

Letter to the Editor**Emergence of *mcr-1* mediated colistin resistant *Escherichia coli* from a hospitalized patient in Bangladesh**

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Abstract

Introduction: The emergence of plasmid mediated *mcr* in bacteria has become global public health threat. Herein, we report a *mcr-1* positive *E. coli* in normal human flora from a patient admitted in Dhaka Medical College Hospital (DMCH).

Methodology: In total, 700 non-duplicate rectal swabs were collected from DMCH during 13th May to 12th June 2018. *E. coli* from rectal swabs were isolated on chromogenic UTI media containing vancomycin 10mg/l (Liofilchem, Italy) and confirmed by MALDI-TOF. Minimum inhibitory concentrations (MIC) were determined by agar dilution and interpreted according to EUCAST breakpoints. Genomic analysis of *mcr* positive *E. coli* (MCRPEC) was performed by Illumina MiSeq sequencing and pulsed field gel electrophoresis (PFGE) using S1 nuclease DNA digests and *bla_{mcr-1}* probing. Transferability of *bla_{mcr-1}* were determined by conjugation assays.

Results: We found one MCRPEC from 700 rectal swab screening which was isolated from the rectal swab culture of a 17-year boy who was admitted to the burns ICU, DMCH with 53% flame burn involving much of the trunk and face. Genome sequencing revealed that *mcr-1* was present on an IncH12 plasmid of 257,243 bp and flanked by ISApal1. The colistin resistance can be transferred to the recipient *Klebsiella varicola* with a frequency of 8.3×10^{-5} . Transconjugants were more resistant to colistin than donor (MIC 32 µg/mL).

Conclusions: This is the first human associated *mcr* in Bangladesh. These data indicate the need for a systematic “one health” surveillance in the country.

Key words: *mcr-1*; human; Bangladesh.

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The Study

The emergence of the plasmid mediated colistin resistance gene, *mcr* in bacteria of environmental, animal, food and human origin has become global public health threat and radically limits therapeutic options for multidrug resistant (MDR) Gram-negative bacterial infections [1,2]. There are few studies on the prevalence of *mcr* in Southeast Asia. Recently, *mcr* has been detected in the food-chain and environment from Pakistan, India and Bangladesh [3-5]. Here, we report the presence of *mcr-1* in an *E. coli* strain retrieved from human faecal flora in a Bangladeshi hospital and provide evidence of its emergence into the clinical sector.

In total, 700 non-duplicate rectal swabs were collected from both inpatients and the outpatient department (OPD) of Dhaka Medical College Hospital (DMCH) from the 13th May to 12th June 2018. Informed consent was taken from each participant and the project was ethically approved by Ethical Review Committee of DMCH (MEU-DMC/ECC/2017122). *E. coli* from rectal swabs were isolated on chromogenic UTI media containing vancomycin 10mg/Liter (Liofilchem, Roseto degli Abruzzi, Italy) and confirmed by MALDI-TOF. Minimum inhibitory concentrations (MIC) were determined by agar dilution and interpreted according to EUCAST breakpoints [6]. *E. coli* resistant to colistin were examined by PCR for *mcr* variants. Genomic analysis of *mcr* positive *E. coli* (MCRPEC) was

