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Genomic insights into colistin-resistant *Escherichia coli* from clinical isolates in Thailand: Diversity of sequence types, plasmid-borne *mcr* variants, and One Health implications

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ABSTRACT

Background: Colistin is a last-resort antimicrobial agent for treating multidrug-resistant Enterobacterales. However, resistance has emerged globally through plasmid-mediated *mcr* genes and chromosomal mutations. Reports of whole-genome sequencing of colistin-resistant *Escherichia coli* from clinical settings in Thailand remain limited.

Objectives: To characterize the molecular and phenotypic features of colistin-resistant *E. coli* isolated from a tertiary hospital in Thailand using whole-genome sequencing (WGS).

Materials and methods: Fourteen colistin-resistant *E. coli* isolates were collected from clinical specimens between 2021 and 2022. Antimicrobial susceptibility testing was performed using broth microdilution. WGS was applied to identify resistance determinants, plasmid replicons, virulence genes, phylogroups, and sequence types.

Results: The prevalence of colistin-resistant *E. coli* was 1.2% (14/1203 isolates). Most isolates exhibited an ESBL-like multidrug-resistant profile with preserved carbapenem susceptibility. WGS revealed diverse sequence types (including ST131, ST95, ST58, ST69) and phylogroups, indicating polyclonal dissemination. Two *mcr* variants (*mcr-1.1* and *mcr-3.5*) were identified on mobile plasmids (IncX4, IncI2, IncFII, IncHI2), with some isolates carrying both variants. Several isolates without *mcr* carried chromosomal mutations in *mgrB*, *phoPQ*, or *pmrAB*. Virulence genes, particularly adhesins, siderophore systems, and capsule-related determinants, were widely distributed. The detection of *mcr-3.5*, previously reported in livestock, within clinical isolates highlights potential zoonotic or foodborne transmission.

Conclusion: Colistin-resistant *E. coli* in Thailand shows significant genetic diversity and frequent coexistence of *mcr* and ESBL genes, often on plasmids with high potential for horizontal transfer. These findings emphasize the importance of antimicrobial stewardship, stricter control of drug use in animals, and integrated genomic surveillance under a One Health framework to mitigate the dissemination of colistin resistance.

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Introduction

An antimicrobial resistance (AMR) has emerged as one of the most global health threats. Among the Enterobacterales, *Escherichia coli* is a pathogen responsible for hospital-acquired infections and is

frequently implicated in multidrug-resistant (MDR) outbreaks. The increasing prevalence of colistin-resistant strains has the widespread use of colistin.[1] a last resort polymyxin antibiotic. However, colistin resistance has rapidly emerged through both chromosomal mechanisms and horizontally transferable plasmid-mediated *mcr* genes.[2]

In Thailand, the emergence of colistin-resistant Enterobacterales is a growing concern for tertiary care hospital[3]. This trend not only compromises empirical options but also a potential in infection control. These aims to prevent the misuse of last-line agents like colistin and strengthen surveillance systems that can detect and respond to emerging resistance trends.[4,5] In Thailand, the emergence of colistin-resistant Enterobacterales is a growing concern for tertiary care hospitals.[6] Specifically, colistin resistance in *Escherichia coli* has been previously documented from various settings, including clinical samples and slaughter pigs in Thailand.[7]

The Whole-genome sequencing (WGS) offers a powerful platform for AMR surveillance, providing detailed insights into resistance mechanisms, clonal relatedness, and gene transmission pathways.[8] Unlike conventional phenotypic tests, WGS can differentiate between plasmid-mediated and chromosomal resistance, identify high-risk sequence types (STs), and trace the distribution of virulence and resistance genes across clinical settings These genomic data not only inform antimicrobial stewardship by guiding empirical treatment guidelines and targeted infection control interventions, but also emphasize a One Health perspective highlighting how resistance genes such as *mcr* can disseminate across humans, animals, and the environment. Integrating genomic surveillance across sectors is therefore crucial to track the evolution and transmission of resistance determinants and to inform coordinated control strategies. In this study, we applied WGS to characterize colistin-resistant *E. coli* isolates obtained from clinical specimens at Lerdsin General Hospital. The findings aim to provide molecular epidemiological evidence that supports local infection control and national AMR policies, and to demonstrate the utility of genomic surveillance in antimicrobial use in Thailand.

