VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms

Amira Djouzi[1*], Abdelheq Barberis[2], Meriem Derkaoui[1], Ismail Lafri[3], Hamza Ait seddik[4], Mohamed Sebaihia [1], Hamza Rahab[2]

[1]*Laboratory of Molecular Biology, Genomics and Bioinformatics, Department of Biology, Faculty of Nature and Life Sciences, University Hassiba Benbouali of Chlef, Algeria - DZ*
[2]*Biotechnology Research Center CRBt , Constantine, Algeria - DZ*
[3]*Department of Microbiology and Veterinary Pathology, Institut Pasteur of Algeria, Algiers 16000, Algeria; Laboratory of Biotechnologies Linked to Animal Reproduction, Institute of Veterinary Sciences, University Blida 1,Blida 09000, Algeria - DZ*
[4]*Laboratory of Valorisation and Bioengineering of Natural Resources LVBRN, Faculty of Sciences, University of Algiers 1 Benyoucef Benkhedda, Algiers, Algeria - DZ*

[*]*Corresponding author at: Laboratory of Molecular Biology, Genomics and Bioinformatics, Department of Biology, Faculty of Nature and Life Sciences, University Hassiba Benbouali of Chlef, Algeria - DZ*
E-mail: a.djouzi@univ-chlef.dz

Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3
Available on line: 07.09.2026

Abstract

Colibacillosis is a highly prevalent bacterial disease in poultry, resulting in the widespread use of antibiotics for both curative and preventive purposes. Consequently, avian pathogenic *Escherichia coli* (APEC) continues to act as a reservoir for antibiotic resistance genes, including the *mcr-1* gene, which codes for resistance to colistin, a crucial antibiotic in human medicine. The aim of this study was to evaluate the antibiotic resistance pattern of APEC and to investigate the genotyping, phylogrouping, and virulence of *mcr-1*-positive isolates. A total of 113 APEC were isolated, of which 92% were multidrug resistant (MDR). The *mcr-1* gene was detected in 41 isolates originating from turkeys and broilers. Two isolates carried bla_{TEM}, one of which also harboured bla_{CTX-M} encoding beta-lactamases. The Clermont phylogrouping revealed that 76% of the isolates belonged to phylogroup B1. Concerning the detection of the virulence-associated genes, 88% of isolates carried at least 3 genes. The ERIC-PCR classified our isolates into 6 different clusters. Our study highlights the emergence of colistin resistance and MDR, which pose a real threat to poultry production and public health. Control of antibiotic use in the poultry sector is urgent and mandatory.

Keywords

APEC, genotyping, mcr-1, phylogrouping, virulance

Introduction

Avian colibacillosis is a major infectious disease affecting poultry. It is caused by avian pathogenic *Escherichia coli* (APEC), which is capable of inducing systemic infections in chickens and turkeys. Avian colibacillosis is associated with high mortality and morbidity rates, leading to a reduction in the productive performance and dramatically affecting the global poultry industry (Dho-Moulin and Fairbrother, 1999; Mellata, 2013; Guabiraba and Schouler, 2015).

APEC can cause primary and secondary infections. Primary colibacillosis is manifested in outbreaks characterised by sudden and high mortality in several flocks that have an epidemiological link (Kathayat et al., 2018, 2021). However, secondary infection presents limited outbreaks and is mostly conditioned by the presence of predisposing factors such as viral infections and impaired biosecurity measures (Monroy et al., 2025; Vougat Ngom et al., 2025). Molecular typing of APEC isolates showed differences between APEC implicated in each type of infection. In primary infections,

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

one clonal lineage is detected in the majority of animals and flocks. However, in secondary infection, APEC reveal heterogeneous backgrounds even within the same flock (Mageiros et al., 2021; Kromann et al., 2022; Biström et al., 2025).

The genetic complexity and heterogeneity of APEC imply that no particular phylogroup is implicated in colibacillosis (Mehat et al., 2021). Some studies have reported the predominance of phylogroup B2 and related lineages (Zhu et al., 2021; Johnson et al., 2022), while others have found predominantly APEC from phylogroups A and B1(Ghanbarpour et al., 2011; Zhu Ge et al., 2014; Rezatofighi et al., 2021; Jalil et al., 2023; Jhandai et al., 2024; Boulbair et al., 2025), indicating that colibacillosis is multifactorial and not restricted to a distinct lineage of *E. coli* (Puterflam et al., 2022). In addition, the repertoire of expressed virulence-encoding genes, such as iron uptake systems, serum survival, and toxins (Amer et al., 2020), makes some APEC lineages more virulent than others (Schouler et al., 2012; van der Westhuizen and Bragg, 2012; Mbanga and Nyararai, 2015).

