PREVALENCE AND MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* ST131 ISOLATES FROM PATIENTS AT A TERTIARY-CARE HOSPITAL IN PHAYAO PROVINCE, THAILAND (MARCH 2015 - JUNE 2017)

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Abstract. *Escherichia coli* is a common cause of infection and its identification and characterization provide appropriate and effective antimicrobial treatment. Phylogenetic group and ST131 strain and subtypes of clinical *E. coli* isolates (n = 58, 59% being urine samples) from patients attending a tertiary care hospital in Phayao Province, northern Thailand during March 2015 and June 2017 were identified using a Clemont protocol and multiplex PCR respectively, and presence of β-Lactamase genes ($bla_{\text{CTX-M}}$, bla_{OXA} and bla_{TEM}) and mutation of *gyr*A and *par*C were determined by multiplex and allele-specific multiplex PCR respectively. Antibiogram profiling showed 95, 66 and 48% of the strains were resistant to ampicillin, four fluoroquinolones (carrying mutations in fluoroquinolone resistance-determining regions of *Gyr*A and *Par*C) and all four fluoroquinolones together with ESBL production (carrying in addition $bla_{\text{CTX-m1}}$ or $bla_{\text{CTX-m9}}$ and integron 1), respectively. While only 31% of the strains were *E. coli* ST131, 67% were from urine specimens and 78% from patients >40 years of age, with 72 % belonging to subtypes A and C2 and 33 % to subtypes C1-M27 and C1-non-M27. Proportion of ST131 strains increased from 10% in 2015 to 30% in 2016 and to 73% in 2017 (in latter period samples were collected only in the first six months). In conclusion, PCR-based techniques allowed rapid and facile detection of antibiotic-resistance genes and virulence genetic markers in clinical *E. coli* isolates allowing prompt and appropriate antimicrobial treatment of patients and, in addition, providing molecular signatures for epidemiological surveillance.

Keywords: *Escherichia coli*, *E. coli* ST131 subtypes, northern Thailand

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INTRODUCTION

Escherichia coli is a common bacterium found in gastrointestinal tracts of humans and existing in a symbiotic relationship (Kaper *et al*, 2004). However, intra-intestinal pathogenic *E. coli* strains can cause diarrheal illness and extra-intestinal strains infection in other organs such as urinary tract (UT) (Kaper *et al*, 2004). UT infection (UTI) is often due to uropathogenic *E. coli* (UPEC) that have distinctive virulent genes and antibiogram profiles (Khairy *et al*, 2019), the majority of which in female patients belong to group B2 or D with sequence types (STs) ST69, ST73, ST95, and ST131 (Banerjee *et al*, 2013).

Pathogenic *E. coli* ST131 in B2 clonal lineage is the most predominant strain, with mainly O25:H4 (specifically O25b) and O16:H5 serotypes, and multidrug resistance phenotypes especially against fluoroquinolones and beta-lactamases (Nicolas-Chanoine *et al*, 2014). *E. coli* ST131 is divided into three major clades according to *fim*H subtype, namely, clade A carrying *fim*H41, clade B *fim*H22 and clade C *fim*H30, the latter being further divided to two subclades, namely, subclade C1/H30-R with fluoroquinolone resistance (FQ-R) and subclade C2/H30-Rx with FQ-R and extended beta-lactamase (ESBL) CTX-M-15 (Nicolas-Chanoine *et al*, 2014). Subclade C2/H30-Rx is predominant and spreads principally through clonal expansion (Clermont *et al*, 2008; Nicolas-Chanoine *et al*, 2008; Johnson *et al*, 2012). C2/H30-Rx clade expressing ESBL CTX-M-15 has emerged in Canada, China and Japan, and that expressing ESBL CTX-M-27 in France, Japan and Switzerland (Peirano *et al*, 2010; Johnson *et al*, 2012; Matsumura *et al*, 2016; Bevan *et al*, 2017). FQ-R in *E. coli* ST131 isolates is primarily due to mutations in QR-determining regions (QRDRs) of *gyr*A and *par*C (Johnson *et al*, 2013).

