

# Occurrence of K 1, K5 and O antigens in *Escherichia coli* isolates from patients with urinary tract infections or bacteraemia

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**Summary.** The distribution of K1, K5 and O antigens was examined in 500 clinical strains of *Escherichia coli*. Of 400 strains from urine, 52% belonged to serogroups O1, O2, O4, O6, O7, O8, O9, O18, O25 and O75; 34% were non-typable (NT) and 14% were autoagglutinable (AA). Antigen K1 was carried by 17% of these strains, and K5 by 15%. The numbers of O-serogroupable, NT and AA strains among 100 strains from blood were 62, 29 and 9, respectively. K1 antigen was detected on 20% of isolates from blood and K5 on 13%. There was no statistically significant difference in the distribution of K1, K5 or O antigens between strains from blood compared with those from urine. The occurrence of K1 and K5 antigens among smooth and AA strains suggested that AA strains were derived primarily from the common O-serogroups.

## Introduction

The K1 antigen is thought by many to be an important virulence factor of *Escherichia coli* because c. 80% of strains isolated from cases of neonatal meningitis carry it.<sup>1</sup> However, there is relatively little information about the prevalence of K1 in *E. coli* strains isolated from other extra-intestinal infections. The few studies from which results are available reveal an unclear picture; some report that K1 occurs significantly more frequently in *E. coli* strains isolated from blood than in those from urine<sup>2,3</sup> whereas others have not found this difference.<sup>1,4</sup>

It has been claimed repeatedly that the K5 antigen occurs frequently amongst *E. coli* strains isolated from extra-intestinal infections, although there is little published work to substantiate this claim. In part, this may be because K5 is a poor immunogen, probably because of its structural similarity to desulphoheparin,<sup>5</sup> and detection by traditional serological means has been difficult. The K1 antigen is also a poor immunogen,<sup>6,7,8</sup> but its detection has been greatly facilitated by the use of K1-specific bacteriophages.<sup>9</sup> A bacteriophage which recognises the K5 antigen has also been isolated.<sup>10,11</sup> These K antigen-specific phages

facilitate investigations into the distribution and prevalence of the K1 and K5 antigens in *E. coli*.

Geographical factors have an important influence on the frequency of occurrence of the O-antigenic types of *E. coli*,<sup>12</sup> and the presence of K1 antigen.<sup>4,13</sup> Most published studies concerning K1 have originated in the USA, and, regrettably, these have rarely given adequate details of the O antigens of the strains. This information is important when comparisons are made between *E. coli* isolates from different clinical conditions.

In this UK study, 500 clinical isolates of *E. coli* were examined for the presence of K1 and K5 antigens and O-antigenic type. The relationships between the serotypes of strains from patients with urinary tract infections (UTI) or bacteraemia were investigated.

## Materials and methods

### Bacteria

Over a period of c. 3 years ending in August 1986, 400 strains of *E. coli* from mid-stream specimens of urine infected with *E. coli* ( $>10^5$  cfu/ml) were obtained from patients attending the urinary tract infection clinics at Charing Cross Hospital; 100 strains of *E. coli* were also isolated from the blood of hospital in-patients. Only one isolate per patient was included in the study unless a second isolate was of a different O-serogroup or of a different K-antigenic type. *E. coli* strains 20896 and 20440

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were supplied by Dr C. Hughes (Institut für Genetik und Mikrobiologie, 8700 Würzburg, West Germany) and were used to propagate K1- and K5-specific bacteriophages respectively. All isolates were stored on nutrient agar slopes at 4°C in the dark.

### Bacteriophages

Phages  $\phi$ K1GS and  $\phi$ K5DG, specific respectively for the K1 and K5 antigens of *E. coli*, were supplied by Dr C. Hughes. To propagate the phages, 20 ml of Nutrient Broth (Oxoid No. 2, CM67) were inoculated with the appropriate propagating strain of *E. coli* and incubated at 37°C for 4 h. Two drops of phage suspension were added and the culture was incubated for a further 15–18 h at 37°C. The culture was then centrifuged and the supernate filtered through a 0.22- $\mu$ m porosity membrane. The resulting phage suspension was stored in the dark at 4°C.

### Detection of K1 and K5 antigens

*E. coli* strains bearing K1 or K5 antigens were identified by sensitivity to the above phages. Test strains of *E. coli* were incubated at 37°C for 4 h in 4 ml of peptone water (Oxoid CM9). A single streak of each culture was then made on Cystine Lactose Electrolyte Deficient Agar (CLED; Oxoid CM301) and allowed to dry. A small drop of each phage suspension, from a wire loop of c. 1 mm internal diameter, was placed on each streak. This size of loop allowed delivery of a volume of phage suspension which gave a zone of lysis of c. 3–4 mm with susceptible strains but caused minimal disruption of the bacterial streak during application. Plates were then incubated at 37°C for 15–18 h. The presence of either the K1 or K5 antigen was indicated by a distinct reduction in growth where the appropriate phage suspension had been placed.

