

# Acquired resistome and plasmid sequencing of *mcr-1* carrying MDR Enterobacteriaceae from poultry and their relationship to STs associated with humans

Antoine Abou Fayad <sup>1,2,3</sup>†, Maria El Azzi<sup>1,2,3</sup>†, Ahmad Sleiman<sup>1,2,3</sup>, Issmat I. Kassem<sup>4,5</sup>, Reema A. Bawazeer<sup>6,7</sup>, Liliane Okdah<sup>6,7</sup>, Michel Doumith <sup>6,7</sup>, Majed F. Alghoribi<sup>6,7</sup> and Ghassan M. Matar<sup>1,2,3\*</sup>

<sup>1</sup>Department of Experimental Pathology, Immunology and Microbiology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon; <sup>2</sup>Center for Infectious Diseases Research, American University of Beirut, Beirut, Lebanon; <sup>3</sup>World Health Organization (WHO) Collaborating Center for Reference and Research on Bacterial Pathogens, Beirut, Lebanon; <sup>4</sup>Department of Nutrition & Food Science, American University of Beirut, Beirut, Lebanon; <sup>5</sup>Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA; <sup>6</sup>Infectious Diseases Research Department, King Abdullah International Medical Research Center, Riyadh, Saudi Arabia; <sup>7</sup>King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

\*Corresponding author. E-mail: [gmatar@aub.edu.lb](mailto:gmatar@aub.edu.lb)

†These authors contributed equally.

Received 1 June 2021; accepted 17 December 2021

**Objectives:** To investigate the acquired resistome in 18 colistin-resistant *Escherichia coli* isolated from different poultry farms in Lebanon, analyse Inc plasmids associated with *mcr* and assess potential transmission to humans.

**Methods:** A total of 18 *E. coli* were recovered from poultry faeces collected from different poultry farms in Lebanon. Broth microdilution (BMD) assay was performed to determine the antimicrobial resistance profiles. WGS was used to identify the genetic determinants behind the resistance in these isolates.

**Results:** BMD results showed that all of the 18 isolates were colistin resistant. Furthermore, resistance to trimethoprim/sulfamethoxazole was the most recorded among the isolates and only one isolate was resistant to cefepime. Sequencing results showed that the isolates were distributed into seven different STs and that the most abundant was ST1140. The number of antimicrobial resistant determinants ranged from 4 to 21 among the 18 isolates, with *tet(A)* and *floR* being the most frequent. Moreover, a total of 15 different plasmid replicon types were identified. The *mcr-1* gene was shown to be predominantly located on IncX4 plasmids. Additionally, two isolates harboured the IncI2-type self-conjugative plasmid.

**Conclusions:** The findings show that *mcr* and other important resistance determinants occur in MDR *E. coli* isolated poultry farms in Lebanon. The occurrence of *mcr* on mobile plasmids and the zoonotic potential and clinical relevance of some strains highlight a risk of transmission to humans.

## Introduction

The emergence of MDR bacteria has resulted in the use of colistin (polymyxin E) as a last resort for treatment of severe Gram-negative bacterial infections.<sup>1</sup> This induced a reliance on this antibiotic and the likely selection of colistin-resistant strains.<sup>2</sup> Initially, colistin resistance was believed to be solely caused by chromosomal mutations. However, the discovery of the plasmid-borne mobile colistin resistance gene, *mcr-1*, in

2015 in China indicated that resistance can also be transmitted laterally. Today, *mcr* is spreading globally and colistin use in animal farming is thought to be a major driver in this dissemination.<sup>3</sup> The *mcr-1* gene has been reported in approximately all food-producing animal species in 57 countries distributed over five continents.<sup>4</sup> Therefore, this study aimed to investigate the acquired resistome, Inc plasmid types, and STs of 18 colistin-resistant *Escherichia coli* isolates from different poultry farms in Lebanon.

