Plasmidome in mcr-1 harboring carbapenem-resistant enterobacterales isolates from human in Thailand

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The emergence of the mobile colistin-resistance genes mcr-1 has attracted significant attention worldwide. This study aimed to investigate the genetic features of mcr-1-carrying plasmid among carbapenem-resistant Enterobacterales (CRE) isolates and the potential genetic basis governing transmission. Seventeen mcr-1 harboring isolates were analyzed based on whole genome sequencing using short-read and long-read platforms. All the mcr-1-carrying isolates could be conjugatively transferred into a recipient Escherichia coli UB1637. Among these 17 isolates, mcr-1 was located on diverse plasmid Inc types, consisting of IncX4 (11/17; 64.7%), IncI2 (4/17; 23.53%), and IncHI/IncN (2/17; 11.76%). Each of these exhibited remarkable similarity in the backbone set that is responsible for plasmid replication, maintenance, and transfer, with differences being in the upstream and downstream regions containing mcr-1. The IncHI/IncN type also carried other resistance genes (blaTEM-1B or blaTEM-132). The mcr-1-harboring IncX4 plasmids were carried in E. coli ST410 (7/11; 63.6%) and ST10 (1/11; 9.1%) and Klebsiella pneumoniae ST15 (1/11; 9.1%), ST336 (1/11; 9.1%), and ST340 (1/11; 9.1%). The IncI2-type plasmid was harbored in E. coli ST3052 (1/4; 25%) and ST1287 (1/4; 25%) and in K. pneumoniae ST336 (2/4; 50%), whereas IncHI/IncN were carried in E. coli ST6721 (1/2; 50%) and new ST (1/2; 50%). The diverse promiscuous plasmids may facilitate the spread of mcr-1 among commensal E. coli or K. pneumoniae strains in patients. These results can provide information for a surveillance system and infection control for dynamic tracing.

The global spread of carbapenem-resistant Enterobacterales (CRE) has become a leading public health concern due to the rapidly increasing prevalence of carbapenemase gene carriage by Enterobacterales, with most carbapenem resistance conferred by carbapenem-degrading enzymes (carbapenemase) such as K. pneumoniae carbapenemase (blaKPC), New Delhi metallo-β-lactamase (blaNDM), and OXA-48-like carbapenemase1,2.

The lack of accessible treatment has resulted in the use of colistin, an outmoded antibiotic, as a last-resort therapeutic drug for human infections by Gram-negative bacteria. The widespread use of colistin in humans and animals has led to the emergence of colistin resistance in Gram-negative bacteria, with rates of resistance continuously increasing3,4. A classic mechanism of colistin resistance is thought to be associated with chromosomal mediation5. The discovery of plasmid-mediated colistin resistance encoded by mcr genes revealed high prevalence in human and animal isolates harboring these genes and the transmission of mcr is of global concern6. Up to the present, 10 variants of mcr (mcr-1 through mcr-10) have been reported7,8. Of particular concern is the spread of mcr genes into CRE, which would create strains that are potentially pan-drug resistant. The coexistence of mcr and carbapenemase genes, such as blaNDM, blaOXA-48-like, and blaIMP in CRE isolates has been described worldwide9,10.

The global prevalence of mcr genes revealed that mcr-1 (4917/5191; 94.7%) is a common gene and has a wider distribution than mcr-2 through to mcr-8. Human infections with CRE isolates carrying mcr-1 have been

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reported\(^{10-14}\) and the prevalence of mcr-1 has been increasing in Thailand\(^{13}\). The mcr-carrying plasmids identified consist of IncX4, IncI2, IncHI2, IncF, IncP, IncY, and ColE10-like ones, most of which are conjugative plasmid\(^{15}\).

Collectively, information regarding the genetic context of mcr-1 plasmid and its organization in the genome is still limited in Thailand. One study revealed the general characteristics of mcr-1 harboring CRE isolated from patients in Thailand\(^{16}\). However, genomic analysis has not yet determined insights to plasmidome in the CRE harboring mcr-1. Thus, this study aimed to determine the complete genomic sequences to provide insight into plasmidome and to compare plasmid harboring mcr-1- among CRE isolates from human patients in Thailand.

### Materials and methods

#### Bacterial isolates.

This study used 17 CRE carrying mcr-1 isolates, consisting of 12 isolates (E. coli = 8; K. pneumoniae = 4)) in a previous study\(^{13}\) and 5 isolates (E. coli = 4; K. pneumoniae = 1) sent by hospitals in Thailand for further confirmation by the Public Health Microbiological Laboratory of the Faculty of Public Health, Kasetsart University Chalermprakiat Sakon Nakhon Province Campus under the Emerging Antimicrobial Resistant Bacterial Surveillance Program (EARB) during 2016–2019 (Table 1). The presence of mcr-1–carrying CRE isolates (blaNDM, blaKPC, blaCTX1, and blaKDC)\(^{17}\) was confirmed in these CRE isolates using Multiplex polymerase chain reaction (PCR), as previously described.

#### Ethical approval.

Ethical review and approval were not required because no human specimens or data were used in the current study.

