A Numerical Taxonomic Study of the Genus *Shigella*

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One hundred and two strains representing the four species of the genus *Shigella* and sixty-four strains representing fourteen other genera of the family Enterobacteriaceae were tested for 192 characters based on their morphology, biochemistry and physiology. The data were subjected to a number of numerical analyses. The results (confirmed by the use of overlap statistics) showed that four phenons can be distinguished within the genus *Shigella*. These correspond with the species *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.

Of the other bacteria studied the Alkaescens-Dispar group was most closely related to the genus *Shigella* and the need for this group to be studied further is noted. The importance of using computer-based matrices for the routine identification of strains of *Shigella* and other enterobacteria is discussed.

Strains of the genus *Providencia* clustered separately from the shigellae. Three distinct species were evident within this genus: *P. stuartii*, *P. alcalifaciens* and a new *Providencia* species. The latter can be equated with the 'BG3' group in the DNA–DNA hybridization studies of Brenner *et al.* (1978).

INTRODUCTION

The present classification of the genus *Shigella* (Carpenter, 1974) is based on that first proposed by Ewing (1949) which was later amended and extended by the International Enterobacteriaceae Subcommittee (Report, 1951, 1953, 1954, 1958). Four species, sometimes referred to as subgroups, are recognized within the genus, *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup D) (Edwards & Ewing, 1972; Carpenter, 1974). The first three species are divided into a number of serotypes. Although each has qualitatively distinct antigens, serological cross reactions do occur between them and also with *Escherichia coli* strains (Edwards & Ewing, 1972; Carpenter, 1974).

The four species are distinguished biochemically mainly on the basis of the production of acid from mannitol and lactose and indole production (Carpenter, 1974). However, in practice the distinction is not clear cut and it is frequently difficult to identify new isolates with confidence. In their work on the construction of a computer-based identification matrix for Gram-negative, fermentative rod-shaped bacteria, Bascomb *et al.* (1973) found that they could not produce a system which would distinguish between reference strains of the species *S. flexneri*, *S. boydii* and *S. dysenteriae*. In addition, strains of *Shigella* species were frequently mis-identified as Alkaescens-Dispar strains, and strains of *Escherichia coli* and of the Alkaescens-Dispar group were mis-identified as shigellae.

Some recent numerical taxonomic studies of representatives of the family Enterobacteriaceae have raised questions about the current phenetic classification of the genus *Shigella*. Johnson *et al.* (1975) found that only strains of *S. sonnei* could be distinguished as a distinct taxon; the remaining shigellae clustered as a diffuse group which could not be equated with any particular species. In the study of Véron & Le Minor (1975), only strains of *S. dysenteriae* formed a distinct taxon. Strains of the other three species fell into two groups, neither of which could be deemed to represent a currently accepted species within the genus *Shigella*. While in a numerical taxono-

mic study of the Proteus-Providence group of bacteria (McKell & Jones, 1976) two strains labelled *S. dysenteriae* clustered in the *Providencia alcalifaciens* taxon.

On the basis of DNA relatedness, guanine plus cytosine content and genome size, Brenner *et al.* (1973) concluded that it was difficult to distinguish between *Shigella* species and *E. coli* but that the Proteus-Providence group was distinct from *Shigella* and *E. coli*.

Several problems are therefore apparent in the taxonomy of the genus *Shigella*. Can the four species of the genus *Shigella* be distinguished on the basis of their biochemical and cultural characteristics: if so, what characters distinguish each species? Can members of the genus *Shigella* be readily distinguished from strains of *E. coli* and the Alkalescens-Dispar group by biochemical and cultural characters? What is the nature, if any, of the apparent phenetic relationship between the genera *Shigella* and *Providencia*? This study was undertaken in an attempt to answer these questions.

METHODS

Strains. These are listed in Table 1. Most were obtained from culture collections but some fresh isolates were included. Type strains were used wherever possible. At the time this study was begun type strains were not designated for the species *S. flexneri*, *S. boydii* or *S. sonnei* (Carpenter, 1974). To determine the reproducibility of the test system 11 cultures were split and the duplicates treated as separate strains throughout the study. These duplicated cultures are suffixed A and B after the study number.

The names of the bacteria used here are those included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) which now determine the nomenclature of bacteria. Where strains bearing names not included in the List are referred to, they are put in quotation marks.

Strains were stored as broth cultures frozen in glycerol at -76°C on beads (Feltham *et al.*, 1978). Additionally, strains were subcultured at three weekly intervals on blood agar base plates and stored at 4°C .

Basal media. The bacteria were grown on Blood Agar Base (BAB; Oxoid) or in Nutrient Broth no. 2 (NB2; Oxoid) when a liquid culture was needed. These were the basal media used for many of the tests. When a less rich medium was required, e.g. when cultures were to be used for inoculating carbon source utilization tests, peptone water agar [1%, w/v Bacteriological peptone (Oxoid); 0.5%, w/v NaCl; 0.5%, w/v Oxoid Agar no. 3] was used. In tests where single colonies were required, the swarming of *Proteus* strains was prevented by the addition of 0.18% (w/v) sterile chloral hydrate which was added to the sterile molten medium before dispensing. Normally, all tests were inoculated from overnight BAB cultures. Unless otherwise stated, incubation was at 37°C overnight (18 to 24 h) except for strain D1514 which required 48 h to produce significant growth.

Colonial morphology. Twenty-four hour cultures on plates of BAB and nutrient agar (NA, Difco) were examined for colonial morphology by eye and under low power magnification ($\times 10$) on a binocular microscope using transmitted and reflected light. The growth of the strains on BAB and NA was compared and the BAB plates were examined for swarming (37°C). The ability to swarm was also tested on BAB at 25°C .

Cell morphology. Smears of strains grown on BAB and NA were made in saline on slides and Gram stained by the method of Baker (1967). Acetone was used as decolouriser. The slides were examined at $\times 1000$ magnification.

Motility. This was examined at 25 and 37°C . NB2 solidified with 0.25% (w/v) agar (Oxoid No. 3) was dispensed in 15 ml amounts into universal bottles containing Craigie tubes (Craigie, 1931). After inoculation of the Craigie tube the bottles were incubated for 3 d and examined daily for growth in the outer tube. All strains giving a positive result were re-examined for motility by the hanging drop method using 6 and 24 h NB2 cultures of the organism. Only those confirmed as motile by this method were scored as positive.

Growth characteristics in broth. Tubes of NB2, preincubated at 37°C , were lightly inoculated and incubated overnight. The broths were examined for the presence of a ring or pellicle, the presence of a deposit and degree of turbidity.

Growth at different temperatures. Growth was examined at 4, 10 and 45°C . Bacteria were lightly inoculated into tubes of NB2, preincubated at the appropriate temperature. The cultures were examined for growth after overnight incubation at 45°C , and daily for 21 d for growth at 4 and 10°C .

Initiation of growth at different pH values. Growth was tested at pH 4, 6, 8 and 10. BAB was adjusted to the required pH before autoclaving and then dispensed into repli dishes (Sterilin). The media were stab inoculated by the multi-inoculation technique (Stevens, 1969) and incubated for 7 d. The plates were examined for growth at 1, 2, 3 and 7 d. Day 2 results were used for coding the data.

Initiation of growth at different NaCl concentrations. Sodium chloride was added to NA to give added concentrations of 0%, 0.5%, 5.0%, and 10% (w/v) NaCl. The tests were carried out as for the pH tests. Day 3 results were used for coding the data.

Growth on desoxycholate citrate agar. Desoxycholate citrate agar (Oxoid) was prepared as instructed by the manufacturer. The medium was dispensed into repli dishes and stab inoculated. Plates were incubated and examined daily for 3 d for the presence of growth and the production of black colonies.

Table 1. *Strains used in study*

Species	Study no.	Source and original designation*	Additional information
<i>Shigella</i>			
<i>S. boydii</i> serotype 1	E12	PHLL 32395/75	
<i>S. boydii</i> serotype 1	E16	NCTC 9327	
<i>S. boydii</i> serotype 2	E18	NCTC 9328	
<i>S. boydii</i> serotype 3	E13	PHLL 37746/75	
<i>S. boydii</i> serotype 3	E20	NCTC 9329	
<i>S. boydii</i> serotype 4	E22	NCTC 9770	
<i>S. boydii</i> serotype 5	E23	NCTC 9733	
<i>S. boydii</i> serotype 6	E24	NCTC 9771	
<i>S. boydii</i> serotype 7	E25	NCTC 9333	
<i>S. boydii</i> serotype 8	E27	NCTC 9353	
<i>S. boydii</i> serotype 9	E28	NCTC 9356	
<i>S. boydii</i> serotype 10	E29	NCTC 9358	
<i>S. boydii</i> serotype 10	E30	NCTC 9784	Mannitol negative
<i>S. boydii</i> serotype 11	E31	NCTC 9360	
<i>S. boydii</i> serotype 12	E32	NCTC 9772	
<i>S. boydii</i> serotype 13	E33	NCTC 9361	
<i>S. boydii</i> serotype 14	E34	NCTC 9766	
<i>S. boydii</i> serotype 15	E35A/B	NCTC 8590	Mannitol negative
<i>S. boydii</i> serotype 15	E36	NCTC 9365	
<i>S. dysenteriae</i> serotype 1	E37	NCTC 4837	Type strain
<i>S. dysenteriae</i> serotype 1	E38	NCTC 6781	
<i>S. dysenteriae</i> serotype 1	E39	Sakazaki 4379-60	(Johnson <i>et al.</i> 1975)
<i>S. dysenteriae</i> serotype 1	E40	NCTC 8571	Rough strain
<i>S. dysenteriae</i> serotype 2	E14	PHLL 36595/75	
<i>S. dysenteriae</i> serotype 2	E41	NCTC 2966	
<i>S. dysenteriae</i> serotype 2	E42	NCTC 8218	
<i>S. dysenteriae</i> serotype 2	E43	NCTC 9718	
<i>S. dysenteriae</i> serotype 2	E44	NCTC 9952	
<i>S. dysenteriae</i> serotype 3	E45	NCTC 9720	
<i>S. dysenteriae</i> serotype 3	E46	NCTC 9953	
<i>S. dysenteriae</i> serotype 4	E47	NCTC 9760	
<i>S. dysenteriae</i> serotype 4	E48	NCTC 9954	
<i>S. dysenteriae</i> serotype 5	E49	NCTC 9761	
<i>S. dysenteriae</i> serotype 5	E50	NCTC 9955	
<i>S. dysenteriae</i> serotype 6	E51A/B/BX	NCTC 9762	
<i>S. dysenteriae</i> serotype 6	E52	NCTC 9956	
<i>S. dysenteriae</i> serotype 7	E53	NCTC 9763	
<i>S. dysenteriae</i> serotype 7	E54	NCTC 9957	
<i>S. dysenteriae</i> serotype 8	E55	NCTC 8599	
<i>S. dysenteriae</i> serotype 8	E56	NCTC 9346	
<i>S. dysenteriae</i> serotype 8	E109	Strain 17	(Véron & Le Minor, 1975)
<i>S. dysenteriae</i> serotype 9	E57	NCTC 9348	
<i>S. dysenteriae</i> serotype 10	E58	NCTC 9351	
<i>S. dysenteriae</i> serotype 3873-50	E59	NCTC 9349	Provisional serotype
<i>S. dysenteriae</i> serotype 2000-53	E60	NCTC 9352	Provisional serotype
<i>S. dysenteriae</i>	E61	ATCC 11456a	Indicator strain for lysogenic Sd 11456b
<i>S. dysenteriae</i>	E62	ATCC 11456b	Lysogenic; carries Phage P2
<i>S. dysenteriae</i>	E63	ATCC 23351	Phage host
<i>S. flexneri</i> serotype 1a	E64	NCTC 3	
<i>S. flexneri</i> serotype 1a	E65	NCTC 8192	
<i>S. flexneri</i> serotype 1a	E66A/B	NCTC 9950	
<i>S. flexneri</i> serotype 1b	E9A/B	PHLL 33272/75	
<i>S. flexneri</i> serotype 1b	E67	NCTC 5	
<i>S. flexneri</i> serotype 2a	E10	PHLL 23364/75	
<i>S. flexneri</i> serotype 2a	E69	NCTC 1	
<i>S. flexneri</i> serotype 2a	E70	NCTC 8519	
<i>S. flexneri</i> serotype 2a	E72	ATCC 25875	
<i>S. flexneri</i> serotype 2b	E74	NCTC 4	

