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Letter to the Editor

Emergence of plasmid-borne colistin resistance gene *mcr-1* in multidrug-resistant *Escherichia coli* isolated from irrigation water in Lebanon

Sir,

Detection and dissemination of the plasmid-borne mobile colistin resistance (*mcr*) genes have raised concerns over the loss of efficacy of colistin in treating multidrug-resistant (MDR) bacterial infections. Despite global attention, colistin resistance and the *mcr* genes remain relatively understudied in countries with weak antimicrobial stewardship and debilitated resources and infrastructure [1]. Given that the aforementioned conditions are associated with an increase in MDR bacterial infections, it can be argued that preserving the efficacy of colistin in these countries is of paramount importance to local national health and to curb the spread of antimicrobial resistance globally.

Lebanon, a small country on the shores of the Mediterranean Sea, has been suffering from well-documented post-civil war infrastructure and waste and water management issues, which have severely challenged the country's resources and its underdeveloped policies on the use of antimicrobial agents in human medicine and agriculture [1]. Recently, the use and importance of colistin-containing drugs in human medicine and animal farming in Lebanon have been documented [1]. Furthermore, three colistin-resistant *Klebsiella pneumoniae* were reported in a hospital in Lebanon in 2015, which were also shown to be negative for *mcr-1* and *mcr-2* [2]. Notably, *mcr-1* was detected in 23 and 88 *Escherichia coli* isolated from Lebanese swine and pre-harvest broiler farms, respectively [3,4]. However, the impact of *mcr-1* in Lebanese farmed animals as well as data on the spread of *mcr* in Lebanon remain not fully characterised. Given the status of the Lebanese infrastructure, we predict that *mcr*-associated colistin resistance might have spread to other niches. Therefore, we investigated the emergence of *mcr* in irrigation water, which was targeted because it is affected by agricultural practices and has the potential to widely disseminate contaminants to other vital milieus.

A total of 27 irrigation water samples (1 L) were collected from the two major agricultural areas in Lebanon, namely South Lebanon and the Beqaa Valley, from April–September 2018 (Table 1; Supplementary Fig. S1). The water was used to irrigate a variety of important crops. Water (100 mL and 500 mL from each sample) was filtered through 0.22-µm Millipore® membranes (Sigma-Aldrich), which were placed onto *E. coli* selective medium (RAPID'*E.coli* 2 agar; Bio-Rad) supplemented with 4 µg/mL colistin (Sigma-Aldrich) [3]. Of the 27 water samples, 12 (44%) yielded *E. coli* colonies, which ranged in number between 1 and 10^4 CFU/100 mL. A total of 22 *E. coli* (1–3 isolates per sample) were successfully purified and their identity was confirmed by targeting an *E. coli*-specific 16S rRNA gene fragment by PCR. The isolates were then analysed for (i) the presence of *mcr-1* to *mcr-8* as well as other genes encoding extended-spectrum β-lactamases (ESBLs) and carbapenemases by PCR; (ii) the colistin minimum inhibitory concentration (MIC) by the broth microdilution method; and (iii) resistance to other antibiotics by the disk diffusion assay as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines [4]. All of the isolates were positive for *mcr-1*, which was further confirmed by sequencing (GenBank accession nos. MK262910–MK262912). The genes *mcr-2*, *-3*, *-4*, *-5*, *-6*, *-7* and *-8* were not detected in any isolate. Plasmids purified from three *mcr-1*-positive isolates (SH-14-A, BQ-5-B and BQ-5-C) representing different geographical regions were successfully transformed into chemically-competent *E. coli* JM109 (QIAGEN) by the heat-shock method. The colistin MIC of *E. coli* JM109 before transformation was <0.25 µg/mL. However, the transformants were positive for *mcr-1* and were resistant to colistin (MIC ≥ 4 µg/mL), confirming that *mcr-1* was plasmid-borne. The colistin MIC for the *mcr-1*-positive water isolates ranged between 4 µg/mL to 64 µg/mL (Table 1). Resistance was also observed against penicillin (100% of isolates), ampicillin (100%), amoxicillin/clavulanic acid (23%), cefepime (18%), cefotaxime (41%), cephalexin (73%), cefixime (32%), doripenem (5%), meropenem (5%), gentamicin (36%), kanamycin (64%), streptomycin (95%), tetracycline (100%), ciprofloxacin (36%), norfloxacin (45%), trimethoprim/sulfamethoxazole (SXT) (68%) and chloramphenicol (45%). Resistance to imipenem was not observed. All of the isolates were MDR (resistant to at least four classes of antimicrobials) (Table 1) and harboured class 1 integron genes and bla_{TEM}, whilst 36% and 41% of the isolates were positive for bla_{CTX-M} and bla_{SHV}, respectively. The high occurrence of bla_{TEM} corroborates previous findings showing that this gene is prevalent in ESBL-producing Gram-negative bacilli in Lebanon [5], whilst the class 1 integron genes highlight the ability of the water isolates to evolve via acquisition and expression of different antimicrobial resistance genes [6]. The two carbapenem-resistant *E. coli* were negative for bla_{KPC} and bla_{IMP} but harboured bla_{NDM-1} and bla_{OXA-48}, respectively. It is important to note that the *mcr-1*-positive transformants were not positive for bla_{NDM-1} or bla_{OXA-48}. BOX-PCR fingerprinting analysis of the *mcr-1*-positive isolates revealed considerable genotypic diversity; specifically, the 22 isolates were grouped into 11 unique profiles based on the number and location of the bands (Supplementary Fig. S2). The *mcr-1*-positive water isolates (SH-14-A and BQ-5-C) were inoculated into South Lebanon and Beqaa Valley irrigation waters that were previously sterilised using multiple autoclave cycles. The density of the isolates was adjusted to ca. 10^4 CFU/100 mL of autoclaved water and the suspension was then incubated at 25°C. Periodically, 100 mL of the suspension was processed using membrane filtration and enrichment on colistin-containing RAPID'*E.coli* 2 agar as described above. Notably, the *mcr-1*-positive isolates survived for >45 days in irrigation water without a significant decrease ($P < 0.05$) in their number. PCR and MIC