#### Antimicrobial susceptibility testing.

The minimal inhibitory concentration (MIC) of colistin was determined in 5 CRE carrying mcr-1 isolates using the broth microdilution method according to 2021 Clinical and Laboratory Standards Institute guidelines\(^{18}\). The broth microdilution method was conducted using cation-adjusted Mueller–Hinton broth (Becton, Dickinson and Company, Sparks, MD, USA). MIC values ≤ 2 µg/ml were interpreted as intermediate susceptibility, whereas an MIC of ≥ 4 µg/ml was considered resistant. Antimicrobial susceptibility to ampicillin, gentamicin, amikacin, amoxicillin/clavulanic acid, amoxicillin/sulbactam, piperacillin–tazobactam, trimethoprim/sulfamethoxazole, cefepime, cefotaxime, ciprofloxacin, levofloxacin, ertapenem, imipenem, meropenem, doripenem, ceftazidime, ceftriaxone, cefoxitin, and netilmicin was performed with a Vitek\textsuperscript{2} automated system (Clinical Microbiology Laboratory, Sakon Nakhon Hospital).

#### Conjugation assay.

Conjugation assay was performed in all 17 mcr-1-carrying CRE isolates consisting of E. coli (n = 12) and K. pneumoniae (n = 5) isolates, as previously described\(^{19,20}\). The mcr-harboring CRE strains (donor) and streptomycin-resistant E. coli UB1637 (recipient) were mixed in a ratio of 1:100. The mixtures were collected and then plated on MacConkey agar containing streptomycin (3200 µg/ml) and colistin (4 µg/ml). The transconjugants harboring mcr genes were confirmed using PCR\(^{19}\).

#### Complete genome sequencing.

Bacterial genomic DNA samples were extracted using ZymoBIOMICS DNA Kits (Zymo Research, CA, USA) according to manufacturer's instructions. Only 12 isolates from the previous study were sequenced by Oxford Nanopore Technologies (ONT)\(^{13}\), while 5 isolates were sequenced using the ONT and Illumina platforms. Library preparation for ONT sequencing followed the rapid barcoding DNA sequencing protocol with the SQK-RBK004 kit without DNA size selection (to preserve the plasmid DNA) and the libraries were sequenced using a single R9.4.1/FLO-MIN106 flow cell on a MinION Mk1B sequencer. We base-called and demultiplexed the raw data using Guppy v3.4.5 (ONT), specifying the high-accuracy model (-c dna_r9.4.1_450bps_hac.cfg). The ONT adapters were trimmed using Porechop v0.2.4 (https://github.com/drwick/Porechop). Quality control of ONT reads was undertaken using Nanoplot v1.28.1 (https://github.com/wdecost/NanoPlot).

For the Illumina platform, the sequencing library was generated using a NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, UK), following the manufacturer's recommendations. The genomic DNA was randomly fragmented to a size of 350 bp and the fragments were A-tailed and ligated with the adapter. Libraries were sequenced using the Illumina HiSeq platform with the 150 paired-end sequencing strategy. We applied Fastp v0.19.2\(^{25}\) with default parameters for the quality filtering of Illumina reads. Adapters were trimmed using Skewer v0.2.2\(^{26}\). The quality checking of Illumina reads was performed using FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Hybrid assemblies with the ONT and Illumina data were performed using Unicycler v0.4.8\(^{30}\) and the genome sequences of all 17 isolates were checked for quality using QUAST v5.0.2\(^{27}\). Genome sequences were submitted to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.12) for annotation. The default parameters were used for all software unless otherwise specified.

#### Bioinformatics analysis.

Identification of antimicrobial resistance genes was analyzed using ResFinder 4.1\(^{17}\) and the Comprehensive Antibiotic Resistance Database (CARD)\(^{38}\). Determination of the mcr-1-carrying plasmid was carried out using PlasmidFinder\(^{27}\). Phylogrouping for E. coli and the KL type of K. pneumoniae were performed using ClermontTyping\(^{28}\) and Kaptive\(^{29}\), respectively. Multilocus sequence typing (MLST) analysis of mcr-1-carrying E. coli and K. pneumoniae was determined using MLST 2.0\(^{39}\).

To search for the genetically closest relatives to the mcr-carrying isolates, a modular single genome analysis was conducted following the core genome multilocus sequence typing approach by BacWGSTDb 2.0\(^{40}\). The genetically closest relatives were chosen for 5–10 strains based on small numbers of allelic differences with selection thresholds of 100–500, depending on the strains under current study. The phylogenetics of the mcr-carrying CRE isolates and the closest relatives selected from BacWGSTDb were conducted using a reference genome-based,
were determined for antimicrobial susceptibility because the other 12 CRE isolates had already been described. Values in the range 4–16 µg/ml, whereas the rest were susceptible (Table 3). This suggested that no. 54715), transferring both. Colistin resistance was also detected in 14 isolates could be conjugatively transferred into (isolate mcr-1 was conducted using the Mashtree program, following the program’s instructions. Genetic analysis of plasmid-harboring mcr-1 was conducted using the Mashtree program, following the program’s instructions. Accession number. The assembled genomic sequences were deposited under the BioProject accession number PRJNA525849. The accession numbers for each mcr-1-harboring isolate are provided in Table 1.

