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Intestinal carriage of *mcr*-1 and *mcr*-5 mediated colistin-resistant *Escherichia coli* in avian species: First report from broiler breeders and laying hens in Algeria

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ABSTRACT

Colistin, a critical last-resort antibiotic in human medicine, is commonly used in veterinary practice both as a growth promoter and for the treatment and prevention of enterobacterial infections in livestock. However, its extensive use has been linked to the emergence of colistin-resistant Gram-negative bacteria. The co-occurrence of *mcr* genes with other multidrug resistance determinants increases the risk of pan-drug-resistant strains, posing a significant threat to public health and potentially limiting therapeutic options. This study aimed to investigate the occurrence of colistin resistance in *Escherichia coli* isolates from apparently healthy poultry in Algeria. A total of 360 *E. coli* isolates collected from broilers, broiler breeders, breeding hens, and laying hens were tested for colistin susceptibility using the broth microdilution method. Phenotypic resistance was observed in 14 isolates (3.9 %) with MICs ranging from 4 to 32 μg/mL. All resistant isolates exhibited a multidrug-resistant phenotype. PCR screening and Sanger sequencing revealed that 78.6 % (11/14) of the colistin-resistant isolates carried the *mcr*-1 gene, and 57.1 % (8/14) harboured both *mcr*-1 and *mcr*-5. The *mcr*-2, *mcr*-3, and *mcr*-4 genes were not detected. Additionally, conjugation assays demonstrated the transferability of the *mcr*-1 gene in one isolate, indicating its potential for horizontal dissemination. This is the first documented report of *mcr*-1 and *mcr*-5 in *E. coli* from broiler breeders and laying hens in Algeria, highlighting its significant public health implications. These findings underscore the need for enhanced surveillance and stringent control measures to prevent the spread of colistin resistance from animal reservoirs to humans.

1. Introduction

The global impact of antimicrobial resistance (AMR) is well documented, with antimicrobial use in the livestock sector recognised as a major contributor [1]. The emergence and spread of colistin resistance in poultry present a critical challenge to both veterinary and public health. Colistin, a polymyxin antibiotic, is considered a last-resort treatment for multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacterial infections in humans [2]. In Algeria, colistin is commonly used in poultry production for therapeutic and prophylactic purposes, as well as a growth promoting feed additive [3]. Its frequent use without veterinary supervision, often at sub-therapeutic doses, has exerted significant selective pressure, driving the emergence of colistin-resistant strains, particularly *Escherichia coli* harbouring plasmid-mediated colistin resistance (*mcr*) genes. Similar practices in neighbouring countries further contribute to the regional spread of colistin [4].

Poultry and livestock are recognised as major reservoirs for *mcr*-positive bacteria and as important sources for the transmission of colistin resistance [5]. Since the first identification of the *mcr*-1 gene in 2015 [6], at least ten distinct variants (*mcr*-1 to *mcr*-10) and more than 37 *mcr*-1 subvariants have been reported globally, predominantly in

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E. coli, *Klebsiella pneumoniae*, and non-typhoidal *Salmonella* strains from animal, environmental, and human sources [7,8]. Among these, *mcr*-1 remains the most prevalent, accounting for approximately 95 % of *mcr* detections worldwide [9]. Whole-genome sequencing (WGS) investigations have provided comprehensive insights into the genetic environment of *mcr* genes and their role in the dissemination of colistin resistance [8].

In Algeria, *mcr*-1 gene was first detected by Olaitan et al., in 2016 [10], followed by Chabou et al. [11] in the same year.

Recent genomic studies have shown that multiple *mcr* variants, such as *mcr*-1.1 and *mcr*-3.5, can co-occur within a single *E. coli* isolate, often located on highly conjugative plasmids, such as IncX4, IncI2, and IncHI2, promote rapid horizontal gene transfer and facilitate the widespread dissemination of colistin resistance across diverse bacterial strains and species [9,12]. The *mcr*-carrying plasmids frequently co-harbour other critical resistance determinants, including extended-spectrum beta-lactamases (ESBLs) (e.g., $bla_{\text{CTX-M-55}}$), fluoroquinolone resistance, and carbapenemases, leading to XDR phenotypes that severely limit treatment options. The co-localisation of multiple resistance genes accelerates the spread of multidrug resistance within poultry microbiota and increases the risk of transmission to humans through direct contact or the food chain [2,6].

Epidemiological studies in North Africa, the Middle East, and other regions have reported moderate to high prevalence rates of *mcr*-1 and other *mcr* variants in poultry farms. This reflects the widespread dissemination of colistin resistance in avian production systems, often exacerbated by the uncontrolled or indiscriminate use of colistin-containing products [4,13]. The detection of *mcr* genes in poultry, farm workers, and environmental sources underscores the potential for horizontal gene transfer and zoonotic transmission, which poses a significant risk to human health.