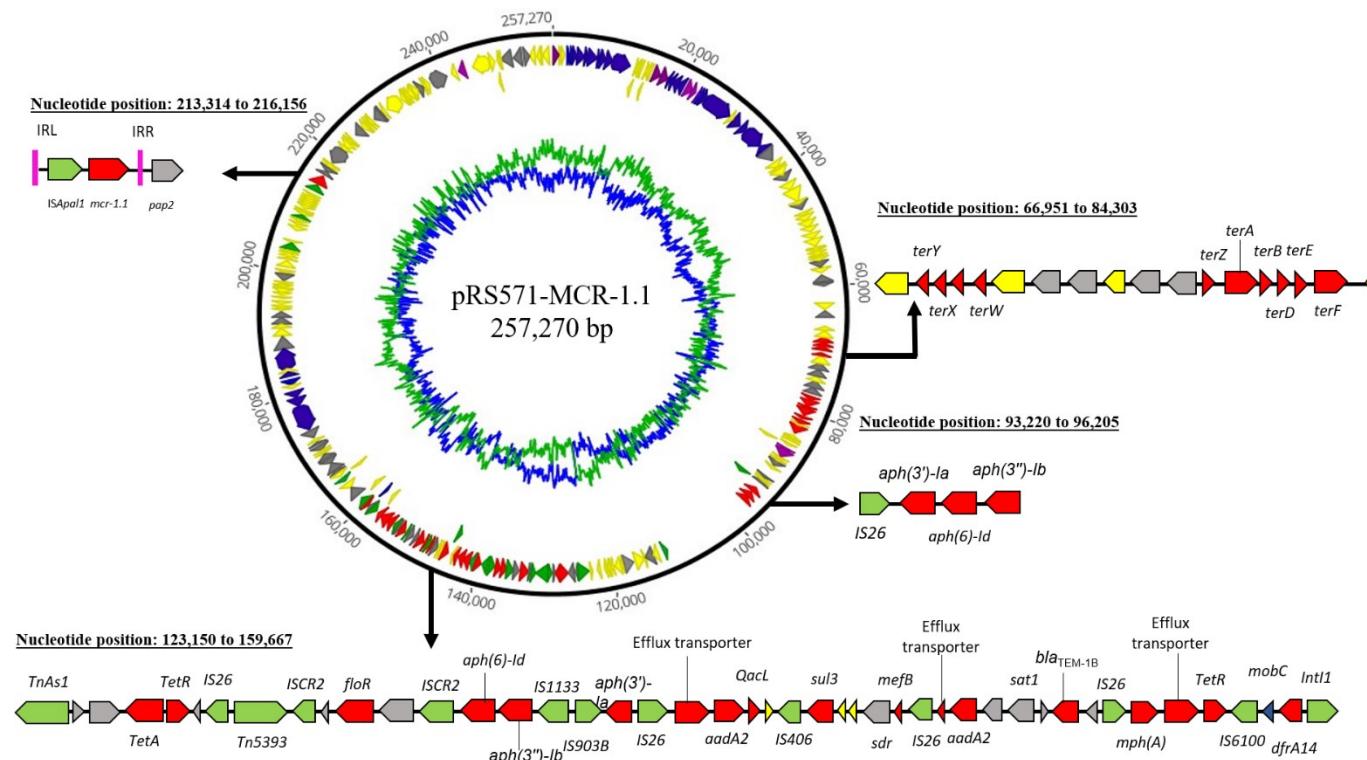
performed by Illumina MiSeq (Illumina Inc., San Diego, CA) (please see Supplementary methods) and minION (Oxford Nanopore technologies, Oxford, UK) sequencing (please see Supplementary methods) and pulsed field gel electrophoresis (PFGE) using S1 nuclease DNA digests and *bla_{mcr-1}* probing. Conjugation assays used a *Klebsiella varicola* strain (accession number: PJQN00000000) as the recipient. Transconjugants were selected on UTI agar (colistin 4mg/Liter) and investigated for *mcr-1* by PCR (please see Supplementary methods).

The MCRPEC (RS571; accession no: CP034389-CP034392) was isolated from the rectal swab culture of a 17-year boy who was admitted to the burn's ICU, DMCH with 53% flame burn involving much of the trunk and face. He suffered a smoke inhalation injury and became severely hypoxic. The patient worked as a laborer in a garment factory in Munshiganj district and the injury was sustained due to a gas pipe leak. Rectal swabs were collected 2 days after admission so RS571 could have been acquired in the community or the hospital. Clindamycin and ceftriaxone treatment were commenced early in the hospital stay. The patient died on day 5 of the admission, with the cause of death given

as septicemia. No clinical specimens were referred for culture during his hospital stay so it is not known whether RS571 was colonising flora only or played a pathogenic role, although it was resistant to all of the antibiotics received during the admission.

