

MICROBIAL PATHOGENICITY

Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*

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A total of 868 isolates was screened from seven different collections of organisms from previous studies – pyelonephritis in children aged 1–24 months; first, second and recurring urinary tract infection (UTI) in women aged 18–39 years; UTI in women aged 40–65 years and peri-urethral and faecal isolates from women aged 18–39 years – for the presence of 10 potential *Escherichia coli* UTI virulence genes. Previously reported differences between the frequency of these genes in UTI compared with faecal isolates were confirmed and extended. A single virulence signature (strains containing *aer*, *kpsMT*, *ompT*, *fim* and *papG_{AD}*) occurred in 29% of the pyelonephritic isolates, but in no more than 11% of the other collections. Peri-urethral isolates were found to have frequencies of these 10 genes that differed from those found for both UTI and faecal isolates.

Introduction

Urinary tract infection (UTI) is one of the most commonly acquired bacterial infections in ambulatory and hospitalised populations; 11% of all women aged ≥ 18 years in the USA have a UTI each year [1]. Most UTIs among otherwise healthy women are caused primarily by *Escherichia coli*. Certain O:K:H serotypes and virulence factors occur more frequently in urinary than faecal isolates, suggesting that uropathogenic *E. coli* are different from normal bowel inhabitants [2]. However, they are also a diverse group: 20 O:K:H serotypes have been associated with pyelonephritis [2]. Furthermore, when first-time UTI isolates were grouped by the presence or absence of nine putative UTI virulence genes, 36 groups were observed [3].

Dot-blot hybridisation was used earlier to screen several *E. coli* isolate collections for the presence or

absence of some genes potentially associated with UTI: aerobactin (*aer*); group II capsule (*kpsMT*); group III capsule (*capIII*); cytotoxic necrotising factor 1 (*cnf1*); Dr-binding adhesins (*drb*); haemolysin (*hly*); outer-membrane protein T (*ompT*); P-pili family of fimbriae (*pff*), further characterised by identification of three subclasses – *papG₁₉₆* (class I), *papG_{AD}* (class II) and *prsG₁₉₆* (class III); S fimbrial adhesin (*sfa*); and type 1 pili (*fim*). A previous report described the genotypic distribution of these virulence genes among urinary *E. coli* isolates from women with their first UTI and the relationships among these factors [3], as well as the 6-month risk of a second UTI according to the presence of each virulence factor [4]. This report describes the distribution of these genes in *E. coli* isolates from seven different collections: (1) pyelonephritis in children aged 1–24 months, (2) urinary isolates from women aged 18–39 years with first-time UTI, (3) urinary isolates from the same women who had a second UTI within a 6-month period, (4) urinary isolates from women aged 18–39 years with recurring UTI (more than three infections in the past year), (5) urinary isolates from women aged 40–65 years with UTI, (6) peri-urethral isolates from women aged 18–39

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years without UTI, and (7) faecal isolates from women aged 18–39 years without UTI.

Materials and methods

Sample collection

Pyelonephritic isolates (n = 153) were collected from children aged 1–24 months from five hospitals in Finland, as described previously [5]. All isolates from women aged 18–39 years were collected at student health services, either at the University of Michigan (UM) or the University of Texas at Austin (UT). Women with first UTI (n = 285) were followed and second UTI isolates were obtained from those who had a second UTI within 6 months of the first (n = 45) [6]. Recurring UTI isolates were collected from women aged 18–39 years at UM Health Services who reported a history of three or more UTIs in the past year (n = 27). Peri-urethral and faecal isolates were obtained from 318 women presenting without UTI to the gynaecology clinic at UM for pelvic examinations. UTI isolates from women aged 40–65 years were collected at Battle Creek Regional Medical Laboratories in Michigan (n = 60) and the Infectious Disease Unit at Ha'Emek Medical Center in Afula, Israel (n = 58) [7].

A UTI was defined by the combination of a urine culture of ≥ 1000 cfu/ml of urine in the presence of one or more urinary symptoms and a clinical diagnosis. Pyelonephritis was defined by the combination of a urine culture of $\geq 10^5$ cfu/ml of urine in the presence of two out of three of the following: pyuria (leucocyte count $>10/\text{mm}^3$), fever $\geq 38.0^\circ\text{C}$ or C-reactive protein (CRP) >25 mg/L.

