UROPATHOGENIC PROPERTIES OF *Escherichia coli* in Recurrent Urinary-tract Infection

Heather J. L. Brooks*, F. O'Grady†, M. Anne McSherry and W. R. Cattell

**Department of Medical Microbiology and Department of Nephrology,**
**St Bartholomew's Hospital, London EC1A 7BE**

*Escherichia coli* is the pathogen most commonly isolated in urinary-tract infections (UTI). It is thought that *E. coli* from the gut colonise the periurethral area, extend into the anterior urethra and are introduced into the bladder during micturition. Imperfections in the host defence mechanisms allow bacteria to multiply in the bladder urine and, in some cases, to cause an ascending infection of the kidney (O'Grady *et al.*, 1970).

Unless urinary-tract infection differs from infection elsewhere in the body it is likely that some strains of *E. coli* possess properties that enable them to overcome host defences more easily. Numerous reports have examined whether certain serotypes of *E. coli* are more common in UTI simply because they are more common in the faeces or because they possess specific uropathogenic properties. The properties that might be implicated in the pathogenesis of UTI include: (1) the ability to colonise the urinary tract by the production of mucinase that enables the organisms to reach the uroepithelium, or the possession of fimbriae that allow adhesion to the mucosal surface; (2) preferential nutritional requirements for substances present in urine or relative resistance to urinary inhibitors such as urea or low pH; (3) resistance to phagocytosis and the serum bactericidal system; (4) the elaboration of toxins.

We studied several properties that may influence the pathogenicity of *E. coli* strains and compared their occurrence in strains isolated from (i) the urine of patients with UTI, (ii) the urethral meatus of nephrourological patients without current UTI and (iii) the urethral meatus of normal subjects.

**Materials and methods**

**Subjects**

One hundred and sixty seven patients and 28 controls were studied. The patients were referred to the Nephrourological Clinic at St Bartholomew's Hospital because of known or suspected UTI; all had been followed for at least 3 months. Intravenous pyelograms were routinely performed and, where necessary, residual urine volumes were measured by the
I\(^{131}\)-hippuran method (Shand et al., 1968). The patients were divided into two groups: (A) *abacteriuric patients* (62) remained free from infection with *E. coli* during the study. Nine patients were asymptomatic throughout; 53 had symptoms of frequency or dysuria or both, and were described as suffering from the "urethral syndrome"; (B) *bacteriuric patients* (105) had at least one episode of *E. coli* bacteriuria, i.e., two consecutive urine specimens contained \( > 10^5 \) bacteria/ml. They were subdivided according to the presence or absence of radiological abnormalities. The control group consisted of 28 healthy female laboratory workers with no history of UTI; none had symptoms of UTI or was taking antibiotics.

**Collection of specimens**

Periurethral swabs and urine specimens were collected and cultured as described by Cattell et al. (1974).

**Identification of strains**

Strains were identified as *E. coli* according to Edwards and Ewing (1972); they were maintained in nutrient-agar stabs at 4°C.

**Sources of strains**

*Bladder urine* strains were isolated from mid-stream specimens of urine from the 105 patients with bacteriuria; one isolate from each patient was randomly selected. Fifteen *upper tract* strains were from ureteric urine specimens from five male and 10 female patients undergoing localisation tests by the method of Stamey et al. (1965); two strains were isolated from the renal tissue of an excised kidney and two were from renal calculi from separate patients. Twenty nine *lower tract* strains were from seven male and 22 female patients undergoing localisation tests; the ureteric urine of these patients was sterile.

*Periurethral strains.* One hundred and fifty six strains were isolated from periurethral swabs. Forty three strains were from bacteriuric patients between episodes of bacteriuria; the patients were abacteriuric for at least 6 weeks before and 6 weeks after the periurethral strain was isolated. None was taking antibiotics during this period. Nine strains were thought to give rise to infection in that an episode of bacteriuria before or after isolation of the periurethral strain was due to an *E. coli* strain of the same serotype. Sixty eight strains were from abacteriuric patients; 58 were from patients with the "urethral syndrome" and 10 strains were from abacteriuric and asymptomatic patients. Forty five strains were from normal subjects.

**Studies of uropathogenic properties**

*O and H serotyping.* O-serotypes were determined with a set of 150 O-antisera (Bettelheim and Taylor, 1969). Motile strains were typed with 52 H-antisera (Chandler and Bettelheim, 1974).

*K antigen* was estimated quantitatively by the method of Glynn and Howard (1970).