Results
Antimicrobial susceptibility of mcr-1-harboring CRE isolates. In a previous study, 4516 (64.5%) CRE were identified from 6996 multidrug-resistant isolates. Of these, 4235 (93.7%) isolates were classified as carbapenemase-producing Enterobacteriales (CPE) and carried carbapenemase genes including blaqae, blaoxa-48-likp, blaoxd, or coexisting carbapenemase genes according to the modified carbapenem inactivation method (mCIM) and PCR results. Of all the CPE isolates, 13 (0.3%) carried mcr genes. This study did not detect mcr-1 in other species of Enterobacteriales except E. coli and K. pneumoniae. In the current study, five additional Enterobacteriales isolates from the EARB program were included. Unfortunately, one isolate of the previous study was unrecovered. Therefore, a total of 17 isolates were conducted to their completed genome and further analysis.

PCR confirmed the presence of mcr-1 in all 17 isolates, with one isolate (no. 54715) coexisting with mcr-3. Five additional mcr-1-carrying isolates in the current study presented carbapenemase genes including blaoxdm1 in three isolates (strain nos. 2509, 2117, and V417), whereas in two isolates (strain nos. P24-5 and P36.8) the carbapenemase genes were not detected (Fig. 1). Only five isolates carrying mcr-1 isolates included in this study were determined for antimicrobial susceptibility because the other 12 CRE isolates had already been described elsewhere. All 17 mcr-1-harbouring E. coli (n = 12) and K. pneumoniae (n = 5) isolates were resistant to colistin (MIC values 4–16 µg/ml), ampicillin and ampicillin/sublactam (Table 2). Fifteen isolates (88.24%) of the mcr-1-harbouring strains were resistant to carbapenems. Among the five isolates carrying mcr-1 isolates included in this study, three were resistant to ampicillin, ampicillin/sublactam, Piperacillin/tazobactam, cepafime, cefotaxime, cefoxitin, imipenem, meropenem, ertapenem, ciprofloxacin, and levofloxacin. Two mcr-1-carrying E. coli isolates (P24-5 and P36.8) were resistant only to ampicillin, ampicillin/sublactam, gentamicin, levofloxacin, and ciprofloxacin whereas they were susceptible to the carbapenems (Table 2).

Conjugative transfer of the mcr-1 gene. As shown in Fig. 2 and Table 3, all 17 mcr-1-carrying CRE isolates could be conjugatively transferred into E. coli UB1637. One coexisted of mcr-1 and mcr-3 E. coli (isolate no. 54715), transferring both. Colistin resistance was also detected in 14 E. coli recipient (82.35%) with MIC values in the range 4–16 µg/ml, whereas the rest were susceptible (Table 3). This suggested that mcr-1 in all 17 CRE isolates carried on the conjugative plasmids.

Genomic characterization of mcr-harboring CRE isolates. Table 1 summarizes the antimicrobial-resistant genes in all 17 mcr-1-carrying isolates. Five additional mcr-1-carrying isolates in the current study presented an associated β-lactamase gene: blactx-M-15, blactx-M-55, blasnv-10a, blactm-2, blatem-18, and blatem-135. Of these, three isolates were coexisting carbapenemase genes including blaoxdm1 (Table 1 and Fig. 1). Other antimicrobial-resistance genes in the five isolates are shown in Table 1. Finally, β-lactamase-encoding genes in mcr-carrying isolates were located on different plasmid replicon types: IncFIA, IncFIB, IncFII, IncC, or IncI1-I (Table 1).

Based on MLST analysis, we detected 4 different STs in five additional mcr-1-carrying isolates; ST410, ST15, ST6726, and new ST (Table 1). Clermont phylotyping of four mcr-harboring E. coli isolates showed phylogroups C (2/4; 50%), A (1/4; 25%), and Clade III (1/4; 25%) while eight E. coli isolates in the previous study revealed 5 phylogroup C and 2 and 1 for phylogroups A and D, respectively. We concluded that the predominant phylogroup in all 17 isolates was C, accounting for 41.18% (7/17).

All 12 mcr-1-harbouring E. coli isolates carried the virulence genes gad (glutamate decarboxylase) and terC (tellurium ion resistance protein). Five mcr-1-harboring K. pneumoniae isolates carried fyuA (siderophore receptor), iutA (ferric aerobactin receptor), and trp2 (iron regulatory protein), as shown in Fig. 3. An additional single K. pneumoniae isolate included in the current study was KL type 28, whereas 3 KL25 and 1 KL15 were detected in K. pneumoniae in the previous study.

The genetic relationships based on the SNPs phylogeny of these mcr-1-harbouring isolates are shown in Fig. 3 and Fig. 4. E. coli strain no. 2117 was closely related with strains from China (accession no. CP035123.1). Isolate no. P36.8 was closely related with the reference strain K12 and clustered with P24-5 (new ST), as shown in Fig. 3. K. pneumoniae strain no. 2509 was closely related with the K. pneumoniae SIKP199 strain from Thailand (accession no. GCA_004833525.1) (Fig. 4).

