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High prevalence of ESBL-producing *E. coli* phylogroup B2 clinical isolates in northeastern Thailand

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Abstract

Background Production of extended-spectrum β-lactamases (ESBLs) is a common resistance mechanism in *Enterobacteriaceae*, leading to serious hospital-acquired infections. This study aimed to assess phenotypic, phylogenetic, and antibiotic resistance patterns among ESBL-producing *Escherichia coli* isolates recovered from two rural tertiary hospitals in Thailand.

Results Among 467 *Enterobacteriaceae* isolates, *E. coli* was the most prevalent 356 (76.2%) followed by *K. pneumoniae* 88 (18.8%), *K. aerogenes* 8 (1.7%), *K. variicola* 3 (0.6%), *K. quasipneumoniae* 1 (0.2%%), *K. oxytoca* 1 (0.2%), and unidentified 9 (1.9%). Of the 202 cephalosporin-resistant *E. coli* isolates, 195 (96.5%) were ESBL-producing and 7 (3.5%) were non-ESBL-producing. Clermont typing revealed that phylogroup B2 was predominant (43.3%), followed by phylogroups F (11.3%), D (10.3%), C (9.7%), and A (8.7%). Among the beta-lactamase-encoding genes, bla_{CTX-M} (83.6%) and bla_{TEM} (81.0%) were widely found among the isolates, and $bla_{CTX-M-1}$ (60.7%) was the most common among the five bla_{CTX-M} subgroups detected. The predominant ESBL was $bla_{CTX-M-15}$ (58.3%). All isolates were resistant to cefotaxime (100%) and ampicillin (100%), followed by ciprofloxacin (91.3%), ceftazidime (72.8%), and tetracycline (64.1%).

Conclusion Our findings show that phylogroup B2 was the most prevalent phylogroup among ESBL-producing *E. coli* isolates in northeastern Thailand. Notably, the isolates mostly carried the bla_{CTX-M} gene(s).

Keywords blaCTX-M, E. coli, Clermont typing, Extended-spectrum β-lactamase, Third-generation cephalosporin resistance

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Background

The global burden of antimicrobial resistance (AMR) is increasing, with transmission frequently occurring in healthcare settings due to poor infection control practices and overuse of antimicrobials. According to the World Health Organization (WHO), a few third-generation cephalosporin-resistant gram-negative bacteria, included in a list of 12 globally important antimicrobial-resistant pathogens, should be considered a critical priority, and new antibiotics should be developed against them [1]. Recently, many studies have shown that multidrug-resistant (MDR) bacteria are becoming more common in humans, birds, cattle, and fish. This shows how important it is to do regular antimicrobial susceptibility testing to find the best antibiotic and check for the new MDR strain [2].

Among these, the strains that produce extended-spectrum β-lactamase (ESBL) are more resistant to cephalosporins than those that produce AmpC cephalosporinases and carbapenemases. This enzyme is capable of hydrolyzing penicillins, 3rd and 4th -generation cephalosporins and monobactams, but not cephamycins and carbapenems. Additionally, they can be inhibited by classical and newly developed β-lactamase inhibitors such as clavulanic acid, avibactam, sulbactam, tazobactam, relebactam or nacubactam [3]. The dissemination of ESBL-producing bacteria worldwide has become a serious public health issue, as they are a frequent cause of infection in healthcare centers and the community. Furthermore, plasmids that easily spread or transmit between bacterial species contain most ESBL-encoding genes [4]. Infections with ESBL-producing strains are responsible for increased morbidity and mortality rates and prolonged hospitalization [5–7].

ESBLs are categorized based on several b-lactamase-encoding genes, including $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} [8]. Among these genes, $bla_{\text{CTX-M}}$ is the most common worldwide [4, 9]. There are many types of $bla_{\text{CTX-M}}$ β-lactamases. Some of the most common types are found in *Escherichia coli* and *Klebsiella pneumoniae*. Other types can be found in *Salmonella* spp. (both typhoidal and non-typhoidal), *Shigella* spp., *Citrobacter freundii*, and *Enterobacter* spp [4]. The $bla_{\text{CTX-M}}$ gene is classified into five subgroups: $bla_{\text{CTX-M-1, -2, -8, -9}}$, and $_{-25}$. Some regional differences in the prevalence of the $bla_{\text{CTX-M}}$ subgroups have been observed; for example, $bla_{\text{CTX-M-9}}$ was detected at a higher rate in Thailand [10].

Escherichia coli is responsible for a wide range of infectious diseases worldwide, including urinary tract infections (UTI), hospital-acquired pneumonia (HAP), surgical site infection (SSI), gastrointestinal tract infections, hemolytic-uremic syndrome (HUS), neonatal and inflammation of meningitis, and bacteremia [11]. Extraintestinal pathogenic *E. coli* (ExPEC) infections are typically more medically serious than *E. coli* intestinal infections. The virulence factors, including adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, types 1 and 3 fimbriae, and invasins are typically encoded on pathogenicity islands (PAIs), plasmids, and other mobile genetic components [12].

