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Escherichia coli Sequence Type 131 as a Prominent Cause of Antibiotic Resistance among Urinary *Escherichia coli* Isolates from Reproductive-Age Women

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The recent emergence of multidrug-resistant *Escherichia coli* sequence type 131 (ST131) has coincided with an increase in general antibiotic resistance of *E. coli*, suggesting that ST131 has a contributing role in resistance. However, there is little information about the contribution of ST131 to different clinical syndromes or the basis for its impressive emergence and epidemic spread. To investigate this, we studied 953 *E. coli* isolates from women of reproductive age in the central west region of New South Wales, Australia, including 623 urinary isolates from patients with cystitis (cystitis isolates) ($n = 322$) or pyelonephritis (pyelonephritis isolates) ($n = 301$) and 330 fecal isolates from healthy controls. The characteristics studied included ST131 clonal group status, resistance to different antibiotics, presence of virulence factor (VF) genes, and biofilm production. As expected, fecal isolates differed significantly from urinary (cystitis and pyelonephritis) isolates in most of the studied characteristics. Antibiotic resistance was significantly more common in ST131 than in non-ST131 isolates. Both antibiotic resistance and ST131 were more common in pyelonephritis than cystitis isolates and least so among fecal isolates. Within each source group, individual VF genes were more prevalent and VF scores were higher for ST131 than for non-ST131 isolates. For ST131 only, the prevalences of most individual VF genes and VF scores were the lowest in the fecal isolates, higher in the cystitis isolates, and highest in the pyelonephritis isolates. Biofilm production was strongly associated with ST131 status and antibiotic resistance. These results clarify the distribution of the ST131 clonal group and its epidemiological associations in our region and suggest that it exhibits both enhanced virulence and increased antibiotic resistance compared with those of other urinary tract infection (UTI) and fecal *E. coli* isolates from women of reproductive age.

Urinary tract infections (UTIs) are among the most common bacterial infections in humans (1). They are usually caused by specialized strains of *Escherichia coli* termed uropathogenic *E. coli* (UPEC), which possess distinct virulence factors (VFs) that can allow them to invade and injure a host. The effective management of UPEC is needed to reduce the associated health care costs and social burden.

Among urinary *E. coli* isolates, the prevalence of antibiotic resistance, especially to commonly used agents, such as fluoroquinolones (FQs), has increased substantially in recent years (2, 3, 4). However, although several studies have examined antibiotic resistance in different populations, most have not examined the related clinical and epidemiological associations. A better understanding of the microbiological basis for the emergence of antibiotic resistance among UPEC isolates is needed to guide the efforts that are aimed at interrupting this process.

The recent emergence of multidrug-resistant *E. coli* sequence type 131 (ST131) has contributed to the rising prevalence of resistance. ST131 is now globally disseminated and is a significant contributor to extraintestinal *E. coli* infections worldwide, which poses further challenges to the management of UTIs (5). In previous studies in the United States and Europe, ST131 has accounted for a large proportion of resistant *E. coli* isolates overall, especially those that are resistant to FQs, trimethoprim-sulfamethoxazole (TMP-SMZ), and extended-spectrum cephalosporins.

Recent surveys of urine and fecal *E. coli* isolates from children and men (6, 7) have shown that the prevalence of ST131 is significantly higher among urinary isolates (especially from patients with pyelonephritis) than in fecal isolates, suggesting heightened urovirulence for this clonal group. If confirmed, this might help to explain the recent emergence of ST131 and the rising prevalence of antibiotic resistance in clinical *E. coli* isolates. However, several studies have suggested that antibiotic-resistant isolates, particularly those that are resistant to quinolones and FQs, possess fewer VF genes than their susceptible counterparts (8–11). Thus, clarification is needed regarding the virulence potential of ST131 compared to other *E. coli* types and any differences in its prevalence between fecal or colonizing isolates and more or less invasive urinary isolates, especially from young women, who account for the greatest proportion of UTI episodes.

Biofilm production by *E. coli* is an important VF that may also protect bacteria from antibiotic action and so contribute to resistance (12, 13). However, little is known about the relationship between antibiotic resistance and biofilm production in urinary *E. coli* isolates according to clinical syndromes or clonal background, including ST131 status.

