PATHOGENICITY

Virulence and other phenotypic characteristics of urinary isolates of cysteine-requiring *Escherichia coli*

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Summary. Urinary isolates of cysteine-requiring *Escherichia coli* were found to be generally lacking in virulence factors commonly associated with uropathogenic strains. The proportion of auxotrophic strains showing type-1 fimbriation, haemolysin production, motility and sensitivity to normal human serum was significantly less than that of a comparable number of urinary isolates of prototrophic *E. coli*, although the proportion in both groups possessing K1 antigen was similar. Furthermore, the biotyping and serogrouping of these and other strains from systemic infections demonstrated a high degree of phenotypic diversity. This is further evidence that infection with these auxotrophs results from a combination of decreased host resistance and a physiological condition conducive to the random selection of these auxotrophs *in vivo*.

Introduction

Cysteine-requiring (Cys⁻) *Escherichia coli* strains are found in about 1.5% of all urinary tract infections (UTI)¹ and in an even higher proportion of infections in older patients with some degree of renal impairment.² These strains, in common with other uropathogens, appear to be of bowel origin.² Their derivation from prototrophs *in vitro* is enhanced under conditions of cysteine and other sulphur compound excess,² a situation which occurs *in vivo* in patients with decreased renal function.^{3–5} Additionally, Cys⁻ *E. coli* may cause septicaemia which usually originates from the urinary tract.⁶

Little attention has been paid to the virulence characteristics associated with Cys⁻ *E. coli*, with only a single report noting the presence of K1 antigen in a Cys⁻ *E. coli* isolate from blood culture.⁷ Blood and urine isolates of Cys⁻ *E. coli* were examined for the presence of certain virulence characteristics commonly associated with uropathogenic strains. Additionally, Cys⁻ *E. coli* from UTI were biotyped and serotyped for somatic antigen to determine the extent of phenotypic similarities amongst Cys⁻ strains. These results were used to explain further the pathogenesis of Cys⁻ *E. coli* UTI and the concentration of this micro-organism in UTI in certain groups of patients.

Materials and methods

Strains

Eighty-eight Cys⁻ *E. coli* strains, isolated from different patients and identified in this laboratory^{6,8} and whose antibiotic sensitivities were determined by previously described methods,⁹ were examined. A further 11 Cys⁻ strains from blood, urine and abscesses of two patients and 34 auxotrophic strains from the urine of 12 patients with chronic or recurrent UTI were studied. The number of strains isolated from each patient in the latter group ranged from two to six collected over 27 months. A sample of 100 prototrophic *E. coli* strains from untreated patients with UTI was selected randomly.

Virulence characteristics

The presence of K1 capsular antigen, type-1 fimbriae, and haemolysin was investigated in the above strains. Monoclonal antibody to *Neisseria meningitidis* serogroup B antigen (Wellcome Diagnostics) was used to detect the presence of K1 antigen by agglutination of bacterial suspensions obtained after overnight growth on Brain Heart Infusion Agar (Oxoid) supplemented with 0.63 mM L-cysteine HCl. Type-1 fimbriation was determined by the method of Duguid and Gillies.¹⁰ Haemolytic properties were examined on bi-layered agar plates comprising a basal layer of

Table I. Arrangement of test results to construct a biocode for biotyping Cys⁻ *E. coli*

Test or substrate examined				Assigned value
Motility	Adonitol	Raffinose	Sorbitol	1
Arginine	Melibiose	Rhamnose	Sucrose	2
Ornithine	Myo-inositol	Salicin	Xylose	4

Columbia Agar (Oxoid) and a surface layer of Tryptone Soya Agar (Oxoid) containing washed horse erythrocytes 5% v/v and a duplicate plate supplemented with 0.63 mM L-cysteine HCl. Each strain was spot inoculated on to each medium and a haemolytic zone larger than the overlying colony after overnight incubation was considered a positive result.¹¹ Cys⁻ strains were examined for motility by observing their growth in semi-solid media¹² and by flagella staining¹³ and were compared with 1382 prototrophic *E. coli* strains from UTI isolated over the period of the study whose motility was assessed by growth in semi-solid media. The sensitivity of 58 Cys⁻ and 56 prototrophic *E. coli* (all from different patients) to the action of normal human serum (NHS) was established by the method of Olling¹⁴ except that phosphate-buffered saline, pH 7.3, was used instead of Hanks's solution. Probability of significant difference between the auxotrophs and prototrophs in the expression of each virulence factor was determined by the χ^2 test with Yates's correction for continuity with a computer software program (Instat® GraphPad™ software, San Diego, CA, USA).

