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Emergence of the Mobile Colistin Resistance Gene *mcr-1* in Multidrug-Resistant *Escherichia coli* Isolated from the Fecal Matter of Toddlers in a Community

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Colistin is one of the few first-line options for treating complicated infections with certain multidrug-resistant bacteria (1). The emergence and global dissemination of *mcr* (mobile colistin resistance) genes have threatened the usefulness of colistin (1, 2). However, the spread of *mcr* in nonclinical communities remains understudied, especially in Lebanon, which is experiencing severe economic and medical crises and challenges in infrastructure and antimicrobial stewardship. In Lebanon, the overreliance on colistin in medicine and animal farming was recently documented along with the emergence of *mcr-1* on poultry farms and in environmental matrices (3–9). Therefore, it was necessary to assess the occurrence of these genes in the community. For this purpose, 72 fecal samples were collected from discarded diapers of toddlers (≤2 years old) in reputable community daycares at five major locations/cities across Lebanon. The daycares were licensed to receive these age groups and followed the protocols mandated by the Lebanese Ministry of Public Health, which required parents to notify the daycare of any sickness or unusual symptoms experienced by the toddlers and resident staff to maintain records of any medication/illness experienced by the toddlers while at the daycare. Ethical approval was not required to conduct the study. In order not to bias the sampling, we instructed the daycare staff to collect fecal samples from healthy/nonsymptomatic, including nondiarrheic, diapers. The samples were suspended in buffered peptone water, and an aliquot (100 μl) was spread onto an *Escherichia coli* selective medium, RAPID'*E. coli* 2 agar (Bio-Rad, USA), supplemented with colistin (4 μg/ml) (Sigma-Aldrich, USA) (2–9). Thirteen (18%) samples yielded *E. coli* (violet to pink colonies). Twenty-four *E. coli* samples (one to four colonies per sample) were purified, and their identity was further confirmed using PCR analysis (2–10), which also showed that the isolates were *mcr-1* positive and *mcr-2* to *mcr-8* negative. *mcr-1* detection was confirmed by commercial sequencing of the amplicons. The MIC of colistin against the isolates ranged between 4 and 32 μg/ml (Table 1). Plasmids were extracted and successfully transformed into chemically competent *E. coli* JM109 using the heat shock method (2–9). The transformants were *mcr-1* positive and colistin resistant (MIC ≥ 4 μg/ml), confirming that the gene was plasmid-borne. PCR-based replicon typing (11) showed that isolates harbored diverse plasmids, including IncI2, IncI1α, IncX1, IncX4, IncF2, and IncFIB, which are associated with worldwide dissemination of *mcr-1* and the spread of other antibiotic resistance genes (12, 13). Using the disk diffusion assay, the isolates were shown to exhibit resistance to penicillin (100% of isolates), ampicillin (83.5%), amoxicillin-clavulanic acid (83.5%), cefepime (33.5%), cefotaxime (75%), cephalexin (87.5%), cefixime (71%), doripenem (8.5%), imipenem (4.2%), meropenem (21%), gentamicin (12.5%), kanamycin (29.2%), streptomycin (29.2%), tetracycline (42%), ciprofloxacin (12.5%), norfloxacin (8.5%), trimethoprim-sulfamethoxazole (50%), and chloramphenicol

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TABLE 1 Antibiotic resistance profiles and sequence types of the colistin-resistant and *mcr-1*-positive *E. coli* isolated from fecal matter of toddlers in different locations in Lebanon

