# Genomic Characterization of Nonclonal *mcr-1*-Positive Multidrug-Resistant *Klebsiella pneumoniae* from Clinical Samples in Thailand

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Multidrug-resistant *Klebsiella pneumoniae* strains are one of the most prevalent causes of nosocomial infections and pose an increasingly dangerous public health threat. The lack of remaining treatment options has resulted in the utilization of older drug classes, including colistin. As a drug of last resort, the discovery of plasmid-mediated colistin resistance by *mcr-1* denotes the potential development of pandrug-resistant bacterial pathogens. To address the emergence of the *mcr-1* gene, 118 gram-negative *Enterobacteriaceae* isolated from clinical samples collected at Queen Sirikit Naval Hospital in Chonburi, Thailand were screened for colistin resistance using automated antimicrobial susceptibility testing and conventional PCR screening. Two *K. pneumoniae* strains, QS17-0029 and QS17-0161, were positive for *mcr-1*, and both isolates were sequenced to closure using short- and long-read whole-genome sequencing. QS17-0029 carried 16 antibiotic resistance genes in addition to *mcr-1*, including 2 carbapenemases, $bla_{NDM-1}$ and $bla_{OXA-232}$. QS17-0161 carried 13 antibiotic resistance genes in addition to *mcr-1*, including the extended-spectrum β-lactamase $bla_{CTX-M-55}$. Both isolates carried multiple plasmids, but *mcr-1* was located alone on highly similar 33.9 Kb IncX4 plasmids in both isolates. The IncX4 plasmid shared considerable homology to other *mcr-1*-containing IncX4 plasmids. This is the first report of a clinical *K. pneumoniae* strain from Thailand carrying *mcr-1* as well as the first strain to simultaneously carry *mcr-1* and multiple carbapenemase genes (QS17-0029). The identification and characterization of these isolates serves to highlight the urgent need for continued surveillance and intervention in Southeast Asia, where extensively drug-resistant pathogens are being increasingly identified in hospital-associated infections.

**Keywords:** MDRO, *mcr-1*, *Klebsiella pneumoniae*, Thailand

## Introduction

**T**HE EMERGENCE AND GLOBAL SPREAD of multidrug-resistant (MDR) gram-negative bacteria has become one of the leading public health threats of the 21st century.[1] Of these, MDR *Klebsiella pneumoniae* are a prevalent cause of nosocomial infections, and can harbor a wide range of antibiotic resistance genes, including extended-spectrum β-lactamases (ESBLs) and/or carbapenemases.[2] MDR *K. pneumoniae* carrying ESBLs and/or carbapenemases have been

described worldwide and are an especially concerning issue in developing regions, such as Southeast Asia, where prevalence is high due to widespread antibiotic misuse and lack of resources to execute thorough surveillance studies and interventions.[3–6] The role of MDR *K. pneumoniae* as a prevalent nosocomial infection makes it a dangerous carrier and disseminator of antimicrobial resistance mechanisms to other bacterial pathogens.

Treatment options for MDR *K. pneumoniae* are limited, and hospital-associated MDR *K. pneumoniae* infections

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frequently generate additional treatment challenges due to previous exposure to multiple antibiotics.[7,8] In underdeveloped regions, patient diagnosis and pathogen characterization are often delayed or not performed, resulting in treatment failure and the development and spread of drug-resistant isolates.[9,10] Older classes of antibiotics that were previously unused due to side effects (*e.g.*, polymyxins such as colistin [Polymyxin E]), are being increasingly prescribed to patients as a treatment of last resort.[11,12] The discovery by Liu *et al.* in 2015 of the plasmid-mediated colistin resistance gene, *mcr-1*, from a clinical *Escherichia coli* isolate in China has quickly led to the reporting of *mcr-1* and commonly associated conserved mobile mechanisms in a range of gram-negative bacterial isolates worldwide.[13–15] The global dissemination of *mcr-1* signals a new era of pandrug-resistant bacterial pathogens.