E. coli ST131 infection in hospitals can be nosocomial or community acquired, probably through direct human-to-human transmission (Woodford *et al*, 2004; Nicolas-Chanoine *et al*, 2014; Day *et al*, 2019). Treatment of *E. coli* ST131 infection is complicated due to presence of resistance to commonly used first-line antibiotics, such as amoxicillin, extended-spectrum cephalosporins (ESC), FQs, and trimethoprim-sulfamethoxazole, possibly acquired by horizontal transfer from drug-resistant *E. coli* and other bacterial species (Evans *et al*, 2020). Although epidemiological studies have documented *E. coli* ST131 as the major prevalent linage in urine from UTI patients in Asia, Europe and North America (Rogers *et al*, 2011), epidemiological surveys of *E. coli* ST131 in Thailand are sparse partly due to the complicated molecular protocol required to identify this strain (Seenama *et al*, 2019).

Here, simple molecular techniques were employed to identify and characterize ST131 strains in clinical *E. coli* isolates obtained from patients at Phayao Ram Hospital, Phayao Province, northern Thailand, from March 2015 to June 2017. The results of the study should provide rapid and facile detection of antibiotic-resistance genes and virulence genetic markers in clinical *E. coli* isolates, which should help in prompt and appropriate antimicrobial treatment of patients and to provide molecular markers for epidemiological surveillance.

MATERIALS AND METHODS

Bacterial isolates

Clinical *E. coli* isolates (n = 58) were cultured from blood, pus, stool, and urine of inpatients at Phayao Ram Hospital, Phayao Province, northern Thailand from March 2015 to June 2017. Samples were streaked onto sterile MacConkey agar (Oxoid, Hampshire, UK) and incubated at 37°C for 12-18 hours. Suspected (red) *E. coli* colonies were inoculated in Triple-Sugar-Iron (TSI) slant (Biomedia, Nonthaburi, Thailand) and subjected to four biochemical tests (citrate utilization, indole production, lysine decarboxylase activity, and urease activity) and a motility assay (Barrow and Feltham, 2009).

E. coli isolates used in this study were leftover specimens collected from the previously approved research protocol entitled "The complete analysis of Salmonella serotype and the current occurrence of drug resistant situation of *Salmonella* spp in Amphoe Mueang, Phayao District" which was approved by the Ethics Committee, University of Phayao (approval no. 57 02 04 0020).

Antibiogram profiling and ESBL production determination

Antibiogram profiling of 11 antibiotics was performed using a disk diffusion method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2017). Each disc (Oxoid, Hampshire, UK) contained amoxicillin/clavulanate (AMC) 20/10 μg, ampicillin (AMP) 10 μg, cefepime (FEP) 30 μg, cefotaxime (CTX) 30 μg, cefoxitin (FOX) 30 μg, ceftazidime (CAZ) 30 μg, ceftriaxone (CRO) 30 μg, ciprofloxacin (CIP) 5 μg, gentamicin (GEN) 10 μg, imipenem (IPM) 10 μg, levofloxacin (LEV) 10 μg, nalidixic acid (NA) 30 μg, norfloxacin (NOR) 10 μg, and sulfamethoxazole/trimethoprim (SXT) 1.25 μg/23.75 μg). *E. coli* ATCC 25922 was used as a negative control strain. ESBL test was performed using a combination disk method according to CLSI guidelines (CLSI, 2017) with individual disc (Oxoid, Hampshire, UK) containing CAZ (30 μg) + clavulanic acid (CLA) (10 μg) and CTX (30 μg) + CLA. In-house ESBL-producing *E. coli* and ESBL-negative *E. coli* ATCC 25922 was used as positive and negative control respectively.

E. coli phylogenetic group and ST131 identification

DNA was extracted from *E. coli* strains as previously described (McNerney *et al*, 2017). In brief, one ml aliquot of an overnight culture was sedimented, washed and resuspended in 400 μl of TE buffer (10 mM Tris HCl, pH 8.0 containing 1 mM EDTA),

incubated at 80°C for 20 minutes, cooled to ambient temperature, then added with 50 µg of lysozyme and incubated at 37°C for one hour. A 75 µl aliquot of 10% SDS/proteinase K (10 mg/ml) solution was added and mixture was incubated at 65°C for 10 minutes prior to addition of 100 µl aliquot of 5 M NaCl and 100 µl of 10% (w/v) N-cetyl-N,N,N,-trimethyl ammonium bromide solution containing 5 M NaCl (at 65°C), followed by incubation at 65°C for 10 minutes, addition of 750 µl aliquot of chloroform:isoamyl alcohol (24:1) mixture, and centrifugation at 11,000 g at 4°C for 5 minutes. DNA was precipitated with ethanol, suspended in 50 µl of double-distilled water and stored at -20°C until used.