Location[a]	Toddler age (yr)	*E. coli* isolate code[b]	Colistin MIC (μg/ml)	Antibiotic resistance profile[c]	Intermediate resistance profile	Additional acquired ABR genes detected by WGS analysis[d]	Sequence type (ST)[e]
Beirut	≤1	Bei-2.1	4	PEN-AMP-AMC-LEX			
		Bei-2.3	16	PEN-TET	KAN		
		Bei-2.4	8	PEN-AMP-AMC-CTX-LEX-CFM-KAN-STR-SXT-CHL	FEP-NOR		
		Bei-2.5	4	PEN-AMP-AMC-FEP-CTX-LEX-CFM-STR-TET-SXT	KAN	*aph(3″)-Ib*, $bla_{CTX-M-15}$, bla_{TEM-1B}, *dfrA1*, *mdf*(A), *qnrS1*, *tet*(B), *tet*(C), *sul2*	Unknown ST
		Bei-6.1	4	PEN-AMP-AMC-CTX-LEX-CFM-GEN-KAN-STR-TET-CIP-NOR-SXT-CHL	FEP	*aac(3)-IId*, *aph(3″)-Ib*, *ant(3″)-Ia*, *aph(3′)-Ia*, *aph(6)-Id*, bla_{CMY-15}, bla_{TEM-1B}, bla_{TEM-1C}, *dfrA1*, *floR*, *fosA6*, *mdf*(A), *tet*(B), *sul2*	ST57
		Bei-6.2	16	PEN-TET	AMP-KAN-STR		
		Bei-6.3	8	PEN-LEX-TET	STR		
		Bei-6.4	4	PEN-AMP-AMC-GEN-KAN-TET-CIP-NOR-SXT-CHL	STR		
Saida	≤1	Sai-7.1	8	PEN-AMP-AMC-CTX-LEX-CFM-MEM-KAN-TET-SXT	FEP-DOR-STR-CIP		
		Sai-10.1	32	PEN-AMP-AMC-FEP-CTX-LEX-CFM-DOR-MEM-IPM-SXT	KAN-STR-TET		
		Sai-12.1	16	PEN-AMP-AMC-FEP-CTX-LEX-CFM-STR-SXT-CHL	KAN		
	≤2	Sai-69.1	16	PEN-AMP-AMC-CTX-LEX-CFM-TET	IPM-GEN-KAN-CIP		
		Sai-71.1	32	PEN-AMP-FEP-CTX-LEX-GEN-KAN-STR-TET-CIP-SXT-CHL	DOR-IPM-NOR	*aac(3)-IId*, *aadA2*, *aph(3″)-Ib*, *aph(6)-Id*, bla_{CFE-1}, bla_{CMY-2}, $bla_{CTX-M-3}$, $bla_{TEM-141}$, *dfrA12*, *erm*(42), *floR*, *fosA3*, *mdf*(A), *mph*(A), *tet*(A), *sul2*	ST1011
Tripoli	≤1	Tri-26.1	8	PEN-AMC-FEP-CTX-LEX-KAN-SXT	AMP-MEM-IPM-STR-TET-CHL	bla_{CFE-1}, *mdf*(A)	Unknown ST
		Tri-26.2	4	PEN-AMP-AMC-FEP-CTX-LEX-CFM-KAN-STR-TET	MEM		
		Tri-35.1	8	PEN-AMP-AMC-CTX-LEX-CFM-SXT	DOR-MEM-IPM-KAN		
Choueifat	≤2	Cho-42.1	4	PEN-AMP-AMC-LEX-CFM-STR	FEP-CTX-DOR-GEN		
		Cho-42.2	32	PEN-AMP-AMC-CTX-LEX-CFM-SXT	MEM-KAN		
		Cho-42.3	16	PEN-AMP-AMC-CTX-LEX-CFM-SXT			
		Cho-43.1	8	PEN-AMP-AMC-CTX-LEX-CFM-CHL	DOR-IPM-KAN-STR-TET		
		Cho-44.1	16	PEN-AMP-AMC-FEP-CTX-LEX-CFM-MEM	DOR-IPM-KAN		
		Cho-44.2	32	PEN-AMP-AMC-FEP-CTX-LEX-CFM-DOR-MEM	IPM-KAN		
Jounieh	≤2	Jou-50.1	16	PEN-AMP-AMC-CTX-LEX-CFM	DOR-MEM-IPM-KAN-STR-CIP	bla_{CFE-1}, bla_{CMY-2}, *mdf*(A), *qnrB19*	ST69
		Jou-50.2	16	PEN-AMP-AMC-CTX-LEX-CFM-MEM	FEP-DOR-IPM-KAN-STR-CIP		