*Serum sensitivity.* Sensitivity to the bactericidal activity of normal human serum was measured by the method of Taylor, Roberts and Gower (1972) except that viable counts were determined by the method of Miles, Misra and Irwin (1938). Serum was collected from four healthy volunteers with no previous history of UTI.

*Haemolysin production.* "Solid" haemolysin production on blood-agar layer plates and 'liquid' haemolysin productions in isotonic peptone water were determined by the method of Cooke (1968).

*Fimbriation.* The presence of fimbriae was detected by the methods of Duguid et al. (1955) and Duguid (1968).

*Fermentation tests.* Strains were incubated for up to one week in sucrose, salicin and dulcitol peptone-water sugars (Oxoid) and examined for acid production on days 1, 2, 3, 4, 5 and 7.

*Sensitivity to serine, spermine and urea.* Minimum inhibitory concentrations (MICs) of DL
serine, spermine and urea were determined with the minimal medium of Davis and Mingioli (1950) solidified with 1% agarose (Miles Servac) for all strains at pH 7.2 and for randomly selected strains at pH 5.5 and 6.0. Twofold dilutions of serine and spermine were incorporated in the plates to give final concentrations of 1–1024 µg/ml and 32–512 µg/ml respectively. Urea was tested at concentrations of 0.5–0.6% in 0.5% steps. Plates were inoculated with 0.001 ml of an overnight broth culture diluted 100-fold (10^2–10^3 organisms) by a multipoint inoculator (Denley Instruments Ltd). The MIC was the lowest concentration that limited growth to < 5 colonies or a barely discernible haze of growth after overnight incubation at 37°C.

Growth requirements. The growth requirements of strains unable to grow on minimal medium were assessed by the method of Holliday (1956).

Mucinase production. Mucinase activity was detected by the method of Ross (1959); 1% cetrimide was the flocculating agent.

RESULTS

Results obtained from 309 test strains are shown in table I.

O and H Serotypes

The differences in prevalence of serotypes between the groups of strains were not significant except with serotypes O75 and H5 which were more common amongst strains isolated from urine than amongst periurethral strains from normal subjects (0.05 > p > 0.02 for both serotypes); 55% of the smooth, typable strains from the upper tract belonged to serotype O75, but the total number of strains in this group was small and the incidence of O75 strains was not significantly higher amongst strains derived from the upper tract than amongst those from infections confined to the lower tract.

Serotypes O2, O4, O6, O8, O18ab and O75 were the most common serotypes amongst strains recovered from mid-stream urine and accounted for 54% of smooth, typable isolates. Only 16% of smooth, typable periurethral strains from normal subjects belonged to these six O-serotypes and this difference was statistically significant (0.01 > p > 0.001). Most (82%) smooth, typable strains from the upper tract belonged to serotypes O2, O4, O6, O8, O18ab and O75 whereas these types were significantly less common (44%) amongst strains from the lower tract (0.05 > p > 0.02).

Sixty percent of H-serotypable urinary strains and 39% of periurethral strains from normal subjects belonged to H-serotypes H1, H4, H5 and H7; this difference was not statistically significant. An excess of particular combinations of O- and H-serotypes was not found in any group of strains but most O75 strains were either non-motile or of type H5.

K-antigen content

The K-antigen titres are shown in table II. Approximately the same proportion of strains in each group had K-antigen titres of ≥ 1024. However, more urinary strains had titres of 32–512 (K-rich) compared with periurethral strains from normal subjects (p = 0.008). More strains from the upper tract
<table>
<thead>
<tr>
<th>Source* of strain</th>
<th>Number of strains total smooth typable</th>
<th>Percentage of smooth typable strains belonging to serogroup</th>
<th>Percentage of strains that possessed the given property</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O75 O2 O4 O6 O8 O18ab O75</td>
<td>K-antigen titre ≥ 32 Resistance to serum Haemolysin production Fimbriation Salicin fermentation Growth on minimal medium Mucinase production</td>
</tr>
<tr>
<td>BU</td>
<td>105</td>
<td>17 54 60</td>
<td>66 39 43 79</td>
</tr>
<tr>
<td>UT</td>
<td>19</td>
<td>55 82 57</td>
<td>65 40 58 79</td>
</tr>
<tr>
<td>LT</td>
<td>29</td>
<td>19 44 39</td>
<td>48 27 27 83</td>
</tr>
<tr>
<td>AP</td>
<td>68</td>
<td>14 32 41</td>
<td>60 23 21 74</td>
</tr>
<tr>
<td>BP</td>
<td>43</td>
<td>21 41 59</td>
<td>58 33 28 70</td>
</tr>
<tr>
<td>CP</td>
<td>45</td>
<td>3 16 39</td>
<td>44 33 9 60</td>
</tr>
</tbody>
</table>