Therefore, we studied urinary *E. coli* isolates from young

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women with UTIs and also fecal isolates from healthy controls who were from the same geographical area and time period, in order to define (i) the prevalence of ST131 and antibiotic resistance in relation to the epidemiological source group, (ii) the contribution of ST131 to resistance, (iii) any association between biofilm production and isolate source, ST131 status, or antibiotic resistance, and (iv) the inferred virulence potential of ST131 compared to other *E. coli* sequence types.

MATERIALS AND METHODS

Study design. This prospective study involved 11 regional hospitals and 23 outpatient centers in the central west region of New South Wales (NSW), Australia (population, 180,000). It was conducted in conjunction with physicians in the participating centers. Each participating physician received a protocol for urine collection and the diagnostic criteria for cystitis and pyelonephritis. Based on the medical history and a physical examination, physicians recorded the following deidentified patient information: age, presence of clinical UTI syndrome, previous UTI history, and any known underlying host conditions.

Strains and study subjects. A total of 953 *E. coli* isolates were studied. They were isolated from the urine specimens of nonpregnant reproductive-age women (i.e., age 15 to 45 years) with cystitis (cystitis isolates) ($n = 322$) or pyelonephritis (pyelonephritis isolates) ($n = 301$) (as defined below) or from the fecal specimens of healthy women without urinary symptoms ($n = 330$). The isolates were collected over a 2-year period (June 2009 to July 2011), and one isolate per subject was included. To allow for a focus on bacterial characteristics with minimal confounding by host characteristics, patients with known diabetes mellitus, diarrhea, antibiotic therapy in the last month, menstruation, or urinary tract abnormalities were excluded.

Urine specimens were collected from qualifying women presenting to the participating centers. A diagnosis of cystitis or pyelonephritis required specific clinical manifestations (described below) plus a midstream urine culture that yielded $\geq 10^8$ CFU/liter of *E. coli*. Cystitis was defined clinically by urinary frequency, dysuria, and/or suprapubic tenderness, without fever or loin pain. Pyelonephritis was defined clinically by urinary symptoms, a fever of ≥38°C, and flank pain, with or without nausea and/or vomiting.

During the same time period, rectal swabs were collected from consenting female volunteers who, according to self-report, lacked UTI-associated manifestations. These women were recruited from family members accompanying patients or women attending reproductive clinics. Written informed consent was obtained from each volunteer. Controls were matched to the UTI subjects for age and (to the extent possible) by center. One arbitrarily chosen *E. coli* colony per specimen was used. Previous data indicate that the probability of an arbitrarily chosen fecal *E. coli* colony representing the quantitatively predominant clone in the sample is 86% (14). Rectal swabs were processed within 10 h of collection for the isolation of *E. coli*, which was identified by conventional biochemical tests.

Ethics approval. The project was approved by relevant institutional review boards (Charles Sturt University and Sydney West Area Health Service research committees). Since clinical information for patients with UTIs was provided anonymously by clinicians, patient consent was not obtained.

Urine cultures. Urine specimens were collected by clinical staff members of the participating health care centers using a standardized protocol. Semiquantitative culture was performed on horse blood, MacConkey, and chromogenic agars, followed by conventional identification. *E. coli* isolates were stored in 5% glycerol in Trypticase soy broth at −70°C.

Antibiotic susceptibility. *E. coli* isolates were tested for susceptibility to 14 antibiotics according to the disk diffusion method specified by the Clinical and Laboratory Standards Institute (CLSI) (15), using Neo-Sensitabs discs (Rosco, Taastrup, Denmark). The antibiotics tested were (disk content) amikacin (30 μg), amoxicillin-clavulanate (60 μg), ampicillin (25 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cephalothin (30 μg), ciprofloxacin (10 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), tetracycline (30 μg), and TMP-SMZ (5 μg). The double-disk diffusion test was used to detect the production of extended-spectrum β-lactamases (ESBLs) (16). Resistant isolates were defined as those that were resistant to ≥1 agent. The resistance score was the number of antibiotic classes (of the 13 classes studied) for which an isolate exhibited resistance to ≥1 representative agent.

Biofilm production. *In vitro* biofilm production was tested using crystal violet retention in microtiter plates, as described elsewhere (17).

