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Emergence of Mobile Colistin Resistance (*mcr-8*) in a Highly Successful *Klebsiella pneumoniae* Sequence Type 15 Clone from Clinical Infections in Bangladesh

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ABSTRACT The emergence of mobilized colistin resistance genes (*mcr*) has become a serious concern in clinical practice, compromising treatment options for life-threatening infections. In this study, colistin-resistant *Klebsiella pneumoniae* harboring *mcr-8.1* was recovered from infected patients in the largest public hospital of Bangladesh, with a prevalence of 0.3% (3/1,097). We found *mcr-8.1* in an identical highly stable multidrug-resistant IncFIB(pQil) plasmid of ~113 kb, which belonged to an epidemiologically successful *K. pneumoniae* clone, ST15. The resistance mechanism was proven to be horizontally transferable, which incurred a fitness cost to the host. The core genome phylogeny suggested the clonal spread of *mcr-8.1* in a Bangladeshi hospital. Core genome single-nucleotide polymorphisms among the *mcr-8.1*-positive *K. pneumoniae* isolates ranged from 23 to 110. It has been hypothesized that *mcr-8.1* was inserted into IncFIB(pQil) with preexisting resistance loci, $bla_{\text{TEM-1b}}$ and $bla_{\text{CTX-M-15}}$, by IS*903B*. Coincidentally, all resistance determinants in the plasmid [*mcr-8.1*, *ampC*, *sul2*, *1d-APH(6)*, *APH(3'')-Ib*, $bla_{\text{TEM-1b}}$, $bla_{\text{CTX-M-15}}$] were bracketed by IS*903B*, demonstrating the possibility of intra- and interspecies and intra- and intergenus transposition of entire resistance loci. This is the first report of an *mcr*-like mechanism from human infections in Bangladesh. However, given the acquisition of *mcr-8.1* by a sable conjugative plasmid in a successful high-risk clone of *K. pneumoniae* ST15, there is a serious risk of dissemination of *mcr-8.1* in Bangladesh from 2017 onwards.

IMPORTANCE There is a marked paucity in our understanding of the epidemiology of colistin-resistant bacterial pathogens in South Asia. A report by Davies and Walsh (Lancet Infect Dis 18:256–257, https://doi.org/10.1016/S1473-3099(18)30072-0, 2018) suggests the export of colistin from China to India, Vietnam, and South Korea in 2016 was approximately 1,000 tons and mainly used as a poultry feed additive. A few reports forecast that the prevalence of *mcr* in humans and livestock will increase in South Asia. Given the high prevalence of $bla_{\text{CTX-M-15}}$ and bla_{NDM} in India, Bangladesh, and Pakistan, colistin has become the invariable option for the management of serious infections, leading to the emergence of *mcr*-like mechanisms in South Asia. Systematic scrutiny of the prevalence and transmission of *mcr* variants in South Asia is vital to understanding the drivers of *mcr* genes and to initiate interventions to overcome colistin resistance.

KEYWORDS *mcr-8.1*, *Klebsiella pneumoniae*, human, Bangladesh

Klebsiella pneumoniae is an opportunistic Gram-negative pathogen that is mostly associated with nosocomial infections (1). Carbapenems have been widely used to treat multidrug-resistant (MDR) *K. pneumoniae* infections, leading to the emergence of

