Emergence of clinical mcr-1-positive Escherichia coli in Lebanon

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Letter to the Editor

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Sir,

Antimicrobial resistance has become a global health crisis, and Lebanon does not escape this situation. A study in Lebanese hospitals reported a critical increase in antimicrobial resistance rates among clinical isolates, mainly Gram-negative bacteria. Moreover, resistance to last-resort antibiotics, including carbapenems, is frequent in healthcare settings in Lebanon, whereas colistin resistance is only rarely identified [1]. Surprisingly, the prevalence of colistin resistance in *Escherichia coli* from Lebanese poultry was found to be among the highest worldwide, and the plasmid-mediated *mcr-1* gene has been detected in Lebanese poultry and swine [2,3]. A recent study also found *mcr-1*-positive *E. coli* isolates in water sources in this country [4]. Conversely, to the best of our knowledge, *mcr* genes have never been reported from human isolates in Lebanon.

In this study, a total of 988 non-duplicate Lebanese clinical Enterobacteriaceae isolates stored in the Lebanese University bacterial bank (CMUL) between 2006–2019 were screened on MacConkey agar (Bio-Rad, Hercules, CA, USA) supplemented with 3.5 mg/L colistin (Sigma-Aldrich, St Louis, MO, USA). Regrettably, colistin use in the patients had not been recorded. All isolates grown on selective agar were purified and were identified by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF). Of the 988 isolates screened, 36 (3.6%) were resistant to colistin, with minimum inhibitory concentrations (MICs) ranging from 4 mg/L to 64 mg/L as determined by broth microdilution according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Among the colistin-resistant isolates, *E. coli* was predominant (26/36; 72.2%), however *Klebsiella pneumoniae* (7/36; 19.4%), *Salmonella enterica* (2/36; 5.6%) and *Enterobacter cloacae* (1/36; 2.8%) were also detected.

By PCR for *mcr-1* to *mcr-5* genes and sequencing, *mcr-1* was detected in six *E. coli* isolates recovered in 2011 ($n = 1$), 2013 ($n = 1$), 2017 ($n = 2$), 2018 ($n = 1$) and 2019 ($n = 1$), whereas no other *mcr* genes were identified [5]. Chromosomal mechanisms conferring colistin resistance, such as mutations in the *mgrB* gene in colistin-resistant *K. pneumoniae*, were not further investigated. Also, since not all variants of the *mcr* gene were searched in this study, we cannot exclude possible additional *mcr*-positive isolates in this collection. As assessed by disk diffusion according to EUCAST guidelines, five out of the six *mcr-1*-positive isolates were resistant to all classes of β-lactams except carbapenems. These isolates also presented associated resistance to sulfonamides (6/6), tetracyclines (5/6), trimethoprim (5/6), streptomycin (4/6), chloramphenicol (4/6), nalidixic acid (4/6), enrofloxacin (4/6), kanamycin (3/6) and gentamicin (2/6) but remained susceptible to amikacin (Table 1). Two *mcr-1*-positive *E. coli* additionally harboured an extended-spectrum β-lactamase (ESBL) (CTX-M-15) and three harboured an AmpC phenotype (CMY-2), as shown by synergy test or cefoxitin resistance, respectively (Table 1).

Multilocus sequence typing (MLST), *E. coli* phylogrouping, and pulsed-field gel electrophoresis (PFGE) using *Xba*I restriction revealed fully different genetic backgrounds for the six *mcr-1*-positive *E. coli* isolates (Table 1; Supplementary Fig. S1), which also differed from the only *mcr-1*-positive animal isolate, a ST515 *E. coli* collected from poultry, for which MLST data are available in Lebanon [6]. This genetic diversity demonstrates horizontal transfer of the *mcr-1* gene rather than clonal spread of a single clone. Using the PCR-based replicon typing scheme (PBRT Kit; Diatheva, Fano, Italy) and Southern blotting on S1-PFGE gels, the *mcr-1* gene was shown to be located on an IncX4 plasmid in all six isolates (Table 1). IncX4 plasmids are self-transferable at high frequency and have been widely reported to disseminate *mcr-1* in Enterobacteriaceae both from human and animal origins [7]. Conjugation experiments using *E. coli* BM21 as recipient were successful for all isolates, showing the capacity of all plasmids to easily disseminate the *mcr-1* gene horizontally. None of the transconjugants harboured an ESBL/AmpC gene as assessed by PCR, demonstrating that *mcr-1* and ESBL/AmpC genes were not located on the same plasmid. Moreover, all transconjugants were fully susceptible to non-β-lactam antibiotics, verifying that the IncX4 plasmid carried only the colistin resistance determinant.

