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Clonal Dissemination of Genetically Diverse Fluoroquinolone-Resistant Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* ST131 in a Veterans Hospital in Southern Taiwan

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Abstract

Uropathogenic *Escherichia coli* is the common pathogen to cause urinary tract infections (UTIs) and have become multidrug-resistant (MDR) extended-spectrum *β*-lactamase (ESBL) producers. The differences in the antimicrobial susceptibility, 5 *bla* genes, 12 virulence genes of 87 clinical ESBL-producing *E. coli* isolates and genomic variations and sequence types of 18 recurrent and repeated isolates from 9 patients were investigated. The 87 MDR-ESBL isolates collected mainly from indwelling urinary catheters (IUCs) and UTIs were highly resistant to fluoroquinolones, with over 50% of the isolates being resistant to cefepime and piperacillin/tazobactam and a few being resistant to carbapenem. These isolates carried at least two of the five *bla* genes examined, with the highest prevalence (87.4%) found for $bla_{\text{CTX-M}}$ ($bla_{\text{CTX-M3-like}}$ and $bla_{\text{CTX-M14-like}}$), followed by $bla_{\text{CMY-2}}$ (80.5%) and bla_{SHV} (56.3%). The predominant virulence genes were the fimbriae gene *fimH* and the toxin genes *cnf*1 and *hlyA* in blood isolates and the capsule gene *kpsMTII* in UTI and blood isolates. Over 80% of the isolates carried yersiniabactin and aerobactin of siderophores. In 18

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isolates, the fluoroquinolone-resistant ST131 isolate of pulsotypes I and II with *bla*$_{CTX-M-15}$ was clonally disseminated in the hospital. The genomic plasticity of these ST131 occurred mainly through the conjugative plasmids with differences in replicon types A/C, I1, FIA, FIB and Y, size and number. In conclusion, MDR ESBL-producing *E. coli* isolates differed in virulence genes of UPEC and antibiotic resistance associated with the sources. Plasmid acquisition and chromosomal variations increase the spread of fluoroquinolone-resistant UPEC ST131 worldwide.

Keywords

***E. coli*, ESBL, Virulence Genes, Antimicrobial Resistance, MLST**

1. Introduction

Genetically diverse *Escherichia coli* is an opportunistic pathogen that causes gastroenteritis, bacteremia, bladder infections, meningitis or pus [1]-[3]. In urinary tract infections (UTIs), *E. coli* can infect from outside the body, resulting in direct urethra inflammation, and transport through the circulation system from a wound, or movement from nearby tissues. As the main pathogen to cause UTI, uropathogenic *E. coli* (UPEC) consists of different pathogenicity islands [4], which encode several virulence factors, including adhesins (such as type 1 fimbriae and P fimbriae), iron-associated siderophores (aerobactin genes *iutA* and *iucDCBA*, salmochelin genes *iroBCDEN*, and yersiniabactin genes *fyuA-irp* operon), toxins (*i.e.*, hemolysin gene *hlyA*, cytotoxin necrotizing factor gene *cnf*1, and secreted autotransporter toxin gene *sat*), the capsule (such as capsule K1/K5), and other factors (*i.e.*, serum resistance gene *traT* and uropathogenic protein gene *usp*) [2] [5] [6].

Genetically related strains with multidrug resistance are the characteristics of UTI- and catheter-associated UTIs (CAUTIs)-associated *E. coli*, which carry similar virulence genes as UPEC [7] and account for more than 40% of all nosocomial infections in hospitals and nursing homes [3] [7]-[9]. Important factors that lead to ESBL-producing *E. coli*-associated nosocomial infection are the use of extended-spectrum cephalosporins and urethral catheterization [10]-[12]. The most prevalent uropathogenic *E. coli* ST131 strains differ in their uropathogenic virulence factors, are associated with phylogroup B2, and carry IncF, IncN, IncA/C, and IncI1 plasmids, with a size ranging from 50 to >200 kb [13]-[16]. The aims of this study were to characterize the ESBL-producing *E. coli* with differences in uropathogenic virulence genes and to investigate the genomic and plasmid variations of *E. coli* ST131 based on nine episodes of recurrent infections and repeated isolation.

