

Sensitivity of some smooth strains of *Escherichia coli* to the bactericidal action of normal human serum

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SYNOPSIS The sensitivity to normal human serum of 144 smooth strains of *Escherichia coli* isolated from cases of urinary tract infection was determined. It was found that serum-sensitive and serum-resistant strains were not randomly distributed throughout the 10 O serogroups commonly associated with urinary infection. A similar distribution was observed when a group of strains isolated from rectal swabs was studied. The results indicate that serum resistance may be related to qualitative characteristics of the O antigen.

Normal human serum possesses bactericidal activity against many strains of Gram-negative bacteria although serum-resistant strains are encountered (Mackie and Finkelstein, 1931; Roantree and Rantz, 1960). The mechanism of serum resistance has yet to be fully elucidated but there is evidence to suggest that in *Escherichia coli* the lipopolysaccharide O antigen or the acidic polysaccharide K antigen may protect the cell against the serum bactericidal system in a way not directly related to the O or K serological specificity of the cell (Roantree, 1971; Glynn and Howard, 1970).

As part of a study of serum resistance in *E. coli* strains isolated from cases of urinary tract infection, it was decided to compare the O and K antigen content of strains which correspond in O serogroup but differ in their response to normal human serum. When a search for such strains was instituted it became evident that serum-resistant strains were not randomly distributed amongst the 10 O serogroups commonly associated with urinary infections. The distribution of serum-resistant and serum-sensitive strains amongst these serotypes was therefore investigated further and the present report describes the results of this survey.

Materials and Methods

ORGANISMS

Strains were isolated from urinary tract infections; the infection was diagnosed on the basis of a suprapubic aspirate of urine or on three consecutive

midstream specimens of urine each containing more than 100000 organisms/ml in apparently pure culture. Strains were identified as *E. coli* by standard biochemical methods (Cowan and Steel, 1965) and typed using antisera prepared against the 10 O serogroups commonly associated with urinary tract infection (01, 02, 04, 06, 07, 09, 011, 018, 039, 075; Grüneberg, Leigh, and Brumfitt, 1968).

For the purposes of comparison *E. coli* strains were also isolated from rectal swabs.

SERUM

Samples of serum were obtained from a panel of healthy hospital and laboratory staff, pooled and used immediately. Sera from all volunteers contained O antibody against the test organisms when examined by the passive haemagglutination technique of Kunin (1962). Complement activity was detected by the ability of serum dilutions to lyse sheep red cells sensitized with antisherp red cell serum.

SERUM BACTERICIDAL REACTION

Bacterial sensitivity to normal human serum was estimated by the technique of Taylor, Roberts, and Gower (1972). An early log phase culture in nutrient broth is washed in 0.06M NaCl and resuspended in 0.05 M Tris-HCl buffer pH 8.4 to a concentration of 1×10^6 organisms/ml. A sample (1 ml) of this suspension is added to 3 ml of serum and viable counts are obtained, using the pour plate technique, at the beginning of each test and after one, two, and three hours' incubation at 37°C. The results are graded into six categories as shown in figure 1.

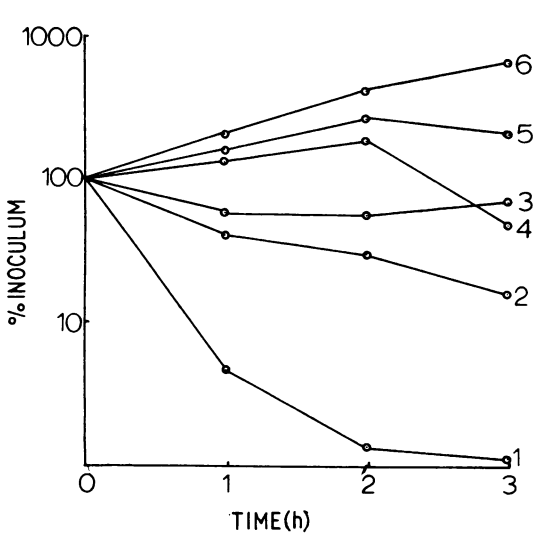


Fig 1 The various grades of bacterial response to normal human serum. Grade 1, progressive decrease in viable count at each hourly interval with a final count equal to or less than 10% of inoculum. Grade 2, progressive decrease in viable count at each hourly interval with a final count greater than 10% of inoculum. Grade 3, viable counts at all intervals less than the inoculum but not showing an overall progressive decrease. Grade 4, viable counts greater and less than the inoculum. Grade 5, viable counts at all intervals greater than the inoculum but not showing an overall progressive increase or showing a progressive increase but with a final count less than 200% of inoculum. Grade 6, progressive increase in viable count at each hourly interval with at least a doubling of the inoculum after three hours.

Results

The sensitivity to normal human serum of 144 urinary strains of *E. coli* was determined: the results are shown in relation to the O serogroup in table I.

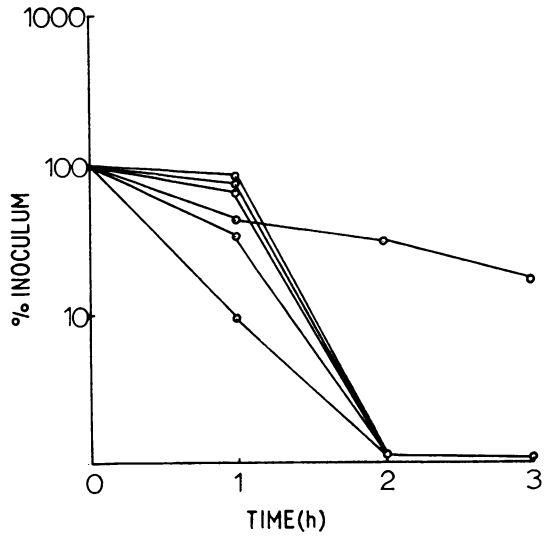


Fig 2 The interaction of some strains of *E. coli* 075 and normal human serum. Four strains were rapidly killed by serum after a delay of one hr; two other strains are shown for comparison.