E. coli is divided into the phylogenetic groups A, B1, B2, C, D, E, F, and clade I [13]. Pathogenic *E. coli* that caused intestinal infections belonging to phylogenetic groups A, B1, or D. *E. coli* causing extraintestinal infections are members of groups B2 and D. Group E is closely related to group D, while group F is associated with the main group B2. Clones of *E. coli* that differ genetically but are phenotypically indistinguishable have been classified into cryptic clade I [12].

ESBL-producing *E. coli* has emerged as a prevalent health concern in hospital settings and is responsible for community-acquired infections worldwide, leading to an increase in the resistance ratio against β-lactam antibiotics. The prevalence of ESBL production among *E. coli* isolates from Indonesia, the Philippines, and Thailand were 71%, 47%, and 55% [14]. Intestinal colonization by ESBL-producing *E. coli* showed a temporal increase from 2.6% in 2003–2005 (95% CI 1.6%-4.0%) to 21.1% in 2015–2018 (95% CI 15.8%-27.0%). Notably, South-East Asia had the highest carriage rate at 27% (95% CI 2.9%-51.3%), while Europe had the lowest rate at 6.0% (95% CI 4.6%-7.5%) [15].

Since 2006, ESBL-producing *E. coli* has been reported in Thailand [16]. The overall contamination rate of ESBL-producing *E. coli* in foods and open water sources in Thailand was 26.4%, while the carriage rate of ESBL-producing *E. coli* in the gastrointestinal tract in Thai adults was 66.5% [17]. Carriers of ESBL-producing *E. coli* isolates increased significantly in rural Thai provinces (Sa Kaeo and Nakhon Phanom Provinces) from 19 to 22% in 2008–2010 to approximately 30% from 2011 to 2014 [18]. Several studies have demonstrated that CTX-M-type ESBL ($bla_{\text{CTX-M}}$) is a common ESBL-producing *E. coli* isolate in Thailand [19–21].

Therefore, continuous surveillance of ESBL-producing *E. coli* is necessary to monitor countermeasures against potential nosocomial infections. This study aimed to characterize third-generation cephalosporin-resistant *E. coli* isolates were collected from two tertiar hospitals in northeastern Thailand between 2019 and 2020. Its objectives were to evaluate the prevalence of ESBL-producing *E. coli* antimicrobial susceptibility profile and to characterize their phenotypic and genetic features, followed by an analysis of phylogenetic groups.

Methods

Bacterial isolation and identification

Between January 2019 to December 2020, a total of 467 *Enterobacteriaceae* strains were isolated from various clinical specimens, including urine (n=221, 47.3%), rectal swab (n=142, 30.4%), pus (n=47, 9.9%), blood (n=35, 7.5%), and sputum (n=22, 4.7%). These specimens were routinely collected from two tertiary hospitals in a rural area of northeastern Thailand. Tertiary hospitals in this region typically have over 800 beds and provide a comprehensive range of services for patients with serious medical conditions.

The isolates were cultured on MacConkey agar plates (HIMEDIA, Mumbai, India) and then incubated at 37 °C for 24 h before undergoing Gram staining. *E. coli* screening was done using multiplex PCR (mPCR) with primers specific for the *uidA* gene, which encodes β-D-Glucuronidase. This mPCR method was used to identify species of *K. pneumoniae*, *K. quasipneumoniae*, *K. varicola* [22], *K. oxytoca* [23], and *K. aerogenese* [24] (see Supplementary Table 1). *E. coli* species confirmation was done using standard biochemical tests, including catalase, oxidase, IMViC (indole, methyl red, Voges-Proskauer, citrate utilization), triple sugar iron, urease, motility, and oxidative fermentation tests.

Phenotypic identification of ESBL-production

All *Enterobacteriaceae* isolates were screened for third-generation cephalosporin resistance using disk diffusion tests with cefotaxime and ceftazidime. Isolates resistant to third-generation cephalosporin (cefotaxime and ceftazidime) were further evaluated for ESBL production. The ESBL production was determined using the disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021) [25]. In brief, bacterial cultures equivalent to a 0.5 McFarland standard were inoculated onto Mueller Hinton agar plates (Merck, Germany). Ceftazidime (30 µg), ceftazidime/clavulanate (30/10 µg), cefotaxime (30 µg), and cefotaxime/clavulanate (30/10 µg) disks (MASTDISCS®AST, Reinfeld, Germany) were placed on the inoculated plates, which were incubated at 37 °C for 18 h. Organisms that exhibited ≥5 mm inhibition zones with combination disks compared to individual antibiotics (without clavulanate) were designated as ESBL producers.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of cephalosporin-resistant isolates was assessed following the CLSI, 2021 guidelines. Testing included 11 antimicrobial agents from various classes, including penicillins: ampicillin (AP 10 µg), β-lactam combination agents: piperacillin-tazobactam (PTZ 110 µg), cephems: cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), aminoglycoside: gentamicin (GM 10 µg), fluoroquinolones: ciprofloxacin (CIP 5 µg), carbapenems: imipenem (IMP; 10 µg), meropenem (MEM; 10 µg), phenicols: chloramphenicol (C 30 µg), tetracycline: tetracycline (T 30 µg), folate pathway antagonist: trimethoprim (TM 15 µg), fosfomycins: fosfomycin (FOT 200 µg), and nitrofurans: nitrofurantoin (NI 300 µg). *Escherichia coli* ATCC 25922 served as the control strain for antimicrobial susceptibility testing. The antibiotic disks were obtained from MASTDISCS®AST, Reinfeld, Germany). The plates were then incubated at 37 °C for 18–24 h. After overnight incubation, the zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant based on CLSI recommendations [25]. Multidrug resistance patterns of the isolates were identified according to the guideline described by Magiorakos et al. [26].

