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Distribution of phylogenetic groups, sequence type ST131, and virulence-associated traits among *Escherichia coli* isolates from men with pyelonephritis or cystitis and healthy controls

T. Kudinha[1,2], J. R. Johnson[3], S. D. Andrew[1], F. Kong[2], P. Anderson[1] and G. L. Gilbert[2,4]

1) *Charles Sturt University, Leeds Parade, Orange,* 2) *Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW, Australia,* 3) *Veterans Affairs Medical Center and University of Minnesota, Minneapolis, MN, USA and* 4) *Sydney Institute for Emerging Infectious Diseases and Biosecurity, University of Sydney, Sydney, NSW, Australia*

Abstract

Urinary tract infections (UTI), which are mostly caused by *Escherichia coli*, are an important public health problem worldwide. Although men experience diverse UTI syndromes, there have been relatively few molecular-epidemiological studies of UTI pathogenesis in men. We studied the distribution of 22 *E. coli* virulence factor (VF) genes, major phylogenetic groups, sequence type ST131, and UTI-associated O antigens among 101 pyelonephritis, 153 cystitis and 135 fecal healthy control *E. coli* isolates from men aged 30–70 years in a regional area of NSW, Australia. Overall, the studied traits exhibited a prevalence gradient across these groups, highest in pyelonephritis, intermediate in cystitis, and lowest among fecal isolates. Differences in virulence gene prevalence between cystitis and pyelonephritis isolates were limited to eight genes. The UTI-associated O antigens were also distributed widely, but types O6, O25 and O75 were significantly associated with pyelonephritis. The ST131 clonal group, which accounted for 13% of isolates overall (22% of group B2 isolates), likewise exhibited a significant descending prevalence gradient from pyelonephritis (36%), through cystitis (8%), to fecal (0%) isolates. These findings contribute to better understanding of the pathogenesis of UTIs in men and identify specific VF genes and O types, and a prominent clonal group (ST131), as being important in UTI pathogenesis in this population.

Keywords: Clonal, *Escherichia coli*, phylogenetic, urinary tract infections, virulence factors
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Corresponding author: G. L. Gilbert, Centre for Infectious Diseases and Microbiology Laboratory Services (CIDMLS), Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Westmead NSW 2145, Australia
E-mail: lyn.gilbert@sydney.edu.au

Introduction

Urinary tract infections (UTIs), which are among the commonest bacterial diseases of humans, are responsible for considerable morbidity, mortality and healthcare costs worldwide [1]. Better understanding of their pathogenesis is needed, to guide the development of preventive measures.

Most UTIs are caused by *Escherichia coli*, a normal constituent of the intestinal microbiota [2]. The distinctive *E. coli* strains that cause most UTIs have been designated uropathogenic *E. coli*. They possess diverse virulence-associated factors (VFs) that assist them in attaching to, invading, and injuring the host, and include adhesins, toxins, siderophores, protective polysaccharide coatings, invasins, and serum resistance-associated proteins. The presence and numbers of such VFs predicts *in vivo* virulence [3].

Most studies, comparing the distribution of VFs among *E. coli* isolates from different uroclinical syndromes, and fecal isolates, have focused on women and girls, among whom UTI is most common [2,4]. However, men also experience UTIs, including cystitis, pyelonephritis, prostatitis, and febrile UTI [5,6]. As a consequence, studies of the *E. coli* strains that cause UTI in men are needed.

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The few such studies available date to 2007 or earlier, and involved small sample sizes, a restricted range of VF genes analysed, lack of consideration for phylogenetic and clonal groups (including the recently emerged disseminated clonal group sequence type ST131), or pooled data from men and women. The specific characteristics of uropathogenic *E. coli* from men, especially in the current era, therefore remain incompletely defined.

We compared the distributions of VF genes, phylogenetic groups, ST131, and UTI-associated O antigens among three groups of recent *E. coli* isolates, including urinary isolates from men with uncomplicated cystitis or pyelonephritis and fecal isolates from healthy controls, with efforts to avoid interference from the confounding effect of host compromise.

