The Colistin Resistance Gene, *mcr-1*, is Prevalent in Commensal *E. coli* Isolated from Lebanese Pre-harvest Poultry

Zaynab Hmede[1] and Issmat I. Kassem[1*]

[1]Department of Nutrition and Food Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon.

*Corresponding author: Issmat I. Kassem, PHD; E-mail: ik12@osu.edu; Phone: +961 1 350000 Ext: 4456; Fax: Fax: +961 1 744460.

Keywords: colistin, *mcr*-1, multidrug resistance, *E. coli*, poultry, pre-harvest, Lebanon

Antimicrobial resistance is a serious global problem. However, antimicrobial stewardship remains deficient in Lebanon (1). National antimicrobial resistance monitoring programs are lacking with negligible attention to agricultural practices, and data are largely restricted to a few clinical studies (1). Notably, colistin (polymyxin E) resistance was recently detected in clinical settings in Lebanon, while the antibiotic is promoted to control diseases in Lebanese poultry (1,2). Since the use of colistin against multidrug-resistant Gram-negative bacteria is jeopardized by the proliferation of the plasmid-borne mobile-colistin-resistance gene (*mcr*-1), we investigated the potential emergence of colistin resistance on Lebanese poultry farms. For this purpose, fresh fecal samples (n = 93) were collected from three major Lebanese broiler chicken farms (31 samples per farm) between September 2017 and March 2018. All samples were screened on an *E. coli* selective medium, RAPID'E.coli 2 Agar (Bio-Rad, USA), which was supplemented with 4 µg/ml colistin (Sigma-Aldrich, USA). Our results showed that 90 samples (97%) yielded *E. coli* colonies (~ 80 - 10^4 CFUs per g fecal matter). Ninety *E. coli* (31, 30, and 29 isolates from the three farms, respectively) were further purified and screened to determine:

1) the colistin minimum inhibitory concentration (MIC) using the broth microdilution method and 2) the presence of *mcr*-1 and an *E. coli*-specific 16S rRNA gene fragment using PCR (3,4). Our results showed that the colistin MIC ranged between 8-32 µg/ml. All the isolates were positive for the 16S rRNA gene, while 88 isolates (~ 98%) harbored *mcr*-1, which was further confirmed by sequencing (GenBank accession numbers: MH759759-MH759761). PCR analysis showed that the two *mcr*-1-negative isolates did not harbor *mcr*-2 or *mcr*-3 (5,6). Antimicrobial susceptibility analysis using the disk diffusion assay (7) showed that the *mcr*-1-positive *E. coli* were also resistant to penicillin (100%), ampicillin (100% of isolates), amoxicillin + calvulanic acid (56%), cefepime (25%), cefotaxime (70%), cephalexin (94%), cefixime (76%), doripenem (1%), imipenem (1%), meropenem (2%), gentamicin (61%), kanamycin (76%), streptomycin (80%), tetracycline (89%), ciprofloxacin (91%), norfloxacin (68%), trimethoprim-sulfamethoxazole (84%), and chloramphenicol (95%). All the tested isolates were multidrug-resistant; exhibiting resistance to 4 or more classes of antimicrobials (Table S1). PCR analysis showed that 99%, 96% and 35.5% of the isolates were positive for Class 1 Integrons (Integ), bla_{TEM} and $bla_{\text{CTX-M}}$ (extended-spectrum β-lactamase genes), respectively (8). Taken together, our results showed a wide-spread resistance to colistin and other important antimicrobials in *E. coli* from Lebanese poultry. *mcr*-1 was also prevalent in these isolates. In the few previous studies in the Middle-East, *mcr*-1 was detected in clinical *E. coli* from Bahrain (n = 2 of seventy-five colistin-resistant *Enterobacteriaceae* strains), Saudi Arabia (n = 1), and the United Arab Emirates (n = 1), while 14 (15.6%) isolates were associated with broiler chickens in Qatar (9,10). While plasmid mediated colistin resistance has increased in the food chain globally (11), resistance in Lebanon might be among the highest reported incidences. Therefore, there is a peremptory national need to revisit antimicrobial stewardship and agricultural practices in order

to restrict access to important antibiotics and control the proliferation of antimicrobial resistance in Lebanon.

Acknowledgments

This study was supported by the AUB-URB program and the FAFS Dean's Seed grants.

References

1- Chamoun K, Farah M, Araj G, Daoud Z, Moghnieh R, Salameh P, Saade D, Mokhbat J, Abboud E, Hamze M, Abboud E, Jisr T, Haddad A, Feghali R, Azar N, El-Zaatari M, Chedid M, Haddad C, Zouain Dib Nehme M, Barakat A, Husni R; Lebanese Society of Infectious Diseases Study Group (LSID study group). 2016. Surveillance of antimicrobial resistance in Lebanese hospitals: retrospective nationwide compiled data. Int J Infect Dis 46:64-70.

2- Okdah L, Leangapichart T, Hadjadj L, Olaitan AO, Al-Bayssari C, Rizk R,Hammoud M, Diene SM, Rolain JM. 2017. First report of colistin-resistant *Klebsiella pneumoniae* clinical isolates in Lebanon. J Glob Antimicrob Resist 9:15-16.

3- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, 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(2):161-168.

4- 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(2):844-849.

5- Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr*-2, in *Escherichia coli*, Belgium. Euro Surveill 7:21(27).

6- Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. 2017. Novel Plasmid-Mediated Colistin Resistance Gene *mcr*-3 in *Escherichia coli*. MBio 8(3):e00543-17.

7- Clinical and Laboratory Standards Institute (CLSI). 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100 (ISBN 1-56238-805-3 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA.

8- Literak I, Dolejska M, Radimersky T, Klimes J, Friedman M, Aarestrup FM, Hasman H, Cizek A. 2010. Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in central Europe: multiresistant *Escherichia coli* producing extended-spectrum beta-lactamases in wild boars. J Appl Microbiol 108(5):1702-11.

9- Sonnevend Á, Ghazawi A, Alqahtani M, Shibl A, Jamal W, Hashmey R, Pal T. 2016. Plasmid-mediated colistin resistance in *Escherichia coli* from the Arabian Peninsula. Int J Infect Dis 50:85-90.

10- Eltai NO, Abdfarag EA, Al-Romaihi H, Wehedy E, Mahmoud MH, Alawad OK, Al-Hajri MM, Al Thani AA, Yassine HM. 2018. Antibiotic Resistance Profile of Commensal *Escherichia coli* Isolated from Broiler Chickens in Qatar. J Food Prot 81(2):302-307.

11- Founou LL, Founou RC, Essack SY. 2016. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. Front Microbiol 7:1881.