Table 1.—(continued)

Species	Study no.	Source and original designation*	Additional information
<i>S. flexneri</i> serotype 2b	E75	NCTC 8518	
<i>S. flexneri</i> serotype 3	E76	ATCC 11836	
<i>S. flexneri</i> serotype 3a	E77	NCTC 9724	
<i>S. flexneri</i> serotype 3a	E78	NCTC 9989	
<i>S. flexneri</i> serotype 4	E79	ATCC 9748	
<i>S. flexneri</i> serotype 4a	E80	NCTC 8296	
<i>S. flexneri</i> serotype 4a	E81	NCTC 7885	Mannitol negative
<i>S. flexneri</i> serotype 4b	E83	NCTC 8336	
<i>S. flexneri</i> serotype 4b	E84	NCTC 9726	
<i>S. flexneri</i> serotype 5	E85A/B	ATCC 9204	
<i>S. flexneri</i> serotype 5a	E11	PHLL	
<i>S. flexneri</i> serotype 5a	E86	NCTC 8523	
<i>S. flexneri</i> serotype 5b	E87	NCTC 9727	
<i>S. flexneri</i> serotype 6	E88	NCTC 9729	
<i>S. flexneri</i> serotype 6	E89	NCTC 4720	Manchester variety
<i>S. flexneri</i> serotype 6	E90	NCTC 9780	Newcastle variety; mannitol negative
<i>S. flexneri</i> serotype 6	E91	NCTC 9781	Newcastle variety; mannitol negative; anaerogenic
<i>S. flexneri</i> serotype 6	E92	NCTC 8525	Boyd 88 variety; dulcitol positive; anaerogenic
<i>S. flexneri</i> serotype 6	E93	NCTC 8526	Boyd 88 variety; dulcitol negative; anaerogenic
<i>S. flexneri</i> serotype X	E94	NCTC 9769	
<i>S. flexneri</i> serotype Y	E95A/B	NCTC 6	Serologically <i>S. flexneri</i> 2
<i>S. flexneri</i> serotype Y	E96	NCTC 9730	
<i>S. flexneri</i>	E97	ATCC 12661	Phage host
<i>S. flexneri</i>	E110	PHLL (Adelaide)	
<i>S. sonnei</i>	E1	PHLL 8471/75	
<i>S. sonnei</i>	E2	PHLL 15805/75	
<i>S. sonnei</i>	E3	PHLL 4406/75	
<i>S. sonnei</i>	E4	PHLL 7622/75	
<i>S. sonnei</i>	E5	PHLL 41703/75	
<i>S. sonnei</i>	E6	PHLL 42312/75	
<i>S. sonnei</i>	E7	PHLL 42314/75	
<i>S. sonnei</i>	E8	PHLL 44783/75	
<i>S. sonnei</i>	E98	NCTC 9774	
<i>S. sonnei</i>	E99	NCTC 8220	
<i>S. sonnei</i>	E100	NCTC 8574	Standard sulphonamide-sensitive strain
<i>S. sonnei</i>	E101A/B	NCTC 8575	Sulphonamide-resistant strain
<i>S. sonnei</i>	E102	NCTC 8586	Mannitol negative
<i>S. sonnei</i>	E103	NCTC 9992	Xylose positive
<i>S. sonnei</i>	E104	NCTC 10352	Salicin positive
<i>Shigella</i> sp.	E105	NCTC 2670	
<i>Shigella</i> sp.	E106	NCTC 8589	Indole positive: serotype CDC 147
<i>Shigella</i> sp.	E107	NCTC 10351	Indole negative: serotype CDC 147
<i>Shigella</i> sp.	E108	NCTC 10361	Serotype CDC 147
<i>Shigella</i> sp.	E111	PHLL	
<i>Citrobacter</i>			
<i>C. freundii</i>	K9	NCTC 9750	Type strain
<i>C. koseri</i>	E122	NCTC 10786	
<i>Edwardsiella</i>			
<i>E. tarda</i>	K88	NCTC 10396	
<i>Enterobacter</i>			
<i>E. aerogenes</i>	K93	NCTC 10006	Type strain
<i>E. cloacae</i>	E132	'Bact-chek' strain	ATCC 13047 derivative
<i>E. cloacae</i>	K14	NCTC 10005	Type strain
' <i>E. liquefaciens</i> '	K16	NCTC 10422	
' <i>E. liquefaciens</i> '	K17	NCTC 10423	

Table 1.—(continued)

Species	Study no.	Source and original designation*	Additional information
<i>Erwinia</i>			
<i>E. carotovora</i>	D1253	NCPPB 312	
<i>Escherichia</i>			
<i>E. coli</i>	K1	NCTC 9001	Type strain
<i>E. coli</i>	P83A/B	NCTC 86	
<i>E. coli</i>	P103	S 149	(McKell, 1977)
<i>E. coli</i>	P104	S 274	(McKell, 1977)
<i>E. coli</i>	E131	NCTC 9704	O type 112
' <i>E. alkalescens</i> '	E15 + K11	NCTC 1601	
' <i>E. alkalescens</i> ' serotype 1	E127	NCTC 7925	
' <i>E. alkalescens</i> ' serotype 2	E128	NCTC 7926	
' <i>E. alkalescens</i> ' serotype 3	E129	NCTC 7927	
' <i>E. alkalescens</i> ' serotype 4	E130	NCTC 7928	
' <i>E. dispar</i> ' type 1	E123	NCTC 7921	
' <i>E. dispar</i> ' type 2	E124	NCTC 7922	
' <i>E. dispar</i> ' type 3	E125	NCTC 7923	
' <i>E. dispar</i> ' type 4	E126	NCTC 7924	
<i>Hafnia</i>			
<i>H. alvei</i>	K63	NCTC 8105	
<i>Klebsiella</i>			
' <i>K. aerogenes</i> '	K38	NCTC 8172	
' <i>K. edwardsii</i> '	K35	NCTC 5054	
<i>K. ozaenae</i>	K37A/B	NCTC 5050	Type strain
<i>K. pneumoniae</i>	K32	NCTC 9633	Type strain
<i>K. rhinoscleromatis</i>	K34	NCTC 5046	Type strain
<i>Plesiomonas</i>			
<i>P. shigelloides</i>	E113	NCTC 10363	
<i>Proteus</i>			
<i>P. mirabilis</i>	P2	NCTC 3177	
<i>P. mirabilis</i>	P5	NCTC 60	
<i>P. morgani</i>	P11	NCTC 235	
<i>P. rettgeri</i>	P18	NCTC 7475	
<i>P. rettgeri</i>	P28	NCTC 10377	Lactose positive
<i>P. vulgaris</i>	P6	NCTC 10376	
<i>P. vulgaris</i>	P7	NCTC 4175	Type strain
<i>Providencia</i>			
<i>P. alcalifaciens</i>	P77	NCTC 10286	Type strain
<i>P. alcalifaciens</i>	E119	ATCC 25828	
<i>P. stuartii</i>	P78	NCTC 10318	
<i>P. stuartii</i>	E120	ATCC 25825	
<i>P. stuartii</i>	E121A/B	ATCC 25826	
<i>Providencia</i> sp.	E114	NCTC 8055	
<i>Providencia</i> sp.	E115	NCTC 8057	
<i>Providencia</i> sp.	E116	NCTC 6932	
<i>Providencia</i> sp.	E117	NCTC 6934	
<i>Providencia</i> sp.	E118	NCTC 6344	
<i>Providencia</i> sp.	P22	NCTC 8056	
<i>Providencia</i> sp.	P23	NCTC 6345	
<i>Providencia</i> sp.	P24	NCTC 8113	
<i>Providencia</i> sp.	P32	NCTC 2481	
<i>Providencia</i> sp.	P75	NCTC 6933	
<i>Providencia</i> sp.	P76	NCTC 10378	
<i>Salmonella</i>			
<i>S. choleraesuis</i>	K49	NCTC 5735	Type strain
' <i>S. paratyphi</i> '	K43	NCTC 8002	
<i>S. typhimurium</i>	K46	NCTC 74	Type strain
<i>Serratia</i>			
<i>S. marcescens</i>	K18A/B	NCTC 1377	
<i>S. marcescens</i>	K19	NCTC 10211	Type strain

Table 1.—(continued)

Species	Study no.	Source and original designation*	Additional information
<i>Vibrio</i>			
<i>V. cholerae</i>	D1865	PHLL	Biotype El Tor; Ogawa serotype
<i>Yersinia</i>			
<i>Y. enterocolitica</i>	D1655	Mollaret 88	
<i>Y. pestis</i>	D1514	NCTC 5923	Avirulent working type
<i>Y. pseudotuberculosis</i>	D1516	NCTC 8315	
'Enterobacterium Sachs A12'	E112	S 139	(Howie & Old, 1976)

* NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection; PHLL, Public Health Laboratory, Leicester; NCPPB, National Collection of Plant Pathogenic Bacteria. All D, P and K strains were obtained from culture collections in the Department of Microbiology, University of Leicester.