## Methods

### Bacterial isolates

The faecal samples were suspended in sterile buffered peptone water and homogenized gently by pipetting. An aliquot (100 µL) from each suspension was spread onto the selective medium RAPID'E. coli 2 agar (Bio-Rad, USA), which was supplemented with 4 mg/L colistin (Sigma-Aldrich, USA). The isolates ( $n=90$ ) that exhibited a typical *E. coli* phenotype on the agar (violet to pink colour) were further purified and their identity was confirmed by PCR targeting a species-specific fragment of the 16S rRNA gene.<sup>5</sup> A total of 18 *E. coli* isolates were included in this study. Ten (56%) and eight (44%) *E. coli* isolates were recovered between 2017 and 2018 from broiler faeces collected from poultry farms located in the north and south of Lebanon, respectively.<sup>6</sup> These animals were housed in three major farms in Lebanon (two farms in the north and one in the south).

### Broth microdilution (BMD) assay

BMD was performed using 19 different antibiotics. Serial dilution ranged from 1024 mg/L to 1 mg/L. The plate was incubated at 37°C for 18 h and the experiments were run in duplicate for each antibiotic. The results were interpreted according to the CLSI M100 guidelines.<sup>7</sup>

### Transformation

Plasmids were extracted using the QIAGEN® Plasmid Mini Kit (QIAGEN, USA) and transformed into recipient chemically competent *E. coli* JM109 cells using the heat-shock method.<sup>8,9</sup> Competent *E. coli* (50 µL) were mixed with 10 µL of the extracted plasmid and incubated on ice for 30 min. In a separate reaction, 10 µL deionized water (no plasmids added) was used as a control. The mixtures were heat shocked at 42°C for 60 s in a water bath and incubated on ice for 2 min. Sterile LB broth (940 µL) was added, and the mixtures were placed in a shaking incubator (180 rpm) for 90 min at 37°C. The mixtures were centrifuged for 2 min at 14 000 rpm, and the pellet was resuspended in 0.1 mL LB broth, which

was spread onto RAPID'E. coli 2 agar plates supplemented with 2 mg/L of colistin. The plates were incubated at 37°C for 18–24 h. Putative transformants were harvested and analysed for the acquisition of *mcr-1* using PCR and colistin resistance using the BMD assay as described above.<sup>6</sup>

### WGS and bioinformatics

Genomic DNA was extracted from overnight cultures using the QIAamp DNA mini kit (QIAGEN, USA) according to manufacturer's instructions. The quality and yield of the extracted DNA were checked on the nanodrop and qubit and then prepared for sequencing using the Nextera XT DNA library preparation kit (Illumina, UK). Sequencing was performed on the MiSeq instrument using the 2 × 300 paired-end protocol. Generated reads were assembled into contigs using shovill 1.0.9 (<https://github.com/tseemann/shovill>) with the default options. MLST were inferred from assembled genomes with the mlst-v2.18.1 software, while antimicrobial resistance genes were identified using Abricate 0.9.8 (<https://github.com/tseemann/abricate>). Chromosomal mutations associated with antimicrobial resistance were detected using gene\_finder ([https://github.com/phe-bioinformatics/gene\\_finder](https://github.com/phe-bioinformatics/gene_finder)). Plasmids were determined using PlasmidFinder on CGE (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>). To confirm the complete structure of the *mcr-1*-bearing IncX4- and IncI2-type plasmids, DNA from isolates ECOL79 and ECOL80 were subjected to low coverage sequencing on the MinION sequencer using the nanopore ligation sequencing kit according to the manufacturer's instructions. Hybrid assembly of short and long reads were performed using Unicycler 0.4.8.<sup>10</sup> Plasmid contigs from assembled genomes using the Illumina reads were compared using BLASTn and BRIG.<sup>11</sup>