The major *E. coli* phylogenetic groups, A, B1, B2, and D, were identified by multiplex PCR (Multiplex 1) (Clermont *et al*, 2000), ST131 was by standard PCR (Monoplex 1) (Doumith *et al*, 2015) and ST131 clades by multiplex PCR (Multiplex 2) (Matsumura *et al*, 2017). Reaction mixture (10 µl) contained 1 µl of DNA, primers (Table 1) and 2 µl of HOT FIREPol Blend Master Mix Plus containing 10 mM $MgCl_2$ (Solis Biodye, Tartu, Estonia). In Multiplex 1, thermocycling (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) was performed as follows: 95°C for 15 minutes; 40 cycles of 95°C for 30 seconds, 55°C for 40 seconds and 72°C for 60 seconds; with a final step at 72°C for 7 minutes. In Monoplex 1 and Multiplex 2, the above described thermocycling conditions were used except annealing step was 60°C for 30 seconds and 57°C for 20 seconds respectively. Amplicons were visualized following 1.5% agarose gel-electrophoresis by staining with RedSafe™ nucleic acid staining solution (iNtRON Biotechnology, Burlington, MA).

E. coli aac, *bla* genes, *gyr*A, integron locus, *mcr* genes, and *par*C detection

Amplification of $aac_{(6')Ib\text{-}cr}$, bla_{CMY2}, $bla_{CTX\text{-}M\ (groups\ 1\ and\ 9)}$, bla_{SHV}, bla_{TEM}, *gyr*A, integron locus, $mcr_{1,\ 3\ and\ 4}$ and *par*C were performed by conventional monoplex (Monoplex 2, 3 and 4) or multiplex PCR (Multiplex 3, 4, 5, 6 and 7). Reaction mixtures were as described above using primers listed in Table 1. In Monoplex 2 and Multiplex 3 and 4 thermocycling conditions were as follows: 95°C for 15 minutes; 40 cycles of 95°C for 40 seconds, 60°C for 40 seconds and 72°C for 60 seconds; with a final step at 72°C for 7 minutes. In Monoplex 3 and 4, the above described thermocycling conditions were used annealing step was 52°C for 60 seconds and 55°C for 60 seconds respectively, and in Multiplex 5, 6 and 7 annealing step was 58°C for 90 seconds, 64°C for 60 seconds and 55°C for 60 seconds, respectively. Amplicons were analyzed as described above.

Statistical analysis

The statistical analysis was performed using R version 3.6.1 software (The R Foundation for Statistical Computing, Vienna, Austria). A chi-square goodness-of-fit test was carried out to compare frequency of observations (for each incident). A p-value <0.050 is considered significant.

RESULTS

Of 58 *E. coli* strains obtained from Phayao Ram Hospital, Phayao Province (March 2015 - June 2017), 54% belonged to phylogenetic group B2 and 31% being ST131 strain (Table 2). The majority of phylogenetic group B2 *E. coli* strains were from female patients >40 years of age (although the number of male and female patients recruited in the study was nearly equal), of *E. coli* strain ST131 from patients of both genders >40 years of age and of specimens from urine, mainly infected with non-ST131 strain, indicating a high proportion of UPEC infection among the patients. The number of patients in 2015 and 2016 were nearly equal and dropped by half in 2017 (during which samples were collected only in the first six months), but, interestingly, in this half-year period there were more ST131 than non-ST131 strains (but this could be a coincidence in view of the shorter study period).