2. Materials and Methods

2.1. Bacterial Sources

A total of 101 clinical *E. coli* isolates were collected consecutively from IUCs, UTIs, blood, sputum, wounds and ears of patients from August 2010 to February 2011 and were identified as ESBL producers in the Medical Laboratory Division of Chiayi Branch, Taichung Veterans General Hospital. After the further identification of *E. coli* by biochemical methods, confirmation of ESBL at National Chiayi University and the removal of genetically identical isolates from same patient, 87 ESBL-producing isolates were analyzed. All of the *E. coli* isolates were routinely incubated on EMB agar for further use. Furthermore, 18 isolates from 9 patients who were infected recurrently or at different sites within a short period were investigated. A recurrent *E. coli* infection was defined as a patient who was infected by a culture-proven *E. coli* more than 1 month after the completion of the initial antimicrobial therapy.

2.2. Antimicrobial Susceptibility Test

Antimicrobial susceptibility to ampicillin (AMP, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), cefotaxine/clavulanic acid (CTX/CLA, 30/10 μg), ceftazidime/clavulanic acid (CAZ/CLA, 30/10 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NAL, 30 μg), levofloxacin (LVX, 5 μg), moxifloxacin (MXF, 5 μg), piperacillin (PIP, 100 μg), piperacillin/tazobactam (TZP, 100/10 μg), cefepime (FEP, 30 μg), ertapenem (ETP, 10 μg), imipenem (IPM, 10 μg) and meropenem (MEM, 30 μg) was determined using the

disc-diffusion method and CLSI standards [17]. The antimicrobial discs were purchased from BD (Becton Dickinson and Company, Sparks, MD, USA). *Escherichia coli* ATCC 25922 was used as the reference strain. ESBL isolates were determined by the diameter differences of the inhibition zones between cefotaxine and cefotaxine/clavulanic acid or between ceftazidime and ceftazidime/clavulanic acid. AmpC production was also examined.

2.3. PCR Detection of Genes for Antimicrobial Resistance and Virulence Factors

The primers used to amplify genes for five *β*-lactamases and 12 virulence factors are listed in **Table 1**. Three multiplex PCR sets were developed by modifying previous primer sequences. Antimicrobial resistance genes were detected by Multiplex PCR I for bla_{SHV}, $bla_{CTX\text{-}M3\text{-}like}$, $bla_{CTX\text{-}M14\text{-}like}$, bla_{TEM} and bla_{DHA} and by single PCR for $bla_{CMY\text{-}2}$. Twelve virulence genes were amplified by two multiplex PCR sets: *hlyA*, *usp*, *sat*, *fyuA*, *ironN*, *iutA* and *iucD* for multiplex PCR II and *fimH*, *cnf*1, *traT*, *kpsMTII* and *papGII* for Multiplex PCR III. All PCR products were separated by electrophoresis on a 2% agarose gel at 50 V for 120 min. After ethidium bromide staining and UV illumination, the gel image was recorded and analyzed.

2.4. Genomic, Plasmid, Multilocus Sequence Typing (MLST), and Sequence Analysis of Recurrent Isolates

The procedure for PFGE was described previously [18]. Briefly, 10 U of *Xba*I were used for the restriction digestion. Isolates were defined as the subgenotype for those with ≤3 fragment differences and as the genotype for those with >3 fragment differences. Plasmid size and number were analyzed using the Kado-Liu method [19], and *Salmonella* Choleraesuis OU7085 (6.6- and 50-kb plasmids) and *S*. Choleraesuis OU7526 (50- and 90-kb plasmids) as a control. Furthermore, the plasmid replicon groups FIA, FIB, FIC, HI1, HI2, I1-Igamma, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA were identified for each isolate by PCR amplification [20]. Full-length $bla_{CTX\text{-}M\text{-}3\text{-}like}$ was sequenced and aligned to the NCBI database. MLST was performed using previously described methods [21]. After PCR amplification and sequencing, MLST ST types were determined at http://mlst.ucc.ie/.

