Accepted Manuscript

Title: Emergence of *mcr-1* mediated colistin resistance in *Escherichia coli* isolates from poultry in Algeria

Authors: Selma Chabou, Jean-Marc Rolain

PII: S2213-7165(18)30256-X
DOI: https://doi.org/10.1016/j.jgar.2018.12.012
Reference: JGAR 817

To appear in:

Author: Hamza Leulmi

PII: S2213-7165(18)30256-X
DOI: https://doi.org/10.1016/j.jgar.2018.12.012
Reference: JGAR 817

To appear in:

Received date: 6 July 2018
Revised date: 10 December 2018
Accepted date: 18 December 2018

Please cite this article as: Hamza Leulmi, Emergence of mcr-1 mediated colistin resistance in Escherichia coli isolates from poultry in Algeria (2018), https://doi.org/10.1016/j.jgar.2018.12.012

Title: Emergence of *mcr-1* mediated colistin resistance in *Escherichia coli* isolates from poultry in Algeria.

Author: Selma Chabou[1], Hamza Leulmi [2] and Jean-Marc Rolain[1]*

[1]Aix-Marseille Univ., IRD, APHM, MEPHI, IHU Méditerranée Infection, Marseille, France, IHU Méditerranée Infection, Marseille, France.

[2] Faculté des Sciences de la Nature et de la Vie, Université Saad Dahleb, Blida, 09000, Algérie.

*Corresponding author

Phone: (33) 4 91 32 43 75. Fax: (33) 4 91 38 77 72,

Email: *jean-marc.rolain@univ-amu.fr*

Text word count = 780

Number of references = 5

Table = 1

Keywords: mcr-1, poultry, colistin, Algeria

To the editor

Since November 2015, the newly identified plasmid-mediated colistin resistant gene (*mcr-1*) has been reported worldwide in Gram negative bacteria and increasingly gaining recognition (1). This newly identified resistance gene, a phospho-ethanolamine transferase gene, triggered an avalanche of retrospective studies investigating the presence of this specific gene from

various isolates, including foods, animals and humans (2). The aim of the present study was to evaluate the occurrence of *mcr-1* encoding gene in feces of poultry from Algeria, a country where colistin is widely used in animal breeding.

In 2016, we collected feces from chicken farms and slaughterhouses in Algeria. Samples were harvested directly from the chicken cloacae, and placed in single sterile and identified tube. One hundred and twenty samples of poultry feces were collected in three regions of Algeria: two slaughterhouses in Algiers (n=79), one broiler chicken farm in Blida (n=22), and one broiler chicken farm in Souk Ahras (n=19). The choice of these regions and farms was based on a good cooperation and permission of the farm owners.

Once samples has been collected, DNA extraction was carried out using an automatic robot EZ1 (Qiagen BioRobot EZ1-, Tokyo, Japan), using the extraction kit (EZ1 DNA, Qiagen, Hilden, Germany), following the manufacturer's instructions. The extracted DNA was used as a template for double quantitative real-time PCR assays, using the PE1 and PE2 systems targeting *mcr-1* encoding genes (3). All real-time PCRs were performed using the CFX96^{Tm} Real Time system C1000^{Tm} Touch thermal cycler (Bio-Rad, Singapore). Results were deemed positive if the Cycle threshold (Ct) value obtained by CFX96^{Tm} was lower than 35. Standard PCR amplification and sequencing were used to confirm the presence of *mcr*-1 as reported previously (3). Positive PCR products were purified and sequenced using a Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Using qPCR, 25 out of 120 (20.8%) chicken fecal samples were positive for the *mcr-1* gene [(21/79 (26.6%) in Algiers and 4/22 (18.2%) in Blida]. We confirmed the 25 positive samples with a second qPCR system (**Table 1**).

The 25 (20.8%) *mcr-1* positive samples were tested by standard PCR, as previously described, prior to their sequencing (3). The obtained sequences were 100% identical to that of the *mcr-1* gene sequence reported by Liu and colleagues (1).

For the selection of bacteria carrying *mcr-1* gene, samples were incubated on a selective culture medium containing colistin for 24 hours at 37°C. Isolated colonies from each sample were identified by MALDI-TOF MS using the Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany). From the 25 positive samples, we were able to isolate eight *E. coli* (7/79 from Algiers and 1/22 from Blida), that were confirmed to harbor the *mcr-1* gene by PCR.