Phylotyping, VF genotyping, and ST131 detection. The isolates were tested in duplicate for the 4 main *E. coli* phylogenetic groups (A, B1, B2, and D) using a triplex PCR (18). A multiplex PCR-reverse line blot assay was used to detect the presence of any of the 22 VF genes, as previously described (19). The VF score of an isolate was defined as the sum of the VFs detected in it, except that multiple *pap* operon genes were counted as a single factor. Such molecular characteristics predict experimental virulence *in vivo* (20).

Group B2 isolates were screened for ST131 status by PCR-based detection of ST131-specific single nucleotide polymorphisms (SNPs) in the *mdh* and *gyrB* genes (21, 22). PCR-based O (somatic antigen) typing was used to detect 2 ST131-associated O types, O16 and O25 (21). The O25 isolates were further characterized as O25a versus O25b by variant-specific PCR (23, 24).

Statistical analysis. Comparisons of proportions were tested using Fisher's exact test. Virulence and resistance score comparisons were tested using the Mann-Whitney U test.

RESULTS

Distribution of antibiotic resistance by source. Overall, a significantly higher proportion of urinary (65%) than fecal (24%) isolates were resistant to >1 antibiotic and also to 12 of 14 individual agents (Table 1). Among the urinary isolates, pyelonephritis isolates were generally more resistant than cystitis isolates. The rates of ESBL production followed a similar gradient (highest among pyelonephritis and lowest among fecal isolates).

Distribution of phylogenetic groups, O types, and ST131 by source. Group B2 predominated among urinary isolates, occurring with similar frequencies in cystitis (66%) and pyelonephritis (70%) isolates (Table 2). In contrast, among the fecal isolates, groups A (40%) and B2 (31%) predominated. Overall, 27% of group B2 isolates belonged to ST131, and of these, 85% were from serogroup O25b and 15% from serogroup O16 (Table 2). The prevalence of ST131 was highest for pyelonephritis isolates (30%), followed by cystitis isolates (13%), and was lowest for fecal isolates (4%).

Distribution of antibiotic resistance in relation to ST131 status and source group. Within each source group, resistance to most individual antibiotics, resistance scores, and ESBL production were significantly more prevalent or higher in ST131 than in non-ST131 isolates (Table 3). Among the ST131 isolates, ESBL production was lowest in fecal isolates, higher in cystitis isolates, and highest in pyelonephritis isolates. Similar gradients were demonstrated across source groups for the contribution of ST131 to individual antibiotic resistances (Table 4). Likewise, within each source group, the prevalence of biofilm production was significantly higher among ST131 isolates than non-ST131 isolates and followed a significant prevalence gradient from the lowest in fecal isolates, higher in cystitis isolates, and highest in pyelonephritis isolates (Table 3). Among non-ST131 isolates, neither ESBL nor biofilm production differed significantly in prevalence by source, but overall resistance to >1 antibiotic was more prevalent among urine isolates than fecal isolates (164/492 [33%] versus 75/326 [23%]) ($P = 0.001$).

TABLE 1 Prevalence of antimicrobial drug resistance among 953 *E. coli* isolates from reproductive-age women with cystitis or pyelonephritis and healthy fecal controls

Resistance parameter[a]	Isolate source			*P* value by isolate source[b]		
	Fecal ($n = 330$)	Cystitis ($n = 322$)	Pyelonephritis ($n = 301$)	Fecal vs cystitis	Fecal vs pyelonephritis	Cystitis vs pyelonephritis
No. (%) of isolates resistant to the following antibiotics						
Ampicillin	59 (18)	135 (42)	160 (53)	<0.001	<0.001	0.006
Amoxicillin-clavulanate	33 (10)	84 (26)	102 (34)	<0.001	<0.001	0.036
Cephalothin	33 (10)	129 (40)	157 (52)	<0.001	<0.001	0.003
TMP-SMZ	17 (5)	64 (20)	87 (29)	<0.001	<0.001	0.009
Nitrofurantoin	3 (1)	10 (3)	9 (3)	0.05	0.05	NS
Gentamicin	3 (1)	19 (6)	21 (7)	<0.001	<0.001	NS
Amikacin	0 (0)	6 (2)	6 (2)	0.010	0.010	NS
Norfloxacin	10 (3)	40 (12)	42 (14)	<0.001	<0.001	NS
Ciprofloxacin	10 (3)	41 (13)	41 (14)	<0.001	<0.001	NS
Imipenem	0 (0)	2 (0.5)	2 (0.5)	NS	NS	NS
Nalidixic acid	13 (4)	68 (21)	72 (24)	<0.001	<0.001	NS
Tetracycline	76 (23)	182 (57)	196 (65)	<0.001	<0.001	0.033
Ceftriaxone	3 (1)	13 (4)	18 (6)	0.011	NS	NS
Ceftazidime	0 (0)	3 (1)	12 (4)	0.016	<0.001	0.017
No. (%) of isolates that were ESBL producers	3 (1)	16 (5)	27 (9)	0.002	<0.001	0.04
No. (%) of isolates resistant to >1 agent	79 (24)	203 (63)	201 (67)	<0.001	<0.001	NS
Resistance score (median [range])	3 (0–9)	5 (0–11)	7 (0–13)	<0.001	<0.001	<0.001