Growth on selenite agar. Selenite broth base (19 g; Oxoid) was suspended in distilled water (800 ml) in which sodium biselenite (4 g; Oxoid) was dissolved. The solution was sterilized in a boiling water bath for 10 min, then molten agar [200 ml of 5% (w/v) solution, Oxoid agar No. 3] was added. Repli dishes containing the medium were stab inoculated by the multi-inoculation technique, incubated and examined daily for 3 d.

Growth on potassium cyanide agar. The medium of Cowan (1974*b*) was used except that agar (1%, w/v Oxoid No. 3) was added to the medium before sterilizing. The KCN agar was dispensed into repli dishes, stab inoculated, then incubated and examined daily for 3 d.

Amino acid decarboxylases. The presence of decarboxylases for four amino acids was tested: L-arginine, L-glutamine, L-lysine and L-ornithine. Method 2 of Cowan (1974*b*) was used with Decarboxylase Medium Base (Difco) as the basal medium.

Arginine decarboxylase. This was tested by Method 1 of Cowan (1974*b*).

Urease. Urease agar (100 tablets; Oxoid) was dissolved in water (950 ml) and the medium autoclaved at 121 °C for 15 min. Sterile urea [50 ml of 40% (w/v) solution; Oxoid] was added after cooling. The medium was dispensed in 3.5 ml amounts into test tubes and slanted. The slants were streaked heavily from overnight cultures and incubated for 5 d. Readings were taken at 4 h and then daily. All tests were read against a urease-negative *E. coli* control.

Catalase. Colonies of an overnight BAB culture were flooded with 20 vol H₂O₂ and observed for the production of effervescence for up to 1 min.

Oxidase. Growth from a fresh BAB culture was rubbed on to Whatman no. 1 filter paper previously soaked in oxidase reagent (Cowan, 1974*b*; solution 1) and observed for the development of a dark purple colour for up to 10 s (Kovacs, 1956).

Phenylalanine deaminase. The medium of Edwards & Ewing (1972) was used and tested by Method 2 of Cowan (1974*b*).

ONPG test. This was carried out by the method described in Cowan (1974*b*).

Phosphatase. This was carried out using Method 1 of Cowan (1974*b*), except that BAB replaced NA as the basal medium.

DNAase. The method was based on that of Smith *et al.* (1969). Plates of DNAase test agar with methyl green (Difco) were streaked for single colonies and incubated for 2 d. Plates were examined at 24 and 48 h for the clearing of the green colour around the growth.

Gelatin hydrolysis. Tubes of Nutrient gelatin (Oxoid) were stab inoculated and examined after 5 and 14 d incubation. Tubes were refrigerated at 4 °C for 2 h before reading.

Tributyryl hydrolysis. Plates of tributyrin agar (Oxoid), streaked for single colonies and incubated for 5 d were examined daily for zones of clearing around the colonies and on day 5 some bacterial growth was removed and the underlying agar examined for clearing.

Casein hydrolysis. The medium described by Cowan (1974*b*) was used except that BAB replaced NA. The test was carried out as for tributyrin hydrolysis.

Eijkman test. Tubes of MacConkey Broth (Oxoid) containing Durham tubes were inoculated and incubated at 44 °C for 48 h. Tubes were examined for turbidity and the production of acid and gas.

Oxidation-fermentation of glucose. Tubes of O.F. basal medium (Difco) were inoculated and incubated according to the method of Hugh & Liefson (1953).

Indole production. Indole production was tested in tubes of Tryptone water (Oxoid) according to Method 2 of Cowan (1974*b*). The production of indole was examined after incubation for 48 h at 37 and 44 °C.

Methyl red and Voges Proskauer tests. MR/VP medium (Oxoid) was dispensed in 1.5 ml amounts in bijou bottles and sterilized. Inoculated bottles were incubated at 37 °C for 2 d and then tested. For the MR tests 10 drops of

methyl red solution (Cowan, 1974*b*) were added. The VP test was carried out according to Method 1 of Cowan (1974*b*).

Hydrogen sulphide production. Two sulphide sources were used: cysteine and thiosulphate. The following medium containing (g l⁻¹): peptone (Oxoid) 15; proteose peptone (Oxoid) 5; brown ferric ammonium citrate (Fisons) 0.5; dipotassium hydrogen phosphate (BDH) 1; agar (Oxoid) 15; either sodium thiosulphate (BDH) 0.08 or cysteine hydrochloride (BDH) 0.5, was dispensed in 4 ml amounts into large test tubes which were then slanted. The media were inoculated by stabbing the butt and streaking the slant. Tubes were incubated for 7 d and examined daily for blackening of the medium.

Gas production from carbohydrates and production of ammonia from peptone. Carbohydrates tested were: adonitol, dulcitol, glycerol, glucose, inositol, maltose, mannitol and starch. To the following basal medium containing (g l⁻¹): Bacteriological peptone (Oxoid) 10; sodium chloride (Fisons) 5; bromothymol blue 0.04, was added the appropriate carbohydrate as a filter-sterilized solution to give a final concentration of 1% (w/v). In the case of the starch-containing medium the carbohydrate was added to the basal medium before sterilizing and autoclaved at 115 °C for 10 min. The media were dispensed (4 ml) into 4 × 0.5 inch tubes containing Durham tubes. Before inoculation these were steamed for 30 min to remove all air from the Durham tubes. The inoculated tubes were examined daily for 3 d and then at 7 and 14 d for the production of gas. The production of ammonia from peptone was tested in tubes of the basal medium containing no carbohydrate. Ammonia production was measured by the change of the indicator colour from green to blue.

Reduction of nitrate. This was carried out by Method 1 of Cowan (1974*b*) with NB2 as the basal medium.

Reduction of nitrite. The method described in Cowan (1974*b*) was used with NB2 as the broth medium.

Carbon source utilization. The basal medium of Véron (1975) was supplemented with yeast extract (Difco) to a final concentration of 0.005% (w/v). The carbon source (5 g) was added to the basal medium before autoclaving except for the following which were added as filter-sterilized solutions to the sterile medium: fructose, cellobiose, lactose, galactose, raffinose and ethanol.

The media were dispensed in 2.5 ml amounts into repli-dishes (Sterilin) and the dishes dried to remove surface water. Overnight cultures on peptone agar were suspended in sterile NaCl (0.9%, w/v) to give approximately 10⁵ organisms per ml. The diluted cultures were used to inoculate the carbon source media. Growth was assessed by eye after 1, 2, 3 and 7 d incubation. In all cases a control dish with no added carbon source was set up and used as a comparison when assessing the growth.

Acid from carbohydrates. This was tested in peptone water agar [peptone (Oxoid), 1% (w/v); NaCl, 0.5% (w/v); agar (Oxoid no. 3), 0.5% (w/v)], containing bromothymol blue indicator at a final concentration 0.004% (w/v). Filter-sterilized carbohydrates (with certain exceptions) were added to the sterilized basal medium to give a final concentration of 1% (w/v). The exceptions were dextrin, starch, aesculin, mucate and glycogen. These substances did not form a solution which was readily filter-sterilized and were therefore added to the medium before autoclaving. The media were dispensed into repli-dishes (Sterilin), inoculated with overnight cultures, incubated and read daily for 8 d, then every 2 d up to 14 d. Acid production on days 1–6 was scored as 'early', on days 7–14 as 'late'.

Antibiotic sensitivities. BAB plates were flooded with overnight broth cultures and the excess carefully removed. Multidisks (Oxoid) containing appropriate antibiotics were then placed on the plates. Sensitivities were recorded after 1 and 2 d.

Dye sensitivity. The medium and method of Serény (1967) were used. Only one dye, basic fuchsin, was tested. Not only did we not obtain the species discrimination noted by Serény (1967), we also found the test to be <15% reproducible. The results of this test were therefore not included in the study.

Coding of characters. The data were coded as characters with either two- or three-states. The two-state characters were coded as positive and negative and scored as 1 and 0, respectively. The three-state characters were coded as strongly positive, weakly positive and negative and were scored as 2, 1 and 0, respectively.

Before coding the results were scrutinized for tests which gave identical results for all the strains studied. Eight tests were not scored for this reason; these were: Gram reaction (negative); shiny colonies on NA; glutamine decarboxylase (negative); acid produced early from glucose (strongly positive) and L-xylose (negative); acid produced late from erythritol and mucate (negative); utilization of formate (negative).

Certain other test results were not included in the coded data because of their lack of reproducibility. These were colonial morphological characters (opacity, elevation, edge, rough or smooth surface and ease with which the colony could be emulsified in saline whether grown on BAB or on NA) and sensitivity to basic fuchsin.

Computation. All computing was done on the University of Nottingham ICL 1900 computer, except for the dendrograms which were produced on the University of Leicester CDC CYBER 72 using the TAXPAK program written by M. J. Sackin. Gower's (1971) similarity coefficient (S_G) was computed and clustered by unweighted pair group average linkage (UPGMA) and single linkage methods (Sneath & Sokal, 1973).

The data were converted to two-state character data by combining 'strongly positive' and 'weakly positive' in the three-state characters, into a single 'positive' state scored as 1 and leaving the two-state characters unchanged. The simple matching coefficient (S_{SM}) and the pattern difference coefficient (D_P) (Sneath & Sokal, 1973) were

then computed on the converted data, and both similarity matrices were clustered by the same two methods. For every clustering the cophenetic correlation was computed between the dendrogram and the similarity matrix from which it was derived.

The IGROUPS (Integer groups) program printed character value statistics on specified groups of OTUs chosen from the sorted matrix. Overlap statistics were computed by means of the OVCLUST program (Sneath, 1979). The program was run on data for every pair of clusters using the intercentroid distance (D) between each pair of clusters and the standard deviation (s_q) of the q -distributions of each cluster of the pair. The D and s_q values were obtained from a revised IGROUPS program. The chosen overlap used was 5%.

RESULTS

Reproducibility

The similarity between duplicate strains varied from 92.4%S to 98.4%S, the average similarity between the duplicates being about 95%. This indicates an estimated average error in testing of 2.46%. Test reproducibility was studied by repeating 28 tests (resulting in 51 characters) for all strains and comparing the two sets of data. The reproducibility obtained varied from 100% to 62%. Sneath & Johnson (1972) have suggested that tests showing errors in reproducibility of 10–15% are acceptable. Fourteen test characters fell outside the 90% reproducibility level and eight of these outside a level of 85% reproducibility.

