Sporadic dissemination of *mcr-8*-ST11 *Klebsiella pneumoniae* isolates in China

Diseminación esporádica de aislados de Klebsiella pneumoniae mcr-8-*ST11 en China*

Dear Editor:

Since the first plasmid-encoded colistin resistance gene (*mcr-1*) was described in *Escherichia coli* in China,[1] 9 others mobile colistin resistance genes have been identified (*mcr-2* to *mcr-10*).[2] A plasmid carrying *mcr-8* was first reported in 2018 but similar mechanisms of action for *mcr-8* and *mcr-2* indicated that *mcr-8* resistance genes may have appeared very early.[2] Notably, the *mcr-8* gene has been detected in patients from intensive care units as well as livestock[2] and *mcr-8*-bearing plasmids have been found primarily in *Klebsiella pneumoniae* and *Raoultella ornithinolytica.* In the current study, an IncFIA plasmid carrying *mcr-8* was identified in an ST11*K. pneumoniae* strain isolated from wastewater in a duckery in Fujian, China. Further genetic analysis of this plasmid and others of the same type from global databases were then compared.

The *mcr-8*-harboring *K. pneumoniae* isolate ZZW20 was identified by PCR amplification and sequencing of the 16S rRNA. Susceptibility testing was performed following the CLSI[3] and the EUCAST[4] methodologies, and this strain was resistant to colistin (MIC 32 mg/L) and 6 other antimicrobials (Table S1). The whole genome sequence of ZZW20 was determined using a combination of Illumina (San Diego, CA, USA) and MinION (Nanopore, Oxford, UK) platforms and assembled using Unicycle.[5] MLST analyses indicated that this *mcr*-8-positive *K. pneumoniae* belonged to the most prevalent clonal type (ST11) that carried virulence factors, that was present in Asia, especially China.[6]

A phylogenetic tree of ZZW20 was generated using 44 additional *K. pneumoniae* strains carrying *mcr-8* on plasmids. The 44 *mcr-8*-harboring *K. pneumoniae* strains were primarily from Asia but were distributed within different clades and isolated from humans ($n = 17$) and animals ($n = 27$), indicating a prevalence in animals. A total of 17 blow flies carrying *mcr-8* were trapped at three different locations in Northern Thailand: a local market in an urban community, a rural area and a suburb of the city Phitsanulok. Importantly, they were grouped in a cluster, suggesting that flies are likely to be one of the key vectors of the *mcr-8*. Compared with other strains carrying *mcr-8*, their ST type was different, indicating that the strains carrying *mcr-8* began to evolve toward diversification. Almost all strains of ST11 from China shared 6588 core-genome single nucleotide polymorphisms (cgSNP). Interestingly, significantly distinct cgSNPs (range 1868–4216) were observed between ZZW20 and other database strains suggesting that they did not share a common transmission event (Fig. 1A).

The *mcr-8* gene was present in strain ZZW20 on an ~88 kb plasmid (pZZW20_80K; Acc. No. CP058962) that was identified by S1-PFGE and southern blotting[7] using the appropriate probes (Figure S1), and was typed as IncFIA using the WGS data. BLAST analysis demonstrated that pZZW20_80K had a 129 kb backbone closely related to the *K. pneumoniae* plasmids p18-29mcr-8.2 (MK262711), p2018C01-046-1MCR8 (CP044369), p2018N16-148-2MCR8 (CP044395), p2018N17-066-2MCR8 (CP044391), pVNKp83 (LC549808) and PK91 (MG736312). Interestingly, there were only 44 nucleotides that differed between the plasmids in this group (>99.96% identity). Additionally, even though the 7 IncFIA ST11 *mcr-8*-positive *K. pneumoniae* isolates were unique (range 1868–4216 cgSNPs), the *mcr-8* plasmids they carried were highly similar, especially for pZZW20_80K with 88 kb identity (81% query coverage and 99.9% identity) to *mcr-8* plasmid p18-29 (MK262711) from a human isolate in China. Then we used these plasmids to make a circular plasmid map with BRIG for further analysis.[8] These plasmids shared the genetic context IS*903B-ampC-hp-hp-hp-Giy-T-dgkA-baeS-copR*-IS*3-mcr-8-Gly-T*-IS*5*. However, only the ZZW20 carried $bla_{\text{CTX-M-3}}$ and $bla_{\text{TEM-1B}}$ genes. There were also 2 copies of ΔTn*2* downstream of $bla_{\text{TEM-1B}}$ as well as an intact Tn*2* in pZZW20_88K indicating that the insertion of $bla_{\text{CTX-M-3}}$ and $bla_{\text{TEM-1B}}$ were most likely mediated by Tn*2*[9] (Fig. 1B).

The pZZW20_80K plasmid also possessed the *tra/trb* gene cluster that mediates plasmid conjugative functions and we therefore conducted conjugation experiments to examine its potential transferability using *E. coli* EC600^{str} as recipient. The transconjugants that were isolated retained most of the antibiotic resistance genes from the plasmid including colistin resistance (Table S1). This was an additional confirmation that IncFIA plasmids carrying *mcr-8* may spread among *K. pneumoniae* strains.

The 6 virulence factors present on the chromosome of strain ZZW20 were identified using the VFDB database (http://www.mgc.ac.cn/VFs/main.htm). The genes necessary for enterobactin synthesis, transport and utilization (*entABCDEF, fepABCDG, fes*) as well as the gene responsible for salmochelin cleavage (*iroE*) were present on the plasmid. The enterobactin siderophore system is the primary iron-sequestering system for *Enterobacterales*. The presence of salmochelin is associated with invasive disease isolates and is common in highly virulent *K. pneumoniae* isolates.[6] Strain ZZW20 also carried a Type VI Secretion System (T6SS) cluster that contributes to bacterial competition, cell invasion and *in vivo* colonization that can sensitize hosts to potentially fatal infections by other bacterial pathogens.[10] These characteristics of ZZW20 indicated a likelihood that it was evolving into a hypervirulent strain.

