Journal of Global Antimicrobial Resistance 24 (2021) 446–447

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

Emergence of *mcr-3*-mediated IncP and IncFII plasmids in Thailand

Sir,

The emergence of *mcr* on a conjugative plasmid among Enterobacteriaceae conferring resistance to colistin is now a global problem. Several studies on *mcr*-positive bacteria in Thailand have been documented. Of these, *mcr-1* is the most prevalent, while *mcr-3* is less frequent. Most *mcr-1* genes are carried on IncX4 plasmids and these sequences have been well described [1,2]. However, detailed genetic analysis of *mcr-3* plasmids has not been well reported. It was recently shown that *mcr-3* from *Klebsiella pneumoniae* was located on an ~148-kb IncC plasmid [2]. The *mcr* variants and their associated plasmids continue to expand, not least of which for *mcr-3* there are now 30 variants. Therefore, to understand the rapid emergence of *mcr-3*-mediated colistin resistance in Thailand, plasmids carrying *mcr-3* and their potential transmission were investigated.

As part of a 'One Health' approach to understanding the spread of colistin resistance in Thailand, we screened 1464 Enterobacteriaceae isolates recovered from our surveillance study on cefotaxime-resistant Enterobacteriaceae in Northern Thailand between 2013–2015, of which 79 isolates (5.4%) were positive for *mcr-3*. We subsequently examined 10 *mcr-3*-positive *Escherichia coli* from faecal samples (3 cows, 2 pigs, 1 chicken and 1 human) and river water ($n = 1$). These isolates were identified to species level by biochemical tests and 16S rDNA sequencing. Additionally, two *mcr-1*-carrying *E. coli* (PN24 and PN42), obtained from the same area [1], were found to possess *mcr-3* and were included in this study. Minimum inhibitory concentrations (MICs), determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines, revealed that all isolates possessed colistin MICs ranging from 4–16 mg/L but remained susceptible to carbapenems. All isolates could transfer *mcr-3* to *E. coli* J53 at high frequency (10^{-6}–10^{-2}). Transconjugants carrying *mcr-3* demonstrated a >32-fold increase in colistin MICs (2–4 mg/L) compared with that of *E. coli* J53 (<0.125 mg/L).

Total DNA from all donors and transconjugants was extracted using a QIAcube automated system (QIAGEN, USA). DNA libraries were prepared using a Nextera XT Kit (Illumina Inc., USA). Sequencing was performed using an Illumina MiSeq v.3 (Illumina Inc.) and MinION technologies (Oxford Nanopore Technologies, Oxford, UK). Raw sequence reads were trimmed using TrimGalore (https://github.com/FelixKrueger/TrimGalore) and were de novo assembled into contigs using SPAdes v.3.9.0. All contigs were searched using Geneious 10.0.9 (Biomatters Ltd., Auckland, New Zealand) and sequence comparisons were analysed using the Center for Genomic Epidemiology (CGE) platform (http://www.genomicepidemiology.org/) and BLAST tool (https://www.ncbi.nlm.nih.gov/).

Among the 10 *mcr-3*-positive *E. coli*, sequence typing showed five different sequence types (STs), namely ST34, ST206, ST359, ST542 (1 isolate each) and ST48 ($n = 4$). PN24 and PN42 had previously been identified as ST3631 and ST744, respectively [1]. *S1* nuclease pulsed-field gel electrophoresis (S1-PFGE) followed by Southern hybridisation and sequence analysis with PlasmidFinder showed that three and seven isolates carried *mcr-3* on IncP (~50 kb) and IncFII (~83–97 kb) plasmids, respectively (Supplementary Table S1).

