



# Genotypic analysis of uropathogenic *Escherichia coli* to understand factors that impact the prevalence of $\beta$ -lactam-resistant urinary tract infections in a community

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## ABSTRACT

**Objectives:** To determine the contribution of specific uropathogenic *Escherichia coli* (UPEC) lineages, drug resistance genes, and plasmid incompatibility/replicon (Inc) groups to the prevalence of  $\beta$ -lactam-resistant urinary tract infections (UTIs) in a university community.

**Methods:** Urine samples were consecutively collected and cultured over a 2-year period from patients presenting to a university health centre with symptoms of UTI. Isolated UPEC were subtyped by multilocus sequence typing and *fimH* typing, and tested by PCR and sequencing for  $\beta$ -lactamase genes and plasmid Inc groups.

**Results:** Among 273 UPEC isolates, 85 (31%) were ampicillin-resistant (AMP-R) and 188 (69%) were susceptible to all  $\beta$ -lactam drugs (AMP-S). Six lineages accounted for two-thirds of the isolates: ST95 (21%), ST69 (11%), ST420 (11%), ST73 (10%), ST127 (8%), and ST404 (3%). ST69 and ST404 were associated with AMP-R ( $P=0.003$ ,  $P=0.0005$ ), while ST420 and ST127 were associated with AMP-S ( $P<0.0001$ ,  $P=0.027$ ). ST95 contained four *fimH* types; the ST95/*f*-6 sublineage was more frequently identified among the AMP-R population ( $P=0.009$ ), while the ST95/*f*-47 sublineage was more frequently identified among the AMP-S population ( $P=0.007$ ). The most common  $\beta$ -lactamase gene was *bla*<sub>TEM</sub>, which was identified in 81 (95%) AMP-R isolates. IncFIB, IncFIA, and IncB/O type plasmids were the most commonly identified types, and were associated with  $\beta$ -lactam resistance ( $P<0.001$  for all).

**Conclusions:** These observations indicate that the prevalence of  $\beta$ -lactam-resistant UTIs in this community was largely determined by a limited set of circulating UPEC STs and sublineages, carrying TEM  $\beta$ -lactamase genes that were likely encoded on one of three Inc type plasmids.

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## 1. Introduction

Urinary tract infections (UTIs) are among the most common types of infection in community and healthcare settings. The main cause of UTIs in both environments is uropathogenic *Escherichia coli* (UPEC), which typically accounts for 75% of community-acquired UTIs (CA-UTIs) and 50–65% of healthcare-associated UTIs [1,2]. Empirical antimicrobial therapy is frequently administered to treat UTIs and prevent the spread of infection to other sites such as the bloodstream. However, selection of an appropriate antimicrobial agent is a growing challenge due to the increasing prevalence of antimicrobial resistance in *E. coli* and other urinary tract pathogens [3,4]. In particular,  $\beta$ -lactam-resistant *E. coli*, especially

those producing extended-spectrum  $\beta$ -lactamases (ESBLs) or carbapenemases, have become a global concern [3–6]. Understanding factors that affect the prevalence and dissemination of antimicrobial resistance may provide insights into strategies to slow the spread of resistance and inform better empirical treatment decisions.

Uropathogenic *E. coli* are a subset of extraintestinal pathogenic *E. coli* (ExPEC), which cause disease outside the intestinal tract [7]. Classic pandemic ExPEC clones, identified through multilocus sequence typing (MLST), include ST393, ST69, ST73, ST95, and ST131 [7]. The predominant clones identified in a population and the proportional composition can vary and depend on the study population [8]. Few studies have fully examined the distribution of ExPEC STs across both antimicrobial-susceptible and antimicrobial-resistant UPEC, and even fewer studies have examined the distribution of sublineages [8–14]. As a result, it is not well understood whether the clonal composition of the

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antimicrobial-resistant UPEC population in a community is distinct from the susceptible population, and whether resistant UPEC emerge from prevalent sublineages in the drug-susceptible population.

To address these questions, the present study compared the distribution of genotypes among  $\beta$ -lactam-resistant and susceptible UPEC isolated from a university community. In addition, isolates were tested for  $\beta$ -lactamase genes and plasmid incompatibility/replicon (Inc/rep) groups to identify the most prevalent resistance genes and plasmid types associated with resistance.

## 2. Materials and methods

### 2.1. Strain collection and susceptibility testing

The CA-UTI *E. coli* were isolated from urine specimens consecutively collected from patients presenting to the University Health Services at the University of California, Berkeley with symptoms of UTI from April 2003 to June 2004 and October 2004 to January 2005 [15,16]. *E. coli* isolated from urine samples demonstrating a viable bacterial count of  $\geq 10^4$  CFU/mL were used in this study. Isolates were tested for susceptibility to antimicrobial agents by broth microdilution, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, which included the following classes and agents:  $\beta$ -lactams (ampicillin (AMP), piperacillin, ampicillin/sulbactam, piperacillin/tazobactam, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, cephalothin, cefazolin, cefoxitin, cefotetan, cefuroxime, ceftriaxone, ceftizoxime, cefotaxime, ceftazidime, aztreonam, cefepime, imipenem, meropenem), folate pathway inhibitors (trimethoprim/sulfamethoxazole (SXT)), aminoglycosides (amikacin, gentamicin, tobramycin), fluoroquinolones (ciprofloxacin, gatifloxacin, levofloxacin), nitrofurantoin, tetracycline (TET), and chloramphenicol (CHL) [15,16]. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more classes of tested drugs. For the  $\beta$ -lactams, isolates resistant to at least ampicillin were designated AMP-R, and isolates susceptible to all tested  $\beta$ -lactams were designated AMP-S.