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Table 1
Antimicrobial resistance profile of *mcr-1*-positive colistin-resistant *Escherichia coli* isolated from two major agricultural areas in Lebanon.

Location	Sample ID code[a]	Antimicrobial resistance profile[b]	Intermediate antimicrobial resistance profile	Colistin MIC (μg/mL)	Antimicrobial resistance genes
South Lebanon	SH-1-A	PEN/AMP/STR/TET/SXT	AMC/FEP	4	*mcr-1*, bla_{TEM}
	SH-1-B	PEN/AMP/STR/TET/SXT	AMC	8	*mcr-1*, bla_{TEM}, bla_{SHV}
	SH-2	PEN/AMP/KAN/STR/TET/NOR/SXT	AMC	8	*mcr-1*, bla_{TEM}
	SH-4	PEN/AMP/LEX/GEN/STR/TET/SXT	CTX	8	*mcr-1*, bla_{TEM}
	SH-6	PEN/AMP/LEX/STR/TET	KAN	8	*mcr-1*, bla_{TEM}, bla_{SHV}
	SH-14-A	PEN/AMP/AMC/MEM/GEN/KAN/STR/TET/CIP/SXT/CHL	NOR	32	*mcr-1*, bla_{TEM}, bla_{NDM-1}
	SH-14-B	PEN/AMP/AMC/LEX/CFM/GEN/KAN/STR/TET/SXT/CHL	FEP/CTX/CIP/NOR	16	*mcr-1*, bla_{TEM}, bla_{SHV}, bla_{CTX-M}
	SH-14-C	PEN/AMP/AMC/LEX/GEN/KAN/STR/TET/SXT/CHL	FEP/CTX/CIP	16	*mcr-1*, bla_{TEM}, bla_{SHV}, bla_{CTX-M}
Beqaa Valley	BQ-1-A	PEN/AMP/FEP/CTX/LEX/CFM/KAN/STR/TET/NOR/SXT	AMC/CIP	8	*mcr-1*, bla_{TEM}, bla_{SHV}, bla_{CTX-M}
	BQ-1-B	PEN/AMP/AMC/FEP/CTX/LEX/CFM/KAN/STR/TET/CIP/NOR/SXT	CHL	64	*mcr-1*, bla_{TEM}, bla_{CTX-M}
	BQ-4-A	PEN/AMP/LEX/STR/TET/CIP/NOR/SXT/CHL	FEP/CTX/KAN	4	*mcr-1*, bla_{TEM}
	BQ-4-B	PEN/AMP/LEX/STR/TET/CIP/NOR/CHL	AMC/KAN	8	*mcr-1*, bla_{TEM}, bla_{SHV}
	BQ-5-A	PEN/AMP/AMC/FEP/CTX/LEX/CFM/GEN/KAN/STR/TET/CIP/NOR/SXT/CHL		32	*mcr-1*, bla_{TEM}, bla_{CTX-M}
	BQ-5-B	PEN/AMP/FEP/CTX/LEX/CFM/DOR/GEN/KAN/STR/TET/CIP//NOR/SXT/CHL	MEM	64	*mcr-1*, bla_{TEM}, bla_{SHV}, bla_{OXA-48}
	BQ-5-C	PEN/AMP/CTX/LEX/CFM/GEN/KAN/STR/TET/CIP/NOR/SXT/CHL	AMC	32	*mcr-1*, bla_{TEM}
	BQ-6-A	PEN/AMP/CTX/LEX/KAN/STR/TET	GEN/NOR/SXT	8	*mcr-1*, bla_{TEM}
	BQ-6-B	PEN/AMP/CTX/LEX/KAN/STR/TET/NOR/SXT/CHL	FEP/CIP	4	*mcr-1*, bla_{TEM}, $bla_{SHV.}$
	BQ-9	PEN/AMP/LEX/TET	KAN/STR	8	*mcr-1*, bla_{TEM}, bla_{CTX-M}
	BQ-11-A	PEN/AMP/CTX/LEX/CFM/KAN/STR/TET	AMC/FEP/GEN	8	*mcr-1*, bla_{TEM}, bla_{SHV}, bla_{CTX-M}
	BQ-11-B	PEN/AMP/STR/TET	FEP/GEN/KAN	16	*mcr-1*, bla_{TEM}
	BQ-11-C	PEN/AMP/GEN/KAN/STR/TET	CTX	16	*mcr-1*, bla_{TEM}
	BQ-14	PEN/AMP/CTX/LEX/KAN/STR/TET/CIP/NOR/SXT/CHL	FEP	16	*mcr-1*, bla_{TEM}, bla_{CTX-M}