The epidemiology and management of APEC are further complicated by the widespread occurence of antimicrobial resistance (AMR). For example, APEC is the most significant reservoir for the plasmid-mediated colistin resistance (*mcr*) genes (Lima Barbieri et al., 2017). This is a cause for concern, given that colistin is considered a last-resort treatment for multidrug resistant (MDR) Gram-negative bacterial infections in humans (Poirel et al., 2018; Valiakos and Kapna, 2021). Among the *mcr* variants, the *mcr-1* gene remains the most prevalent worldwide in poultry (Badr et al., 2022).

In Algeria, where chicken meat is the main source of animal protein, various studies have been carried out on antibiotic resistance in APEC (Hammoudi and Aggad, 2006; Halfaoui et al., 2017; Meguenni et al., 2019; Lounis et al., 2020; Aberkane et al., 2023). However, studies on colistin resistance and the emergence of the *mcr-1* gene in APEC strains are limited (Halfaoui et al., 2024).

Given the global concern over APEC zoonotic risks, the spread of the *mcr-1* gene in poultry, and the lack of data from Algeria, further investigation is needed. Understanding the phylogenetic distribution, virulence profiles, and antimicrobial resistance patterns of *mcr-1* carrying APEC isolates is essential to assess risks related to poultry production and potential public health implications.

Hence, this study was undertaken to characterize APEC isolates from Algerian poultry farms, with a particular focus on the detection and characterization of *mcr-1* positive isolates, their phylogenetic background, and their antimicrobial resistance profiles.

Materials and methods

Study setting

From June 2023 to March 2024, 46 farms were sampled in different provinces of Algeria: Algiers, Boumerdes, Bouira, Tizi Ouzou, Blida, Medea, Ain Defla, Batna, and M'Sila (Figure 1). The farms included between 2,000 and 50,000 broilers, 26,000 to 30,000 layers, and 4,000 turkeys, aged between 4 days and 43 weeks. Various lines of poultry have been sampled, including Cobb 500 (n = 92) and Efficiency (n = 52) for broilers, Isabrown (n = 10) for layers, and BIG (n = 10) for turkeys.

The poultry were generally treated with antibiotics, the most reported ones being amoxicillin, colistin, erythromycin, doxycycline, tylosine, lincomycin, spectinomycin, norfloxacin, and erythromycin. Some farms use anticoccidials and vitamins such as vitamin C, K3, and sorbitol. Most farms were vaccinated against avian influenza virus (AIV), Newcastle disease virus (NDV), and infectious bronchitis virus (IBV). In addition, some farms were vaccinated against infectious bursal disease virus (IBDV) and Marek's disease.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