The best reproducibilities were for antibiotic sensitivities (95–100% reproducible), and many of the biochemical tests such as gelatin hydrolysis, catalase, indole production, fell within this range of reproducibility also. The production of acid from carbohydrates showed good reproducibility although the range here was greater (99.4%–88.7%), the poorer reproducibility being for the late production of acid. Results for the utilization of carbon sources were reproducible within the range of 85–95%, although one carbon source (2-oxoglutarate) showed reproducibility of 72%.

Characters based on colonial morphology showed the poorest reproducibility. Although a few gave high scores of 99% (red colony colour, shiny surface, presence of striations), most were below 85% reproducible and two (flat colony, entire colony edge) were less than 65% reproducible. The reading of colonial morphology characters is subjective and only where the categories were well defined was there good reproducibility. Where the dividing line between categories was less specific (e.g. between a flat and a low convex colony) and more subjective, reproducibility was poor. This was a particular problem in this study as most of the bacteria under investigation were all very similar in their colony morphology and only small differences in appearance were being determined. Because of their poor reproducibility most of the colonial morphology data (see *Coding of characters* in Methods) were excluded from the coded data matrix.

Cluster analysis

The results of the different computations gave broadly similar groupings. The classification which resulted from UPGMA clustering on the Gower coefficient (Figs 1 and 2) will be described in detail. Reference will be made to the pattern coefficient/UPGMA analysis which makes allowances for differences in growth rates and other factors which can distort similarity values (Sneath, 1968).

In the dendrogram derived from UPGMA clustering on the Gower coefficient (Figs 1 and 2) all the strains grouped into six Areas at a similarity level of 75%. Area I contains all the strains received as *Shigella* spp., strains of the *Alkalescens-Dispar* group, *Providencia* spp., two strains of *Proteus rettgeri* and single strains received as 'enterobacterium Sachs' type A12', *E. coli*, *Pleisomonas shigelloides*, *Edwardsiella tarda* and *Vibrio cholera*. Area II contains strains received as members of the genera *Citrobacter*, *Escherichia*, *Salmonella* and of the species *Enterobacter liquefaciens*, *Hafnia alvei* and *Erwinia carotovora*. Area III contains all the *Proteus* species studied with the exception of *Prot. rettgeri*. Area IV contains mainly members of the genera *Klebsiella* and *Enterobacter*. Area V contains other members of the genus *Klebsiella*. Area VI contains representatives of the genus *Serratia*. At a similarity level of approximately 80% all but four of the strains clustered into 14 phenons (Figs 1 and 2).

In the dendrogram derived from the UPGMA clustering based on the pattern coefficient only five Areas were evident at a similarity level of 75%. With the exception of *Ent. liquefaciens* (strains K16, K17) and *Erw. carotovora* (strain D1253) all the strains in Area II (Figs 1 and 2) moved into Area 1. The only other major change in classifications based on the different coefficients was the position of phenons D and E which moved out of Area 1 (Figs 1 and 2) and grouped with phenon K to form Area III. The strain composition of the phenons was almost identical in both computations. One strain in phenon E (*Prot. rettgeri*, P28) grouped with phenon O in Area VI.

Description of the phenons

Phenon A (82.5%S) contains all the strains received as members of the genus *Shigella*, seven of the eight *Alkalescens-Dispar* strains, one *E. coli* strain and one 'enterobacterium Sachs' type A12'. Eight subphenons (A1–A8) are evident within the cluster.

Subphenon A1 (89.5%S) contains all the *S. sonnei* strains (16) studied and one strain of '*E. dispar*'. The subphenon corresponds to the species *S. sonnei*. The eight strains of *S. sonnei* which were received as new isolates ('*S. sonnei* clinical', Fig. 1) show a high degree of relatedness (94.5%S) to each other. However, no single character or set of characters was unique to this 'clinical' group of *S. sonnei*, although certain of them did show resistance to a higher proportion of the antibiotics tested.

The '*E. dispar*' strain (E126) which clusters with the *S. sonnei* strains is quite typical of the subphenon in its biochemical reactions. It does not ferment xylose, dulcitol, sorbitol or salicin; rhamnose and maltose are fermented early; lactose and sucrose are fermented late; lysine and ornithine are not decarboxylated. Wheeler identified this strain as *S. sonnei* (Catalogue of the National Collection of Type Cultures, 1972) and from the clustering of E126 here it appears that this is the correct classification of this strain. The serological relationship of this strain to *S. sonnei* is not known to us.

Subphenon A2 (87.5%S) contains three *Shigella* strains (E106–E108) and one '*E. dispar*' strain. The three *Shigella* strains are closely related at 94.5%S. These three strains share the same serotype (147 or 145/46 Carpenter, Ewing *et al.*, 1958) which is antigenically identical to *S. dysenteriae* serotype 3, and to *E. coli* O group 124. Many authors (Manolov, 1959; Manolov & Trifanova, 1962; Tekelieva, 1975) would include such strains as part of the genus *Shigella* whilst others (Rowe *et al.*, 1977) uphold the present classification of such strains as *E. coli* variants. The serotype 147 strains studied by us differed from *E. coli* in that they did not decarboxylate lysine, nor ferment salicin nor produce acid early from lactose. However, they did ferment sorbitol (unusual amongst shigellae) and utilize sodium acetate. This latter characteristic alone would lead to their exclusion from the genus *Shigella* by the criteria of Edwards & Ewing (1972).

Subphenon A3 (89.5%S) contains 34 strains. All but one of the *S. flexneri* strains of serotypes 1–5 (31 strains) cluster here together with one strain (E28) received as *S. boydii* and two received as *Shigella* species. This subphenon corresponds to the species *S. flexneri*. There is some evidence of subgrouping within the cluster but this does not seem to be strongly correlated with serotype (Fig. 1).

The *S. boydii* strain (E28) clustering in subphenon A3 is of serotype 9. Strains of this serotype often show features not characteristic of the species *S. boydii*, viz. late lactose fermentation and rhamnose fermentation. However, strain E28 did not show these characteristics. Serological cross-reactions between *S. boydii* serotype 9 and certain serotypes of *S. flexneri* are known (Edwards & Ewing, 1972).

Shigella strain E105 was known previously as Gettings' bacillus (Andrewes & Inman, 1919). These authors stated that the strain was typical of the '*S. paradyenteriae*' group but was not serologically related to the five serotypes of Andrewes & Inman (1919). In the Catalogue of the National Collection of Type Cultures (1972) this strain is listed as a *Shigella* sp. with a side listing as *S. dysenteriae* (though no reason is given for this side listing). On the evidence produced in our study, strain E105 would appear to be part of the species *S. flexneri*. Its serological reactions are not known to us, thus we do not know if it is serologically related to the 'flexneri' group.

Subphenon A4 (89%S) contains 27 strains. These comprise all but five of the *S. boydii* strains studied, eight strains of *S. dysenteriae*, three strains of *S. flexneri* serotype 6 and a strain of

Fig. 1. Dendrogram derived from UPGMA clustering on the Gower coefficient (S_G). Cophenetic correlation coefficient is 0.91.

Fig. 2. Simplified dendrogram showing the main clusters in Fig. 1.

'enterobacterium Sachs' type A12'. The subphenon may be equated with the species *S. boydii*.

Too few strains of *S. boydii* are present to determine if there is any relation between the formation of subgroups and serotypes. The two *S. boydii* serotype 10 strains (E29 and E30) do cluster together, as do the two *S. boydii* serotype 3 strains (E20 and E34); however, the *S. boydii* serotype 15 strains (E35A/B and E36) do not show a close association.

The *S. dysenteriae* strains clustering with *S. boydii* are of serotypes 3, 5, 6, 9, 10 and provisional serotype 3873-50. Except for the strain of serotype 10, these strains show a very high similarity on the basis of pattern. *S. dysenteriae* serotype 3 is unusual in being the only *S. dysenteriae* serotype to frequently produce acid from mannitol; however, neither of the *S. dysenteriae* serotype 3 strains studied was mannitol positive. *Shigella dysenteriae* serotype 5 strains are unusual in fermenting dulcitol; a character generally found amongst shigellae only in *S. boydii* strains of certain serotypes and in some *S. flexneri* serotype 6 strains. Serological cross-reactions are known between *S. dysenteriae* serotype 5 and serotype 4 of *S. boydii* (Edwards & Ewing, 1972). The strains of *S. dysenteriae* serotypes 9, 10 and provisional serotype 3873-50 all fermented D-xylose, a characteristic found restricted primarily to the strains of subphenon A4 amongst the shigellae studied here. Serological cross-reactions are also known between *S. boydii* serotype 1 and *S. dysenteriae* serotype 10 (Edwards & Ewing, 1972).

The clustering of the 'enterobacterium Sachs' A12' type with the *S. boydii* cluster supports the suggestion of various workers (Carpenter, 1961; Szturm-Rubinsten *et al.*, 1962; Howie & Old, 1976; Regina *et al.*, 1981) that such strains are aerogenic, mannitol negative variants of *S. boydii* serotype 14 with which 'Sachs' A12' strains are antigenically identical.

The three *S. flexneri* serotype 6 strains clustering in subphenon A4 are of the Boyd 88 variety (which produce only acid from glucose and mannitol). Two (strains E88 and E92) ferment dulcitol and the other strain (E93) does not. Only two of these *S. flexneri* strains (E92, E93) cluster together; strain E88 shows the closest relationship to *S. boydii* serotype 4 (strain E22).

Subphenon A5 (90% S) contains 20 strains all but one of which were received as strains of *S. dysenteriae* and includes the type strain of the species (E37, NCTC 4837). The cluster corresponds to the species *S. dysenteriae*. Three distinct subgroups within this subphenon are evident, and these are related to the serotypes of the strains (Fig. 1).

Subgroup A5(a) (93.5% S) contains five strains of *S. dysenteriae* serotype 2 (92.5% S) and two mannitol negative strains of *S. boydii* serotype 15. Ewing *et al.* (1958) noted that mannitol positive *S. boydii* serotype 15 strains could be regarded as a subserotype and biotype within *S. dysenteriae* serotype 2, but preferred to consider these strains as a new serotype of *S. boydii*. The clustering of the mannitol negative variants of *S. boydii* serotype 15 in our study suggests that at least this variety of *S. boydii* serotype 15 should be considered for re-classification as *S. dysenteriae* serotype 2.