Materials and Methods

Study design

This prospective study, which involved 11 regional hospitals and 23 outpatient centres, in the central west region of New South Wales, Australia (human population, 180 000), was conducted in conjunction with physicians in the participating centres. Hospitalized patients were not included. Each participating physician received a protocol on urine collection, and the diagnostic criteria for cystitis or pyelonephritis. In addition to performing physical examination of patients, physicians collected and reported anonymously, the following information for patients with UTI (defined below): age, clinical UTI diagnosis, previous UTI and known underlying host conditions.

E. coli isolates and study subjects

Contemporaneous *E. coli* isolates from 288 men, with UTI, one per subject, were collected at the participating centres over a 2-year period (June 2009 to June 2011). The subjects had cystitis (n = 153), or pyelonephritis (n = 101). Subjects were aged from 30 to 70 years, with a median age of 47 years.

A diagnosis of cystitis or pyelonephritis required specific manifestations, as recorded by the treating medical practitioner, and a midstream urine culture yielding $\geq 10^8$ CFU/L of *E. coli*. Cystitis-defining manifestations included dysuria, frequent urination, and suprapubic tenderness, without fever or loin pain. Pyelonephritis-defining manifestations included urinary symptoms plus, fever of $\geq$38°C and flank pain, with or without nausea/vomiting. Patients with diabetes and known urinary tract abnormalities were excluded.

Urinary tract abnormality was defined based on the attending physician's assessment at the time of the index patient encounter. Urological evaluation to exclude inapparent structural or functional genitourinary abnormalities was not done.

During the same 2-year period, fecal isolates were collected from 135 consenting male volunteers (30–70 years old) who, according to self-report, lacked UTI-associated manifestations. These controls were matched with UTI patients by age and, as closely as possible, by place of residence within the region. Control participants provided written informed consent and a rectal swab, which was processed within 10 h of collection. One arbitrarily chosen *E. coli* colony per specimen was studied [7].

Ethics approval

The Sydney West Area Health Service Human Ethics Research Committee and Charles Sturt University Ethics Committee approved the study protocol. Guidelines for experimentation at the authors' institutions were followed in the conduct of this clinical research. As clinical information for patients with UTI was collected anonymously, patient consent was not obtained.

Urine culture

Clinical staff at the participating healthcare centres collected the urine specimens, using a provided standardized protocol. Semi-quantitative culture was performed on horse blood, MacConkey and chromogenic agars, followed by conventional identification. The *E. coli* isolates were stored in 5% glycerol in trypticase soy broth at −70°C.

Virulence factor genes

Twenty-two VF genes (Table 1) were identified using a multiplex PCR reverse line-blot hybridization assay [8]. The aggregate VF score for an isolate was the number of unique VFs detected, counting multiple *pap* operon genes as one. Such molecular characteristics predict experimental virulence *in vivo* [3].

Phylogenetic grouping, ST131 detection and O typing

Major *E. coli* phylogenetic groups (A, B1, B2 and D) were identified by triplex PCR [9]. Group B2 isolates were screened for ST131 by PCR-based detection of ST131-specific single nucleotide polymorphisms in *mdh* and *gyrB* [10,11]. PCR-based O (somatic antigen) typing was used to detect 12 UTI-associated O types: O1, O2, O4, O6, O7, O12, O15, O16, O17, O18, O25 and O75 [10]. ST131 isolates exhibiting O-type 25 were further characterized as O25a versus O25b by variant-specific PCR [11,12].

Quality control

All molecular testing was performed in duplicate using independently prepared DNA lysates of the test strain and appropriate positive and negative controls.

