Serological grouping of urinary *Escherichia coli*

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SYNOPSIS Using only 12 antisera it has been possible to group 56·6% of all strains. Forty-four per cent of all strains were typable in the first eight of the serological groups used. Of the primary isolates from symptomatic infections, 65·7% were typable; 48·6% of all strains of the serological groups 01, 02, 04, and 06 occurred in these primary symptomatic isolates.

There was a good correlation in the serological typing of five colonies isolated from the initial isolation plates. There was complete agreement in all the typable strains, 325 of the 350 colonies. Twenty-five strains were not typable due to roughness. It is considered that this reproducibility confirms the validity of using one colony for serotyping of urinary isolates.

Statistically there was no significant difference in the number of typable strains from specimens with high bacterial counts and specimens with low bacterial counts. Nor was there any difference in frequency of the typability of strains in patients with primary symptomatic infection compared with those with associated urinary tract abnormalities. There was a possibly significant increased frequency of typable strains in the primary infection compared with the recurrent infection. There was no significant difference in the frequency of specific serological groups in the high and low bacterial count groups. There was no statistical difference in the frequency of individual serological groups isolated from the two sexes.

Where *Escherichia coli* has been found epidemiologically associated with disease, as in infantile gastroenteritis, the pathogenic significance of certain serological types has been noted. Kauffmann (1947) suggested that certain serological groups of *Escherichia coli* were isolated more frequently from the urine than from the faeces. Vahlne (1945) and Sjöstedt (1946) considered that although certain serological groups were found more commonly in human infections these were not limited to the urinary tract infections. More recently Rantz (1962) suggested that certain serological groups were more invasive for the urinary tract than other types and that these caused clinically evident pyelonephritis more frequently than other serological groups. Turck and Petersdorf (1962) confirmed that certain groups were associated with non-enteric infections but suggested that this may be due to an increased prevalence in the environment. The majority of patients studied in this paper were not exposed to the risk of nosocomial infection; thus there is little possibility of the incidence of serological groups being biased by the inclusion of environmentally prevalent strains.

MATERIALS AND METHODS

Five hundred and thirty-four strains of *Escherichia coli*

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were investigated; 316 were from urines with bacterial counts more than 100,000 per ml., 86 from the intermediate count group, and 132 from counts below 10,000 organisms per ml. They were all identified biochemically as *Escherichia coli* by routine methods (Edwards and Ewing, 1962).

PREPARATION OF SPECIFIC 'O' ANTISERA The following strains of *Escherichia coli* were obtained from Dr. Franz Ørskov of the Statens Serum-institut, Copenhagen.

01	U5/41	01:K1:H7
02	U9/41	02:K1:H4
04	U4/41	04:K3:H5
05	U1/41	05:K4:H4
06	Bi7458/41	06:K2a, 2c:H1
07	Bi7509/41	07:K1:H—
08	G3404/41	08:K8:H4
09	Bi316/42	09:K9:H12
011	Bi623/42	011:K10:H10
018	F10018/41	018:K76:H14
039	H7	039:K?:H—
075	E3b	075:K74:H5

These strains were grown on digest nutrient agar for 18 hours and the cultures emulsified in saline and placed in a Koch steamer for two hours to destroy the 'K' antigen (Vahlne, 1945). The deposits were alcohol-treated and acetone-dried, then reconstituted in 20 ml. of saline. Rabbits were inoculated twice weekly with graded doses of the suspension, starting with 0·5 ml. (1/40th of the culture) intramuscularly for the first week, then two 1-ml. amounts intraperitoneally in the second week. For the next fortnight 1-ml. amounts were inoculated intra-

muscularly. Blood samples were removed from an ear vein for antibody level. When the titre was greater than 1 in 2,000 the animal was exsanguinated by carotid section.

PREPARATION OF *ESCHERICHIA COLI* STRAINS FOR TYPING
The organisms to be tested were grown on digest nutrient agar for 18 hours and a smooth colony was removed and inoculated into nutrient broth for six to eight hours. The suspension was then placed in the Koch steamer for two hours to remove the 'K' antigen. If the suspension appeared rough two further attempts were made to obtain smooth suspensions by subculturing on to blood agar plates. If these failed to produce smooth suspensions the organism was termed 'rough' and designated 'non-typable rough' (N.T.R.); rough suspensions were found in 35 strains. Where no agglutination occurred with the antisera the strain was designated 'non-typable smooth' (N.T.S.); 197 smooth strains were untypable.

TYPING PROCEDURE The strains were screened using three pools of antisera: Pool A contained antisera 01, 02, 04, 07; pool B 05, 06, 08, 09; and pool C 011, 018, 039, and 075.

The antiserum dilution for each component was 1 : 150. Where agglutination occurred the strain was tested against monovalent antisera of the pool at a dilution of 1 : 300. The tubes were incubated at 50°C. for 18 to 24 hours and examined at four and again at 18 to 24 hours. The four-hour examination of the pooled antisera screen often enabled the specific antisera to be used the same day.