Among the genetic mechanisms driving the dissemination of antimicrobial resistance and virulence genes, conjugation is considered the primary route. As a major form of horizontal gene transfer (HGT) both within and between species, conjugation plays a key role in the rapid dissemination of antibiotic resistance genes [14]. This process is well documented in *Enterobacteriaceae* and occurs in the intestinal microbiota of humans and animals, as well as in the environment [15].

The persistence and dissemination of colistin resistance in poultry threaten the efficacy of one of the last therapeutic options for treating MDR infections in human medicine. This highlights the urgent need for stringent antimicrobial stewardship, stronger regulatory control of antibiotic use in animal husbandry, and robust surveillance programs to monitor resistance trends. Addressing colistin resistance in poultry is imperative to safeguard both animal and human health under the One Health framework [5].

In view of the rapid global spread of plasmid-mediated colistin resistance genes, comprehensive data on their prevalence and epidemiological are essential for developing effective prevention and control strategies. This study aimed to investigate the occurrence of *mcr* genes, using both phenotypic and molecular analyses, in *E. coli* isolates previously collected from the gut microbiota of chicken in Algeria [16].

2. Materials and methods

2.1. Study Overview

This study is part of a comprehensive project carried out from December 2021 to October 2024, investigating ESBL/pAmpC and MDR *E. coli* in healthy poultry in Algeria. The broader project focuses on risk factors associated with these resistant strains, and related findings have been published previously [16].

2.2. Bacterial collection

A total of 360 *E. coli* strains were previously isolated from cloacal samples of healthy poultry, including broilers, laying hens, broiler breeders, and breeding hens [16]. The isolates were identified using MALDI-TOF, stored at −80 °C, and subsequently included in this study for further characterisation.

2.3. Antimicrobial susceptibility testing

The resistance profile of these strains against fourteen antibiotics was previously investigated by Akkari et al., 2024 [16]. Colistin Minimum inhibitory concentration (MIC) using the broth micro-dilution method in Mueller-Hinton broth (HiMedia, Mumbai, India) supplemented with cations (Ca^{+2} and Mg^{+2}), using colistin sulfate (Sigma-Aldrich, USA) in 96-well microtiter plates, following the EUCAST guidelines (2022) [17]. The experiment was repeated in triplicate for each strain. *E. coli* ATCC 25922 (colistin-sensitive) and *E. coli* NCTC 13846 (colistin-resistant) served as negative and positive controls, respectively.

2.4. Detection and sequencing of mcr genes

Isolates exhibiting colistin MIC values exceeding 2 mg/L according to Smelikova et al. [18] were subjected to plasmid DNA extraction and were screened for *mcr*-1 to *mcr*-5 genes by conventional PCR using primers detailed in Supplementary material Table S 1. PCR products positive for *mcr-1* (n = 11) and *mcr-5* (n = 8) were confirmed by Sanger sequencing [19], using the same primers listed in (Table S1). Sequences were compared against reference sequences available in the NCBI database (http://www.ncbi.nlm.nih.gov).

2.5. Phylogenetic analysis

Phylogenetic analysis of *mcr* gene sequences were performed as described by Li et al., 2019 [20]. Both study and GenBank reference sequences of *mcr*-1 and *mcr*-5 were aligned individually using MEGA11 software to assess their genetic relatedness.

2.6. Conjugation assays

Conjugation experiments were conducted on plasmids from 14 colistin-resistant isolates using the broth-mating method, with sodium azide-resistant *E. coli* J53-2 as the recipient strain, following established protocols [21]. Plasmid DNA was initially extracted from overnight Luria-Bertani (LB) broth via a modified alkaline lysis technique [22]. Transconjugants were selected on LB broth supplemented with 3 μg/mL of colistin and 200 μg/mL of rifampicin. Colistin resistance was confirmed by MIC determination. Antimicrobial susceptibility testing and *mcr* gene detection by PCR followed by Sanger sequencing were performed on transconjugants to verify concordance with donor strains.

3. Results

3.1. Determination of phenotypic colistin susceptibility

Of the 360 *E. coli* strains tested, 346 (96.1 %) were susceptible to colistin, with relatively low MIC values ranging from 0.125 to 1 μg/mL. In contrast, 14 strains (3.9 %) exhibited resistance to colistin, showing MIC values between 4 and 32 μg/mL (Table 1). Most resistant isolates were recovered from broiler chickens (n = 8), followed by broiler breeders (n = 3), breeding hens (n = 2), and laying hens (n = 1). All resistant isolates displayed a multidrug-resistant phenotype. The antimicrobial susceptibility profiles of colistin-resistant *E. coli* isolates to other antibiotics are shown in (Fig. 1).

3.2. Genes encoding colistin resistance

Among the 14 colistin-resistant strains, 11 (78.57 %) carried the *mcr*-1 gene, and 8 (57.14 %) harboured the *mcr*-5 gene (Fig. 2). None of the

Table 1
Antimicrobial resistance characteristics of *E. coli* strains from healthy poultry.