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TABLE 1. Distribution of virulence-associated genes by source among 389 *Escherichia coli* isolates from men with cystitis or pyelonephritis and healthy controls

Functional category	Trait/gene	Prevalence, no. (column %)				p value[a]		
		Total (*n* = 389)	Fecal (*n* = 135)	Cystitis (*n* = 153)	Pyelo[b] (*n* = 101)	Fecal vs cystitis	Fecal vs pyelo	Pyelo vs cystitis
Adhesins	*afa/draBC*	33 (8)	1 (1)	17 (11)	15 (15)	<0.001	<0.001	NS[c]
	bmaE	2 (0.5)	0 (0)	0 (0)	2 (2)	NS	NS	NS
	sfaS	73 (19)	10 (7)	40 (26)	23 (23)	<0.001	0.001	NS
	fimH	363 (93)	119 (88)	147 (96)	97 (97)	0.014	0.035	NS
	focG	151 (39)	6 (4)	89 (58)	56 (56)	<0.001	<0.001	NS
	papGI	14 (4)	1 (1)	9 (6)	4 (4)	0.022	NS	NS
	papGII	115 (30)	15 (11)	47 (30)	53 (53)	<0.001	<0.001	<0.001
	papGIII	97 (25)	13 (10)	43 (28)	41 (41)	<0.001	<0.001	0.042
	papGI + III	6 (1.5)	0 (0)	5 (3)	1 (1)	NS	NS	NS
	papGII + III	22 (6)	0 (0)	12 (8)	10 (10)	<0.001	<0.001	NS
	papGI + II + III	3 (1)	0 (0)	2 (1)	1 (1)	NS	NS	NS
	papAH	213 (55)	41 (30)	93 (61)	79 (79)	<0.001	<0.001	0.004
	papC	220 (57)	45 (33)	93 (61)	82 (82)	<0.001	<0.001	<0.001
	papEF	233 (60)	49 (35)	97 (63)	87 (87)	<0.001	<0.001	<0.001
	gafD	4 (1)	0 (0)	2 (1)	2 (2)	NS	NS	NS
Toxins	*cnf1*	177 (46)	14 (10)	80 (52)	83 (83)	<0.001	<0.001	<0.001
	hlyA	214 (55)	25 (19)	113 (74)	76 (76)	<0.001	<0.001	NS
Siderophores	*iutA*	232 (60)	21 (16)	127 (83)	84 (84)	<0.001	<0.001	NS
	fyuA	254 (65)	36 (27)	127 (83)	91 (91)	<0.001	<0.001	NS
	iroN	222 (57)	25 (19)	115 (75)	82 (82)	<.001	<0.001	NS
Protectins	*kpsMT II*	201 (52)	24 (18)	101 (66)	76 (76)	<0.001	<0.001	NS
	kpsMT III	35 (9)	0 (0)	18 (12)	17 (17)	<0.001	<0.001	NS
	traT	252 (65)	43 (32)	125 (82)	84 (84)	<0.001	<0.001	NS
	ompT	227 (58)	25 (19)	113 (74)	89 (89)	<0.001	<0.001	0.007
Bacteriocin	*usp*	222 (57)	31 (23)	107 (70)	84 (84)	<0.001	<0.001	0.018

[a]p values (by Fisher's exact test) are shown where $p < 0.05$ comparing cystitis with fecal isolates. The 22 virulence factors analysed were; *papA*, P fimbriae structural subunit; *papC*, p fimbriae assembly; *papEF*, fimbriae tip pilins; *papG*, p fimbriae adhesin (and alleles I, II, and III); *sfaS*, S fimbriae; *focG*, F1C fimbriae; *afa/draBC*, Dr-binding adhesins; *fimH*, type 1 fimbriae; *hlyA*, haemolysin; *cnf1*, cytotoxic necrotizing factor type 1; *fyuA*, ferric yersiniabactin receptor; *iutA*, aerobactin receptor; *iron*, salmochelin receptor; *kpsM* II, group 2 capsule (with K1 and K2 variants); *kpsMT* III, group 3 capsule; *traT*, serum-resistance associated; *ompT*, outer membrane protein T (protease); *bmaE*, M fimbriae; *gafD*, G fimbriae.
[b]Pyelo, pyelonephritis.
[c]NS, not significant ($p \geq 0.05$).

Statistical analysis

Comparisons of proportions were tested using Fisher's exact test. Virulence score comparisons were tested using the Mann–Whitney *U*-test. The significance criterion was $p < 0.05$.