### 2.2. DNA extraction

Bacteria were subcultured from frozen glycerol stocks and grown overnight in LB broth in a 37 °C shaker. The next day, 1 mL of

culture was pelleted in a microfuge tube by spinning in a microcentrifuge at maximum speed for 1 min. Pellets were resuspended in 300  $\mu$ L of sterile water and boiled for 10 min. Samples were pelleted, and the supernatant was collected and stored at  $-20^\circ\text{C}$ .

### 2.3. Multilocus sequence typing

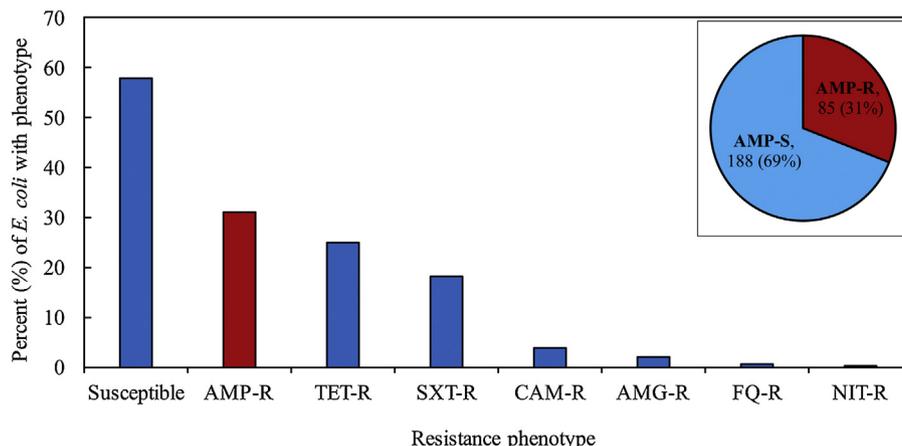
Primer sequences and protocols for PCR amplification of seven housekeeping genes for MLST – *adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* – were obtained from the EnteroBase website ([https://enterobase.warwick.ac.uk/species/ecoli/allele\\_st\\_search](https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search)) [17]. Amplicons were cleaned and sequenced by Sanger sequencing at the University of California, Berkeley DNA Sequencing Facility (Berkeley, CA). Geneious® v.9.1.3 (Biomatters Ltd., New Zealand) was used to visually inspect, edit, then align forward and reverse sequences to obtain a consensus sequence. Trimmed consensus sequences were submitted to the EnteroBase MLST database curator for sequence type (ST) and sequence complex assignment.

### 2.4. fimH typing

Single nucleotide polymorphism (SNP) analysis of *fimH* can be used to further differentiate isolates within lineages identified by MLST. The PCR for *fimH* SNP analysis was performed according to Tartof et al. [18]. Amplicons were cleaned, sequenced, then analysed with Geneious®. The known *fimH* alleles, corresponding to bp positions 360 to 781, were obtained from GenBank then aligned with trimmed *fimH* consensus sequences to type the alleles [18–20]. A FASTA file of the known *fimH* alleles can be found in the Supplementary section.

### 2.5. Identification of $\beta$ -lactamase genes

PCR for  $\beta$ -lactamase genes – *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>KPC</sub>, and *bla*<sub>CMY</sub> – was performed according to Dallenne et al. [21]. Amplicons were cleaned, sequenced, then analysed with Geneious®. DNA sequence variants for *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, and *bla*<sub>CTX-M</sub> genes were obtained from the database of K. Bush, T. Palzkill, and G. Jacoby (<http://www.lahey.org/Studies/>) and GenBank, then aligned with the current  $\beta$ -lactamase consensus sequences to identify the  $\beta$ -lactamase variants present in the current collection.



**Fig. 1.** Distribution of antimicrobial resistance among CA-UTI *Escherichia coli* isolates.

Susceptible, isolates were susceptible to all agents tested. AMP-R, resistant to at least ampicillin for the  $\beta$ -lactams tested; TET-R, tetracycline-resistant; SXT-R, trimethoprim/sulfamethoxazole-resistant; CAM-R, chloramphenicol-resistant; AMG-R, resistant to at least one tested aminoglycoside; FQ-R, resistant to at least one tested fluoroquinolone; NIT-R, resistant to nitrofurantoin. The figure inset displays the number and percent of isolates that are AMP-S (susceptible to all  $\beta$ -lactams tested) and AMP-R.

2.6. Plasmid incompatibility/replicon (Inc/rep) typing

The PCR for plasmid Inc/rep typing for Inc groups A/C, B/O, F, FIA, FIB, FIC, FIHA, HI2, I1-Iγ, L/M, N, P, and Y was performed according to Carattoli et al. [22]. Amplicons were cleaned, sequenced, then analysed with Geneious®. The Inc/rep consensus sequences were compared with sequences deposited in the NCBI database using the BLAST program.

2.7. Statistical analysis

Categorical variables were compared with a  $\chi^2$  or Fisher's exact test (two-tailed). A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Antimicrobial susceptibility profile of E. coli causing CA-UTIs

Of the 273 consecutively collected CA-UTI E. coli isolates, 85 (31%) were AMP-R (resistant at least to ampicillin for the  $\beta$ -lactams tested) and 188 (69%) were AMP-S (susceptible to all  $\beta$ -lactams tested) (Fig. 1). In addition, 68 (25%) isolates were resistant to TET, 48 (18%) were resistant to SXT, 11 (4%) were resistant to CHL, six (2%) were resistant to an aminoglycoside, two (0.7%) were resistant to a fluoroquinolone, and one (0.4%) was resistant to nitrofurantoin.

Of 188 AMP-S isolates, 157 (84%) were susceptible to all tested antimicrobial agents; the other 31 (16%) were mainly resistant to SXT (16%), TET (52%), or both (29%). Of 85 AMP-R isolates, 54 (64%) were resistant to another class of antimicrobial agent, which typically included SXT (20%), TET (37%), or both (43%).