PCR-based detection of β-lactamase genes

DNA extraction from the bacteria was performed using the heat lysis method [27]. The presence of the genes encoding bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, and the $bla_{\text{CTX-M}}$ subgroup ($bla_{\text{CTX-M}}$ groups 1, 2,8, 9, and 25) in the isolates was determined by multiplex PCR, following previously described protocols [28–30]. All primers were manufactured by Integrated DNA Technologies (Singapore). The target genes, primers sequence, cycle conditions, and amplicon size are described in Supplementary Table 1. Each reaction mixture (25 µl) contained DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific, Vilnius, Lithuania), 0.3 µM of each primer, sterile deionized (DI) and 2 µl of bacterial DNA. The PCR product was visualized and photographed under UV transillumination (SYNGENE, Cambridge, UK). Amplified PCR products for the full-length bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ genes were sent to be analyzed by an automated DNA sequencing system at Genewiz services company (China).

Phylogenetic grouping of the third-generation cephalosporin-resistant *E. coli* isolates

The phylogenetic groups of the cephalosporin-resistant *E. coli* isolates were determined using quadruplex PCR, following the method outlined by Clermont et al. [31]. This method enabled the assignment of *E. coli* isolates to eight phylogroups (A, B1, B2, C, D, E, F, and cryptic clade) based on the presence or absence of four genes: *arpA*, *chuA*, *yjaA*, and the *TspE*4.*C*2 DNA fragment.

Results

Characteristics of the recovered *E. coli* isolates

Among the 467 *Enterobacteriaceae* clinical isolates, *E. coli* was the predominant species, found 356 isolates (76.2%). Moreover, multiplex PCR used for confirmed *E. coil* led to *K. pneumoniae* followed in with 88 isolates

(18.8%). The remaining isolates included *K. aerogenes* (8 isolates; 1.7%), *K. variicola* (3 isolates; 0.6%), *K. quasipneumoniae* (1 isolate; (0.2%%), *K. oxytoca* (1 isolate; 0.2%), and unidentified species (9 isolates; 1.9%). Phenotypically, *E. coli* a gram-negative bacterium, rod-shaped, motile, growing on MacConkey agar (with red or colorless colonies), exhibiting lactose fermentation, indole positivity, urease negativity, citrate negativity, gas production, hydrogen sulfide negativity, and lysine decarboxylase positivity.

Phenotypic determination for ESBL producing *E. coli* isolates

The two hundred and two isolates were determined to be resistant to third-generation cephalosporins (cefotaxime and/or ceftazidime). Among these, all 202 *E. coli* isolates exhibited resistance to cefotaxime, while 146 isolates demonstrated resistance to both cefotaxime and ceftazidime. Furthermore, analysis using the combination disk method revealed that 96.5% (195 out of 202 isolates) produced ESBL (Fig. 1).

Antibiotic susceptibility pattern of ESBL-producing *E. coli* isolates

The antimicrobial resistance profile of these *E. coli* isolates is depicted in Fig. 2. It was observed that all isolates exhibited complete resistance to ampicillin and cefotaxime, followed by relatively high resistance rates to ciprofloxacin (91.3%, 178/195), ceftazidime (72.9%, 142/195), and tetracycline (64.1%, 125/195). Conversely, these isolates were generally susceptible to carbapenem, fosfomycin, and nitrofurantoin. Among the 195 ESBL-producing *E. coli* isolates, (98.5%, 192/195) were classified as multidrug-resistant (MDR) demonstrating resistance to at least one agent in three or more antimicrobial categories (Supplementary Table 2).