Antibiogram profiling using a disc diffusion assay of clinical *E. coli* strains revealed all strains were resistant to 10 of 11 antibiotics tested (the exception being imipenem), with highest frequency against ampicillin (95%), followed by ciprofloxacin (84%) and trimethoprim/sulfamethoxazole (81%), with 60% of the strains demonstrating ESBL production (Table 3). Eighty-six percent of strains were resistant to fluoroquinolones with 66% resistant to all four fluoroquinolones tested and 40% resistant to all four fluoroquinolones together with ESBL production. Resistance to fluoroquinolones was due to mutations in QRDRs of *gyr*A and *par*C, and to a lesser extent to $aac_{(6')Ib\text{-}cr}$, and ESBL-producing strains carried $bla_{CTX\text{-}m1}$ or $bla_{CTX\text{-}m9}$. As expected, the majority of *E. coli* strains (67%) harbored bla_{TEM} (responsible for ampicillin resistance). Additionally, 62% of strains harbored integron 1 (1,500 bp) (linked to *dfr*A17 and *aad*A5 determinants). The majority of strains having the above described phenotypes and genotypes were found (as expected) among *E. coli* group B2.

E. coli ST131 strains were primarily from urine samples (72%) and from patients >40 years of age (78%). Proportion of ST131 strains increased from 10% in 2015 to 30% in 2016, with a significant increase to 73% in the first half of 2017 (Table 2). ST131 strains are significantly associated with resistance to antibiotics, fluoroquinolone resistance combined with ESBL production and carriage of antibiotic resistance genes, except for resistance to ampicillin, cefoxitin and colistin, and for carriage of bla_{TEM}, bla_{CMY2}, and $mcr_{1 \text{ and } 3}$ (Table 3). ST131 strains harboring $bla_{CTXm\text{-}9}$ significantly increased from 1 (in 2015) to 5 (in 2017) compared to that of $bla_{CTXm\text{-}1}$ from 1 (in 2015) to 2 (in 2017), although samples were collected only in the first six months of 2017) (data not shown). ST131 strains resistant to four fluoroquinolones in combination with ESBL-production principally carried $bla_{CTX\text{-}m9}$ (50%) compared to non-ST131 strains (18%) (p-value = 0.005)

ST131 strains belonged mainly to subtypes A and C2, while no B subtype was found (Table 3). ST131 subtype A harbored either $bla_{CTX\text{-}m1}$ or $bla_{CTX\text{-}m9}$ while

Table 1

Primers used in the study

Primer	Gene	Sequence (5′ → 3′)	Amplicon size (bp)	Primer concentration (μM)	Reference
Escherichia coli typing					
Multiplex 1 (phylogenetic group)					
*fljB*_f	*chu*A	GACGAACCAACGGTCAGGAT	279	1.0	Clermont *et al* (2000)
*fljB*_r		TGCCGCCAGTACCAAAGACA		1.0	
gyrB_f	*yja*A	TGAAGTGTCAGGAGACGCTG	211	1.0	
gyrB_r		ATGGAGAATGCGTTCCTCAAC		1.0	
ycfQ_f	*tspe4.c2*	GAGTAATGTCGGGGCATTCA	310	1.0	
ycfQ_r		CGCGCCAACAAAGTATTACG			
Monoplex 1 (ST131)					
ST131_f		GACTGCATTTCGTCGCCATA	310	0.2	Doumith *et al* (2015)
ST131_r		CCGGCGGCATCATAATGAAA		0.2	

Table 1 (cont)

Primer	Gene	Sequence (5′ → 3′)	Amplicon size (bp)	Primer concentration (μM)	Reference
Multiplex 2 (ST131 clade)					
A_f	Clade A	TGACGGGACGTGAGCAAATTA	707	0.15	Matsumura *et al* (2017)
A_r		AGTCAGACCTAGCCACCCTT		0.15	
ST131M_f		AGCAACGATATTTGCCCATT	580	0.15	
ST131M_r		GGCGATAACAGTACGCCATT		0.15	
B_f	Clade B	CAACGTTGAAGCAGTGTATGAG	442	0.08	
B_r		TGACAATCGACGGCTTTAGA		0.08	
C1_f	Clade C1	GGCCCCACAAATTGCTT	337	0.1	
C1_r		CGCACCTCCGATACCAAA		0.1	
C1-M27_f	Clade C1-M27	TGAATCAAAGGTCCGAGCTG	232	0.08	
C1-M27_r		TATGGCTGGCAGATGCTTTA		0.08	
C2_f	Clade C2	ACGGATTCAGGTAGACGATT	164	0.25	
C2_r		CCTCACCAAAGTTGCGATTAC		0.25	
C_f	Clade C	CGCTGGCCAGTTATCTGAAAT	103	0.2	
C_r		CCTTTCACCAACTGGGTTACT		0.2	