Bacterial strains, DNA probes and dot blots

The digoxigenin-labelled DNA gene probes and controls for DNA hybridisation have been described previously [3, 4, 8, 9]. PCR was used to generate probes specific to the *papG_{AD}*, *papG_{J96}* and *prsG_{J96}* P-pilus adhesin subtypes as described previously [9, 10]. Also, as in previous studies, the presence or absence of virulence factors was determined by dot-blot hybridisation [3].

Data analysis

Frequencies of virulence factors were described by means of sample proportions. Differences in proportions among collections were tested by the χ^2 test. All analyses were performed with SAS version 6.10 [11].

Results and discussion

This study screened isolates for nine virulence genes thought to be important in *E. coli*-mediated UTI: *aer*, *kpsMT*, *cnf1*, *drb*, *hly*, *ompT*, *pff*, *sfa* and *fim*. The

P-pili were further subtyped into the three classes of adhesins, *papG_{AD}*, *papG_{J96}* and *prsG_{J96}*. The study also tested for the presence of *capIII* genes, a factor of unknown importance in UTI. The *aer*, *kpsMT* and *papG_{AD}* genes occurred significantly more often ($p < 0.001$ – 0.004) among pyelonephritic isolates than in any of the other collections (Table 1). The *ompT* gene occurred significantly more frequently among pyelonephritic isolates than among first ($p < 0.001$) or second ($p < 0.01$) UTI, peri-urethral ($p < 0.001$), or faecal ($p < 0.001$) isolates. The *fim* gene occurred in virtually all isolates from all collections, with *ompT* and *kpsMT*, respectively, as the next most common genes in all collections.

The distributions observed in this study compare favourably to those of similar clinical populations collected in France [12] and Spain [13]. In France the prevalence of *pff* in 27 children with pyelonephritis was quite similar to this pyelonephritis collection (85.2 versus 82.4%), but *drb* occurred more frequently (18.5% versus 5.9%) and *sfa* less frequently (11.1% versus 31.4%). The French cystitis isolates from women included both first and recurring UTI. If the first, second and recurring UTI isolates in the present study are pooled, the frequencies for *pff*, *drb* and *sfa* (49%, 14.7%, 29.1%) are quite similar to the frequencies observed in the French study (44%, 11.9%, 16.9%). By contrast, among 121 isolates from women in Spain with cystitis [13], expression of haemolysin and CNF1 occurred more frequently than predicted by genotype among first UTI isolates in the present study (50% versus 37.6% and 40% versus 26.6% respectively), but P-fimbrial expression was half the frequency of *pff* genes in the isolates in the present study (22% versus 49.8%). However, *pff* gene expression is under phase variation control, so strains containing P-fimbrial genes often do not express them. The differences in frequencies observed among the Spanish and UM/UT collections might be due to the differences seen in phenotypic versus genotypic detection techniques, but also geographic variation, and the Spanish collection included recurring UTI isolates.

The peri-urethral isolates from women aged 18–39 years without UTI constitute a group distinct from all UTIs (first, second, recurring) and faecal isolates from women in the same age range (Table 1). The *aer* gene occurred less frequently in peri-urethral than in UTI ($p = 0.02$) or faecal ($p = 0.04$) isolates. The *drb* gene occurred slightly less frequently in peri-urethral isolates ($p = 0.45$) than in faecal isolates, and much less frequently than in UTI isolates ($p = 0.02$). The *cnf1* gene occurred slightly less frequently in peri-urethral ($p = 0.59$) than in UTI isolates, but significantly more often when compared with faecal isolates ($p = 0.003$). The *hly* gene also occurred less frequently in peri-urethral than in UTI isolates ($p = 0.12$), but more frequently than in faecal isolates ($p = 0.04$). Genes encoding *ompT* ($p = 0.17$) and *pff* ($p = 0.04$) were less

Table 1. Distribution of potential UTI virulence genes among isolates belonging to different collections