Materials and methods

Collection and identification of bacterial isolates

All 1,203 isolates of *Escherichia coli* were collected from clinical specimens. Of these, 14 colistin-resistant *Escherichia coli* isolates were subsequently collected from residual clinical specimens processed at the Clinical Microbiology Laboratory, Lerdsin Hospital, Bangkok, Thailand. between October 2021 and September 2022. Species identification was performed using MALDI-TOF MS (Sirius system; Bruker Daltonik, Germany). All isolates were recoded to ensure patient anonymity and preserved at -80 °C in tryptic soy broth supplemented with 20% glycerol. Prior to testing, isolates were subculture on MacConkey agar containing colistin (4 µg/mL) and incubated at 37 °C for 18 hours.

Antimicrobial susceptibility testing

All isolates were tested for susceptibility to 16 antibiotics. The minimum inhibitory concentrations (MICs) were determined by broth microdilution using the automated Sensititre™ THAN2F system (Thermo Fisher, UK), following the manufacturer's protocol.[9] Quality control was performed using *E. coli* ATCC 25922. The MICs were interpreted according to CLSI guidelines (M100-S31, 2021 Edition)[10] Isolates with colistin MICs ≥4 µg/mL were classified as colistin-resistant and included in the study.

Whole genome sequencing (WGS)

Genomic DNA was extracted using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, USA) and quantified with a NanoDrop spectrophotometer (Biotek, USA). Whole-genome sequencing was performed at MicrobeNG (Birmingham, UK) using paired-end 2 × 250 bp Illumina NextSeq technology with a minimum mean coverage of 30×. Raw reads (FASTQ format) were trimmed using Trimmomatic and assessed for quality using FastQC. Genome assembly was performed using SPAdes v3.15.5. The assembly were checking quality by filter sequence by length minimum 200 bp and QUAST for assembly quality (https://usegalaxy.eu, Galaxy Europe version 25.0, access on 31 Aug 2025). Read and assembly were used to bioinformatics analysis. Antibiotic resistance genes were identified using ResFinder (https://genepi.food.dtu.dk/resfinder), access on 24 Aug 2025[5] and the CARD RGI tool (https://card.mcmaster.ca/analyze/rgi, access on 24 Aug 2025).[11] Mobile genetic elements were identified using MGE in (https://cge.food.dtu.dk/services/MobileElementFinder, access on 25 Aug 2025).[5] Chromosomal mutations associated with colistin resistance, including *mgrB, pmrAB* and *phoPQ* were analyzed using BLASTn and ClustalW, aligned with *E. coli* K-12 MG1655 reference genomes.[5] Amino acid substitutions were predicted using BLASTx, and insertion sequences were identified using ISfinder (https://isfinder.biotoul.fr/blast.php, access on 24 Aug 2025).[12] Virulence genes were identified using VirulenceFinder (https://cge.food.dtu.dk/services/VirulenceFinder, access on 25 Aug 2025).[5] Plasmid replicons were detected using PlasmidFinder (https://cge.food.dtu.dk/services/PlasmidFinder, access on 25 Aug 2025.[5] Phylogrouping of *E. coli* was performed using Clermont Typing (https://ezclermont.hutton.ac.uk, access on 30 Aug 2025),[13] and sequence types (STs) were assigned using MLST based on the (https://pubmlst.org, access on 30 Aug 2025).[5]

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the study isolates. Categorical data,

such as specimen type, department of admission, and gender, were presented as counts and percentages. The association between colistin-resistant and non-colistin-resistant *E. coli* isolates and various categorical variables was tested using the Chi-square test or Fisher's exact test, where appropriate, to determine the *p*-values shown in Table 1. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 29.0.1.0.