Analysis of mcr-1-bearining plasmids. Three different plasmid replicon types were identified in the 17 mcr-1-harboring isolates (Figs. 5 and 6). The most frequent plasmid replicons were IncX4 (11/17; 64.7%), IncI2 (4/17; 23.53%), and IncHI2/IncN (2/17; 11.76%), respectively (Figs. 5 and 6). The sizes of the 11 IncX4 carrying mcr-1 plasmids were in the range 33,309–45,011 bp, whereas the 4 IncI2 carrying mcr-1 plasmids were in the range 60,960–67,526 bp. The 2 IncHI1/IncN were 270,820 bp and 273,765 bp. As shown in Fig. 5, there was high similarity among the IncX4 plasmids, although some of had different positions of the mcr-1 gene (nos. 56511 and 59990). In contrast, IncI2 and IncHI2/IncN, each had mcr-1 positions. We found that IncX4-type plasmids were carried on E. coli STs 410 (7/11; 63.6%) and ST10 (1/11; 9.1%) and on K. pneumoniae ST15 (1/11; 9.1%).
Table 1. Distribution of sequence types and antimicrobial-resistant genes in mcr-1-carrying E. coli and K. pneumoniae strains.
The high prevalence of human Enterobacterales isolates harboring \textit{mcr}\textsuperscript{-1} genes is of global concern. A recent report revealed the overall average prevalence of \textit{mcr}\textsuperscript{-1} genes was 4.7% (0.1–9.3%) in 47 countries across 6 continents\textsuperscript{4}; as many as 10 variants of the \textit{mcr}\textsuperscript{-1} genes (\textit{mcr}\textsuperscript{-1} through to \textit{mcr}\textsuperscript{-10}) have been documented\textsuperscript{7,8}. A recent study reported 1.03% and 0.12% \textit{mcr}-harboring carbapenem-resistant \textit{E. coli} and \textit{K. pneumoniae}, respectively\textsuperscript{13}. Up to the present, 15 Inc-type \textit{mcr}\textsuperscript{-1}-carrying plasmids have been documented, consisting of IncFII, IncHI1, IncHI2, IncI2, IncP1, IncX4, IncY, IncFIB, IncFIC, IncI1-1Y, IncN, IncFII\textsubscript{S}, IncO1, and syncretic\textsuperscript{35}. Most plasmids carrying \textit{mcr}\textsuperscript{-1} are transferable and IncX4, IncHI2, and IncI2 are predominant worldwide\textsuperscript{34–38}. In the current study, \textit{mcr}\textsuperscript{-1} was located on 3 different plasmids (IncX4, IncI2, and IncHI/IncN), mainly on IncX4 and IncI2 that was concordant with previous reporting\textsuperscript{39}. Our single stain of \textit{K. pneumoniae} carrying \textit{mcr}\textsuperscript{-1} on the IncX4 plasmid was genetically almost identical to the \textit{mcr}\textsuperscript{-1}-carrying IncX4 plasmid pMCR\textsubscript{WCHEC1618} recovered from \textit{K. pneumoniae} in healthy adults\textsuperscript{40}. According to several reports in Thailand, the major plasmid types carrying \textit{mcr}\textsuperscript{-1} in Enterobacterales isolates were IncX4 and IncI2, although other plasmid replicons have been described.

**Figure 1.** Agarose gel electrophoresis of PCR-amplified products of carbapenemase and \textit{mcr}\textsuperscript{-1} genes from five \textit{mcr}\textsuperscript{-1}-carrying \textit{E. coli} and \textit{K. pneumoniae} isolates. Positive control of \textit{bla}\textsuperscript{IMP} (lane 1), \textit{bla}\textsuperscript{OXA-48-like} (lane 2), \textit{bla}\textsuperscript{NDM} (lane 3), \textit{bla}\textsuperscript{KPC} (lane 4), \textit{mcr}\textsuperscript{-1} (lane 5), \textit{K. pneumoniae} strain no. 2509 (lane 6), \textit{E. coli} strain no. 2117 (lane 7), V417 (lane 8), P24-5 (lane 9), and P36.8 (lane 10), negative control (lane 11). A 100-bp DNA ladder is shown in lane M.

**Table 2.** Antimicrobial susceptibility of \textit{mcr}\textsuperscript{-1}-harboring carbapenem-resistant \textit{E. coli} and \textit{K. pneumoniae} strains.

