Proposal to Recognise Seroovar 145/46 (Synonyms: 147, *Shigella* 13, *Shigella sofia*, and *Shigella manolovii*) as a New *Escherichia coli* O Group, O164

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A biochemically and serologically homogeneous group of bacterial strains is described. The strains were selected because of their biochemical and serological similarity to strain number 145/46, which was isolated from a patient with the symptoms of clinical dysentery in the British Isles in 1946. Similar strains, sometimes called "serotype 147," have been described as biochemically atypical members of *Escherichia coli* O group 25. Other studies have suggested, on the basis of biochemical tests and pathogenicity tests, that such strains should be classified as *Shigella* and the names *Shigella* 13, *S. sofia*, *S. manolovii*, and *S. metadysenteriae* have been proposed for them. Some workers have proposed that these strains should be regarded as intermediate between *Shigella* and *E. coli*. There is little doubt that these organisms have caused outbreaks of bloody diarrhoea resembling bacillary dysentery, but pathogenicity cannot be considered as a criterion for inclusion in the genus *Shigella*. The present study shows that the organisms can be classified biochemically as *E. coli* but they are atypical in being nonmotile, anaerogenic, and non-lactose-fermenting; serological studies show that these organisms can easily be distinguished from all currently recognised *E. coli* O groups (O1 to O163). It is proposed that these strains should be regarded as members of a new *E. coli* O group, O164.

Strain number 145/46 was isolated in 1946 from a patient with the symptoms of clinical dysentery in a prison in the British Isles. The patient had suffered previous attacks of dysentery in North Africa. The organism could not be classified readily because its biochemical reactions did not conform to those of a typical *Shigella* or to those of typical *Escherichia coli*.

Organisms resembling strain number 145/46 biochemically and serologically have been examined in several studies during the last 30 years and although their epidemiological importance is undoubted, their taxonomic position has remained in doubt (5-7, 10-14). In the present study, 100 isolates of serovar 145/46 were examined in detail and a definitive classification was proposed.

MATERIALS AND METHODS

Bacterial strains. The 100 strains examined were obtained from the culture collection of the *Salmonella* and *Shigella* Reference Laboratory. They had been submitted by laboratories in various parts of the world during the years 1946 to 1974. The source of the strains and relevant epidemiological information is described below.

Epidemiology. One of the 100 strains was isolated in the United Kingdom from the diarrhoeal stool of a gibbon imported from Thailand. The remaining 99

strains were isolated from human faeces; 64 strains were isolated in the United Kingdom, 23 were isolated in Bulgaria, 3 were isolated in Australia, 3 were isolated in Malta, 2 were isolated in Yugoslavia, and 1 each was isolated in Germany, Hong Kong, Hungary, and Kuwait. Two of the strains were isolated from infants less than 3 years old, 31 were isolated from children between the ages of 3 and 15, and 37 were isolated from people more than 15 years old. In 29 cases the age of the patient was not known. Sixty-seven patients had diarrhoea, and in many cases there was blood and mucus in the stool; 6 patients were free of symptoms, and in 26 cases no clinical information was obtained.

The strains studied included representatives from several outbreaks. (i) Outbreak 1: 11 adult patients in a psychiatric unit in England developed acute diarrhoea with blood and mucus in the stools and mild fever, the symptoms persisting for 24 to 48 h. An organism resembling strain number 145/46 biochemically and serologically was isolated from the faeces of 10 of the 11 patients. Seven of these isolates were included in the present study. (ii) Outbreak 2: three children in the same family in England suffered diarrhoea and vomiting. The family had not been abroad recently nor was there any connection with a psychiatric hospital. Strains from all three children were examined in the present study. (iii) Outbreak 3: two of the strains studied were representatives of an organism isolated from the inmates of a home for mothers and babies in Sydney, Aus-

tralia, during an outbreak of diarrhoea (8). The organism was isolated from 6 of 17 babies with diarrhoea and from 2 of 3 mothers with diarrhoea. Symptomless inmates were also examined and the organism was isolated from 3 of 10 babies and 4 of 5 mothers but was not isolated from 9 members of staff examined. (iv) Outbreak 4: 18 of the strains examined were from an outbreak of enteritis in a unit housing mentally retarded children in England. Diarrhoea occurred in patients in several wards and faecal specimens were examined from all the patients in the unit. All patients with serovar 145/46 in their faeces were accommodated in a separate ward and barrier nursing was instituted. As a result of these measures the outbreak was brought under control.

Biochemical tests. The biochemical reactions of the 100 strains were determined using the methods of Cowan and Steel (2).