Subgroup A5(b) (92% S) contains two strains of *S. dysenteriae* serotype 4, three ('split') strains of *S. dysenteriae* serotype 6 and one strain of *S. flexneri* serotype 6. The *S. dysenteriae* serotype 6 strains grouping here (E51A/B/BX) do not show a close relationship to the other *S. dysenteriae* serotype 6 strain (E52). This is the only *S. dysenteriae* serotype included in this study for which strains of the same serotype did not cluster together. However, strain E51 showed certain unusual growth characteristics not found amongst the other shigellae. In particular this strain could not grow on NA without the addition of 0.5% NaCl. This may explain why the two strains of *S. dysenteriae* serotype 6 did not cluster in the same subphenon.

The *S. flexneri* serotype 6 strain clustering with *S. dysenteriae* is of the aerogenic Newcastle variety (fermentation of glucose and delayed fermentation of dulcitol, with the production of acid and gas; mannitol not fermented). This was the only *S. flexneri* strain of those studied which did not decarboxylate arginine. When originally isolated, strains of this type ('*S. newcastle*') were classified with the mannitol negative shigellae (later *S. dysenteriae*), but were subsequently transferred to *S. flexneri* because of their antigenic relationship to mannitol positive *S. flexneri* serotype 6 strains. The biochemical similarity of such strains to *S. dysenteriae* is evident here.

Subgroup A5(c) (92% S) contains four strains of *S. dysenteriae* serotype 1 and three *S. dysenteriae* strains of unknown serotype. This cluster includes the type strain (NCTC 4837). The characters distinguishing the three subgroups of subphenon A5 are shown in Table 2.

Table 2. *Characters of discriminatory value in differentiating the subgroups of subphenon A5 (S. dysenteriae)*

Subgroup No. of strains	<i>S. dysenteriae</i> 2 A5(a) 7	<i>S. dysenteriae</i> 4 A5(b) 6	<i>S. dysenteriae</i> 1 A5(c) 7
Catalase	+	— (67%)	—
Indole at 37 °C	+	— (83%)	—
Indole at 44 °C	+	—	—
Acid from glycerol late*	+	+(50%)	+
Acid from ribose early*	+(57%)	+	—
Acid from ribose late	+(86%)	+	—

+, 100% of strains gave positive reaction; —, 100% of strains gave negative reaction. Figures in parenthesis indicate the percentage of strains giving the indicated reaction for variable characters.

* Late, after 6 d; early, 1–6 d.

Subphenon A6 (89% S) contains six *S. dysenteriae* strains and one *S. flexneri* strain. The *S. dysenteriae* strains comprise two strains of serotype 7, three strains of serotype 8 and one strain of the provisional serotype 2000-53. Again, *S. dysenteriae* strains having the same serotype are more closely related than those having different serotypes. The exclusion of *S. dysenteriae* serotype 2000-53 strains from the genus *Shigella* has been suggested by Edwards & Ewing (1972) because strains of this serotype utilize sodium acetate, may be aerogenic, and decarboxylate ornithine. The clustering of strain E60 in our study does not support this proposal. The *S. flexneri* strain clustering in subphenon A6 (strain E81) is a mannitol negative variant of serotype 4a. Such strains have been described by a number of species epithets, viz. '*S. rio*' (Weil *et al.*, 1948); '*S. saigonensis*' (Rubinsten *et al.*, 1950) and '*S. rabaulensis*' (Mumford & Mohr, 1944), and were at one time classified with the mannitol negative shigellae. Although strain E81 is described as a mannitol negative variant of *S. flexneri* serotype 4a, it does not differ merely in not fermenting mannitol. Strain E81 does not ferment trehalose, but does ferment D-xylose and is ONPG positive. In all these characteristics the strain differs from the *S. flexneri* strains of subphenon A3. Their differences lie not only in the loss of some characters by strain E81 but also in its possession of characters not found in the *S. flexneri* subphenon. This suggests that a more fundamental difference exists between mannitol negative and mannitol positive varieties of *S. flexneri* serotype 4a.

Subphenon A7 (88.5% S) contains only three strains, two *S. flexneri* serotype 6 strains and one strain of *S. boydii*. The *S. flexneri* serotype 6 strains are strain E89 which is of the Manchester variety (which produce acid and gas during fermentation of glucose or mannitol and may ferment dulcitol), and strain E91 which is of the anaerogenic Newcastle variety (only acid is produced on fermentation of glucose or delayed fermentation of dulcitol). It is interesting that the latter does not cluster with the aerogenic Newcastle strain, E90.

The *S. boydii* strain (E31) is of serotype 11. Both it and strain E91 were unusual in being sensitive to the majority (23/24) of antibiotics against which they were tested. Strain E31 ferments both maltose and dulcitol, characters it shares with the two *S. flexneri* serotype 6 strains and which are found only in strains of certain serotypes amongst the species *S. boydii* (Edwards & Ewing, 1972).

Subphenon A8 (84% S) contains eight strains. Six of these are strains of '*E. alkalescens*', one is an '*E. dispar*' strain and one is an *E. coli* strain. This cluster could be equated with the Alkalescens-Dispar group or, alternatively, as all the strains named '*E. alkalescens*' cluster here the cluster may correspond to a species for which the name *E. alkalescens* could be re-proposed.

The *E. coli* strain (E131) belongs to O antigen group 112 and is of the type originally designated '*S. guanabara*' by de Assis (1948). Ewing & Kauffman (1950) showed a serological relationship (though not complete identity) between '*S. guanabara*' strains and those of the *E. coli* O group 112 and re-designated '*S. guanabara*' strains as anaerogenic variants of *E. coli* O112. The biochemical resemblance of these strains to those of the Alkalescens-Dispar group was noted by Ewing & Kauffman (1950); the clustering of strain E131 in Fig. 1 supports this finding.

One strain is ungrouped in phenon A; this strain is *S. boydii* serotype 13 (strain E33). Serotype 13 strains are unusual in possessing an ornithine decarboxylase system (generally only found in *S. sonnei* amongst the shigellae) and aerogenic forms are known. The DNA-DNA hybridization studies of Brenner *et al.* (1973) showed that *S. boydii* strains of serotype 13 formed a homogeneous group and were unlike the other shigellae. The ungrouped position of strain E33 (Fig. 1) supports this finding and suggests that strains of this serotype may constitute a separate species.

The characteristics of the subphenons of phenon A are given in Table 3. Phenon B (82% S) contains two strains, one *Plesiomonas shigelloides* and one *Edwardsiella tarda*. The taxonomic validity of this cluster cannot be assessed on this information. Phenon C (81.5% S) contains two of the three *Yersinia* strains studied. The cluster may be equatable with the genus *Yersinia* but again the taxonomic validity of the group cannot be assessed. Phenon D (84% S) contains all the *Providencia* strains and corresponds to the genus *Providencia*. Three subphenons are evident within this group.

Subphenon D1 (89% S) contains two *Providencia alcalifaciens* strains (including the type strain, P77 (NCTC 10286)) and five *Providencia* species. This may be equated with the species *P. alcalifaciens*.

Subphenon D2 (91% S) contains five *Providencia* strains. Three of these strains (P24, P32 and P75) were included in the study of McKell & Jones (1976) and in that study formed part of the *P. alcalifaciens* cluster. Brenner *et al.* (1978) in DNA-DNA hybridization studies found two hybridization groups among strains named *P. alcalifaciens*. One group termed by them 'BG1, 2' was characteristically adonitol positive and was typified by the type strain of *P. alcalifaciens*.

Table 3. Characteristics of the subphenons of phenons A, D and phenon E, expressed as percentage of positive reactions

Subphenon No. of strains	A1 17	A2 4	A3 34	A4 27	A5 20	A6 7	A7 3	A8 8	D1 7	D2 5	D3 5	E 2
Colony diameter <1 mm on BAB	0	0	0	4	0	29	0	0	29	40	20	0
Shiny colonies on BAB	100	100	100	100	85	100	100	100	100	100	100	100
Colony diameter <1 mm on NA	0	0	3	7	5	14	0	0	29	80	0	50
Colonies striated on NA	0	0	0	0	0	0	0	0	14	0	0	0
Deposit formed at 37 °C	100	100	100	100	100	100	100	100	100	100	80	100
Ring/pellicle at 37 °C	0	0	0	0	0	0	0	13	14	0	0	0
Growth at 4 °C	6	25	3	4	5	43	0	13	57	100	40	0
Growth at 10 °C	100	100	76	93	65	100	33	100	100	100	100	100
Growth at 45 °C	94	100	74	89	70	100	67	100	14	20	100	50
Motility at 25 °C	0	0	0	0	0	0	0	0	100	20	80	100
Motility at 37 °C	0	0	0	0	0	0	0	0	57	0	80	100
Better growth on BAB than NA	18	25	38	12	50	14	67	0	29	100	20	50
Growth on DOC agar	94	100	94	100	90	100	0	100	100	100	100	100
Black colonies on DOC agar	0	0	0	0	0	0	0	0	0	0	0	50
Growth on selenite agar	82	100	21	96	50	100	33	88	100	100	100	100
Growth on KCN agar	100	100	100	100	100	100	67	100	86	100	100	100
Growth on 0% added NaCl	100	100	100	100	95	100	100	100	100	100	100	100
Growth on 5% added NaCl	100	100	88	96	75	100	67	100	100	80	100	100
Growth in 10% added NaCl	0	0	0	0	0	0	0	0	0	0	40	0
Growth initiation at pH 4	12	0	0	0	0	0	0	0	29	0	0	0
Arginine decarboxylase	65	75	29	81	40	86	67	88	0	0	0	0
Lysine decarboxylase	0	0	0	0	0	0	0	75	0	0	0	0
Ornithine decarboxylase	88	25	0	7	10	14	0	13	0	20	0	0
Arginine dihydrolase	0	0	0	0	0	0	0	0	0	0	40	0
Urease	0	0	0	0	0	0	0	0	0	0	0	100
Catalase	100	100	100	78	45	71	100	100	100	100	100	100
Phenylalanine deaminase	0	0	0	0	0	0	0	0	100	100	40	100
ONPG	88	25	3	19	20	14	0	75	0	0	20	50
Phosphatase	18	0	0	22	15	57	33	100	100	40	100	100
DNAase	0	0	0	0	0	0	0	0	100	80	100	100
Tributylin hydrolysis	94	100	68	67	25	86	0	63	86	40	60	100
Casein hydrolysis	0	25	0	4	0	0	0	25	0	0	0	0

Table 3.—(continued)