Sample ID	Sampling origin	Location	Phylogenetic groups	MIC of Colistin (μg/mL)	bla_{TEM}	bla_{CMY-2}	bla_{SHV}	bla_{CTX-M}	*mcr*-1	*mcr*-5	*tet*(A)	*tet*(B)	*aac-(6')-Ib*	*qnrA*	*qnrB*	*qnrS*	*catA1*	*sul1*	*sul2*
DZEC 3	Breeding hens	Biskra	B1	32			+	+											
DZEC 4	Breeding hens	Biskra	B2	32			+	+								+			
DZEC 7	Broiler breeders	Biskra	A	4							+								
DZEC 102*	Broilers	Batna	D	32					+	+									
DZEC 103*	Broilers	Batna	A	4	+				+	+							+		
DZEC 106	Broilers	Batna	D	4	+				+		+				+	+	+	+	+
DZEC 109	Broilers	Batna	A	4					+		+							+	
DZEC 139*	Broiler breeders	Biskra	B2	32	+			+	+	+	+	+			+	+	+	+	+
DZEC 194*	Broilers	Batna	D	8		+			+	+	+	+				+		+	
DZEC 199*	Layin hens	Batna	D	4					+	+	+		+					+	
DZEC 206*	Broilers	Batna	D	32					+	+	+					+		+	
DZEC 237	Broilers	Batna	D	4	+				+			+				+		+	
DZEC 238*	Broilers	Batna	B2	4	+				+		+	+				+			
DZEC 277*	Broiler breeders	Batna	D	4	+				+	+	+	+				+	+	+	

Note: The colored boxes indicate the AMR genes associated with various classes of antimicrobials. * *E. coli* isolates harbouring both *mcr*-1 and *mcr*-5.

β-Lactams Colistin Tetracycline Aminoglycoside Quinolone Phenicol Sulfonamide

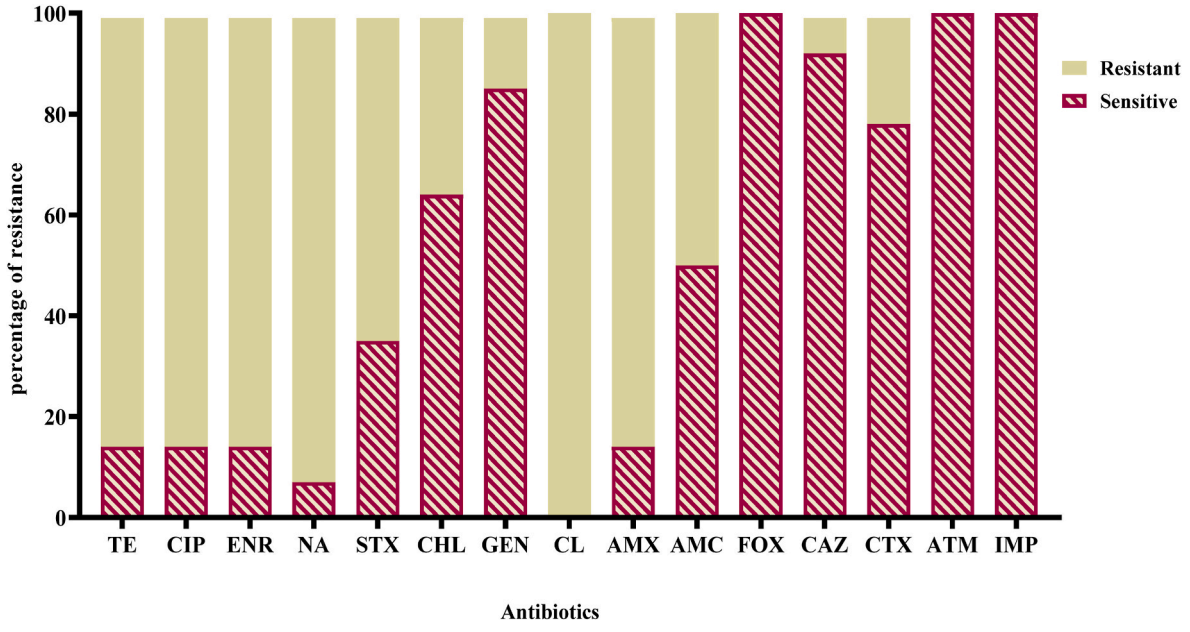


Fig. 1. Antimicrobial resistance profiles of colistin-resistant *E. coli* strains recovered from healthy poultry. AMX; amoxicillin, TE; tetracycline, ENR; enrofloxacin, CIP; ciprofloxacin, STX; sulfamethoxazole, NA; nalidixic acid, CHL; chloramphenicol, CL; colistin, GEN; gentamicin, AMC; amoxicillin/clavulanic acid, CAZ; ceftazidime, CTX; cefotaxime, FOX; cefoxitin, ATM; aztrreonam, IMP; imipenem.

E. coli isolates tested carried the other colistin resistance genes, namely *mcr*-2, *mcr*-3, or *mcr*-4.

3.3. Sequence analysis

Sequencing analysis of the *mcr*-1 and *mcr*-5 genes revealed 100 % identity with the reference sequences deposited in the NCBI GenBank database. The obtained sequences were submitted to GenBank and assigned specific accession numbers, as shown in (Figs. 3 and 4).