Whole genome sequencing revealed a 1626 bp open reading frame (ORF) encoding putative phosphoethanolamine transferase, consisting of 541 amino acids which exhibited 100% nucleotide sequence identity to *mcr-1.1* (Supplementary Figure 1) [1]. *E. coli* (RS571) belonged to sequence type 648. The strain was resistant to amoxicillin-clavulanate (MIC >256 µg/mL), piperacillin-tazobactam (MIC 8 µg/mL), ceftazidime (MIC 32 µg/mL), cefotaxime (MIC >256 µg/mL), cefepime (MIC 32 µg/mL), ciprofloxacin (MIC 256 µg/mL), gentamicin (MIC 256 µg/mL), trimethoprim-sulfamethoxazole (MIC 256 µg/mL) and colistin (MIC 16 µg/mL). *E. coli* RS571 was susceptible to imipenem (MIC 0.25 µg/mL), meropenem (MIC 0.25 µg/mL), amikacin (MIC 8 µg/mL), fosfomycin (MIC ≤ 1 µg/mL) and tigecycline (MIC 0.25 µg/mL) (Table 1).

Figure 1. Structural organization of plasmid, pRS571-MCR-1.1 (Accession no: CP034390).

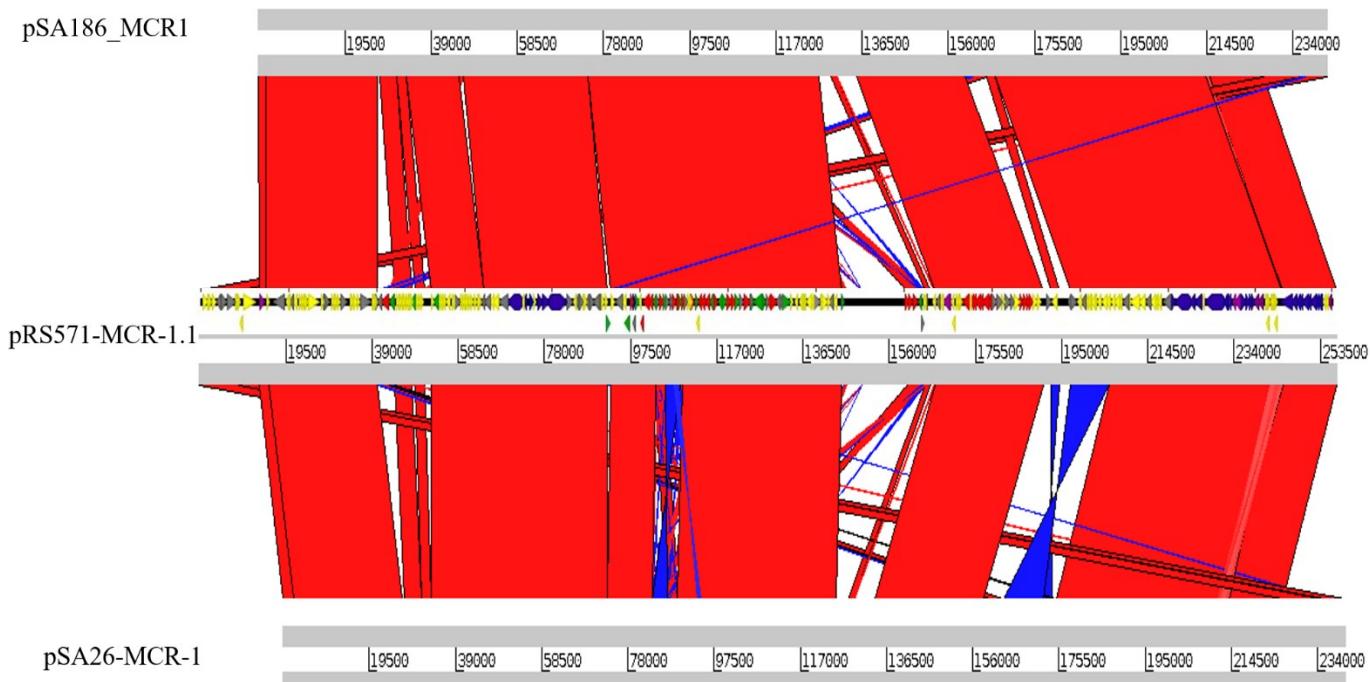


Schematic representation of genetic context of resistance loci are represented in further detail. Arrows represent the position and transcriptional direction of the open reading frames. Resistance genes are represented by red, transposase elements in green, genes associated with conjugation in blue, replication-associated genes in purple, regulatory/accessory genes in grey, hypothetical proteins in yellow and inverted repeats (IRs) in pink.