2.5. Plasmid Conjugation

Sodium azide-susceptible clinical recurrent *E. coli* isolates and sodium azide-resistant *E. coli* strain J53 were used as donors and recipient, respectively, for conjugation experiments. After the co-culture of recipient and donor strains on filter paper on MHA agar overnight, the mixture was plated onto MacConkey agar with the appropriate antibiotic and sodium azide. Plasmid profile of the transconjugants was analyzed using the Kado-Liu method. The replicons of the conjugation plasmid were examined as above [20].

3. Results

These ESBL-producing *E. coli* isolates were predominantly collected from IUC (43%) and urine (26%), followed by blood and sputum, wounds (**Table 2**).

3.1. Antimicrobial Resistance

In general, all isolates were multidrug resistant to AMP, CRO and CTX and PIP with the least antimicrobial resistance for the urine group and the highest antimicrobial resistance for the IUC group (**Table 2**). The average resistance rate was 54% to TZP (range: 42.3% for the urine group to 66.7% for the wound group); 76% to CAZ (range: 65.4% in the urine group to 91.7% in the blood group); 60.9% to FEP (range: 57.7% for the urine group to 70% for the sputum group); less than 10% to carbapenems, including ETP, IPM and MEM, with the value observed highest in the urine group; near 90% to fluoroquinolones; over 95% to nalidixic acid; and near 90% to CIP, LVX, and MXF, particularly differing between the urine and IUC groups, with the lowest found in the blood and wound groups.

3.2. ESBL-Related *bla* Genes

The *bla* genes differed in prevalence among the isolation source (**Table 3**). The most prevalent *bla* gene was $bla_{CTX\text{-}M}$ (87.4%; 54.0% for the $bla_{CTX\text{-}M3\text{-}like}$ gene and 33.3% for $bla_{CTX\text{-}M14\text{-}like}$), followed by $bla_{CMY\text{-}2}$ (80.5%),

Table 1. Primer sequences and PCR product size of genes for antimicrobial resistance and virulence factors.

PCR	Gene	Primer	Nucleotide sequence（5’→3’）	Product size (bp)
Multiplex I			Class A *β*-lactamase	
	$bla_{\text{CTX-M-3}}$	F	AATCACTGCGCCAGTTCACGCT	479
		R	GAACGTTTCGTCTCCCAGCTGT	
	$bla_{\text{CTX-M-14}}$	F	TACCGCAGATAATACGCAGGTG	355
		R	CAGCGTAGGTTCAGTGCGATCC	
	bla_{SHV}	F	AACGGAACTGAATGAGGCGCT	141
		R	TCCACCATCCACTGCAGCAGCT	
	bla_{TEM}	F	GAAGATCAGTTGGGTGCACGAGT	520
		R	CAACTTTATCCGCCTCCATCCAGT	
			Class C *β*-lactamase	
	bla_{DHA}	F	AACTTTCACAGGTGTGCTGGGT	405
		R	CCGTACGCATACTGGCTTTGC	
Simplex	$bla_{\text{CMY-2}}$	F	CTGACAGCCTCTTTCTCCACA	1100
		R	CTACGTAGCTGCCAAATCCAC	
Multiplex II	*hlyA*	F	AACAACGATAAGCACTGTTCTGG	1177
		R	CCATATAAGCGGTCATTCCCG	
	usp	F	GGAAAATGGTCGCTCAGTGG	992
		R	CTGTAGTGAATCTCATCGTGTAGTC	
	sat	F	TCAGAAGCTCAGCGAATCATTG	931
		R	CATTATCACCAGTAAAACGCACC	
	fyuA	F	GGCTTTATCCTCTGGCCTT	878
		R	GAAAACCCAGTCATCGGTGG	
	iron	F	CTCTGGTGGTGGAAGCC	815
		R	TGTCGGTACAGGCGGTTC	
	iutA	F	CACTCCGGTACTCCAGTCA	688
		R	CCTCCAACCAGATGTTCTTCG	
	iucD	F	CCGGAGAAGCCTGAAATATATTCA	584
		R	CCGGATTGTCATATGCAGACC	
Multiplex III	*fimH*	F	GTTTATAATTCGAGAACGGATAAGCC	491
		R	GTGCATAATTTGCCGTTAATCCC	
	cnf1	F	TTCTTCTGTACTTCCCCCAG	408
		R	TGAGCGGCATCTACTATGAAGT	
	traT	F	CATAACCACGGTTCAGCCATC	328
		R	TTGCACTGGTCAGTTCCAC	
	kpsMTII	F	CATCAGACGATAAGCATGAGCA	269
		R	TGCGCATTTGCTGATACTGT	
	papG II	F	GGGCCCCCAAGTAACTC	189
		R	GGATGAGCGGGCCTTTG	

Table 2. Antimicrobial resistance of *E. coli* isolates from different sources.