[a]Location refers to the city in Lebanon where the sampling occurred.
[b]The isolate code indicates the location/city, diaper number, and isolate number. Therefore, Bei-2.1 is isolate number 1 from diaper number 2 that was sampled in Beirut.
[c]Antibiotic resistance profiles were determined using the disk diffusion assay as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines (21). Ampicillin (AMP), amoxicillin plus clavulanic acid (AMC), cefepime (FEP), cefotaxime (CTX), cephalexin (LEX), cefixime (CFM), doripenem (DOR), imipenem (IPM), meropenem (MEM), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim-sulfamethoxazole (SXT), and chloramphenicol (CHL).
[d]ABR, antibiotic resistance; WGS, whole-genome sequencing.
[e]Unknown STs are those that could not be matched to previously known STs, potentially indicating that the unknown STs were novel and/or unavailable in the MLST database.

(25%). All the isolates were multidrug resistant (resistance to ≥3 classes of antibiotics). Five random isolates were selected for whole-genome sequencing (WGS) analysis. Using ResFinder (version 3.0) database (14), *mcr-1.1* was detected in the genomes with 2 to 16 other acquired genes that encode resistance to important antibiotics (Table 1). Multilocus

sequence type (MLST) analysis showed that three isolates belonged to ST57, ST69, and ST1011 (Table 1), which are associated with *mcr-1*-positive *E. coli* isolated from diverse sources, including clinical samples, chickens, and food (raw cheese) (15–19), perhaps indicating transmission to humans via the food chain and/or environment, which corroborates previous findings about the occurrence of *mcr-1* in these matrices in Lebanon.

To our knowledge, this is the first study on *mcr*-mediated colistin resistance in toddlers in the Middle East and North Africa (MENA) region, which includes several countries with challenges in antimicrobial stewardship and catastrophic medical and humanitarian crises. Assessment of the available literature showed that the occurrence of *mcr-1*-positive *E. coli* in Lebanese toddlers ranks second after Bolivia (38.3%) (20). In conclusion, there is a paramount need to regulate the use of colistin, especially in agricultural practices, in order to restrict the spread of *mcr* in the Lebanese population.

Data availability. Sequencing data reported in this study can be found under SRA accession numbers SRX7741060, SRX7741058, SRX7741057, SRX7741056, and SRX7741055.

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We declare that we have no conflicts of interest.