While resistance to different classes of antimicrobial agents typically occurred together,  $\beta$ -lactam resistance was the most common phenotype identified among isolates resistant to a single class of drugs; 31 (36%) of 85  $\beta$ -lactam-resistant, 15 (22%) of 68 TET-resistant, five (10%) of 48 SXT-resistant, and one (9%) of 11 CHL-resistant isolates were resistant only to the indicated drug class. Thirty (11%) isolates in this collection were MDR;  $\beta$ -lactam/SXT/TET resistance was the most common MDR phenotype (76% of MDR isolates). One AMP-R isolate was resistant to third-generation cephalosporins (cefotaxime and ceftriaxone); this isolate was also MDR.

3.2. Distribution of MLSTs and identification of  $\beta$ -lactamase genes in E. coli

All isolates were genotyped by MLST, and 57 STs were identified (Table 1). Six STs accounted for 175 (64%) of 273 isolates: ST95 (21%), ST69 (11%), ST420 (11%), ST73 (10%), ST127 (8%), and ST404 (3%). The pandemic lineages ST95, ST69, and ST73 were common in both AMP-R and AMP-S isolates, but they accounted for 51 (60%) isolates in the former and 63 (34%) isolates

**Table 1**  
Distribution of MLSTs among AMP-S and AMP-R E. coli, and identification of  $\beta$ -lactamase genes in  $\beta$ -lactam-resistant isolates.

MLST (complex)	No. (%) of isolates	No. (%) of isolates that are:		P value <sup>c</sup>	bla genes in AMP-R isolates (no. of isolates) <sup>d</sup>
		AMP-S <sup>b</sup>	AMP-R		
ST95 (ST95 cplx)	56 (20.5)	34 (18.1)	22 (25.9)	0.140	bla <sub>TEM</sub> (22)
ST69 (ST69 cplx)	31 (11)	14 (7.4)	17 (20.0)	<b>0.003</b>	bla <sub>TEM</sub> (17), bla <sub>OXA</sub> (1)
ST420	31 (11)	31 (16.5)	0	<b>&lt;0.0001</b>	
ST73 (ST73 cplx)	27 (10)	15 (8.0)	12 (14.1)	0.116	bla <sub>TEM</sub> (11), bla <sub>SHV</sub> (1)
ST127	21 (8)	19 (10.1)	2 (2.4)	<b>0.027</b>	bla <sub>TEM</sub> (2)
ST404 (ST14 cplx)	9 (3)	1 (0.5)	8 (9.4)	<b>0.0005</b>	bla <sub>TEM</sub> (8)
ST10 (ST10 cplx)	8 (3)	5 (2.7)	3 (3.5)	0.707	bla <sub>TEM</sub> (3)
ST12 (ST12 cplx)	8 (3)	5 (2.7)	3 (3.5)	0.707	bla <sub>TEM</sub> (1), bla <sub>SHV</sub> (2)
ST62	6 (2)	5 (2.7)	1 (1.2)	0.669	bla <sub>TEM</sub> (1)
ST141	5 (2)	5 (2.7)	0	0.329	
ST131 (ST131 cplx)	4 (1.5)	2 (1.1)	2 (2.4)	0.591	bla <sub>TEM</sub> (2)
ST144	4 (1.5)	3 (1.6)	1 (1.2)	1.0	bla <sub>TEM</sub> (1)
ST405 (ST405 cplx)	4 (1.5)	1 (0.5)	3 (3.5)	0.091	bla <sub>TEM</sub> (3)
ST14 (ST14 cplx)	3 (1)	2 (1.1)	1 (1.2)	1.0	bla <sub>TEM</sub> (1)
ST372	3 (1)	3 (1.6)	0	0.555	
ST555 (ST538 cplx)	3 (1)	2 (1.1)	1 (1.2)	1.0	bla <sub>TEM</sub> (1)
ST58 (ST155 cplx)	2 (0.8)	1 (0.5)	1 (1.2)	0.527	bla <sub>TEM</sub> (1)
ST88 (ST23 cplx)	2 (0.8)	1 (0.5)	1 (1.2)	0.527	bla <sub>TEM</sub> (1)
ST106 (ST69 cplx)	2 (0.8)	2 (1.1)	0	1.0	
ST297	2 (0.8)	2 (1.1)	0	1.0	
ST393 (ST31 cplx)	2 (0.8)	1 (0.5)	1 (1.2)	0.527	bla <sub>TEM</sub> (1)
ST410 (ST23 cplx)	2 (0.8)	2 (1.1)	0	1.0	
ST491	2 (0.8)	2 (1.1)	0	1.0	
ST706	2 (0.8)	2 (1.1)	0	1.0	
ST998	2 (0.8)	1 (0.5)	1 (1.2)	0.527	bla <sub>TEM</sub> (1)
ST38 (ST38 cplx)	1 (0.4)	0	1 (1.2)	0.311	bla <sub>TEM</sub> (1), bla <sub>CTX-M</sub> (1)
ST394 (ST394 cplx)	1 (0.4)	0	1 (1.2)	0.311	bla <sub>OXA</sub> (1)
ST1604	1 (0.4)	0	1 (1.2)	0.311	bla <sub>TEM</sub> (1)
ST2328	1 (0.4)	0	1 (1.2)	0.311	bla <sub>TEM</sub> (1)
ST2617	1 (0.4)	0	1 (1.2)	0.311	bla <sub>TEM</sub> (1)
Other STs <sup>a</sup>	27 (10)	27 (14.4)	0		
Total (% of total)	273 (100)	188 (69)	85 (31)		bla <sub>TEM</sub> (81; 95%), bla <sub>SHV</sub> (3; 4%), bla <sub>OXA</sub> (2; 2%), bla <sub>CTX-M</sub> (1; 1%)

AMP-S, susceptible to ampicillin/all  $\beta$ -lactams tested; AMP-R, resistant to at least ampicillin.