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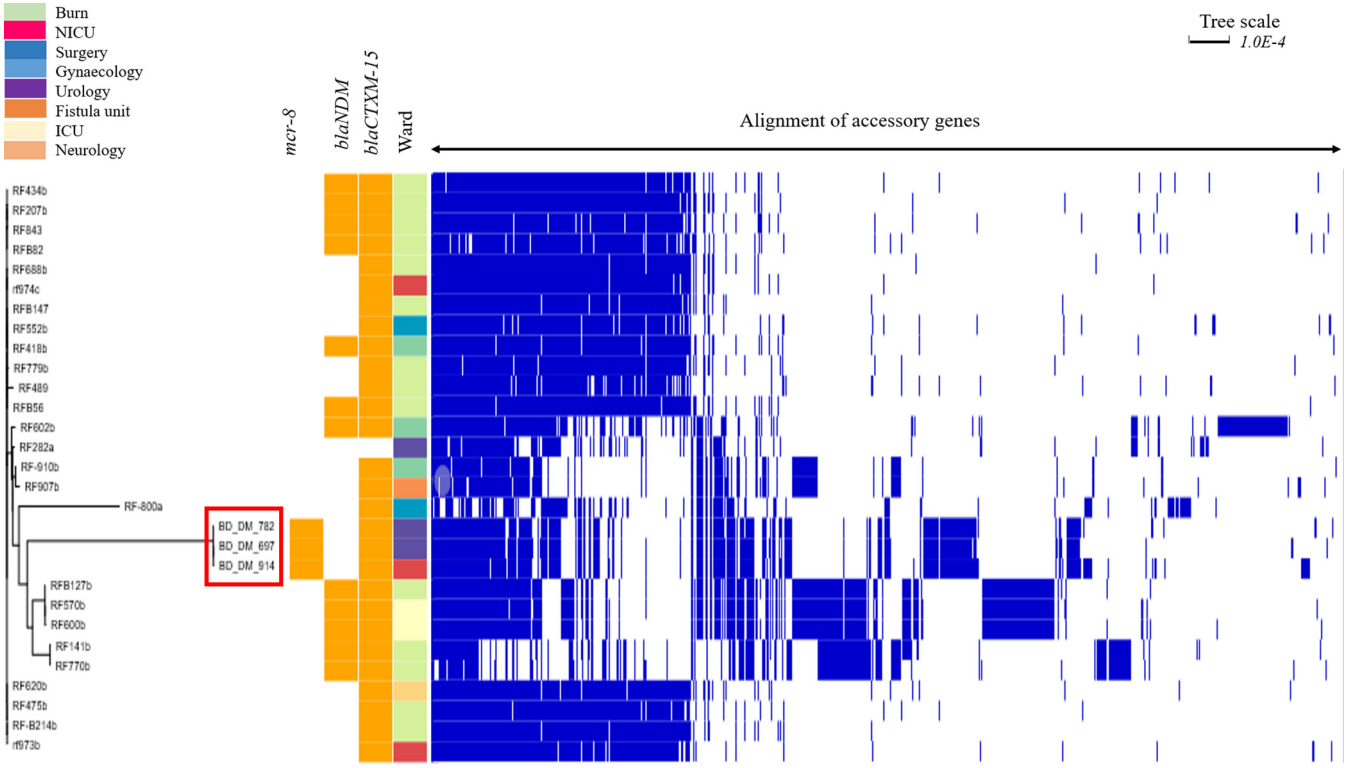

FIG 1 Phylogenetic tree of *K. pneumoniae* ST15 identified in this study ($n = 29$). Shown is a maximum likelihood (ML) phylogenetic tree constructed using a pangenome alignment. Strains were grouped together based on the similarity of genes and the presence of genes in the accessory genome using Roary (v3.12.0). Epidemiologically important resistance genes are indicated by orange cells, accessory genes by blue cells, and the absence of genes by white cells. NICU, neonatal intensive care unit; ICU, intensive care unit.

carbapenem resistance, where colistin is one of a few viable options (2, 3). The prevalence of colistin resistance is rapidly expanding in South Asia (4–10). Colistin resistance is either mediated by mutational disruption or insertional inactivation of *mgrB* (11) or via the acquisition of MCR plasmid-mediated resistance (12, 13). Here, we characterize a clinical epidemic *K. pneumoniae* clone harboring *mcr-8.1* from a Bangladeshi hospital.

A pilot antimicrobial resistance (AMR) survey was conducted from 21 October 2016 to 23 September 2017 at Dhaka Medical College Hospital (DMCH), which included 1,097 culture-positive clinical specimens. The project was approved by the Ethical Review Committee of DMCH (MEU-DMC/ECC/2017122). *K. pneumoniae* was recovered on chromogenic UTI containing vancomycin (10 mg/liter) and identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper; Bruker Daltonics, Inc., Billerica, MA, USA). MICs of relevant antimicrobials were determined by agar dilution and the MIC of colistin by broth microdilution. Susceptibility patterns of antimicrobials were interpreted according to EUCAST breakpoints. Sequencing was performed using Illumina MiSeq (Illumina Inc., San Diego, CA) and Nanopore (Oxford Nanopore Technologies, Oxford, UK) platforms. We adopted a hybrid strategy to assemble draft genomes using Unicycler (v0.4.0) (see Text S1 in the supplemental material). Pangenome analysis was performed using Roary (v3.12.0). A maximum likelihood phylogenetic tree was built using FastTree (v2.1.0) and visualized using Phandango and iTOL (v5.3). Intraclade single-nucleotide polymorphisms (SNPs) were identified using Snippy (v4.4.5). Plasmid size was confirmed by pulsed-field gel electrophoresis (PFGE) of S1 nuclease DNA digests and *mcr-8.1* probing. Conjugation assays were performed using *Escherichia coli* J53 as the recipient (9). Serial passaging of MCR-positive *K. pneumoniae* (MCRPKP) was performed in a colistin-free medium up to 12 days, and genomic DNA (gDNA) was extracted on days 0, 3, 6, 9, and 12. Plasmid stability was