Data availability statement

The complete genomes were deposited in GenBank under Bioproject Accession No. PRJNA1312245.

Results

Clinical isolates from clinical specimens

A total of 14 colistin-resistant isolates were collected from residual clinical specimens between October 2021 and September 2022. The isolates originated from various specimen types, including urine, blood, sputum, pus, and other sources, and were derived from multiple hospital departments including intensive care unit (ICU) and non-ICU Table 1. Among 1,203 *E. coli* isolates, 14 isolates (1.2%) were colistin-resistant *E. coli*. The distribution of resistant isolates did not significantly differ from non-colistin resistant isolates with respect to specimen type (p=0.318), department of admission (p=0.850), or gender (p=0.925). The median age of patients with colistin-resistant isolates was 70 years (IQR 60-78), compared with 65 years (IQR 55-73) among those with non-resistant isolates.

Table 1. Comparison of specimen sources, departments, and demographic characteristics between colistin-resistant and non-resistant *Escherichia coli* isolates.

	Number of isolates		
	Colistin resistant *E. coli* N=14 (1.2%)	**Non-colistin resistant *E. coli* N=1189 (98.8%)**	***p*-value**
Specimens			0.318
Blood	1 (7.1%)	183 (15.4%)	
Sputum	1 (7.1%)	118 (9.9%)	
Urine	9 (64.3%)	713 (59.9%)	
Pus	3 (21.4%)	95 (8.0%)	
Department			0.850
ICU	0	52 (4.4%)	
Non-ICU	14 (100%)	1,057 (95.6%)	
Gender			0.925
Male	6 (42.9%)	452 (38%)	
Female	8 (57.1%)	737 (62.0%)	
Age (years)			
Median age	70 (60-78)	65 (55-73)	

Phenotypic antimicrobial resistance profiles

Among the 14 colistin-resistant *E.coli* isolates, a high prevalence of multidrug resistance (MDR) was observed. All 14 colistin-resistant *E.coli* isolates exhibited ESBL phenotypes and met the MDR definition. The most frequent resistance pattern included ß-lactams, aminoglycosides, and fluoroquinolones, with six isolates also resistant to trimethoprim–sulfamethoxazole. Resistances to ampicillin and third-generation cephalosporins (including ceftazidime, ceftriaxone, and cefotaxime) were common, indicating the presence of an extended-spectrum ß-lactamase (ESBL)-like phenotype in all isolates. In addition, resistance to fluoroquinolones (ciprofloxacin and levofloxacin) was frequently detected among the same isolates, further supporting their classification as MDR strains. The full antimicrobial susceptibility profile is presented in Table 2.

Table 2. Antibiotic susceptibility pattern of colistin-resistant *E. coli* 14 isolates.

Isolates	Colistin (µg/mL)	Year	Sample	Resistant mechanism	MDR status	AK	CN	AP	AUG	TZP	CAZ	CRO	CTX	FOX	CIP	LEV	ETP	IMP	MEM	SXT
EC1	8	2021	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	S
EC2	8	2021	Sputum	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	S
EC3	8	2021	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	S
EC4	8	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	R
EC5	8	2022	Pus	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	R
EC6	>8	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	R
EC7	4	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	S	S	S	S	S	R
EC8	8	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	S	S	S	S	S	S
EC9	4	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	R
EC10	8	2022	Pus	ESBL	MDR	S	R	R	S	S	R	R	R	S	R	R	S	S	S	S
EC11	>8	2022	Urine	ESBL	MDR	S	S	R	S	S	R	R	R	R	R	R	S	S	S	S
EC12	>8	2022	Pus	ESBL	MDR	S	S	R	S	S	R	R	R	S	R	R	S	S	S	R
EC13	4	2022	Blood	ESBL	MDR	S	S	R	S	S	R	R	R	S	R	R	S	S	S	R
EC14	>8	2022	Urine	ESBL	MDR	S	R	R	S	S	R	R	R	S	S	S	S	S	S	R

Note: S: susceptible, I: intermediate, R: resistance, CT: colistin, AK: amikacin, CN: gentamicin, AP: ampicillin, AUG: amoxicillin-clavulanic acid, TZP: piperacillin-tazobactam, CAZ: ceftazidime, CRO: ceftriaxone, CTX: cefotaxime, FOX: cefoxitin, CIP: ciprofloxacin, LEV: levofloxacin, ETP: ertapenem, IMP: imipenem, MEM: meropenem, SXT: trimethoprim-sulfamethoxazole.