Sources[1]	No	Resistance to [N (%)][2]													
		Penicillin			Cephalosporin				Carbapenem			Quinolone	Fluoroquinolone		
		AMP	PIP	TZP	CTX	CAZ	CRO	FEP	ETP	IPM	MEM	NAL	CIP	LVX	MXF
Blood	12	12 (100.0)	12 (100.0)	6 (50.0)	12 (100.0)	11 (91.7)	12 (100.0)	8 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	12 (100.0)	10 (83.3)	10 (83.3)	10 (83.3)
IUC	32	32 (100.0)	32 (100.0)	19 (59.4)	32 (100.0)	29 (90.6)	32 (100.0)	19 (59.4)	1 (3.1)	4 (12.5)	2 (6.3)	32 (100.0)	32 (100.0)	31 (96.9)	31 (96.9)
Urine	26	26 (100.0)	26 (100.0)	11 (42.3)	26 (100.0)	17 (65.4)	26 (100.0)	15 (57.7)	2 (7.7)	0 (0.0)	0 (0.0)	25 (96.2)	22 (84.6)	22 (84.6)	22 (84.6)
Sum	58	58 (100.0)	58 (100.0)	30 (51.7)	58 (100.0)	46 (79.3)	58 (100.0)	34 (58.6)	3 (5.2)	4 (6.9)	2 (3.4)	57 (98.3)	54 (93.1)	53 (91.4)	53 (91.4)
Sputum	10	10 (100.0)	10 (100.0)	6 (60.0)	10 (100.0)	8 (80.0)	10 (100.0)	7 (70.0)	0 (0.0)	1 (10.0)	0 (0.0)	10 (100.0)	10 (100.0)	9 (90.0)	10 (100.0)
Ear	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Wound	6	6 (100.0)	6 (100.0)	4 (66.7)	6 (100.0)	5 (83.3)	6 (100.0)	4 (66.7)	2 (33.3)	1 (16.7)	0 (0.0)	5 (83.3)	5 (83.3)	5 (83.3)	5 (83.3)
Total	87	87 (100.0)	87 (100.0)	47 (54.0)	87 (100.0)	71 (81.6)	87 (100.0)	53 (60.9)	5 (5.7)	6 (6.9)	2 (2.3)	85 (97.7)	80 (92.0)	78 (89.7)	79 (90.8)

[1]: IUC = indwelling urinary catheter. [2]: AMP = ampicillin; CAZ = ceftazidime; CIP = ciprofloxacin; CTX = cefotaxime; CRO = ceftriaxone; ESBL = extended-spectrum beta-lactamase; ETP = ertapenem; FEP = cefepime; IPM = imipenem; LVX = levofloxacin; MEM = meropenem; MXF = moxifloxacin; NAL = nalidixic acid; PIP = piperacillin; TZP = piperacillin/tazobactam.

Table 3. Prevalence of *bla* genes in ESBL-producing *E. coli* isolates from different sources.

