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Short Communication

Emergence of an *Escherichia coli* strain co-harbouring *mcr-1* and bla_{NDM-9} from a urinary tract infection in Taiwan

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ABSTRACT

Objectives: Multidrug-resistant bacteria have become a serious threat worldwide. In particular, the coexistence of carbapenemase genes and *mcr-1* leaves few available treatment options. Here we report a multidrug-resistant *Escherichia coli* isolate harbouring both *mcr-1* and bla_{NDM-9} from a patient with a urinary tract infection.
Methods: Antimicrobial susceptibility and resistance genes of the *E. coli* isolate were characterised. Furthermore, the assembled genome sequences of *mcr-1*- and bla_{NDM-9}-carrying plasmids were determined and comparative genetic analysis with closely related plasmids was carried out.
Results: Three contigs were assembled comprising the *E. coli* chromosome and two plasmids harbouring *mcr-1* (p5CRE51-MCR-1) and bla_{NDM-9} (p5CRE51-NDM-9), respectively. Whole-genome sequencing revealed that the two antimicrobial resistance genes are located on individual plasmids.
Conclusions: The emergence of coexistence of carbapenemase genes and *mcr-1* in Enterobacteriaceae highlights a serious threat to antimicrobial therapy.

1. Introduction

In the last decade, carbapenem-resistant Enterobacteriaceae (CRE) have become one of the most important pathogens responsible for nosocomial infections, especially among intensive care unit patients [1]. CRE have created a challenge for appropriate therapy owing to their resistance to multiple antibiotics such as aminoglycosides, fluoroquinolones, cephalosporins and carbapenems.

Colistin (polymyxin E), belonging to the polymyxins, a family of cationic polypeptide antibiotics with broad-spectrum antimicrobial activities, has become one of the last effective antimicrobials for treating infections caused by CRE. The World Health Organization (WHO) has included colistin in the list of critically important antimicrobials. However, increasing use of colistin has led to the emergence of colistin-resistant bacteria, and their colistin resistance mechanisms are usually based on chromosomal mutations [2]. Recently, Liu et al. reported the first plasmid-mediated colistin resistance mechanism, MCR-1, in animal and human isolates of *Escherichia coli* and *Klebsiella pneumoniae* in China [3]. MCR-1 is a member of the phosphoethanolamine transferase enzyme family that catalyses the addition of phosphoethanolamine to lipid A resulting in reduced affinity of colistin for lipopolysaccharide (LPS). The proportion of *mcr-1*-carrying isolates of human origin is relatively lower than that of animal origin, suggesting that MCR-1-mediated colistin resistance may have originated from animals and spread to humans [3].

Infections with carbapenem-resistant bacteria represent a major therapeutic challenge since these isolates are susceptible to very few antibiotics, converting these remaining antibiotics into last-resort agents. Co-existence of carbapenemases and MCR-1 leaves clinicians with no reserve antibiotics for treating fatal bacterial infections. In this study, we report a carbapenem-resistant *E. coli* clinical isolate co-harbouring *mcr-1* and bla_{NDM-9} in Taiwan and analysed its whole genome sequence.

2. Materials and methods

2.1. Identification of the mcr-1-positive strain

Carbapenem-resistant *E. coli* strain 5CRE51 was isolated from a urine sample collected from a 79-year-old female patient in southern Taiwan in December 2015 as previously described [4]. The strain was identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS)

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using a microflex™ LT MALDI-TOF MS system (Bruker Daltonics, Billerica, MA). Antimicrobial susceptibility was determined using a BD Phoenix™ Automated Microbiology System with a BD Phoenix™ NMIC/ID-72 panel (Becton Dickinson & Co., Sparks, MD) and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [5].

Molecular characterisation of carbapenemase resistance genes, including bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM} and bla_{OXA-48}, as well as the colistin resistance gene *mcr-1* was performed by PCR amplification followed by Sanger sequencing as previously described [3,6].

2.2. Whole-genome sequencing

DNA of *E. coli* strain 5CRE51 was extracted and was sequenced using PacBio RSII SMRT (single-molecule, real-time) technology (Pacific Biosciences, Menlo Park, CA). De novo assembly was performed using the SMRT Portal (Pacific Biosciences). Furthermore, the DNA was also sequenced via Illumina MiSeq (Illumina Inc., San Diego, CA) with 150-bp paired-end reads, and these Illumina short reads were used to correct errors of PacBio-assembled sequences for hybrid assembly. Whole-genome sequencing of *E. coli* strain 5CRE51 resulted in three contigs that have been deposited in the National Center for Biotechnology Information (NCBI) database under accession nos. **CP021175** (chromosome, complete sequence), **CP021176** (plasmid, complete sequence) and **CP021177** (plasmid).

