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Four New Provisional Serovars of *Shigella*

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Four bacterial strains are described that possess the biochemical characteristics of *Shigella* species but do not belong to any of the established *Shigella* serovars or to any previously described provisional serovar. One strain fermented mannitol, and it is proposed that this be the type strain for a new provisional serovar of *Shigella boydii*. The remaining strains did not ferment mannitol and belonged to three different serovars. These strains are proposed as type strains for three new provisional serovars of *Shigella dysenteriae*. All four strains were invasive in a HEp-2 cell tissue culture test, but only one was invasive in the guinea pig eye test and might therefore be expected to cause dysenterylike illness in humans. It is important that the designation of such strains remain provisional until other reference laboratories have had the opportunity to search for additional isolates and the possible pathogenicity of these strains for humans can be further assessed.

Bacterial strains that give the biochemical reactions of *Shigella* species but do not belong to any of the recognized serovars are occasionally described. Once ratified by the International Collaborating Center for Shigella (WHO), Centers for Disease Control, Atlanta, Ga., they may be considered provisional serovars and remain sub judice until reference laboratories have had the opportunity to assess their epidemiological importance. Recently, five of these provisional serovars have been incorporated into the serotyping scheme as *Shigella dysenteriae* serovars 11 and 12 and *Shigella boydii* serovars 16, 17, and 18 (2). In the study presented here, four *Shigella* strains are described and the establishment of four new provisional serovars is proposed.

MATERIALS AND METHODS

Bacterial strains. Three strains were isolated from the feces of patients with diarrhea. E670/74 was isolated from a patient in Britain who developed diarrhea while in Bangladesh, E22383 was from a Swedish patient who developed diarrhea while traveling in Saudi Arabia, and E23507 was from a Swedish patient who developed diarrhea while in India. Strain E28938 was isolated at a zoo in England from the feces of a mouflon (a species of wild southern European sheep).

Biochemical tests. The biochemical reactions of the strains were determined by the methods of Cowan and Steel (3). The strains were also tested for mutative fermentation of lactose, sucrose, and salicin and for mutative utilization of Christensen citrate (5).

Serological tests. Strains E670/74, E22383, E23507, and E28938 were used as antigens for the preparation of O antisera in rabbits by a method described previously (9). Agglutination tests 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%.

Antisera for the vaccine strains were tested against O-antigen suspensions of all *Escherichia coli* O groups O1 to O170 and all of the established and provisional *Shigella* serovars, including the new serovars *S. dysenteriae* 11 and 12, *S. boydii* 16, 17, and 18, and provisional *S. boydii* E16553 (6). O-antigen suspensions of the four strains were tested for agglutination in O antisera for all *E. coli* O groups and the established and provisional *Shigella* serovars and against the four new antisera. When serological cross-reactions were found, reciprocal absorptions were performed to determine the extent of the antigenic relationship.

Test for invasiveness. The four organisms were tested for invasive potential by means of the HEp-2 tissue culture test (4). 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. The four organisms were also tested for invasive potential in a guinea pig eye test based on the method of Serény (11) as modified by Scotland et al. (10).

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

RESULTS

Biochemical tests. All of the strains were nonmotile gram-negative rods. They were oxidase negative and fermentative in the Hugh and Leifson test and reduced nitrate. Other reactions (Table 1) were those of *Shigella* species. They did not mutatively ferment lactose, sucrose, or salicin, and they did not mutatively utilize Christensen citrate.

