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Raoudha Grami, Wejdene Mansour, Wahib Mehri, Olfa Bouallègue, Noureddine Boujaâfar, Jean-Yves Madec, Marisa Haenni

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R Grami [1,3], W Mansour [2,3], W Mehri [4], O Bouallègue [3], N Boujaâfar [3], J Madec [1], M Haenni [1]
1. Unité Antibiorésistance et Virulence Bactériennes, ANSES Site de Lyon, Lyon, France
2. Institut Supérieur des Sciences Appliquées et de Technologie de Mahdia, Tunisia
3. Unité Résistances Bactériennes Emergentes et Sécurité des Soins, UR12SP37, Laboratoire de Microbiologie, Hôpital Universitaire Sahloul, Sousse, Tunisia
4. Commissariat Régional au Développement Agricole, Sousse, Tunisia

Correspondence: Marisa Haenni (marisa.haenni@anses.fr)

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We report a high prevalence of MCR-1 and CTX-M-1-producing *Escherichia coli* in three Tunisian chicken farms. Chickens were imported from France or derived from French imported chicks. The same IncHI2-type plasmid reported to carry those genes in cattle in France and in a food sample in Portugal was found in Tunisian chickens of French origin. This suggests a significant impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance in Europe.

Horizontal transfer was found to play a major role in the spread of colistin resistance in *Enterobacteriaceae* when a plasmid-located *mcr-1* gene was reported to be circulating in livestock, foodstuff and human beings in China in late 2015 [1]. A few weeks later, *mcr-1* was recognised in Europe among extended-spectrum beta-lactamase (ESBL)- or AmpC-producing *Escherichia coli* isolated from chicken meat and humans [2]. In January 2016, the worldwide distribution of the *mcr-1* gene was highlighted [3,4].

The plasmid type first identified as a *mcr-1* vehicle in China was an IncI2-like plasmid, but several different *mcr-1*-positive plasmids have now been reported, including IncHI2-type plasmids. Indeed, Tse et al. reported *mcr-1* on an IncHI2-type plasmid in a *Salmonella enterica* isolate from a food sample in Portugal in 2011 [5]. Interestingly, IncHI2-type plasmids were also recognised to spread $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* in *E. coli* in food animals in France [6]. These data suggest a specific epidemiology of *mcr-1* plasmids in the European animal reservoir that pose a risk for humans. This prompted us to investigate 37 *E. coli* strains recovered from 29 Tunisian chickens imported from France or derived from French imported chicks and harbouring resistance to colistin and broad-spectrum cephalosporins.

Detection of the $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* genes in healthy chickens in Tunisia