In Southeast Asia, plasmid-mediated colistin resistance by *mcr-1* has been detected in *E. coli* from isolates collected in Cambodia, Vietnam, Malaysia, Singapore, and Thailand.[16–24] *mcr-1*-carrying *K. pneumoniae* isolates have been described in Laos and Singapore.[20,23] In Thailand, *mcr-1* positive *E. coli* has been observed in nonclinical human isolates that were archived in 2012 and two *E. coli* human clinical isolates collected in 2015 and 2016, respectively.[16–18] Environmental samples of *mcr-1* positive *E. coli* isolates have also been reported from canal water samples collected in 2015 and at rural pig farms in isolates archived as early as 2012.[18,19]

The current study characterizes the antimicrobial resistance profiles of two nonclonal *K. pneumoniae* strains isolated from individual patients at the Queen Sirikit Naval Hospital in Chonburi, Thailand. Both patients were housed in separate wards within the hospital, and the two isolates displayed unique antimicrobial susceptibility profiles. The isolates were shown to carry the plasmid-mediated colistin resistance gene, *mcr-1*, along with additional antibiotic resistance genes from multiple enzymatic classes.

## Materials and Methods

### Bacterial strains and media

Surveillance was performed at Queen Sirikit Naval Hospital during 2017 to identify MDR bacterial strains from routinely collected clinical samples (*e.g.*, Pus, urine, rectal swabs, sputum, and blood). A total of 118 gram-negative *Enterobacteriaceae* showing MDR characteristics were identified and transferred to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand for further analysis. *K. pneumoniae* QS17-0029 and QS17-0161 strains were cultured from clinical samples collected during this active surveillance. Isolates were grown on MacConkey agar plates, trypticase soy agar plates, blood agar plates, or Mueller Hinton agar plates in preparation for analysis. Phenotypic screening for carbapenemase activity was performed using the Carba NP test with appropriate controls as described previously.[25]

### Biochemical identification and antimicrobial susceptibility testing

Biochemical identification and antimicrobial susceptibility testing was performed with the BD Phoenix™ 50 using the NMIC/ID 4 panel, according to the manufacturer's in-structions (BD Diagnostics, Sparks, MD). The colistin minimum inhibitory concentration (MIC) values were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[26,27]

### ESBL and carbapenemase gene screen

Molecular analysis to determine the presence of ESBL and carbapenemase antimicrobial resistance genes was performed using conventional multiplex and real-time PCR as described previously in detail.[28,29]

### Screening for mcr-1

DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (Germantown, MD). Detection of *mcr-1* was performed by conventional PCR, as described previously.[13]

### Conjugation of mcr-1 *plasmid into recipient strain*

Conjugation between colistin-resistant *K. pneumoniae* QS17-0029 or QS17-1616 and a recipient *E. coli* strain J53 (sodium azide resistant) was performed.[23,30] In brief, overnight cultures of the *K. pneumoniae* strains were grown in tryptic soy broth (TSB) +2 mg/L colistin, and *E. coli* J53 was grown in TSB +100 μg/ml sodium azide at 37°C, 150 rpm. Overnight cultures were centrifuged and resuspended in TSB to a final dilution of ∼0.6 McFarland. *K. pneumoniae* QS17-0029 or QS17-0161 were mixed with *E. coli* J53 at a 1:2 ratio. The mixed cultures were incubated for 24 hours with no shaking at 37°C, and subsequently plated on MacConkey agar +100 μg/ml sodium azide +2 mg/L colistin. Conjugation frequency was calculated as the number of transconjugates per initial donor cell. Resulting transconjugates were isolated and tested further. The colistin MIC values of the donor *K. pneumoniae* strains, recipient *E. coli* J53 strain, and transconjugates were performed as recommended by the joint CLSI-EUCAST polymyxin breakpoint working group with MIC values of >2 μg/ml considered resistant for *Enterobacteriaceae*.[26,27,31] Broth microdilution panels in untreated polystyrene microplates (SARSTEDT AG&Co., Nümbrecht, Germany) were prepared by making serial two-fold dilutions with cation adjusted Mueller Hinton broth to obtain a colistin sulfate (U.S. Pharmacopeial Convention) concentration range of 0.125 to 64 μg/ml.[31,32] The parent and transconjugate were diluted to a final concentration of ∼5×10⁵ CFU/ml per well. The panels were incubated at 35°C±2°C for 16–20 hours. Interpretation was performed based on CLSI and EUCAST guidelines. The bacterial strains *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control standards.[26]