Source	No	ESBL phenotype [N (%)]		*bla* genes [N(%)]						
		CTX	CAZ	TEM	CMY-2	SHV	DHA	CTX-M	CTX-M-3 like	CTX-M-14-like
Blood	12	10 (83.3)	8 (66.7)	4 (33.3)	9 (75.0)	6 (50.0)	0 (0.0)	10 (83.3)	6 (50.0)	4 (33.3)
IUC	32	27 (84.4)	25 (78.1)	11 (34.4)	28 (87.5)	20 (62.5)	0 (0.0)	27 (84.4)	18 (56.3)	9 (28.1)
Urine	26	23 (88.5)	18 (69.2)	14 (53.8)	20 (76.9)	13 (50.0)	2 (7.7)	24 (92.3)	13 (50.0)	11 (42.3)
Sum	58	50 (86.2)	43 (74.1)	25 (43.1)	48 (82.8)	33 (56.9)	2 (3.4)	51 (87.9)	31 (53.4)	20 (34.5)
Sputum	10	8 (80.0)	6 (60.0)	2 (20.0)	7 (70.0)	5 (50.0	0 (0.0)	9 (90.0)	6 (60.0)	3 (30.0)
Ear	1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Wound	6	4 (66.7)	6 (100.0)	1 (16.7)	5 (83.3)	4 (66.7)	0 (0.0)	6 (100.0)	4 (66.7)	2 (33.3)
Total	87	72 (82.8)	63 (72.4)	32 (36.8)	70 (80.5)	49 (56.3)	2 (2.3)	76 (87.4)	47 (54.0)	29 (33.3)

1: IUC = indwelling urinary catheter.

bla_{SHV} (56.3%), bla_{TEM} (36.8%) and bla_{DHA} (2.3%). Overall, 67.4% of the isolates carried at least 3 *bla* genes. For example, $bla_{CTX\text{-}M\text{-}3\text{-}like}$, bla_{CMY}, and bla_{SHV} were observed in 31.6% of the isolates. The prevalence of bla_{TEM} ranged from 16.7% for the wound group to 53.8% in the urine group; the prevalence of $bla_{CMY\text{-}2}$ ranged from 70.0% for the sputum group to 85.4% for the IUC group. The prevalence of bla_{SHV} ranged from 50% for the blood and sputum group to 66.7% in the wound group. The prevalence of $bla_{CTX\text{-}M3\text{-}like}$ ranged from 52% for the urine group and blood group to 66.7% for the wound group. The prevalence of $bla_{CTX\text{-}M14\text{-}like}$ ranged from 28.1% in the IUC group to 42.3% in the urine group. The $bla_{CTX\text{-}M3\text{-}like}$ and $bla_{CTX\text{-}M14\text{-}like}$ that were present were mutually exclusive. The gene bla_{DHA} was identified in only two urine isolates.

3.3. Differences in Virulence Genes in Pathogenicity Islands (PAI_{536}) of UPEC

In contrast to the more than 80% of isolates that carried type I fimbrae *fimH*, the prevalence of the pathogenicity island-associated fimbrial gene *papGII* ranged from 22% for the wound group to 67% for the blood group (**Table 3**). Over 50% of the urine and bacteremia isolates carried the capsular gene *kpsMTII*. The toxin genes *cnf*1 and *hlyA* were more highly observed in bacteremia isolates. Among the three siderophore types, aerobactin genes (*iutA* and *iucD*) and the yersiniabactin gene (*fyuA*) were present in over 80% of the isolates, whereas the salmochelin gene *iroN* was nearly absent. The *fyuA* gene was present in over 90% of the wound and urine groups and in only 56% of the sputum group. The uropathogenic protein gene *usp* was found in more bacteremia and UTI groups (75% and 68%) than sputum group (55%). The gene *sat* was most often found in the IUC group (**Table 4**).

3.4. Genetic Variations in 18 *E. coli* Isolates from Recurrent Infections and Different Locations in Same Patient

These isolates were obtained mostly from urine and IUC and belonged to uropathogenic ST131 of pulsotypes I (IA and IB) and II (IIA and IIB), except ST95 of pulsotype III for patient 5 (**Table 5**). Furthermore, all pulsotype

Table 4. Prevalence of virulence genes in ESBL-producing *E. coli* isolates from different sources.