Subphenon	A1	A2	A3	A4	A5	A6	A7	A8	D1	D2	D3	E
Acid from aesculin late	0	0	3	0	0	0	0	25	84	100	60	100
Acid from D-arabinose early	35	100	0	4	0	14	0	88	0	0	0	50
Acid from D-arabinose late	88	75	0	7	5	14	0	75	0	0	0	0
Acid from L-arabinose early	100	100	68	96	10	100	100	100	0	0	0	0
Acid from L-arabinose late	100	100	56	96	20	100	100	88	0	0	0	0
Acid from arabitinol early	0	0	0	0	0	0	0	0	0	0	0	100
Acid from arabitinol late	0	0	0	0	0	0	0	0	0	0	0	50
Acid from arbutin early	35	0	3	0	0	0	0	50	0	0	0	100
Acid from arbutin late	94	75	6	11	5	0	0	88	0	0	20	100
Acid from cellobiose early	6	0	0	4	0	0	0	0	0	0	100	0
Acid from cellobiose late	12	0	0	4	0	0	0	13	0	0	100	50
Acid from dextrin early	0	0	3	0	0	0	0	0	0	0	0	0
Acid from dextrin late	0	25	3	0	0	14	0	13	0	0	0	0
Acid from dulcitol early	0	0	0	30	5	0	67	88	0	0	0	0
Acid from dulcitol late	0	0	0	33	5	0	100	38	0	0	0	0
Acid from erythritol early	0	0	0	0	0	0	0	0	0	0	0	100
Acid from fructose late	100	100	100	100	100	100	100	100	71	100	100	100
Acid from galactose early	100	100	100	100	100	100	100	100	0	100	100	100
Acid from galactose late	100	100	100	100	100	100	100	100	43	100	100	50
Acid from glycerol early	100	100	38	96	80	100	100	100	100	100	100	100
Acid from glycerol late	100	100	73	96	85	86	100	100	100	100	40	50
Acid from inositol early	0	0	0	0	0	0	0	0	0	0	100	100
Acid from inositol late	0	0	0	0	0	0	0	0	0	0	20	50
Acid from inulin early	0	0	0	0	0	0	0	0	0	0	40	0
Acid from inulin late	0	0	0	0	0	0	0	0	0	0	40	0
Acid from lactose early	0	0	0	0	0	0	0	13	0	0	20	50
Acid from lactose late	82	25	0	7	5	0	0	25	0	0	20	50
Acid from maltose early	100	100	50	11	0	71	67	100	0	0	0	0
Acid from maltose late	100	100	44	11	35	86	100	50	0	0	0	0
Acid from mannitol early	94	100	100	59	0	0	67	100	0	0	0	100
Acid from mannitol late	94	100	100	63	0	0	67	100	0	0	0	0
Acid from mannose late	100	100	97	100	100	86	100	100	100	100	100	100
Acid from melibiose early	12	75	76	7	10	14	0	38	0	0	0	0
Acid from melibiose late	100	75	74	7	5	14	0	0	0	0	0	0
Acid from mucate early	0	25	0	0	0	0	0	25	0	0	0	0
Acid from raffinose early	6	0	38	0	0	0	0	25	0	0	20	0
Acid from raffinose late	76	0	47	0	0	0	0	13	0	0	0	0
Acid from rhamnose early	100	100	6	0	25	57	0	63	0	0	0	50
Acid from rhamnose late	71	25	3	0	15	43	0	38	0	0	0	50
Acid from ribose early	100	100	62	93	45	100	100	100	100	100	100	100
Acid from ribose late	100	100	79	97	60	100	100	100	100	100	100	100
Acid from salicin early	0	0	0	0	0	0	0	13	0	0	0	100
Acid from salicin late	0	0	3	0	0	0	0	25	0	0	40	100
Acid from sorbitol early	0	100	24	85	50	14	67	88	0	0	100	0
Acid from sorbitol late	6	0	15	93	65	14	100	75	0	0	60	0
Acid from L-sorbose early	0	0	0	33	0	43	0	88	0	0	0	0
Acid from L-sorbose late	6	25	0	30	0	43	0	25	0	0	0	0
Acid from starch early	0	0	3	0	0	0	0	0	0	0	0	0
Acid from starch late	53	25	3	0	0	0	0	13	0	0	0	0
Acid from sucrose early	12	25	3	0	5	0	0	25	57	60	100	50
Acid from sucrose late	82	25	32	4	5	0	0	25	100	80	80	50
Acid from trehalose early	100	100	97	93	75	57	33	100	0	0	100	0
Acid from trehalose late	100	100	91	81	85	43	100	25	14	0	20	0
Acid from D-xylose early	6	100	0	18	0	43	0	88	0	0	0	50
Acid from D-xylose late	6	0	0	56	0	57	33	38	0	0	0	0

All of the strains grew at 37 °C; in the presence of 0.5% added NaCl; initiated growth at pH 6, pH 8, pH 10; were sensitive to nalidixic acid, gentamicin, kanamycin and neomycin; produced acid from fructose and mannose early; produced acid from glucose aerobically and anaerobically; produced acid from *N*-acetylglucosamine early and late; produced ammonia from peptone; utilized pyruvate.

None of the strains produced red pigment on BAB or on NA; exhibited striations on colonies on BAB; exhibited swarming at 25 °C or 30 °C; produced gas from inositol, starch or maltose; liquefied gelatin; gave a positive oxidase reaction; gave a positive result in the Eijkman test; utilized adipate, benzoate, hippurate, oxalate or D-tartrate; produced acid from amygdalin, glycogen, melezitose, α -methyl-D-glucoside or L-xylose.

Table 4. Characters differentiating subphenons of phenon D (*Providencia*) and phenon E (*Prot. rettgeri*)

Phenon/subphenon No. of strains	D1 7	D2 5	D3 5	E 2
Growth at 4 °C	+(57%)	+	+(40%)	—
Motility at 37 °C	+(57%)	—	+(80%)	+
Urease	—	—	—	+
Indole at 44 °C	+(29%)	—	+	—
Sensitivity to tetracycline	+(43%)	+	—	+(50%)
Sensitivity to chlortetracycline	+(43%)	+	—	+(50%)
Sensitivity to oxytetracycline	+(43%)	+	—	+(50%)
Sensitivity to sulphamethoxazole/trimethoprim	+	+	+(60%)	—
Utilization of carnitine	+(57%)	—	+	+(50%)
Acid from adonitol early	+	—	—	+
Acid from aesculin early	—	—	—	+
Acid from arabinol early	—	—	—	+
Acid from arbutin early	—	—	—	+
Acid from arbutin late	—	—	+(20%)	+
Acid from cellobiose early	—	—	+	—
Acid from cellobiose late	—	—	+	+(50%)
Acid from erythritol early	—	—	—	+
Acid from galactose early	—	+	+	+
Acid from inositol early	—	—	+	+
Acid from mannitol	—	—	—	+
Acid from salicin early	—	—	—	+
Acid from salicin late	—	—	+(40%)	+
Acid from sorbitol early	—	—	+	—
Acid from trehalose early	—	—	+	—

+, 100% strains gave positive reactions; —, 0% strains gave positive reactions. Figures in parentheses indicate the percentage of positive reactions for variable characters.

(P77, NCTC 10286, ATCC 9886). Brenner *et al.* (1978) suggested *P. alcalifaciens* strain ATCC 12013, as the designated type strain for their other hybridization group 'BG3'. This strain is strain NCTC 8113 (i.e. strain P24) which in our study groups in subphenon D2. The two subphenons D1 and D2 therefore appear to be equatable with the two hybridization groups found among *P. alcalifaciens* strains by Brenner *et al.* (1978).

Subphenon D3 (88% S) contains four strains of *P. stuartii* (including NCTC 10318, designated as *P. stuartii* by Ewing 1963), and one received as a *Providencia* species. It thus corresponds to the species *P. stuartii*. The characteristics of these three subphenons and the characters distinguishing them are shown in Tables 3 and 4, respectively. It should be noted that strains of subphenon D1 are adonitol positive, as was the 'BG1, 2' hybridization group of Brenner *et al.* (1978), and this character distinguishes it from subphenon D2.

Phenon E (81.5% S) contains two strains of *Prot. rettgeri*. These show a very close association with the *Providencia* cluster (Phenon D) from which it is separated at the 80% S level (Figs 1 and 2; Tables 3 and 4). It is questionable whether the *Prot. rettgeri* strains should be regarded as constituting a separate taxon or whether they should be considered to be part of the *Providencia* cluster. The close relationship of *Prot. rettgeri* strains to the genus *Providencia* was seen in the numerical study of McKell & Jones (1976) and in the molecular studies of Brenner *et al.* (1978). Indeed, both names, *Prot. rettgeri* and *Prov. rettgeri*, are included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980). Phenon F (84.5% S) contains two *Citrobacter* strains and corresponds to the genus *Citrobacter*. Phenon G (84% S) contains two of the three *Salmonella* strains in the study. The type strain of the type species is present in the cluster and so the phenon corresponds to this genus. Phenon H (84% S) contains five *E. coli* strains including the type strain, and one strain of '*E. dispar*'. The cluster can be equated with the genus *Escherichia*. Phenon J (89.5% S) contains two strains received as '*Enterobacter liquefaciens*'. Phenon K (82.5% S) contains representatives of three species of the genus *Proteus*: *P. vulgaris* (two strains including the type strain); *P. morganii* (one strain) and *P. mirabilis* (two strains). The cluster thus

Table 5. *Characters of greatest discriminatory value in distinguishing phenon A from the other groups in the study*