| Isolate No | Oganism | E. coli | E. coli | E. coli | E. coli | E. coli | K. pneumoniae | K. pneumoniae | E. coli | K. pneumoniae | E. coli | K. pneumoniae | E. coli | K. pneumoniae | E. coli | K. pneumoniae | E. coli | K. pneumoniae | E. coli |
|------------|----------|--------|--------|--------|--------|--------|---------------|---------------|--------|---------------|--------|---------------|--------|---------------|--------|---------------|--------|---------------|--------|
| 53360      | Penicillin | Ampicillin | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R |
| 54881      | β-Lactam  | Amoxicillin/Clavulanic Acid | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R |
| 56511      | Combination | Ampicillin/Sulbactam | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | 64 I | > 256 R | > 256 R | > 256 R | > 256 R |
| 58967      | 3rd Generation Cephalosporin | Cefepime | > 256 R | > 256 R | > 256 R | > 256 R | 0.125 S | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | 16 I | > 256 R | > 256 R | > 256 R | > 256 R |
| 62122      | Carbapenem | Imipenem | > 256 R | > 256 R | > 256 R | > 256 R | 8 S | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 256 R | > 64 R | > 256 R | > 256 R | > 256 R | > 256 R |
| 60000      | | | | | | | | | | | | | | | | | | | | |
| 54715      | | | | | | | | | | | | | | | | | | | | |
| 53037      | | | | | | | | | | | | | | | | | | | | |
| 59990      | | | | | | | | | | | | | | | | | | | | |
| 60220      | | | | | | | | | | | | | | | | | | | | |
| 61843      | | | | | | | | | | | | | | | | | | | | |
| 2414–18    | | | | | | | | | | | | | | | | | | | | |
| 2509       | | | | | | | | | | | | | | | | | | | | |
| 2117       | | | | | | | | | | | | | | | | | | | | |
| P24.5      | | | | | | | | | | | | | | | | | | | | |
| P36.8      | | | | | | | | | | | | | | | | | | | | |
| V417       | | | | | | | | | | | | | | | | | | | | |

**Discussion**

The high prevalence of human Enterobacterales isolates harboring \textit{mcr} genes is of global concern. A recent report revealed the overall average prevalence of \textit{mcr} genes was 4.7% (0.1–9.3%) in 47 countries across 6 continents\textsuperscript{4}; as many as 10 variants of the \textit{mcr} genes (\textit{mcr}-1 through to \textit{mcr}-10) have been documented\textsuperscript{3,8}. A recent study reported 1.03% and 0.12% \textit{mcr}-harboring carbapenem-resistant \textit{E. coli} and \textit{K. pneumoniae}, respectively\textsuperscript{13}. Up to the present, 15 Inc-type \textit{mcr}-1-carrying plasmids have been documented, consisting of IncFII, IncHI1, IncHI2, IncI2, IncP1, IncX4, IncY, IncFIB, IncFIC, IncI1-1Y, IncN, IncFII\textsubscript{S}, IncO1, and syncretic\textsuperscript{35}. Most plasmids carrying \textit{mcr}-1 are transferable and IncX4, IncHI2, and IncI2 are predominant worldwide\textsuperscript{34–38}. In the current study, \textit{mcr}-1 was located on 3 different plasmids (IncX4, IncI2, and IncHI/IncN), mainly on IncX4 and IncI2 that was concordant with previous reporting\textsuperscript{39}. Our single stain of \textit{K. pneumoniae} carrying \textit{mcr}-1 on the IncX4 plasmid was genetically almost identical to the \textit{mcr}-1-carrying IncX4 plasmid pMCR\textsubscript{WCHEC1618} recovered from \textit{K. pneumoniae} in healthy adults\textsuperscript{40}. According to several reports in Thailand, the major plasmid types carrying \textit{mcr}-1 in Enterobacterales isolates were IncX4 and IncI2, although other plasmid replicons have been observed.
documented, including IncI, IncFIB, IncFrepB, IncY13,40–44. These results suggested that IncX4 and IncI2 bearing mcr-1 mediated major transmission of colistin resistance in Enterobacterales in Thailand.

The conjugation experiment in the current study revealed that all mcr-1-harboring plasmids were successfully transferred from the donor to the E. coli recipient; according to the plasmid Inc types, they are conjugative plasmids39. Among such plasmids in our study, the IncX4 and IncI2 plasmid types were genetically similar, with the least variability, whereas the IncHI2/IncN plasmid type was divergent due to the fact that this type of double-Inc type plasmid contains multiple antimicrobial-resistant genes. This was consistent with the results of the two plasmids merging to perhaps increase the range of host species, plasmid fitness, and/or the acquisition of multiple antimicrobial-resistant genes45. Another study demonstrated that IncHI2-type plasmids are genetically divergent due to containing an MDR region which comprises a variable combination of antimicrobial-resistance genes and insertion sequences, such as Tn6330, in the IncHI2 type that is still highly active and is often transposable37. Our IncHI2/IncN plasmid also showed multiple antimicrobial-resistant genes. In addition, mcr-1 was stably located on IncX4 and IncI2 without cut-paste transposition37, which could explain that why mcr-1 was commonly distributed in these plasmids.

Table 3. Profiles of antimicrobial-resistance genes of donors E. coli, K. pneumoniae and tranconjugants.

| Code | Strain     | mcr | MIC  | Donor | Tranconjugant |
|------|------------|-----|------|-------|---------------|
| 53037| E. coli    | mcr-1| 8    | 8     |
| 53360| E. coli    | mcr-1| 8    | 8     |
| 54715| E. coli    | mcr-1, mcr-3| 8 | 8     |
| 54881| E. coli    | mcr-1| 8    | 8     |
| 56511| E. coli    | mcr-1| 8    | 8     |
| 58967| E. coli    | mcr-1| 8    | 8     |
| 59990| K. pneumoniae | mcr-1| 8   | 8     |
| 60000| E. coli    | mcr-1| 4  | 16    |
| 60220| K. pneumoniae | mcr-1| 16 | 16    |
| 61843| K. pneumoniae | mcr-1| 16 | 8     |
| 62122| E. coli    | mcr-1| 8  | 16    |
| 2514-18| K. pneumoniae | mcr-1| 16 | 8     |
| 2117 | E. coli    | mcr-1| 8  | 2     |
| 2509 | K. pneumoniae | mcr-1| >8  | 4     |
| P24.5| E. coli    | mcr-1| >8  | 1     |
| P36.8| E. coli    | mcr-1| >8  | 1     |
| V417 | E. coli    | mcr-1| 8  | 16    |