### Detection of O antigens

Isolates were O-serogrouped with *E. coli* O1, O2, O4, O6, O7, O8, O9, O18, O25 and O75 antisera. Isolates which failed to agglutinate with any of these sera were defined here as being non-typable (NT), whereas those which agglutinated in all sera and in saline (NaCl 0.85% w/v) were termed autoagglutinable (AA). Because of the report of an epidemic of multiresistant *E. coli* O15,<sup>14</sup> all NT isolates were also examined for the presence of this antigen with *E. coli* O15 antiserum.

## Results

Of the 400 urinary isolates (table I), 52% (208 strains) were O-serogroupable. A further 34% (138 strains) were NT, and 14% (54 strains) were AA. Three isolates carried the O15 antigen but none was multiresistant. The K1 antigen was associated with 17% (66) and the K5 antigen with a further 15% (61) of the strains from urine.

**Table I.** The occurrence of O, K1 and K5 antigens in *E. coli* strains isolated from patients with urinary tract infections

O antigen of strain	Number of strains	Number of strains with	
		K1 antigen	K5 antigen
O1	14	11	1
O2	20	13	2
O4	19	0	0
O6	39	0	6
O7	10	8	0
O8	18	0	0
O9	16	0	0
O18	40	11	24
O25	10	0	6
O75	22	1	16
Number (% of total) of:			
O-serogroupable strains	208 (52)	44 (11)	55 (14)
Autoagglutinating strains	54 (14)	14 (4)	3 (<1)
Non-typable strains	138 (34)	8 (2)	3 (<1)
Total number of strains (%)	400 (100)	66 (17)	61 (15)

**Table II.** The occurrence of O, K1 and K5 antigens in *E. coli* strains isolated from patients with bacteraemia

O antigen of strain	Number of strains	Number of strains with	
		K1 antigen	K5 antigen
O1	6	6	0
O2	4	2	1
O4	5	0	0
O6	11	0	1
O7	7	6	0
O8	9	0	0
O9	4	0	0
O18	5	1	3
O25	3	0	1
O75	8	1	5
Number of:			
O-serogroupable strains	62	16	11
Autoagglutinating strains	9	0	2
Non-typable strains	29	4	0
Total number of strains	100	20	13

Of the 100 isolates from blood (table II), 62% were O-serogroupable. NT strains accounted for 29% and AA strains for 9% of isolates; no isolate carried the O15 antigen. The K1 antigen was carried by 20% of the isolates from blood and the K5 antigen by 13%.

Neither the K1 nor the K5 antigen was randomly

distributed among strains belonging to the common O-serogroups. For *E. coli* strains from both urine and blood, the K1 antigen occurred in most strains belonging to serogroups O1 (17 of 20) and O7 (14 of 17), but in smaller proportions of isolates of serogroups O2 (15 of 24), O18 (12 of 45) and O75 (2 of 30). However, the K5 antigen was found to be associated relatively frequently with strains of serogroups O18 (27 of 45), O25 (7 of 13) and O75 (21 of 30), and less frequently with strains of serogroups O1 (1 of 20), O2 (3 of 24) and O6 (7 of 50).

The difference in the proportion of AA strains between *E. coli* from urine compared with those from blood did not reach statistical significance ( $\chi^2$  with Yates' correction = 1.0908;  $0.3 > p > 0.2$ ). Similarly, when the proportion of O-serogroupable and NT isolates was considered between strains from urine and blood, no significant difference was found ( $\chi^2 = 1.6361$ ;  $0.3 > p > 0.2$ ). However, because different proportions of the two groups of strains were O-serogroupable (52% of strains from UTI, and 62% from bacteraemia), it was considered valid to compare only the distribution of individual O-serogroups with other O-serogroupable strains. In no instance did the difference in distribution of individual O-serogroups reach statistical significance. The maximum value for  $\chi^2$  was found in comparing strains belonging to serogroup O18 (40 of 208 from urine, 5 of 62 from blood;  $\chi^2 = 3.5215$ ;  $0.1 > p > 0.05$ ).

For all strains, the difference in proportion of K1 antigen-bearing strains between isolates from urine and blood was not statistically significant ( $\chi^2 = 0.4646$ ;  $0.5 > p > 0.4$ ). Similarly, although in some cases the numbers were small, no significant difference was found in the distribution of the K1 antigen amongst AA strains, NT strains or O-serogroupable strains (maximum  $\chi^2 = 2.0485$ ;  $0.2 > p > 0.1$ ). A similar comparison of the distribution of the K5 antigen between strains from urine and blood showed that there was no significant difference (maximum  $\chi^2 = 1.5148$ ;  $0.3 > p > 0.2$ ).

Of the total of 270 O-serogroupable strains, 126 (47%) possessed either the K1 or the K5 antigen, compared with only 9% (15 of 167) of NT strains. This difference was highly significant ( $\chi^2 = 65.334$ ;  $p < 0.001$ ). Of the 63 AA strains 19 (30%) had one or other of these antigens. This proportion, compared with that for the O-serogroupable strains, was probably significant ( $\chi^2 = 5.011$ ;  $p < 0.05$ ), and when compared with that for the NT isolates was highly significant ( $\chi^2 = 14.646$ ;  $p < 0.001$ ). It indicates that the AA strains were derived primarily from the common O-serogroup strains.