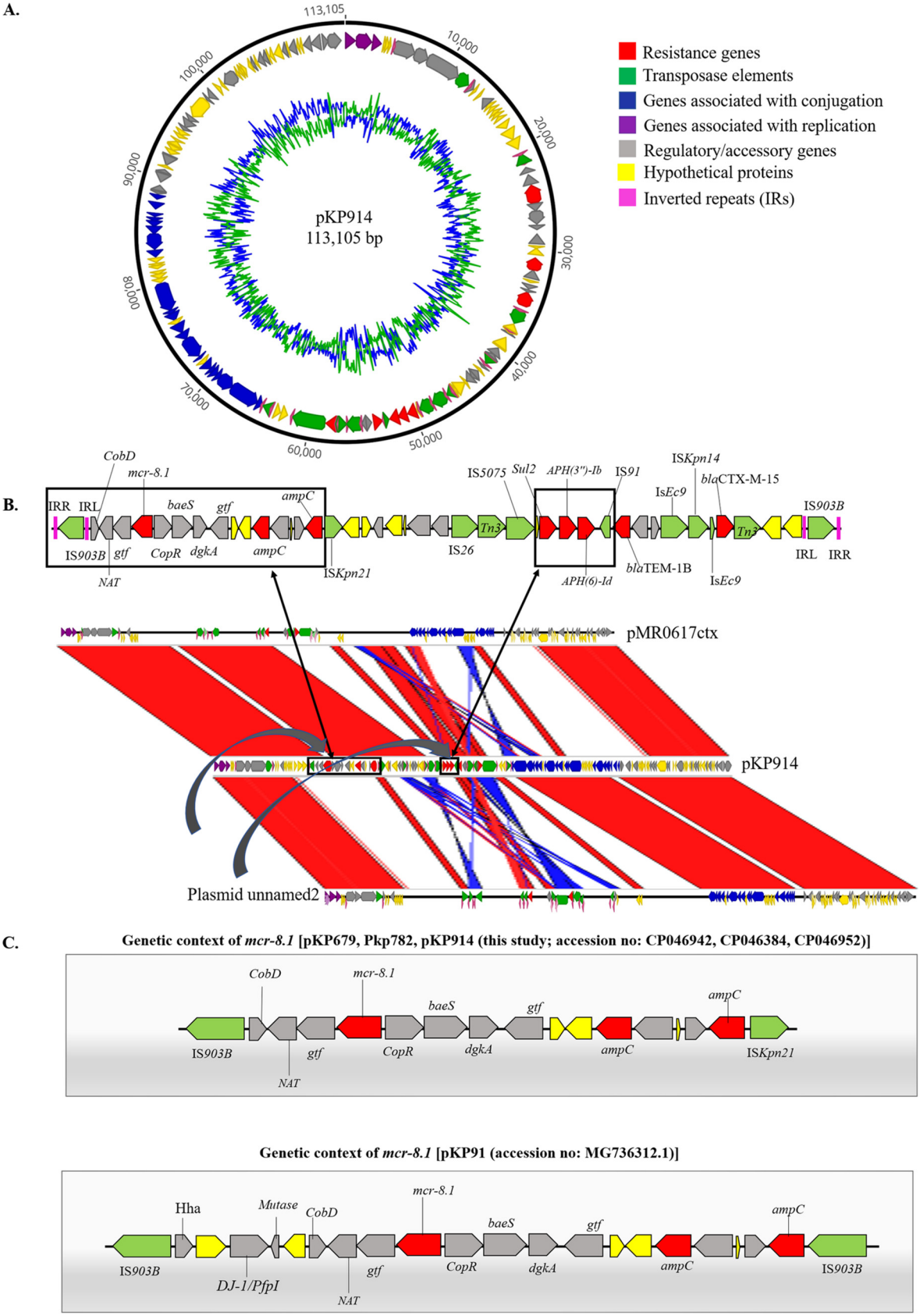

FIG 2 Genetic organization of plasmid harboring *mcr-8.1*. (A) Circular view of pKP914 (accession no. CP046952). (B) Schematic layout of sequence comparison of pKP914 (accession no. CP046952) against FDAARGOS_440 plasmid unnamed2 (accession no. CP023922.1) and