* BU = Bladder urine; UT = upper tract; LT = lower tract only; AP = periurethral area of abacteriuric patients; BP = periurethral area of bacteriuric patients between infections; CP = periurethral area of normal subjects (controls).
TABLE II

K-antigen titres of strains of Escherichia coli from urine and periurethral areas of patients and normal subjects

<table>
<thead>
<tr>
<th>Source of strain*</th>
<th>Percentage of strains in each group with K-antigen titres of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0   1  2  4  8  16  32  64  128  256  512  1024</td>
</tr>
<tr>
<td>BU</td>
<td>28  2  1  0  1  10  9  21  14  1</td>
</tr>
<tr>
<td>UT</td>
<td>26  5  5  0  11  16  11  21</td>
</tr>
<tr>
<td>LT</td>
<td>48  4  0  0  17  17  10  4</td>
</tr>
<tr>
<td>AP</td>
<td>38  2  2  0  16  15  18  7</td>
</tr>
<tr>
<td>BP</td>
<td>37  3  5  3  16  16  9   9</td>
</tr>
<tr>
<td>CP</td>
<td>54  2  0  0  2  4   7  9  11</td>
</tr>
</tbody>
</table>

* See footnote to table I.

(64%) had K-antigen titres of \( \geq 32 \) than strains from the lower tract (48%), but this difference was just outside the accepted level of statistical significance \( p=0.06 \). There was no difference in K-antigen titres between strains from the lower-tract and periurethral strains from normal subjects.

Serum sensitivity

Most rough strains (82%) were sensitive to the bactericidal activity of serum. *E. coli* strains may develop rough colonies on storage and these strains were excluded from analysis. The serum sensitivity of smooth urinary strains was similar to that of periurethral strains from normal subjects. Strains from the lower tract were slightly more sensitive than those from the upper tract but this was not statistically significant. The K-antigen content of smooth strains was not directly related to their serum sensitivity but a few strains (15) had K-antigen titres of \( < 32 \) and were resistant to the bactericidal activity of serum (table III). Only seven rough strains were serum resistant, of which two strains had K-antigen titres of \( < 32 \), but not all rough strains were devoid of K antigen (table III).

TABLE III

Relationship of K-antigen titre to serum sensitivity

<table>
<thead>
<tr>
<th>Reaction with serum</th>
<th>Type of agglutination reaction</th>
<th>Number of strains with K-antigen titres of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \leq 1/32 )</td>
</tr>
<tr>
<td>Resistant</td>
<td>smooth</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>rough</td>
<td>2</td>
</tr>
<tr>
<td>Sensitive</td>
<td>smooth</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>rough</td>
<td>18</td>
</tr>
</tbody>
</table>
Haemolysin production

Most haemolytic strains produced both "liquid" and "solid" haemolysins; six strains produced only "liquid" haemolysin, but none produced only "solid" haemolysin. The number of urinary strains that were haemolytic (43%) was significantly greater than the number of haemolytic periurethral strains from normal subjects ($p < 0.001$). The majority of strains from the upper tract (58%) produced haemolysin compared with 27% of strains from the lower tract but this difference just failed to reach statistical significance ($0.1 > p > 0.05$). The proportion of haemolytic strains amongst lower-tract strains was significantly greater than amongst periurethral strains from normal subjects ($0.02 > p > 0.01$). Haemolysin production was associated with serogroups O4 and O6; 82% of haemolytic strains belonged to serotype O4 and 85% of serotype O6 strains were haemolytic.

Fimbriation

Most strains in all groups produced fimbriae, but a significantly higher proportion of urinary strains (79%) were fimbriate compared with periurethral strains from normal subjects (60%; $0.02 > p > 0.01$). There was no difference between the proportions of fimbriate strains from the upper and lower tracts.

Fermentation of sucrose, salicin and dulcitol

There were no significant differences between strains from different sources in their ability to ferment sucrose and dulcitol but a significantly greater proportion of urinary strains (80%) fermented salicin compared with periurethral strains from normal subjects (53%; $p < 0.001$). There was no difference between the fermentative abilities of strains from the upper and lower tracts.