2.3. Comparative analysis of the sequences

Comparison of the *mcr-1*-harbouring plasmid p5CRE51-MCR-1 with pP111 (**KY120365**) and pHNSHP45 (**KP347127**) was performed using GView Interactive Genome Viewer (http://www.gview.ca). Pairwise comparison of the bla_{NDM-9}-harbouring plasmid p5CRE51-NDM-9 with pHNYJC8 (**KY019259**) and pC629 (**CP015725**) was made using the BLASTn plug-in in Easyfig [7] to reveal relationships.

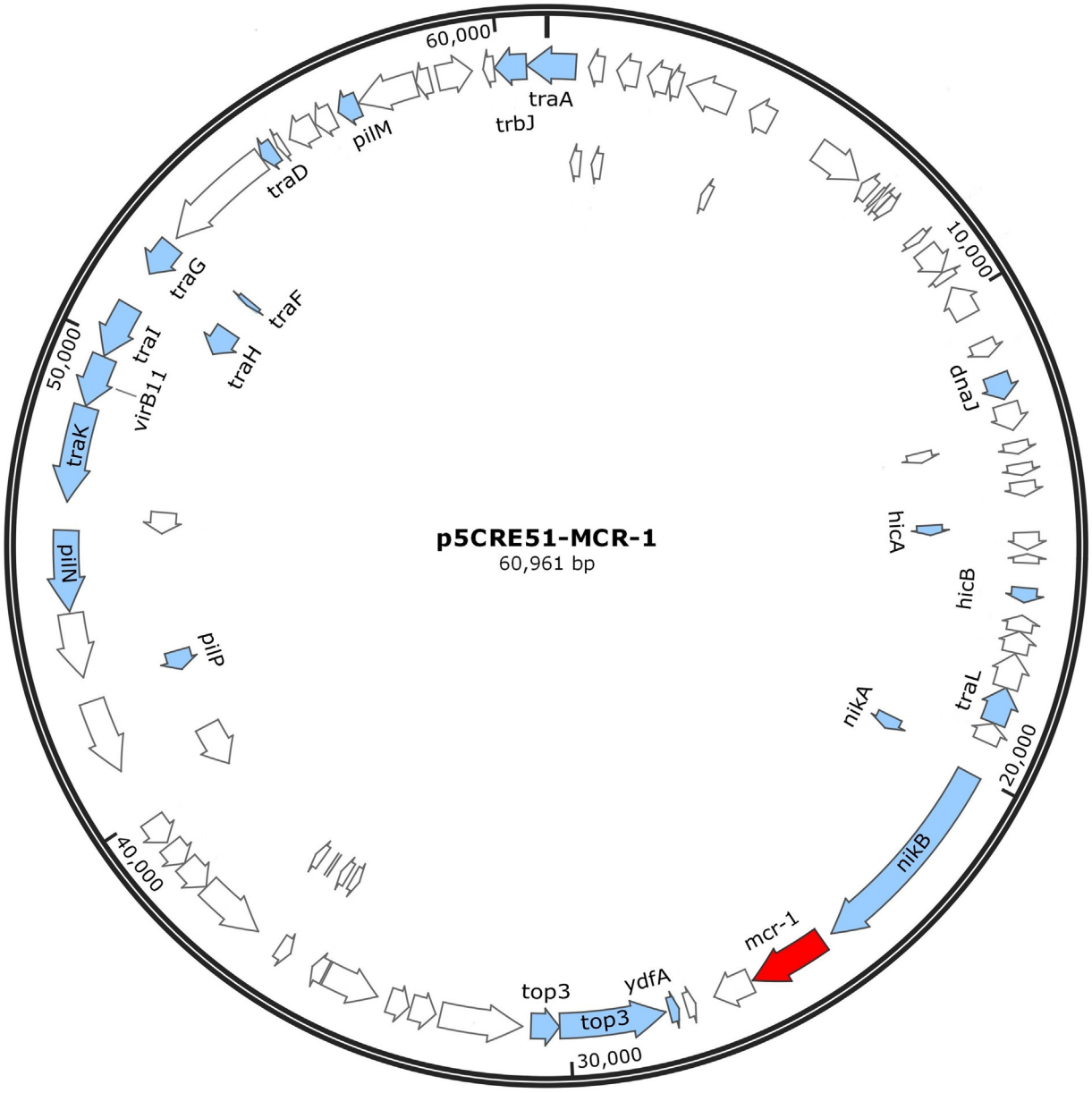

Fig. 1. Circular representation of the *mcr-1*-carrying plasmid p5CRE51-MCR-1. Arrows indicate coding sequences (CDSs); blue arrows with annotation, white arrows without annotation, and the red arrow indicates the *mcr-1* sequence.