## Results

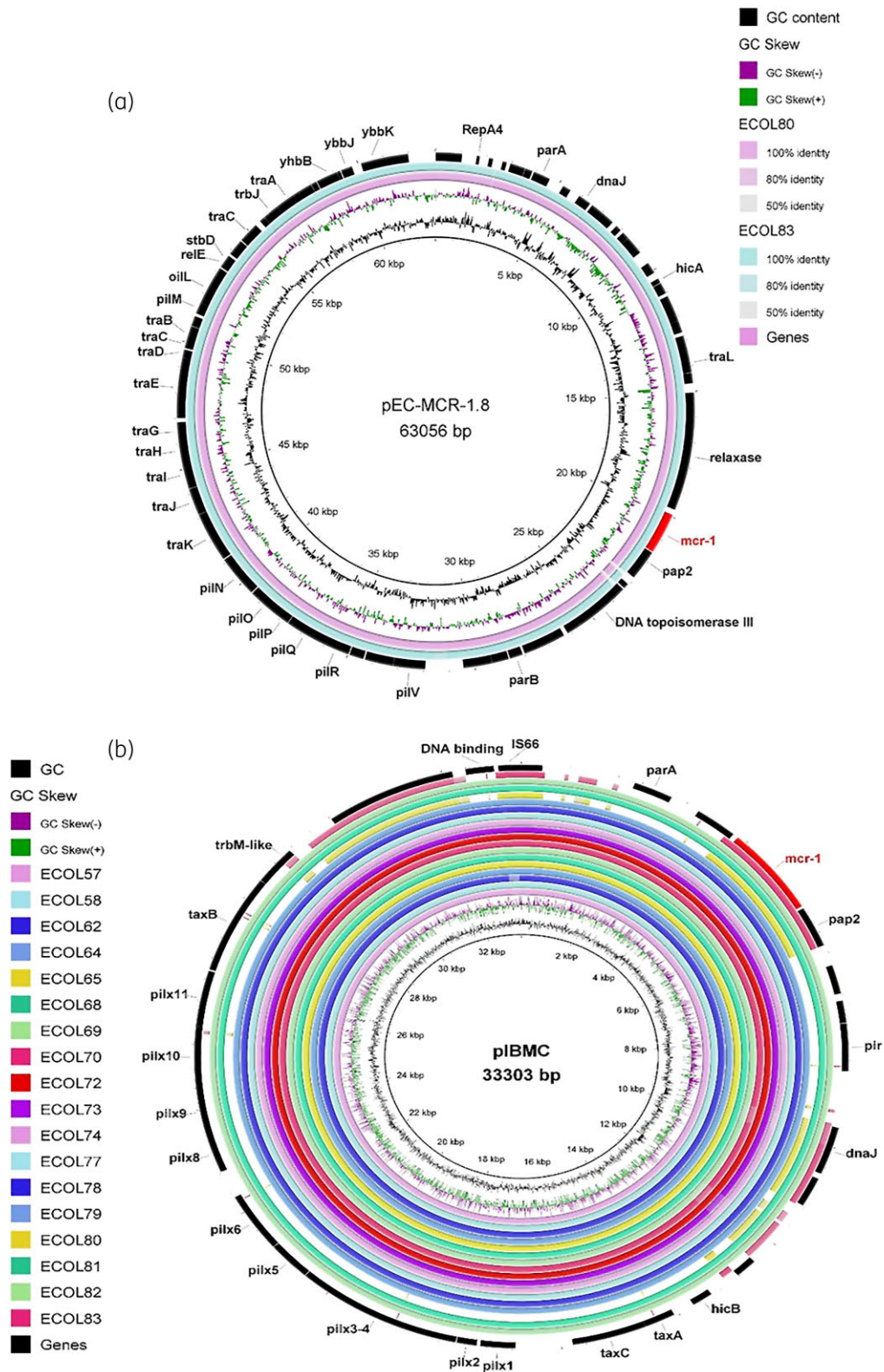
### Antimicrobial susceptibility testing

BMD results showed that all of the 18 (100%) *E. coli* isolates were resistant to colistin with MICs ranging between 4 and 8 mg/L.