Among the three *mcr-3* IncP plasmids, sequences of pPN143 and pPN150 enabled complete assembly and showed plasmid sizes of 50 379 bp and 50 525 bp, respectively, with a GC content of 47.1%. Sequence analysis showed that these two IncP plasmids were identical and carried *mcr-3.1* in the same genetic context of ΔTn*3*–*mcr-3.1*–*dgkA*–IS*6* (Fig. 1A). No other antibiotic resistance genes were found (Supplementary Table S2). The pPN143 and pPN150 plasmids were almost identical (>99%) to pMCR3_025943 in an *E. coli* recovered from hospital sewage in China [3]. The same genetic context was found in the three plasmids, except that the *mcr-3* variant in pMCR3_025943 was identified as *mcr-3.5*. These results suggest that pMCR3_025943, pPN143 and pPN150 may derive from a common ancestor.

Nucleotide sequences of three *mcr-3* IncFII plasmids (pPN24, pPN42 and pPN156) were fully assembled and confirmed plasmids of 97 423 bp, 89 141 bp and 83 316 bp, respectively, with a GC content of 51.8%. The *mcr-3* variant of pPN24/pPN42 was *mcr-3.1*, while that of pPN156 was identified as *mcr-3.5*. (Fig. 1B). Other antibiotic resistance genes, including *catA2*, $bla_{\text{CTX-M-55}}$ and *qnrS1*, were found on the three plasmids. Moreover, *aac(3)-IId*, was additionally present in pPN24/pPN42. Plasmid sequences from pPN24 revealed 91% identity with an *mcr-3* IncFII plasmid in *E. coli* CHL5009T (Fig. 1B) [4]. The genetic arrangements of *mcr-3* (ΔIS*6*–ΔTn*3*–*mcr-3*–*dgkA*–IS*3*–IS*6*) on pCHL5009T and pPN24/pPN42 were identical. In contrast, *mcr-3.5* on pPN156 was flanked by *traX*–*finO*–ΔTn*3* and *dgkA*–IS*6* (Supplementary Table S2).

pCHL5009T was found in an *E. coli* recovered from a New Zealander patient in 2017, while pPN24-carrying *E. coli* were isolated in Thailand in 2014 [1]. It has been suggested that this patient acquired *mcr-3*-positive *E. coli* during her holiday in Thailand 4 months before her admission [4]. The fact that $bla_{\text{CTX-M-55}}$ residing on the same plasmid is highly prevalent in Thailand suggests that pPN24 might have been transferred to *E. coli* CHL5009T. Compared with pPN24, there is an additional 4-kb region in pCHL5009T suggesting that insertion of genetic elements had occurred.

Recently, *mcr-3* on IncP and IncFII plasmids in *K. pneumoniae* in Laos, a neighbouring country, have been reported [5]. The *mcr-3* IncP plasmid is similar to pPN143/pPN150 (83% identity), whereas *mcr-3* IncFII plasmid differed markedly from pPN24, showing only 42% identity. In addition, the differences in plasmid size, *mcr-3* variant and genetic environment surrounding *mcr-3* were clearly observed (Fig. 1).

Our study reports *mcr-3* IncP and IncFII plasmids spreading in a Thai community. A limitation of this study is that *mcr-3*-positive *E. coli* isolates were obtained from a group of cefotaxime-resistant Enterobacteriaceae, however the existence of *mcr-3* plasmids in cefotaxime-susceptible isolates cannot be excluded. Therefore, the numbers and types of *mcr-3* plasmids may be underestimated. To the best of our knowledge, this report represents the first association between *mcr-3* and IncP/IncFII plasmids in Thailand. Both IncP and IncFII are the broad-host range plasmids that can disseminate to a variety of Gram-negative bacilli. Importantly, our *mcr-3* plasmids show high transfer rates, indicating that these plasmids may play an important role in the dissemination of *mcr-3* in Thailand and Southeast Asia.