Molecular characterization of β-lactamase genes

The predominant genes identified in our study were bla_{TEM} and $bla_{\text{CTX-M}}$ in combination (65.13%, 127/195 isolates), followed by $bla_{\text{CTX-M}}$ alone (15.9%, 31/195 isolates), bla_{TEM} alone (13.3%, 26/195 isolates), and the combined presence of $bla_{\text{TEM+}}bla_{\text{CTX-M+}}bla_{\text{SHV}}$ (2.6%, 5/195 isolates). The 163 isolates carrying $bla_{\text{CTX-M}}$, the majority belonged to the $bla_{\text{CTX-1}}$ group (45.4%, 74/163 isolates), followed by the $bla_{\text{CTX-9}}$ group (28.2%, 46/163 isolates), and the combined $bla_{\text{CTX-1}}$ and $bla_{\text{CTX-9}}$ group (15.3%, 25/163 isolates). The major β-lactamase genes found in all *E. coli* isolates were bla_{TEM}+$bla_{\text{CTX-M-1}}$ (26.7%, 52/195 isolates), followed by bla_{TEM}+$bla_{\text{CTX-M-9}}$ (14.9%, 29/195 isolates). In addition, we detected plasmid-mediated AmpC cephalosporinase-encoding genes

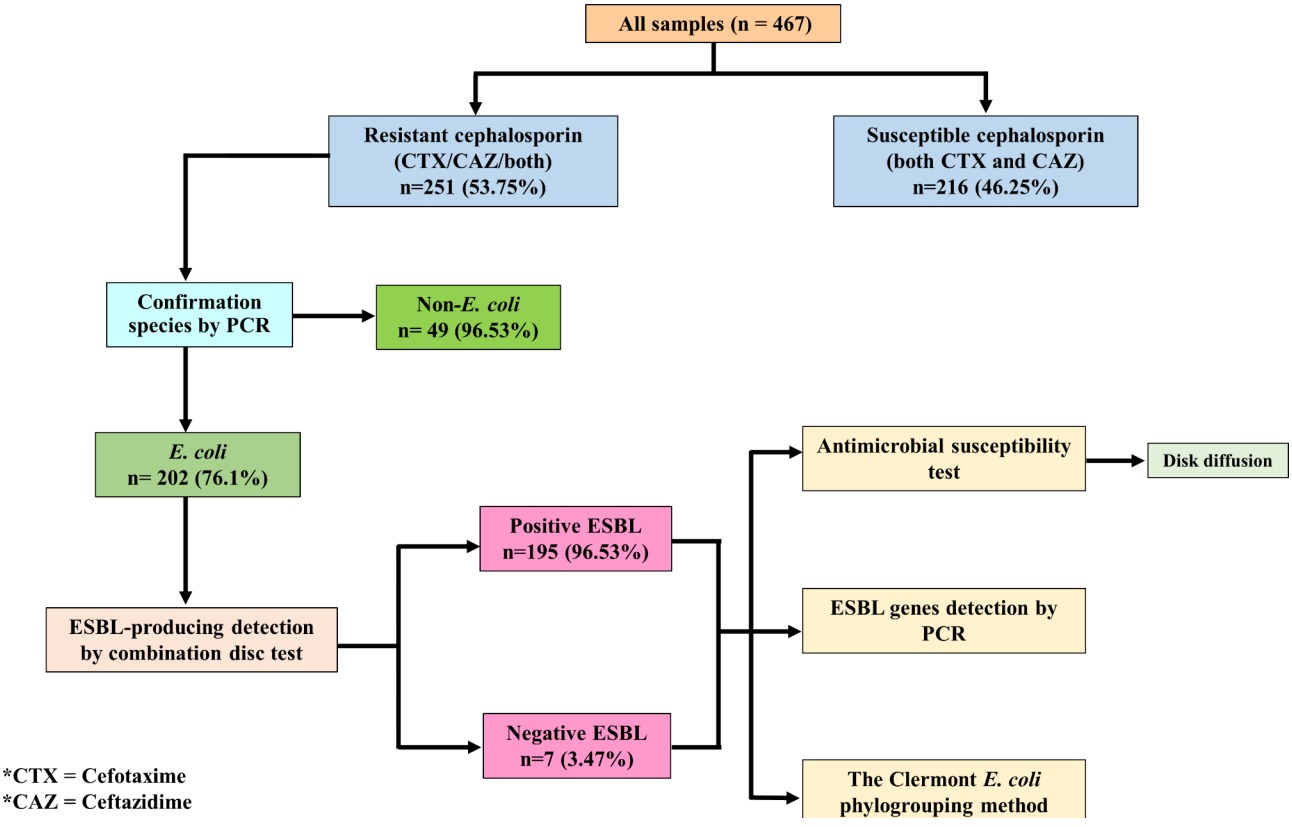

Fig. 1 Flow chart of the study. Characterization of Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*