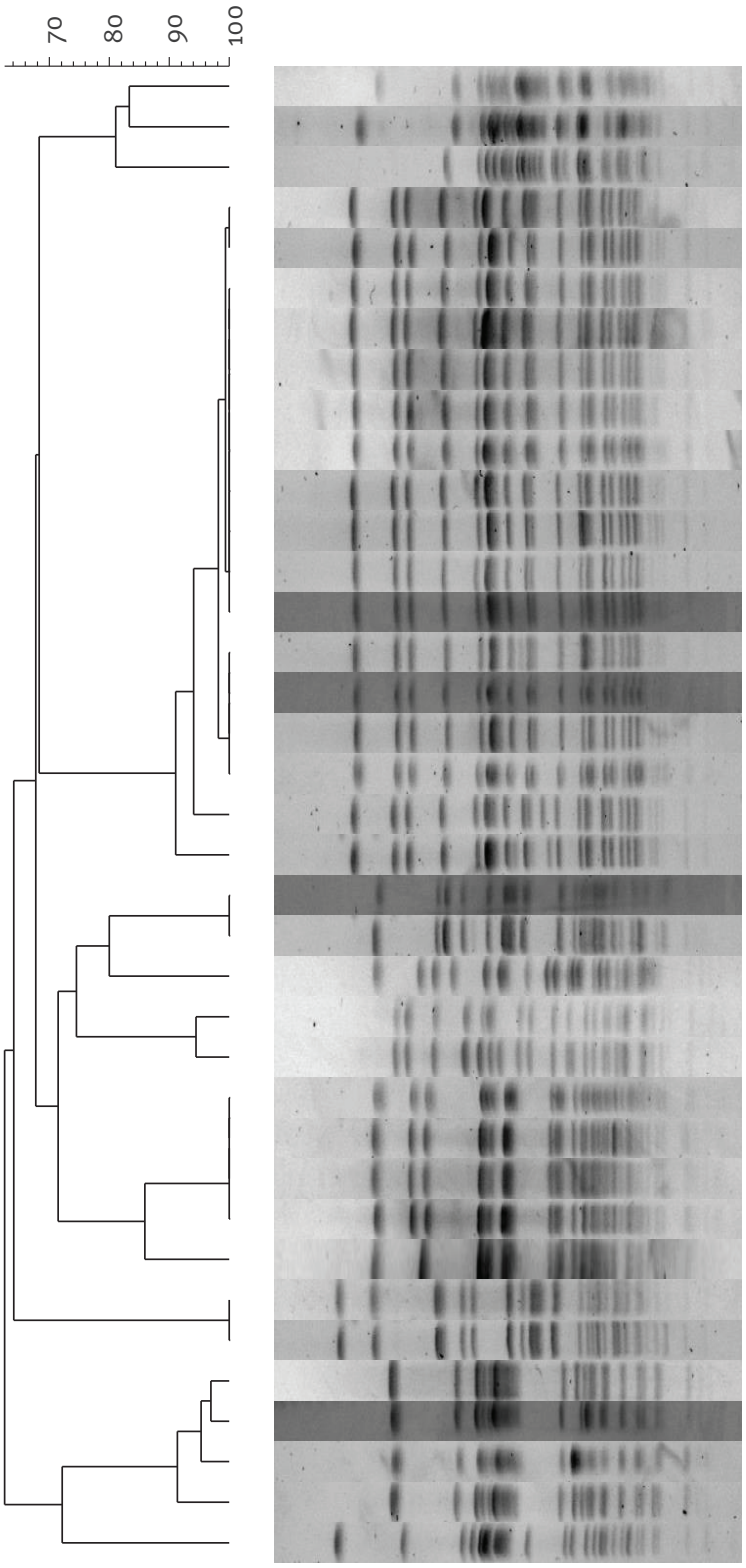
In July 2015, 52 randomly chosen healthy birds were collected on three different Tunisian farms: 10 on farm A, 12 on farm B and 30 on farm C with the initial purpose to investigate the prevalence of ESBL-positive chickens. A faecal sample of each individual was plated on MacConkey agar containing 4 mg/L cefotaxime and one colony per morphology was picked up. This resulted in the identification of 37 *E. coli* isolates harbouring resistance to broad-spectrum cephalosporins and originating from 29 birds (Table).

Those 29 birds were from farm A (2/10), farm B (2/12) and farm C (25/30). All 37 isolates produced an ESBL as attested by the synergy test, and the $bla_{CTX\text{-}M\text{-}1}$ gene was identified in all isolates by PCR and sequencing. All isolates expressed additional co-resistances to phenicols, tetracyclines, sulfonamides, trimethoprim, quinolones and fluoroquinolones as determined by disk diffusion against 32 antibiotics. Surprisingly, disk diffusion also revealed small colistin diameters (16–17 mm). We were in the course of investigating these non-susceptible isolates when the publication by Liu et al. [1] drew a different light on our results and prompted us to further investigate colistin resistance.

All isolates presented a minimum inhibitory concentration (MIC) of 2–6 µg/mL to colistin by E-test. PCR and sequencing using published primers [1] revealed the newly described *mcr-1* gene in all of the ESBL-positive *E. coli* with 100% homology to the published sequence (GenBank: KP347127.1). Isolates from farm A presented two closely related but not identical *Xba*I pulsed-field gel electrophoresis (PFGE) patterns (one band difference) belonging to cluster G, while isolates from farm B presented two distinct patterns belonging to the clusters F and K (Figure 1).

Figure 1

Pulsed-field gel electrophoresis-based dendrogram and *Xba*I macrorestrictions, Tunisia, July 2015 (n = 37)