Results

VF gene distribution by source group

Among the 389 total *E. coli* isolates, the prevalence of individual VF genes, within the three source groups, ranged from 0% (*bmaE*, *gafD*, *kpsMTIII*) to 97% (*fimH*) (Table 1). Nineteen VF genes were detected in at least one isolate per source group, the exceptions being *bmaE*, *gafD* and *kpsMTIII*, which were absent from fecal isolates. The most frequently encountered VF genes overall were *fimH*, *fyuA*, *ompT*, *papEF*, *traT*, *iutA*, *usp*, *hlyA* and *iroN* ($\geq$55% each).

Compared with cystitis and pyelonephritis isolates, fecal isolates exhibited a significantly lower prevalence of 20 VF genes (Table 1). Apart from *fimH* (88%), only four genes (*traT*, *papE*, *papC* and *papAH*) occurred in >30% of fecal isolates. Accordingly, VF scores were much lower among fecal isolates than cystitis and pyelonephritis isolates (Fig. 1).

In contrast, differences between cystitis and pyelonephritis isolates for VF gene prevalence were confined to eight genes

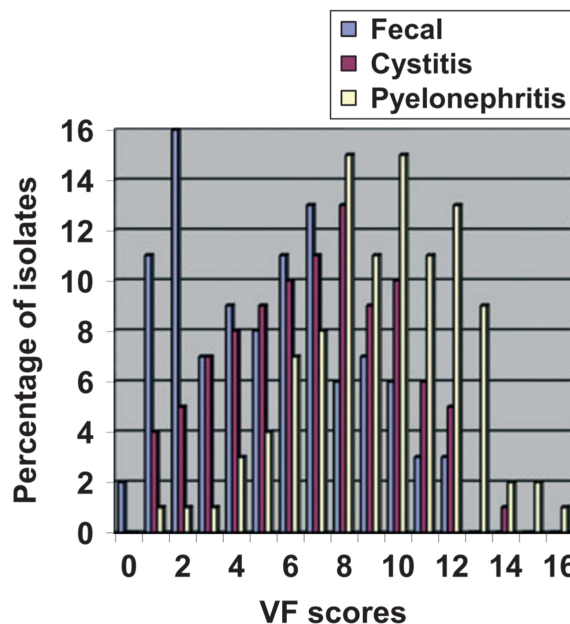

FIG. 1. Distribution of virulence factor (VF) scores among 389 *Escherichia coli* isolates from men with cystitis or pyelonephritis and healthy controls. The coloured columns indicate, for each source group, the percentage of isolates (*y*-axis) with a given VF score (*x*-axis). Median VF scores (range), by group: fecal, 3 (0–13); cystitis 7 (1–14); pyelonephritis 9 (1–16). p values for fecal versus cystitis, fecal versus pyelonephritis, cystitis versus pyelonephritis: $p \leq 0.001$ for all comparisons.

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(*papAH*, *papC*, *papEF*, *papGII*, *papGIII*, *cnf1*, *ompT* and *usp*), all of which were more common among pyelonephritis isolates. Accordingly, pyelonephritis isolates had significantly higher median virulence scores (p <0.001) (Fig. 1).

Phylogenetic groups

Of the 389 isolates, 225 (58%) belonged to group B2, followed by groups A (21%), D (16%) and B1 (6%) (Table 2). Group B2 dominated among clinical isolates (70%), but accounted for only 34% of fecal isolates (p <0.001), among which group A was the most prevalent (42% versus 9%). Phylogenetic group distribution did not differ significantly between the cystitis and pyelonephritis isolates.

Distribution of VF genes by phylogenetic group and source

Overall, most VF genes were most prevalent among group B2 isolates, followed by group D isolates (Table 3). Accordingly, VF scores followed a descending gradient, from group B2, through group D to groups A and B1 (Table 4).