By contrast, susceptibility to carbapenems (ertapenem, imipenem, and meropenem) was retained in all isolates, suggesting the absence of carbapenemase producers in this collection. Among aminoglycosides, amikacin susceptibility was common, whereas gentamicin resistance was observed in several isolates.

Notably, three isolates (EC6, EC8, and EC14) exhibited largely susceptible phenotypes, with resistance confined to ampicillin or a limited number of agents, and two isolates EC9 and EC11 demonstrated intermediate susceptibility to cefoxitin or fluoroquinolones. Overall, the resistance pattern highlights the predominance of ESBL-producing MDR *E.coli* with preserved carbapenem susceptibility, while a minority of isolates displayed non-ESBL or susceptible phenotypes. The co-existence of ESBL-mediated multidrug resistance with colistin resistance indicates that these isolates represent high-risk lineages with limited therapeutic options.

Genotypic features of colistin-resistant E. coli isolates

The 14 colistin-resistant *E. coli* isolates represented diverse sequence types (STs) including high-risk extraintestinal *E. coli* lineages ST131 and ST95, as well as ST58, ST69, ST117, ST457. Multiple plasmid replicons were detected, predominantly IncF variants (FIA, FIB, FII) in combination with Col-type replicons. Plasmid backbones such as IncX4, IncI2, IncHI2, and IncFII carried *mcr* determinants. Two *mcr* variants were identified, namely *mcr*-1.1 and *mcr*-3.5. Several isolates carried both *mcr*-variants (EC3, EC8, EC14) with *mcr*-1.1 located on IncX4 or IncI2 plasmids replicon and *mcr*-3.5 on IncFII or IncX1 plasmids replicons. The co-existence of two *mcr* genes within single isolates suggests possible plasmid co-residence of multiple plasmids. In contrast, three isolates (EC9, EC11, EC12, EC13) lacked *mcr* genes, implying colistin resistance was mediated by chromosomal mutations. Point mutations were identified in multiple chromosomal loci implicated in colistin resistance. Variants in *mgrB* (e.g., N36S, N3K, Y12H), *phoPQ* (e.g., I44L, R6H, S138T), and *pmrAB* (e.g., S29G, D283G, H2R, V351I) were observed across isolates. These mutations were especially relevant in isolates without *mcr* genes, supporting their role in chromosomal-mediated colistin resistance.

An acquired resistance determinants were detected. The ESBL-associated genes, including $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-24}}$, and $bla_{\text{CTX-M-55}}$, were predominant. The other β-lactamases such as $bla_{\text{OXA-1}}$ and $bla_{\text{CMY-42}}$ were also present. Aminoglycoside resistance genes included *aac(3)-IId*, *aac(3)-VIa*, and *aac(6')-Ib-cr*, while fluoroquinolone resistance was shown *to be the qnrS1* gene. The co-carriage of ESBLs and aminoglycoside or fluoroquinolone resistance genes highlights the multidrug-resistant *E. coli* of many isolates. The details of genomic features are in Table 3.

Table 3. Genomic features of colistin-resistant *E. coli* 14 isolates.