Serological tests. Only heterologous agglutinations that occurred at titers of 1/16 or more of the homologous titer were considered to be significant. Strain E670/74 gave significant cross-reactions with *E. coli* O87, O137, and O170 but not with any *Shigella* serovars. Absorption studies (Table 2) showed that strain E670/74 was antigenically identical to *E. coli* O170. Strain E22383 had minor antigenic relationships with five *E. coli* O antigens and with *S. boydii* 6 (Table 3). Strain E23507 was antigenically identical to a provisional *E.*

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TABLE 1. Biochemical reactions of provisional *Shigella* strains

Test (days of incubation)	Result for strain:			
	E670/74	E22383	E23507	E28938
Catalase	+	+	+	+
Oxidase	−	−	−	−
Methyl red				
37°C (2)	+	+	+	+
20°C (5)	+	+	+	+
Voges-Proskauer				
37°C (2)	−	−	−	−
20°C (5)	−	−	−	−
Christensen citrate (7)	−	−	−	−
Simmons citrate (7)	−	−	−	−
Sodium acetate (7)	−	−	−	−
Malonate (1)	−	−	−	−
Mucate (14)	−	−	−	−
Growth in potassium cyanide (2)	−	−	−	−
Indole (Kovacs) (2)	+	−	−	+
H_2S (TSI[a]) (7)	−	−	−	−
Gluconate (2)	−	−	−	−
Christensen urea (7)	−	−	−	−
Phenylalanine deaminase (1)	−	−	−	−
Arginine decarboxylase (4)	−	−	−	−
Lysine decarboxylase (4)	−	−	−	−
Ornithine decarboxylase (4)	−	−	−	−
β-Galactosidase (ONPG[b]) (2)	−	−	−	−
D-Adonitol (14)	−	−	−	−
L-Arabinose (7, 1, 2, 14)[c]	+	+	+	−
Cellobiose (14)	−	−	−	−
Dulcitol (14)	−	−	−	−
D-Glucose				
Acid (1)	+	+	+	+
Gas (14)	−	−	−	−
Glycerol (14, 2, 7, 4)	−	+	+	+
myo-Inositol (14)	−	−	−	−
Inulin (14)	−	−	−	−
Lactose (14)	−	−	−	−
Maltose (14, 14, 10, 1)	−	−	+	+
D-Mannitol (14, 14, 14, 1)	−	−	−	+
Raffinose (14)	−	−	−	−
L-Rhamnose (1, 14, 14, 14)	+	−	−	−
Salicin (14)	−	−	−	−
D-Sorbitol (14, 3, 3, 14)	−	+	+	−
L-Sorbose (14)	−	−	−	−
Sucrose (14)	−	−	−	−
Trehalose (1, 1, 1, 4)	+	+	+	+
D-Xylose (14, 14, 14, 1)	−	−	−	+

[a] TSI, Triple sugar iron agar.
[b] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[c] Respective days of incubation for the four strains as listed.

coli serovar, E11362 (Table 4). The provisional *E. coli* type strain E11362 produces heat-labile enterotoxin and was isolated in Australia from a patient with diarrhea (unpublished results). Strain E23507 had no other significant cross-reactions with *E. coli* or *Shigella* serovars. Strain E28938 had minor cross-reactions with *E. coli* O2 and O74 (Table 5).

Test for invasiveness. The four strains were all invasive in the HEp-2 tissue culture test, but only strain E670/74 was invasive in the guinea pig eye test. Guinea pigs challenged with the three remaining strains showed no signs of conjunctivitis after 3 days.

Test for drug resistance. Strains E670/74 and E28938 were susceptible to all antimicrobial drugs tested. Strain E22383 was resistant to sulfonamide, and strain E23507 was resistant to streptomycin and sulfonamide.

TABLE 2. Antigenic relationships of provisional *S. dysenteriae* E670/74

Antiserum	Absorbing suspension	Titer vs antigen suspension			
		S. dysenteriae E670/74	*E. coli*		
			O87	O137	O170
S. dysenteriae 670/74	Nil	3,200	—[a]	—	3,200
	O170	—	—	—	—
E. coli O87	Nil	800	3,200	—	—
	E670/74	—	3,200	—	—
E. coli O137	Nil	800	—	800	—
	E670/74	—	—	800	—
E. coli O170	Nil	800	200	200	800
	E670/74	—	—	—	—

[a] —, <100.