	<i>Shigella</i> Alkalescens-Dispar		<i>Yersinia</i>	<i>Providencia</i>	<i>Prot. rettgeri</i>	<i>Citrobacter</i>	<i>Salmonella</i>	<i>Escherichia</i>	<i>Ent. liquefaciens</i>	<i>Proteus</i>	<i>Enterobacter</i>	<i>Klebsiella</i> I	<i>Klebsiella</i> II	<i>Serratia</i>
Phenon No. of strains	A 121	B 2	C 3	D 17	E 2	F 2	G 2	H 6	J 2	K 5	L 2	M 4	N 4	O 3
Growth at 4 °C	—	.	+	.	.	.	.	.	+	.	.	.	.	+
Swarming at 25 °C	—	.	.	.	.	.	.	.	.	+	.	.	.	.
Motility at 25 °C	—	+	.	.	+	.	.	.	+	+	+	.	.	+
Motility at 37 °C	—	+	.	.	+	.	.	.	+	.	+	.	.	+
Growth in 5% NaCl	+	—	.	.	.	.	.	.	.	.	.	.	.	.
Growth at pH 4	—	.	.	.	.	.	.	.	.	.	.	+	.	.
Lysine decarboxylase	—	+	.	.	.	.	+	.	+	.	.	+	.	+
Arginine dihydrolase	—	.	.	.	.	+	.	.	.	.	+	.	.	.
Urease	—	.	+	.	+	+	.	.	.	+	+	+	.	.
Phenylalanine deaminase	—	.	.	.	+	.	.	.	.	+	.	.	.	.
DNAase	—	.	.	+	+	.	.	.	.	+	.	.	.	+
Gelatin hydrolysis	—	.	.	.	.	.	.	.	.	.	.	.	.	+
Casein hydrolysis	—	.	.	.	.	.	.	.	.	.	+	.	.	+
VP	—	.	.	.	.	.	.	.	.	.	+	+	.	+
H ₂ S from cysteine	—	.	.	.	.	+	.	.	.	+	.	.	.	.
Gas from adonitol	—	.	.	.	.	.	.	.	.	.	.	+	.	.
Gas from glucose	—	.	.	.	.	+	+	.	+	.	.	.	.	.
Gas from glycerol	—	.	.	.	.	+	.	.	.	.	.	.	.	.
Gas from maltose	—	.	.	.	.	+	+	.	.	.	.	.	.	.
Gas from mannitol	—	.	.	.	.	+	+	.	.	.	.	.	.	.
Sensitivity to Ampicillin	+	.	.	.	.	.	.	.	.	.	—	.	.	—
Sensitivity to Carbenicillin	+	.	.	.	.	.	.	.	.	.	—	.	—	.
Sensitivity to Colistin sulphate	+	.	.	.	—	.	.	.	.	.	.	.	.	—
Sensitivity to Polymyxin B	+	.	.	.	—	.	.	.	.	.	.	.	.	—
Sensitivity to Tetracycline	+	.	.	.	.	.	.	.	.	.	.	.	.	—
Sensitivity to Cephaloridine	+	.	.	.	—	.	.	.	.	.	—	.	.	—
Sensitivity to Chlortetracycline	+	.	.	.	.	.	.	.	.	.	.	.	.	—
Sensitivity to Oxytetracycline	+	.	.	.	.	.	.	.	.	.	.	.	.	—
Sensitivity to Streptomycin	+	.	.	.	.	.	.	.	.	.	—	.	.	.
Utilization of 3-hydroxybenzoate	—	.	.	.	.	+	.	.	.	.	.	.	.	.
Utilization of 4-hydroxybenzoate	—	.	.	.	.	+	.	.	.	.	.	.	.	.
Utilization of cellobiose	—	.	.	.	.	+	.	.	+	.	+	+	+	+
Utilization of citrate	—	.	.	+	+	+	+	.	+	.	+	+	.	+
Utilization of ethanol	—	.	.	.	.	.	.	.	+	.	.	.	.	+
Utilization of hippurate	—	.	.	.	.	.	.	.	+	.	.	.	.	+
Utilization of lactose	—	.	.	.	.	+	.	+	+	.	+	+	.	+
Utilization of malate	+	.	—	.	.	.	.	.	.	.	.	.	.	.
Utilization of phenylalanine	—	.	.	.	.	.	.	.	+	.	.	.	.	.
Utilization of propionate	—	.	.	.	.	+	+	.	.	.	.	.	.	+
Utilization of aesculin	—	.	+	.	.	.	.	.	+	.	+	.	.	.
Acid from adonitol early	—	.	.	.	+	.	.	.	.	.	.	+	+	+
Acid from aesculin early	—	.	.	.	+	.	.	.	+	.	.	+	+	+
Acid from aesculin late	—	.	.	.	+	.	.	.	+	+	.	+	+	+
Acid from amygdalin early	—	.	.	.	.	.	.	.	.	.	+	+	.	.
Acid from amygdalin late	—	.	.	.	.	.	.	.	.	.	+	+	+	.
Acid from arabinol early	—	.	.	.	+	.	.	.	.	.	.	+	+	.
Acid from arbutin early	—	.	+	.	+	+	.	.	+	.	.	+	+	+
Acid from cellobiose early	—	.	.	.	.	+	.	.	.	.	+	+	+	.
Acid from cellobiose late	—	.	.	.	.	.	.	.	+	.	.	.	.	+
Acid from erythritol early	—	.	.	.	+	.	.	.	.	.	.	.	.	+
Acid from fructose late	+	.	.	.	.	.	.	.	.	.	.	—	.	—
Acid from galactose late	+	.	.	.	.	.	.	.	.	.	—	—	.	—

Table 5.—(continued)

Phenon	A	B	C	D	E	F	G	H	J	K	L	M	N	O
Acid from glucose late	+	.	.	.	.	.	.	.	.	.	—	—	.	—
Acid from inositol early	—	.	.	.	+	+	.	.	+	.	+	+	+	+
Acid from inositol late	—	.	.	.	.	.	.	.	+	.	.	.	+	.
Acid from lactose early	—	.	.	.	.	+	.	+	.	.	+	+	+	.
Acid from α -methyl-D-glucoside early	—	.	.	.	.	+	.	.	.	.	.	+	.	.
Acid from <i>N</i> -acetylglucosamine late	+	.	.	.	.	.	.	.	.	.	—	—	.	—
Acid from salicin early	—	.	+	.	+	+	.	.	+	.	+	+	+	+
Acid from salicin late	—	.	+	.	+	+	.	+	+	.	+	+	+	+
Acid from starch early	—	.	.	.	.	.	.	.	.	.	.	.	+	.
Acid from starch late	—	.	.	.	.	.	.	.	+	.	.	.	+	.
Acid from sucrose early	—	.	.	.	.	+	.	.	+	+	+	+	.	+

+, $\geq 90\%$ strains give positive reactions; —, $\leq 10\%$ gives positive reactions.

corresponds to the genus *Proteus* but the species distinction is not good. The clustering of *Prot. morganii* with *Prot. vulgaris* and *Prot. mirabilis* supports the findings of Johnson *et al.* (1975). It is not in agreement with the results of the numerical study of McKell & Jones (1976), which resulted in the clustering of *Prot. morganii* as a distinct taxon well separated from *Prot. vulgaris* and *Prot. mirabilis*. The study of McKell & Jones (1976) included many more strains of the genus *Proteus* and so is likely to be a more accurate picture of the interrelationships in the Proteus-Providence region than this present study. The hybridization studies of Brenner *et al.* (1978) also indicate that *Prot. morganii* is different from the genus *Proteus* (*Prot. vulgaris* and *Prot. mirabilis*) and from members of the genus *Providencia*. Brenner *et al.* (1978) suggested that *Prot. morganii* strains be reclassified in the genus *Morganella* as first suggested by Kauffman (1954).

Phenon L (88.5% S) contains two strains including the type strain of *Enterobacter cloacae*. The other *Enterobacter* strains studied do not cluster here, but since *Ent. cloacae* is the type species of the genus *Enterobacter*, the phenon may be equated with this genus. Phenon M (80.5% S) contains two *Klebsiella* strains, one strain of *Enterobacter* and one *Salmonella* strain. The clustering of the strain of *Sal. paratyphi* with the *Klebsiella* strains and not in phenon G with the other *Salmonella* strains, was unexpected. It is probable that the culture tested (K43) was not as originally described but at some time had been contaminated or confused with a *Klebsiella* strain. Attempts to compare our culture of this strain with another freeze-dried culture of K43 maintained at Leicester proved impossible as the stored strain failed to grow.

The two *Klebsiella* strains clustering in phenon M are '*K. aerogenes*' and '*K. edwardsii*'. The close association of these two species was seen in the study of Bascomb *et al.* (1971) who considered them to be the same species. The association of *Ent. aerogenes* with these strains was also seen in the study of Bascomb *et al.* (1971) and led these authors to suggest that *Ent. aerogenes* be included in the genus *Klebsiella* as *K. mobilis*. The strain of *Ent. aerogenes* (NCTC 10006) is the designated type strain of *K. mobilis* (Skerman *et al.*, 1980). Phenon N (82% S) contains three *Klebsiella* strains representing the species *K. pneumoniae* (capsular type 3) and *K. ozaenae*. Related to this phenon at just below 80% S is a strain of *K. rhinoscleromatis* it is considered to be part of phenon N. The association of *K. pneumoniae* with *K. ozaenae* and *K. rhinoscleromatis* is not in accord with the findings of Bascomb *et al.* (1971).

The clustering of klebsiellae in our study resembles most closely the groupings suggested by Henriksen (1954). He proposed two groups for the *Klebsiella* strains based on their biochemical characteristics, one containing *K. pneumoniae* capsular types 1 and 2 (equivalent to the species '*K. aerogenes*' and '*K. edwardsii*'), and the other containing *K. pneumoniae* capsular type 3, *K. ozaenae* and *K. rhinoscleromatis*. These would be equivalent to phenons M and N, respectively, in this study. Phenon O (89% S) contains three strains including the type strain of *S. marcescens*, and this cluster corresponds to the genus *Serratia*. The characters distinguishing each of the phenons B–O from phenon A are shown in Table 5.

DISCUSSION

The purpose of the study was to determine the number and nature of the groupings which could be defined phenotypically within the genus *Shigella* and to ascertain whether they could be distinguished from each other and from other taxa within the family Enterobacteriaceae, notably the Alkalescens-Dispar group, *E. coli* and the genus *Providencia*.

Within the genus *Shigella* at least four taxospecies (Ravin, 1963) are evident. In our opinion subphenons A5 and A6 correspond to the species *S. dysenteriae*, the type species of the genus *Shigella*. Although subphenon A6 exhibits an overall similarity of only 86% to subphenon A5 and a similar similarity to subphenon A3 (Fig. 1), the overlap statistics (Table 6) indicate that subphenon A6 is not a robust cluster. It is of interest that the subgroups discernible within subphenons A5 and A6 correspond to different serotypes (Fig. 1). The study of Goullet (1980) showed that strains of *S. dysenteriae* serotypes 1, 2 and 4 constitute different esterase zymotypes. In particular, the electrophoretic mobilities of the esterases of *S. dysenteriae* serotype 1 strains were markedly different from those of the other *S. dysenteriae* strains studied.

Mannitol negative strains of *S. boydii* serotype 15 appear to be closely related to *S. dysenteriae* serotype 2 strains and our results suggest they should be included within *S. dysenteriae*. Similarly, the clustering of the mannitol negative variant of *S. flexneri* serotype 4a (strain E61), together with its biochemical characteristics noted earlier, indicate that this strain is not merely a variant of *S. flexneri* serotype 4a which has lost the ability to produce acid from mannitol, but is probably a serotype of *S. dysenteriae*. However, further work is necessary to confirm this and to investigate the systematic position of the mannitol fermenting strains of *S. flexneri* serotype 4a. It is interesting that all the strains received as *S. flexneri* which clustered outside subphenon A3 (*S. flexneri*, Fig. 1) are of the serotypes and varieties cited by Reavis & Ewing (1958) as consistently negative for mannitol fermentation.