(Continued on next page)

assessed by relative abundance of *mcr-8.1* compared to that of housekeeping genes (HKGs) using quantitative PCR (Bio-Rad, USA) (Text S2). The *in vitro* growth rate of *E. coli* J53 and transconjugants (TDM697b, TDM782, and TDM914b) was determined by optical density (OD) in 30-min intervals for 24 h using FLUOstar Omega (BMG Labtech Ltd., Aylesbury, UK). The growth rate of each transconjugant was compared to that of *E. coli* J53 by unpaired two-tailed *t* test using GraphPad Prism (v7.04) (Text S3).

Description of cases with infections by MCRPKP. In this study, 3 *K. pneumoniae* isolates (3/1,097, 0.3%) were phenotypically resistant to colistin. MCRPKP isolates were recovered from the urine of two patients admitted under urology and the blood of a third patient in the neonatal intensive care unit (NICU). Case 1 (BD_DM_697) was a 55-year-old male with benign enlargement of the prostate with diabetes mellitus and a history of catheterization for 13 days. Case 2 (BD_DM_782) was a 63-year-old male patient with a left renal tumor, a history of catheterization for 15 days, and hematuria. These patients were discharged on days 20 and 35 of hospitalization, respectively. Case 3 (BD_DM_914) was a 5-day preterm low-birth-weight neonate with late-onset neonatal sepsis who died within 18 days after hospital admission. We did not observe any overlapping of hospital stay among the MCRPKP cases. MCRPKP isolates were coresistant to amoxicillin-clavulanate, piperacillin-tazobactam, cephalosporins (ceftazidime and cefotaxime), ciprofloxacin, levofloxacin, gentamicin, trimethoprim-sulfamethoxazole, and colistin and susceptible to carbapenems, amikacin, fosfomycin, and tigecycline. Although MCRPKP cases initially were shown to be treated with inappropriate antimicrobials, we have no data on whether the antibiotic therapy was subsequently changed based on the sensitivity report from the local laboratory.

Clonal spread of *mcr-8.1*. *In silico* genome-wide analysis of MCRPKP detected a 1,698-bp open reading frame (ORF), encoding a phosphoethanolamine transferase, showing 100% nucleotide identity to *mcr-8.1*. The prevalence of *K. pneumoniae* among all clinical isolates from this study was 21% (228/1097), of which 13% (29/228) belonged to ST15. *K. pneumoniae* ST15 harboring *mcr-8.1* was clustered in one clade (Fig. 1), suggesting the clonal spread of MCRPKP. SNP mapping found 110 and 107 SNPs in BD_DM_782 and BD_DM_914, respectively, compared to BD_DM_697, and 23 SNPs in BD_DM_782 compared to BD_DM_914. MCR-8 was described previously in *K. pneumoniae* ST1, of human origin, and *K. pneumoniae* ST42, of animal origin (14). *K. pneumoniae* ST15 has been regarded as a successful clone in disseminating $bla_{\text{CTX-M-15}}$ globally (15). The draft genome sequences and S1 PFGE indicate that *mcr-8.1* elements in *K. pneumoniae* were located on identical IncFIB(pQil) plasmids of ~113 kb (GenBank accession no. CP046384, CP046952, and CP046942) (Fig. 2 and Fig. S1). The gene *mcr-8.1* was stable after serial passaging without any antibiotic challenge. Compared to that at day 0, the abundance of *mcr-8.1* versus HKG was static up to day 12 (Fig. S2). Yang et al. (16) reported that colistin susceptibility could be attenuated after serial passaging of *mcr-1*-positive strains in antibiotic-free medium. Our findings demonstrate that the IncFIB(pQil) plasmid harboring *mcr-8.1* was remarkably stable, suggesting adaptive plasmid-host evolution (17). *K. pneumoniae* ST15 can be a vector capable of spreading *mcr*-mediated colistin resistance, particularly in a setting with suboptimal infection control practices (18).