Table 1. MIC of antimicrobials against transconjugants obtained in this study along with donor (RS571, accession no: CP034389- CP034392) and recipient (BD_DM_07, accession no: PJQN00000000).

Antibiotics	Donor (RS571) ¹	Recipient (BD_DM_07) ¹	Transconjugant ¹
Piperacillin-tazobactam	8	4	8
Ceftazidime	32	0.125	0.25
Cefotaxime	>256	0.125	0.25
Cefepime	32	0.125	0.125
Imipenem	0.25	0.125	0.25
Meropenem	0.25	≤0.06	≤0.06
Ciprofloxacin	256	4	4
Levofloxacin	32	0.5	1
Amikacin	8	4	4
Gentamicin	256	4	128
Trimethoprim-sulfamethoxazole	256	64	256
Fosfomycin	1	8	8
Minocycline	1	16	16
Tigecycline	2	2	4
Colistin	16	0.5	32

¹MIC values for the antibiotics are in µg/mL.

Figure 2. Linear comparison of plasmid sequence pRS571-MCR-1.1 (Accession no: CP034390), pSA186_MCR1 (Accession no. CP022735.1) and pSA26-MCR-1 (Accession no. KU743384.1).

Forward matches are indicated by red and reverse matches by blue. Region of sequence with homology to the other genomes are separated by black lines. Genes associated with pRS571-MCR-1.1 are represented by colour. Resistance genes are represented by red, transposase elements in green, genes associated with conjugation in blue, replication-associated genes in purple, regulatory/accessory genes in grey and hypothetical proteins in yellow. Genomic comparison was performed by artemis comparison tool (ACT) (v.18.0.1).

In addition to *mcr-1*, other resistance genes such as *bla_{TEM-1B}*, *aac(3')-IId*, *aph(3'')-Ib*, *aph(6)-Id*, *aph(3')-Ia*, *aadA1*, *aadA2*, *sul3*, *dfrA14*, *mph(A)*, *tet(A)*, *catB3*, *cml* and *floR* (plasmid mediated) and *aac(6')-Ib-cr*, *aac(3')-IIa*, *bla_{TEM-1B}*, *bla_{OXA-1}* and *bla_{CTXM-15}* (chromosomal) were present.

The draft genome sequence of *E. coli* strain RS571 was assembled into 4 contigs with an N_{50} length of 5,085,958 bp. A 257,243 bp long plasmid which carried *mcr-1.1* was named pRS571-MCR-1.1 (accession no: CP034390) and belongs to the IncH12-ST3 replicon type. One copy of *mcr-1* (nucleotide 214,483 to 216,108) was found in pRS571-MCR-1.1, flanked by *ISApalI* (nucleotide 213,314 to 214,287) at the 5' end (Figure 1). Other resistance genes such as *tet(A)*-*floR*-*aph(6)-Id*-*aph(3'')-Ib*-*aph(3')-Ia*-*aadA1*-*sul3*-*aad2*-*bla_{TEM-1B}*-*mph(A)*-*dfrA14* were carried elsewhere (Figure 1), suggesting independent capture of *mcr-1* by *ISApalI*. The variable region of pRS571-MCR-1.1 shared the most identity with pSA186_MCR1 (CP022735.1) and pSA26-MCR-1 (KU743384.1) (88% coverage and 99% nucleotide identity for both), isolated from the Arabian Peninsula (Figure 2) [7]. The colistin resistance in *E. coli* can be transferred to the recipient *Klebsiella varicola* and with a transfer frequency of 8.3×10^{-5} . Transconjugants were more resistant to colistin than donor (MIC 32 µg/mL) and conferred resistance to gentamicin and trimethoprim-sulfamethoxazole as well (Table 1).