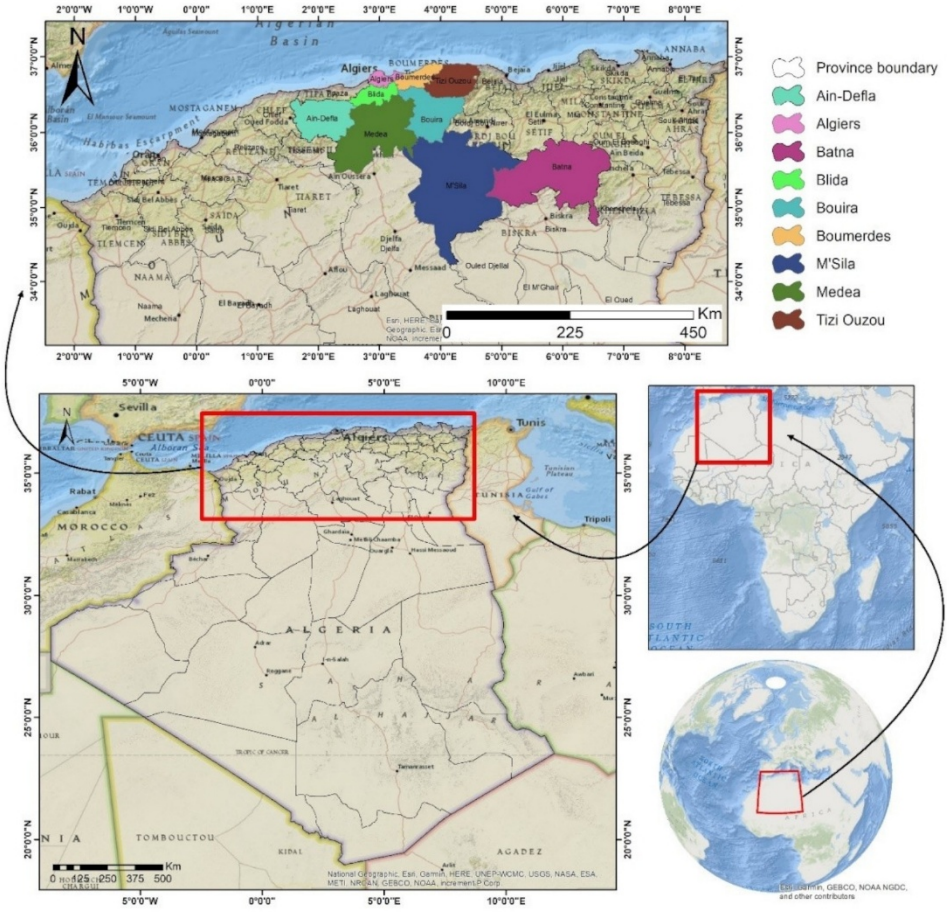

Figure 1. The map of Algeria showing the different provinces of sample collection (cartography created using ArcGIS10.2).

Sample collection and preparation

Samples were collected from poultry showing symptoms associated with colibacillosis, such as diarrhoea, inappetence, rales, snoring, and weakness. The number of animals collected from each farm, including freshly dead and euthanized moribund poultry, varied from farm to farm and ranged from 5 to 10 subjects.

A total of 250 organs were collected. Among them, 155 showed several lesions associated with colibacillosis (pericarditis, perihepatitis, airsacculitis, tracheitis, splenitis, and hepatic hypertrophy) (Figure 2). These organs originated from 144 broilers, 10 layers, and 10 turkeys, and included lungs (n = 39), livers (n = 38), hearts (n = 36), spleens (n = 21), air sacs (n = 14), tracheas (n = 6), and yolk sacs (n = 1). The samples were transported in Brain Heart Infusion Broth (BHIB) supplemented with glycerol (30%) and stored at -20°C until analysis.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

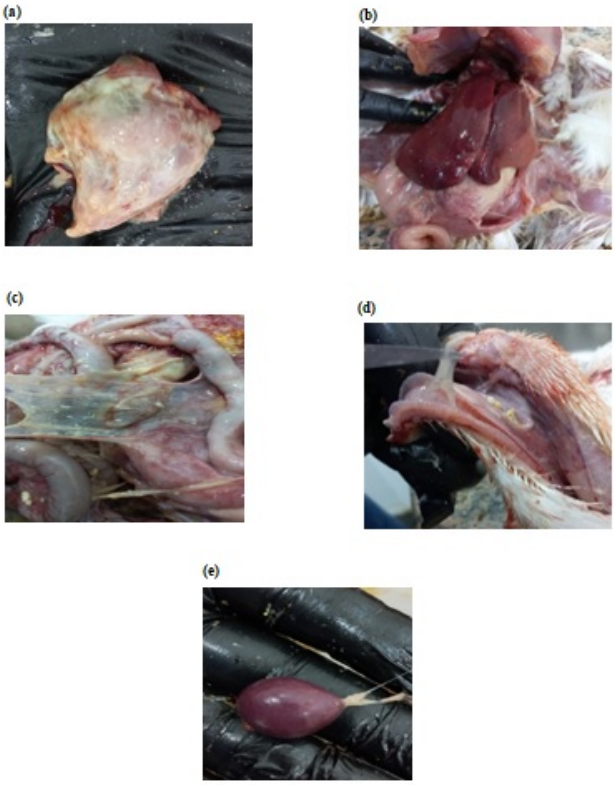

Figure 2. Organs showing lesions due to colibacillosis. (a) pericarditis; (b) hepatic hypertrophy; (c) airsacculitis; (d) tracheitis; and (e) spleen inflammation.