Genetic context and dynamics of plasmids harboring *mcr-8.1*. Genome-wide analyses demonstrated that the plasmids recovered from the MCRPKP were almost identical to each other (Fig. S3). Complete plasmid sequences were determined for pKP782 (accession no. CP046384) and pKP914 (accession no. CP046952) by hybrid assembly, while pKP697 (accession no. CP046942) was not successfully closed. One

pMR0617ctx (accession no. CP024040.1). Arrows represent the position and transcriptional direction of the open reading frames. Genomic comparison was performed by Artemis Comparison Tool (ACT) (v.18.0.1). (C) Comparison of genetic environments of *mcr-8.1*. *APH*, aminoglycoside phosphotransferase; *baeS*, histidine-protein kinase; *bla*, beta-lactamase; *Cob*, cobalamin biosynthesis; *Cop*, copper homeostasis transcription factor; *dgkA*, diacylglycerol kinase; *DJ-1/PfpI*, cysteine peptidase; *gtf*, glucosyltransferase; Hha, hemolysin expression-modulating protein; IRL, inverted repeat left; IRR, inverted repeat right; IS, insertion sequence; *mcr-8.1*, mobilized colistin resistance; *NAT*, *N*-acetyltransferase; *Sul2*, dihydropteroate synthase.

copy of the IncFIB(pQil) plasmid with an identical resistance profile was recovered from each MCRPKP isolate and shared 99.72% nucleotide identity at 70% coverage with previously described plasmids (accession no. CP023922.1 and CP024040.1). However, those plasmids (CP023922.1 and CP024040.1) were absent from *mcr*-like genes (Fig. 2B). The genetic environment around *mcr-8.1* in IncFIB(pQil)-MCR-8.1 (pKP697, pKP782, and pKP914) shares identity with a previously described *mcr-8.1*-containing plasmid isolated from pigs in China (accession no. MG736312.1), although the plasmids harboring *mcr-8.1* in this study are truncated at the 5′ end and IS*903B* at the 3′ end was replaced by ISKpN14 (14) (Fig. 2C). It is possible that *mcr-8.1* originally was transposed to the IncFIB(pQil) plasmid by an IS*903B* composite transposon (Fig. 2B and C). An array of AMR genes ($bla_{\text{TEM-1b}}$ and $bla_{\text{CTX-M-15}}$) was in a composite transposon, flanked by insertion sequences (Fig. 2B). Incidentally, all resistance components in IncFIB(pQil) plasmids in this study were bracketed by IS*903B* from nucleotide position 20590 to 64656, demonstrating the potential for the transposition of the entire intervening DNA segment. The conjugation assay confirmed the transferability of the plasmid containing *mcr-8.1* to *E. coli* J53 with a frequency range of 3.1×10^{-2} to 8×10^{-2}. Phenotypically, the transconjugants were resistant to ampicillin, amoxicillin-clavulanate, 3rd-generation cephalosporins, trimethoprim-sulfamethoxazole, and colistin (Table S1).

The acquisition of a resistance plasmid may impose a fitness cost, depending on the host and plasmid backbones (19, 20). We found a significantly lower growth rate over time in TDM697b and TDM914b relative to that of *E. coli* J53 ($P < 0.0001$) (Fig. S4), implying a significant fitness cost owing to the acquisition of a plasmid harboring *mcr-8.1*. Compared to that of *E. coli* J53, a lower growth rate was also observed in TDM782b; however, the fitness cost was not statistically significant (Fig. S4).

This is the first report of transferable colistin resistance associated with human infections from Bangladesh. Given the acquisition of *mcr-8.1* on a conjugative plasmid, with good stability in ST15, a successful high-risk clone of *K. pneumoniae*, there is a serious risk of dissemination of *mcr-8.1* in South Asia.

Accession number(s). The nucleotide sequences of MCRPKP isolates are available under NCBI accession no. CP046939 to CP046947, CP046381 to CP046385, and CP046939 to CP046947.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TEXT S1, DOCX file, 0.02 MB.

TEXT S2, DOCX file, 0.02 MB.

TEXT S3, DOCX file, 0.02 MB.

FIG S1, TIF file, 0.9 MB.

FIG S2, TIF file, 0.1 MB.

FIG S3, TIF file, 0.4 MB.

FIG S4, TIF file, 0.3 MB.

TABLE S1, DOCX file, 0.01 MB.

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