Isolates	Sequence types	Colistin (µg/mL)	*mcr* variant (location)	Plasmid replicon type	*mgrB*	*phoP*	*phoQ*	*pmrA*	*pmrB*	Acquired resistance genes
EC1	131	8	*mcr*-3.5 (IncFII)	Col-type, IncF (FIA, FIB, FIC), IncFII	N36S	I44L	WT	S29G, T31S	V351I, H2R, E123D, D283G	*bla* $_{CTX-M-15}$, *bla* $_{OXA-1}$, *aac(6)-Ib-cr, aac(3)-IIa*
EC2	648	8	*mcr*-3.5 (IncFII)	Col-type, IncF (FIA, FIB, FIC), IncFII	WT	I44L	L467M	S29G	A360V, H2R, D283G	*bla* $_{TEM-1B}$, *bla* $_{CTX-M-55}$, *qnS1, aac(3)-IId*
EC3	4679	8	*mcr*-1.1 (IncX4), *mcr*-3.5 (IncX1)	Col-type, IncF (FIB, FIC), IncI1-I, IncQ1, IncR, IncX1, IncX4	WT	I44L	WT	S29G	Y358N, D283G	*bla* $_{CTX-M-55}$, *bla* $_{TEM-1B}$, *aac(3)-IId*
EC4	58	8	*mcr*-1.1 (IncX4)	Col-type, IncF (FIA, FIB, FIC), IncI1-I, IncQ1, IncX1, IncX4, IncY	WT	I44L	WT	S29G	Y358N, D283G	*bla* $_{TEM-1B}$, *bla* $_{CTX-M-55}$, *qnS1, aac(3)-IId*
EC5	69	8	*mcr*-3.5 (IncFII)	Col440, ColRNAI, IncFII, IncQ1	WT	I44L	S138T, A482T	S29G	D283G, H2R, S138N	*bla* $_{TEM-1B}$, *qnS1, aac(3)-VIa*
EC6	117	>8	*mcr*-1.1 (IncHI2)	Col-type, IncB, IncF (FIB, FIC), IncHI2	WT	I44L	WT	S29G	A360V, H2R, D283G	*bla* $_{TEM-1B}$, *bla* $_{CTX-M-55}$, *qnS1, aac(3)-IId*
EC7	95	4	*mcr*-3.5 (IncFII)	Col-type, IncFIB, IncFII, IncQ1	WT	I44L	R6H	S29G, G144S, T31S, L128N	V351I, H2R, E123D, D283G	*bla* $_{TEM-1B}$, *aac(3)-IId*
EC8	58	8	*mcr*-1.1 (IncX4), *mcr*-3.5 (IncFII)	Col-type, IncB, IncF (FIB, FIC), IncHI2, IncFII, IncI1, IncX4	WT	I44L	WT	S29G	Y358N, D283G	*bla* $_{TEM-1B}$, *qnS1, aac(3)-IId, aac(3)-VIa*
EC9	457	4	-	Col-type, IncF (FIA, FIB, FII), IncX1, IncY	N3K, Y12H, N36S	I44L	L467M	S29G	D283G	*bla* $_{TEM-1B}$, *bla* $_{CTX-M-55}$, *qnS1, aac(3)-IId*
EC10	58	8	*mcr*-3.5 (IncFII)	Col-type, IncF (FIA, FIB, FIC), IncFII	WT	I44L	WT	S29G	Y358N, H2R, D283G	*bla* $_{TEM-1B}$
EC11	11021	>8	-	Col-type, IncF (FIA, FIB, FII), IncI	WT	I44L	WT	S29G, A105P	H2R	*bla* $_{OXA-1}$, *bla* $_{CMY-42,}$ *aac(6)-Ib-cr*
EC12	10	>8	-	IncFIB, IncI	WT	WT	WT	S29G	H2R	*bla* $_{TEM-1B,}$ *aac(3)-IId*
EC13	131	4	-	Col-type, IncF (FIA, FIB, FII)	N36S	I44L	WT	S29G, T31S	V351I, E123D, D283G	*bla* $_{CTX-M-14}$, *bla* $_{CTX-M-24}$
EC14	58	>8	*mcr*-1.1 and *mcr*-3.5 (IncI2)	Col-type, IncF (FIA, FIB, FII), IncI2	WT	I44L	WT	S29G	Y358N, H2R, D283G	*bla* $_{TEM-1B}$, *aac(3)-IId*