Serological tests. Strain number 145/46 was selected as the vaccine strain and an O antiserum was prepared. The vaccine suspension was prepared by harvesting the growth from two nutrient agar slopes in 0.9% saline and heating at 100°C for 2.5 h. After centrifugation the heated organisms were suspended in 15 ml of saline, and commercial Formalin was added to a final concentration of 0.3%. A rabbit was immunised by intravenous injection of 0.5 ml, 1.0 ml, 2.0 ml, 2.0 ml, and 2.0 ml at 5-day intervals; 40 ml of blood was taken at 5 and 10 days after the final injection and the rabbit was exsanguinated 5 days later. The products of all three bleedings were pooled.

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; commercial Formalin was added to a final concentration of 0.3%.

Antiserum for strain number 145/46 was tested against O antigen suspensions of all *E. coli* O groups (O1 to O163) and all the established and provisional *Shigella* serovars (3). O antigen suspension of strain number 145/46 was tested for agglutination in O antisera for all *E. coli* O groups and all established and provisional *Shigella* serovars. Where serological cross-reactions were found, reciprocal absorptions were performed to determine the extent of the antigenic relationships.

The O antigen suspensions of the remaining 99 strains were tested for agglutination in antisera for all *E. coli* O groups and all established and provisional *Shigella* serovars as well as in antiserum for strain number 145/46.

RESULTS

Biochemical tests. All the 100 strains were gram-negative rods. They were oxidase negative, catalase positive, fermentative in the Hugh and Liefson test, and reduced nitrate. Their remaining reactions (Table 1) were those of an *E. coli* but differed from the usual pattern in that they were nonmotile, anaerogenic, and, with one exception, failed to ferment lactose.

Serological tests. Serological cross-reactions

TABLE 1. *Biochemical reactions of 100 isolates of serovar 145/46^a*

Test	No. positive	Sign
Motility (Craigie tube)	0	—
Methyl red test:		
37°C (2 days)	100	+
20°C (5 days)	100	+
Voges-Proskauer test:		
37°C (2 days)	0	—
20°C (5 days)	0	—
Christensen citrate	0 (100)	— (+)
Simmons citrate	0	—
Sodium acetate	9 (81)	— (+)
Malonate	0	—
Mucate	0	—
Growth in potassium cyanide	0	—
Indole (Kovac)	99	+
H ₂ S (T.S.I.)	0	—
Gluconate	0	—
Christensen urea	0	—
Phenylalanine deaminase	0	—
Gelatin (stab)	0	—
Arginine decarboxylase	0 (1)	—
Lysine decarboxylase	0	—
Ornithine decarboxylase	0	—
β -Galactosidase (ONPG)	1	—
Glucose (acid)	100	+
Glucose (gas)	0	—
Lactose	1	—
Mannitol	100	+
Sucrose	0	—
Salicin	1 (2)	—
Dulcitol	0	—
Inositol	0	—
Adonitol	0	—
Raffinose	0	—
Sorbitol	99 (1)	+
Arabinose	100	+
Rhamnose	81 (19)	d (+)
Xylose	86 (14)	d (+)
Trehalose	100	+
Inulin	0	—
Glycerol	0 (100)	— (+)
Cellobiose	0	—
Sorbose	0	—
Maltose	100	+

^a Figures and signs in parentheses indicate reactions delayed beyond 24 h of incubation. Symbols: +, 90% or more positive; (+), 90% or more positive after more than 24 h of incubation; d, between 10 and 90% positive; —, less than 10% positive.

were considered significant only if the cross-reaction titre was greater than or equal to one-sixteenth of the homologous titre. Several insignificant cross-reactions were also found, and this may indicate that the test strain is partly autoagglutinable.

Antiserum for strain number 145/46 gave significant cross-reactions with O antigen suspensions of *S. dysenteriae* 3 and with *E. coli* O12

TABLE 2. Relationship between serovar 145/46, *S. dysenteriae* 3, and *E. coli* O124

Serum	Absorbing suspension	Agglutination titre with antigen suspension no.		
		145/46	<i>S. dysenteriae</i> 3	<i>E. coli</i> O124
Serovar 145/46		25,600	6,400	1,600
	<i>S. dysenteriae</i> 3	12,800	<100	<100
	O124	25,600	<100	<100
<i>S. dysenteriae</i> 3		800	6,400	25,600
	O124	<100	<100	<100
	145/46	<100	1,600	1,600
<i>E. coli</i> O124		800	6,400	6,400
	<i>S. dysenteriae</i> 3	<100	<100	<100
	145/46	<100	1,600	1,600

TABLE 3. Relationship between serovar 145/46 and *E. coli* O25

Serum	Absorbing suspension	Agglutination titre with antigen suspension no.	
		145/46	O25
Serovar 145/46	O25	25,600	200
		12,800	<100
<i>E. coli</i> O25	145/46	400	6,400
		<100	6,400

and O124. O antigen suspension of strain number 145/46 gave significant cross-reactions with O antisera for *S. dysenteriae* 3 and *E. coli* O23, O25, O65, O82 and O124.