Table 1 (cont)

Primer	Gene	Sequence (5′ → 3′)	Amplicon size (bp)	Primer concentration (μM)	Reference
Antibiotic resistance gene					
Multiplex 3 (beta-lactam)					
TEM_f	bla_{TEM}	CATTTCCGTGTCGCCCTTATTC	800	0.4	
TEM_r		CGTTCATCCATAGTTGCCTGAC		0.4	
SHV_f	bla_{SHV}	AGCCGCTTGAGCAAATTAAAC	713	0.4	
SHV_r		ATCCCGCAGATAAATCACCAC		0.4	
OXA_f	bla_{OXA}	GGCACCAGATTCAACTTTCAAG	564	0.4	
OXA_r		GACCCCAAGTTTCCTGTAAGTG		0.4	
Multiplex 4					
CTX 1_f	$bla_{\text{CTX-M group 1}}$	TTAGGAARRTGTGCCGCTGYYA	668	0.4	
CTX 1_r		CGATATCGTTGGTGGTRRCCAT		0.2	
CTX 9_f	$bla_{\text{CTX-M group 9}}$	TCAAGCCTGCCGATCTGGT	561	0.4	
CTX 9_r		TGATTCTCGCCGCTGAAG		0.4	

Table 1 (cont)

Primer	Gene	Sequence (5′ → 3′)	Amplicon size (bp)	Primer concentration (μM)	Reference
Monoplex 2					
CMY_f	bla_{CMY}	GTTCAGGAGAAAACGCTCCA	87	0.2	
CMY_r		CCAGCCTAATCCCTGGTACA			
Multiplex 5 (colistin)					
mcr 1_f	mcr_1	AGTCCGTTTGTTCTTGTGGC	320	0.25	
mcr 1_r		AGATCCTTGGTCTCGGCTTG		0.25	
mcr 3_f	mcr_3	AAATAAAAATTGTTCCGCTTATG	929	0.25	Rebelo *et al* (2018)
mcr 3_r		AATGGAGATCCCCGTTTTT		0.25	
mcr 4_f	mcr_4	TCACTTTCATCACTGCGTTG	1,116	0.25	
mcr 4_r		TTGGTCCATGACTACCAATG		0.25	
Multiplex 6 (fluoroquinolone)					
gyrA_f	*gyr*A	TACACCGGTCAACATTGAGG	647	0.1	Onseedaeng and Ratthawongjirakul (2016)
gyrA_r		TTAATGATTGCCGCCGTCGG		0.1	
gyrA83_f	*gyr*A83	TACCATCCCCATGGTGACTC	440	0.1	
gyrA87_r	*gyr*A87	GCCATGCGGACAATCGTGTC	255	0.1	
uspA_f	*usp*A	CCGATACGCTGCCAATCAGT	289	0.1	
uspA_r	*usp*A4	ACGCAGACCGTAGGCCAGAT	135	0.1	

Table 1 (cont)

Primer	Gene	Sequence (5′ → 3′)	Amplicon size (bp)	Primer concentration (μM)	Reference
Multiplex 7 (fluoroquinolone)					
parC_f	*par*C	AAACCTGTTCAGCGCCGCATT	395	0.04	
parC_r	*par*C	GTGGTGCCGTTAAGCAAA		0.04	
parC80_f	*par*C80	AATACCATCCGCACGGCGATAG	289	0.04	
parC84_r	*par*C84	CGCCATCAGGACCATCGGTT	135	0.04	
uspA_f	*usp*A	CCGATACGCTGCCAATCAGT	289	0.04	
uspA_r	*usp*A4	ACGCAGACCGTAGGCCAGAT	135	0.04	
Monoplex 3 (mutant $aac_{(6')Ib\text{-}cr}$)					
aac(6')Ib-cr_f	$aac_{(6')Ib\text{-}cr}$	TTGGAAGCGGGGACGGAM^{M}	260	0.5	Wareham *et al* (2010)
aac(6')Ib-cr_r		ACACGGCTGGACCATA	260	0.5	
Integron locus					
InvA_f	Integron locus	TCTCGGGTAACATCAAGG	1,000-1,700	0.025	Leverstein-Van Hall *et al* (2002)
InvA_r		AGGAGATCCGAAGACCTC			