<sup>a</sup> Includes ST59, ST80, ST93, ST101, ST126, ST136, ST155, ST348, ST357, ST550, ST641, ST847, ST906, ST963, ST1147, ST1155, ST1633, ST1859, ST1946, ST2178, ST2227, ST2562, ST2567, ST4044, ST6092, and two unknown STs.

<sup>b</sup> 157 (84%) of 188 AMP-S isolates were susceptible to other tested agents.

<sup>c</sup> The P value compares the AMP-S column and AMP-R column. Bolded values are statistically significant.

<sup>d</sup> Sequences identified include bla<sub>OXA-1</sub>, bla<sub>SHV-1</sub>, and bla<sub>CTX-9/51</sub>, and of the 36 bla<sub>TEM</sub> sequences typed, 8% were bla<sub>TEM-1A</sub>, 69% were bla<sub>TEM-1B</sub>, and 22% were bla<sub>TEM-1C</sub>.

in the latter group ( $P < 0.0001$ ). ST69 and ST404 were more frequently identified among  $\beta$ -lactam-resistant isolates, accounting for 17 (20%) and eight (9%) AMP-R isolates, compared with 14 (7%) and one (0.5%) AMP-S isolate ( $P = 0.003$ ,  $P = 0.0005$ , respectively). In contrast, ST420 and ST127 were more frequently identified among  $\beta$ -lactam-susceptible isolates, accounting for 31 (17%) and 19 (10%) AMP-S isolates, but zero and two (2%) AMP-R isolates ( $P < 0.0001$ ,  $P = 0.027$ , respectively). In addition to  $\beta$ -lactam resistance, ST69 was also more frequently identified as resistant to other antimicrobial agents compared with other STs; of 31 ST69 isolates, 19 (61%) were SXT-R ( $P < 0.0001$ ), 14 (45%) were TET-R ( $P = 0.0056$ ), five (16%) were CHL-R ( $P = 0.0039$ ), and 11 (35%) were MDR ( $P < 0.0001$ ).

All AMP-R isolates were tested for  $\beta$ -lactamase genes (Table 1). Eighty-one (95%) of 85 AMP-R isolates tested positive for  $bla_{TEM}$ . Thirty-six (44%)  $bla_{TEM}$  sequences were analysed and found to be  $bla_{TEM-1}$ ; three (8%) were  $bla_{TEM-1A}$ , 25 (69%) were  $bla_{TEM-1B}$  (also aligned with  $bla_{TEM-104, 122, 163, 198, 206, \text{ or } 214}$ ), and eight (22%) were  $bla_{TEM-1C}$ . In addition, three (4%) AMP-R isolates carried  $bla_{SHV-1}$ , two (2%) AMP-R isolates carried  $bla_{OXA-1}$ , and one ST38 AMP-R isolate carried  $bla_{CTX-M-9/51}$ ; this was the only ESBL gene that was identified.

### 3.3. Distribution of *fimH* genotypes among different MLSTs, and identification of prevalent sublineages of AMP-S and AMP-R E. coli

Of the 273 isolates, 269 (99%) tested positive for *fimH*, and 35 *fimH* SNP types were identified (Table 2). Six *fimH* types accounted for 180 (65%) isolates: *f-1* (20%), *f-6* (15%), *f-29* (11%), *f-2* (8%), *f-47* (6%), and *f-3* (5%). The *f-1* and *f-6* SNP types were more frequently

identified among  $\beta$ -lactam-resistant isolates, accounting for 30 (35%) and 20 (24%) AMP-R isolates, compared with 25 (13%) and 21 (11%) AMP-S isolates ( $P < 0.0001$ ,  $P = 0.008$ , respectively). In contrast, *f-29* and *f-2* were more frequently identified among  $\beta$ -lactam-susceptible isolates, accounting for 30 (16%) and 20 (11%) AMP-S isolates, but zero and two (2%) AMP-R isolates ( $P < 0.0001$ ,  $P = 0.017$ , respectively). The six main *fimH* types – *f-1*, *f-6*, *f-29*, *f-2*, *f-47*, and *f-3* – were well represented among the six major STs (Table 2). One or two *fimH* types typically predominated in an ST group; the main exception was in ST73, where the most prevalent *fimH* type (*f-8*) represented 10 (37%) of 27 isolates in the group.

**Table 3**

Subtype distribution among prevalent STs that demonstrate more than one *fimH* type.

Subtype	Number (%) of ST	No. (%) of ST that are:		
		AMP-S	AMP-R	P value <sup>a</sup>
ST95/ <i>f-1</i>	2 (4)	2 (5.9)	0	0.514
ST95/ <i>f-6</i>	36 (64)	17 (50.0)	19 (86.4)	<b>0.009</b>
ST95/ <i>f-9</i>	1 (2)	0	1 (4.5)	0.393
ST95/ <i>f-47</i>	17 (30)	15 (44.1)	2 (9.1)	<b>0.007</b>
ST69/ <i>f-1</i>	29 (94)	12 (85.7)	17 (100)	0.196
ST69/ <i>f-43</i>	2 (6)	2 (14.3)	0	0.196
ST73/ <i>f-3</i>	9 (33)	7 (46.7)	2 (16.7)	0.217
ST73/ <i>f-8</i>	10 (37)	4 (26.7)	6 (50.0)	0.257
ST73/ <i>f-9</i>	6 (22)	4 (26.7)	2 (16.7)	0.662
ST73/ <i>f-60</i>	2 (7)	0	2 (16.7)	0.188

<sup>a</sup> The P value compares the AMP-S group with the corresponding AMP-R group for each subtype. Bolded values are statistically significant.