**Table 1.** Antimicrobial susceptibility testing results of the 18 *E. coli* isolates

Antimicrobial family	Antibiotic	No. resistant (%)	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
Cephalosporins	Cefuroxime	11 (61%)	2 to 1024	64	1024
	Ceftazidime	8 (44%)	≤ 1 to 64	≤ 1	32
	Cefepime	1 (6%)	≤ 1 to 32	≤ 1	8
Carbapenems	Meropenem	0 (0%)	≤ 1	≤ 1	≤ 1
	Imipenem	0 (0%)	≤ 1 to 2	≤ 1	≤ 1
	Ertapenem	0 (0%)	≤ 1	≤ 1	≤ 1
Monobactams	Aztreonam	7 (39%)	≤ 1 to 64	8	32
Fluoroquinolones	Ciprofloxacin	14 (78%)	≤ 1 to 32	16	32
	Levofloxacin	14 (78%)	≤ 1 to 32	8	32
Aminoglycosides	Gentamicin	12 (67%)	≤ 1 to 512	64	512
	Amikacin	0 (0%)	≤ 1 to 4	≤ 1	4
Tetracyclines	Tetracycline	15 (83%)	≤ 1 to 256	128	256
	Tigecycline	13 (72%)	0.25 to 2	0.5	1
Polymyxins	Colistin	18 (100%)	4 to 8	4	8
Macrolides	Azithromycin	9 (50%)	≤ 0.125 to > 128	16	> 128
Fosfomycin	Fosfomycin	10 (56%)	2 to > 1024	1024	> 1024
Sulphonamides	Trimethoprim/sulfamethoxazole	16 (89%)	≤ 0.25 to > 256	> 256	> 256
β-lactam + β-lactamase inhibitor	Piperacillin/tazobactam	0 (0%)	≤ 1 to 16	8	16
	Ceftolozane/tazobactam	9 (50%)	≤ 1 to 8	2	8

MIC<sub>50</sub>, MIC value at which 50% of the 18 *E. coli* isolates were inhibited; MIC<sub>90</sub>, MIC value at which 90% of the 18 *E. coli* isolates were inhibited.



**Figure 1.** Circular graphical map showing BLAST comparisons of the *mcr-1* carrying plasmids identified in (a) Isolates 80 and 83 against IncI2-type plasmid pEC-MCR1.8 (accession number: KY792081) and (b) the remaining isolates against IncX4-type plasmid pIBMC\_mcr1 (accession number: MF449287.1). The *mcr-1* gene location is shown in the figure with all the other genes. The colour intensity in each ring represents the BLASTn match identity as shown in the legend of (a).

In total, 16 (89%) isolates were resistant to trimethoprim/sulfamethoxazole, 15 (83%) to tetracycline, 14 (78%) ciprofloxacin and levofloxacin, 13 (72%) to tigecycline, 12 (67%) to gentamicin, 11 (61%) to cefuroxime, 10 (56%) to fosfomycin, 9 (50%) to azithromycin and ceftolozane/tazobactam, 8 (44%) to ceftazidime and 7 (39%) to aztreonam, and only 1 (6%) isolate showed resistance to ceftazidime. None of the isolates was resistant to piperacillin/tazobactam, amikacin, ertapenem, meropenem or imipenem (Table 1 and Table S1, available as [Supplementary data](#) at JAC-AMR Online). Moreover, all the isolates were MDR (Table S1).

### Transmissibility of the *mcr-1*-carrying plasmids

Plasmids extracted from the *mcr-1*-positive *E. coli* were successfully transformed into recipient *E. coli* cells, which was evident by the formation of transformant colonies on the RAPID'E. coli 2 agar plates supplemented with colistin. The transformants were found positive for *mcr-1* using PCR analysis. Additionally, the tested transformants were colistin resistant (MIC > 2 mg/L).