[a] TMP-SMZ, trimethoprim-sulfamethoxazole; ESBL, extended-spectrum β-lactamase.
[b] *P* values are shown where $P < 0.05$, as calculated by Fisher's exact test; in the last row, the Mann-Whitney U test was used. NS, nonsignificant.

Association of ST131 with VF genes. Individual VF gene prevalences and VF scores were significantly higher for ST131 than non-ST131 isolates (Table 5). The VF scores of both ST131 and non-ST131 isolates followed statistically significant ascending gradients from fecal, to cystitis, to pyelonephritis isolates. Four VF genes (*iutA*, *usp*, *traT*, and *ompT*) were present in every ST131 isolate.

DISCUSSION

In recent years, antibiotic resistance among urinary *E. coli* isolates, particularly to FQs, TMP-SMZ, and cephalosporins, has increased and has coincided with the emergence of ST131. In this study, we assessed the contribution of ST131 to antibiotic resistance in UTI-associated *E. coli* isolates from women of reproductive age and provided novel insights into its epidemiology.

Increasing antibiotic resistance can be due to the acquisition of resistance by previously susceptible strains and/or the expansion and dissemination of resistant clones. Our results show that the ST131 clonal group contributed substantially to *E. coli* resistance among the isolates studied, especially those causing pyelonephritis; it accounted for 33% of all isolates that were resistant to one or more antibiotics and for considerable proportions of isolates that were resistant to most individual agents. Antibiotic resistance and ESBL production were significantly more common in ST131 than in non-ST131 isolates.

ST131 accounted for 30% of pyelonephritis isolates overall and 88% of those that were resistant to FQs. This is remarkable considering that *E. coli* is generally a diverse species, and it indicates that ST131 is a major clonal component among antibiotic-resistant (especially FQ-resistant) *E. coli* isolates in women of reproductive age in this region of NSW, Australia. Even among the cystitis isolates, the prevalence of ST131 in the FQ-resistant isolates was high (38%) and was comparable with that (35%) reported by Cagnacci et al. (25) in FQ-resistant cystitis isolates col-

TABLE 2 Distribution of phylogenetic groups and sequence type 131 among 953 *E. coli* isolates from reproductive-age women with cystitis or pyelonephritis and healthy fecal controls

Phylogenetic/clonal group[a]	No. (%) of isolates by source			*P* value by isolate source[b]		
	Fecal ($n = 330$)	Cystitis ($n = 322$)	Pyelonephritis ($n = 301$)	Fecal vs cystitis	Fecal vs pyelonephritis	Pyelonephritis vs cystitis
A	132 (40)	25 (8)	9 (6)	<0.001	<0.001	NS
B1	30 (9)	13 (4)	12 (5)	0.011	0.01	NS
B2	102 (31)	213 (66)	211 (70)	<0.001	<0.001	NS
D	66 (20)	64 (20)	57 (19)	NS	NS	NS
ST131	13 (4)	42 (13)	89 (30)	<0.001	<0.001	<0.001
O25b ST131	13 (4)	37 (12)	73 (24)	<0.001	<0.001	<0.001
O16 ST131	0 (0)	6 (2)	15 (5)	0.014	<0.001	0.044

[a] ST131 is a subset of phylogenetic group B2. O25b and O16 are subsets of ST131.
[b] *P* values (by Fisher's exact test) are shown where $P < 0.05$. NS, nonsignificant.