**Table 2**

Distribution of *fimH* alleles among AMP-S and AMP-R isolates in different MLST groups.

MLST(no. of isolates)	Isolates that have <i>fimH</i> type (no.):	
	AMP-S	AMP-R
ST95 (56)	<i>f-1</i> (2), <i>f-6</i> (17), <i>f-47</i> (15)	<i>f-6</i> (19), <i>f-9</i> (1), <i>f-47</i> (2)
ST69 (31)	<i>f-1</i> (12), <i>f-43</i> (2)	<i>f-1</i> (17)
ST420 (31)	<i>f-29</i> (30), <i>f-29+3bp del</i> (1)	0
ST73 (27)	<i>f-3</i> (7), <i>f-8</i> (4), <i>f-9</i> (4)	<i>f-3</i> (2), <i>f-8</i> (6), <i>f-9</i> (2), <i>f-60</i> (2)
ST127 (21)	<i>f-2</i> (18), U (1)	<i>f-2</i> (2)
ST404 (9)	<i>f-1</i> (1)	<i>f-1</i> (8)
ST10 (8)	<i>f-1</i> (1), <i>f-4</i> (1), <i>f-7</i> (1), <i>f-15</i> (1), <i>f-28</i> (1)	<i>f-4</i> (2), <i>f-15</i> (1)
ST12 (8)	<i>f-1</i> (4), <i>f-6</i> (1)	<i>f-1</i> (1), <i>f-12</i> (1), <i>f-51</i> (1)
ST62 (6)	<i>f-5</i> (5)	<i>f-5</i> (1)
ST141 (5)	<i>f-12</i> (5)	0
ST131 (4)	<i>f-6</i> (1), <i>f-17</i> (1)	<i>f-6</i> (1), <i>f-17</i> (1)
ST144 (4)	<i>f-1</i> (2), <i>f-4</i> (1)	<i>f-4</i> (1)
ST405 (4)	<i>f-6</i> (1)	<i>f-1</i> (2), <i>f-13</i> (1)
ST14 (3)	<i>f-1</i> (2)	<i>f-1</i> (1)
ST372 (3)	<i>f-3</i> (3)	0
ST555 (3)	U (2)	U (1)
ST58 (2)	Neg (1)	<i>f-15</i> (1)
ST88 (2)	<i>f-1</i> (1)	<i>f-1</i> (1)
ST106 (2)	<i>f-43</i> (2)	0
ST297 (2)	<i>f-10</i> (2)	0
ST393 (2)	<i>f-4</i> (1)	<i>f-4</i> (1)
ST410 (2)	<i>f-15</i> (2)	0
ST491 (2)	<i>f-12</i> (2)	0
ST706 (2)	<i>f-14</i> (2)	0
ST998 (2)	<i>f-3</i> (1)	<i>f-3</i> (1)
ST38 (1)	0	<i>f-12</i> (1)
ST394 (1)	0	Neg (1)
ST1604 (1)	0	<i>f-4</i> (1)
ST2328 (1)	0	<i>f-15</i> (1)
ST2617 (1)	0	<i>f-24</i> (1)
Other STs (27)	Neg, <i>f-2</i> , <i>f-5</i> , <i>f-7</i> , <i>f-9</i> , <i>f-26</i> (2's); <i>f-3</i> , <i>f-4</i> , <i>f-6</i> , <i>f-11</i> , <i>f-12</i> , <i>f-16</i> , <i>f-45</i> , <i>f-57</i> (1's); U (7)	0
Prevalent <i>fimH</i> types (total; % of group) <sup>a</sup>	<b><i>f-1</i></b> (25; <b>13%</b> ), <b><i>f-6</i></b> (21; <b>11%</b> ), <b><i>f-29</i></b> (30; <b>16%</b> ), <b><i>f-2</i></b> (20; <b>11%</b> ), <i>f-47</i> (15; 8%), <i>f-3</i> (12; 6%)	<b><i>f-1</i></b> (30; <b>35%</b> ), <b><i>f-6</i></b> (20; <b>24%</b> ), <b><i>f-29</i></b> (0; <b>0%</b> ), <b><i>f-2</i></b> (2; <b>2%</b> ), <i>f-47</i> (2; 2%), <i>f-3</i> (3; 4%)

U, unique *fimH* type; Neg, tested negative for *fimH* PCR.

<sup>a</sup> Bolded values indicate that the P value is statistically significant ( $P < 0.05$ ) for the comparison of the indicated *fimH* type between the AMP-S and AMP-R group.

Alleles *f*-47 and *f*-29 were only found among ST95 and ST420 isolates, respectively.

*fimH* typing allowed for subtyping of isolates within an ST (Table 3). ST95 contained four subtypes: *f*-1, *f*-6, *f*-9, and *f*-47. The ST95/*f*-47 subtype was more frequently identified among β-lactam-susceptible isolates: 44% of AMP-S ST95 were *f*-47, but 9% of AMP-R ST95 were *f*-47 ( $P=0.007$ ). The ST95/*f*-6 subtype was more frequently identified among β-lactam-resistant isolates: 86% of AMP-R ST95 were *f*-6, while 50% of AMP-S ST95 were *f*-6 ( $P=0.0093$ ). ST73 also contained four subtypes: *f*-3, *f*-8, *f*-9, and *f*-60. While 50% of AMP-R ST73 isolates were subtype ST73/*f*-8, and nearly 50% of AMP-S ST73 isolates were subtype ST73/*f*-3, this difference in subtype distribution was not statistically significant.