M^{M} = A plus C, R^{R} = A or G and Y^{Y} = C or T where A, C, G and T refer to Adenine, Cytosine, Guanine and Thymine, respectively

bp: base pair; μM: micromolar

Table 2

Demographic profile of patients, source of specimens and clinical *Escherichia coli* strains classification from Phayao Ram Hospital, Phayao Province, northern Thailand (March 2015 - June 2017)

Demographic profile (patients and specimens)	*E. coli* phylogenetic group, number of isolates					*p*-value*	*E. coli* sequence type 131 subtypes, number of isolates					Non-ST131
	All isolates (*n*=58)	A (*n*=15)	B1 (*n*=6)	B2 (*n*=31)	D (*n*=6)		All isolates (*n*=18)	A (*n*=6)	C1-M27 (*n*=1)	C1-nM27 (*n*=5)	C2 (*n*=6)	*n*=40
Age (years)												
<2	16	6	1	7	2	0.089	3	1	1	0	1	13
2-40	9	0	3	3	3	NA	1	1	0	0	0	8
>40	*33*	9	2	21	1	<0.001	14	4	0	5	5	19
Gender												
Male	28	8	4	13	3	0.031	8	3	0	4	1	20
Female	30	7	2	18	3	<0.001	10	3	1	1	5	20
Specimen source												
Pus	8	2	2	3	1	0.801	2	1	0	0	1	6
Urine	34	7	2	21	4	<0.001	13	5	0	3	5	21
Stool	10	5	1	3	1	0.221	1	0	1	0	0	9
Blood	4	1	1	2	0	NA	1	0	0	1	0	3
Sputum	2	0	0	2	0	NA	1	0	0	1	0	1
Year												
2015[a]	20	8	2	8	2	0.065	2	0	1	0	1	18
2016	27	6	4	14	3	0.011	8	3	0	2	3	19
2017[b]	11	1	0	9	1	NA	8	3	0	3	2	3

[a]March – December; [b]January – June; *Significant at p <0.050, phylogenetic group B2 compared to others; NA: not applicable

Table 3

Antibiogram profiles of clinical *Escherichia coli* strains from patients at Phayao Ram Hospital, Phayao Province, northern Thailand (March 2015 - June 2017)

Characteristics of isolates	*E. coli* phylogenetic group, number of isolates					*p*-value*	*E. coli* sequence type 131 subtypes, number of isolates					Non-ST131
	All isolates (*n*=58)	A (*n*=15)	B1 (*n*=6)	B2 (*n*=31)	D (*n*=6)		All isolates (*n*=18)	A (*n*=6)	C1-M27 (*n*=1)	C1-nM27 (*n*=5)	C2 (*n*=6)	*n*=40
Antibiotic resistance phenotype												
ESBL	35	6	3	20	3	<0.001	16	6	1	5	4	10
Non ESBL	23	9	3	11	3	0.014	2	0	0	0	2	11
AMPC	*7*	4	2	1	0	NA	1	0	0	0	1	5
ESBL + AMPC	4	2	1	1	0	NA	1	0	0	0	1	3
ESBL + fluoroquinolones[a]	30	3	3	19	2	<0.001	16	6	1	5	4	9
ESBL +AMPC + fluoroquinolones	2	0	1	1	0	NA	1	0	0	0	1	1
Fluoroquinolone (number of types[a])												
4	38	6	5	23	4	< 0.001	14	3	1	4	6	24
3	2	1	0	1	0	NA	0	0	0	0	0	2
2	5	1	0	4	0	NA	4	4	0	0	0	1
1	5	2	1	1	1	0.896	0	0	0	0	0	5
0	8	5	0	2	1	NA	0	0	0	0	0	8