3. Results and discussion

Antimicrobial susceptibility testing showed that *E. coli* strain 5CRE51 was resistant to nearly all antimicrobial drugs tested, including colistin, imipenem, meropenem, ertapenem, gentamicin, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, cefalexin, aztreonam, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, piperacillin/tazobactam, ciprofloxacin, chloramphenicol and tetracycline but was susceptible to amikacin and tigecycline (Supplementary Table S1). Additional PCR analysis and Sanger sequencing confirmed that this strain harboured both the *mcr-1* and bla_{NDM-9} genes, which account for the colistin and carbapenem resistance, respectively.

Genome assembly was constructed on the basis of next-generation sequencing data from PacBio and was corrected with Illumina MiSeq 150-bp paired-end reads to resolve the genetic information of *E. coli* strain 5CRE51. Three contigs have been assembled, comprising the *E. coli* chromosome and two plasmids harbouring *mcr-1* (p5CRE51-MCR-1) and bla_{NDM-9} (p5CRE51-NDM-9), respectively.

The complete chromosome of *E. coli* strain 5CRE51 is ca. 5 Mbp in size (contig 1) with serotype O89:H10 and sequence type (ST) 617. The *E. coli* ST617 clone carrying bla_{NDM} group genes has been reported by several countries, including China [8], Switzerland [9] and Mexico [10]. Contig 2 from plasmid p5CRE51-MCR-1 carries the *mcr-1* gene, with a 60 961-bp circular sequence, and belongs to incompatibility group IncI2. The genetic map of p5CRE51-MCR-1 is shown in Fig. 1. Contig 3 from plasmid p5CRE51-NDM-9 carries bla_{NDM-9} with partial sequences of 154 099 bp. In addition to bla_{NDM-9}, the resistance genes $bla_{CTX-M-65}$, *aadA2*, *dfrA12*, *floR*, *fosA* and *sul1* were also identified on p5CRE51-NDM-9.

The complete sequence of p5CRE51-MCR-1 showed high similarity to six *mcr-1*-carrying plasmids isolated from livestock and humans in the NCBI database from locations including Taiwan (*Salmonella enterica* plasmid pP111 from a pig), China (**CP022452**, *S. enterica* plasmid pD90-2 from a chicken), China (**MF175189**, *E. coli* plasmid pColR598_2 from a human), South Korea (**KY657476**, *E. coli* plasmid pCREC-527-4), Malaysia (**CP016187**, *E. coli* plasmid pS2.14-2) and Australia (**KY795978**, *E. coli* plasmid pJIE3685-1 from a human). None of these isolates carried additional antimicrobial resistance genes on the *mcr-1*-carrying plasmids. These plasmids were aligned with pHNSHP45, the first published *mcr-1*-carrying plasmid in China, as a reference plasmid [3] (Fig. 2). These six plasmids shared a common backbone with high similarity, and all of them lacked an IS*Apl1* insertion element upstream of *mcr-1* compared with pHNSHP45. IS*Apl1* has been shown to play a key role in the mobilisation and dissemination of *mcr-1*,and the localisation of ISApl1 upstream of *mcr-1* may facilitate the translocation of *mcr-1* onto the chromosome [11]. Moreover, an *mcr-1* gene flanked by IS*Apl1* and IS*Apl1*-like elements has been reported directly upstream of the multidrug resistance (MDR) region in the same plasmid [12]. Therefore, it is of great concern that the *mcr-1* gene disseminates by illegitimate recombination of the transposon to MDR plasmids or chromosomes.