### 3.4. Identification of plasmid Inc groups in AMP-S and AMP-R *E. coli*

*E. coli* were tested for plasmid Inc groups commonly found in Enterobacteriaceae [22]. Of 273 isolates, 52 (19%) tested negative by the Inc/rep typing PCR (Fig. 2). The IncFIB replicon was the most common replicon identified, detected in 204 (75%) isolates. IncFIA and IncB/O replicons were detected in 30 (11%) and 27 (10%) isolates, respectively. The prevalent Inc groups were more frequently identified in AMP-R isolates than in AMP-S isolates (Fig. 2). Among the 85 AMP-R isolates, 75 (88%) harboured IncFIB, 20 (24%) harboured IncFIA, and 21 (24%) harboured IncB/O replicons, while among the 188 AMP-S isolates 129 (69%) harboured IncFIB, 10 (5%) harboured IncFIA, and six (3%) harboured IncB/O replicons ( $P=0.0006$ ,  $P<0.0001$ , and  $P<0.0001$ , respectively). In addition to β-lactam resistance, the IncFIA replicon was more frequently identified among isolates resistant to other classes of antimicrobial agents. Fifteen (31%) of 48 SXT-R isolates harboured IncFIA compared with 15 (6%) of 225 SXT-S isolates ( $P<0.0001$ ); 22 (32%) of 68 TET-R isolates harboured IncFIA compared with eight (4%) of 205 TET-S isolates ( $P<0.0001$ ); five (45%) of 11 CHL-R isolates harboured IncFIA compared with 25 (10%) of 262 CHL-S isolates ( $P=0.0033$ ); and 17 (57%) of 30 MDR isolates harboured IncFIA compared with 13 (5%) of 243 non-MDR isolates ( $P<0.0001$ ). All 10 isolates with IncP replicons were resistant to TET.

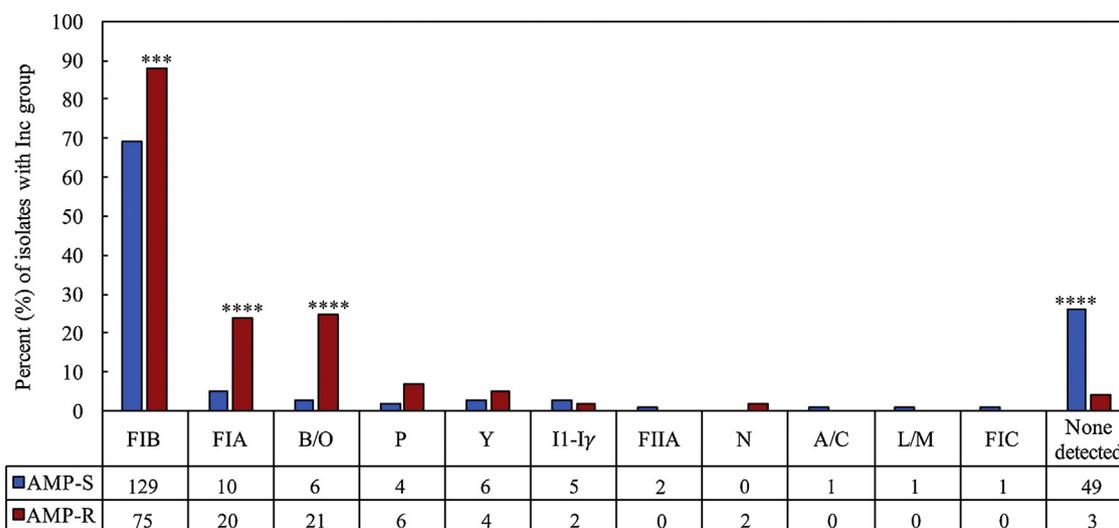
The distribution of plasmid Inc groups among the prevalent STs was different (Fig. 3). While 10 (67%) of 15 AMP-S ST73 tested negative for the Inc types, two (17%) of 12 AMP-R ST73 tested

negative for the Inc types examined ( $P=0.0185$ ). Over half of the 27 IncB/O containing isolates were ST95, with IncB/O observed in 15 (68%) of 22 AMP-R ST95 compared with two (6%) of 34 AMP-S ST95 isolates ( $P<0.0001$ ). The IncFIB replicon was nearly ubiquitous in some STs. While all (100%) of 31 ST420 isolates and 55 (98%) of 56 ST95 isolates contained the IncFIB replicon, 118 (63%) of 186 non-ST95/420 isolates contained this replicon ( $P<0.0001$ ). Additionally, IncFIB was less frequently identified in SF73 compared with other STs: 13 (48%) of 27 ST73 isolates contained IncFIB compared with 191 (78%) of 246 non-ST73 isolates ( $P=0.0008$ ). The IncFIA replicon was more frequently identified in ST69 and ST73 than other STs; while seven (23%) of 31 ST69 and six (22%) of 27 ST73 isolates contained IncFIA, 17 (8%) of 215 non-ST69/73 isolates contained this replicon ( $P=0.0017$ ).