Biotyping

The 45 strains which were multiple isolates from 14 patients were biotyped by determining their ability to utilise adonitol, D (+) melibiose, myo-inositol, raffinose, L (+) rhamnose, salicin, D-sorbitol, sucrose, D-xylose, L-arginine HCl and L-ornithine HCl (Sigma). Carbohydrate fermentation was tested in peptone water base¹⁵ and Moeller's method¹⁶ was used to determine amino-acid utilisation. These substrates were chosen because of the differences in their utilisation by different *E. coli*.¹⁷ A biocode was derived by

arranging the results for each test as shown in table I and Hunter and Gaston's application of Simpson's index of diversity¹⁸ was applied to determine the degree of separation of strains from the same and different patients.

Somatic serotyping

One hundred Cys⁻ *E. coli* strains were serotyped with antisera prepared in rabbits against O types 1–30, 32–46; 48–66; 68–92; 95–121 and 123–164 by techniques described previously.^{12, 19} An index of diversity was also calculated by the above method.

Results

The numbers of auxotrophic and prototrophic strains from UTI in which type-1 fimbriation, haemolysin, motility and K1 antigen virulence factors were detected are shown in table II. Results for haemolysin production were identical on both un-supplemented and cysteine-supplemented media. Similarly, motility as detected in a semi-solid medium correlated without exception with a positive flagella stain. The numbers and proportion of Cys⁻ *E. coli* which were motile, produced haemolysin and exhibited type-1 fimbriation were significantly less than the numbers of prototrophic strains with the same characteristics ($p \leq 0.0006$). Furthermore, auxotrophs were more sensitive to the action of NHS than prototrophs ($p = 0.0399$). In contrast, K1 antigen was present in approximately equal proportions of auxotrophic and prototrophic strains ($p = 0.8890$). K1 antigen was not present in any of the Cys⁻ strains resistant to killing by NHS but was present in 11 of 39 isolates sensitive or intermediately sensitive to NHS. Five of 30 prototrophic strains resistant to the action of NHS carried the K1 antigen as did nine of 26 sensitive or intermediately sensitive strains. K1 antigen was present in one of 11 Cys⁻ blood-culture isolates.

Twelve different biocodes were obtained with 45 auxotrophic strains isolated sequentially from 14 patients (table III). Two different biocodes were common to strains from four patients and the isolates from each of the remaining 10 patients had unique

Table II. Virulence factors present in Cys⁻ and prototrophic *E. coli* isolated from UTI

Virulence factor	Cys ⁻ <i>E. coli</i>		Prototrophic <i>E. coli</i>		p value
	n	Positive (%)	n	Positive (%)	
Type-1 fimbriation	86	50 (58)	100	84 (84)	0.0002
Haemolysin	88	9 (10)	100	32 (32)	0.0006
Motility	88	37 (42)	1382	951 (69)	0.0001
K1 antigen	87	19 (22)	100	21 (21)	NS
SBC activity	58	39 (67)	56	26 (46)	0.0399

NS, not statistically significant; SBC, serum bactericidal activity (number of strains sensitive to NHS).

Table III. Biotyping of cysteine-requiring strains of *E. coli* isolated from recurrent or co-existing infections in 14 patients

Patient	Number of isolates	Origin of auxotroph	Isolation period	Biocode
1	2	Urine	6 months	0235
2	3	Urine	1 months	7065*
3	2	Urine	4 days	2365
4	4	Urine	5 months	7065*
5	3	Urine	22 days	0277
6	3	Urine	8 months	7236
7	9	Urine, blood and wounds	8 months	4237
8	2	Urine	7 days	7265**
9	6	Urine	27 months	7277
10	2	Urine	24 months	7265**
11	2	Urine and blood	1 day	6237
12	2	Urine	8 days	2217
13	3	Urine	14 months	4027
14	2	Urine	1 day	3277

*, **Common biocodes.