To clarify the relationship between phylogenetic groups and VF genes, VF scores were compared among the four phylogenetic groups, stratified by source group, and among the source groups, stratified by phylogenetic group (Table 4). There was a descending VF score gradient from pyelonephritis, through cystitis, to fecal isolates and, within each source group, from phylogenetic groups B2, through group D to groups A and B1 (Table 4). Increments in VF score between source groups (within a given phylogenetic group) were similar in magnitude to those between phylogenetic groups (within a given source group). Consequently, VF scores were highest among group B2 pyelonephritis isolates (median, 14), lowest among group A and B1 fecal isolates (median, 2), and intermediate (but quite similar) among group A and B1

TABLE 2. Distribution of phylogenetic groups and sequence type ST131 among 389 *Escherichia coli* isolates from men with cystitis or pyelonephritis and healthy controls

Phylogenetic/clonal group	Prevalence, no. (column %)				p value[a]		
	Total (*n* = 389)	Fecal (*n* = 135)	Cystitis (*n* = 153)	Pyelo[b] (*n* = 101)	Fecal vs cystitis	Fecal vs pyelo	Pyelo vs cystitis
A	80 (21)	57 (42)	15 (10)	8 (8)	<0.001	<0.001	NS[c]
B1	24 (6)	12 (9)	8 (5)	4 (4)	NS	NS	NS
B2	225 (58)	46 (34)	104 (68)	75 (75)	<0.001	<0.001	NS
D	63 (16)	24 (18)	26 (17)	13 (13)	NS	NS	NS
ST131[d]	49 (13)	0 (0)	13 (8)	36 (36)	<0.001	<0.001	<0.001
O25b ST131[e]	44 (11)	0 (0)	13 (8)	31 (31)	<0.001	<0.001	<0.001
O16 ST131[e]	5 (1)	0 (0)	0 (0)	5 (5)	NS	0.014	0.014

[a]p values (by Fisher's exact test) are shown where p <0.05.
[b]Pyelo, pyelonephritis.
[c]NS, not significant (p ≥0.05).
[d]ST131, sequence type 131: a subset of phylogenetic group B2.
[e]O25b and O16, the corresponding – O-type-based subsets of ST131.

TABLE 3. Phylogenetic group distribution of virulence genes among 389 *Escherichia coli* isolates from men with cystitis or pyelonephritis and healthy controls

Virulence gene	Prevalence of gene, no. (column %)				p value[a]	
	A (*n* = 80)	B1 (*n* = 24)	B2 (*n* = 225)	D (*n* = 63)	B2 vs D	B2 vs A & B1
afa/dra	4 (5)	0 (0)	27 (12)	2 (3)	NS[b]	<0.001
bmaE	0 (0)	0 (0)	2 (1)	0 (0)	NS	NS
sfaS	0 (0)	1 (4)	67 (30)	5 (8)	<0.001	<0.001
fimH	60 (75)	18 (75)	223 (99)	62 (99)	NS	NS
focG	2 (2)	1 (3)	52 (23)	2 (3)	<0.001	<0.001
papGI	0 (0)	0 (0)	13 (6)	1 (2)	NS	<0.001
papGII	0 (0)	0 (0)	99 (44)	15 (24)	<0.001	<0.001
papGIII	0 (0)	1 (1)	90 (40)	6 (9)	<0.001	<0.001
papAH	8 (10)	1 (3)	163 (72)	41 (65)	NS	<0.001
papC	9 (11)	1 (5)	170 (76)	40 (63)	NS	<0.001
papEF	10 (13)	2 (8)	182 (81)	39 (62)	0.002	<0.001
gafD	0 (0)	0 (0)	4 (16)	0 (0)	<0.001	<0.001
cnf1	0 (0)	1 (3)	157 (70)	19 (31)	<0.001	<0.001
hlyA	3 (4)	1 (5)	185 (82)	25 (40)	<0.001	<0.001
iutA	14 (18)	2 (8)	173 (76)	43 (68)	NS	<0.001
fyuA	11 (14)	4 (17)	205 (91)	34 (54)	<0.001	<0.001
iroN	8 (10)	8 (33)	172 (76)	34 (53)	0.002	<0.001
traT	20 (25)	3 (13)	180 (80)	49 (77)	NS	<0.001
kpsMT II	2 (2)	1 (4)	171 (75)	20 (32)	<0.001	<0.001
kpsMT III	0 (0)	0 (0)	23 (10)	0 (0)	<0.001	<0.001
ompT	5 (6)	8 (33)	188 (84)	31 (49)	<0.001	<0.001
usp	1 (1)	4 (17)	185 (82)	32 (51)	<0.001	<0.001

[a]p values (by Fisher's exact test) are shown where p <0.05.
[b]NS, not significant (p ≥0.05).