Source[1]	No	Fimbriae			Capsule	Toxins			Siderophores[2]					*traT*	*usp*	*sat*
		fimH	*papGII*	Both	*kpsMTII*	*cnf*1	*hlyA*	Both	*iroN*	*iutA*	*iucD*	Both	*fyuA*			
Blood	12	11[3] (91.7)	7 (58.3)	6 (50.0)	7 (58.3)	5 (41.7)	5 41.7	5 (41.7)	2 (17)	11 (91.7)	11 (91.7)	10 (83)	9 (75.0)	11 (91.7)	9 (75.0)	8 (66.7)
IUC	32	31 (96.9)	18 (56.3)	18 (56.3)	10 (31.3)	15 (46.9)	12 (37.5)	11 (34.4)	1 (4)	30 (93.8)	29 (90.6)	22 (88)	27 (84.4)	29 (90.6)	21 (65.6)	23 (71.9)
Urine	26	26 (100.0)	11 (42.3)	11 (42.3)	13 (50.0)	7 (26.9)	4 (15.4)	4 (15.4)	1 (3)	23 (88.5)	23 (88.5)	33 (92)	24 (92.3)	23 (88.5)	17 (65.4)	14 (53.8)
Sum	58	57 (98.3)	29 (50.0)	29 (50.0)	23 (39.7)	22 (37.9)	16 (27.6)	15 (25.9)	2 (3)	53 (91.4)	52 (89.7)	55 (90)	51 (87.9)	52 (89.7)	38 (65.5)	37 (63.8)
Sputum	10	8 (80.0)	5 (50.0)	3 (30.0)	2 (20.0)	3 (30.0)	3 (30.0)	3 (30.0)	0 (0)	9 (90.0)	9 (90.0)	8 (80)	5 (50.0)	10 (100.0)	5 (50.0)	5 (50.0)
Ear	1	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Wound	6	5 (83.3)	2 (33.3)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0)	5 (83.3)	5 (83.3)	5 (83)	6 (100.0)	5 (83.3)	4 (66.7)	3 (50.0)
Total	87	82 (94.3)	44 (50.6)	41 (47.1)	34 (39.1)	32 (36.8)	25 (28.7)	24 (27.6)	4 (4.5)	79 (90.8)	78 (89.7)	79 (89)	72 (82.8)	79 (90.8)	57 (65.5)	54 (62.1)

[1]: IUC = indwelling urinary catheter. [2]: *iroN* for salmochelin, *iutA* and *iucD* for aerobactin, and *fyuA* for yersiniabactin. [3]: 11 (91.7) means N (%).

Table 5. Characteristics of antimicrobial resistance, genome types, virulence genes, and conjugation plasmid of ESBL-producing *E. coli* strains from patients with recurrent infection.

Patient	Isolate	Ward[1]	Source[2]	Pulsotype	ST type	PIP/TZP	Cephalosporins	Fluoquinolone	Virulence gene	Siderophore			*traT*	*usp*	*sat*	Conjugation plasmid (kb)				
							CRO/CTX/CAZ/FEP	CIP/LVX/MXF	*fimH/papGII/kpsMTII/cnf1/hlyA*	*iutA*	*iucD*	*fyuA*				≥90	50~<90	<50	Antibiogram	Replicon
1	16	22W	Urine	IA	131	R/R	R/R/R/R	R/R/R	+/+/-/+/+	+	+	+	+	+	+		1		A	I1
																	1		K	FIA/FIB
	55	ICU	IUC										-	+	+		1		A	I1
																	1		K	FIA/FIB
2	5	RCW	Urine	IB	131	R/S	R/R/R/R	R/R/R	+/+/-/+/+	+	+	+	+	+	+	1	1		S	A/C
																1	1		K	FIA/FIB
	9	SCU	Blood						+/+/+/+/+							1	1		S	A/C
																1	1		K	FIA/FIB
3	31	22W	Urine	IA	131	R/R	R/R/R/R	R/R/R	+/+/-/+/+	+	+	+	+	+	-		1		A	I1
	82		Blood			R/I										1	1		A	I1/RepB
4	13	ER	IUC	IIA	131	R/S	R/R/R/S	R/R/R	+/-/+/-/-	+	+	+	+	+	+	1	1		S	A/C
	74	22W		IA		R/R	R/R/R/R		+/+/-/+/+							1		1	A	I1/RepB
5	36	21W	Urine	III	95	R/R	R/R/R/S	S/S/S	+/+/+/-/-	-	-	+	-	+	-		1		A	I1
	59	ER	Wound			R/I	R/R/I/S		+/+/+/-/-								1		A	I1
6	3	OPD	Blood	IIB	131	R/R	R/R/R/S	R/R/R	+/-/+/-/-	+	+	+	+	+	+	1			T	A/C
	69	ER					R/R/R/I									1			T	A/C
7	10	21W	Blood IUC	IB	131	R/R	R/R/R/R	R/R/R	+/+/-/+/+	+	+	+	+	+	+		1		A	FrepB
																	1		K	A/C
	11																1		A	FrepB
																	1		K	A/C
8	17	ER	Urine	IB	131	R/S	R/R/I/R	R/R/R	+/-/-/-/-	+	+	+	+	+	+	1		1	K	FIA/FIB/Y
	32	21W				R/I	R/R/R/R									1		1	K	FIA/FIB/Y
9	67	RCW	IUC	IC	131	R/S	R/R/R/I	R/R/R	+/-/-/-/-	+	+	+	+	+	+	1			K	FIA/FIB
	72	SCU	Wound	IB			R/R/R/R		+/+/-/+/+							1			K	FIA/FIB