3.4. Screening for antimicrobial resistance genes

Among the 11 *mcr*-1-positive *E. coli* isolates, six harbour β-lactam resistance genes such as bla_{TEM}. Of these, one isolate carried extended-spectrum β-lactamase (ESBL) genes, specifically $bla_{CTX-M-1}$. A plasmid-mediated AmpC β-lactamase gene, bla_{CMY-2}, encoding the CMY-2 enzyme, was identified in one isolate. In addition to colistin resistance genes, the *E. coli* isolates most frequently carried resistance genes to fluoroquinolones (8/11, 72.7 %), tetracyclines (9/11, 81.8 %), aminoglycosides (1/11, 9.1 %), chloramphenicol (5/11, 45.5 %), and sulfonamides (8/11, 72.7 %). Furthermore, class 1 integrons were detected in 10 out of the 11 isolates (Table 1).

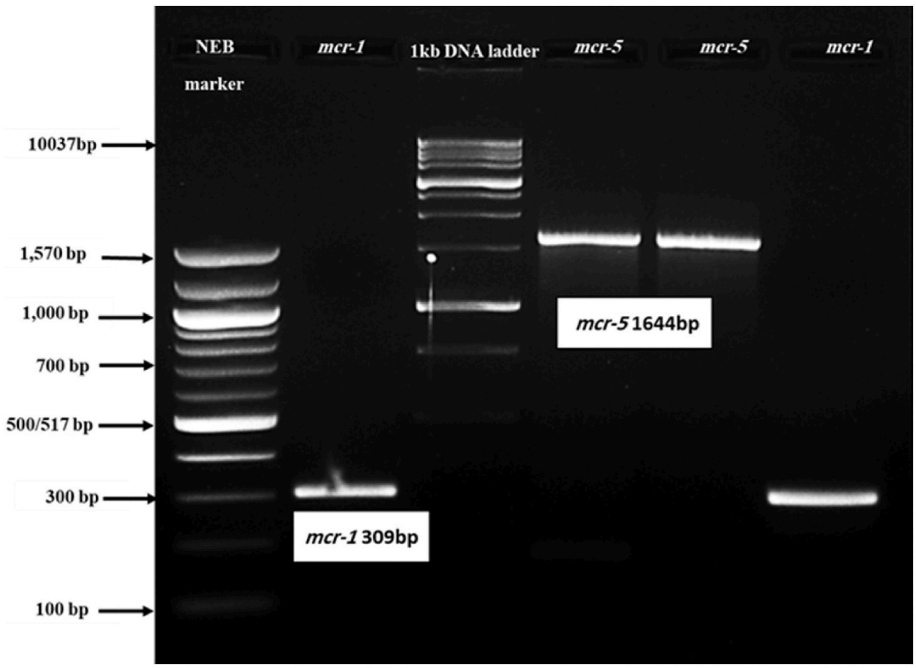


Fig. 2. PCR products of *mcr*-1 and *mcr*-5 genes.

