,8)[a]				
	I, II, III, V, VI	IV	3,4	6	7,8
4a	−	+	+	−	−
4b	−	+	+, −	+	−
4 (IV:7,8)	−	+	−	−	+

[a] Symbols: +, +++ or ++++; −, no agglutination.

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