New Provisional Serovar of *Shigella boydii*

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Ten bacterial strains are described that give biochemical reactions characteristic of the genus *Shigella* but do not belong to any of the established or provisional serovars. The strains ferment mannitol and are antigenically indistinguishable. They do not possess any of the group antigens of *Shigella flexneri*, and it is therefore proposed that they be regarded as members of a new provisional serovar of *Shigella boydii*. Strain E16553 is designated as the test strain for the new serovar.

No new serovars have been added to the internationally accepted serotyping scheme for *Shigella* since 1958 (5). Nevertheless, certain provisional serovars have been described and remain sub judice (6). In the present paper, 10 strains are described that give the biochemical reactions of *Shigella* but do not belong to any of the recognized or provisional serovars. The establishment of a new provisional serovar, E16553, is proposed.

MATERIALS AND METHODS

The 10 strains studied are listed in Table 1. Nine strains were isolated from the feces of patients, six of whom were known to have diarrhea. The details of the remaining strain were not known.

Biochemical tests. The biochemical reactions of the strains were determined by using the methods of Cowan and Steel (2). The strains were also tested for mutative fermentation of lactose, sucrose, and salicin and for mutative utilization of Christensen's citrate, by the methods of Ewing et al. (6).

Serological tests. Using strain E16553 as a vaccine strain, an O antiserum was prepared in a rabbit by a method described previously (10). Agglutination tests were performed in plastic agglutination trays and were incubated at 50°C for 16 h. O-antigen suspensions for agglutination tests were prepared by heating overnight broth cultures at 100°C for 30 min, followed by the addition of commercial Formalin to a final concentration of 0.3%.

Antiserum for the vaccine strain was tested against O-antigen suspensions of all *Escherichia coli* O groups O1 to O169 and all the established and provisional *Shigella* serovars, including provisional *Shigella boydii* E10163 (4, 8). O-antigen suspensions of the 10 strains were tested for agglutination in O antisera for all *E. coli* O groups, all established and provisional *Shigella* serovars, and in the antiserum prepared by using E16553 as a vaccine. Where serological cross-reactions were found, reciprocal absorptions were performed to determine the extent of the antigenic relationship.

Tests for invasiveness. The 10 organisms were tested for invasive potential by means of the HEP-2 tissue culture test (3). Approximately 10^7 bacteria in suspension were added to HEP-2 cells grown as monolayers on glass cover slips. After incubation at 37°C for 2.5 h, the monolayers were washed and then incubated for a further 3 h in tissue culture growth medium containing gentamicin and lysozyme.

The monolayers were washed, fixed in 100% methanol, and stained with 10% Giemsa. The cover slips were examined microscopically, and 300 cells were counted for the presence of intracellular bacteria.

Tests for drug resistance. The strains were tested for resistance to the following antimicrobial drugs by using methods described previously: ampicillin, chloramphenicol, neomycin, streptomycin, sulfonamides, gentamicin, tetracyclines, furazolidone, nalidixic acid, trimethoprim, and cephaloridine (1).

RESULTS

All the strains were gram-negative rods and were nonmotile.

Biochemical tests. All strains were oxidase negative, fermentative in the Hugh and Liefson test, and reduced nitrate. Their other reactions (Table 2) were those of *S. boydii*. They did not mutatively ferment lactose, sucrose, or salicin, and they did not mutatively utilize Christensen's citrate.

Serological tests. Only those heterologous agglutinations that occurred at titers of $1/16$ or more of the homologous titer were considered to be significant.

Strain E16553 showed minor antigenic relationships with seven *E. coli* O antigens (Table 3), but there were no cross-reactions with any of the established or provisional serovars of *Shigella*. A specific antiserum for the O antigen of strain E16553 could easily be prepared. The 10 organisms in the study were shown by agglutination and by absorption studies to belong to the same

TABLE 1. Sources of strains

Country of isolation	Country visited	Age of patient (yr)
Britain	India	23
Britain	India	Not known
Britain	Not known	9
Finland	India	Adult
Britain	India	18
Iceland	Not known	25
Japan	Not known	Not known
Britain	Ghana	38
Britain	India	Adult
Sweden	Pakistan	3

O serogroup, to be referred to as provisional *S. boydii* E16553.

Test for invasiveness. Nine of the strains gave a positive result in the HEp-2 tissue culture test. The negative strain was that isolated in Iceland.

