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Genome Note

Draft genome sequence of a colistin-resistant *Escherichia coli* ST226: A clinical strain harbouring an *mcr-1* variant

Watcharaporn Kamjumphol[a], Warawan Wongboot[b], Namfon Suebwongsa[a], Pipat Kluabwang[c], Siriporn Chantaroj[b], Kazuhisa Okada[a,d,*]

[a] *Thailand–Japan Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI), Nonthaburi 11000, Thailand*
[b] *National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand*
[c] *Department of Pediatrics, Maesot General Hospital, Tak 63110, Thailand*
[d] *Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan*

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ABSTRACT

Objectives: Escherichia coli isolates carrying the *mcr-1* gene are rarely reported in diarrhoeal patients. Here we report the draft genome sequence of a colistin-resistant *E. coli* isolated from a hospitalised patient with acute diarrhoea in Thailand.

Methods: Whole genomic DNA of the colistin-resistant *E. coli* isolate (MSF11) was extracted and was sequenced using an Ion Torrent sequencer with 400-bp read chemistry. The draft genome sequence of MSF11 was analysed with regard to multilocus sequence type (ST), serotype, acquired antimicrobial resistance genes, plasmid replicon types and virulence genes using tools from the Center for Genomic Epidemiology.

Results: E. coli strain MSF11 was serotype OUT:H10 and ST226. Acquired antimicrobial resistance genes [$bla_{CTX\text{-}M\text{-}15}$, *qnrS1*, *catA2*, *mdf(A)* and *mcr-1.1*] and virulence-related genes (*astA* and *gad*) were identified. The *mcr-1* gene contained a single nucleotide polymorphism at position 27 (C → T) of the prototype, and the variant gene was associated with an IncX4-type plasmid. This plasmid-borne colistin resistance mediated by the *mcr-1* variant has been observed among colistin-resistant strains from humans, animals and the environment previously reported in Thailand, although the STs and serotypes of the *E. coli* strains were different.

Conclusions: An *mcr-1* variant was identified in an *E. coli* isolate harbouring the EAST1 (enteroaggregative *E. coli* heat-stable toxin 1) gene (*astA*) from a human diarrhoeal stool specimen. This study highlights the potential risk of dissemination of colistin-resistant *E. coli* in view of the prevalence of the variant gene on IncX4-type plasmids.

Colistin-resistant *Escherichia coli* harbouring the *mcr-1* gene was first reported in China in November 2015 [1] and, currently, *E. coli* harbouring *mcr-1* and its variants are continuously emerging and being recognised in several countries, including Thailand [2–5].

A colistin-resistant *E. coli* isolate (MSF11) was isolated from a stool specimen of a 60-year-old woman with watery diarrhoea (10 times/24 h), abdominal pain and low-grade fever (37.8 °C). Antimicrobial susceptibility testing of strain MSF11 using the VITEK®2 Compact system (bioMérieux, USA) showed resistance to cephalosporins (cefotaxime, ceftazidime, cefpirome) and fluoroquinolones (moxifloxacin) but susceptibility to carbapenems (imipenem, doripenem, meropenem), tigecycline and trimethoprim/sulfamethoxazole. The broth microdilution method performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI document M100, 28th ed, 2018) showed resistance to colistin with a minimum inhibitory concentration (MIC) of 4 mg/L (Table 1).

Whole genomic DNA was extracted using a DNeasy® Blood and Tissue Kit (QIAGEN, USA) and was sequenced on an Ion Torrent PGM™ system (Life Technologies) following the manufacturer's protocols for a 400-bp genomic DNA fragment library and template

* Corresponding author. Present address: Thailand–Japan Research Collaboration Center on Emerging and Re-emerging Infections, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand.
E-mail address: kazuhisa@biken.osaka-u.ac.jp (K. Okada).

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Table 1
Characteristics of *Escherichia coli* isolates harbouring the plasmid-borne colistin resistance gene *mcr-1*.