### Short- and long-read whole-genome sequencing

Short- and long-read whole-genome sequencing (WGS) was performed on an Illumina MiSeq Benchtop sequencer and Pacific Biosciences RSII instrument, respectively. DNA extraction, library preparation, sequencing, assembly, and analysis of the short- and long-read data were performed as described previously.[33] In brief, high-quality DNA was extracted using the Ultra-Clean Microbial DNA Isolation Kit (MoBio, Inc. Carlsbad, CA). For short-read sequencing,

libraries were constructed using the KAPA HyperPlus Library Preparation Kit (Roche Diagnostics Corporation, Indianapolis, IN) and quantified using the KAPA Library Quantification Kit–Illumina/Bio-Rad iCycler™ (Roche Diagnostics Corporation) on a CFX96 real-time cycler (Bio-Rad, Hercules, CA). Libraries were normalized to 2 nM, pooled, denatured, and diluted to 20 pM. The pooled samples were further diluted to a final concentration of 13 pM. Samples were sequenced using MiSeq Reagent Kit v3 (600 cycle; 2 × 300 bp) (Illumina, San Diego, CA). For long-read sequencing, 20K libraries were constructed using the PacBio Template Preparation 1.0 kit (Pacific Biosciences, Menlo Park, CA) and size selected on Blue Pippin (Sage Sciences, Beverly, MA). Sequencing was performed on individual cells on the RSII using 240 minute movies.

### Analysis of WGS data

Short-read sequencing data were quality trimmed when the average quality score dropped below 15 (Phred), using a sliding 5 bp window. Sequence reads were then adaptor trimmed and *de novo* assembled using Newbler (V2.7). Minimum thresholds for contig size and coverage were set at 100 bp and 50×, respectively. Long-read sequencing data were *de novo* assembled using HGAP 2.0 in the SMRT Analysis Portal (Pacific Biosciences). Overlapping contig ends were removed to circularize individual PacBio contigs, and short-read data were mapped to circularized contigs to detect/correct errors.

Comparative genomic analyses were performed using Geneious (Biomatters, Auckland, New Zealand).[34] Antimicrobial resistance genes were annotated using ResFinder 2.0.[35] Genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline version 4.1. Single-nucleotide polymorphism (SNP)-based dendrograms were generated using Geneious and RAxML.[36]

### Accession numbers

Complete genome sequences *K. pneumoniae* QS17-0029 (MRSN479404) and QS17-0161 (MRSN480738) and their attendant plasmids have been deposited in the NCBI nucleotide database under accession numbers CP024038-CP024044 and CP024458-CP024465, respectively.

## Results

### Phenotypic testing of isolates

The selected 118 MDR *Enterobacteriaceae* (74 *E. coli*, 39 *K. pneumoniae*, 3 *Proteus mirabilis*, 1 *Enterobacter aerogenes*, and 1 *Enterobacter cloacae*) underwent automated antimicrobial susceptibility testing to detect colistin resistance. Two isolates, *K. pneumoniae* QS17-0029 and QS17-0161, had MIC values of colistin >4 µg/ml and were deemed resistant to colistin according to CLSI guidelines.[32] Both isolates underwent further characterization. QS17-0029 was cultured from a rectal swab sample of a 94-year-old male patient (Table 1). Antimicrobial susceptibility testing of QS17-0029 showed extensive resistance to 18/21 antibiotics tested, with the exception of susceptibility to amikacin and gentamicin, and intermediate susceptibility to chloramphenicol (Table 1). QS17-0161 was cultured from a sputum sample of an 88-year-old male patient (Table 1). QS17-0161 was resistant to 14/21 antibiotics tested and demonstrated susceptibility to amikacin, ciprofloxacin, all carbapenems, and trimethoprim-sulfamethoxazole, with intermediate susceptibility to moxifloxacin (Table 1). Carbapenemase production was identified in QS17-0029, using the Carba NP test, but not in QS17-0161, which was consistent with the carbapenem susceptibility profile (Table 1).