TABLE 3 Distribution of antimicrobial drug resistance in relation to source group and ST131 status among 953 *E. coli* isolates from reproductive-age women with cystitis or pyelonephritis and from healthy fecal controls

Antibiotic resistance or other trait[a]	No. (column %) with trait by isolate source and ST131 status						*P* values by isolate source (vs ST131)[b]		
	Fecal		Cystitis		Pyelonephritis				
	ST131 ($n = 4$)	Non-ST131 ($n = 326$)	ST131 ($n = 42$)	Non-ST131 ($n = 280$)	ST131 ($n = 89$)	Non-ST131 ($n = 212$)	Fecal	Cystitis	Pyelonephritis
Antibiotics									
Ampicillin	4 (100)	78 (24)	42 (100)	93 (33)	89 (100)	71 (33)	0.004	<0.001	<0.001
Amoxicillin-clavulanate	4 (100)	42 (13)	42 (100)	48 (17)	89 (100)	13 (6)	<0.001	<0.001	<0.001
Cephalothin	1 (25)	46 (14)	23 (55)	106 (38)	54 (61)	103 (49)	NS	0.043	NS
TMP-SMZ	2 (50)	23 (7)	22 (52)	42 (15)	52 (58)	35 (17)	NS	<0.001	<0.001
Nitrofurantoin	0 (0)	3 (1)	4 (10)	6 (2)	9 (10)	0 (0)	NS	0.029	<0.001
Gentamicin	1 (25)	3 (1)	9 (21)	10 (4)	13 (15)	8 (4)	0.039	<0.001	0.002
Amikacin	0 (0)	0 (0)	3 (6)	3 (1)	5 (6)	1 (<1)	NS	0.031	0.010
Norfloxacin	1 (25)	13 (4)	15 (36)	25 (9)	37 (42)	5 (2)	NS	<0.001	<0.001
Ciprofloxacin	1 (25)	13 (4)	15 (35)	26 (9)	36 (41)	5 (2)	NS	<0.001	<0.001
Imipenem	0 (0)	0 (0)	1 (2)	1 (<1)	1 (1)	1 (<1)	NS		
Nalidixic acid	1 (25)	16 (5)	24 (58)	44 (16)	58 (65)	14 (6)	NS	<0.001	<0.001
Tetracycline	4 (100)	104 (32)	37 (88)	166 (59)	84 (94)	112 (53)	0.011	<0.001	<0.001
Ceftriaxone	0 (0)	3 (1)	4 (10)	9 (3)	11 (12)	7 (3)	NS	NS	0.006
Ceftazidime	0 (0)	0 (0)	1 (2)	2 (1)	3 (3)	9 (4)	NS	NS	NS
ESBL production	0(0)	3 (1)	5 (11)	9 (3)	17 (19)	10 (5)	NS	0.002	<0.001
Resistance to >1 agent	4 (100)	75 (23)	42 (100)	93 (33)	89 (100)	71 (33)	NS	<0.001	<0.001
Biofilm production	4 (100)	52 (16)	36 (86)	151 (53)	89 (100)	128 (60)	NS	<0.001	<0.001
Resistance score (median [range])	5 (5–9)	2 (0–8)	7 (5–11)	5 (0–9)	9 (7–13)	8 (3–10)	<0.001	<0.001	<0.001

[a] TMP-SMZ, trimethoprim-sulfamethoxazole; ESBL, extended-spectrum β-lactamase.
[b] *P* values are shown where $P < 0.05$, as calculated by Fisher's exact test; in the last row, the Mann-Whitney U test was used. *P* values for ST131 by source group of fecal versus pyelonephritis and cystitis versus pyelonephritis were nonsignificant. NS, nonsignificant.

lected from 8 European countries in 2003 to 2006 and by other investigators (26, 27). These results imply that interrupting the dissemination of the ST131 clonal group might slow the rise in *E. coli* resistance in UPEC, particularly with FQs.

The higher prevalence of ST131 among the more-invasive isolates (30% in pyelonephritis) than in less-invasive (13% in cystitis) clinical and colonizing (4% in fecal) isolates provides epidemiological evidence for the increased virulence of ST131. This has been assumed but not previously demonstrated, except in our previous study of isolates from males (6) from the same geographical region and time period, in which ST131 was found in 0%, 8%, and 36% of fecal, cystitis, and pyelonephritis isolates, respectively ($P < 0.001$) (6). Our study of isolates from children (7) showed similar results.