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pyelonephritis isolates, group D cystitis isolates, and group B2 fecal isolates (medians, 5–6) (Table 4).

O types

Overall, the most commonly identified O types, in descending rank order, were O2, O25, O16 and O6 (>10% of isolates each) (Table 5). A significantly higher proportion of fecal (32%) than cystitis (18%) or pyelonephritis (14%) isolates, belonged to none of the defined uropathogenic *E. coli*-related O-types. Nine of the defined O types were significantly distributed by source group. Most of these were significantly more prevalent among cystitis and/or pyelonephritis isolates than fecal isolates (e.g. O6, O16, O25, O25b and O75), whereas others were significantly more prevalent among fecal isolates (O4), cystitis isolates (O12, O15), or cystitis and fecal isolates (O7) than among pyelonephritis isolates.

Prevalence and clinical correlates of ST131

Forty-nine (22%) of the 225 group B2 isolates belonged to ST131 (Table 2). ST131 was strongly associated with clinical source, exhibiting a marked descending prevalence gradient from pyelonephritis (36%), through cystitis (8%) to fecal (0%). A similar (albeit less marked) source-related ST131 prevalence gradient was apparent even among group B2 isolates. Specifically, of the 225 group B2 isolates, 36 (16%) were ST131 isolates from pyelonephritis cases, compared with 13 (6%) from cystitis and 0 (0%) from fecal isolates.

According to O-type PCR, 44 (90%) of the 49 ST131 isolates were O25b and five (10%) were O16 (Table 2). Interestingly, all five (100%) of the O16 ST131 isolates were pyelonephritis isolates compared with 70% of the O25b ST131 isolates.

Discussion

In this molecular-epidemiological study, we examined the distribution of VF genes, phylogenetic groups, ST131 status and UTI-associated O types among *E. coli* isolates from men with cystitis or pyelonephritis and healthy controls. We

TABLE 4. Distribution of aggregate virulence factor (VF) scores by phylogenetic group and source among 389 *Escherichia coli* isolates from men

	Aggregate VF score, median (range)				p value[a]			
Phylogenetic group	Total	Fecal	Cystitis	Pyelo[b]	Fecal vs cystitis	Fecal vs pyelo	Cystitis vs pyelo	Fecal vs (cystitis + pyelo)
A (*n* = 80)	4 (0–10)	2 (0–5)	4 (1–9)	6 (1–10)	NS[c]	<0.001	0.03	<0.001
B1 (*n* = 24)	4 (0–8)	2 (0–5)	4 (1–8)	5 (3–8)	NS	<0.001	NS	<0.001
B2 (*n* = 225)	9 (3–16)	6 (3–13)	9 (5–14)	14 (8–16)	<0.001	<0.001	<0.001	<0.001
D (*n* = 63)	7 (2–13)	4 (2–11)	6 (3–11)	9 (5–13)	0.02	<0.001	<0.001	<.0 001

[a]p values by Mann–Whitney test are shown where p <0.05.
[b]Pyelo, pyelonephritis.
[c]NS, not significant (p ≥ 0.05).