### WGS

Genome sequence analyses showed that the 18 *E. coli* isolate were distributed into seven different STs, which included ST1140 ( $n=7$ ), ST226 ( $n=3$ ), ST2705 ( $n=3$ ), ST162 ( $n=2$ ), ST2936 ( $n=1$ ), ST3288 ( $n=1$ ) and ST6448 ( $n=1$ ). The *E. coli* isolates recovered from poultry faeces from the south belonged to ST1140 ( $n=5$ ), ST162 ( $n=2$ ) and ST6448 ( $n=1$ ). Furthermore, *E. coli* isolates obtained from poultry faeces from farms in the north belonged to ST2705 ( $n=3$ ), ST226 ( $n=3$ ), ST1140 ( $n=2$ ), ST2936 ( $n=1$ ) and ST3288 ( $n=1$ ). The number of antimicrobial resistance determinants ranged from 4 to 21 among the 18 isolates. The *tet(A)* and *floR* genes were the most frequent, and they were detected in 15 (83%) out of the 18 *E. coli* isolates. Moreover, several  $\beta$ -lactamase genes were detected, including: *bla*<sub>TEM-1</sub> ( $n=12$ ) and *bla*<sub>TEM-122</sub> ( $n=5$ ) as penicillinases, *bla*<sub>CTX-M-3</sub> ( $n=4$ ) and *bla*<sub>CTX-M-55</sub> ( $n=1$ ) as ESBLs, and *bla*<sub>CMY-2</sub> ( $n=7$ ) as plasmid-borne cephalosporinase. Furthermore, resistance to fluoroquinolones was either due to two mutations in the *gyrA* gene (S83L and D87N) and one in the *parC* gene (S80I) ( $n=13$ ) or one mutation in the *gyrA* gene (S83L) ( $n=1$ ). In addition, 12 out of the 18 *E. coli* harboured the macrolide resistance genes *Inu(F)* and *mph(A)*. Finally, eight different aminoglycosides resistance genes were detected (Table S2).

A total of 15 different plasmid replicon types were detected in the isolates; distributed as follows: IncX4 ( $n=16$ ), IncFII(pCoo) ( $n=10$ ), IncI2(Delta) ( $n=7$ ), IncHI2 ( $n=6$ ), IncHI2A ( $n=6$ ), IncFIB(K) ( $n=6$ ), IncFIB(AP001918) ( $n=5$ ), IncFIA ( $n=4$ ), IncI1-I(Alpha) ( $n=4$ ), IncFII ( $n=4$ ), IncFIC(FII) ( $n=3$ ), pO111 ( $n=3$ ), IncFII(29) ( $n=2$ ), IncI2 ( $n=1$ ) and IncFIB(pLF82-PhagePlasmid) ( $n=1$ ) (Table S2).

The *mcr-1.1* gene in the majority of sequenced isolates (16/18, 88.9%) was located on a 33.3 kb circular contig sharing high similarity with IncX4-type plasmids harbouring *mcr* variants (coverage 97%–100%, identity 99%–100%) that were previously reported in various Enterobacteriales species, including *Salmonella enterica*, *E. coli* and *Klebsiella pneumoniae* (Figure 1b).<sup>12</sup> The draft genome of Isolate 80 located *mcr-1* on a 64.7 kb circular contig that was most similar to the IncI2-type self-conjugative plasmid bearing *mcr-1.8* (92% coverage with 99.46% identity, accession number:

KY792081.2). Sequence comparison confirmed that Isolate 83 had the same IncI2-type *mcr-1*-harbouring plasmid (Figure 1a). Moreover, no other resistance genes were detected on both plasmids.<sup>13</sup>

## Discussion

In 2017, an *E. coli* isolate belonging to ST2705 was recovered from a stool sample of a patient admitted to Hôtel-Dieu de France hospital in Beirut.<sup>14</sup> This matches with the STs of three of the *E. coli* in this study. Moreover, in the University Hospital of Larissa-Central Greece an *E. coli* isolate belonging to ST1140 was detected in a patient,<sup>15</sup> and this is the most abundant ST in this study. These findings show that MDR strains harbouring *mcr* with zoonotic potential occur in poultry farming in Lebanon. Urgent action is needed to control the dissemination of these determinants and strains on poultry farms in order to control the emergence of pandrug resistant bacteria that can affect both humans and animals in a One Health approach.