Table 3 (cont)

Characteristics of isolates	*E. coli* phylogenetic group, number of isolates					*p*-value*	*E. coli* sequence type 131 subtypes, number of isolates					Non-ST131
	All isolates (*n*=58)	A (*n*=15)	B1 (*n*=6)	B2 (*n*=31)	D (*n*=6)		All isolates (*n*=18)	A (*n*=6)	C1-M27 (*n*=1)	C1-nM27 (*n*=5)	C2 (*n*=6)	*n*=40
Ampicillin (AMP)	55	14	6	29	6	<0.001	18	6	1	5	6	37
Cefotaxime (CTX)	40	10	4	23	3	<0.001	16	6	1	5	4	24
Cefoxitin (FOX)	7	4	2	1	0	NA	1	0	0	0	1	6
Imipenem (IPM)	0	0	0	0	0	NA	0	0	0	0	0	0
Nalidixic acid (NA)	46	8	5	28	5	<0.001	17	6	1	4	6	29
Norfloxacin (NOR)	40	7	5	24	4	<0.001	13	2	1	4	6	27
Ciprofloxacin (CIP)	49	10	6	29	4	<0.001	17	6	1	4	6	32
Levofloxacin (LEV)	38	6	5	23	4	<0.001	13	2	1	4	6	25
Gentamicin (GEN)	39	10	3	22	4	<0.001	16	6	0	4	6	23
Trimethoprim/ sulfamethoxazole (STX)	47	9	4	28	6	<0.001	16	6	1	4	5	31
Colistin	3	3	0	0	0	NA	0	0	0	0	0	3

Table 3 (cont)

Characteristics of isolates	*E. coli* phylogenetic group, number of isolates					*p*-value*	*E. coli* sequence type 131 subtypes, number of isolates					Non-ST131
	All isolates (*n*=58)	A (*n*=15)	B1 (*n*=6)	B2 (*n*=31)	D (*n*=6)		All isolates (*n*=18)	A (*n*=6)	C1-M27 (*n*=1)	C1-nM27 (*n*=5)	C2 (*n*=6)	*n*=40
Antibiotic resistance gene												
bla_{TEM}	39	9	5	20	5	<0.001	9	7	0	2	0	30
bla_{SHV}	0	0	0	0	0	NA	0	0	0	0	0	0
bla_{OXA}	8	1	0	6	1	NA	6	0	0	0	6	2
$bla_{CTX\text{-}m\ group\ 1}$	20	8	2	8	2	0.065	7	3	0	0	4	13
$bla_{CTX\text{-}m\ group\ 9}$	15	1	1	12	1	<0.001	9	4	1	2	0	8
bla_{CMY2}	10	5	3	2	0	NA	1	0	0	0	1	9
*gyr*A83 (mutant)	44	10	4	26	4	<0.001	17	6	1	5	6	27
*gyr*A87 (mutant)	41	9	4	24	4	<0.001	15	3	1	5	6	26
*par*C80 (mutant)	39	8	4	23	4	<0.001	14	2	1	5	6	25
$aac_{(6')Ib\text{-}cr}$	9	1	0	7	1	NA	3	0	0	0	3	6
Integron locus	36	6	2	23	5	<0.001	16	6	1	4	5	20

*Significant at *p*-value <0.050, phylogenetic group B2 compared to others

[a]number of quinolone drug resistance (4 resistant to Nalidixic acid, Norfloxacin, Ciprofloxacin, and Levofloxacin; 0: susceptible to all)

subtype C2 exclusively harbored $bla_{CTX\text{-}m1}$ (except two samples that carried no bla_{CTX}) and subtype C1 (both M27 and non-M27) $bla_{CTX\text{-}m9}$. Only ST131 subtype A strains exhibited resistance to two types of fluoroquinolones. bla_{OXA} was exclusively present in ST131 subtype C2.

Seasonal incidence of *E. coli* infection from admitted patients during a calendar year (2016) demonstrated two major episodes in May and July during the summer period (April to August) with predominance of ESBL-producing *E. coli* and, interestingly, a high proportion of ST131 strains (Fig 1).