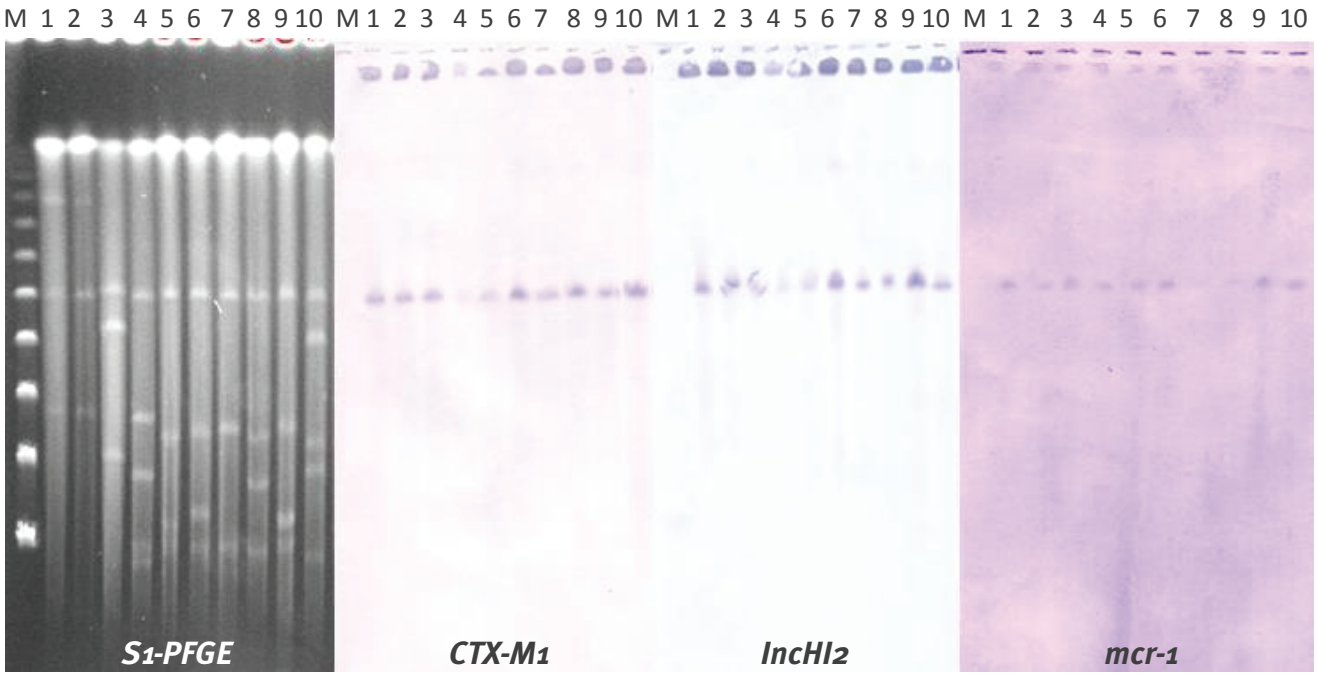
Isolate	Farm	MIC (µg/mL)	PFGE cluster
24B	C	3	A
33B	C	6	B
39B	C	3	C
19A	C	3	D
33A	C	3	D
14	C	6	D
18B	C	4	D
22	C	3	D
23	C	3	D
24A	C	3	D
26	C	4	D
32B	C	2	D
40	C	5	D
41	C	5	D
32A	C	3	D
42A	C	5	D
36A	C	3	D
39A	C	3	D
25A	C	6	D
30	C	4	D
21A	C	4	E
25B	C	3	E
10	B	6	F
MF5	A	3	G
MF8	A	5	G
15	C	3	H
16A	C	4	H
17A	C	3	H
20	C	4	H
18A	C	3	I
16B	C	3	J
31A	C	3	J
12	B	3	K
42B	C	3	K
36B	C	3	K
38B	C	4	K
38A	C	3	L

MIC: minimum inhibitory concentration; PFGE: pulsed-field gel electrophoresis.

Analysis was performed using the Dice coefficient with optimisation set at 0.5% and tolerance at 1.5%.

Figure 2

Southern blot hybridisations on S1 nuclease-pulsed-field gel electrophoresis gels using specific probes for the detection of $bla_{CTX\text{-}M\text{-}1}$, IncHI2 and *mcr-1*, Tunisia, July 2015 (n = 10)



PFGE: pulsed-field gel electrophoresis

M: size marker (Lambda ladder 0.05-1 Mb, Bio-Rad); Lane 1: isolate MF5; Lane 2: isolate MF8; Lane 3: isolate 10; Lane 4: isolate 12; Lane 5: isolate 14; Lane 6: isolate 16A; Lane 7: isolate 16B; Lane 8: isolate 18A; Lane 9: isolate 21A; Lane 10: isolate 24B.

All 37 isolates presented the same profile, so that only a subset of 10 isolates is presented here.

Isolates from farm C presented one main cluster (cluster D encompassing 17 isolates presenting patterns with › 90% similarity) and nine additional clusters (A–C, E, H–L) presenting patterns with ‹ 90% similarity. Antibiotics used were colistin, sulfonamides and enrofloxacin on farms A and C, and chloramphenicol and enrofloxacin on farm B.