The sequence of p5CRE51-NDM-9 shared an ca. 150-kb backbone with the non-bla_{NDM-9}-carrying plasmid pHNYJC8 in *E. coli* (Fig. 3). The bla_{NDM-9} gene of p5CRE51-NDM-9 was embedded in a class 1 integron, forming a composite cassette that was bracketed by two IS*26* elements with the same orientation, similar to *S. enterica* plasmid pC629 (Fig. 3). In addition, the fosfomycin resistance gene *fosA* was located close to the $bla_{CTX-M-65}$ gene, and both of them were flanked by IS*26*. It has been suggested that *fosA* and $bla_{CTX-M-65}$ genes may transmit together [13]. These observations suggested that insertion element IS*26* could recruit antimicrobial resistance genes into the mobile gene pool by forming transposons carrying many different resistance genes in Gram-negative bacteria [14].

To the best of our knowledge, this is the first characterisation of an IncI2-type plasmid carrying the *mcr-1* gene from a carbapenem-

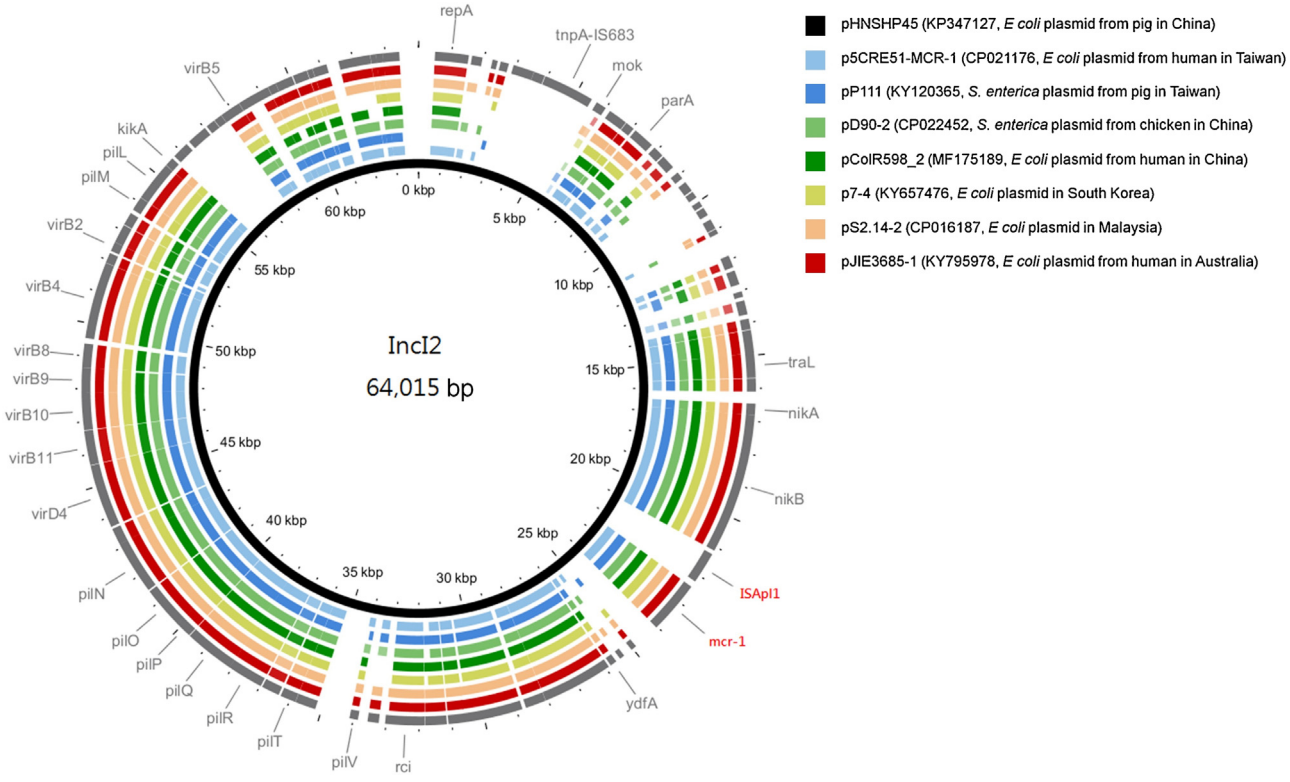

Fig. 2. Alignment of *mcr-1*-carrying plasmids. The first *mcr-1*-carrying plasmid pHNSHP45 (**KP347127**) was used as reference plasmid (black circle). The grey circle shows the annotations of the reference plasmid. The gaps show regions that were missing in the respective plasmid compared with the reference plasmid.