DISCUSSION

E. coli is often resistant to many first line drugs such as cephalosporins and fluoroquinolones widely used in governmental hospitals in Thailand (Lestari *et al*, 2012). Molecular characterization provides a rapid method for identification of drug resistance markers and virulent strains, pivotal data for prompt effective treatment and epidemiological surveillance. The study employed PCR-based techniques to identify genes responsible for antibiotics resistance and virulence genotypes present in *E. coli* strains isolated from various clinical samples of patients admitted to a tertiary-care hospital in northern Thailand over a period of 28 months.

Standard antimicrobial tests showing a high prevalence of multidrug resistance agreed with PCR-based identification of the cognate resistance genes, confirming the usefulness of this rapid and generally accessible molecular platform as an adjunct to standard microbiological

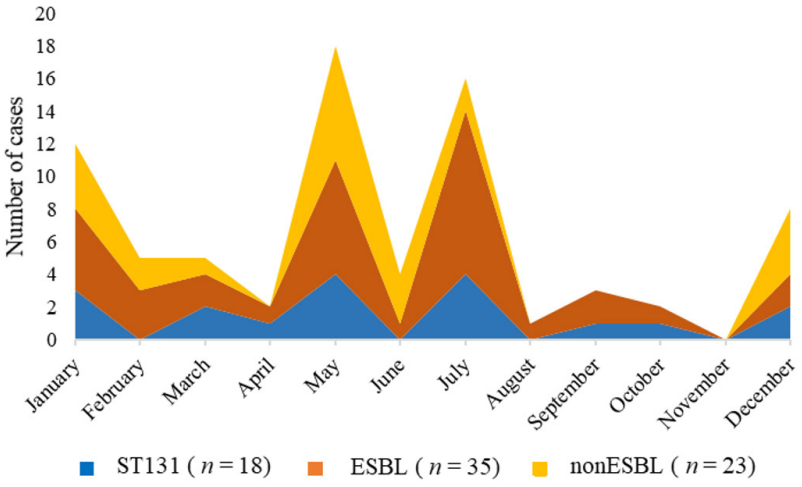

Fig 1 - Seasonal variations in number of *Escherichia coli* isolates from 58 patients admitted to Phayao Ram Hospital, Phayao Province, Thailand (2016)

assays. The major source of the pathogenic *E. coli* strains was urine, indicating this bacterium was a major causative agent of UTI in northern Thailand (Parikumsil *et al*, 2017). The PCR-based method allows facile identification of fluoroquinolone- and beta-lactam-resistant ST131 strains in waste water (Finn *et al*, 2020), which accounted for nearly half of *E. coli* strains isolated from urine, suggesting avoidance of these two classes of antimicrobials in empirical treatment of UTI in this region of the country.

The sudden rise in 2017 of *E. coli* ST131 carrying higher bla_{CTXm-9} :bla_{CTXm-1} was unexpected and needs to be confirmed in more recently isolated clinical samples, although this trend has been reported elsewhere (Matsumura *et al*, 2016; Birgy *et al*, 2017; Ghosh *et al*, 2017). In Thailand, the clonal spread of *E. coli* ST131 carrying bla_{CTXm-9} was previously suggested to have originated from backyard poultry farms, farmers and environment sources (Tansawai *et al*, 2019). Although ST131 subtypes A and C2 were predominant, presence of subtypes C1-M27 and C1-non-M27 indicated possible future spread of these C1 subtypes in northern Thailand. A recent report in Japan of ST131 subtype C1-M27 among residents in long-term-care is a cause of public health concern because there was the possible evidence of virulent plasmid (blaCTX-M-27/ F1:A2:B20) dissemination (Matsuo *et al*, 2020). In addition, ST131 subtype C2 harboring bla_{OXA} was probably nosocomial and originated from a SEA-C2 clone currently circulating elsewhere in Southeast Asia (Chen *et al*, 2019), but this need further confirmation.

In conclusion, the PCR-based protocols described in the study demonstrate utility of this rapid and facile molecular platform in the identification of antibiotic resistance genes and virulent types of *E. coli* isolated from clinical specimens, which should assist in appropriate and timely empirical drug treatment and in surveillance studies, not only in northern Thailand and elsewhere in the country.

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