Conclusion

This is the first comprehensive report of *mcr* in humans from South-Asia, and detected on a MDR IncH12 plasmid that is widespread throughout China [8,9]. However, *mcr* positive strains were detected from the food-chain in India and Pakistan those were related to IncH12 and IncI2 plasmids, respectively [3,4]. These data indicate the need for a “one health” surveillance system to prevent the spread of mobile colistin resistance.

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Annex – Supplementary Items

Supplementary Methods

Transferability of the mcr-1 gene by conjugation

Conjugation experiment was performed using isolates carrying the *mcr-1* gene as donors (RS571) and *Klebsiella variicola* (BD_DM_07) as recipient. Donor and recipient strains were grown in Luria broth (Sigma-Aldrich, Darmstadt Germany) until they reached the exponential growth phase (optical density at 620 nm [OD₆₂₀] of 0.6). Conjugation experiments were performed on chromogenic UTI agar (Sigma-Aldrich, Darmstadt, Germany) plates, using a 1:1 donor-recipient mix, and plates were incubated at 37°C for 6 hours. The conjugation mixture was suspended in 1 mL of phosphate-buffered saline, and dilutions were plated on chromogenic UTI agar containing colistin (4 µg/mL) for selection. Blue colonies on chromogenic UTI agar containing colistin (4 µg/mL) were considered as transconjugants. Transferability of *mcr-1* in transconjugants was confirmed by PCR. Transfer frequencies were calculated by colony forming unit (cfu) count of transconjugants against the cfu count of donor.

Illumina MiSeq sequencing and bioinformatics

Whole genome sequencing (WGS) was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Briefly, gDNA was extracted from overnight culture using the QIAcube (Qiagen, Hilden, Germany), and resulting gDNA was quantified using the Qubit 3.0 (Thermos Fisher Scientific). DNA libraries were prepared for paired end sequencing (2×301 cycles) using Nextera XT. Quality control of raw reads included fastqc (0.11.2), and quality and adaptor trimming was performed using Trimgalore (0.4.3). Reads were assembled in contigs using the *de novo* assembler SPAdes (3.9.0) (fasta) and were aligned to the original fastq reads using BWA aligner (0.7.15). Any error was corrected using Pilon (1.22). Assembly metrics were evaluated using Quast (2.1).

MinION sequencing and bioinformatics

MCR-1 positive strain was sequenced by MinION sequencing (Oxford Nanopore Technologies Ltd., Oxford Science Park, UK). Large scale bacterial gDNA was extracted. Two loop full bacterial colony in 9.5 mL of TE buffer was mixed with 50 µL proteinase K (20mg/mL) and 0.5 mL of 10% SDS. After incubation at 37°C for 1 hour, 1.8 mL heated 5M NaCl was added and was incubated at 65°C for 5 minutes. The suspension was treated with 1.5 mL of heated CTAB/NaCl and was incubated at 65°C for 20 minutes. Then equal volume of chloroform was added into the mixture followed by gently shaking for 1 hour. The mixture was centrifuged at 13,000 rpm for 15 minutes. Supernatant was taken and equal volume of isopropanol was added until visualizing the clumping of gDNA. DNA was washed with 70% ethanol and 200 µL of water was added to dissolve the gDNA. DNA library was prepared by pooling of all barcoded samples to aim a final DNA concentration >500ng/µL and 1 µL of RAD was added to DNA. A final mixture of 75 µL (34 µL sequencing buffer, 30 µL water and 11 µL DNA library) was loaded to flow cell. MinION device was connected to MinKNOW GUI to obtain the reads. Demultiplexing of Nanopore reads was performed by Porechop (0.2.3). Unicycler (0.4.4) was used to yield hybrid assembly using both Illumina short reads and minION long reads.

Supplementary Figure 1. Phylogenetic tree of the amino-acid sequences of phosphoethanolamine transferases MCR variants (MCR1-8) using Geneious (10.2; Biomatters Ltd.).