Antibiotic susceptibility and E-test was determined on Mueller–Hinton agar using the standard disc diffusion procedure as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk diffusion susceptibility testing showed that a total of eight *E. coli* strains were resistant to colistin and amoxicillin. Minimum inhibitory concentrations (MICs) of colistin confirmed *E.coli* resistance with MIC ranging from 3 to 4 mg / l. Using Multi-Locus Sequence Typing (MLST) on the isolate strains, based on the use of seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA and recA*) from the MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli), we drew a phylogenetic tree using the MEGA6 program and the neighbor-joining method. This analysis showed that the eight *E. coli* isolates belonging to the same genotype (ST 48) which has already been reported in Algeria from chickens and humans (4).

In this investigation, we clearly showed the emergence and high prevalence (20.3%) of the *mcr-1* plasmid-mediated colistin resistant gene in *E. coli* isolates from poultry in Algeria. In Algeria, *mcr-1* was firstly detected in chicken feces (3) but only in one region (Skikda). This plasmid was also detected in *E. coli* from human samples in two hospitals in Algeria. In both studies, MLST indicated that two *E. coli* samples belonged to the same type of sequence, (ST 405).

Our results highlight the potentially spreading of the *mcr-1* gene in poultry from Algeria that seems to be related to an uncontrolled use of colistin in animals. Recently, in Vietnam, a study has demonstrated the relationship between colistin use on farms and the presence of the *mcr-1* gene in animals (5). The increased and inappropriate use of colistin has led inexorably to the

worldwide emergence of colistin-resistant bacteria. In Algeria, colistin is used in animals as a therapeutic agent and also as prophylactic and growth promoter. In addition, our findings suggest that poultry can serve as a reservoir for colistin-resistant *E. coli*, adding another layer of complexity to the rapidly evolving epidemiology of plasmid-mediated colistin resistance in the community.

Acknowledgment

We thank TradOnline for English corrections

Declarations

Funding: This work was partly funded by the CNRS and IHU Fondation Méditerranée Infection.

Competing Interests: None to declare

Ethical Approval: Not required

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 16:161–8.
2. Baron S, Hadjadj L, Rolain J-M, Olaitan AO. 2016. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents 48:583–591.
3. Chabou S, Leangapichart T, Okdah L, Le Page S, Hadjadj L, Rolain JM. 2016. Real-time quantitative PCR assay with Taqman probe for rapid detection of *MCR-1* plasmid-mediated colistin resistance. New Microbes New Infect. Elsevier Ltd.
4. Berrazeg M, Hadjadj L, Ayad A, Drissi M, Rolain J-M. 2016. First Detected Human Case in Algeria of *mcr-1* Plasmid-Mediated Colistin Resistance in a 2011 *Escherichia coli* Isolate. Antimicrob Agents Chemother 60:6996–6997.
5. Trung NV, Matamoros S, Carrique-Mas JJ, Nghia NH, Nhung NT, Chieu TTB, Mai HH, van Rooijen W, Campbell J, Wagenaar JA, Hardon A, Mai NTN, Hieu TQ, Thwaites G, de Jong MD, Schultsz C, Hoa NT. 2017. Zoonotic Transmission of *mcr-1* Colistin Resistance Gene from Small-Scale Poultry Farms, Vietnam. Emerg Infect Dis 23:529–532.

Table 1. Results of positive MCR-1 stool

Samples	qPCR using PE1 system	qPCR using PE2 system	PCR standard	Sequencing	culture	COL MIC (mg/L)	qPCR using PE1 system	qPCR using PE2 system	MLST results
A27	25,37	25,49	+	+	*E. coli*	3	17,75	17,54	ST48
A26	25,63	26,03	+	+	*E. coli*	3	17,53	17,41	ST48
A24	27,16	27,40	+	+	*E. coli*	4	18,24	19,01	ST48
A16	27,57	28,18	+	+	*E. coli*	4	18,53	18,19	ST48
A21	28,20	28,82	+	+	*E. coli*	3	18,65	18,24	ST48
A34	28,95	28,91	+	+	*E. coli*	4	20,31	20,56	ST48
A15	29,45	29,68	+	+	*E. coli*	4	21,17	21,47	ST48
B5	30,16	30,47	+	+	*E. coli*	4	21,50	21,22	ST48
A28	30,90	31,10	+	+	-				
A30	29,23	30,37	+	+	-				
A32	29,34	30,40	+	+	-				
A25	30,19	31,07	+	+	-				

A33	30,48	31,03	+	+	-
A14	31,42	31,02	+	+	-
A23	31,59	32,01	+	+	-
A31	32,25	32,20	+	+	-
A36	30,59	31,95	+	+	-
A29	31,57	32,50	+	+	-
A22	31,63	32,53	+	+	-
A37	31,80	32,18	+	+	-
A35	32,28	33,37	+	+	-
A38	32,72	35,18	+	+	-
B6	30,21	30,97	+	+	-
B7	31,32	31,94	+	+	-
B8	31,50	30,96	+	+	-