REFERENCES

1. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J. 2016. 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 16:161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.
2. Nang SC, Li J, Velkov T. 2019. The rise and spread of *mcr* plasmid-mediated polymyxin resistance. Crit Rev Microbiol 45:131–161. https://doi.org/10.1080/1040841X.2018.1492902.
3. Kassem II, Hijazi MA, Saab R. 2019. 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 16:162–164. https://doi.org/10.1016/j.jgar.2019.01.019.
4. Hmede Z, Kassem II. 2018. The colistin resistance gene *mcr-1* is prevalent in commensal *Escherichia coli* isolated from preharvest poultry in Lebanon. Antimicrob Agents Chemother 62:e01304-18. https://doi.org/10.1128/AAC.01304-18.
5. Hmede Z, Sulaiman AAA, Jaafar H, Kassem II. 2019. Emergence of plasmid-borne colistin resistance gene *mcr-1* in multidrug-resistant *Escherichia coli* isolated from irrigation water in Lebanon. Int J Antimicrob Agents 54:102–104. https://doi.org/10.1016/j.ijantimicag.2019.05.005.
6. Alhaj Sulaiman A, Kassem II. 2019. 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 30:117–120. https://doi.org/10.1016/j.tmaid.2019.06.014.
7. Sourenian T, Mann D, Li S, Deng X, Jaafar H, Kassem II. 2020. The dissemination of multidrug resistant E. coli harboring the mobile colistin resistance gene, *mcr-1.1*, on transmissible plasmids to the Mediterranean Sea. J Glob Antimicrob Resist 22:84–86. https://doi.org/10.1016/j.jgar.2020.05.007.
8. Hassan J, Eddine RZ, Mann D, Li S, Deng X, Saoud IP, Kassem II. 2020. The mobile colistin resistance gene, *mcr-1.1*, is carried on IncX4 plasmids in multidrug resistant E. coli isolated from rainbow trout aquaculture Microorganisms 8:1636. https://doi.org/10.3390/microorganisms8111636.
9. Alhaj Sulaiman AA, Kassem II. 2020. First report of the plasmid-borne colistin resistance gene (*mcr-1*) in *Proteus mirabilis* isolated from domestic and sewer waters in Syrian refugee camps. Travel Med Infect Dis 33:101482. https://doi.org/10.1016/j.tmaid.2019.101482.
10. Sabat G, Rose P, Hickey WJ, Harkin JM. 2000. Selective and sensitive method for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. Appl Environ Microbiol 66:844–849. https://doi.org/10.1128/aem.66.2.844-849.2000.
11. Carloni E, Andreoni F, Omiccioli E, Villa L, Magnani M, Carattoli A. 2017. Comparative analysis of the standard PCR-based replicon typing (PBRT) with the commercial PBRT-KIT. Plasmid 90:10–14. https://doi.org/10.1016/j.plasmid.2017.01.005.
12. Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A, Dorai-Schneiders T, Weinert LA, Iqbal Z, Didelot X, Wang H, Balloux F. 2018. The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. Nat Commun 9:1179. https://doi.org/10.1038/s41467-018-03205-z.
13. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J. 2018. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. J Antimicrob Chemother 73:1121–1137. https://doi.org/10.1093/jac/dkx488.
14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
15. Liu BT, Song FJ, Zou M, Zhang QD, Shan H. 2017. High incidence of *Escherichia coli* strains coharboring *mcr-1* and bla_{NDM} from chickens. Antimicrob Agents Chemother 61:e02347-16. https://doi.org/10.1128/AAC.02347-16.
16. Elnahriry SS, Khalifa HO, Soliman AM, Ahmed AM, Hussein AM, Shimamoto T, Shimamoto T. 2016. Emergence of plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate from Egypt. Antimicrob Agents Chemother 60:3249–3250. https://doi.org/10.1128/AAC.00269-16.
17. Hammad AM, Hoffmann M, Gonzalez-Escalona N, Abbas NH, Yao K, Koenig S, Allué-Guardia A, Eppinger M. 2019. Genomic features of colistin resistant *Escherichia coli* ST69 strain harboring *mcr-1* on IncHI2 plasmid from raw milk cheese in Egypt. Infect Genet Evol 73:126–131. https://doi.org/10.1016/j.meegid.2019.04.021.
18. Anyanwu MU, Okpala CO, Chah KF, Shoyinka VS. 2021. Prevalence and traits of mobile colistin resistance gene harboring isolates from different ecosystems in Africa. BioMed Res Int 2021:6630379. https://doi.org/10.1155/2021/6630379.
19. Maciuca IE, Cummins ML, Cozma AP, Rimbu CM, Guguianu E, Panzaru C, Licker M, Szekely E, Flonta M, Djordjevic SP, Timofte D. 2019. Genetic features of *mcr-1* mediated colistin resistance in CMY-2-producing *Escherichia coli* from Romanian poultry. Front Microbiol 10:2267. https://doi.org/10.3389/fmicb.2019.02267.
20. Giani T, Sennati S, Antonelli A, Di Pilato V, di Maggio T, Mantella A, Niccolai C, Spinicci M, Monasterio J, Castellanos P, Martinez M, Contreras F, Balderrama Villaroel D, Damiani E, Maury S, Rocabado R, Pallecchi L, Bartoloni A, Rossolini GM. 2016. High prevalence of carriage of *mcr-1*-positive enteric bacteria among healthy children from rural communities in the Chaco region, Bolivia. Euro Surveill 23:1800115. https://doi.org/10.2807/1560-7917.
21. Clinical and Laboratory Standards Institute (CLSI). 2017. Performance standards for antimicrobial susceptibility testing, 27th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.