TABLE 5. Distribution of various O types by source among 389 *Escherichia coli* isolates from men with cystitis or pyelonephritis and healthy controls

	Prevalence of O type, no. (column %)				p value[a]		
O type	Total (*n* = 389)	Fecal (*n* = 135)	Cystitis (*n* = 153)	Pyelo[b] (*n* = 101)	Fecal vs cystitis	Fecal vs pyelo	Cystitis vs pyelo
O1	17 (4)	4 (3)	8 (5)	5 (5)	NS[c]	NS	NS
O2	55 (14)	20 (15)	18 (12)	17 (17)	NS	NS	NS
O4	11 (3)	11 (8)	0 (0)	0 (0)	<0.001	0.003	NS
O6	37 (10)	8 (6)	8 (6)	15 (15)	NS	0.027	0.012
O7	17 (4)	8 (6)	9 (6)	0 (0)	NS	0.011	0.013
O12	26 (7)	7 (5)	18 (12)	1 (1)	NS	NS	0.001
O15	7 (2)	1 (1)	6 (4)	0 (0)	NS	NS	NS
O16	41 (11)	4 (3)	23 (15)	14 (14)	<0.001	<0.001	NS
O17	14 (4)	9 (7)	5 (3)	0 (0)	NS	0.011	NS
O18	11 (3)	4 (3)	6 (4)	1 (1)	NS	NS	NS
O25	48 (12)	3 (2)	14 (9)	31 (31)	NS	<0.001	<0.001
O25a	2 (<1)	0 (0)	1 (1)	1 (1)	NS	NS	NS
O25b	46 (12)	0 (0)	13 (8)	33 (33)	<0.001	<0.001	<0.001
O75	22 (6)	4 (3)	5 (3)	13 (13)	NS	0.005	0.005
Untypable	85 (24)	43 (32)	28 (18)	14 (14)	0.009	0.002	NS

[a]p values (by Fisher's exact test) are shown where p <0.05.
[b]Pyelo, pyelonephritis.
[c]NS, not significant (p ≥ 0.05).

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documented numerous syndrome-specific differences in the distribution of many of these traits, but conservation across source groups for others, particularly the most prevalent VFs. Overall, compared with fecal isolates, pyelonephritis and cystitis isolates contained more VF genes, and were more likely to belong to UTI-associated O types, phylogenetic group B2 and ST131.

The identified syndrome-specific differences among *E. coli* clinical and fecal isolates from men are consistent with findings from other studies, mostly involving women, which have shown a gradient of virulence from *E. coli* strains causing more invasive UTI syndromes, such as pyelonephritis and febrile UTI, through those causing cystitis to fecal strains [2,13]. However, compared with many studies in women, including our own, in the same geographic area and time period [8], the present cystitis isolates appear more virulent and closer to the pyelonephritis isolates, and farther from the fecal isolates. This suggests that men are less likely than women or girls to develop cystitis because of low-virulence strains, which is consistent with a previous observation that isolates from men with febrile UTI appeared relatively virulent, even in the presence of host compromise [14].

Of the three source groups, the pyelonephritis isolates exhibited the highest prevalences of many individual VFs, had the highest VF scores, and, were the most likely to belong to phylogenetic group B2, ST131 and a UTI-associated O type. Eight VF genes were significantly more prevalent among pyelonephritis than cystitis isolates, including five from the *pap* operon (*papAH*, *papC*, *papEF*, *papGII*, *papGIII)* and three that encode non-adhesin traits, including a toxin (*cnf1*), an outer membrane protease (*ompT*), and a bacteriocin (*usp*). Most of these genes have also been associated with pyelonephritis in females [15–17], and conceivably could be targets for interventions against pyelonephritis in males.

The higher prevalence of *pap* operon genes (encoding P fimbriae) in pyelonephritis than in cystitis isolates is in agreement with previous findings [15,17] and correlates with increased tropism, for the kidney, of P fimbriated strains. *papGII* has also been shown, experimentally to contribute to the pathogenesis of pyelonephritis [18,19], and *ompT* is strongly associated with febrile UTI in men [6,14]. However, it is not clear whether these VFs act individually or in concert with other known or unknown VFs in causing pyelonephritis. Additionally, although these findings suggest that cystitis can be caused by less virulent strains than those causing pyelonephritis, they could also mean that other VF genes, not included in the present study, are specifically associated with cystitis in men. Also, the simple presence/absence of VFs may be insufficient to define differences in virulence between strains. Notably, we did not explore other factors, such as VF gene polymorphisms [20,21], and variations in *fimH* sequences [20,21] or *fim* operon regulation [22], that also play a role in UTI pathogenesis.