Virulence factor and gene name	Percentage of strains harbouring the gene						
	Pyelonephritis age ≤2 years (n = 153)	First UTI aged 18–39 years (n = 237)	Second UTI aged 18–39 years (n = 42)	Recurring UTI aged 18–39 years (n = 27)	UTI aged 40–65 years (n = 87)	Peri-urethral aged 18–39 years (n = 53)	Faecal (no UTI); aged 18–39 years (n = 269)
Aerobactin (<i>aer</i>)	69.3	46.0	33.3	40.7	37.9	26.4	41.3
Capsule, group II (<i>kpsMT</i>)	93.5	81.9	78.6	74.1	75.9	73.6	63.6
Capsule, group III	2.0	5.9	4.8	3.7	3.4	7.6	5.2
Cytotoxic necrotising factor 1 (<i>cnf1</i>)	28.1	26.6	28.6	40.7	29.9	24.5	10.0
Dr family of adhesins (<i>dtb</i>)	5.9	15.2	16.7	7.4	10.3	3.8	5.6
Haemolysin (<i>hly</i>)	43.8	37.6	31.0	48.2	44.8	26.4	14.9
OmpT (<i>ompT</i>)	94.8	83.1	83.3	85.2	88.5	75.5	67.7
P-pili family (<i>pff</i>)	82.4	49.8	38.1	59.3	57.5	34.0	34.2
(i) <i>papG</i> ₉₆ (class I)	0.0	2.1	0.0	3.7	2.3	0.0	0.0
(ii) <i>papG</i> _{AD} (class II)	67.3	27.0	14.3	37.0	31.0	11.3	23.1
(iii) <i>prsG</i> ₉₆ (class III)	19.0	21.1	19.1	29.6	24.1	20.8	8.6
S-fimbriae (<i>sfa</i>)	31.4	27.9	26.2	44.4	41.4	24.5	12.6
Type 1 pili (<i>fim</i>)	99.3	99.6	100	100	100	100	99.3

common in peri-urethral isolates than in UTI isolates. Finally, *papG*_{AD} occurred at lower frequencies in peri-urethral isolates than in either UTI ($p = 0.02$) or faecal isolates ($p = 0.06$), while *prsG*₉₆ ($p = 0.008$) and *sfa* ($p = 0.03$) occurred more often in peri-urethral than in faecal strains. As peri-urethral *E. coli* were somewhat different from uropathogens, it is possible that they cause UTI only if something facilitates their movement, for example, if pushed into the bladder by catheterisation. Peri-urethral *E. coli* might also protect against colonisation by more virulent strains if urethral colonisation is a necessary prelude to UTI.

Each isolate was assigned a virulence signature [3, 4, 8] based on the presence or absence of each gene tested. When these virulence signatures were compared by collection, only a few virulence signatures made up at least 5% of any collection (Table 2). Results from previous studies showed that *E. coli* isolated from the same or different sites within the same individual that had the same virulence signature also had identical pulsed-field gel electrophoresis (PFGE) patterns. However, a single virulence signature from a study population may contain many genetically different strains as determined by PFGE (data not shown). Furthermore, the ability of a virulence signature to provide information regarding genetic similarity among strains depends on the number of virulence genes present. For example, virulence signature 0000000010000 contains all isolates with the *fim* gene, but lacking all other genes tested. As strains in this signature are mostly defined by what they lack, they are likely to represent a very heterogeneous collection of strains. In contrast, strains with virulence signature 1111110010100 contain *sfa*, *aer*, *kpsMT*, *ompT*, *hly*, *fim* and *papG*_{AD}, and thus are likely to be more similar to each other. With these constraints in mind, two of the virulence signatures listed in Table 2 seem to be of particular interest. Signature 1011100010100, containing *aer*, *kpsMT*, *ompT*, *fim* and *papG*_{AD}, is found twice as often – prevalence ratio (PR) = 2.0; 95% confidence interval (CI) = 1.08–3.81 – in first UTI as in faecal isolates, and nearly six times as often (PR = 5.7; CI = 3.21–9.95) in pyelonephritis as in faecal isolates. Signature 1101111010001, containing *sfa*, *kpsMT*, *ompT*, *hly*, *cnf1*, *fim* and *prsG*₉₆, is found three times more often in isolates from periurethral (PR = 3.4; CI = 1.45–7.78), first UTI (PR = 3.1; CI = 1.65–5.90), recurring UTI (PR = 3.3; CI = 1.15–9.59) and UTI among older women (PR = 3.4; CI = 1.59–7.07) than in faecal isolates. The frequency of this virulence signature is less than twice that of faecal isolates in pyelonephritis (PR = 1.9; CI = 0.89–4.07) and second UTI (PR = 1.6; CI = 0.47–5.43) isolates, even though it is not statistically significant.