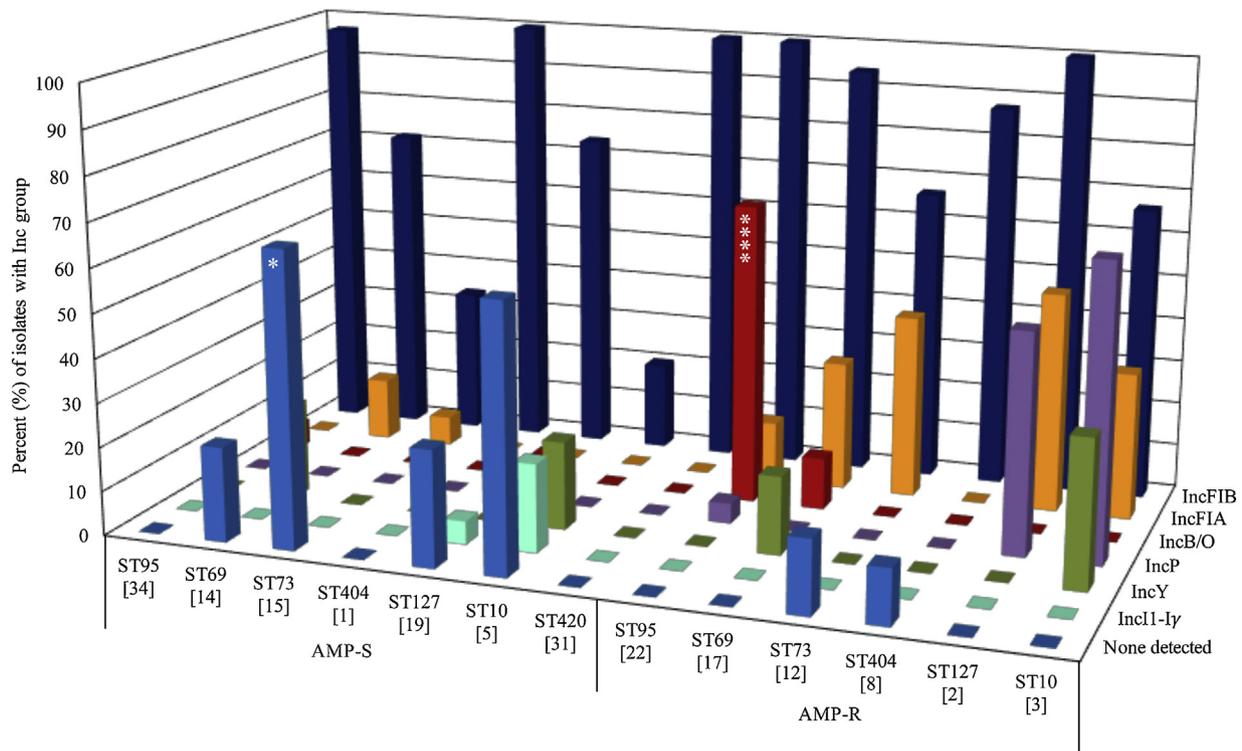
## 4. Discussion

The prevalence of antimicrobial resistance in ExPEC is a global concern and major obstacle to empirical treatment of infections caused by these organisms. To better understand the factors that drive the prevalence of β-lactam resistance in a community, this study genetically characterised consecutively collected *E. coli* isolated from individuals with CA-UTI at a university health centre over two intervals between 2003 and 2005. By comparing β-lactam-resistant (AMP-R) and β-lactam-susceptible (AMP-S) UPEC, it was found that the prevalence of β-lactam resistance was multifactorial, and that a specific set of ST/*fimH* sublineages carrying specific plasmid Inc types and β-lactamase genes contributed to a large proportion of β-lactam-resistant CA-UTIs in the study community. These findings are in accordance with previous studies that used *fimH* SNP analysis to subtype *E. coli* MLST groups, which demonstrated that there are sublineages within specific STs that are associated with antimicrobial susceptibility or resistance [19,23,24].

Only four of the six most prevalent STs appreciably contributed to the AMP-R population (representing 69%): ST95, ST69, ST73, and ST404. Within ST95 and ST73, *fimH* typing enabled the identification of multiple sublineages, revealing differences in sublineage distribution among AMP-R and AMP-S isolates, indicating that the prevalence of β-lactam resistance in the study community is dependent, in part, on the prevalence of several sublineages that are AMP-R. Sublineage ST95/*f*-6 represented 22% of the AMP-R



**Fig. 2.** Distribution of plasmid Inc groups among AMP-S and AMP-R *Escherichia coli*. The bar graph represents the percent of isolates within each group that contain the Inc type, while the values under the graph are the number of isolates that contain the Inc type. The *P*-value compares the AMP-S group with the corresponding AMP-R group for each Inc type. \*\*\* $P<0.001$ . \*\*\*\* $P<0.0001$ .



**Fig. 3.** Distribution of plasmid Inc groups among AMP-S and AMP-R *Escherichia coli* from seven prevalent STs. Data for IncFIB, IncFIA, IncB/O, IncP, IncY, IncI1-Iy, and no Inc detected are shown. The bar graph represents the percent of isolates within each group that contain the Inc type, while the values in parenthesis are the number of isolates per group. The *P*-value compares the AMP-S group with the corresponding AMP-R group for each ST. \**P* < 0.05. \*\*\*\**P* < 0.0001.

population, and sublineage ST73/*f*-8 represented 7% of the AMP-R population. Interestingly, *fimH* typing did not offer further discriminatory power among other prevalent STs – including ST69, ST404, ST420, and ST127 – indicating either that isolates that cause UTI from these lineages are more homogeneous than ST95 and ST73, or that another subtyping method would be more appropriate.

Interestingly, ST420 was tied for second-most prevalent ST in this study community, but none were resistant to the tested antimicrobial agents. In other studies that have identified ST420 among human clinical isolates, it is often reported as a low-prevalence clone that is susceptible to all antimicrobial agents; however, several antimicrobial-resistant ST420 *E. coli* have been isolated from animal sources [10,12,13,19,25,26]. ST95 was previously identified as a lineage associated with a low prevalence of antimicrobial resistance [19]. Stephens et al. used whole-genome sequence analysis to examine ST95 isolates for factors associated with a lack of acquired antimicrobial resistance genes [27]. Carriage of a 114-kb IncFIB/IncFII plasmid called pUTI89 was significantly associated with a pan-susceptible ST95 sublineage in their study. Interestingly, the ST420 isolates in this study all contained IncFIB-type plasmids. Further genomic analysis of ST420 could lead to identification of additional factors that limit the spread of antimicrobial resistance genes.