Figure 2. Agarose gel electrophoresis of PCR-amplified products from the tranconjugants E. coli UB1637. Conjugation assay was performed in mcr-1-carrying CRE isolates. The tranconjugants were collected nine colonies in each sample and confirmed the mcr-1 gene using PCR. Positive control (lane 1), E. coli strain no. 58967 (lane 2–10), negative control (lane 11), E. coli strain no. 56511 (lane 12–20), negative control (lane 22). A 100-bp DNA ladder is shown in lane M.
Plasmid phylogenetic analysis in the current study showed that most of our IncX4-type plasmids carrying \textit{mcr-1} were grouped, although some were diverse. Notably, 5 isolates carrying \textit{mcr-1} on the IncX4-type plasmid were clustered with \textit{mcr-1}-IncX4 plasmids from either \textit{E. coli} or \textit{K. pneumoniae} from Thailand, indicating that they are close relatives and this type of plasmid is circulating in Thailand. In contrast, the other \textit{mcr-1} plasmid replicon types in our study were mostly related to several plasmid carrying \textit{mcr-1} types from China, perhaps suggesting that they are widely distributed in this region and they may have originated from the same source or ancestor.

The STs of \textit{mcr-1}-harboring \textit{E. coli} isolates in this study were mainly disseminated through local clonal expansion with a high-risk international clone ST410 that can cause several types of infection highly resistant to antibiotics and a global distribution. The \textit{mcr-1}-carrying IncX4 plasmids have also been identified in \textit{E. coli} ST410 recovered from human blood. This may suggest a possible association between \textit{E. coli} ST410 and the carriage of \textit{mcr-1}-IncX4 plasmids. In contrast, the most globally common ST of \textit{E. coli} carrying \textit{mcr-1} is ST104. However, previous study in Thailand revealed the \textit{mcr-1} carrying \textit{E. coli} isolates from humans had diverse STs.

In the current study, \textit{K. pneumoniae} ST336 was predominant. ST336 belongs to clonal complex 17, predominant in carbapenem-resistant \textit{K. pneumoniae} and is considered an international clone frequently associated with global spread. The \textit{K. pneumoniae} ST15 isolates associated with the spread of multiple drug-resistance genes include ESBLs and \textit{mcr-1}.

\textit{mcr-1} was widely distributed in many bacterial species such as \textit{E. coli}, \textit{K. pneumoniae}, \textit{Salmonella enterica}, \textit{Shigella} spp., \textit{Enterobacter cloacae}, \textit{Pseudomonas} spp., \textit{Aeromonas} spp., \textit{Citrobacter freundii}, \textit{Kluyvera ascosbarta}, and others.

**Figure 3.** Phylogenetic tree based on single nucleotide polymorphisms (SNP) using the neighbor-joining method, sequence types (STs) and virulence gene patterns in \textit{E. coli}. Virulence genes are represented by respective blue-colored shapes. The tree was visualized and annotated using Interactive Tree of Life (iToL).

**Figure 4.** Phylogenetic tree based on SNP, STs, and virulence gene patterns in \textit{K. pneumoniae}. Virulence genes are represented by respective red-colored shapes. The tree was visualized and annotated using iToL.
E. coli is the most prevalent species among the mcr-harboring isolates, accounting for approximately 91% of the total mcr-carrying isolates, followed by Salmonella enterica (~7%) and K. pneumoniae (~2%)\(^1\). In Thailand, mcr harboring E. coli and K. pneumoniae has been reported approximately 1.03–2% and 0.12–1% of isolates during 2014–2019, respectively\(^1\). The mcr genes have been reported high prevalence rate (3.3%, 24/724) in Salmonella clinical isolates associated with mcr-3 (91.67%, 22/24) and mcr-1 (8.33, 2/24) in Thailand\(^5\). A previous study reported that the dissemination of 26 mcr-1-carrying enterobacterial strains (23 E. coli and 3 K. pneumoniae) isolated from contact surfaces (such as handrails and vending machines) on public transportation routes suggested a possible transmission vector of these organisms from one location to another, thereby posing a broader public health risk\(^5\). These results demonstrated that plasmids are the major vehicle involved in the dissemination of resistance or virulence genes. Notably, mcr-1-carrying enterobacterial strains were recovered from samples collected from hospitals in the current and the previous studies\(^1\), indicating that these isolates could be of nosocomial origin and thus highlighting the need for strong infection control implementation to prevent transmission of mcr-gene-containing bacteria capable of causing potential outbreaks.