The pyelonephritic strains from children aged 1–24 months were previously shown to contain predominantly the *papG*_{AD} (class II) allele [5]; additionally, the present study has shown that a substantial portion (29%

Table 2. Distribution of the most common *E. coli* virulence signatures among isolates originating from different collections

Virulence signature psa2ohcdE3gl*	Number of strains (%) with the virulence signature							All isolates (n = 868)
	Pyelonephritis age ≤2 years (n = 153)	First UTI aged 18–39 years (n = 237)	Second UTI aged 18–39 years (n = 42)	Recurring UTI aged 18–39 years (n = 27)	UTI aged 40–65 years (n = 87)	Peri-urethral aged 18–39 years (n = 53)	Faecal (no UTI); 18–39 years (n = 269)	
000000010000	1 (0.6)	13 (5.5)	2 (4.8)	2 (7.4)	7 (8.0)	10 (18.9)	43 (16.0)	78 (9.0)
0000100010000	2 (1.3)	10 (4.2)	1 (2.4)	0	5 (5.7)	0	8 (3.0)	26 (3.0)
0001100010000	1 (0.6)	20 (8.4)	9 (21.4)	3 (11.1)	7 (8.0)	11 (20.8)	29 (10.8)	80 (9.2)
0011100010000	3 (1.9)	17 (7.2)	2 (4.8)	0	3 (3.4)	3 (5.7)	30 (11.2)	58 (6.7)
0011100110000	5 (3.2)	20 (8.4)	4 (9.5)	1 (3.7)	4 (4.6)	2 (3.8)	7 (2.6)	43 (5.0)
1001100010100	13 (8.4)	11 (4.6)	3 (7.1)	1 (3.7)	6 (6.9)	3 (5.7)	24 (8.9)	61 (7.0)
1011100010100	45 (29.4)	25 (10.6)	1 (2.4)	3 (11.1)	4 (4.6)	2 (3.8)	14 (5.2)	94 (10.8)
1101110100001	13 (8.4)	33 (13.9)	3 (7.1)	4 (14.8)	13 (14.9)	8 (15.1)	12 (4.5)	86 (9.9)
1111110010100	7 (4.5)	3 (1.3)	0	2 (7.4)	7 (8.0)	0	4 (1.5)	23 (2.6)

*p, *pfi*; s, *sfa*; a, *aer*; 2, *kpsMT*; o, *ompT*; h, *hly*; e, *cnfI*; d, *drb*; f, *fim*; 3, *capIII*; g, *papG_{AD}*; j, *papG₉₆*; r, *prsG₉₆*; 1, presence of gene; 0, absence of gene.

of these isolates have a specific virulence signature (1011100010100 containing *aer*, *kpsMT*, *ompT*, *fim* and *papG_{AD}*; Table 2). The same virulence signature was found in 10–11% of first UTI and recurring UTI isolates, but in only ≤5% of strains in the other four collections. In comparison with these frequencies, the virulence signature 1100111011011 (containing *sfa*, *ompT*, *hly*, *cnfI*, *fim*, *capIII*, *papG₉₆* and *prsG₉₆*) of the best studied pyelonephritic strain, J96 [14], is very rare and was present in <1% of all isolates. Strain CFT073, a pyelonephritic strain that is highly virulent in the CBA mouse model of ascending UTI [15], has virulence signature 1111110010100 (containing *sfa*, *aer*, *kpsMT*, *ompT*, *hly*, *fim* and *papG_{AD}*), which occurred in <7% of strains in the different collections (Table 2). Interpreting the differences between the pyelonephritic and other UTI isolates is problematic, as it was not possible to determine how much of the variation was due to patient age or geographic differences in the sample collections, rather than the differences in the site of disease. To better assess this, future studies should focus on determining the virulence gene distribution among adult pyelonephritic isolates within the same geographic region.