Analysis of virulence genes revealed that all 14 colistin-resistant *E. coli* isolates possessed multiple virulence determinants across diverse functional categories shown in Table 4. Adhesin-associated genes were universally present (14/14, 100%), including *fimH*, *pap* family genes *papA, papC, fdeC*, *yeh*, and *yfcV,* indicating a strong potential for host colonization and epithelial attachment. Iron acquisition systems were detected in almost all isolates (13/14, 92.9%), with siderophore-related gene *chuA, fyuA,* and *iutA* frequently observed, reflecting the ability to thrive in iron-limited host environments. Capsule and serum resistance genes *kps, iss, ompT, traT* were also common (12/14, 85.7%), supporting mechanisms for immune evasion and serum survival.

Table 4. Virulence genes distribution in colistin-resistant *E. coli*.

Functional group	Virulence genes	No. positive isolates (%)
Adhesins	*fimH, papA/papC, fdeC, focC, sfaD, hra, iha, lpfA, yeh, yfcV, csgA*	14/14 (100%)
Iron acquisition	*chuA, fyuA, iroN, ireA, irp2, iucC, iutA, sitA, katP*	13/14 (92.9%)
Capsule/Serum resistance	*kpsE, kpsMII_K1/K5/K11, neuC, iss, ompT, traT*	12/14 (85.7%)
Toxins	*hlyA/E/F, cnf1, sat, vat, usp, pic, tsh, cma, cib*	9/14 (64.3%)
Invasins	*tia, eilA, AslA*	5/14 (35.7%)
Other/Regulators	*anr, gad, hha, nlpI, terC, shiA/B, capU, etsC, cvaC, mchF, traJ*	14/14 (100%)

Toxin-associated genes *hlyA, cnf1, sat*, and *vat* showed variable distribution (9/14, 64.3%), indicating heterogeneity in cytotoxic potential among the isolates. Invasion gene *tia, eilA, AslA* were less prevalent (5/14, 35.7%), suggesting that invasive capacity is limited to a subset of strains. Conversely, regulatory and stress-response genes *anr, gad, hha, nlpI, terC, shiA, capU, etsC, cvaC*, *mchF, traT* were consistently present in all isolates (14/14, 100%), underscoring the genomic stability of stress-adaptive and plasmid maintenance systems in this MDR *E. coli* collection.

Discussion

The prevalence of colistin-resistant *E. coli* has been reported worldwide, including in livestock from farms in Japan[14,15] and China.[1] In Thailand, previous studied have identified strains in slaughter pigs from farms[7] and in clinical samples from tertiary hospitals.[8] This study highlights the complex epidemiology of colistin-resistant *E. coli* in Lerdsin General Hospital a tertiary hospital in Thailand. The isolates belonged to diverse sequence types including high-risk extrapathogenic *E. coli* lineages such as ST131[16] and ST648, as well as virulent but emerging high risk lineages like ST95. Community and animal-associated STs, including ST58 and ST69,[17] were also detected. Together, these findings indicate polyclonal dissemination, suggesting multiple introductory events from both clinical and non-clinical reservoirs rather than clonal expansion within the hospital.

Phenotypic testing revealed that most isolates displayed an ESBL-like multidrug-resistant profile with additional fluoroquinolone resistance, although carbapenem susceptibility was preserved. A report in this study is that most colistin-resistant *E. coli* isolates also exhibited an ESBL phenotype. This co-occurrence may be explained by the carriage of both resistance determinants on the same or compatible plasmid backbones. In several isolates, *mcr* genes were located on IncF or IncI-type plasmids, which are also known to frequently harbor ESBL genes such as bla_{CTX-M}. The genetic linkage of *mcr* and ESBL-associated genes within the same plasmid increases the likelihood of co-selection.[18] Although most colistin-resistant *E. coli* isolates in this study exhibited an ESBL phenotype, phenotypic testing showed that several antimicrobial agents remained effective. All isolates were susceptible to carbapenems, and most retained susceptibility to amikacin. The clinicians may still consider carbapenems or selected aminoglycosides as therapeutic options. However, the broad-spectrum activity of these agents carries a risk of further resistance, underscoring the need for antimicrobial stewardship and ongoing genomic surveillance.