RELIABILITY OF SEROLOGICAL TYPING FROM STROKE PLATE
To consider the validity of serological typing of one colony from the original diagnostic plate it was decided to test the serology of five colonies from each plate.

FIG. 1. This plate has been inoculated with urine from a specimen containing more than 100,000 organisms per ml.

These colonies were removed from different sides of the box-like inoculum of the original urine culture (Fig. 1); two samples were taken from the first side. In the low counts the samples were removed from colonies as widely separated as possible. Seventy culture plates were examined in this way, 63 in the high bacterial count group, three in the intermediate group, and four in the low count group.

RESULTS

CROSS AGGLUTINATION WITH UNABSORBED ANTISERA
Using unabsorbed antisera at a dilution of 1 : 150, 42 strains showed cross agglutination with two antisera. These cross reactions were predominantly between strains of 04 and 018 or 01 and 075 serological groups. By diluting the antisera to 1 : 2,000 or by using absorbed antisera the serological identity of the strain was confirmed. Similar cross-reactions have been noted by Knipschildt (1945), Vahlne (1945), and by Kauffmann (1947).

REPRODUCIBILITY OF SEROLOGICAL TYPING Three hundred and fifty colonies from the initial isolation plates of 70 specimens of urine were examined. With 325 smooth strains there was complete agreement; with the remaining 25 precise typing was not possible due to roughness of the strains. There was no difference noted in the various bacterial count groups.

PREVALENCE OF 'O' GROUPS Of the 534 strains, 302 (56.6%) were typable by the 12 antisera and 35 (6.5%) were not typable due to the roughness of

TABLE I
FREQUENCY OF SEROLOGICAL GROUPS OF *Escherichia coli* IN THE BACTERIAL COUNT GROUPS OF ISOLATION FROM URINE

Serological Group	Bacterial Count (organisms per millilitre urine)			Total
	More than 100,000	10,000 to 100,000	Less than 10,000	
01	32	5	17	54
02	19	2	3	24
04	38	12	16	66
05	7	4	4	15
06	35	7	13	55
07	6	—	4	10
08	2	3	2	7
09	1	—	4	5
011	4	1	3	8
018	21	6	7	34
039	4	—	—	4
075	17	1	2	20
N.T.R.	22	4	9	35
N.T.S.	108	41	48	197
Total	316	86	132	534

N.T.R. = not typable due to roughness of strain
N.T.S. = smooth but not typable

the strains (Table I). The strains most frequently isolated were 01, 02, 04, 06, 018, 075 which accounted for 47.4% of all the strains. Forty-four per cent were typable in the first eight serological groups, namely, 01, 02, 04, 05, 06, 07, 08, 09.

Of the 182 primary isolates, 119 were typable (65.4%) and nine (4.9%) were rough and untypable (Table II). The most frequently isolated serological

TABLE II

FREQUENCY OF SEROLOGICAL GROUP IN PRIMARY AND RECURRENT ISOLATES FROM PATIENTS WITH SIGNIFICANT BACTERIURIA

Serological Group	Primary Isolates	Recurrent Isolates	Total
01	22	10	32
02	15	4	19
04	26	12	38
05	5	2	7
06	24	11	35
07	5	1	6
08	1	1	2
09	1	—	1
011	3	1	4
018	5	16	21
039	3	1	4
075	9	8	17
N.T.R.	9	13	22
N.T.S.	54	54	108
Total	182	134	316

groups were 01, 02, 04, 06, which accounted for 47.8% of the isolates. In the first eight serological groups 54.4% of the strains were found. The majority, 140, of the primary isolates were from patients with symptomatic infections without any associated urinary tract lesions, 92 (65.7%) of whom had strains isolated which were typable and six had strains which were untypable due to roughness of the colonies. The prevalent groups were 01, 02, 04, and 06 which accounted for 48.6% of the total strains isolated within these four serological groups (Table III).

Of the recurrent isolates (Table II), 67 (50%) were typable. There is, of course, an increase in frequency of serological groups 018 and 075 which were persistently isolated from two patients. The serological group of 02 does not appear frequently as a recurrent isolate although it does account for nearly 10% of the typable strains from the symptomatic patients without renal lesions (Table III).

In the bacterial count of less than 10,000 organisms per ml. urine the distribution of serological groups was similar to that of the high bacterial count group: groups 01, 04, 06, and 018 were the most frequently isolated, accounting for 70.6% of the typable strains (Table I). As with the recurrent isolates in the high bacterial counts, serological group 02 does not appear as a frequent isolate.

