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Letter to the Editor

Genomic characterization of plasmid-borne colistin resistance variants, *mcr-1.1* and *mcr-1.26*, in multidrug-resistant *Escherichia coli* isolated from backyard farm animals

Editor: Stefania Stefani

Colistin remains a high-priority, critically important antimicrobial that is used for treating severe infections caused by multidrug-resistant (MDR) Gram-negative bacteria. However, colistin resistance and the emergence of the mobile colistin resistance (*mcr*) genes have been increasingly documented in clinical and community settings, including in humans, animals, and the environment [1–3], which severely limits available antimicrobial options for addressing critical infections. Previous studies have reported that the wide dissemination of *mcr-1* across Lebanon, an East Mediterranean country, is a major concern, especially in agricultural areas. Recently, we have identified a novel *mcr* variant, *mcr-1.26*, which appears to be spreading in Lebanon and is rarely reported abroad. This variant has been detected in hospitalised patients [4], otherwise healthy people [2], and birds [3,5] in Lebanon. *Mcr-1.26*–harbouring bacteria appear to concurrently exhibit resistance to other clinically and agriculturally important antimicrobials. Subsequently, we aimed to assess the faecal carriage and antimicrobial susceptibility patterns of colistin-resistant *E. coli* in backyard food animals in largely disenfranchised areas of North and South Lebanon.

Many individuals in Lebanese rural and agricultural areas own backyard food animals that constitute an important source of nutrition and income. This is vital for disenfranchised communities that face critical socioeconomic challenges and have limited resources that are further weakened by the ongoing economic crisis in Lebanon. The latter has resulted in a lack of government subsidies and restricted access to affordable veterinary supportive care [6]. This prevailing situation often compels individuals to resort to self-prescribing practices, driven by the need to reduce costs and cope with limited access to affordable veterinary services. Consequently, it is necessary to investigate the spread of resistance to colistin and other antimicrobials in these settings [6].

Thirty-three fresh faecal swab samples were collected from different animals from 16 farms in July 2022. The samples were homogenised and spread onto RAPID'*E. coli* 2 Medium (Bio-Rad, Hercules, CA, USA) plates supplemented with colistin (3.5 mg/L) **(Supplementary data 1)**. After incubation for 24 hours at 37 °C under aerobic conditions, putatively colistin-resistant *E. coli* colonies were selected and purified. The identity of the isolates was further confirmed using MALDI-TOF VITEK MS (bioMérieux, Marcy L'Etoile, France). We successfully detected the presence of the MCR-1 protein in four colistin-resistant *E. coli* isolates using the NG-Test® MCR-1 (NG Biotech, Guipry, France) lateral flow immunoassay (LFA). These isolates were characterised by minimum inhibitory concentrations (MICs) of colistin that ranged between 4 and 8 mg/L. The isolates were also found to be resistant to more than three different antimicrobial classes, indicating that they were multi-drug resistant (MDR) (Table 1). Given that the *mcr-1* is the most prevalent mobile colistin resistance determinant, the NG-Test® MCR-1 LFA provides a valuable diagnostic tool in Lebanon and other low- and middle-income countries. In these settings, the Kirby–Bauer disk diffusion method has been commonly used for testing colistin susceptibility, but it lacks precision in distinguishing susceptible from resistant isolates [7].

Most of the farms investigated, including those contaminated with colistin-resistant *E. coli*, rely heavily on antimicrobials that are easily accessible in local agricultural drug stores [8,9]. Animal owners belong to disenfranchised populations who are unable to afford veterinary consultations and generally lack awareness about infectious diseases, appropriate antimicrobial use, and antimicrobial resistance [6]. Moreover, we observed a notable lack of adherence to good hygiene practices and an inadequate infrastructure (e.g., waste- and water-management issues, and absence of sustainable access to clean water and electricity) across the farms, which could contribute to the spread of infectious agents. These factors, in turn, may exacerbate the reliance on antimicrobials that are easily accessible off shelf and without prescriptions.

Whole-genome sequencing (Illumina, San Diego, CA, USA) analysis showed that the isolate DD063 harboured *mcr-1.1* and belonged to ST1431. Notably, an *mcr-1.1*–positive *E. coli* strain belonging to ST1431 had been isolated previously from the semen of a Lebanese patient from the same geographical area in Lebanon [7]. However, the remaining three isolates carried *mcr-1.26* and belonged to ST2207 (P6 and P50) and ST6856 (DD064). In comparison, *mcr-1.26*–positive *E. coli* that were previously reported in Lebanon belonged to ST69 carried by otherwise healthy university students [2], and ST2207 and ST3107 were isolated from a domesticated pigeon [5] and fresh chicken meat, respectively (Fig. 1) [3]. Notably, the MCR-1.26 variant features a Met1Thr substitution in its protein sequence when compared with MCR-1.1 [10].