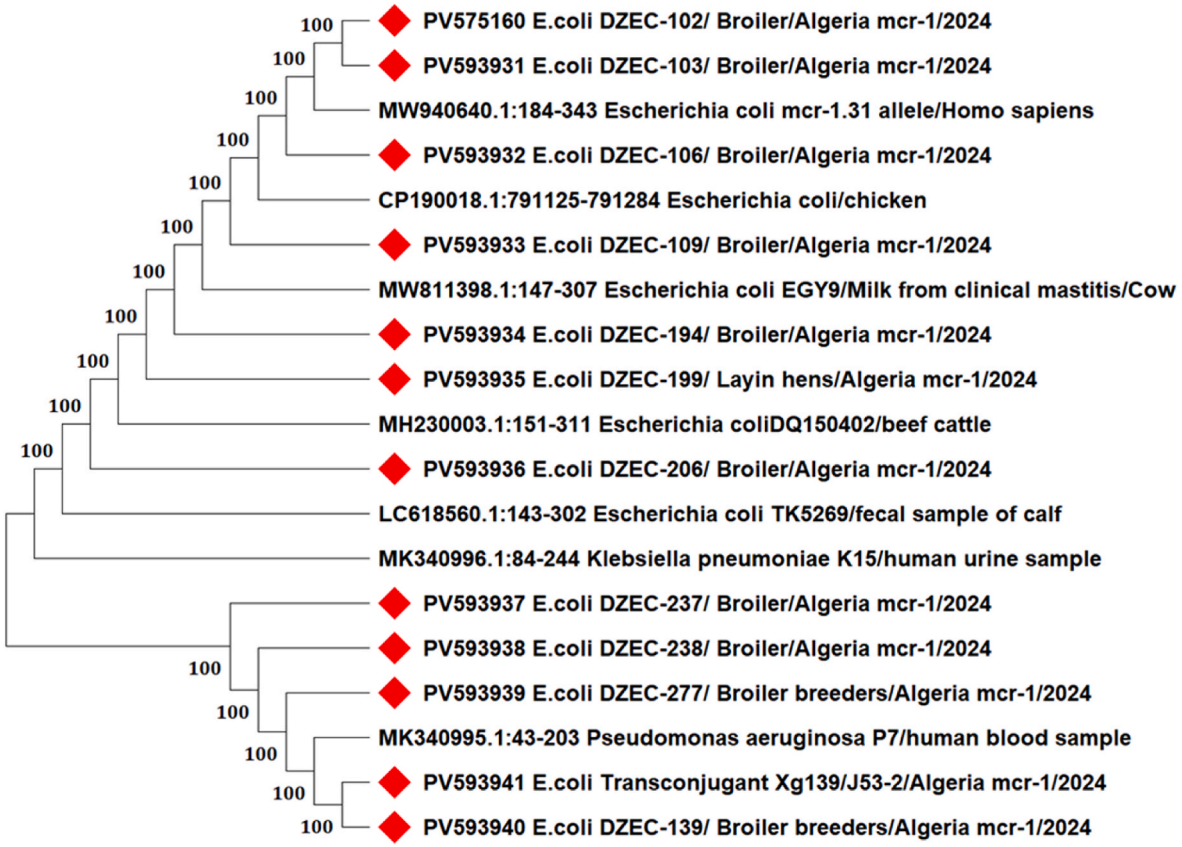


Fig. 3. Phylogenetic tree illustrating the relationships among partial sequences of the *mcr-1* gene. The sequences analysed in this study are indicated with red rhomboids.

3.5. Conjugation

The *mcr*-1 gene was successfully transferred via conjugation in one strain. Additionally, four transconjugants were obtained that exhibited co-transfer of other resistance genes; however, the *mcr*-1 gene was not acquired in these cases, as expected (Table 2). All *mcr-5* positive isolates were also tested in the conjugation assay, but none showed transferable colistin resistance.

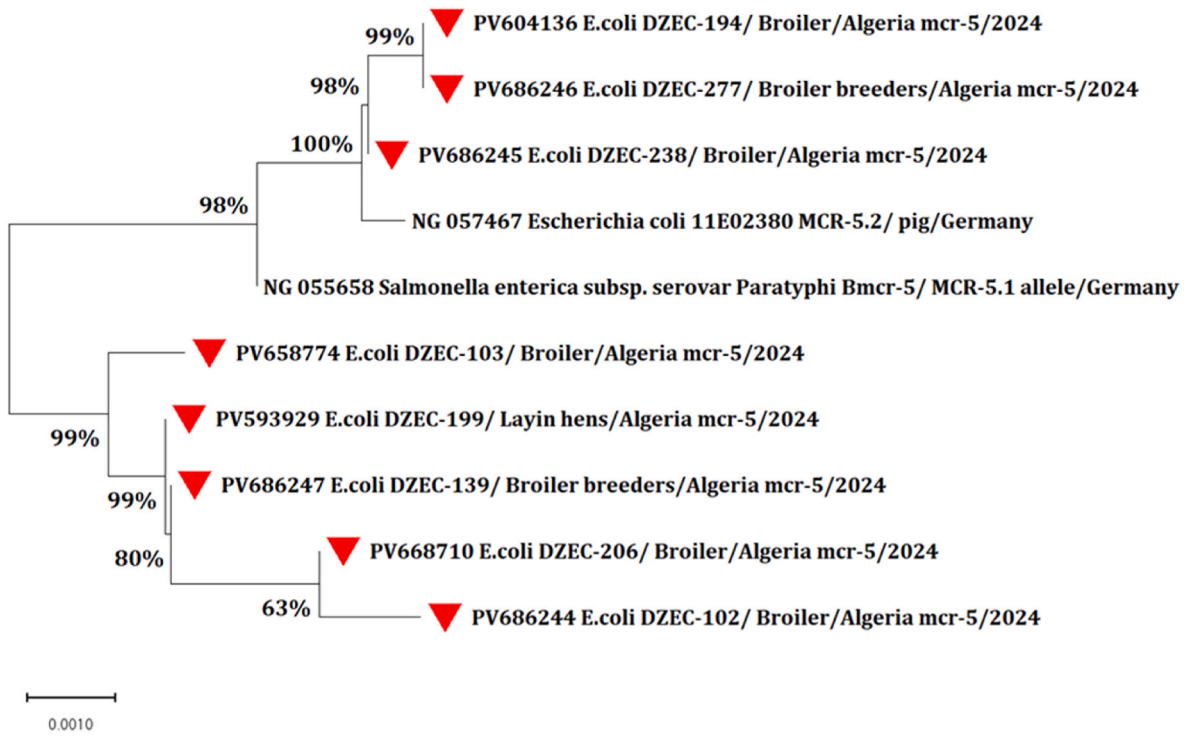


Fig. 4. Phylogenetic tree of *mcr*-5 gene sequences (n = 8) using the Neighbor-Joining method in MEGA 11. Study sequences are highlighted with red triangles.