Table IV. Somatic serotypes of 64 Cys⁻ *E. coli* isolated from UTI

Group	n	K1*	Group	n	K1
O1	2	2	O2	1	1
O4	1	0	O6	10	0
O7	5	2	OG7	1	0
O12	1	1	O16	2	2
O18ac	1	1	O21	1	0
O25	2	0	O66	1	0
O75	15	3	O90	11	5
O91	1	0	O118	1	0
O129	1	0	O132	1	0
O135	1	0	O139	1	0
O141	1	0	O143	1	0
O148	1	0	O153	1	0

*Number of strains with K1 antigen.

biocodes. Multiple and sequential isolates from each patient had the same biocode, virulence factors and antimicrobial susceptibility pattern. Application of Simpson's index of diversity indicated that the biotyping system had a high discriminatory power to distinguish between unrelated strains ($D = 0.907$) and strains from unrelated patients ($D = 0.978$).

Of the 100 strains tested for O antigen, only 64 were serotypable. These were classed into 24 serogroups (table IV) with 16, 17 and 23 % of strains typed as O6, O90 and O75, respectively. The presence of capsular K1 antigen was not prominent within these groups. The distribution of these strains amongst 24 serogroups showed diversity in expression of this phenotype as was also indicated by a high Simpson's index ($D = 0.896$).

Strains from recurrent or persistent infections did not carry more virulence factors. A strain isolated six times from one patient over 27 months (biocode 7277) showed type-1 fimbriation and resistance to killing by NHS, it was motile and haemolytic. However, another

strain isolated from blood, abscesses and urine nine times over 8 months (biocode 4237) had type-1 fimbriation only. Multiple isolates from a further three patients (biocodes 7065, 2365 and 0235), isolated over shorter intervals, lacked type-1 fimbriae and K1 antigen, were non-motile and non-haemolytic and sensitive to killing by NHS.

Discussion

Cys⁻ *E. coli* strains account for only a small proportion of all UTI caused by this species. Previous studies have indicated that a host predisposition is often found in patients with a Cys⁻ infection, either local (usually mechanical) or systemic (such as diabetes mellitus).^{2, 6, 20} The relative absence of virulence factors in the Cys⁻ strains examined here reinforces the view that infection by these organisms is a result of decreased host resistance rather than any pathogenic capability of the organism. However, infection with Cys⁻ strains has been noted to occur more frequently in those older patients who as a group have a disproportionate amount of renal impairment when compared with age-matched controls.² These observations led to the suggestion that the retention of sulphur-containing compounds, which occurs in conditions of decreased renal function,³⁻⁵ predisposes to infection with Cys⁻ strains by providing conditions conducive to their selection and maintenance *in vivo*.² It has been demonstrated *in vitro* that cysteine auxotrophic *E. coli* can be derived from wild-type prototrophs, albeit at a low frequency.²

We have shown previously that Cys⁻ *E. coli* strains belong to the phenotype *cys* ACDH of Jones-Mortimer.⁶ We have also observed that the clinical isolates we have studied emerge following the repression of the *cys* HIJ operon under conditions of cysteine excess.² Despite these similarities amongst Cys⁻ *E. coli*, this study indicated that distinct strains of *E. coli* are involved in Cys⁻ infections when markedly dissimilar isolates were found in patients with chronic or recurrent infections. Whereas the biotyping system devised to examine differences between strains confirmed that sequential isolates from the same patient were identical in regard to the phenotypic characteristics studied, the same system differentiated isolates from 14 patients into 12 distinct biotypes. Furthermore, the serotyping of 64 strains into 24 serogroups with a high diversity index also indicates the heterogeneity of the auxotrophs.

Thus, it seems that conditions that favour the derivation of Cys⁻ strains serve to select these auxotrophs from a variety of prototrophic *E. coli*, but that these conditions occur more frequently in certain patient subsets. However, these strains are not particularly virulent and, consequently, appear to infect predominantly those patients more susceptible to UTI. This hypothesis would explain both the low but constant overall incidence of Cys⁻ UTI and its greater

frequency in a group in which both selection and infection are enhanced.

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