APEC isolation

Collected samples were streaked onto the surface, cut into small pieces, and incubated in BHIB at 37°C for 18-24 h. The cultures were then plated onto Hektoen, Eosin Methylene Blue (EMB), and CHROMagar™ Orientation. After incubation at 37°C for 24 h, colonies showing an orange or salmon colour on Hektoen, a metallic green sheen on EMB, and a pink colour on CHROMagar™ Orientation medium were presumptively identified as APEC isolates. Initial characterisation of APEC was based on clinical diagnosis of infected poultry, and these presumptive isolates were subsequently subjected to Gram staining and biochemical tests (catalase, triple sugar iron, urea, indole, and tryptophan deaminase). These tests were complemented by identification using the API 20E system, for some isolates showing atypical profiles.

Antimicrobial susceptibility testing (AST)

The disc diffusion method was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). For this purpose, 11 antibiotics belonging to 8 different families, were tested: ampicillin (AMP) 10 µg, amoxicillin-clavulanic acid (AMC) 20/10 µg, cefotaxime (CTX) 30 µg, tetracycline (TET) 30 µg, flumequine (UB/FLM) 30 µg, enrofloxacin (ENR) 5 µg, trimethoprim-sulfamethoxazole (SXT) 25/23.75 µg, neomycin (NEO) 30 µg, gentamicin (GEN) 10 µg, nitrofurantoin (F) 300 µg, and chloramphenicol (CHL) 30 µg. *E. coli* ATCC 25922 was used as a quality control. Isolates that were resistant to at least 3 antibiotic families were considered MDR (Basak et al., 2016). Extended-spectrum beta-lactamase (ESBL) production was detected using the double-disc synergy test (DDST) (Badr et al., 2022).

The broth microdilution method was employed to determine the MIC for colistin, using successive twofold dilutions from 16 to 0.03 µg/mL (CLSI, 2012). Colistin resistance was considered when the MIC was > 2 µg/mL (EUCAST, 2022). The *E. coli* ATCC 25922 strain was used for quality control.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Biofilm formation assay

Biofilm production was assessed using Congo red agar (CRA) and crystal violet staining (CVS). The CRA test is a qualitative method to evaluate the ability of the isolates to produce biofilm. CVS is a quantitative method used to measure the level of biofilm production.

In the CRA method, the isolates were streaked onto CRA medium, and incubated at 37°C for 24 h. Biofilm production was determined based on the phenotypic characteristics of the colonies. Colonies that appeared black were considered to be biofilm producers. However, red colonies indicated no biofilm production (Osman et al., 2012).

In the CVS method, 20 µL of bacterial suspension of 10^9CFU/mL were added to 180 µL of BHIB, and dispensed into 96-well plates, then incubated at 28°C for 24 h. Negative control wells consisted of 180 µL of BHIB without inoculation. The bacterial culture was removed and then washed twice with saline water before it was fixed using 200 µL of methanol for 15 min. The methanol was then removed and the microplate was left to dry on air for 30 min before being stained with 0.2% crystal violet for 5 min. The microplate was then washed using sterile water. Next, 160 µL of glacial acetic acid was added and the plate was read at 550 nm using an ELISA reader (BioTek). The cut-off value was determined by calculating the average optical density (ODc) of the negative control from three independent replicates, and then used to classify each isolate. Four categories of biofilm formation were identified: strong (OD > 4 × ODc), moderate (4 × ODc > OD > 2 × ODc), weak (2 × ODc > OD), and non-forming (ODc > OD) (Sivaranjani et al., 2022).

Haemolytic activity

Haemolytic activity was determined using a spot test on 5% fresh blood agar. The presence of a transparent halo indicated total haemolysis (β-haemolysis). A greenish halo indicates partial haemolysis (α-haemolysis), while the absence of a halo indicated no haemolytic isolates (γ-haemolysis). The haemolyticstrain *Staphylococcus aureus* ATCC 6538 was used as a positive control (Saha et al., 2020).