### Detection of antibiotic resistance genes using PCR

Limited preliminary antimicrobial resistance gene screening using conventional and real-time PCR assays indicated that both QS17-0029 and QS17-0161 harbored *mcr-1* as well as the $bla_{CTX-M}$ group-1 ESBL gene. In addition, only QS17-0029 was positive for the carbapenemase genes $bla_{NDM}$-like and $bla_{OXA-48}$-like.

### Multilocus sequence type and isolate relationship

*In silico* multilocus sequence type (MLST) of both isolates was generated from the WGS data, and sequence type was assigned according to the *K. pneumoniae* MLST scheme hosted at the Pasteur Institute.[37,38] QS17-0029 and QS17-0161 were shown to be nonclonal strains of *K. pneumoniae* with sequence types 16 and 45, respectively (Table 1). This was confirmed using a core genome SNP-based analysis, which

TABLE 1. DEMOGRAPHIC AND PHENOTYPIC DATA OF QS17-0029 AND QS17-0161

| Designation | Age/gender | MLST[a] | CarbaNP[b] | MIC (mg/L)[c] |
|---|---|---|---|---|
| QS17-0029 | 94/M | 16 | Pos | CHL (I = 16), CIP (>2), GEN (S ≤ 2), IPM (>8), LVX (>8), MEM (>8), MXF (>4), STX (>2/38) |
| QS17-0161 | 88/M | 45 | Neg | CHL (>16), CIP (S = 1), GEN (>8), IMP (S ≤ 1), LVX (S = 2), MEM (S ≤ 1), MXF (I = 4), STX(S ≤ 0.5/9.5) |

[a]Sequence type generated from *in silico* multilocus sequence type.
[b]CarbaNP test was performed as described by Nordmann *et al.*[25]
[c]Both isolates were susceptible to amikacin. In addition to colistin, both isolates were resistant to amoxicillin–clavulanate, ampicillin, ampicillin–sulbactam, aztreonam, cefazolin, cefepime, cefotaxime, ceftazidime, piperacillin, piperacillin–tazobactam, and tetracycline using CLSI guidelines.[26]
MIC, minimum inhibitory concentration; M, male; Pos, positive, Neg, negative; S, susceptible; I, intermediate; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, Meropenem; MXF, moxifloxacin; STX, trimethoprim–sulfamethoxazole; MLST, multilocus sequence type.

**FIG. 1.** Core genome phylogeny. Dendrogram generated using Geneious and RAxML from the whole genome of QS17-0029 and QS17-0161 (*blue text*) comparing their relationship to one another and to other *Klebsiella pneumoniae* genomes deposited at GenBank (*black text*). Branch lengths are indicative of relatedness.
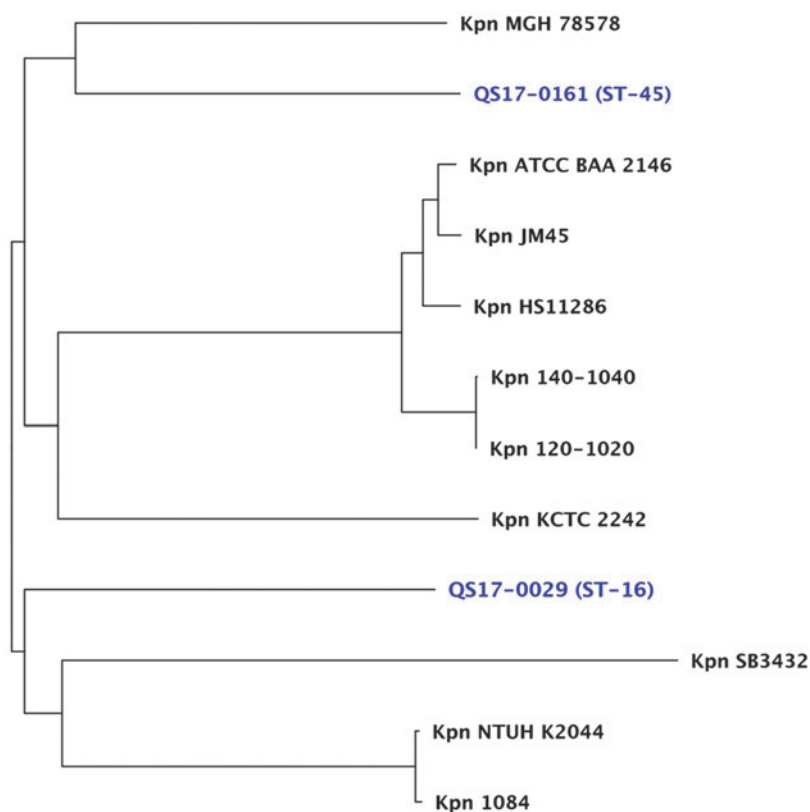
TABLE 2. QS17-0029 AND QS17-0161 PLASMID CHARACTERISTICS, ANTIBIOTIC RESISTANCE GENES, AND GENE LOCATION