Sensitivity to serine, spermine and urea

The mean MICs of serine, spermine and urea were almost identical for each group. Reducing the pH of the medium resulted in an increase in the mean MIC of serine and almost total abolition of the antibacterial activity of spermine (fig. 1). In general, strains were more sensitive to urea at the lower pH, but a few strains were not affected by pH changes (fig. 2).

Growth requirements of strains

Significantly fewer strains from the upper tract (32%) grew on unsupplemented minimal medium compared with strains from the lower tract (69%; $0.05 > p > 0.02$). There were no other significant differences between the groups. Strains unable to grow on minimal medium varied in nutritional requirements; a few failed to grow on any of the supplemented plates.
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FIG. 1.—Effect of pH on the inhibition of Escherichia coli by serine.

FIG. 2.—Effect of pH on the inhibition of Escherichia coli by urea.
Mucinase production

Mucinase was not detected in most strains. There was no correlation between the mucinase titres of periurethral strains and the degree of colonisation as reflected in the number of colonies recovered from the periurethral swabs.

Combinations of uropathogenic properties

Five properties were significantly more common amongst urinary strains than amongst periurethral strains from normal subjects. Strains were awarded a score of 1 for each of the following properties: (i) O-serotypes 2, 4, 6, 8, 18ab or 75; (ii) K-antigen titre >32; (iii) haemolysin production; (iv) fimbriation; (v) salicin fermentation. The results are shown in table IV. Urinary strains had significantly higher scores than periurethral strains from bacteriuric patients between infections (p = 0.0018), abacteriuric patients (p = 0.0018) and normal subjects (p = <0.001). Periurethral strains from bacteriuric patients between infections were not significantly different from those from abacteriuric patients but strains from both groups had significantly higher scores than those from normal subjects (p < 0.001). The exclusion of periurethral strains from bacteriuric patients which subsequently gave rise to infection did not significantly alter the mean score for that group. Periurethral strains from patients with the "urethral syndrome" did not differ from those recovered from asymptomatic, abacteriuric patients. Strains from the upper tract had significantly higher scores than strains from the lower tract (p = 0.03) and strains from the lower tract had significantly higher scores than periurethral strains from normal subjects (p = 0.006).

DISCUSSION

Five of the potentially uropathogenic properties of E. coli that we examined were more common amongst strains from urinary infections and significantly

<table>
<thead>
<tr>
<th>Source of strains*</th>
<th>Number of strains</th>
<th>Percentage of strains with a score† of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt; 1</td>
</tr>
<tr>
<td>BU</td>
<td>105</td>
<td>99</td>
</tr>
<tr>
<td>UT</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>LT</td>
<td>29</td>
<td>93</td>
</tr>
<tr>
<td>AP</td>
<td>68</td>
<td>96</td>
</tr>
<tr>
<td>BP</td>
<td>43</td>
<td>98</td>
</tr>
<tr>
<td>CP</td>
<td>45</td>
<td>89</td>
</tr>
</tbody>
</table>

* See footnote to table I.
† See text.
less common amongst periurethral strains from normal subjects. These were:
(1) O-serotypes 2, 4, 6, 8, 18ab and 75, (2) high K-antigen titre, (3) haemolysin
production, (4) production of fimbriae, (5) salicin fermentation. Some of
these properties may confer resistance to host defence mechanisms; possession
of K-antigen confers resistance to phagocytosis, antibody binding and killing
by complement, and the degree of resistance depends upon the amount of
antigen (Glynn and Howard, 1970; Howard and Glynn, 1971). In the present
study, the high incidence of K-rich urinary strains suggests that this property is
important in the pathogenicity of E. coli, but we were unable to confirm the
relationship between serum-resistance and K-antigen content observed by
Howard and Glynn (1971). Moreover, we did not find that this property
influenced the localisation of infection, although Glynn, Brumfitt and Howard
(1971) found that patients with renal infection were usually infected with
K-rich strains whereas patients with lower tract infection were not.

The prevalence of haemolytic strains in urinary infections suggests that
haemolysin production is related to the ability to invade and infect the urinary
tract. A correlation was also observed between haemolysin production and
localisation of infection and it seems likely that haemolytic strains are selected
during ascending invasion of the urinary tract. This supports the hypothesis
that haemolytic strains have a superior ability to infect the kidney, possibly due
to the inhibitory effect of free haemoglobin on the serum bactericidal system
(Bullen and Rogers, 1969)

Eden et al. (1978) suggested that fimbriae enable E. coli to adhere to the
epithelial cells lining the urinary tract and that fimbriate strains might resist
removal by hydrokinetic clearance. Fimbriate strains were isolated frequently
from urinary infections in the present study, but the relationship between
fimbriation and uropathogenicity was not strong because fimbriae could not be
demonstrated in 21% of urinary isolates.