Detection of ESBL genes

The boiling method was performed for DNA extraction as described previously by Zhu et al. (2021). The extracted DNA was analysed using a spectrophotometer (NanoDrop 8000, Thermo Scientific), and stored at -20°C until use.

The presence of CTX-M type extended spectrum beta-lactamases genes ($bla_{\text{CTX-M}}$) and extended spectrum beta-lactamases genes (bla_{TEM}) was investigated in DDST-positive isolates by PCR, as described previously by Bougouizi et al. (2024) (Table I).

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Gene	Primer sequence 5'-3'	Conditions of PCR amplification	Size (pb)	References
Colistin and ESBL resistance genes				
***mcr*-1**	F:AGTCCGTTTGTTCTTGTGGC R:AGATCCTTGGTCTCGGCTTG	94°C 15 min, 25 cycles (94°C 30 s, 58°C 90 s, 72°C 60 s),72°C 10 min	320	(Rebelo et al., 2018)
bla_{CTX-M}	F:TTTGCGATGTGCAGTACCAGTAA R:CGATATCGTTGGTGGTGCCATA	95°C 15 min, 30 cycles (94°C 30 s, 50°C 30 s, 72°C 2min),72°C10min	500	(Kiiru et al., 2012;Bougouizi et al., 2024)
bla_{TEM}	F : ATGAGTATTCAACAT TTC CG R : CCAATGCTTAATCAG TGA GG		840	
Virulence genes				
***hyl*F**	F : GGCGATTTAGGCATTCCGATACTC R : ACGGGGTCGCTAGTTAAGGAG	94 °C 5 min, 30 cycles (94° 30s, 60°C 30s, 72°C 3min), 72 °C 10min	599	(Johnson et al., 2006)
***iro*N**	F: AAGTCAAAGCAGGGGTTGCCCG R: GACGCCGACATTAAGACGCAG		667	
***omp*T**	F: ATCTAGCCGAAGAAGGAGGC R: CCCGGGTCATAGTGTTCATC		559	
iss	F: CAGCAACCCGAACCACTTGATG R: AGCATTGCCAGAGCGGCAGAA		323	
***iut*A**	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	95°C 12 min, 25 cycles (94°C 30 s, 63°C 30 s, 68°C 3 min), 72°C 10 min	302	(Johnson & Stell, 2000)
Phylogrouping				
***yja*A**	F:CAAACGTGAAGTGTCAGGAG R:AATGCGTTCCTCAACCTGTG	94°C 5min, 30 cycles (94°C 5s, 59°C 20 s, 72°C 60 s), 72°C 5 min	211	(Clermont et al., 2012)
TspE4.C2	F:CACTATTCGTAAGGTCATCC R:AGTTTATCGCTGCGGGTCGC		152	
***arp*A**	F:AACGCTATTCGCCAGCTTGC R:TCTCCCCATACCGTACGCTA		400	
***arp*A (group E)**	F:GATTCCATCTTGTCAAAATATGCC R:GAAAAGAAAAAGAATTCCCAAGAG		301	
***trp*A (group C)**	F:AGTTTTATGCCCAGTGCGAG R:TCTGCGCCGGTCACGCCC		219	
***trp*A (internal control)**	F:CGGCGATAAAGACATCTTCAC R:GCAACGCGGCCTGGCGGAAG		489	
Genotyping				
ERIC	AAGTAAGTGACTGGGGTGACGC	95°C 3 min, 40 cycles (92°C 30 s, 52°C 1 min, 65°C 8 min), 65°C 16min	/	(Decré et al., 2004)

Table I. Primers and conditions of PCR amplification of investigated genes and ERIC-PCR.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Screening of *mcr-1* genes

All isolates were subjected to *mcr-1* detection as described previously by Rebelo et al. (2018) (Table I). *mcr-1* positive isolates were analysed for genotyping, phylogrouping, and virulence genes.

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR)

The genetic similarity between isolates was investigated using ERIC-PCR. The reaction was performed as described by Decré et al. (2004) (Table I).

Electrophoresis was performed on a 1.8% agarose gel stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Spain), at 100 V for 3 h (Kiersten et al., 2003). The results were read using a UV transilluminator (Gel Doc XR+ Gel Documentation System, Bio-Rad, USA/Thermo Fisher Scientific).