| Strain | Location | Name[a] | Size (kb) | Inc[b] | Antibiotic resistance genes[c] |
|---|---|---|---|---|---|
| QS17-0029 | | | | | |
| | Plasmid | pMRAF0417-NDM | 125.3 | FII(F22:A1:B20) | $bla_{NDM-1}$, *ant(3″)-IA (aadA2)*, *sul1*, *tet(B)*, *dfrA12* |
| | Plasmid | pMRAF0417-MCR | 33.9 | X4 | *mcr-1* |
| | Plasmid | pMRAF0417-OXA | 6.1 | ColKp3 | $bla_{OXA-232}$ |
| | Plasmid | pMRAF0417-CTX | 122.1 | F1B(K2:A-:B-) | $bla_{CTX-M-15}$, $bla_{OXA-9}$, $bla_{TEM-1A}$, *aac(6′)1b C (aacA4)*, *ant(3″)-Ia (aadA1)*, *aac(6′)1b-cr* |
| | Plasmid | N/A | 5.3 | ColRNA1 | — |
| | Plasmid | N/A | 4.7 | ColRNA1 | — |
| | Chromosome | — | — | — | $bla_{CTX-M-15}$, $bla_{SHV-1}$, *fosA*, *oqxA*, *oqxB* |
| QS17-0161 | | | | | |
| | Plasmid | pMRAF0517-FLO | 125.3 | F1B(K5:A-:B-) | *strA*, *strB*, *aac(3)-IIa*, *floR*, *sul2*, *tet(A)* |
| | Plasmid | pMRAF0517-MCR | 33.9 | X4 | *mcr-1* |
| | Plasmid | pMRAF0517-CTX | 77.3 | Unknown | $bla_{CTX-M-55}$, $bla_{TEM-1B}$, *qnrS1*, *tet(A)* |
| | Plasmid | N/A | 112.7 | F1B | — |
| | Plasmid | N/A | 10.0 | ColRNA1 | — |
| | Plasmid | N/A | 2.8 | ColRNA1 | — |
| | Plasmid | N/A | 1.6 | Col(MG828) | — |
| | Chromosome | — | — | — | $bla_{SHV-1}$, *fosA*, *oqxA*, *oqxB* |

[a]Assigned plasmid name, where appropriate.
[b]Incompatibility group was determined using the protocol developed by Carattoli *et al.*[40] Unknown indicates that the plasmid could not be assigned to an Inc group using this scheme.
[c]$Bla_{CTX-M-15}$ is present in two copies, one on plasmid pMRAF0147-CTX and one on the chromosome in strain *Klebsiella pneumoniae* QS17-0029. *Tet(A)* is present in two copies, one on plasmid pMRAF0517-FLO and the other on plasmid pMRAF0517-CTX, in *K. pneumoniae* QS17-0161.
N/A, not assigned.

indicated that the two *K. pneumoniae* isolates are distinct strains (Fig. 1).