The detection of *mcr*-1.1 and *mcr*-3.5 on highly mobile plasmids (IncX4, IncI2, IncFII, IncHI2) underscores their potential for horizontal transfer across *E. coli* lineages and possibly other Enterobacterales.[19] The co-existence of both *mcr* variants in single isolates reflects ongoing plasmid creates opportunities for accelerated dissemination. Notably, both *mcr* variants have been widely reported in livestock, especially swine, raising the likelihood of zoonotic or foodborne transmission.[1,20]

Virulence gene profiles, including adhesins, siderophore systems, and capsule-associated genes were widely distributed, suggesting that these resistant strains also retain significant pathogenic potential. The convergence of virulence and resistance is particularly concerning lineages such as ST131, which are globally linked to invasive infections.[21]

Our findings highlight the connection of patients in hospitals, animals, and the community in the emergence of colistin resistance. The core evidence supporting the one health implication lies in the molecular characteristics of the isolates. Specifically, the detection of *mcr*-3.5 within clinical isolates is a key finding. The *mcr*-3 group, and particularly *mcr*-3.5 has been widely reported in livestock, especially swine,[22,23] in Thailand and globally suggesting a strong non-human reservoir linkage.[24] The identification of this specific variant in human clinical samples strongly emphasizes the potential for zoonotic or foodborne transmission from the animal/agricultural sector or the community into the hospital setting. Furthermore, the colistin-resistant isolates belonged to diverse Sequence Types (STs), including community and animal-associated lineages such as ST58 and ST69. This polyclonal dissemination suggests multiple introduction events from both clinical and non-clinical reservoirs rather than clonal expansion within the hospital. The co-existence of these community/animal-associated STs alongside high-risk clinical lineages (e.g., ST131) further strengthens the argument for cross-sectoral movement of colistin resistance genes, necessitating a One Health approach.[25] The mechanism facilitating this cross-sectoral spread is likely the carriage of *mcr* determinants on highly mobile plasmids (IncX4, IncI2, IncFII, IncHI2).[26] These plasmids can circulate widely and transfer resistance genes across different bacterial hosts and lineages. These observations underscore the urgent need for tighter regulation of antimicrobial use in animals and the integration of genomic surveillance with stewardship programs under a One Health framework to guide preventive interventions across clinical, agricultural, and community settings.

Conclusion

This study demonstrates that colistin-resistant *E. coli* isolated from a tertiary hospital in Thailand are genetically diverse, encompassing high-risk extrapathogenic *E. coli* lineages and community-associated strains. The frequent coexistence of colistin resistance with ESBL phenotypes, often mediated by plasmid-borne determinants, underscores the threat of multidrug resistance. Notably, the detection of *mcr*-3.5, previously reported in livestock, within clinical isolates highlights the possibility of zoonotic or foodborne transmission. Plasmids carrying *mcr* genes possess strong potential for horizontal transfer across bacterial hosts, facilitating rapid dissemination. These findings emphasize the need for rational antimicrobial use, continuous genomic surveillance, and integrated stewardship efforts under a One Health framework to mitigate the spread of colistin resistance across human, animal, and environmental sectors.

Ethical approval

This study was exempted from ethical review for human research by the Ethics Committee for Human Research at Lerdsin Hospital, Department of Medical Services, under project code LH661016.Laboratory work complied with national biosafety and biosecurity regulations under supervision of the institutional Biosafety and Biosecurity Committee.

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Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Kwanchon Jearakitiwanich: conceptualization, methodology, data curation, formal analysis, writing: original draft, review and editing, project administration; **Anusak Kerdsin**: data analysis guidance, bioinformatics supervision; **Monchai Siribamrungwong:** clinical supervision, review of antimicrobial resistance aspects; **Panan Ratthawongjirakul**: funding acquisition, resources, project support.

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