Fingerprinting band analysis and phylogenetic tree construction was performed using GelJ v2.3 software based on unweighted pair group method with arithmetic mean (UPGMA) analysis.

Phylogrouping

Phylogrouping was performed using quadriplex PCR to detect *arpA*,*chuA*,*yjaA*, *trpA* and TspE4.C2 (Clermont et al., 2012) in order to classify the isolates to one of eight phylogroups (A, B1, B2, C, D, E, F, or I). The primers used and PCR conditions are provided in Table I.

Detection of virulence-associated genes (VAGs) by PCR

Five plasmid-carried VAGs: haemolysin F (*hylF*), siderophore receptor (*iroN*), ferric aerobactin receptor (*iutA*), increased serum survival (*iss*), and outer membrane protease (*ompT*), were screened in *mcr-1* positive isolates using PCR (Table I). These genes are frequently identified in APEC strains and have been suggested as predictors for distinguishing APEC from non-pathogenic *E. coli* (Johnson et al., 2008).The presence of the *iss*, *ompT*, *hylF*, and *iroN* genes was investigated according to the method described by Johnson et al. (2006). Detection of *iutA* was performed as described by Johnson and Stell (2000).

Results

APEC isolates

Of the 155 organs analysed, 113 isolates were recovered and distributed as follows: 108 from 62 broilers, 2 from 1 layer, and 3 from 1 turkey. The frequency of isolation varied depending on the organ, as follows: 76.92% (n = 30/39) from the lungs, 72.2% (n = 26/36) from the heart, 63.2% (n = 24/38) from the liver, 80.95% (n = 17/21) from the spleen, 71.4% (n = 10/14) from the air sac, and 100% (n = 6/6) from the trachea.

Antimicrobial susceptibility

Our isolates showed high resistance to AMP, 93% (n = 105/113) and TET, 89% (n = 101/113), followed by ENR, 75% (n = 85/113), UB/FLM, 73% (n = 82/113), and SXT, 71% (n = 80/113). Average resistance was observed to NEO, 51% (n = 58/113) and CHL, 42% (n = 47/113) and low resistance to AMC, 12% (n = 3/113). Most isolates were sensitive to CTX, GEN, and FUR, at 98% (n = 2/113), 95% (n = 5/113), and 92% (n = 5/113), respectively (Figure 3). In addition, 3.5% (n = 4/113) of the isolates were positive for DDST and were presumptive ESBL producers.

Most of the isolates 95% (n = 107/113) were resistant to at least three classes of antibiotics and were therefore considered MDR. The dominant profile of MDR isolates was AMP, NEO, SXT, UB/FLM, ENR, and TET.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