MIC, minimum inhibitory concentration; PEN, penicillin; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; FEP, cefepime; CTX, cefotaxime; LEX, cephalexin; CFM, cefixime; DOR, doripenem; MEM, meropenem; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; TET, tetracycline; CIP, ciprofloxacin; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol.

[a] In the sample ID code, the numbers indicate different sites (source of irrigation water) in each geographical area, whilst letters designate different isolates from the same site.

[b] Antibiotics in the resistance profile were arranged according to the order of antibiotics/classes listed in the Clinical and Laboratory Standards (CLSI) guidelines.

analyses showed that the isolates maintained *mcr-1* and colistin resistance throughout the experiment, highlighting a potential low fitness cost for *mcr-1* and its ability to persist in this matrix. Therefore, these results showed that MDR *E. coli* harbouring plasmid-borne *mcr-1* and other important resistance markers were prevalent in Lebanese irrigation waters. Notably, bla_{NDM-1} was not previously reported in environmental samples from Lebanon.

To our knowledge, this is the first study to report the occurrence and potential persistence of *mcr-1* in irrigation water in Lebanon as well as the Middle East and North Africa (MENA) region. The prevalence of *mcr-1*-positive *E. coli* detected in Lebanese irrigation water is higher in comparison with other countries. Specifically, *mcr-1* was previously detected in only two isolates from well water in China and in one isolate from river water in Switzerland [7,8]. Since Lebanese irrigation water might affect a variety of environments, including the Mediterranean Basin, the widespread occurrence of *mcr-1* and other resistance markers in irrigation water might be a cause for concern. We highlight the need to enhance and implement efficient antimicrobial stewardship and surveillance programmes to control the proliferation of resistance to colistin and other critical antibiotics via agricultural practices and environmental matrices.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.05.005.

References

[1] Kassem II, Hijaz MA, Saab R. On a collision course: the availability and use of colistin-containing drugs in human therapeutics and food-animal farming in Lebanon. J Glob Antimicrob Resist 2019;16:162–4. doi:10.1016/j.jgar.2019.01.019.
[2] Okdah L, Leangapichart T, Hadjadj L, Olaitan AO, Al-Bayssari C, Rizk R, et al. First report of colistin-resistant *Klebsiella pneumoniae* clinical isolates in Lebanon. J Glob Antimicrob Resist 2017;9:15–16.
[3] Dandachi I, Fayad E, El-Bazzal B, Daoud Z, Rolain JM. Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacilli and emergence of *mcr-1* colistin resistance gene in Lebanese swine farms. Microb Drug Resist 2019;25:233–40. doi:10.1089/mdr.2018.0110.
[4] 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 pii: e01304-18.
[5] Dandachi I, Sokhn ES, Dahdouh EA, Azar E, El-Bazzal B, Rolain JM, et al. Prevalence and characterization of multi-drug-resistant Gram-negative bacilli isolated from Lebanese poultry: a nationwide study. Front Microbiol 2018;9:550. doi:10.3389/fmicb.2018.00550.
[6] Gillings M, Boucher Y, Labbate M, Holmes A, Krishnan S, Holley M, et al. The evolution of class 1 integrons and the rise of antibiotic resistance. J Bacteriol 2008;190:5095–100. doi:10.1128/JB.00152-08.
[7] Sun P, Bi Z, Nilsson M, Zheng B, Berglund B, Stålsby Lundborg C, et al. Occurrence of bla_{KPC-2}, bla_{CTX-M}, and *mcr-1* in Enterobacteriaceae from well water in rural China. Antimicrob Agents Chemother 2017;61 pii: e02569-16.
[8] Zurfuh K, Poirel L, Nordmann P, Nüesch-Inderbinen M, Hächler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-β-lactamase-producing Enterobacteriaceae in river water and imported vegetable samples in Switzerland. Antimicrob Agents Chemother 2016;60:2594–5. doi:10.1128/AAC.00066-16.

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