Table 2
Comparison of AMR profiles between donor strains and their transconjugants using *E. coli* J53-2 as the recipient.

Col-R strain (Donor)	Resistance phenotypes of donors	Resistance genotype of donors	Transconjugant	Resistance phenotypes of transconjugants	Co-transferred genes
DZEC 139	AMX-CTX- AMC -CAZ- TE-ENR-STX- CIP -NA-CHL-CL	*tet*(A)- *tet*(B)- *sul*1- *sul*2 *qnrS*-*qnr*B- *catA*1 -bla_{TEM}- bla_{CTX-M}- *mcr*-1	J53-2 (pDZ139)	AMX-TE-STX-CHL-NA-CL	bla_{TEM} -*qnrS*-*intI*1-*sul*1-*sul*2-*mcr*-1
DZEC 106	AMX-AMC-TE-ENR-STX CIP- C-NA -CL	*tet*(A)- *tet*(B)-*qnrS*-*sul*1-bla_{TEM}-*intI*1-*mcr*-1	J53-2 (pDZ106)	AMX-TE	*tet*(B)
DZEC 109	AMX-AMC-TE-ENR-CIP-NA -STX-CL	*tet*(A)-*sul*1-bla_{TEM}-*intI*1-*mcr*-1	J53-2 (pDZ109)	AMX-TE-STX	*sul*1-bla_{TEM}
DZEC 238	AMX-TE-ENR-CIP- NA-CL	*tet*(A)-*tet*(B)-*qnrS*-bla_{TEM}-*intI*1- *mcr*-1	J53-2 (pDZ238)	AMX-TE	*tet*(A)-bla_{TEM}

AMX; amoxicillin, TE; tetracycline, ENR; enrofloxacin, CIP; ciprofloxacin, STX; sulfamethoxazole, NA; nalidixic acid, CHL; chloramphenicol, AMC; amoxicillin/clavulanic acid, CAZ; ceftazidime, CTX; cefotaxime, CL; colistin.

4. Discussion

The present investigation aimed to provide a molecular characterisation of the prevalence and genetic profiles of colistin-resistant *E. coli* isolates collected from healthy poultry in Algeria, considering colistin usage patterns in the poultry sector.

Colistin remains an important therapeutic option for managing multidrug-resistant Gram-negative bacterial infections in humans. In veterinary medicine, it is widely used for the treatment and prevention of enteric infections caused by *Enterobacterales*, and in some cases, for prophylactic purposes in livestock. However, the emergence of the *mcr* gene in multidrug-resistant bacteria poses a serious threat to the effectiveness of colistin, which is considered a last-line antimicrobial for difficult-to-treat Gram-negative infections. The extensive use of colistin in livestock, particularly for the treatment of colibacillosis in poultry, is recognised a key driver in the emergence and dissemination of plasmid-mediated colistin resistance, especially via the *mcr*-1 gene [23].

In the present study, *E. coli* isolates from cloacal samples collected from broilers, broiler breeders, and laying hens, showed a notable prevalence of colistin resistance, with detection of both *mcr*-1 and *mcr*-5 genes. These genes were identified in healthy poultry from two of the four Algerian provinces surveyed, with broilers showing the highest frequency of the *mcr*-1.

Among the 14 colistin-resistant *E. coli* isolates, 57.14 % (8/14) harboured both *mcr*-1 and *mcr*-5, a co-occurrence previously reported in bacteria of animal origin [12].

Other *mcr* variants (*mcr*-2, *mcr*-3, and *mcr*-4) were not detected. Interestingly, three isolates with colistin MICs of 4 μg/mL and 32 μg/mL tested negative for all five *mcr* genes screened, suggesting the possible involvement of additional variants (such as *mcr-6* to *mcr-8)* not included in this study, or chromosomal mutations as previously reported [25].

The *mcr*-1 gene was first reported in Algeria, North Africa, in 2016, with Olaitan et al. [10] describing a prevalence of 2.3 %, followed by Chabou et al. [11] in the same year. Subsequent reports have documented its presence in Tunisia, with an 8 % prevalence in chicken isolates [26], and in Egypt with a prevalence of 2.9 % in a human clinical isolate [23]. In Algeria, the *mcr*-1 gene has been detected in ESBL-producing *Salmonella enterica* from ready-to-eat chicken dishes [27], aquaculture-associated ESBL-producing *E. coli* [28], and in 20.8 % of broiler chicken faecal samples [11]. Environmental isolates carrying *mcr*-1 have also been recovered from agricultural soils, animal waste, and irrigation water in the Oran region of northwestern Algeria [29]. To date, the *mcr*-1 has not been reported in broiler breeder flocks or laying hens in Algeria; our study provides the first such documentation.