TABLE 4 Proportional contribution of ST131 to resistance by antimicrobial agent and source group

Antimicrobial agent	No. of ST131-resistant isolates/total no. of isolates (%) by isolate source		
	Fecal	Cystitis	Pyelonephritis
Ampicillin	4/59 (7)	42/135 (31)	89/160 (56)
Amoxicillin-clavulanate	4/33 (12)	42/90 (47)	89/102 (87)
Cephalothin	1/33 (3)	23/129 (18)	54/157 (34)
Trimethoprim-sulfamethoxazole	2/17 (12)	22/64 (34)	52/87 (60)
Nitrofurantoin	0/3 (0)	4/10 (40)	9/9 (100)
Gentamicin	1/3 (33)	9/19 (47)	13/21 (62)
Amikacin	0/3 (0)	3/6 (50)	5/6 (83)
Norfloxacin	1/10 (10)	15/40 (38)	37/42 (88)
Ciprofloxacin	0/3 (0)	15/41 (37)	36/41 (88)
Imipenem	1/13 (7)	1/2 (50)	1/2 (50)
Nalidixic acid	4/76 (5)	24/68 (35)	58/72 (81)
Tetracycline	0/3 (0)	37/193 (19)	84/196 (43)
Ceftriaxone	0/3 (0)	4/13 (31)	11/18 (61)
Ceftazidime	0/3 (0)	1/3 (33)	3/12 (25)
All drug-bacterium combinations	17/265 (6.4)	247/826 (29.9)	554/943 (58.7)

Our present findings suggest possible explanations for the remarkable success of ST131 as an emerging pathogen, including information about its phylogenetic background, virulence characteristics, and antibiotic resistance capacities. Specifically, ST131 isolates had more VF genes and antibiotic resistance markers, had a higher prevalence of biofilm production, and were more likely to be from group B2 than other *E. coli* isolates, even within the same source group. ST131 isolates derive from the phylogenetic group B2, which is associated epidemiologically and experimentally with extraintestinal virulence (10, 11).

The combination of increased antibiotic resistance and virulence provides a plausible explanation for the remarkable success of ST131 as an emerging pathogen by providing a competitive advantage over other *E. coli* strains and promoting clonal expansion and dominance within niches that are traditionally occupied by less virulent and/or more susceptible clones. However, our findings apparently contradict those of other studies (8, 10, 24, 27, 28) that have reported reduced virulence among antibiotic-resistant *E. coli* isolates, although few have specifically studied the role of ST131. Furthermore, a study by Lavigne et al. (29) showed that CTX-M-15-producing ST131 isolates were less virulent than other phylogenetic group B2 isolates, although the number of isolates tested was small (30).

Nevertheless, antibiotic resistance has been generally more common among *E. coli* isolates from non-B2 phylogenetic groups, and resistant isolates generally have fewer virulence traits than susceptible ones (8, 24, 27). Although some investigators have

TABLE 5 Prevalence of virulence traits in relation to source group and ST131 status