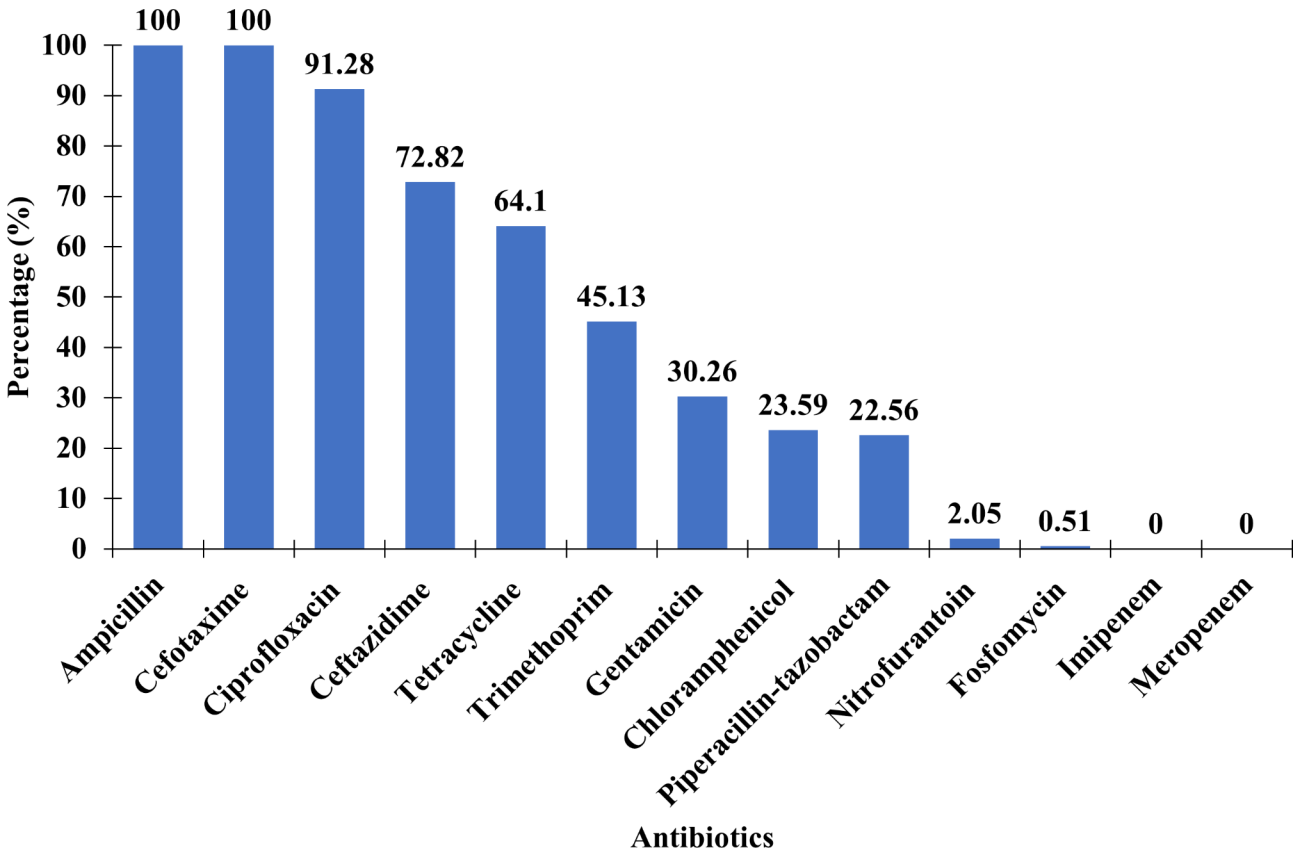

Fig. 2 Antibiotic resistance profile of third generation cephalosporin-resistant *Escherichia coli* isolates ($n = 202$)

in 7 non-ESBL-producing *E. coli* and 19 ESBL-producing *E. coli*.

The full-length $bla_{\text{CTX-M}}$ gene from 163 isolates revealed the presence of various subtypes. $bla_{\text{CTX-M-15}}$ was the most prevalent subtype, identified in 58.8% (96/163) of isolates. Other detected subtypes include $bla_{\text{CTX-M-27}}$ (33.7%, 55/163), $bla_{\text{CTX-M-3}}$ (1.2%, 2/163), $bla_{\text{CTX-M-14}}$ (1.8%, 3/163), $bla_{\text{CTX-M-161}}$ (1.8%, 3/163), and $bla_{\text{CTX-M-202}}$ (2.5%, 4/163). Additionally, among the ESBL-producing isolates, 26 were identified as carrying bla_{TEM}, comprising $bla_{\text{TEM-1}}$ (57.7%, 15/26) and $bla_{\text{TEM-213}}$ (42.3%, 11/26).

Phylogroup of ESBL-producing *E. coli*

Among the 195 ESBL-producing *E. coli* isolates, we observed that phylogroup B2 was the most prevalent (43.6%, 85/195 isolates), followed by phylogroups F (11.3%, 22/195 isolates), D (10.3%, 20/195 isolates), C (9.7%, 19/195 isolates), A (8.7%, 17/195 isolates), B1 (6.2%, 12/195 isolates), E (2.6%, 5/195 isolates), clade I (1.0%, 2/195 isolates), and unknown (6.7%, 13/195 isolates). Organisms carrying both bla_{TEM} and $bla_{\text{CTX-M-1}}$ genes were found in all the phylogroups. Phylogroup B2 predominantly harbored the following genes: bla_{TEM} and $bla_{\text{CTX-M-1}}$, bla_{TEM} and $bla_{\text{CTX-M-9}}$, and $bla_{\text{CTX-M-1}}$. Phylogroup F mainly contained bla_{TEM}, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-9}}$, while phylogroup A carried bla_{TEM} and $bla_{\text{CTX-M-1}}$. The phylogroup C isolates exhibited higher resistance levels to many antimicrobials compared to other phylogroups. Additionally, we observed that 5.9% and 20% of isolates from phylogroups A and E, respectively, showed resistance to nitrofurantoin, while isolates from other phylogroups were highly susceptible to these antibiotics (Fig. 3; Table 1).