The prevalence and dissemination of mcr-1-harboring Enterobacterales isolates from animals (food animals, pet animals, and wildlife), humans (healthy populations and patients) and the environment (farms, urban and rural communities, and natural environments) have been mentioned globally\(^2\). Control of their dissemination among humans, animals, and the environment based on the “One-health approach” is necessary. In addition, the judicious use of antibiotics is advisable to minimize the development and dissemination of colistin resistance in human isolates.
Figure 6. Schematic partial representation of coding sequences or genes surrounding mcr-1 among 17 mcr-carrying plasmids in E. coli and K. pneumoniae. The coding sequences are represented by arrows pointing toward their respective orientation.
Data availability

The assembled genomic sequences during the current study were deposited under the BioProject with accession number JAJBZQ0000000000, JAJBZP0000000000, JAJGBP0000000000, JAJBZO0000000000, and JAJGBO0000000000.

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References

1. Kumarasamy, K. K. et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect. Dis.* **10**, 597–602 (2010).
2. Munoz-Price, L. S. et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect. Dis.* **13**, 785–796 (2013).
3. Kempf, I., Jouy, E. & Chauvin, C. Colistin use and colistin resistance in bacteria from animals. *Int. J. Antimicrob. Agents* **48**, 598–606 (2016).
4. Elbedawi, M. et al. Global burden of colistin-resistant bacteria: Mobilized colistin resistance genes study (1980–2018). *Microorganisms* **7**, 1461 (2019).
5. Meletis, G. & Skoura, L. Polymyxin resistance mechanisms: From intrinsic resistance to *mcr* genes. *Recent Pat. Antiinfect. Drug Discov.* **13**, 198–206 (2018).
Scientific Reports | (2022) 12:39052 | https://doi.org/10.1038/s41598-022-21836-7

6. Liu, Y. Y. et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect. Dis.* **16**, 161–168 (2016).

7. Gharaiheb, M. H. & Shatnawi, S. Q. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: A review. *Vet. World.* **12**, 1735–1746 (2019).

8. Wang, C. et al. Identification of novel mobile colistin resistance gene *mcr-10*. *Emerg. Microbes Infect.* **9**, 508–516 (2020).

9. Huang, H. et al. Colistin-resistance gene *mcr* in clinical carbapenem-resistant Enterobacteriaceae strains in China, 2014–2019. *Emerg. Microbes Infect.* **9**, 237–245 (2020).

10. Mediavilla, J. R. et al. Colistin- and carbapenem-resistant *Escherichia coli* harboring *mcr-1* and *blaNDM-1*: Causing a complicated urinary tract infection in a patient from the United States. *MBio* **7**, e01191-e1116 (2016).

11. Arabaci, Ç., Dal, T., Başyiğit, T., Genişel, N. & Durmaz, R. Investigation of carbapenemase and carbapenem-resistant *Klebsiella pneumoniae* isolates. *J. Infect. Dev. Ctries.* **13**, 504–509 (2019).

12. Khanawapee, A. et al. Whole-genome sequencing of clinically isolated carbapenem-resistant Enterobacterales harboring *mcr* genes in Thailand, 2016–2019. *Front. Microbiol.* **11**, 3393 (2021).

13. Paveenkittiporn, W., Kamjumphol, W., Ungcharoen, R. & Kerdsin, A. Antimicrobial resistance plasmid reservoir in food and food-producing animals. *Microorganisms* **9**, 2436 (2021).

14. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560 (2018).

15. Wu, R. et al. Whole-genome sequencing of *Klebsiella pneumoniae* isolated from slaughtered pigs in Thailand. *Microb. Drug Resist.* **24**, 762–766 (2018).

16. Madec, J. Y. & Haenni, M. Antimicrobial resistance plasmid reservoir in food and food-producing animals. *Plasmid* **99**, 72–81 (2018).

17. Khanawapee, A. et al. Distribution and molecular characterization of *Escherichia coli* harboring *mcr* genes isolated from slaughtered pigs in Thailand. *Microb. Drug Resist.* **27**, 971–979 (2021).

18. Hatrongnit, R., Kerdsin, A., Akeda, Y. & Hamada, S. Detection of plasmid-mediated colistin-resistant and carbapenem-resistant genes by multiplex PCR. *MethodsX.* **5**, 532–536 (2018).

19. Clinical Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing—the 31st edition. *CLSI document M100-S30. Clinical and Laboratory Standards Institute, Wayne PA.* (2021).

20. Mobasseri, G., The, C. S. J., Ooi, P. T. & Thong, K. L. The emergence of colistin-resistant *Klebsiella pneumoniae* strains from swine in Malaysia. *J. Glob. Antimicrob. Resist.* **17**, 227–232 (2019).

21. Phetburom, N. et al. *Escherichia coli* Complex harboring *mcr-1*, *mcr-7*, and *mcr-8* isolates from slaughtered pigs in Thailand. *Microorganisms* **9**, 2436 (2021).

22. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560 (2018).

23. Wu, R. et al. Whole-genome sequencing of *Klebsiella pneumoniae* isolated from slaughtered pigs in Thailand. *Microb. Drug Resist.* **24**, 762–766 (2018).

24. Caratto, A. et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* **58**, 3895–3903 (2014).

25. Alcock, B. P. et al. CARD 2020: Antibiotic resistance surveilance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **48**, 517–520 (2020).