### WGS analysis of QS17-0029

QS17-0029 carried a total of 17 antimicrobial resistance genes located on the chromosome and/or plasmids (Table 2). Six unique plasmids were identified, four of which were shown to carry 12 antibiotic resistance genes (Table 2). The remaining resistance genes were located on the chromosome. The colistin resistance gene *mcr-1* was carried on a 33.9 Kb IncX4 plasmid (pMRAF0417-MCR) that shared >99% homology to other *mcr-1* carrying IncX4 plasmids.[39] Two carbapenemase genes, *bla*$_{NDM-1}$ and *bla*$_{OXA-232}$, were identified on two separate plasmids (pMRAF0417-NDM and pMRAF0417-OXA). Notably, the isolate carried two copies of the ESBL gene, *bla*$_{CTX-M-15}$, one located on plasmid pMRAF0417 and one integrated into the chromosome. pMRAF0417 carried two additional β-lactamases (*bla*$_{TEM-1A}$ and *bla*$_{OXA-9}$), while a third β-lactamase (*bla*$_{SHV-1}$) was also present on the chromosome (Table 2).

### WGS analysis of QS17-0161

QS17-0161 carried a total of 15 antimicrobial resistance genes on the chromosome and/or plasmids (Table 2). The isolate carried seven unique plasmids, of which three carried a combined total of 11 antimicrobial resistance genes (Table 2). The remaining four genes were located on the chromosome. The plasmid identified carrying *mcr-1*, pMRAF0517-MCR, was identical to the IncX4 plasmid pMRAF0417-MCR in QS17-0029. The ESBL gene, *bla*$_{CTX-M-55}$, was identified on plasmid pMRAF0517; the plasmid was untypable according to the typing scheme of Carattoli *et al.*[40] Two additional β-lactamases were identified, *bla*$_{TEM-1B}$ located on the same plasmid and *bla*$_{SHV-1}$ located on the chromosome.

### Conjugation of mcr-1 into E. coli J53

Conjugation assays were performed to determine mobility potential of the identified *mcr-1*-positive plasmids. Conjugation frequencies of the *mcr-1*-positive plasmids from QS17-0029 and QS17-0161 to *E. coli* J53 were calculated to be $2.12 \times 10^{-6}$ and $1.25 \times 10^{-6}$, respectively. QS17-0029 and QS17-0161 parent strains were confirmed resistant with a colistin MIC value of 8 μg/ml. Colistin susceptibility testing of the resulting transconjugates (*E. coli* J53 QS17-0029-C and *E. coli* J53 QS17-0161-C) showed that both were resistant to colistin, with MIC values of 4 μg/ml. The negative control strain, *E. coli* J53, demonstrated a colistin MIC value of 0.5 μg/ml, indicating colistin susceptibility as expected. Transfer of the *mcr-1* gene to the transconjugates was confirmed by PCR, indicating the *mcr-1*-positive plasmid in both *K. pneumoniae* isolates is mobile.

### Discussion

Implementation of active surveillance screening for colistin-resistant bacteria at Queen Sirikit Naval Hospital in Chonburi, Thailand identified two resistant *K. pneumoniae* isolates (QS17-0029 and QS17-0161) from clinical samples. Phenotypic and genetic testing identified that colistin resistance was mediated by the gene *mcr-1*, with numerous other antibiotic resistance genes present as well (Table 2). WGS and subsequent analysis showed that QS17-0029 and QS17-0161 are two distinct strains of *K. pneumoniae*, despite both carrying the plasmid-mediated colistin-resistant gene *mcr-1* on identical 33.9 Kb IncX4 plasmids. To the best of our knowledge, this is the first report from Thailand characterizing *mcr-1*-postive *K. pneumoniae* isolates.