Tests for drug resistance. Four strains were sensitive to all the antimicrobial drugs tested. Four strains were resistant to streptomycin, sulfonamides, and tetracyclines. Two of these were isolated from patients returning to Britain from India and one from a patient returning from Ghana; one strain had been isolated in Iceland. One strain isolated in Britain was resistant to ampicillin, streptomycin, and sulfonamides. It was not known whether this patient had traveled abroad. The strain isolated in Sweden from a child returning from Pakistan was resistant to ampicillin, streptomycin, sulfonamides, and tetracycline.

DISCUSSION

The strains studied formed a biochemically and antigenically homogeneous group. They fermented mannitol and might therefore be classified as either *Shigella flexneri* or *S. boydii* on the basis of biochemical tests. However, since they did not possess any of the group antigens of *S. flexneri*, they are correctly classified as members of *Shigella* subgroup C, *S. boydii*. Furthermore, nine strains resembled other *Shigella* strains in that they were invasive in tissue culture tests and might therefore be expected to cause bacillary dysentery (9).

Infections due to *Shigella dysenteriae* and *S. boydii* are uncommon in the United Kingdom and are usually acquired abroad (7). The fact that six strains of E16553 isolated from travelers returning to the United Kingdom have been referred to this laboratory since September 1979 suggests that such strains may not be uncommon in some countries, particularly in India. The identification of *Shigella* strains is difficult because of their similarity to many biochemically atypical *E. coli* strains and because of the numerous antigenic relationships between *Shigella*

and *E. coli*. For this reason, clinical laboratories need to use biochemical testing and serotyping methods together for the accurate identification of *Shigella*. It is therefore important that new serovars of *Shigella* should be recognized and that their identity and prevalence should rapidly be assessed by a number of laboratories so that they can be incorporated into the internationally accepted serotyping scheme with a minimum of delay. The test strain, E16553, has been deposited in the National Collection of Type Cultures, Colindale, London, England, and has reference number NCTC 11462.

TABLE 2. Biochemical reactions of strains

Test	Reaction (days of incubation)
Catalase	+
Oxidase	-
Methyl red test	
37°C (2 days)	+
20°C (5 days)	+
Voges-Proskauer test	
37°C (2 days)	-
20°C (5 days)	-
Christensen citrate	- (7)
Simmons citrate	- (7)
Sodium acetate	- (7)
Malonate	- (1)
Mucate	-(14)
Growth in potassium cyanide	- (2)
Indole (Kovacs)	- (2)
H ₂ S (TSI ^a)	- (7)
Gluconate	- (2)
Christensen urea	- (7)
Phenylalanine deaminase	- (1)
Arginine decarboxylase	+ (1-4)
Lysine decarboxylase	- (4)
Ornithine decarboxylase	- (4)
B-Galactosidase (ONPG ^b)	- (4)
Glucose (acid)	+ (1)
Glucose (gas)	-(14)
Lactose	-(14)
Mannitol	+ (1)
Sucrose	-(14)
Salicin	-(14)
Dulcitol	-(14)
Inositol	-(14)
Adonitol	-(14)
Raffinose	-(14)
Sorbitol	+ (2-7)
Arabinose	+ (1)
Rhamnose	-(14)
Xylose	+ (3-14)
Trehalose	+ (1)
Inulin	-(14)
Glycerol	+ (2-7)
Cellobiose	-(14)
Sorbose	-(14)
Maltose	-(14)

^a TSI, Triple sugar iron agar.

^b ONPG, O-Nitrophenyl-β-D-galactopyranoside.

TABLE 3. Antigenic relationships of *S. boydii* E16553

Antiserum	Absorbing suspension	Titer versus antigen suspension						
		<i>S. boydii</i> E16553	<i>E. coli</i>					
			O6	O10	O18ac	O23	O38	O52
<i>S. boydii</i> E16553	None	1,600	— ^a	—	200	200	200	200
	O18ac	1,600	—	—	—	—	—	—
	O23	1,600	—	—	—	—	—	—
	O38	1,600	—	—	—	—	—	—
	O52	1,600	—	—	—	—	—	—
<i>E. coli</i> O6	None	200	1,600	—	—	400	—	—
	E16553	—	1,600	—	—	400	—	—
<i>E. coli</i> O10	None	200	—	800	—	—	—	—
	E16553	—	—	800	—	—	—	—
<i>E. coli</i> O18ac	None	200	—	—	3,200	1,600	—	—
	E16553	—	—	—	3,200	800	—	—

^a —, <100.

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