Strain	Nucleotide variation[a]	Inc type[b]	Colistin MIC (mg/L)	MLST	Source	Country	Isolation year	Accession no.
SHP45	–	I2	8	N/D	Pig	China	2013	**KP347127**
PN42	C27T	X4	4	ST744	Human faeces	Thailand	2013	**MG557854**
PN23	C27T	X4	8	ST1121	Duck faeces	Thailand	2014	**MG557852**
PN25	C27T	X4	8	ST101	Duck faeces	Thailand	2014	**MG557853**
PN24	C27T	X4	4	ST3631	Duck faeces	Thailand	2014	**MG570222**
W1_3_ERB3	C27T	X4	4	ST6624	Canal	Thailand	2015	**ERR1218643**
A434-59	C27T	N/D	8	N/D	Human urine	Thailand	2016	**KX242348**
PY1	C27T	X4	N/D	N/D	Pork	China	2012	**KX711708**
CRE1493	C27T	X4	N/D	N/D	Human faeces	China	2013	**CP019072**
GZ49269	C27T	X4	N/D	N/D	Human urine	China	2015	**MG210939**
MSF11	C27T	X4	4	ST226	Human faeces	Thailand	2017	**RIZD00000000**

MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; N/D, not determined.
[a] Synonymous mutation at position 27 (C → T).
[b] Incompatibility (Inc) group was determined using PlasmidFinder 2.0.

preparation and for sequencing (Ion PGM™ Hi-Q View Chef 400 Kit). The obtained reads were trimmed using CLC Genomics Workbench v.10.1.1 (CLC bio, Aarhus, Denmark) with the following parameters: minimum length of sequence reads, 49 nucleotides; limit of low-quality sequence, 0.01; and maximum ambiguous nucleotides, 2. After trimming, the qualified reads were used for de novo assembly using CLC algorithm with the following parameters: mapping mode, map reads back to contigs; update contigs, yes; word size, 25; automatic bubble size, yes; mismatch cost, 2; insertion cost, 3; deletion cost, 3; length fraction, 0.5; similarity fraction, 0.8; and minimum contig length, 200 bp. The genome size was estimated to be 5 109 548 bp with ca. 97× coverage. The G + C content of this strain was 50.5%. In total, 5387 protein-coding sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The sequence type (ST), serotype, plasmid replicon types, antimicrobial resistance genes and virulence genes were identified using MLST 2.0, SeroTypeFinder 2.0, PlasmidFinder 2.0, ResFinder 3.0 and VirulenceFinder 2.0, respectively, from the Center for Genomic Epidemiology (http://genomicepidemiology.org/). *E. coli* MSF11 belongs to ST226 and serotype OUT:H10, in agreement with the results of serotyping obtained using the slide agglutination assay with commercial antisera (Denka Seiken, Tokyo, Japan). ST226 has been detected both from human and animal sources (https://enterobase.warwick.ac.uk/). Five plasmid incompatibility groups [IncX4, IncFIA, IncFII, IncFII(29) and IncY] as well as acquired antimicrobial resistance genes [$bla_{CTX\text{-}M\text{-}15}$, *qnrS1*, *catA2*, *mdf(A)* and *mcr-1.1*] were detected in the genome. Furthermore, the strain harboured the EAST1 (enteroaggregative *E. coli* heat-stable toxin 1) gene (*astA*) and the glutamate decarboxylase gene (*gad*).

An *mcr-1* variant (C → T at position 27, synonymous substitution) was detected in a 31 430-bp contig that also included the replicon sequence of IncX4. Approximately 1.3% of sequence reads (average length 225 bp) from the whole-genome sequence of MSF11 mapped to 97.9% of the sequence region of the reference IncX4-type plasmid PN25 (accession no. **MG557853**). This plasmid-borne colistin resistance mediated by the *mcr-1* variant was detected in certain *E. coli* strains isolated from human, animal and environmental sources via a BLAST search (Table 1), although these strains showed different STs and serotypes [3,4]. This implied that the IncX4 plasmid carrying the *mcr-1* variant has disseminated in diverse *E. coli* in and around Thailand. Accumulation and comparison of data, including those regarding single nucleotide polymorphisms of *mcr* genes, will facilitate investigation of the dissemination dynamics of this resistance-associated gene.

The whole genome sequence of *E. coli* strain MSF11 has been deposited at GenBank with accession no. **RIZD00000000.**

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand [reference no. 44/2558] and the Institutional Review Board of the Mae Sot General Hospital (Tak, Thailand).

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