[1]:ER = emergency room; OPD = outpatient department; SCU = surgical intensive care unit; RCW =: respiratory care ward, 21W and 22 W = internal wards. [2]:IUC = indwelling urinary catheter.

IA isolates were collected from ward 22W. Among the virulence genes, these isolates carried the siderophore genes *iutAB* and *fyuA* and *usp* (**Table 5**). Except for isolate 9, all isolates missed at least one virulence gene from *fimH*, *papGII*, *kpsMTII*, *cnf*1, and *hlyA*. The ST 95 isolates from patient 5 carried fewer siderophore genes, lacked the genes *traT* and *sat* and were sensitive to fluoroquinolone compared to the ST131 isolates, which were all fluoroquinolone-resistant and resistant to cefotaxime (CTX), ceftazidime (CAZ), and ceftriaxone (CRO), of the third-generation cephalosporins. Furthermore, few isolates were resistant to fourth-generation cefepime (FEF). Among the four *bla* genes examined by PCR and sequencing, $bla_{\text{CTM-M15}}$ was the most prevalent *bla* gene and was potentially located on the IncF plasmid (**Figure 1**). Furthermore, the AmpC gene $bla_{\text{CMY-2}}$ was detected with high prevalence (15/18).

Plasmid profile differed in size and number, ranging from 6.6 kb to larger than 90 kb (**Figure 1**). Four plasmids were common, with sizes less than 50-kb (1), 50 - 90 kb (2) and larger than 90 kb (1). Among the 18 plasmid replicon types tested, only five replicon types A/C, I1, FIA, FIB and Y were identified, and the plasmid with these replicons could be conjugated (**Table 5**). Replicon types combined with PFGE, ST, and plasmid analysis demonstrated identical isolates in patients 2, 5, 6, 7, and 8 and different isolates in patients 1, 3, 4, and 9.

4. Discussion

E. coli is the major pathogen for clonal community-acquired and nosocomial UTIs [1] [22]-[24]. In addition, CAUTIs are a major nosocomial infection in hospitals and nursing homes [8]. In this study, in a nursing home in this hospital, CAUTIs (IUCs) were more common than UTIs (**Table 2**). We confirmed that fluoroquinolone-resistant ESBL-producing *E. coli* infections caused more than 80% of the UTIs and bacteremia observed in older patients [25]. The pathogenicity island (PAI) I_{536} – IV_{536} carries genes for UPEC to cause bacteremia [6]. Indeed, virulence genes of PAI-I_{536} – IV_{536} were found to be more common in the isolates from urine, IUC and blood than in those from sputum and wounds, but these genes differed among the UTI, IUC and blood groups. For example, *papGII* and *kpsMTII* were more common in the blood group than in IUCs and UTIs, and the fimbriae genes *fimH* and *papGII*, toxin genes *cnf*1, *hlyA*, and *sat* were more common in the IUC group than in the UTI group (**Table 3**). These results suggest the different origins of blood, IUC and urine groups. Our study also demonstrated that the ESBL-producing isolates and recurrent UPEC ST131 mostly consisted of yersiniabactin and aerobactin genes, and lacked the salmochelin gene (**Tables 3-5**), which confirmed that yersiniabactin and aerobactin are more prevalent in relapsed UPEC isolates [26]. Additionally, yersiniabactin are more common in