## Discussion

Only a few other studies, none originating in the UK, have examined the frequency of occurrence of the K1 antigen in *E. coli* isolates from patients with urinary tract infections (UTI). In this study, 17% of the isolates from urine had the K1 antigen. In American studies, Evans *et al.*<sup>2</sup> also found that 17% of 395 isolates from urine carried the K1 antigen, whereas others reported only 11% of 209 isolates to have the antigen.<sup>1, 4, 15</sup>

In this study, 20% of the *E. coli* isolates from blood carried the K1 antigen. Previously reported values of the frequency of *E. coli* isolates from cases of adult bacteraemia bearing the K1 antigen have varied from 8–12%<sup>1, 4, 16</sup> to between 18% and 32%.<sup>2, 17, 18, 19</sup> In the only other UK study, Cheasty *et al.*<sup>20</sup> found that 13% of *E. coli* isolates from patients above 3 years old with bacteraemia carried the K1 antigen.

Both the K1 and K5 antigens were associated with a limited number of O-serogroups. Sometimes almost all strains of a single O-serogroup carried them. In view of this, since the rates of isolation of specific O-serogroups vary geographically,<sup>12</sup> it is not surprising that the same has been found when studying the occurrence of *E. coli* K1 antigen.<sup>4, 13</sup> The strong association between K antigens and particular O antigens means that it is important to consider them together, particularly when comparisons are being made between the occurrence of specific K antigens in strains isolated from different sites.

There was no statistically significant difference in the occurrence of K1, K5 or O antigens in strains of *E. coli* isolated from urine compared with those from blood. Previous studies have reported similar findings concerning the K1 antigen.<sup>1, 4</sup> Because it is generally accepted that most bacteraemias arise from infected urinary tracts, this is perhaps what could be expected. However, there have been reports of significantly different isolation rates of *E. coli* strains carrying the K1 antigen from these infected sites<sup>2, 3</sup> and it may be that not all strains causing UTI are capable of initiating bacteraemia.

Following the discovery that strains of *E. coli* bearing the K1 antigen are associated very frequently with cases of *E. coli* neonatal meningitis, the possible role of the K1 antigen as a virulence factor in other infections has been examined. High frequency of occurrence has been found consistently only among strains isolated from neonates with bacteraemia or meningitis,<sup>1, 4, 15, 21, 22</sup> so clearly neonates comprise a special group. In other infections, it has been suggested that no single bacterial

property is responsible for the pathogenicity of *E. coli* but rather, specific combinations of O, K and H antigens, as well as haemolysin production and possession of fimbriae, are thought to be important.<sup>3, 20, 22, 23, 24, 25</sup> Furthermore, host factors must not be discounted, as the acquisition and outcome of bacteraemia may depend upon the nature of the underlying disease.<sup>26, 27</sup>

Although it has been stated frequently that *E. coli* strains carrying the K5 antigen are often found among isolates from extra-intestinal infections,<sup>5, 10, 11, 28</sup> supporting data were not given. Detection of this poorly immunogenic antigen was facilitated in this study by the use of a K5-specific bacteriophage.<sup>10</sup> The K5 antigen was detected in 15% of strains from urine, and in 13% of strains from blood, a difference which was not statistically significant. Kaijser and Jodal<sup>29</sup> detected the K5 antigen in 6–12% of strains from urine and in 17% from blood. The present study showed that, like the K1 antigen, the K5 antigen was also strongly associated with particular O-serogroups and must, therefore, also be expected to vary geographically in the frequency of its occurrence. Interestingly, almost all the O75 strains in this study carried the K5 antigen, and this serogroup showed the greatest variation in isolation rate in the study by Grüneberg *et al.*<sup>12</sup>

Among the urinary isolates we examined, the K1 and K5 antigens occurred with similar frequency—17% and 15% respectively. In the strains from blood, however, the K1 antigen occurred in 20% of strains but the K5 antigen in only 13% of strains. This difference is almost entirely accounted for by the increased rate of isolation of O1 and O7 strains (high frequency of K1), and the reduced rate of isolation of O18 strains (high frequency of K5) among the isolates from blood compared with those from urine. Indeed, the difference would have been even greater but for the increased frequency of O75 strains (high frequency of K5) in the isolates from blood.

The K1 and K5 antigens occurred in almost half the isolates belonging to the common O-serogroups and are clearly associated with these groups. Only rarely did they occur in other smooth strains. The frequency of occurrence of these K antigens in AA strains provides further evidence that such strains are largely derived from the common O-serogroups.<sup>30, 31</sup>

The epidemic strain of multiresistant *E. coli* O15 in the London area<sup>14</sup> was first isolated in October 1986. However, only three isolates (UTI) among the 500 strains collected up to August 1986 carried the O15 antigen and none was multiresistant.

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