DISCUSSION

E. coli and *Shigella* species are closely related biochemically and serologically, and intermediate strains may cause difficulties in identification. It is therefore essential that strains under consideration as new *Shigella* serovars be subjected to rigorous biochemical testing, including tests for mutative fermentation of sugars. The strains included in this study could not be excluded from the genus *Shigella* even after such detailed biochemical testing.

Strain E28938 fermented mannitol and might therefore be classified biochemically as either *Shigella flexneri* or *S. boydii*. However, the strain did not possess the group antigens of *S. flexneri*. Furthermore, this strain fermented xylose, a reaction that is found more commonly among strains of *S. boydii* than in other *Shigella* strains. For these reasons, it is proposed that strain E28938 be classified as *S. boydii*. The source of this strain was unusual in that it was isolated from a mouflon at a zoo. The remaining three strains failed to ferment mannitol and had no serological cross-reactions with *S. flexneri*. It is therefore proposed that these strains (E670/74, E22383, and E23507) be classified as *S. dysenteriae*.

Two of the strains were antigenically identical to *E. coli*. This is not surprising, since it is known that many *Shigella* O antigens are identical to *E. coli* O antigens and that others are closely related (8). For this reason, clinical laboratories need to use both biochemical and serotyping tests for the accurate identification of *Shigella*.

The four strains resembled other shigellae in that they were invasive in tissue culture tests, but only strain E670/74 was invasive in the guinea pig eye test and might therefore be expected to cause bacillary dysentery (7). They are designated as test strains for the four new provisional serovars and have been deposited in the National Collection of Type Cultures, Colindale, England, so that reference laboratories in other countries may recognize such strains and their pathogenicity may be further assessed. Their reference numbers are NCTC 11311 (E670/74), NCTC 11867 (E22383), NCTC 11868 (E23507), and NCTC 11869 (E28938).

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TABLE 3. Antigenic relationships of provisional *S. dysenteriae* E22383

Antiserum	Absorbing suspension	Titer vs antigen suspension						
		S. dysenteriae E22383	*S. boydii* 6	*E. coli*				
				O4	O16	O17	O27	O52
S. dysenteriae E22383	Nil	1,600	200	200	—[a]	—	—	400
	S. boydii 6	1,600	—	—	—	—	—	—
	O4	1,600	—	—	—	—	—	—
	O52	1,600	—	—	—	—	—	—
E. coli O4	Nil	1,600	—	12,800	200	—	—	—
	E22383	—	—	12,800	200	—	—	—
E. coli O16	Nil	200	—	200	6,400	—	—	—
	E22383	—	—	—	6,400	—	—	—
E. coli O17	Nil	200	200	—	—	12,800	—	—
	E22383	—	—	—	—	12,800	—	—
E. coli O27	Nil	400	200	200	—	—	800	—
	E22383	—	—	—	—	—	800	—

[a] —, <100.

TABLE 4. Antigenic relationships of provisional *S. dysenteriae* E23507

Antiserum	Absorbing suspension	Titer vs antigen suspension		
		S. dysenteriae E23507	*Shigella* sp. strain 1621-54	*E. coli* E11362
S. dysenteriae E23507	Nil	25,600	—[a]	12,800
	E11362	—	—	—
Shigella sp. strain 1621-54	Nil	200	1,600	—
	E23507	—	1,600	—
E. coli E11362	Nil	12,800	—	12,800
	E23507	—	—	—

[a] —, <100.

TABLE 5. Antigenic relationships of provisional *S. boydii* E28938

Antiserum	Absorbing suspension	Titer vs antigen suspension		
		S. boydii E28938	*E. coli*	
			O2	O74
S. boydii E28938	Nil	3,200	800	800
	O2	3,200	—[a]	400
	O74	3,200	200	—
E. coli O2	Nil	400	1,600	800
	E28938	—	1,600	800
E. coli O74	Nil	400	1,600	1,600
	E28938	—	—	1,600

[a] —, <100.

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