Group or virulence factor	No. (%) of isolates with trait by isolate source and ST131 status						*P* value by isolate source (vs ST131)[a]		
	Fecal		Cystitis		Pyelonephritis				
	ST131 ($n = 4$)	Non-ST131 ($n = 326$)	ST131 ($n = 42$)	Non-ST131 ($n = 280$)	ST131 ($n = 89$)	Non-ST131 ($n = 212$)	Fecal	Cystitis	Pyelonephritis
Groups									
A	0 (0)	130 (40)	0 (0)	28 (10)	0 (0)	13 (6)	NS	0.035	NS
B1	0 (0)	29 (9)	0 (0)	17 (6)	0 (0)	13 (6)	NS	0.007	0.012
B2	4 (100)	98 (30)	42 (100)	174 (62)	89 (100)	138 (65)	<0.001	<0.001	<0.001
D	0 (0)	68 (21)	0 (0)	62 (22)	0 (0)	49 (23)	NS	<0.001	<0.001
Virulence factors[b]									
afa/draBC	1 (25)	19 (6)	6 (14)	39 (14)	19 (21)	19 (9)	NS	NS	0.007
bmaE	0 (0)	0 (0)	2 (5)	1 (<1)	6 (7)	0 (0)	NS	NS	<0.001
sfaS	4 (100)	19 (6)	9 (21)	75 (27)	17 (19)	48 (22)	<0.001	NS	NS
fimH	4 (100)	286 (88)	42 (100)	275 (98)	89 (100)	206 (97)	NS	NS	NS
focG	3 (75)	30 (9)	19 (45)	142 (51)	35 (39)	119 (56)	0.003	NS	0.008
papGI	0 (0)	0 (0)	1 (2)	1 (<1)	5 (6)	4 (2)	NS	NS	NS
papGII	1 (25)	6 (2)	16 (38)	76 (27)	36 (40)	84 (40)	<0.001	NS	NS
papGIII	1 (25)	16 (5)	15 (36)	106 (38)	35 (39)	33 (37)	NS	NS	NS
papAH	4 (100)	65 (20)	24 (48)	169 (60)	52 (58)	136 (64)	0.002	NS	NS
papC	4 (100)	125 (38)	18 (43)	155 (55)	46 (52)	129 (61)	0.023	NS	NS
papEF	4 (100)	112 (34)	21 (50)	184 (66)	50 (56)	142 (67)	0.015	NS	NS
gafD	0 (0)	0 (0)	3 (7)	6 (2)	9 (10)	9 (4)	NS	NS	NS
cnf1	1 (25)	62 (19)	15 (36)	117 (42)	37 (42)	114 (53)	NS	NS	NS
hlyA	1 (25)	91 (28)	16 (38)	135 (48)	34 (40)	126 (59)	NS	NS	0.001
iutA	4 (100)	52 (16)	42 (100)	174 (62)	89 (100)	145 (68)	<0.001	<0.001	<0.001
fyuA	4 (100)	98 (30)	37 (88)	234 (84)	76 (85)	168 (79)	0.009	NS	NS
iroN	2 (50)	84 (26)	35 (83)	152 (54)	78 (88)	136 (64)	NS	<0.001	<0.001
kpsMTII	1 (25)	75 (23)	25 (59)	188 (67)	54 (61)	151 (71)	NS	NS	NS
kpsMTIII	0 (0)	3 (1)	3 (7)	26 (9)	4 (5)	17 (8)	NS	NS	NS
traT	4 (100)	138 (42)	42 (100)	206 (74)	89 (100)	137 (65)	0.034	<0.001	<0.001
ompT	4 (100)	108 (33)	42 (100)	161 (58)	89 (100)	155 (73)	0.013	<0.001	<0.001
usp	4 (100)	227 (70)	42 (100)	125 (45)	89 (100)	167 (79)	0.002	<0.001	<0.001
Virulence score (median [range])	6 (7–12)	2 (0–12)	8 (8–16)	6 (1–13)	9 (10–17)	7 (1–17)	<0.001	<0.001	<0.001

[a] *P* values are shown where $P < 0.05$, calculated by Fisher's exact test, except for last row, where Mann-Whitney U test was used. NS, nonsignificant.
[b] The 22 virulence factors analyzed were *afa/draBC*, Dr-binding adhesins; *bmaE*, M fimbriae; *sfaS*, S fimbriae; *fimH*, type 1 fimbriae; *focG*, F1C fimbriae; *papG*, P fimbria adhesin (and alleles I, II, and III); *papA*, P fimbria structural subunit; *papC*, P fimbria assembly; *papEF*, fimbria tip pilins; *gafD*, G fimbriae; *cnf1*, cytotoxic necrotizing factor type1; *hlyA*, hemolysin; *iutA*, aerobactin receptor; *fyuA*, ferric yersiniabactin receptor; *iroN*, salmochelin receptor; *kpsMTII* group 2 capsule (with K1 and K2 variants); *kpsMTIII*, group 3 capsule; *traT*, serum resistance associated; *ompT*, outer membrane protein T (protease); *usp*, uropathogenic specific protein.

postulated that ST131 has enhanced virulence compared with other *E. coli* strains, this was not supported by the data from experimental animal models (30).

Thus, although increased virulence and antibiotic resistance in *E. coli* organisms have been traditionally regarded as inversely related (31), our study provides support from a new patient population for the concept that ST131 may have both increased virulence and resistance compared with other strains (32, 33). However, we consider these findings to be hypothesis generating and exploratory, requiring confirmation in the future.

Few studies have reported ST131 colonization of the fecal flora (34). We detected ST131 in 4% of fecal isolates in this study and a small proportion (<1%) of those from children (7), signifying an appreciable dissemination of this clone in the community. These findings have major public health significance and indicate a need for research into the methods to interrupt the spread of ST131.