The observed correlations of pathogenicity with O-serotype and salicin
fermentation are less readily explained in terms of resistance to host defence
mechanisms. Although six O-serotypes (O2, O4, O6, O8, O18ab and O75) were
responsible for the majority of urinary infections, a considerable propor-
tion were due to other O-serotypes. Other authors (Vosti, Goldberg and
Rantz, 1965; Gruneberg, Leigh and Brumfitt, 1968; Ganguli, 1970; Dootson,
Maclaren and Titcombe, 1973) have reported similar findings, but different
O-serotypes have been predominant in the various series. The prevalence of
O-serotypes 4 and 6 amongst urinary strains may be related to haemolysin
production. Most of our strains that belonged to serotypes O4 and O6 were
haemolytic and this association has been reported by several other authors
(Vahlne, 1945; Sjöstedt, 1946; Cooke and Ewins, 1975).

McCabe and Jackson (1960) observed that strains from patients with
pyelonephritis often failed to ferment sucrose and salicin, but most of our
urinary strains fermented salicin. We were unable to demonstrate any differ-
ence in the fermentative abilities of strains recovered from the upper and lower
tracts and the reported relationship between fermentation of certain sugars
and ability to cause urinary infection may be fortuitous.
Previous workers had suggested that serum resistance, mucinase production and ability to grow in the presence of serine, spermine and urea were related to uropathogenicity but these properties were not more common in our urinary strains. The decrease in inhibitory activity of spermine and serine at low pH indicates that these components of normal urine have little inhibitory activity \textit{in vivo}. However, urea was more inhibitory at low pH. The conditions of low pH and high urea content in urine at night may inhibit bacterial growth at a time when hydrokinetic clearance is minimal.

Our observations on strains from human infections contrast with those of Guze et al. (1973) who correlated virulence of \textit{E. coli} for the mouse kidney with ability to multiply in minimal medium; many of our strains from upper-tract infections were unable to grow on minimal medium. Similarly there was little correlation between H-serotype and site of isolation; the excess of H5 strains in urinary infections probably reflects the prevalence of O75 strains.

The relationship between ability to cause infection and possession of any one of the five properties that were more common in urinary strains was not particularly strong. However, the possibility that pathogenicity is a multifactorial phenomenon was examined with a scoring system that gave each property equal weight. Strains with high scores for the combined properties were common amongst urinary isolates and rare amongst periurethral isolates from normal subjects. This indicates that strains rich in the combination of these pathogenic properties possess a greater ability to invade the urinary tract. However, strains poor in the combination of pathogenic properties are not necessarily harmless commensals; more than half of the urinary strains possessed fewer than four of the properties. This may reflect the state of the host defence mechanisms; infection with strains of low pathogenicity might occur when host defences are compromised. The localisation of infection also appears to be influenced by the properties of the infecting organism. Strains from upper-tract infections had higher scores for the combination of pathogenic properties than strains confined to the lower tract. It was noticeable that bacteriuric patients between episodes of infection were more often colonised by strains rich in pathogenic properties than were normal subjects, although the majority of these strains did not give rise to infection. These strains may have been the colonising strains of a previous infection or may have been seeded onto the periurethral area during a previous infection and persisted after the infection was eliminated.

**Summary**

Properties of \textit{Escherichia coli} considered to be important in the pathogenesis of urinary-tract infection were investigated. The following properties were more common in \textit{E. coli} strains isolated from urinary infections than in periurethral strains from healthy individuals: (i) O serogroups 2, 4, 6, 8, 18ab and 75; (ii) high K-antigen titre; (iii) production of haemolysin; (iv) production of fimbriae; (v) fermentation of salicin. The correlation between isolation of a strain from the urinary tract and possession of any single property was not
strong; however, strains rich in a combination of these pathogenic properties were rarely isolated from the periurethral area of healthy subjects but were common in urinary infections. Nevertheless, a significant proportion of urinary strains had few pathogenic properties. Strains rich in pathogenic properties were more commonly isolated from upper urinary-tract infections than from lower-tract infections; this indicates that the properties of the invading organism may influence the localisation of infection.

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