Absorption studies showed that the O antigen of strain number 145/46 was not identical to that of any of the cross-reacting strains and could be easily distinguished from them.

The O antigen relationships between strain number 145/46, *E. coli* O124, and *S. dysenteriae* 3 are of particular interest because of the problems of identification which they cause (Table 2). Strain number 145/46 has been described as a member of *E. coli* O group 25 (5); this relationship is shown in Table 3.

Strain number 145/46 was sent to I. Ørskov and F. Ørskov at the Collaborative Centre for Reference and Research on Escherichia (W.H.O.), Copenhagen, Denmark, where our serological findings were confirmed and the strain was designated as the test strain for a new *E. coli* O group O164.

The remaining 99 strains were confirmed by agglutination tests as members of the new O group.

DISCUSSION

In 1958 Ewing et al. (5) examined 10 serologically similar strains which they described as

"serotype 147." Among these were four strains from the Dysentery Reference Laboratory, Colindale, England, including strain number 145/46. These authors concluded on the basis of biochemical tests that the strains could be classified as *E. coli*, and because of a serological cross-reaction with strains of *E. coli* O25 they were further classified as members of that O group.

In 1959, Manolov (6) examined strains of serovar 145/46 and showed that they caused kerato-conjunctivitis in guinea pigs. It was suggested that this test should be the main taxonomic criterion for inclusion in the genus *Shigella* and on this basis he proposed that the strains be named *Shigella* 13 and included in a new subgroup of *Shigella*, subgroup E; Manolov and Trifanova (7) subsequently proposed the name *S. sofia* for these strains. Stenzel in 1962 (11) and 1964 (12) supported the suggestion that tests for kerato-conjunctivitis should be an important taxonomic criterion and proposed that strains of serovar 145/46, which he named *S. manolovii*, should be included in an enlarged subgroup D of *Shigella* and that this subgroup should be renamed *S. metadysenteriae*.

The proposals of Manolov and Stenzel were considered by the Enterobacteriaceae Subcommittee of the International Committee on Bacteriological Nomenclature in 1962 (1). The Subcommittee unanimously agreed that pathogenicity tests, such as the kerato-conjunctivitis test, should not be considered in deciding whether an organism should be classified in any particular genus. The taxonomic position of serovar 145/46 nevertheless remained in doubt. Szturm-Rubinsten and Piechaud in 1971 (13) drew attention to the importance of classifying such organisms and described them as intermediary between *Shigella* and *Alkalescens-Dispar*, a group of organisms now generally considered as anaerogenic *E. coli* (3). In 1974, Shmilovitz et al. (10) reported the epidemiological importance of serotype 147 as a cause of acute

dysentery in Israel and proposed that such strains should be included in a new group to be called Intermediate *Shigella Coli Alkalescens-Dispar* (ISCAD).

Although strains of serovar 145/46 share with *Shigella* the ability to cause kerato-conjunctivitis in guinea pigs and to cause bloody diarrhoea in man, these characteristics are also found in strains of several accepted *E. coli* O groups (9). The taxonomic position of these strains is defined primarily on the basis of their biochemical reactions without consideration of their pathogenic properties. In a study of more than 2,000 *Shigella* strains, Ewing (4) showed that members of this genus do not utilise citrate in Christensen medium. In the present study all the strains gave a positive reaction in this test. On this basis, these strains can be excluded from the genus *Shigella* and should be included in the species *E. coli*, many serovars of which include nonmotile, anaerogenic, and non-lactose-fermenting biotypes (9) which are biochemically similar to strains of serovar 145/46.

In 1975, Tekelieva (14) used immunochemical techniques to show that the lipopolysaccharides of *E. coli* O25 and serovar 145/46 belonged to different chemotypes whereas those of *S. dysenteriae* 3 and serovar 145/46 contained the same sugars. Our serological results are in accord with these findings. The O antigens of *S. dysenteriae* 3 and *E. coli* O124 are identical; the O antigen of serovar 145/46 is related to them but can easily be distinguished from them (Table 2). The relationship between serovar 145/46 and *E. coli* O25 is slight (Table 3).

According to currently accepted criteria, the strains examined in the present study can be classified as *E. coli*. It is proposed that they be regarded as a new *E. coli* O group, O164.

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REPRINT REQUESTS

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