Four VF genes (*iutA*, *ompT*, *usp*, and *traT*) were present in all the ST131 isolates examined in this study and so might represent potential targets for vaccines or other interventions, particularly if a functional role in virulence or dissemination can be demonstrated for them. As was previously reported (35), most (85%) of our ST131 isolates exhibited the O25b variant, and the rest were from type O16. These findings are comparable to those of a previous study in Japan and our own study of isolates from men (6, 36). The origin, relationship to other ST131 strains, and significance of the O16 subgroup within ST131 remain to be elucidated.

The resistance of ST131 to extended-spectrum cephalosporins is often due to the production of ESBLs. The initial descriptions of ST131 emphasized its association with CTX-M-15, but subsequent studies have shown that it is more commonly ESBL negative and FQ resistant (36–38). Here, ESBLs were produced by 16% of ST131 isolates compared with 3% of non-ST131 isolates, indicating a substantial clonal spread of ESBL-producing *E. coli* in women of reproductive age in this region of Australia (39). Our results are consistent with a recent study in Japan, which showed that the ST131-O25b, ST131-O16, and group D-ST405 clonal groups contributed to the spread of ESBL-producing *E. coli* (36). The dominant ESBL in *E. coli*, both globally and in Australia (40), is CTX-M-15,

which is frequently carried on plasmids found in the ST131 pandemic clonal group.

We found strong associations between biofilm production, the ST131 clonal group, and antibiotic resistance in both cystitis and pyelonephritis isolates. This conflicts with a previous study in which biofilm production in *E. coli* was negatively associated with quinolone resistance, although that study involved bacteremic isolates (17). Biofilm production by ST131 may protect bacteria from exposure to high levels of antibiotics, thereby encouraging the development of resistance from low-level exposures. A recent study found that antibiotic resistance and ESBL production were associated with the lack of expression of the biofilm components, curli and cellulose (41). It is important that these apparently contradictory findings be further investigated using isolates from different infection sites (e.g., blood versus urine), since biofilm formation may be a potential target for novel intervention for UTI treatment or the prevention of antibiotic resistance.

The prevalences of resistance to most antibiotics were lower in the present study than has been reported from North America and Europe (3, 10, 11, 24, 27, 31). In addition, contrary to our findings, higher prevalences of quinolone and FQ resistance have been reported in cystitis *E. coli* isolates than in pyelonephritis isolates (10, 42). Over the last 10 to 20 years, antibiotic surveillance programs have shown reductions in the use of antibiotic drugs in Australia (43), Slovakia (44), and Sweden (45). Our results probably reflect more conservative antibiotic prescribing and consumption practices in Australia than in many other countries.

The strengths of this study include its large sample size, geographical and temporal matching of the 3 source groups, clinically well-defined and recent-case isolates, inclusion of fecal controls, and extensive array of bacterial characteristics assessed, including screening for the ST131 clonal group. A study limitation is that certain subgroups, particularly within the fecal group, were small, increasing the probability of type II errors, whereas the use of multiple comparisons increased the likelihood of type I errors. Additionally, the VFs studied represent only a fraction of those that have been identified (42). It is not certain that all the markers we studied contribute to virulence, and an analysis of additional or different VFs might conceivably lead to different conclusions. Although we tried to avoid the confounding effects of host compromise on VF genes, phylogenetic groups, and antibiotic resistance by excluding isolates from patients with known urinary tract abnormalities, previous UTI, pregnancy, and diabetes, it is possible that some patients with these and other host-compromising factors were included. Lastly, we only picked one random fecal *E. coli* colony per patient, which is considered a bias. It is possible that an analysis based on multiple-colony fecal analysis would yield different results.

In summary, we documented a high prevalence of the ST131 clonal group and its substantial contribution to the prevalence of antibiotic resistance among recent urinary and fecal *E. coli* isolates from women of reproductive age in the central west region of NSW, Australia. The distinctive combination of the extensive antibiotic resistance and seemingly enhanced virulence in ST131 isolates may provide a competitive advantage of this clonal group, contributing to its rapid worldwide dissemination. These findings indicate a significant public health threat and therefore a need for additional studies to determine the distribution and risk factors for acquisition of the ST131 clonal group so that effective control measures can be devised.

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