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 papGIII) 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 (drh); haemolysin (hly); outer-membrane protein T (ompT); P-pili family of fimbriae (pilF), further characterised by identification of three subclasses – papGIII (class I), papGII (class II) and pssGIII (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 ≥10⁵ cfu/ml of urine in the presence of two out of three of the following: pyuria (leucocyte count >10/mm³), fever ≥38.0°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 papaGAD, papaG96 and prsG96. P-pili 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 χ² 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: a eer, kpsMT, cnf1, dfr, hly, ompT, pff, sfa and fim. The P-pili were further subtyped into the three classes of adhesins, papaGAD, papaG96 and prsG96. The study also tested for the presence of capIII genes, a factor of unknown importance in UTI. The a eer, kpsMT and papaGAD genes occurred significantly more often (p < 0.001–0.004) among pyelonephric isolates than in any of the other collections (Table 1). The ompT gene occurred significantly more frequently among pyelonephric 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 dfr 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, dfr 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 a eer gene occurred less frequently in peri-urethral than in UTI (p = 0.02) or faecal (p = 0.04) isolates. The dfr 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
common in peri-urethral isolates than in UTI isolates. Finally, \textit{papGAD}_{3} occurred at lower frequencies in peri-
urethral isolates than in either UTI (\(p = 0.02\)) or faecal
isolates (\(p = 0.06\)), while \textit{prG} (\(p = 0.008\)) and \textit{sfa}
(\(p = 0.03\)) occurred more often in peri-urethral than in
faecal strains. As peri-urethral \textit{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 catheterisa-
tion. Peri-urethral \textit{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 \textit{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 popu-
lation may contain many genetically different strains as
determined by PFGE (data not shown). Furthermore,
the ability of a virulence signature to provide informa-
tion regarding genetic similarity among strains depends
on the number of virulence genes present. For example,
virulence signature 0000000010000 contains all iso-
lates with the \textit{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 1111110100100 contain \textit{sfa},
\textit{aer}, \textit{kpsMT}, \textit{ompT}, \textit{hly}, \textit{fim} and \textit{papGAD}_{3},
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 101111010100, containing \textit{aer}, \textit{kpsMT},
\textit{ompT}, \textit{fim} and \textit{papGAD}_{3}, 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 11011110100001, containing \textit{sfa}, \textit{kpsMT},
\textit{ompT}, \textit{hly}, \textit{csgF1}, \textit{fim} and \textit{prG}] are 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 predomi-
antly the \textit{papGAD}_{3} class II allele [3]; additionally, the
present study has shown that a substantial portion (29%
of these isolates have a specific virulence signature (1011100010100 containing aer, kpsMT, ompT, fim and papGPaC3) (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 1100111010111 (containing sfa, ompT, hly, cnf1, fim, capII, papGPaC3, and papGPaC7) of the best studied pyelonephritic strain, J96 [14], is very rare and was present in <1% of all strains. 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 papGPaC3), 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

3. Foxman B, Zhang L, Palen K, Tallman P, Marrs CF. Bacterial