Pairwise single-nucleotide polymorphism distances were calculated from core-genome alignments between the *mcr-1.26*–positive *E. coli* isolates from this study and previously reported cases. The analysis indicated potential clonal transmission of *mcr-1.26* among the domesticated pigeons. In comparison, the other *mcr-1.26*–positive *E. coli* isolated from humans, cattle, and chickens revealed potential polyclonal transmission of this variant gene in the human–animal continuum in Lebanon (Table S1). Using PlasmidFinder v2.1, we found that the *mcr* genes were carried on IncX4 plasmids. Furthermore, conjugation assays were successful with all isolates, and confirming that *mcr-1.1* and *mcr-1.26* were carried on transmissible IncX4 plasmids, conferring colistin resistance to otherwise naïve *E. coli*.

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Table 1
Genome analyses and antimicrobial susceptibility patterns of colistin-resistant *Escherichia coli* isolated from backyard farm animals in Lebanon.

Strain	Source	Colistin MIC (μg/mL)	Non-susceptibility to selected antimicrobials	Susceptibility to selected antimicrobials	Acquired antimicrobial resistance genes[a]	QRDR[b] mutations	Chromosome-mediated colistin resistance	Plasmid replicons (identity%)	ST	Virulence genes[c]	Human pathogen (probability)[d]
DD063	**Chicken**	4	AMX, AMC, TIC, PIP, TZP, CXN, FOX, CTX, TIO, CAZ, FEP, CFT, BPR, ATM, GMN, TMN, CHL, TET, SXT, FLO, NOR, CIP, LVX, CST	TEM, CAZ/AVI, ATM/AVI, ERT, IMP, IMP/REL, MEM, MEM/VAB, MEC, FDC, STR, NEO, AMK, APR, TGC, ERV, FUR, FSF	*mcr-1.1*[¶], bla_{TEM-1B}, bla_{CMY-2}, *aadA1, tet(A), dfrA1, qnrS13, qacE, sitABCD*	*gyrA* (D87N), *gyrA* (S83L), *parC* (S80I), *parE* (S458A)	*pmrA* (S29G), *pmrB* (D283G), *pmrB* (Y358N), *phoP* (I44L)	IncFIB (99.38%); Incl1-I (100%); IncX4 (100%)[¶]	ST1431	*capU, cib, cma, csgA, cvaC, fimH, gad, hlyE, hlyF, iroN, iss, ipfA, nlpI, ompT, sitA, terC, traJ, traT,*	93.2%
DD064	**Cattle**	4	AMX, AMC, TIC, PIP, TPZ, CXN, FOX, CTX, TIO, CAZ, CFT, BPR, ATM, TET, SXT, NOR, CIP, LVX, CST	TEM, FEP, CAZ/AVI, ATM/AVI, ERT, IMP, IMP/REL, MEM, MEM/VAB, MEC, FDC, STR, NEO, GMN, TMN, APR, AMK, TGC, ERV, FUR, CHL, FLO, FSF	*mcr-1.26*[¶], bla_{TEM-1B}, bla_{SHV-12}, bla_{OXA-10}, *aadA1, aadA5, aac(3)-IIa, sul1, sul3, tet(A), dfrA1, dfrA17, floR, cmlA1, arr-2, qnrS1, mph(A), qacE, sitABCD*	*parE* (R458A)	*pmrA* (S29G), *pmrA* (V129L), *pmrB* (H2R)	Col(BS512); IncFIB (99.38%); IncX4 (100%)[¶]	ST6856	*capU, cea, cma, csgA, cvaC, fimH, hlyE, hlyF, iroN, iss, ipfA, nlpI, terC, traJ, traT*	92.2%
P6	**Pigeon**	8	AMX, TIC, PIP, GMN, STR, NEO, TET, SXT, CHL, CST	AMC, TZP, CTX, TIO, CAZ, CAZ/AVI, FEP, CFT, BPR, ATM, ATM/AVI, ERT, IMP, IMP/REL, MEM, MEM/VAB, MEC, NOR, APR, AMK, TGC, ERV, FLO, FUR, NOR, CIP, LVX, FSF	*mcr-1.26*[¶], *aac(3)-IId, dfrA12*	–	*pmrA* (S29G)	IncFII(29) (100%); IncX1 (96.5%); IncX4 (100%)[¶]; P0111 (98.53%)	ST2207	*csgA, gad, hra, nlpI, ompT, terC, traT, yehD*	92.5%
P50	**Pigeon**	4	AMX, TIC, PIP, GMN, STR, TET, SXT, CHL, CST	AMC, TZP, CTX, TIO, CAZ, CAZ/AVI, FEP, CFT, BPR, ATM, ATM/AVI, ERT, IMP, IMP/REL, MEM, MEM/VAB, MEC, NOR, APR, AMK, TGC, ERV, FLO, NEO, FUR, NOR, CIP, LVX, FSF	*mcr-1.26*[¶], *aac(3)-IId, dfrA12*	–	*pmrA* (S29G)	IncFII(29) (100%); IncX1 (96.5%); IncX4 (100%)[¶]; P0111 (98.53%)	ST2207	*csgA, gad, hra, nlpI, ompT, terC, traT, yehD*	92.5%