In conclusion, ST11*K. pneumoniae* strains possessing *mcr-8* IncFIA plasmids have been sporadically disseminated in China. These plasmids were highly similar and pZZW20_80K -*mcr-8* and were able to transfer to *E. coli* by conjugation. The presence of the highly efficient enterobactin system along with 5 other known virulence factors indicated a potential threat to public health. In particular, we found multiple copies of Δ IS*66* transposases on the *mcr-8* plasmid revealing the potential transferability of *mcr-8*. Thus, there is an urgent need for further surveillance to understand the prevalence and dissemination of *mcr-8*-positive *K. pneumoniae* and prevent future disease outbreaks.

Accession number. Sequence of pZZW20-88K has been assigned GenBank accession number CP058962.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eimc.2021.01.007.

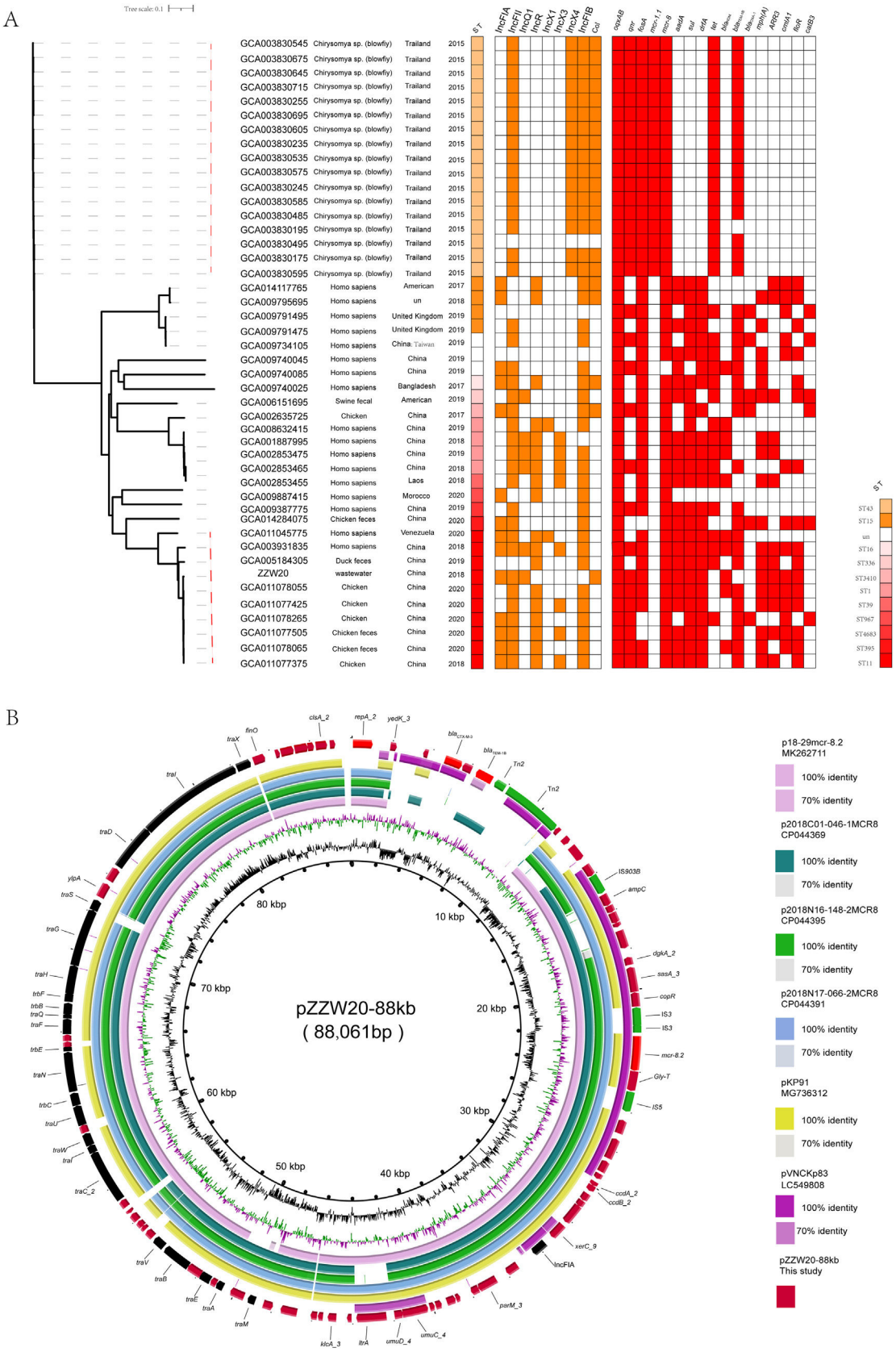

Fig. 1. (A) Midpoint-rooted tree generated from the core-genome sequences of the *mcr-8*-positive ST11 *K. pneumoniae* identified in this study as well as another 43 *mcr-8*-positive *K. pneumoniae* isolates. The collection dates, hosts and country of isolation are indicated for each isolate. Major antibiotic resistance genes, ST carried by the *K. pneumoniae* isolate are indicated. (B) Comparative ananlysis of *mcr-8*-positive plasmid identified in this study. The circular plasmid map was generated using BRIG. Arrows indicate orientation of open reading frames. Regions of homology are marked by shading.

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