Co-localisation of $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* on IncHI2-type plasmids

Replicon typing and hybridisation experiments proved that $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* co-localised in all isolates on a single and large (250–280 kbp) IncHI2-type plasmid (Figure 2).

According to the plasmid double locus sequence typing (pDLST) scheme [7], these IncHI2-type plasmids belonged to the ST4 subtype and presented positive amplification of the *hipA* gene and no amplification of the *smr092* and *smr0183* genes [7]. Interestingly, the IncHI2-type plasmids recently found in food animals in France also belonged to the very same ST4 subtype (data not shown) [6]. Hence, IncHI2-type plasmids were responsible for the spread of $bla_{CTX\text{-}M\text{-}1}$ and *mcr-1* in different chicken farms in Tunisia, in the bovine sector in France and in foodstuff in Portugal.

High prevalence of *mcr-1*-positive chickens on Tunisian farms

Data on *mcr-1* from the poultry reservoir are lacking except for one single case in Algeria [8]. However, *mcr-1* reports from chicken meat have been recurrent [1,2,9,10]. Here, farms A and C (counting 7,500 and 8,500 chickens, respectively) host grandparent flocks and import one-day-old chicks from France (Table). Farm B is located 80 km apart from the others and rears 200,000 broilers deriving from one-day-old chicks sold by a Tunisian hatchery also importing birds from France. Thus, the estimated true prevalence (with confidence intervals at 95%) of *mcr-1*-positive chickens reaches 20% (3–56%) on farm A, 17% (4–49%) on farm B and 83% (65–94%) on farm C. This last figure is even higher than recently reported from food animals in China [1].

Conclusion

From this study, we conclude that the live chicken population in Tunisia is heavily colonised by *mcr-1*-positive *E. coli* with subsequent possible contamination

Table

Epidemiological and molecular features of $bla_{CTX\text{-}M\text{-}1}$/mcr-1-positive *Escherichia coli*, Tunisia, July 2015 (n = 37)

Farm	Location	Number of birds on farm	Age of birds	Origin of the birds	Number of birds sampled	Number of ESBL-positive birds	Number of *mcr1* and bla_{CTXM_1}-positive *E. coli*	Plasmid type carrying bla_{CTXM_1} and *mcr1*
Farm A	Moknine	8,500	17–18 weeks	France	10	2	2	IncHI2/ST4
Farm B	Enfidha	200,000	35 days	Tunisia/France	12	2	2	IncHI2/ST4
Farm C	Moknine	7,500	62 weeks	France	30	25	33[a]	IncHI2/ST4

ESBL: extended-spectrum beta-lactamase.

[a] One colony per morphology was picked up, resulting in a higher number of *E. coli* isolates than the number of samples.

of chicken products [11,12]. Multilocus sequence typing (MLST) was not performed in this study since PFGE demonstrated the presence of numerous clusters of *E. coli* (A to L) so that the *mcr-1* dissemination was clearly a consequence of the spread of the unique IncHI2/ST4 plasmid in various *E. coli* backgrounds.

Contamination of both the poultry production pyramid and the food chain is undoubtedly of public health relevance. It is now crucial to determine the prevalence of the *mcr-1* gene in poultry and poultry meat as well as in other livestock (live animals or meat) in Tunisia and other African countries in order to estimate the risk to human health.

In addition, the finding of a single IncHI2-type plasmid spreading the $bla_{CTX\text{-}M\text{-}1}$/*mcr-1* genes in the food sector in different European and non-European countries makes us believe that global imports and exports of food animals and foodstuff are a major determinant of *mcr-1* dissemination. Global chicken meat production is forecast to dramatically increase in the future because of rising demands worldwide and subsequent rising production volumes in the major exporting countries. European countries already faced a major spread of ESBL/pAmpC genes in animals that subsequently became ESBL sources for humans, mostly as a result of poultry trades [13,14]. Worryingly, genes providing resistance to broad-spectrum cephalosporins and colistin have been shown to be tightly linked on the same plasmids, indicating that urgent international attention is necessary on the global market of veterinary drugs for food animals.

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Conflict of interest

None declared.

Authors' contributions

RG collected the isolates, collected the data and performed the molecular analysis. MH, WM, and JYM coordinated the work and participated to the data analysis. MH and JYM drafted the manuscript, WM and RG participated in the writing of the manuscript, and all authors have read and accepted the submitted manuscript.

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