The high degree of heterogeneity found among *E. coli* colonising the urinary tract, peri-urethra and bowel when classified by the presence or absence of 10 putative uropathogenic genes raises many questions about UTI pathogenesis. Of the virulence genes tested, only *fim* may be universally required for UTI virulence [16]; however, it is not sufficient on its own, as *fim* is present in most *E. coli* but only a subset cause UTI. A better understanding of which *E. coli* genes contribute to UTI and their role in pathogenesis is required to prevent this common disease.

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References

1. Foxman B, Barlow R, d'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. *Ann Epidemiol* 2000; **10**: 509–515.
2. Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* 1991 **4**: 80–128.
3. Foxman B, Zhang L, Palin K, Tallman P, Marrs CF. Bacterial

- virulence characteristics of *Escherichia coli* isolates from first-time urinary tract infection. *J Infect Dis* 1995; **171**: 1514–1521.
4. Foxman B, Zhang L, Tallman P *et al.* Virulence characteristics of *Escherichia coli* causing first urinary tract infection predict risk of second infection. *J Infect Dis* 1995; **172**: 1536–1541.
 5. Jantunen ME, Siitonen A, Koskimies O *et al.* Predominance of class II *papG* allele of *Escherichia coli* in pyelonephritis in infants with normal urinary tract anatomy. *J Infect Dis* 2000; **181**: 1822–1824.
 6. Foxman B, Gillespie B, Koopman J *et al.* Risk factors for second urinary tract infection among college women. *Am J Epidemiol* 2000; **151**: 1194–1205.
 7. Foxman B, Somsel P, Tallman P *et al.* Urinary tract infection among women aged 40 to 65: behavioral and sexual risk factors for acquisition. *J Clin Epidemiol* 2001 (in press).
 8. Zhang L, Foxman B, Tallman P, Cladera E, Le Bouguenec C, Marrs CF. Distribution of *drb* genes coding for Dr binding adhesins among uropathogenic and fecal *Escherichia coli* isolates and identification of new subtypes. *Infect Immun* 1997; **65**: 2011–2018.
 9. Foxman B, Zhang L, Tallman P *et al.* Transmission of uropathogens between sex partners. *J Infect Dis* 1997; **175**: 989–992.
 10. Manning SD, Zhang L, Foxman B, Spindler A, Tallman P, Marrs CF. Prevalence of known P-fimbrial G-alleles in *Escherichia coli* and identification of a new adhesin class. *Clin Diagn Lab Immunol* 2001; **8**: 637–640.
 11. SAS Institute Incorporated: SAS system for Windows release 6.10. (1994) SAS Campus Drive, Cary, NC 27513, USA.
 12. Archambaud M, Courcoux P, Labigne-Roussel A. Detection by molecular hybridization of *pap*, *afa*, and *sfa* adherence systems in *Escherichia coli* strains associated with urinary and enteral infections. *Ann Inst Pasteur Microbiol* 1988; **139**: 575–588.
 13. Blanco M, Blanco JE, Alonso MP, Blanco J. Virulence factors and O groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur J Epidemiol* 1996; **12**: 191–198.
 14. Hull RA, Gill RE, Hsu P, Minshew BH, Falkow S. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. *Infect Immun* 1981; **33**: 933–938.
 15. Mobley HLT, Jarvis KG, Elwood JP *et al.* Isogenic P-fimbrial deletion mutants of pyelonephritogenic *Escherichia coli*: the role of α Gal(1-4) β Gal binding in virulence of a wild-type strain. *Mol Microbiol* 1993; **10**: 143–155.
 16. Connell H, Agace W, Klemm P, Shembri M, Mårild S, Svanborg C. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. *Proc Natl Acad Sci USA* 1996; **93**: 9827–9832.