Discussion

The WHO and the US Centers for Disease Control and Prevention have highlighted the severe threat posed by ESBL-producing organisms to public health [1, 32]. ESBL-producing *E. coli* is a major pathogen in hospitals and communities, leading to longer hospital stays and higher morbidity and mortality rates. Studies in Thailand have shown a growing prevalence of ESBL-producing *E. coli* in clinical specimens, ranging from 21 to 51.8% [18, 33–35]. Our study found a prevalence of 54.8% ESBL-producing *E. coli* consistent with these trends. Our study showed an MDR phenotype in 54.2% of *E. coli* isolates, similar to the findings reported by Lim et al. (58%) in Thailand [36], but lower than the rate reported in Bangladesh (69%) Mazumder et al. [37]. In northeastern

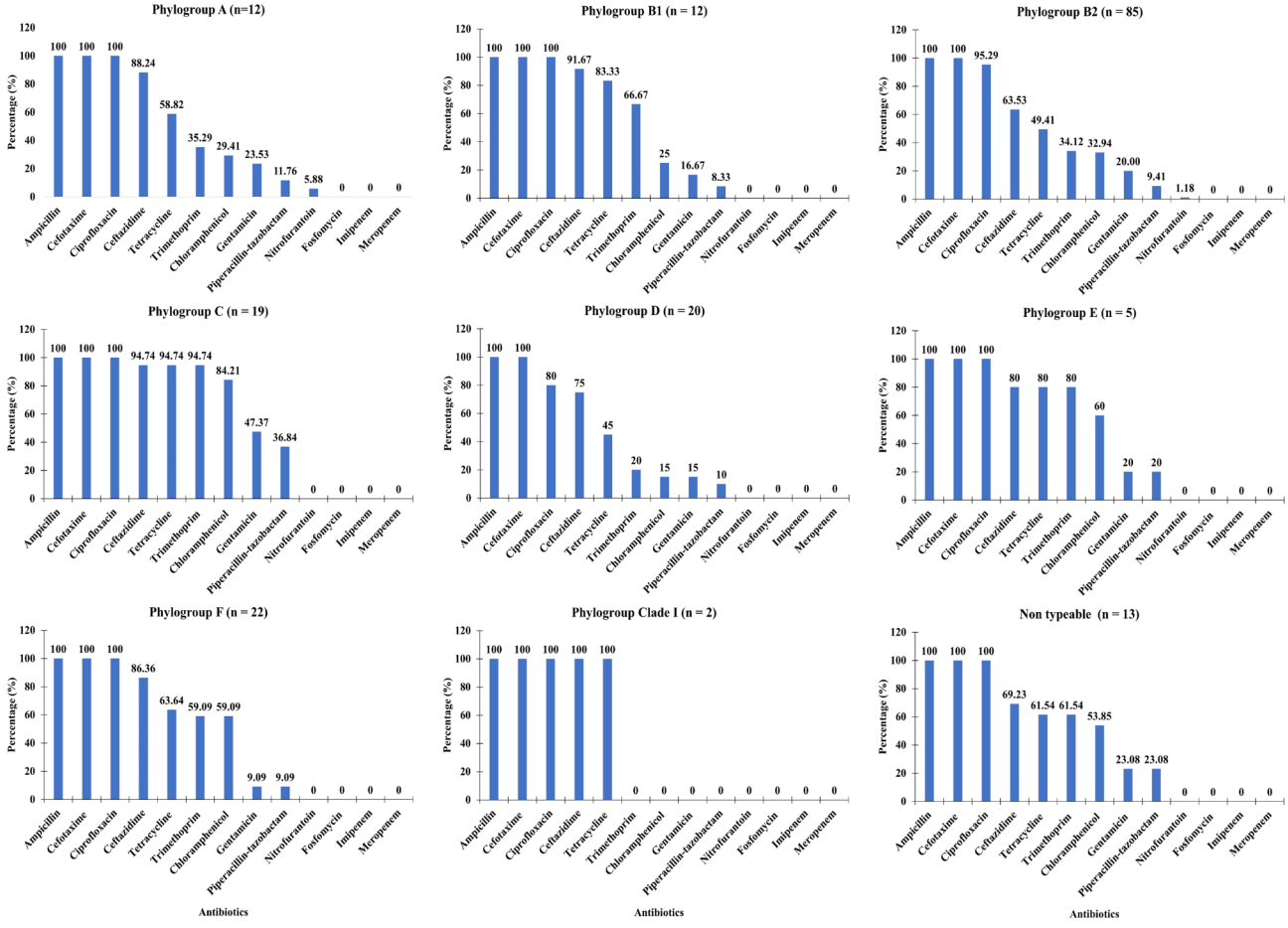

Fig. 3 Phylogroup-wise antibiotic resistance profile of *E. coli* isolates

Thailand, 96.53% of third-generation cephalosporin-resistant hospital isolates were ESBL-producing E. coli, showing high resistance to several antibiotics (ampicillin, cefotaxime, ciprofloxacin, and ceftazidime) [21, 38–40]. The co-occurrence of ESBL and MDR in Gram-negative bacilli is a well-documented phenomenon. Notably, a prior study reported that 32.7% of MDR *E. coli* isolates exhibiting resistance to over seven antimicrobials were also ESBL-positive [41, 42].

Our study found a high prevalence of $bla_{\text{CTX-M}}$ (83.6%, 163/195) in ESBL-producing *E. coli*, with $bla_{\text{CTX-M-1}}$ (45.4%, 74/163) and $bla_{\text{CTX-M-9}}$ (28.2%, 46/163) being the most common variants. In northern Thailand, 76.5% of $bla_{\text{CTX-M}}$ was found in *E. coli* or *K. pneumoniae* isolates [19], while central Thailand showed a high prevalence of $bla_{\text{CTX-M}}$ (99.6%) in ESBL-producing *E. coli* [20]. From 2014 to 2015, the most common strains in Thailand carried $bla_{\text{CTX-M-1}}$ (71.2%) and $bla_{\text{CTX-M-9}}$ (38.9%) among ESBL-producing *E. coli* isolates [21]. Understanding the genetic characteristics of ESBL genes is essential because they can horizontally transfer to other bacterial species, leading to the extensive spread of ESBL activity among diverse pathogens within hospital sittings.

Our study found that $bla_{\text{CTX-M-15}}$ was the most prevalent β-lactamase gene among ESBL-producing *E. coli* isolates, consistent with findings from previous studies [37, 43, 44]. Additionally, the presence of $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27,}}$ and $bla_{\text{CTX-M-14}}$, as identified in these studies and reported in Thailand [45], which are known to exhibit enhanced ceftazidime hydrolytic