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Letter to the Editor

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

Sir,

Since November 2015, the newly identified plasmid-mediated colistin resistance gene *mcr-1* has been reported worldwide in Gram-negative bacteria and is increasingly gaining recognition [1]. This newly identified resistance gene, encoding a phosphoethanolamine transferase, triggered an avalanche of retrospective studies investigating the presence of this specific gene from various isolates, including from food, animals and humans [2]. The aim of the current study was to evaluate the occurrence of the *mcr-1* gene in faeces of poultry from Algeria, a country where colistin is widely used in animal breeding.

In 2016, faeces was collected from chicken farms and slaughterhouses in Algeria. Samples were harvested directly from chicken cloacae and were placed in single, sterile, identified tubes. A total of 120 poultry faeces samples were collected in three regions of Algeria, including 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 good co-operation and permission of the farm owners.

DNA was extracted from the samples using an automated QIAGEN® BioRobot EZ1 (QIAGEN, Tokyo, Japan) with an EZ1 DNA Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was used as a template for double quantitative real-time PCR (qPCR) assays using the PE1 and PE2 systems targeting the MCR-1-encoding gene [3]. All real-time qPCRs were performed using a CFX96™ Real-Time System/C1000™ Touch Thermal Cycler (Bio-Rad, Singapore). Results were deemed positive if the cycle threshold (Ct) value obtained by CFX96™ was <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 BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

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

The 25 *mcr-1* positive samples were tested by standard PCR as previously described prior to sequencing [3]. The obtained

Table 1
Quantitative real-time PCR (qPCR) results of *mcr-1*-positive poultry faeces samples.

Sample	qPCR using PE1 system	qPCR using PE2 system	Standard PCR	Sequencing	Culture	COL MIC (mg/L)	qPCR using PE1 system	qPCR using PE2 system	MLST
A27	25.37	25.49	+	+	*Escherichia 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	+	+	–				

COL, colistin; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing.

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sequences were 100% identical to that of the *mcr-1* gene sequence reported by Liu et al. [1].

For the selection of bacteria carrying the *mcr-1* gene, samples were incubated on selective culture medium containing colistin for 24 h at 37 °C. Isolated colonies from each sample were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using a microflex™ LT spectrometer (Bruker Daltonik GmbH, Bremen, Germany). From the 25 positive samples, it was possible to isolate eight *E. coli* (7/79 from Algiers and 1/22 from Blida) that were confirmed to harbour the *mcr-1* gene by qPCR.

Antimicrobial susceptibility testing and Etest was performed on Mueller–Hinton agar using the standard disk diffusion procedure as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk diffusion susceptibility testing showed that eight *E. coli* strains were resistant to colistin and amoxicillin. Minimum inhibitory concentrations (MICs) of colistin confirmed *E. coli* resistance, with MICs ranging from 3–4 mg/L. Using multilocus sequence typing (MLST) of the isolated strains based on seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) from the MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli), a phylogenetic tree was constructed using the MEGA6 program and the neighbour-joining method. This analysis showed that the eight *E. coli* isolates belonging to the same sequence type (ST48), which has already been reported in Algeria from chickens and humans [4].

In this study, we clearly showed the emergence and high prevalence (20.8%) of the *mcr-1* plasmid-mediated colistin resistance gene in *E. coli* isolates from poultry in Algeria. In Algeria, *mcr-1* was first detected in chicken faeces [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 sequence type (ST405).

The current results highlight the potential spread of the *mcr-1* gene in poultry from Algeria that appears to be related to the uncontrolled use of colistin in animals. Recently, a study in Vietnam has demonstrated the relationship between colistin use on farms and the presence of the *mcr-1* gene in animal isolates [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 a prophylactic and growth promoter. In addition, the current 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.

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Competing interests

None declared.

Ethical approval

Not required.

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