In Lebanon, the frequency of colistin prescription in the clinic remains unclear even though it has most likely increased in parallel to the growing prevalence of carbapenem-resistant bacteria in hospitals. In addition, the amount of colistin imported for human use has increased between 2010 and 2017, and drugs containing colistin are widely and legally available without prescription for veterinary purposes. Of note, all six colistin-resistant *E. coli* had been wrongly reported to physicians as susceptible to this drug. The majority of Lebanese diagnostic laboratories still use the Kirby–Bauer disk diffusion method, which cannot clearly discriminate isolates as susceptible or resistant to colistin, thus predisposing patients to treatment failures.

To the best of our knowledge, this is the first report of *mcr-1*-positive Enterobacteriaceae in humans in Lebanon. Coupled with the low awareness of the antimicrobial resistance threat in the Lebanese community, these findings highlight the need for better infection control and antimicrobial stewardship programmes in this country, including the implementation of reliable tests to be used in clinics before treatment with colistin. A One Health approach should be urgently considered by Lebanese authorities to promote the responsible use of antibiotics and to address risk factors associated with antimicrobial resistance in all sectors.

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Table 1
Characteristics of six clinical *mcr-1*-positive *Escherichia coli* of human origin in Lebanon.

Isolate	Source	Patient age (years)	Hospital (region)	Isolation date	Phylogenetic group	MLST	β-Lactam enzyme	Colistin MIC (mg/L)	Plasmid content[a]	Additional resistance
CMUL371	Semen	18	Nini Hospital (Tripoli)	2011	B1	ST1431	CTX-M-15	8	I1/FIB/Y/FII/X4	GEN, STR, TET, SUL, TET, NAL, ENR
CMUL1076	Urine	1	Nini Hospital (Tripoli)	2013	B2	ST1011	CMY-2	4	FIB/X4	KAN, STR, CHL, TET, SUL, NAL, ENR
CMUL834	Stool	77	Hôtel-Dieu de France (Beirut)	2017	A	NT	CMY-2	4	FIA/FIB/W/T/X1/Y/FII/X4	STR, CHL, TET, SUL, TET
CMUL832	Stool	84	Hôtel-Dieu de France (Beirut)	2017	A	ST2705	CMY-2	8	I1/X1/FII/FIB/X4	TET, SUL, TET
CMUL1075	Urine	65	Hamidi Medical Center (Tripoli)	2018	D	NT	–	8	FII/Y/X4	KAN, CHL, SUL, TET, NAL, ENR
CMUL1030	Stool	50	El Youssef Hospital Center (Halba)	2019	A	NT	CTX-M-15	6	I1/X4	GEN, KAN, STR, CHL, TET, SUL, TET, NAL, ENR

MLST, multilocus sequence typing; NT, not typeable (combination of alleles not available in the Achtman database); MIC, minimum inhibitory concentration; GEN, gentamicin; STR, streptomycin; TET, tetracycline; SUL, sulfonamides; TMP, trimethoprim; NAL, nalidixic acid; ENR, enrofloxacin; KAN, kanamycin; CHL, chloramphenicol.
[a] *mcr-1*-carrying plasmids are underlined. Incompatibility groups were determined using a PBRT Kit (Diatheva, Fano, Italy).

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Competing interests

None declared.

Ethical approval

This study was approved by the Azm Center/Lebanese University Ethical Committee [document CE-EDST-3-2018], authorised by the Lebanese Ministry of Public Health.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.08.019.

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