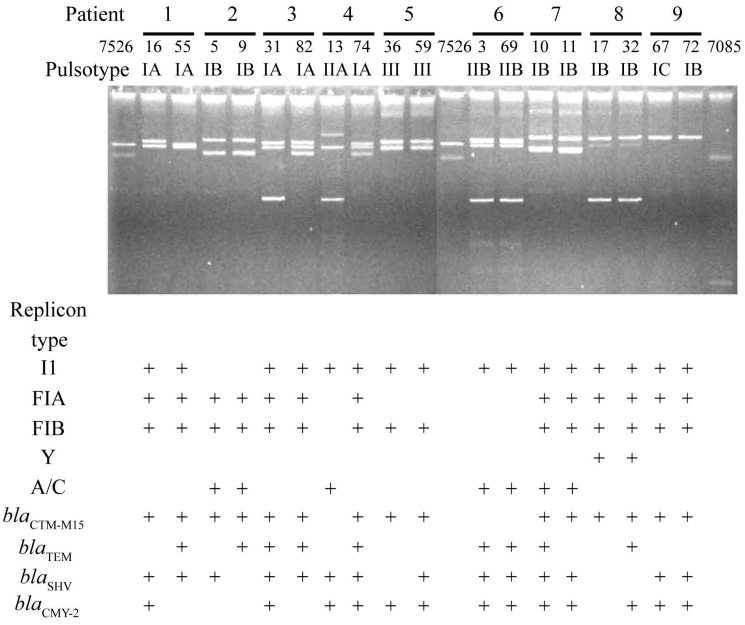

Figure 1. Plasmid profile and replicon type and bla genes in recurrent *E. coli* isolates. A: Plasmid analysis. Size standards are 90-kb and 50-kb plasmids of OU7526 and the 50-kb and 6.6-kb plasmids of OU7085.

ST131 than non-ST131 strains [27], implying different strains between sputum isolates and the blood and UTI isolates (**Table 3**).

E. coli ST131 and ST95 isolates have been the major UPEC isolates causing UTI in Canada and the USA [28] [29]. Our study demonstrated clonal dissemination of ST131 with $bla_{\text{CTX-M-15}}$, such as pulsotype IA isolates in ward 22W and most pulsotype IB isolates in 21W and SCU (**Table 5**, **Figure 1**), which confirmed that the clonal dissemination of UPEC infection is often responsible for recurrent infection in patients due to ineffective treatment [26] as well as for recurrent bloodstream infection in elderly patients [30]. In addition, these isolates frequently carry the CTX-M ESBL enzyme [31].

Fluoroquinolone-resistant *E. coli* ST131 carried a plasmid that was capable of conjugation in which replicon types IncF were the most prevalent. Inc I1-Iγ, K, HI2 and B/O [32] and *E. coli* ST131 were isolated in UK river sediment and carried $bla_{\text{CTX-M-15}}$, which is associated with the replicon IncF plasmid [33]. Furthermore, $bla_{\text{CTX-M-15}}$ was identified in 100-335-kb Inc FII, IncHI2, IncY plasmids and other non-typeable plasmids, with a size ranging from 70 - 330 kb [34]. Carbapenem-resistant *E. coli* ST131with bla_{KPC} on the conjugable plasmid has been identified [35]. However, we only observed reduced susceptibility to carbapenem in this study. In the present study, fluoroquinolone-resistant ESBL-producing *E. coli* ST131 with $bla_{\text{CTX-M-15}}$ evolved differently via an increase in genomic variation (**Figure 1**) and the transfer of plasmids that differed in size, number and replicon types, suggesting a genome plasticity in both the plasmid and chromosome that have resulted in the greater pathogenicity of ST131 and its rapid dissemination worldwide.

5. Conclusion

In conclusion, fluoroquinolone-resistant ESBL-producing *E. coli* differed in the virulence genes of PAI-I_{536}-IV_{536} of UPEC. Uropathogenic *E. coli* ST131 was dominant in this hospital, and it evolves genetically via plasmid transfer and chromosome variations.

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