While the isolates examined in this study were collected between 2003–2005, comparison of these UPEC with those obtained from the same university health service in 2016–2017 demonstrated several important points. Four of the six most prevalent STs found in this study have remained prevalent clones in this community over time: ST95, ST127, ST73, and ST69. The latter two have remained prevalent clones in the AMP-R population [28]. Three significant changes include: a) the emergence of ST131 as a more prevalent ST in this community

(*P* = 0.02); the lack of ST404 (*P* = 0.005), which was a prevalent AMP-R clone in 2003–2005; and the near disappearance of ST420 (*P* < 0.0001), which nearly dominated the AMP-S population in 2003–2005. While some prevalent clones demonstrate stability over time, the emergence and disappearance of other prevalent clones in a population underscores the need for routine monitoring of the clonal composition of UPEC, since these changes can impact the prevalence of antimicrobial resistance in a community. For example, the frequency of fluoroquinolone resistance in the UPEC population increased from 0.7% (2003–2005) to 5.1% (2016–2017), and the occurrence of third-generation cephalosporin resistance increased from 0.4% to 4.3%, both largely due to the emergence of ST131 in this community. The reasons why some prevalent lineages remain stable over time, while others emerge or disappear, are unknown; the introduction of ingestible vehicles into the community, contaminated or not contaminated with particular lineages, is a possibility.

A recent study analysing UPEC from Switzerland found that ST131 was the most frequently occurring ST (equal to ST69) causing CA-UTI, while ST95 was not identified as a cause of CA-UTI in their community [29]. This is in contrast with the current community, where ST95 has been identified as the predominant UPEC ST causing CA-UTIs at multiple points in time over the past 18 years, with ST131 more recently emerging in prevalence [28]. One reason for this difference in clonal composition may be due to differences in patient demographics; the median age of patients in this study representing a university community was 22 years (range 18–44), while the median age of patients from Nüesch-Inderbinen et al. [29] representing a suburban community was 53 years (range 16–87). Interestingly, ST95 was also identified as the predominant clone causing CA-UTI among children seen at a university hospital in South Korea [14]. Thus, while ST95 is a well-recognised pandemic lineage of ExPEC, it

appears to represent a more prevalent cause of CA-UTI in certain patient populations and geographic regions, including the USA, South Korea, and Australia [8,14,28,30].

Non-ESBL *bla*<sub>TEM</sub> genes were a major driving factor for  $\beta$ -lactam resistance in this population, across STs and sublineages; it was the sole  $\beta$ -lactamase gene identified in 93% of AMP-R isolates. In fact, *bla*<sub>TEM</sub> genes have remained the most prevalent  $\beta$ -lactam resistance determinant in this community, where *bla*<sub>TEM</sub> alone accounted for 65% of AMP-R isolates in 2016–2017 [28]. TEM is likely to persist as the main cause of early-generation  $\beta$ -lactam resistance across communities;  $\beta$ -lactamase gene analysis of other recent collections of ExPEC have demonstrated its continued importance [10,30]. CTX-M genes were rare in the study collection: one isolate produced CTX-M-9/51. By 2016–2017, CTX-M-producing UPEC became more frequent in this community (10% of AMP-R isolates), again mostly due to the emergence of ST131 containing CTX-M group 1 genes [28].

In *E. coli*, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, and *bla*<sub>CTX-M</sub> are horizontally acquired  $\beta$ -lactamase genes. Plasmids substantially contribute to the acquisition and dissemination of these and other resistance genes in ExPEC. Based on the strong association between specific replicons and an AMP-R phenotype, it is speculated that the acquired  $\beta$ -lactamase genes are localised on IncFIB, IncFIA, or IncB/O replicon-containing plasmids in the majority of isolates. While these plasmids/replicons appear to be major factors in the dissemination of  $\beta$ -lactam resistance in these isolates, as were IncF-type plasmids in other studies [31], it is important to note that these replicons do not exclusively occur in antimicrobial-resistant isolates [27,32]. The current study found these Inc replicons in AMP-S strains, although at a significantly lower frequency.

The association between IncFIA replicons and  $\beta$ -lactam, SXT, TET and CHL resistance, along with the finding that these resistance phenotypes frequently occurred together, suggests that these resistance genes may be colocalised on IncFIA replicon-containing plasmids in some isolates. Although TET and CHL are not generally used to treat UTIs, these agents are commonly used to treat other infections and conditions. For instance, tetracyclines are the most common oral antibiotics used to treat acne vulgaris [33]. Thus, one significance of TET and CHL resistance in the CA-UTI isolates from this college community is the possibility that antibiotic selection from TET or CHL treatment of other conditions helps maintain resistance to agents used to treat UTIs, in the absence of the agents themselves (i.e.  $\beta$ -lactams and SXT) [34]. While the current Inc analysis was fairly comprehensive for common Inc groups found in Enterobacteriaceae, it was not exhaustive; other Inc replicons that were not properly screened for may exist in the isolates. Additionally, the current Inc analysis may have underestimated the presence of different types of IncFII replicons.

In conclusion, this study found that a limited set of circulating UPEC STs, *fimH* sublineages, TEM  $\beta$ -lactamase genes, and Inc-type plasmids likely harbouring these genes accounted for a substantial proportion of AMP-R UPEC infections in this study community. The distribution of STs and sublineages among  $\beta$ -lactam resistant and susceptible UPEC was not the same, indicating that the selective pressure from  $\beta$ -lactam drugs is not equally exerted on all UPEC. This observation may suggest that the AMP-R strains do not evolve from AMP-S strains that acquire antimicrobial resistance genes in this community; rather, these strains may become introduced into the community by vehicles contaminated with specific lineages of UPEC that colonise the intestine and subsequently cause UTI. If these observations are found to be generalisable by similar studies elsewhere, such information could support targeting specific sublineages with vaccines or other anti-infective modalities.

## Competing interests

None declared.

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## Ethical approval

Not required.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jgar.2019.03.002>.

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