TABLE III

FREQUENCY OF SEROLOGICAL GROUPS OF *Escherichia coli* STRAINS PRIMARILY ISOLATED FROM PATIENTS WITH SIGNIFICANT BACTERIURIA

Serological Group	Symptomatic Infection	Asymptomatic Infection	Renal Lesion	Total
01	17	3	2	22
02	9	1	5	15
04	20	1	5	26
05	4	—	1	5
06	22	1	1	24
07	4	1	—	5
08	—	—	1	1
09	1	—	—	1
011	3	—	—	3
018	2	1	2	5
039	2	—	1	3
075	8	—	1	9
N.T.R.	6	3	—	9
N.T.S.	42	3	9	54
Total	140	14	28	182

COMPARISONS OF FREQUENCY OF SEROLOGICAL GROUPS IN THE BACTERIAL COUNT GROUPS There was no significant difference in the frequency of typable strains from the counts of more than 100,000 organisms per ml. of urine and those below 10,000 organisms per ml. (Table I). No significant difference in the frequency of typable strains was detected between the isolates from patients with symptoms, but without urinary tract abnormalities, and the isolates from patients with such lesions (Table III). There was a possibly significant increased frequency of typable strains in the primary isolates compared with recurrent isolates from the patients with significant bacteriuria (χ^2 6.9217, P between 0.01 and 0.001) as shown in Table II).

Overall there was no significant difference in the frequency of typable strains in the primary symptomatic group compared with the low bacterial count group. With the individual serological groups there was no significant difference in the frequency of 01, 02, 04, 05, 06, or 075. The numbers in the other groups were too small for adequate comparison.

COMPARISON OF THE SEROLOGICAL GROUPS FROM FEMALES AND MALES There was no significant difference in the frequency of serological groups occurring in the sexes in the total primary isolates of more than 100,000 organisms per ml. urine (Table IV).

DISCUSSION

Although there are now 145 recognized serological 'O' groups of *Escherichia coli*, relatively few are associated with urinary infections. In this study more than two-thirds of the primary isolated strains from patients with significant bacteriuria were typable using 12 antisera. Vahlne (1945), using 25 antisera,

TABLE IV
FREQUENCY OF SEROLOGICAL GROUPS OF THE
PRIMARY ISOLATES FROM FEMALES AND MALES
WITH SIGNIFICANT BACTERIURIA

Serological Group	Female	Male	Total ±
01	21	1	22
02	13	2	15
04	22	4	26
05	5	—	5
06	22	2	24
07	4	1	5
08	1	—	1
09	1	—	1
011	3	—	3
018	5	—	5
039	3	—	3
075	7	2	9
Total	107	12	119

typed 61% of his strains, and Rantz (1962), using 126 antisera, found three-quarters of his coliform infections to have typable isolates, although these fell into only 28 serological groups. Turck and Petersdorf (1962), using 137 antisera, typed 76.8% which occurred in only 49 of the serological groups. Indeed the majority of pathological strains occur in the first 10 of Kauffmann's serological groups (Kauffmann, 1947). Vahlne (1945), Ujváry (1958), and Rantz (1962) found that 47.2%, 48%, and 49.2% respectively of their strains occurred in these first 10 groups and, in this present study, 54.4% of the primary isolates were found in eight of these 10 groups: antisera for groups 03 and 010 were not used.

PREVALENCE OF SEROLOGICAL GROUPS It is clear that the majority of isolates fall within a narrow spectrum of serological groups, which in this series consisted of 01, 02, 04, and 06. These types accounted for 73% of the typable strains. Vahlne (1945) found that 01, 02, 04, 07, 018 occurred in 84% of his typable strains, Rantz (1962) had a spectrum of 02, 04, 06, 075 (60.7%), and Turck and Petersdorf (1962) had 02 replaced by 01, resulting in 01, 04, 06, 075 being found in 57% of their significant bacteriurias.

The most frequently encountered serological groups in this study have been 04 and 06; these accounted for one-third of all the strains, and nearly 50% of the typable strains found in the symptomatic significant bacteriurias. Similar figures were obtained by Rantz (1962) and Turck and Petersdorf (1962).

REPRODUCIBILITY OF SEROLOGICAL GROUPING The validity of using one colony for serological typing was evaluated and confirmed. If one excludes the difference in typing due to roughness of the strain then there was complete agreement in the typing of five widely dispersed colonies for the plates inoculated initially from the urine. Indeed in one instance 30 colonies were examined from a patient over a period of five days and the serological type remained constant. This finding would suggest the homogeneity of the infecting organism in urinary infection, that is, one serotype being responsible for each infection. Turck and Petersdorf (1962) found a single serotype in the urine but noted that faecal isolates tended to belong to more than one group. Vahlne (1945) found that there was only one serotype present in 95% of his urinary isolates; similar figures were obtained by Vosti, Monto, and Rantz (1962).

COMPARISON OF THE SEROLOGICAL GROUPS IN THE BACTERIAL COUNT GROUPS In the present study the frequency of serological groups isolated from patients in the high and low bacterial count groups have not shown any demonstrable statistically significant difference. Since the presence of organisms in concentrations of less than 10,000 per ml. of urine is generally accepted as indicating urethral contamination, this lack of difference would suggest that the origin of the urinary infection is from the urethral flora. It has been shown by Helmholz (1950) and by Shakman and Messent (1954) that *Escherichia coli* are commonly present in the urethra and it may be that these assume an invasive role, the mechanism of which is still open to speculation.

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