GenBank accession numbers

The complete nucleotide sequences of five plasmids were submitted to the GenBank database and assigned accession numbers MT449718–MT449722.

http://dx.doi.org/10.1016/j.jgar.2021.02.006

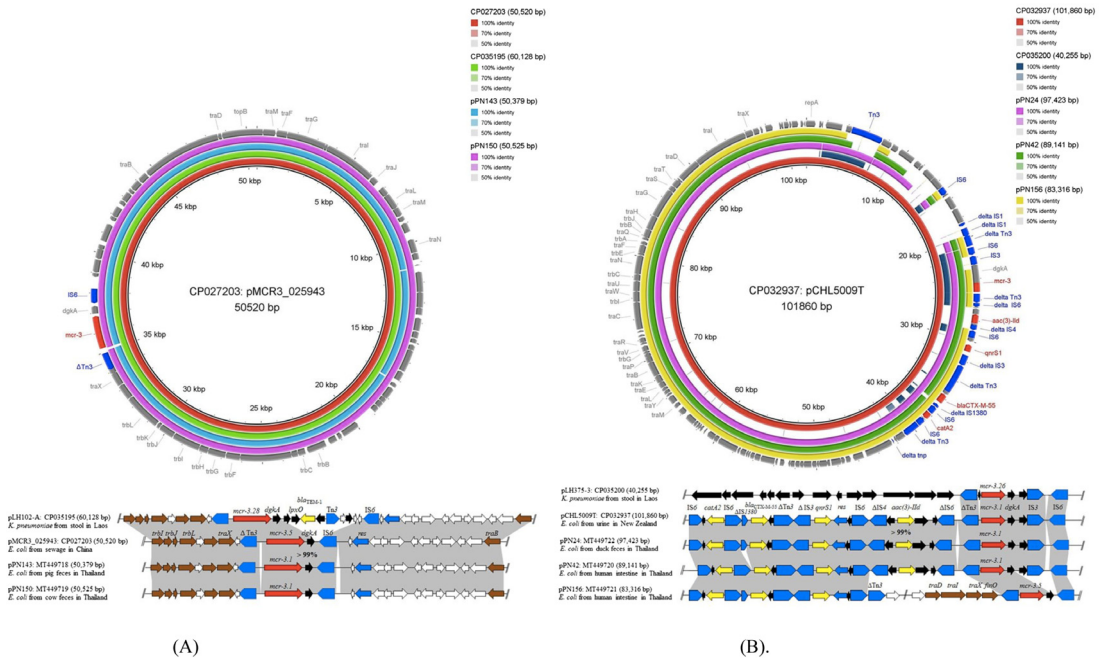

Fig. 1. BLAST Ring Image Generator (BRIG v.0.95555) analysis and genetic context of *mcr-3* IncP and IncFII plasmids. Open-reading frames (ORFs) are illustrated by arrows pointing in the direction of their respective orientations. Important genes are indicated above the plasmid sequences. *mcr-3* is indicated in red, while other antimicrobial resistance genes are in yellow. Mobile genetic elements are in blue. Regions of ≥99% nucleotide sequence identity are indicated by grey shading. (A). Comparison of *mcr-3* IncP plasmids using pMCR3_025943 as a central reference plasmid. The same genetic contexts (ΔTn*3*–*mcr-3*–*dgkA*–IS*6*) were observed in pMCR3_025943, pPN143 and pPN150, except that the *mcr-3* variant in pMCR3_025943 was identified as *mcr-3.5*. (B). Comparison of *mcr-3* IncFII plasmids using pCHL5009T as a central reference plasmid. The genetic arrangements of *mcr-3* on pCHL5009T, pPN24 and pPN42 are identical (ΔIS*6*–ΔTn*3*–*mcr-3*–*dgkA*–IS*3*–IS*6*). In contrast, *mcr-3.5* on pPN156 was flanked by *traX*–*finO*–ΔTn*3* and *dgkA*–IS*6*.

Funding

This work was funded by the National Science and Technology Development Agency (NSTDA), Thailand [FDA-CO-2561-6029-TH]. UT, AK and KA are financially supported by the RGJ-PhD program from the Thailand Research Fund [PHD/0054/2555, PHD/0181/2557 and PHD/0123/2560]. UT is also partly supported by the British Council Newton Fund [GA/PhD/Scholar/Year3/012].

Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2021.02.006.

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Received 20 July 2020

Available online 19 February 2021