Our results do not support the exclusion of strains of *S. dysenteriae* provisional serotype 2000-53 from the genus *Shigella* (Edwards & Ewing, 1972). These strains are phenotypically *S. dysenteriae* (Fig. 1). The characteristics of the species *S. dysenteriae* are depicted in Tables 2 and 3.

Subphenon A3 corresponds to the species *S. flexneri*. Only strains of *S. flexneri* serotypes 1 to 5 cluster here. *Shigella flexneri* serotype 6 strains do not. This is in accord with the findings of many workers that *S. flexneri* serotype 6 strains differ from other strains of the species in their biochemical reactions. They are known to differ in their phage and colicin sensitivity patterns, their lipopolysaccharide structures, in the gene loci determining their antigens and in their esterases (Timakov *et al.*, 1972; Simmons, 1971; Katzenellenbogen *et al.*, 1976; Goullet, 1980). Various workers have suggested the removal of strains designated *S. flexneri* serotype 6 from the species *S. flexneri* (Timakov *et al.*, 1972; Petrovskaya & Bondarenko, 1977; Slopek & Mulczyk, 1967; Gekker *et al.*, 1965; Simmons, 1971; Goullet, 1980). Our findings support this suggestion. The taxonomic position of the *S. flexneri* serotype 6 strains is however difficult to ascertain as the strains examined by us do not cluster within one taxon (Fig. 1).

Petrovskaya & Bondarenko (1977) suggested that *S. flexneri* serotype 6 strains should be considered as a biovar of *S. boydii* and this is supported by the work of Goullet (1980) which showed that the esterases of *S. flexneri* serotype 6 strains examined by him were identical to those from strains of *S. boydii* serotypes 2 and 4. The clustering of three of the *S. flexneri* serotype 6 strains (E88, E92 and E93) in our study supports this proposal, particularly as one of the strains, E88, is most closely related to strains of *S. boydii* serotypes 2 and 4 (strains E18 and E22). Slopek & Mulczyk (1967) proposed that *S. flexneri* serotype 6 strains should be considered as a new species in the genus *Shigella*. The nucleus of such a taxon could be represented by subphenon A7 (Fig. 1). However, the overlap statistics indicate that this grouping has dubious validity as a separate taxon. The clustering of the *S. flexneri* 6 aerogenic Newcastle variety strain (E90) is more consistent with this strain being a serotype of *S. dysenteriae* (Fig. 1). Strains designated *S. flexneri* serotype 6 require further study before any recommendation can be made about their taxonomic status. It appears that although they are serologically homogeneous they are biochemically diverse.

The apparent relationship of *S. boydii* serotype 9 strains to the species *S. flexneri* (Fig. 1) also

Table 6. *Overlap results calculated for chosen cluster pairs*

Pair of clusters	Observed overlap, $V_{(G)}$ (%)	Probability level (P) at which the overlap < 5%
A1 and A2	0.5	$P < 0.90$
A1 and A6	1.4×10^{-4}	$P > 0.99$
A1 and A8	4.8×10^{-3}	$P > 0.99$
A2 and A8	2.15	$P < 0.90$
A3 and A4	0.4	$P > 0.99$
A3 and A5	0.34	$P > 0.99$
A3 and A7	4.1	$P < 0.90$
A4 and A5	0.27	$P > 0.99$
A4 and A6	2.4	$P < 0.90$
A4 and A7	6.1	†
A5 and A6	2.9	$P < 0.90$
D1 and D2	7.0×10^{-4}	$P > 0.99$
D1 and D3	6.7×10^{-11}	$P > 0.99$
D2 and D3	1.0×10^{-21}	$P > 0.99$
D and E	0.6	†

* $P < 0.90$ means that the overlap between the pair of clusters is not significantly less than 5% at the 0.90 level of significance; $P > 0.99$ means that the overlap is significantly greater than 5% at the 0.99 level of significance.

† † test unreliable due to inequality of cluster sizes.

needs further investigation. In the study of Goullet (1980), *S. boydii* serotype 9 strains constituted a distinct zymotype within the species *S. boydii*, but their esterases were not identical with those of *S. flexneri* serotypes 1 to 5.

The species *S. boydii* (subphenon A4, Fig. 1) is quite distinct and can be distinguished from members of the species *S. flexneri* (subphenon A3, Fig. 1) on the basis of biochemical and cultural characteristics (see Table 3). This is contrary to the views of workers who have claimed that these two species can be distinguished only by the use of serological methods (Cowan, 1974a).

The clustering of the 'enterobacterium Sachs' Type 12' strain (E112) within the *S. boydii* cluster supports the suggestion of Carpenter (1961) and Szturm-Rubinsten *et al.* (1962), that such strains be regarded as biochemical variants of *S. boydii* serotype 14 and is in agreement with the view of Regina *et al.* (1981) that Sachs' 'Enterobacterium A12' is an aerogenic variant of *Shigella boydii* 14.

The clustering of certain strains of *S. dysenteriae* with the species *S. boydii* was unexpected. However, only certain serotypes of *S. dysenteriae* cluster here; all the strains of serotypes 3, 5, 9, 10 and provisional serotype 3873-50, but only one strain of serotype 6 (see Fig. 1). This again indicates a close correlation between the serotypes of strains of *S. dysenteriae* and their biochemical and cultural characteristics. Further work is required to confirm the apparent relationships of these strains of *S. dysenteriae* to the species *S. boydii*. As subphenons A4 and A5 (Fig. 1) show little overlap (Table 6), it appears that the serotypes of *S. dysenteriae* which cluster in subphenon A4 (Fig. 1) are distinct from the *S. dysenteriae* strains represented by subphenons A5 and A6 (Fig. 1) and should be designated as new serotypes of *S. boydii*. The species *S. boydii* would then be described as variable for mannitol fermentation.

Subphenon A1 (Fig. 1) corresponds to the species *S. sonnei*. Only one strain not received as *S. sonnei* clusters here (strain E126, *E. dispar*). The clustering of the newly isolated *S. sonnei* strains into a subgrouping is of particular interest. Such a subgrouping within the species cluster was formed by clinical isolates of *Serratia marcescens* in the study of Grimont & Dulong de Rosnay (1972). These strains were found to be multiply resistant to antibiotics and to contain R plasmids. In our study multiple antibiotic resistance (indicating that R plasmids could be present) was seen in only certain of the 'clinical' *S. sonnei*, suggesting that other characters are responsible for the close association of these strains. These findings are illustrative of a frequently observed phenomenon that strains may display quite different features after they have been repeatedly subcultured, from those shown when they were originally isolated.

The strains which cluster in subphenon A2 (Fig. 1) require more detailed investigation. Only a few strains of serotype 147 (Table 1) were studied. It is therefore not possible to decide whether

they should be included in the genus *Shigella* as part of the species *S. sonnei*, or whether they should be left in the species *E. coli* as at present. The result of the overlap statistic analysis (Table 6) indicates that subphenon A2 overlaps with the strains in subphenon A8 – the Alkalescens-Dispar group (Fig. 1).

Shigella boydii serotype 13 (strain E33) shows little phenotypic similarity to any of the main *Shigella* clusters. This correlates with the distinctness of the strains of this serotype indicated by the DNA–DNA hybridization studies of Brenner *et al.* (1973) and suggests that *S. boydii* strains of this serotype may constitute a new species. Further study of these strains is required.

Of the strains received as members of the Alkalescens-Dispar group, those received as '*E. alkalescens*' together with one strain received as '*E. dispar*' and one received as *E. coli*, constitute subphenon A8, while those strains received as '*E. dispar*' encompass a range of types with different biochemical characteristics (Fig. 1, Tables 3 and 5). Alkalescens-Dispar strains are presently classed as non-motile, biochemical variants of *E. coli*. While the overlap statistics indicate that subphenon A8 is distinct from subphenon A1 (*S. sonnei*), they do not indicate that subphenon A8 is distinct from phenon H (*E. coli*). Therefore, although subphenon A8 appears distinct from *E. coli* in Figs 1 and 2, it may not actually be so. When the pattern coefficient was used phenon H (*E. coli*) moved into Area 1 which contains all the *Shigella* strains and those of the Alkalescens-Dispar group. If the strains clustering in subphenon A8 do constitute a distinct group they may represent a new species in either the genus *Shigella* or the genus *Escherichia*.

There is no evidence at all to indicate that the genus *Providencia* is closely related to any of the shigellae. In the genus *Providencia* (phenon D, Figs 1 and 2) three groupings can be discerned: *P. stuartii* (D3), *P. alcalifaciens* (D1) and an as yet un-named species (subphenon D2). The validity of this new species is supported by the results of the overlap statistics (Table 6) and by the DNA–DNA hybridization studies of Brenner *et al.* (1978). The *Prot. rettgeri* strains clustering in phenon E probably represent yet another species of the genus *Providencia* as suggested by the molecular studies of Brenner *et al.* (1978). The overlap statistics indicate that phenons D and E are not separate clusters (Table 6).

The family Enterobacteriaceae is subdivided into far more name bearing taxa than any other bacterial group (see Edwards & Ewing, 1972; Cowan, 1974*a*). This situation has arisen largely due to the clinical importance of many of the taxa. On the basis of DNA–DNA hybridization studies the taxa designated the genera *Shigella* and *Escherichia* could be combined (Brenner *et al.*, 1978). However, as noted by Brenner *et al.* (1978) there is still a need for purely practical purposes to be able to identify a bacterium in a clinical laboratory as *E. coli* or *S. dysenteriae*. Thus, although the *Shigella*–*Escherichia*–Alkalescens-Dispar complex may represent a genospecies as defined by Rabin (1963), the taxospecies into which the complex is now subdivided will probably be used in clinical laboratories for a long time, if not always.

The results presented here indicate that it is possible to distinguish phenotypically at least four taxospecies within the genus *Shigella* viz. *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*, and to differentiate them from each other and from *E. coli* and strains labelled '*E. alkalescens*'. However, each of these taxospecies exhibits a variation of phenotypic characters within the cluster, as wide as that which is exhibited between taxospecies. Thus, if identification of an unknown is based on a few characters only, misidentification can result. However, if classification or grouping is based on the results of many tests then the taxospecies can be reasonably well defined on the basis of their overall properties. Such an exercise is of course not practicable in a clinical laboratory but on the basis of the data presented here reproducible characters can be selected to form the basis of a computer-based identification scheme such as that devised by Bascomb *et al.* (1973). Such a scheme combined with serological data could be a valuable asset to the clinical laboratory.

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