activity [46]. Furthermore, $bla_{\text{TEM-1}}$, widely disseminated worldwide, was likely detected in our study. Notably, our study identified $bla_{\text{TEM-213}}$ for the first time in Thailand, representing the latest subtype of bla_{TEM} [47]. Our study revealed a high prevalence of multidrug resistance among ESBL-producing *E. coli* isolates, with over half of them carrying multiple resistance genes, potentially conferring resistance to a broader spectrum of antimicrobial agents. The most frequently co-occurring genes observed were $bla_{\text{CTX-M}}$ and bla_{TEM}, consistent with findings reported in other studies [48–50]. The presence of multiple ESBL resistance genes within single isolates leads to an elevated resistance profile, posing challenges for treating infections with conventional antibiotics.

The Clermont phylotyping method rapidly groups E. coli phylogenetically and is used worldwide. However,

Table 1 Distribution of beta-lactamase genes and phylogroups among third generation cephalosporin resistant *Escherichia coli* ($n=202$)

Cephalosporin resistant E. coli ($n=202$)	Resistance gene profile	Phylogroup									Total
		A	B1	B2	C	D	E	F	Clade I	NT	
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
ESBL-producing ($n=195$)	bla_{TEM}	2 (10.5)	3 (15.8)	7 (36.8)	1 (5.3)	2 (10.5)	1 (5.3)	1 (5.3)		2 (10.5)	19
	bla_{TEM}+bla_{CIT}				5 (83.3)					1 (16.7)	6
	bla_{TEM}+bla_{DHA}		1 (100)								1
	bla_{TEM}+ $bla_{CTX-M\ (unknown\ group)}$	3 (20)		3 (20)	2 (13.3)	3 (20)		3 (20)		1 (6.7)	15
	bla_{TEM}+ $bla_{CTX-M\ (unknown\ group)}$ + bla_{CIT}				1 (100)						1
	bla_{TEM}+$bla_{CTX-M-1}$	10 (19.2)	2 (3.9)	22 (42.3)	3 (5.8)	7 (13.5)	3 (5.8)	1 (1.9)	1 (1.9)	3 (5.8)	52
	bla_{TEM}+$bla_{CTX-M-9}$			22 (75.9)		4 (13.8)		3 (10.3)			29
	bla_{TEM}+$bla_{CTX-M-9}$+bla_{DHA}	1 (10)	6 (60)					1 (10)		2 (20)	10
	bla_{TEM}+$bla_{CTX-M-1}$+$bla_{CTX-M-9}$			5 (26.3)			1 (5.3)	11 (57.9)		2 (10.5)	19
	bla_{TEM}+$bla_{CTX-M-1}$+$bla_{CTX-M-9}$+bla_{DHA}			1 (100)							1
	$bla_{CTX-M\ (unknown\ group)}$			1 (100)							1
	$bla_{CTX-M-1}$			10 (52.6)	2 (10.5)	3 (15.8)		2 (10.5)	1 (5.3)	1 (5.3)	19
	$bla_{CTX-M-9}$			5 (83.3)						1 (16.7)	6
	$bla_{CTX-M-1}$+$bla_{CTX-M-9}$			5 (100)							5
	bla_{TEM}+ $bla_{CTX-M\ (unknown\ group)}$ + bla_{SHV}				1 (100)						1
	bla_{TEM}+$bla_{CTX-M-1}$+bla_{SHV}				3 (100)						3
	bla_{TEM}+$bla_{CTX-M-9}$+bla_{SHV}					1(100)					1
	Not detected	1 (16.7)		4 (66.7)	1 (16.7)						6
ESBL-non-producing ($n=7$)	bla_{TEM}+bla_{CIT}		1 (11.1)			1 (11.1)	2 (22.2)				4
	bla_{TEM}+bla_{DHA}								1 (100)		1
	bla_{CIT}				1 (50)		1 (50)				2
	Total	17	13	85	20	21	8	22	3	13	202