To facilitate the comparison of results between serogroups, strains falling into grades 1 and 2 were considered to be serum-sensitive and strains falling into grades 5 and 6 were regarded as serum-resistant; grades 3 and 4 were considered as intermediate. It is clear that serum-sensitive and serum-resistant strains are not randomly distributed throughout the common urinary O serogroups of *E. coli*. All 20 strains of *E. coli* 075 were serum-sensitive. No serum-resistant strains of *E. coli* 01 were found. In contrast, only three of 20 *E. coli* 07 strains, four of 20 *E. coli* 018 strains, and five of 20 *E. coli* 06 strains were sensitive to serum. Some serum-sensitive strains were unusual in that they were killed rapidly

Serotype	Number of Strains			
	Sensitive Grades 1, 2	Intermediate Grades 3, 4	Resistant Grades 5, 6	Total
01	13	7	0	20
02	8	3	1	12
04	12	5	3	20
06	5	8	7	20
07	3	8	9	20
09	4	0	1	5
011	1	5	0	6
018	4	5	11	20
039	1	0	0	1
075	20	0	0	20
Totals	71	41	32	144

Table I Distribution of serum-sensitive and serum-resistant urinary strains of *E. coli* in relation to O serotype

Serotype	Number of Strains			
	Sensitive Grades 1, 2	Intermediate Grades 3, 4	Resistant Grades 5, 6	Total
01	3	7	0	10
02	6	0	0	6
04	5	3	0	8
06	3	3	1	7
07	1	2	3	6
09	1	1	0	2
011	0	3	0	3
018	2	5	4	11
075	10	0	0	10
Totals	31	24	8	63

Table II *Distribution of serum-sensitive and serum-resistant rectal strains of E. coli in relation to O serotype*

by serum but only after a delay of one hour. A number of *E. coli* 075 strains responded in this way (fig 2).

As the response to serum of these urinary strains may have been unrepresentative of these serotypes in general, rectal isolates of these O serogroups were also studied (table II). Although many of these strains were from individuals free of urinary infection, some were isolated from patients with urinary infection when the rectal strain did not correspond in O serogroup to the strain causing the infection. With the possible exception of *E. coli* 06, the distribution of serum-sensitive and serum-resistant strains was similar to that of the urinary strains.

Discussion

The results of this survey confirmed the initial impression that serum-sensitive and serum-resistant urinary strains of *E. coli* are not randomly distributed throughout the 10 O serogroups commonly associated with urinary tract infection (table I). Serogroups 075, 01, 02, 04, and 09 appear to be composed predominantly of serum-sensitive strains. All *E. coli* 075 strains examined proved to be serum-sensitive and strains from serogroup 01 were either sensitive to serum or of intermediate sensitivity. Although serum-resistant strains belonging to serogroups 02, 04, and 09 were detected, the majority of strains in these three groups were susceptible to human serum. All but one of the six strains in serogroup 011 gave an intermediate grade of response. Serogroups 06, 07, and 018 were basically serum-resistant, only 12 of 60 strains being sensitive to serum.

In general, the rectal isolates responded to serum in a similar manner to the urinary strains (table II). Serogroups 075, 01, 02, 04, and 09 were mainly serum-sensitive, 07 and 018 were largely serum-resistant, and all three 011 strains were of intermediate sensitivity. *E. coli* 06 may represent an

exception to this correspondence; whereas urinary *E. coli* 06 strains were frequently serum-resistant, only one of seven rectal strains was found to be resistant to serum.

The results of the present study appear to contradict those of Henkel (1970). This author examined six urinary strains of *E. coli* from each of serogroups 01, 02, 04, and 07 and found no correlation between O serogroup and sensitivity to serum. In the present study, however, no serum-resistant *E. coli* 01 strains were found. Although the other three O serogroups contained both serum-sensitive and serum-resistant strains, the distribution was not random; *E. coli* 02 and 04 were generally sensitive while *E. coli* 07 strains were largely resistant. These trends may not be apparent if few strains are studied.

Differences in the distribution of serum-sensitive and serum-resistant strains amongst the various urinary O serogroups have been demonstrated in previous studies (Kimball, Garcia, and Petersdorf, 1964; Vosti and Randall, 1970). However, differences between these studies and the present study are apparent. In the study of Kimball *et al* (1964) resistant strains were frequently found in serogroups 01, 04, and 075. However, strains were classed as serum-resistant if more than 50% of cells survived for 90 minutes in a bactericidal system containing a large proportion of nutrient broth. It has been established that certain broth constituents may interfere with the serum bactericidal reaction (Michael and Braun, 1959). In addition, some strains used in the present study were only killed by serum after a delay of one hour (fig 2); in the bactericidal system of Kimball *et al* these strains may have been classed as serum-resistant. Vosti and Randall (1970) estimated the survival of stationary phase cultures of *E. coli* strains after incubation for two hours in normal human serum. In contrast to the findings of the present study many *E. coli* 01 and 075 strains were found to be serum-resistant. However, stationary phase cultures are known to be less sensitive to

serum than cultures in the log phase of growth (Rowley and Wardlaw, 1958).

The present study demonstrates that resistance of *E. coli* strains to the serum bactericidal system is related, at least in part, to the qualitative character of the O antigen. Any study attempting to correlate quantitative aspects of this antigen with resistance must therefore compare serum-resistant and serum-sensitive strains of the same O serogroup.

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