Since initial discovery in 2015, *mcr-1* has been detected worldwide, most commonly in *Enterobacteriaceae* species such as *E. coli*, *K. pneumoniae*, *Salmonella enterica*, *E. aerogenes*, and *E. cloacae*.[13,14] In human clinical samples, *mcr-1* has been most frequently identified in *E. coli*, but reported prevalence is increasing in other bacterial species, such as *K. pneumoniae*.[13,14,41] In Thailand, *mcr-1* has previously been detected in both *E. coli* environmental samples and *E. coli* human clinical samples.[16–19] Environmentally, *E. coli* isolates positive for *mcr-1* carriage were identified in 2013 from a pig farm in Chonburi Province, the same geographic location as Queen Sirikit Naval Hospital.[19] A recent study by Runcharoen *et al.* also reported the identification of colistin-resistant, *mcr-1*-positive *E. coli* isolates collected in 2015 from canal water in Chachoengsao Province, a neighboring province to Chonburi.[18] Additional reports have demonstrated *mcr-1* in hospital-associated human *E. coli* clinical samples from various geographic locations in Thailand.[16–18] These collective findings indicate that circulation of *mcr-1* may be a more common occurrence in both environmental and hospital settings in Thailand than previously recognized.

An increasing number of reports are characterizing colistin-resistant *E. coli* or *K. pneumoniae* clinical isolates that demonstrate coresistance to β-lactams or carbapenems, and a number of reports have described coresistance to all three antibiotic classes.[14,17,42–48] However, there is evidence that these extensively drug-resistant (XDR) isolates carrying multiple types of antibiotic genes are disseminating in Thailand. An XDR clinical *E. coli* strain carrying, *bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, and *mcr-1* was isolated in early 2016 from a urine sample of a clinical patient in Petchabun Province located in Northern Thailand.[17] As demonstrated in the current study, *K. pneumoniae* strain QS17-0029 is also XDR, and carries multiple β-lactamases, two carbapenemases, and *mcr-1*. As far as the authors are aware, this is the first report of a clinical *K. pneumoniae* strain simultaneously harboring two unique carbapenemases in addition to *mcr-1*.

A majority of *mcr-1* genes that have been detected are located on plasmids, specifically IncX4 and IncI2 plasmids.[39,49,50] Both plasmid types have been detected in concert with *mcr-1* from countries in South and Southeast Asia.[39] IncX4 is naturally found in host *Enterobacteriaceae* species and has an especially high prevalence rate in *E. coli*.[39] The plasmid type IncX4 is thought to be easily transferrable between *Enterobacteriaceae* species, a theory supported by the finding of conjugation between *K. pneumoniae* strains and *E. coli* J53 in the current report. Both *K. pneumoniae* strains isolated from individual patients at Queen Sirikit Naval Hospital carry identical *mcr-1*-positive IncX4 plasmids. The two isolates are not clonally related, which suggests the possibility of an initial common donor strain for *mcr-1* acquisition. Additional studies are necessary to determine the possible source of a Queen Sirikit Naval Hospital *mcr-1*-positive strain reservoir.

The characterization of MDR, including colistin-resistant, bacterial strains identified from Queen Sirikit Naval Hospital is an ongoing effort that is vitally important. Thailand appears to harbor a higher number of strains that carry plasmid-mediated colistin resistance in both environmental and hospital-associated isolates than previously determined. It is important to note that at the time of article publication, the Queen Sirikit Naval Hospital surveillance efforts have confirmed one additional *mcr-1*-positive *K. pneumoniae* strain and three *mcr-1*-positive *E. coli* strains from the clinical samples of individual patients. Additional studies are underway to further investigate this observation.

Alarmingly, *mcr-1*-mediated colistin resistance is being detected with increasing frequency within isolates that also carry an extensive number of other antibiotic resistance genes. Multiple XDR strains that harbor colistin resistance have now been characterized from hospital-associated infections in different geographic locations within Thailand. It is imperative that constant surveillance and characterization of hospital-associated MDR bacterial pathogens continue to be conducted to combat the spread of mobile antibiotic resistance mechanisms, and to inform treatment strategies that will result in positive patient outcomes. Continual analysis and awareness regarding the extent of plasmid-mediated colistin resistance are necessary to determine the prevalence of XDR, and potentially pandrug-resistant, bacterial pathogens, currently circulating in South and Southeast Asia.

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## Disclaimer

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its publication. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting true views of the Department of the Army of the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70-25.

## Disclosure Statement

No competing financial interests exist.

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