26. Bartels, F., Silander, O. K., Pachkov, M., Rainey, P. B. & Van Nimwegen, E. Automated reconstruction of whole-genome phylogenies from short-sequence reads. *Mol. Biol. Evol.* **31**, 1077–1088 (2014).

27. Letunic, I. & Bork, P. Interactive tree of life (iTOL) v3: An online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* **44**, W242–W245 (2016).

28. Katz, L. S. et al. MashTree: A rapid comparison of whole genome sequence files. *J. Open Source Softw.* **4**, 1762 (2019).

29. Xiaoamin, S. et al. Global impact of *mcr*-1-positive Enterobacteriaceae bacteria on “one health”. *Crit. Rev. Microbiol.* **46**, 565–577 (2020).

30. Li, R. et al. Fitness advantage of *mcr-1*-bearing IncI2 and IncX4 plasmids in vitro. *Front. Microbiol.* **9**, 331 (2018).

31. Feng, Y., Zou, S., Chen, H., Yu, Y. & Ruan, Z. BacWGSSGib 2.0: A one-stop repository for bacterial whole genome sequence typing and source tracking. *Nucleic Acids Res.* **49**, D64–D650 (2020).

32. Bertolet, B. et al. Multilocus sequence typing of total genome-sequenced bacteria. *J. Clin. Microbiol.* **56**, e00197–e218 (2018).

33. Larsen, M. V. et al. Multilocus sequence typing of total genome-sequenced bacteria. *J. Clin. Microbiol.* **50**, 13755–13612 (2019).

34. Li, R. et al. Evaluation of plasmid- and carbapenem-resistance genes in *Escherichia coli* isolate of animal origin. *J. Antimicrob. Chemother.* **72**, 696–699 (2017).

35. Doumith, M. et al. Detection of the plasmid-mediated *mcr-1* gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. *J. Antimicrob. Chemother.* **71**, 2300–2305 (2016).

36. Lu, J. et al. Evidence for intrapatient and interspecies transmission events. *J. Antimicrob. Chemother.* **75**, 2485–2494 (2020).

37. Li, R. et al. Colistin resistance and plasmid-mediated *mcr* genes in *Escherichia coli* and *Salmonella* isolated from pigs, pig carcass and pork in Thailand, Lao PDR and Cambodia border provinces. *J. Vet. Sci.* **22**, e68 (2021).

38. Strepis, N. et al. Genetic Analysis of *mcr-1*-carrying plasmids from Gram-negative bacteria in a Dutch tertiary care hospital: Evidence for intrapatient and interspecies transmission events. *Front. Microbiol.* **12**, 2570 (2021).

39. Roer, L. et al. *Escherichia coli* sequence type 410 is causing new international high-risk clones. *mSphere* **3**, e00337–e418 (2018).

40. Rocha, I. V. et al. Ciprofloxacin-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* ST410 strain carrying the *mcr*-1 gene associated with bloodstream infection. *Int. J. Antimicrob. Agents.* **49**, 655–656 (2017).

41. Rodrigues, C., Machado, E., Ramos, H., Peixe, L. & Novais, Á. Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: A successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFIK). *Int. J. Med. Microbiol.* **304**, 1100–1108 (2014).
49. Novović, K. et al. Molecular epidemiology of colistin-resistant, carbapenemase-producing Klebsiella pneumoniae in Serbia from 2013 to 2016. *Antimicrob. Agents Chemother.* **61**, e02550-e2616 (2017).
50. Palmieri, M. et al. Genomic epidemiology of carbapenem- and colistin-resistant *Klebsiella pneumoniae* isolates from Serbia: Predominance of ST101 strains carrying a novel OXA-48 plasmid. *Front. Microbiol.* **11**, 294 (2020).
51. Anyanwu, M. U., Jaja, I. F. & Nwobi, O. C. Occurrence and characteristics of mobile colistin resistance (*mcr*) gene-containing isolates from the environment: A review. *Int. J. Environ. Res. Public Health* **17**, 1028 (2020).
52. Nang, S. C., Li, J. & Velkov, T. The rise and spread of *mcr* plasmid-mediated polymyxin resistance. *Crit. Rev. Microbiol.* **45**, 131–161 (2019).
53. Luk-In, S. et al. Occurrence of *mcr*-mediated colistin resistance in *Salmonella* clinical isolates in Thailand. *Sci. Rep.* **11**, 1–10 (2021).
54. Eiamphungporn, W. et al. Prevalence of the colistin resistance gene *mcr*-1 in colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from humans in Thailand. *J. Glob. Antimicrob. Resist.* **15**, 32–35 (2018).
55. Shen, C. et al. Transmission of *mcr*-1-producing multidrug-resistant Enterobacteriaceae in public transportation in Guangzhou China. *Clin. Infect. Dis.* **67**, S217–S224 (2018).

**Author contributions**
P.B. was the primary investigator and wrote the main manuscript text. T.W. and P.J. assisted with whole genome sequencing using short-read and long-read platforms and analyzed *mcr*-1-bearing plasmids. P.C., R.H., and S.J. provided assistance with data interpretation. A.K. was the senior author for the manuscript and edited the manuscript. All authors reviewed the manuscript.

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**Competing interests**
The authors declare no competing interests.

**Additional information**

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