[a] Acquired antimicrobial drug resistance genes detected by ResFinder v4.4.2 (http://genepi.food.dtu.dk/resfinder) using 99% identity as cut-off.
[b] QRDR, quinolone resistance-determining region.
[c] Virulence genes detected by VirulenceFinder v2.0 (https://cge.food.dtu.dk/services/VirulenceFinder/) using 98% identity as cut-off.
[d] Using PathogenFinder v1.1 (https://cge.food.dtu.dk/services/PathogenFinder/), the isolates were predicted to be a human pathogen.
[¶] The *mcr* gene was located on an IncX4 plasmid and induced colistin resistance in azide–resistant *E. coli* J53 or JM109 strains. The transmissibility of *mcr* genes in pigeon isolates was demonstrated via conjugation with a rifampicin-resistant *Salmonella enterica* serovar Enteritidis, as described previously [3]. Our results showed the ability of the IncX4 plasmids to transfer the *mcr-1* genes horizontally.

AMX, amoxicillin; AMC, amoxicillin-clavulanate; TIC, ticarcillin; PIP, piperacillin; TZP, piperacillin-tazobactam; CXN, cephalexin; FOX, cefoxitin; CTX: cefotaxime, TIO, ceftiofur; CAZ, ceftazidime; FEP, cefepime; CFT, ceftaroline; BPR, ceftobiprole; ATM, aztreonam; GMN, gentamicin; TMN, tobramycin; CHL, chloramphenicol; NOR, norfloxacin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole, CST, colistin; TGC, tigecycline; TEM, temocillin; CAZ/AVI, ceftazidime-avibactam; ATM/AVI, aztreonam-avibactam; ERT, ertapenem; IMP, imipenem; IMP/REL, imipenem-relebactam; MEM, meropenem; MEM/VAB, meropenem-vaborbactam; MEC, mecillinam; STR, streptomycin; NEO, neomycin; TET, tetracycline; FDC, cefiderocol; AMK, amikacin; APR, apramycin; ERV, eravacycline; FUR, nitrofurantoin; FLO, florfenicol; FSF, fosfomycin; MIC, minimum inhibitory concentration.

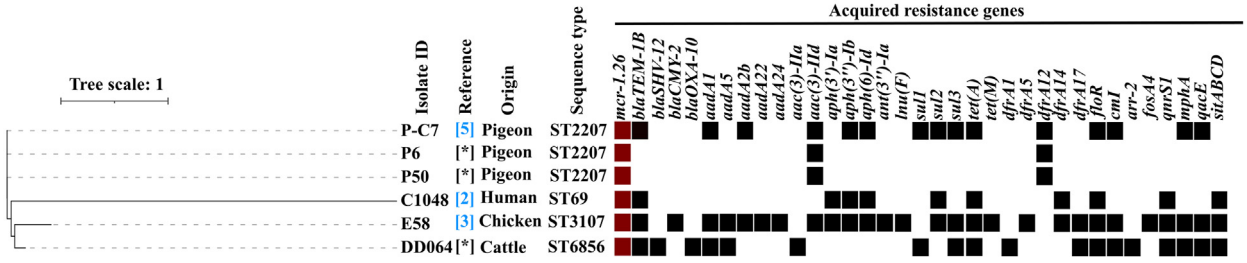

Fig. 1. Maximum likelihood phylogeny of *mcr-1.26*–positive *Escherichia coli* isolates. The phylogenetic tree was constructed based on pairwise single-nucleotide polymorphism distances calculated from core-genome alignments. [*] P6, P50, and DD064 are isolated in this study. The remaining *mcr-1.26*–positive *E. coli* (i.e., C1048, P-C7, and E58) were previously reported in humans and animals in Lebanon. The genome assemblies were downloaded from the NCBI database (GenBank assembly accession: GCF_028616385.1 [2], GCA_017163495.1 [5], and GCF_021228555.1 [3]).

Our findings highlight serious concerns about the potential spread of *mcr-1.26* and other antimicrobial resistance determinants in backyard food animals that extend beyond the commercial agricultural sector in Lebanon. The *mcr-1.26* gene has been identified in various samples in Lebanon, indicating that this variant is undergoing selection. Although the factors driving this selection are not yet understood, the emergence and widespread dissemination of this variant, along with other AMR genes, are a significant cause for concern. Our research also provides evidence supporting the pivotal role of IncX4 plasmids in spreading this variant among virulent *E. coli* strains at the human–animal interface. Hence, there is a pressing need for effective One Health interventions to mitigate the silent spread of colistin resistance genes in vital hosts and niches in Lebanon and beyond.

Ethical Approval

This investigation is a component of a larger research initiative that has been granted approval (CE-EDST-1–2020) by the Azm Center/Lebanese University ethical committee (authorised by the Lebanese Ministry of Public Health).

Accession Numbers

The assembled genomes were deposited in GenBank (GCA_034110445.1, GCA_034110425.1, GCA_036923795.1, and GCA_036923775.1). In addition, the NCBI Reference Sequence is available under accession: JAWCWB000000000, JAWCWC000000000.1, JAYKFQ000000000, and JAYKFP000000000.

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Competing interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.06.009.

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