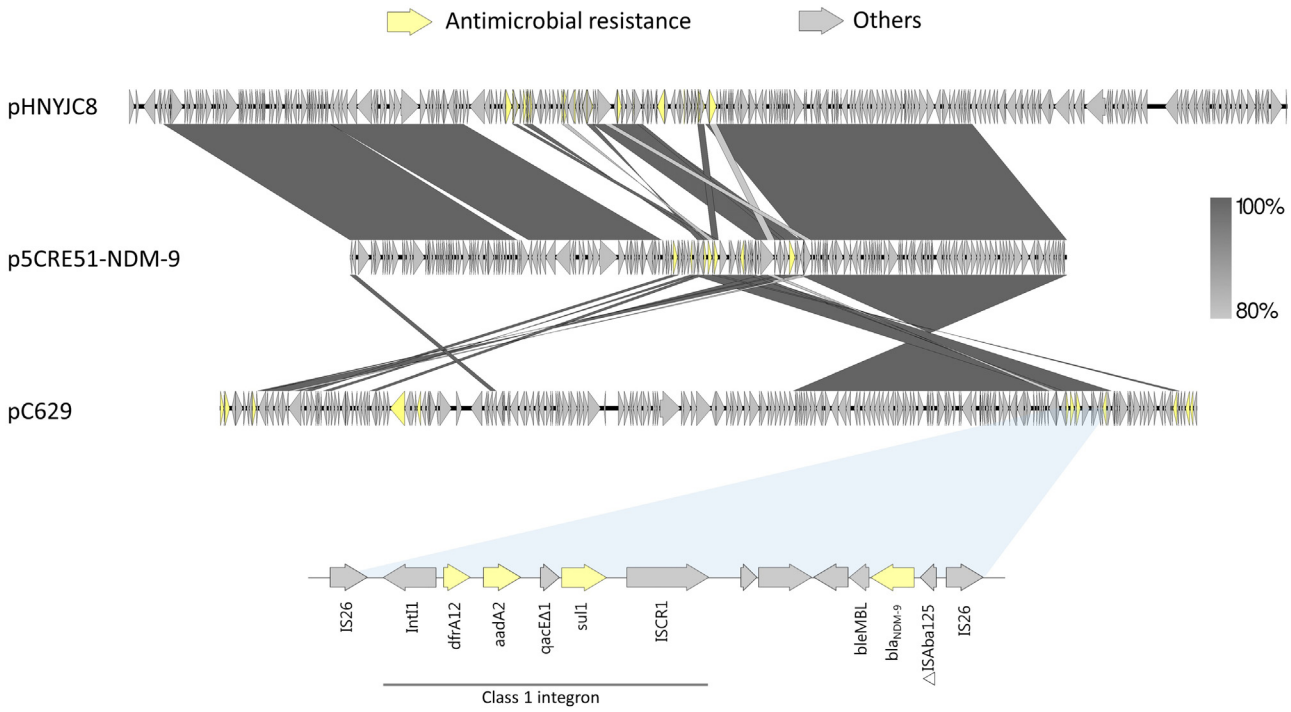

Fig. 3. Pairwise comparison of the p5CRE51-NDM-9 sequence using BLASTn within Easyfig against the pHNYJC8 and pC629 plasmids, respectively. Arrows indicating coding sequences (CDSs) are drawn to scale, and yellow arrows represent antimicrobial resistance genes. The percentages of similarity profiles are indicated in grayscale.

resistant *E. coli* clinical isolate from a human in Taiwan. Noticeably, p5CRE51-MCR-1 was highly similar to another *mcr-1*-carrying plasmid pP111 in *S. enterica* from a pig. The high similarity between these two *mcr-1*-carrying plasmids from distinct sources and different bacteria in the same country suggest that transmission of the *mcr-1* gene may be accounted for by the transfer plasmids rather than a single specific host strain [15]. Similarly, the prevalence of *mcr-1*-positive *E. coli* of different STs recovered from humans and food animals in Taiwan has increased since 2010 [16]. Thus, these observations suggest that *mcr-1* may have been widespread in human and food animals in Taiwan. Owing to the variety of possible transmission routes, coexistence of both bla_{NDM-9} and *mcr-1* may accelerate the dissemination of these two genes. Furthermore, Enterobacteriaceae co-producing MCR-1 and NDM-9 have also been reported from geographically dispersed countries, including the USA [17], Venezuela [18], China [19] and Japan [20]. Further studies and surveillance are necessary to identify the coexistence of carbapenemase- and *mcr-1*-carrying plasmids in Enterobacteriaceae.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.10.003.

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