## Funding

This work was funded by the Medical Practice Plan at the American University of Beirut Medical Center.

## Transparency declarations

None to declare.

## Supplementary data

Tables S1 and S2 are available as [Supplementary data](#) at JAC-AMR Online.

## References

- 1 Ahmed ZS, Elshafie EA, Khalefa HS *et al.* Evidence of colistin resistance genes (*mcr-1* and *mcr-2*) in wild birds and its public health implication in Egypt. *Antimicrob Resist Infect Control* 2019; **8**: 197.
- 2 Islam S, Urmi UL, Rana M *et al.* High abundance of the colistin resistance gene *mcr-1* in chicken gut-bacteria in Bangladesh. *Sci Rep* 2020; **10**: 17292.
- 3 Liu Y-Y, Wang Y, Walsh TR *et al.* Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016; **16**: 161–8.
- 4 Nagy Á, Székelyhidi R, Hanczné Lakatos E *et al.* Review on the occurrence of the *mcr-1* gene causing colistin resistance in cow's milk and dairy products. *Heliyon* 2021; **7**: e06800.
- 5 Kassem II, Mann D, Li S *et al.* Draft genome sequences and resistome analysis of multidrug-resistant *mcr-1*-harbouring *Escherichia coli* isolated from pre-harvest poultry in Lebanon. *J Glob Antimicrob Resist* 2021; **25**: 114–6.
- 6 Hmede Z, Kassem II. The colistin resistance gene *mcr-1* is prevalent in commensal *Escherichia coli* isolated from preharvest poultry in Lebanon. *Antimicrob Agents Chemother* 2018; **62**: e01304–18.
- 7 CLSI. Performance Standards for Antimicrobial Susceptibility Testing—Thirtieth Edition: M100. 2020.
- 8 Sulaiman AAA, Kassem II. First report on the detection of the plasmid-borne colistin resistance gene *mcr-1* in multi-drug resistant *E. coli*



isolated from domestic and sewer waters in Syrian refugee camps in Lebanon. *Travel Med Infect Dis* 2019; **30**: 117–20.

**9** Hmede Z, Sulaiman AAA, Jaafar H *et al*. Emergence of plasmid-borne colistin resistance gene *mcr-1* in multidrug-resistant *Escherichia coli* isolated from irrigation water in Lebanon. *Int J Antimicrob Agents* 2019; **54**: 102–04.

**10** Wick RR, Judd LM, Gorrie CL *et al*. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595.

**11** Alikhan N-F, Petty NK, Ben Zakour NL *et al*. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* 2011; **12**: 402.

**12** Sun J, Fang L-X, Wu Z *et al*. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the *mcr-1* acquisition. *Sci Rep* 2017; **7**: 424.

**13** Li R, Xie M, Zhang J *et al*. Genetic characterization of *mcr-1*-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. *J Antimicrob Chemother* 2017; **72**: 393–401.

**14** Al-Mir H, Osman M, Azar N *et al*. Emergence of clinical *mcr-1*-positive *Escherichia coli* in Lebanon. *J Glob Antimicrob Resist* 2019; **19**: 83–4.

**15** Mavroidi A, Miriagou V, Liakopoulos A *et al*. Ciprofloxacin-resistant *Escherichia coli* in Central Greece: mechanisms of resistance and molecular identification. *BMC Infect Dis* 2012; **12**: 371.