Additionally, we report *E. coli* strains carrying both the *mcr*-1 and *mcr*-5 genes in the intestinal microbiota of laying hens and broiler breeders, including in one isolate resistant to third-generation cephalosporins (ESCs). The co-occurrence of *mcr-1* and *mcr-5* within the same isolate is particularly alarming compared with single-gene carriage, as the coexistence of multiple *mcr* determinants may enhance the stability and persistence of colistin resistance while facilitating its horizontal dissemination. This observation is consistent with a recent study reporting that the high plasticity of plasmid genomes promotes the accumulation and co-transfer of multiple antimicrobial resistance genes (ARGs) within bacterial populations [24].

Globally, the prevalence of *mcr*-1 among poultry-derived *E. coli* varies widely. In Lebanon, Mikhayel et al., 2021 [30] reported a prevalence of 10.78 % in colistin-resistant faecal *E. coli* isolates, while in Egypt, 7.9 % of *E. coli* isolates carried *mcr*-1 [31]. In China, prevalence ranged from 1.1 % in isolates from pigs and chickens [32] to 18.42 % in *E. coli* from diseased swine [24], and up to 31.8 % in chickens [33]. Furthermore, rates of 19.5 % have been documented in chicken meat and liver samples in Brazil [34], and 8.7 % among isolates from chickens in northeast Vietnam [35]. In contrast, Tunisia reported markedly higher rates, with 92.29 % of *E. coli* isolates from chicken faeces and meat carrying *mcr*-1 gene [25], and Lebanon reported 84.4 % [13]. Such likely reflects differences in antimicrobial usage policies, farming practices, biosecurity measures.

Although *E. coli* strains carrying the *mcr*-1 and/or producing ESBL or pAmpC enzymes are generally considered commensal in healthy broiler chickens, recent studies have shown that some harbour various virulence genes associated with avian pathogenic *E. coli* (APEC) or extra-intestinal pathogenic *E. coli* (ExPEC), potentially increasing their ability to colonise the gut [36]. The predominance of *mcr*-mediated colistin resistance in food-producing animals, particularly poultry, highlights the role of livestock as a reservoir for critical antimicrobial resistance determinants [30].

Our study represents the first report of the *mcr*-5 gene in *E. coli* isolates in Algeria. This gene has not been previously documented in bacterial isolates from animals or humans in the country, indicating a potential emergence of novel resistant strains with clinical relevance. The *mcr*-5 sequence showed high nucleotide identity with variants previously reported in Europe. Although its genetic context could not be resolved without whole-genome sequencing, a previous study demonstrated that *mcr-5* is typically associated with Tn3-family transposons on diverse plasmid backbones, facilitating horizontal transfer [37]. Its emergence in Algerian poultry may be linked to the poultry trade, particularly through the importation of breeding hens or feed from Europe, given the country's reliance on such imports, or through environmental dissemination. The association of *mcr-5* with mobile genetic elements underscores its potential for spread and co-resistance, posing a threat to both animal and public health [38].

The detection of *mcr-1* and *mcr*-5 in isolates with MIC values of 4 μg/mL, 8 μg/mL, and 32 μg/mL aligns with findings from Malaysia [39]. Previously, *mcr*-5 has been reported in colistin-resistant *E. coli* from poultry in Nigeria [40] and in *Salmonella* spp. from Malaysia [39]. The public health concern lies in the potential for these resistant pathogens to spread to humans via contaminated poultry products or direct animal contact.

It is noteworthy that some *mcr*-positive strains reported in the literature have MIC values below 2 mg/L, categorising them as phenotypically susceptible. Such observations highlight the possibility of silent dissemination of *mcr* genes within *Enterobacteriaceae* populations [41]. In our study, all colistin-resistant *E. coli* isolates carried at least one additional AMR gene, with 13 distinct genotypic resistance profiles observed. One isolate harboured nine different resistance genes (bla_{TEM}, $bla_{CTX-M-1}$, *tet*(A), *tet*(B), *qnr*S, *qnr*B, *catA*1, *sul*1, *and sul*2) along with *mcr*-1 and *mcr*-5 (Table 1). The most frequently detected AMR gene was *tet*(A), followed by *qnr*S, *sul*1, and bla_{TEM}. Only a few isolates carried *qnr*B, *sul*2, $bla_{CTX-M-1}$, bla_{CMY-2} or *aac-(6′)-Ib*, and none carried bla_{SHV}. These findings are consistent with previous reports highlighting the widespread multidrug resistance among foodborne pathogens and the growing threat of transferable antimicrobial resistance across animal and human health sectors. The detection of *mcr*-1 and other last-resort resistance determinants in this study aligns with global concerns about the dissemination of AMR through the food [32,42].

The consistent presence of multiple AMR genes in all colistin-resistant isolates suggests potential co-transfer of resistance determinants, often involving tetracycline, β-lactams, and fluoroquinolone resistance genes. Given that fluoroquinolones and β-lactams are critically important in both veterinary and human medicine, their co-selection and dissemination via mobile genetic elements represents a significant public health threat [43]. Moreover, this co-resistance may further restrict available therapeutic options and increase the risk of treatment failure in both poultry and human infections. Healthy poultry may thus act as a reservoir for resistance genes that can be transferred to other bacteria.