*NT: non typeable

some *E. coli* isolates could not be assigned to one of the eight recognized phylogroups [31]. Our study successfully identified and assigned approximately 94% of our *E. coli* isolates to one of the eight reported phylogroups. The remaining 6% of isolates were unclassified, possibly due to rare phylogroups or recombination between different *E. coli* phylogroups [51]. Among the assigned phylogroups, the majority of the isolates belonged to phylogroup B2, followed by groups F, D, C, and A (Table 1). Virulent *E. coli* isolates are mainly in phylogroups B2 and D, while less virulent and commensal isolates are in phylogroups B1 and A [52]. In our study, phylogroup B2 mainly harbored $bla_{CTX-M-1}$ and $bla_{CTX-M-9}$. Similar findings were reported in Egypt, where phylogroup B2 was prevalent among ESBL-producing uropathogenic *E. coli* (UPEC) isolates carrying $bla_{CTX-M-1}$ and $bla_{CTX-M-2}$ [53], as

well as Iran and Pakistan, phylogroup B2 was frequently detected among third-generation cephalosporin-resistant *E. coli* isolates associated with urinary tract infections [51, 54]. Thus, in UPEC isolates, phylogroup B2 isolates more frequently produce ESBL than other phylogroups [55]. Notably, the majority of phylogroup F isolates carried bla_{TEM}, $bla_{\mathrm{CTX-M-1}}$, and $bla_{\mathrm{CTX-M-9}}$ genes, representing a novel combination of the *bla* gene (Table 1). A study from Egypt also reported phylogroup F UPEC isolates carrying bla_{TEM} and/or $bla_{\mathrm{CTX-M-1}}$ [53]. Phylogroup F was reclassified from Phylogroup D and is closely relative to B2, sharing close evolutionary relationships and possibly associated with extra-intestinal pathogens due to the presence of antibiotic-resistance genes, replicons, and pathogenicity-related genes [56, 57]. Our study found that most isolates of phylogroup B2 were highly resistant to ampicillin, cefotaxime, ceftazidime, and ciprofloxacin, but more susceptible to other examined antimicrobials (Fig. 2). This finding contrasts with previous studies from Iran [51], and Spain [58], which found that phylogroup B2 is highly resistant to antibiotics. In our study, however, phylogroup C exhibited the highest resistance levels to many antimicrobials. Overall, the distribution of phylogroups and ESBL-encoding genes varied among isolates, isolation sites, and geographic locations.

Conclusion

Our findings highlight a high prevalence of ESBL-producing *E. coli* (41%) among Enterobacteriaceae clinical specimens, with most of these isolates carrying both bla_{TEM} and $bla_{\mathrm{CTX-M}}$ (62.9%). Phylogroup B2 mainly harbored beta-lactamase gene patterns: '$bla_{\mathrm{TEM}}bla_{\mathrm{CTX-M-1}}$, bla_{TEM}+$bla_{\mathrm{CTX-M-9}}$, and $bla_{\mathrm{CTX-M-1}}$'. Further research should investigate plasmidome genomics, virulence determinants, evolutionary relationships among ESBL-producing strains, and clinical outcomes. Moreover, implementing a rapid diagnostic approach to identify ESBL-producing microorganisms, along with robust surveillance efforts, is recommended to control their dissemination between humans and the environment. Public health initiatives are crucial for raising awareness about multidrug resistance and promoting the judicious use of antimicrobial agents.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
AK, PC, and YA conceived and designed the experiment; SC, PK, NP, and PB performed the experiments; AK, PC, DT, and YA analyzed and interpreted the data; AK, SC, PC and SH wrote and edited the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval
Not applicable. Stored isolates used in the study were collected by routine public health surveillance and therefore not considered to involve human subject research.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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