Despite differences in VF gene prevalence values and VF scores, phylogenetic group distribution did not differ significantly between pyelonephritis and cystitis isolates. However, within each phylogenetic group, VF scores exhibited a gradient across source groups, implying the presence of source group-specific subsets with different levels of virulence. This agrees with previous work [16] and suggests that VF repertoire is as, or more, important than phylogenetic background for predicting pathogenic behavior in extraintestinal *E. coli.*

Escherichia coli ST131, within virulence-associated phylogenetic group B2, has emerged recently as a globally disseminated cause of multidrug-resistant extraintestinal infections in humans and companion animals, including in Australia [23]. To our knowledge, the prevalence of ST131 in different types of UTI has not been assessed previously, even among women and girls, let alone men.

Here we report two novel findings regarding ST131. First, ST131 exhibited a marked prevalence gradient across source groups, accounting for 36% of pyelonephritis and 8% of cystitis isolates ($p < 0.001$), but no fecal isolates ($p < 0.01$), consistent with increased urovirulence, compared with other *E. coli*. This provides to our knowledge, the first epidemiological evidence of increased virulence for ST131, which has been presumed but is not supported by available evidence from experimental animal models [24,25]. ST131 is associated with CTX-M-15 extended-spectrum β-lactamase production, which makes it resistant to extended cephalosporins. The resistance advantage, in combination with the possible presence of enhanced virulence, could explain the recent worldwide emergence of ST131.

Second, although most ST131 isolates exhibited the O25b *rfb* variant, as expected, a substantial minority exhibited type O16, and this subset (which, notably, would not be detected by screening for the O25b *rfb* variant) [26] was confined to pyelonephritis, suggesting an enhanced ability to cause kidney infection. Further studies of ST131, including, specifically, its O25b and (newly reported here) O16 variants, are needed to clarify its anatomical site and syndrome tropism and the basis for its impressive emergence and epidemic spread.

UTI-associated O antigen types were widely distributed among the three sources studied, with significant differences in the syndrome-related distribution of certain O types. Of note, three O types (O6, O25 and O75) were significantly associated with pyelonephritis, suggesting that O typing could contribute to intervention strategies.

Strengths of this study include the large number of well-characterized cystitis, pyelonephritis and fecal isolates from

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the same geographical region and time period. This is important because human-associated *E. coli* strains can vary dramatically by region and over time [6,27]. Other strengths include the extensive array of bacterial traits studied (including identification of the epidemic ST131 clonal group), and the analysis of their distribution by phylogenetic group and syndrome.

Study limitations include the use of multiple comparisons, which can increase the chance of type one errors [28]. However, we regard our analysis as being exploratory and hypothesis generating rather than definitive, requiring confirmation in future studies.

Additionally, virulence was inferred based on molecular traits, not *in vivo* assessment, and presence–absence testing for a defined set of genes risks overlooking other potentially important determinants of cystitis and pyelonephritis, including unrecognized VF genes [29], minor sequence variants of known VFs [21], or differences in VF expression [30].

Finally, although our aim was to study uncomplicated UTI without interference from the confounding effect of host compromise, we cannot be certain that no patients had significant urinary tract abnormalities, because studies to rule out structural or functional abnormalities were not performed. However, patients were judged clinically to have uncomplicated UTI based on the absence of known predisposing conditions.

In conclusion, our study, which to our knowledge is the first to compare *E. coli* isolates from men with cystitis or pyelonephritis and from healthy fecal controls, from the same locale and time period, identified a gradient of molecularly inferred virulence, with pyelonephritis isolates appearing the most virulent, followed closely by cystitis isolates, then distantly by fecal isolates. The ST131 clonal group exhibited a similar prevalence gradient, suggesting enhanced virulence compared with other *E. coli.* These findings, which significantly advance our understanding of the distinctive *E. coli* strains that cause UTI in men, may help in designing future diagnostic tests and possibly preventive measures.

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Conflicts of Interest

The authors have no conflict of interest to declare.

Transparency Declaration

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