Bacterial conjugation plays a major role in the horizontal transmission of antimicrobial resistance, particularly through conjugative plasmids [44]. Our conjugation assays confirmed the transferability of *mcr*-1, consistent with findings from Touati et al., 2020 [29] in Algeria and studies from China [15], Bangladesh [45], Egypt [23], and Singapore [43]. However, none of the *mcr-5* positive isolates were transferable under the same experimental conditions. Since plasmid typing to determine the incompatibility groups and host range of *mcr*-carrying plasmids was not performed, we hypothesise that the low transfer frequency of *mcr*-positive plasmids, except for *mcr*-1, could be attributed to factors such as low conjugation efficiency, plasmid instability, host–plasmid incompatibility, and potential fitness costs imposed on the bacterial host [46], as well as experimental limitations, including mating conditions and the sensitivity of transconjugant detection, which may influence the observed transfer frequency and underestimate the occurrence of transfer under natural settings.

Genomic investigations from eastern China based on whole-genome sequencing (WGS) of *E. coli* isolates from poultry and swine have revealed that *mcr* genes are associated with large conjugative plasmids and other clinically relevant resistance determinants [24,42]. Poultry farms are environments where antibiotics are used intensively, creating favourable conditions for the selection and dissemination of resistance genes. The identification of identical resistance genes in multidrug-resistant and environmental isolates suggests horizontal spread, potentially facilitated by integrons located on conjugative plasmids.

The co-existence of multiple resistance genes in poultry-derived *E. coli* is of serious concern, as it enables the rapid development of multidrug resistance and the emergence of highly pathogenic strains. Previous studies from Canada [47] and Ghana [48] have demonstrated potential transmission of resistant bacteria from poultry to humans, with evidence of high genetic similarity between poultry and human isolates. In our study, most colistin-resistant *E. coli* isolates belonged to phylogroup D (n = 7), followed by B2 (n = 2) and A (n = 2). Notably, phylogroups D and B2 are often associated with pathogenic *E. coli* strains [49]. The association of resistance with these phylogroups may reflect virulent *E. coli* lineages that have acquired resistance determinants, as well as the potential adaptation of commensal strains toward increased pathogenicity. This observation highlights the complex interplay between virulence and antimicrobial resistance evolution within the poultry microbiota.

Since the continued use of colistin in veterinary medicine, rigorous and regular surveillance of mobile colistin resistance determinants is essential. Such monitoring will support the early detection of emerging *mcr* variants and allow a more accurate assessment of the potential public health risks posed by foodborne antimicrobial resistance [4].

5. Limitations

This study has certain limitations. First, sampling was restricted to healthy poultry from four provinces, which may not fully represent the situation in other regions or production systems across Algeria; therefore, it does not allow inference of causality or determination of transmission direction between animal and environmental reservoirs. Second, only five *mcr* variants were screened, leaving the possibility that other determinants, such as *mcr*-6 to *mcr*-8 or chromosomal mutations, were missed. Third, plasmid typing to identify the specific incompatibility groups carrying *mcr* genes and their host range was not performed, which could have provided valuable epidemiological insights. Finally, whole-genome sequencing was not conducted, limiting the ability to assess the complete resistome, virulome and the localisation of *mcr* genes in the studied isolates.

6. Conclusion

Poultry farms represent a significant reservoir of antibiotic resistance genes that poses potential risks for public health. This study provides a comprehensive assessment of the prevalence and genetic characteristics of colistin-resistant *E. coli* isolates from healthy poultry in Algeria. This investigation highlights for the first time the detection of *mcr*-1 and *mcr*-5 genes in *E. coli* from the intestinal carriage of broiler chickens, broiler breeders, and laying hens in the country.

The rapid dissemination of these superbugs is alarming, as it is driven by horizontal gene transfer through highly mobile plasmids, enabling their spread within microbial communities and potentially into clinical settings. Furthermore, the considerable genetic diversity among the isolates suggests widespread dissemination of resistance genes within the intestinal microbiota of poultry in Algeria. Future studies should incorporate whole-genome sequencing to enhance the monitoring of *mcr* gene dissemination and genetic context. In parallel, strict antibiotic stewardship, improved on-farm biosecurity, and farmer education on responsible antimicrobial use are essential. Expanding surveillance to include diverse geographic areas, all poultry pyramid levels, and both healthy and diseased birds, as well as poultry products and farm environments, would provide a more comprehensive understanding of resistance spread. Regional collaboration through data sharing and harmonised "One Health" surveillance frameworks would further strengthen antimicrobial resistance control efforts.

CRediT authorship contribution statement

Hafsa Akkari: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Nouzha Heleili:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Osman Birol Ozgumus:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Neslihan Akarsu:** Data curation. **Ali Osman Kiliç:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2025.108180.

Data availability

Data will be made available on request.

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