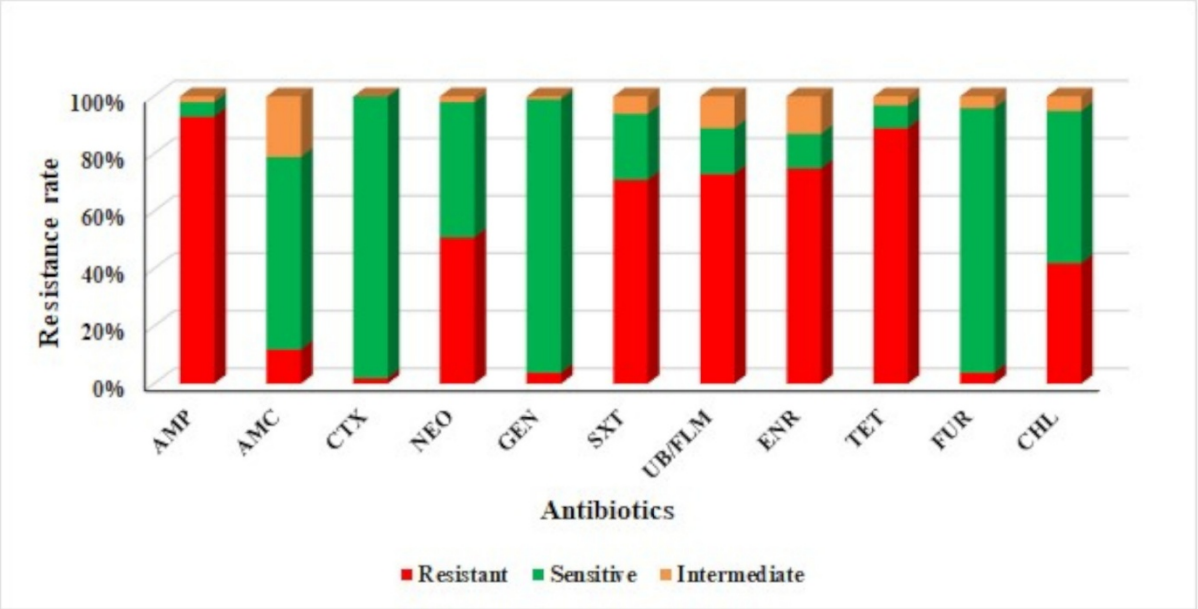

Figure 3. Antibiotic resistance profile of the APEC isolates. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; NEO, neomycin; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; UB/FLM, flumequine; ENR, enrofloxacin; TET, tetracycline; FUR, nitrofurantoin; CHL, chloramphenicol.

Biofilm formation and haemolysis activity

The CRA test showed that 83% (n = 94/113) of the isolates had the capacity to form biofilm (Figure 4). However, CVS revealed that 91% (n = 103/113) of the isolates produced biofilm, while 9% (n = 10/113) did not reveal biofilm formation. Among CVS positive isolates, 55% (n = 56/103) were strong biofilm formers, 23% (n = 24/103) were moderate formers, and 22% (n = 23/103) were weak formers.

Regarding haemolytic activity, 96% (n = 109/113) of the isolates were non-haemolytic (γ-haemolytic), and only 4% (n = 4/113) showed partial red blood cell degradation (α-haemolytic).

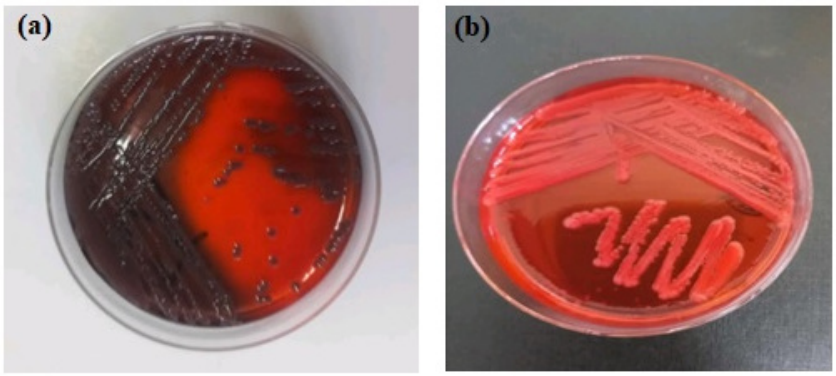

Figure 4. APEC aspect on CRA medium. (a) black colonies, biofilm-producing isolates; (b) red colonies, no biofilm-producing isolates.

Detection of ESBL genes

Among the 4 isolates exhibiting an ESBL phenotype (DDST positive), the bla_{TEM} gene was detected in all isolates. As sequencing was not performed, the TEM variants could not be confirmed. One isolate also carried the $bla_{CTX\text{-}M}$ gene.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Screening of the *mcr-1* gene

The *mcr-1* gene was observed in 41 isolates (36%), including 40 isolates from broilers and 1 from turkeys. All *mcr-1* positive isolates were MDR (Table II). Among the *mcr-1* positive isolates, two from broilers were beta lactamase producers, one carried bla_{TEM}, while the other harboured both the bla_{TEM} and bla_{CTX-M} genes.

The 41 isolates carrying the *mcr-1* gene were tested for their colistin MIC. The results revealed that 80% of isolates were resistant, with an MIC of 4 g/mL for 70% (n = 23/41), and 8 g/mL for 30% (n = 10/41). However, 20% (n = 8/41) of isolates were sensitive to colistin, with a MIC of 0.03 g/mL (12%; n = 1/8), 0.25 g/mL (25%; n = 2/8), 0.5 g/mL (12%; n = 1/8), 1 g/mL (39%; n = 3/8), and 2g/mL for (12%; n = 1/8) (Table II).

Isolates	Antibiotic resistance profile												Genes encoding ESBL		VAGs					Phylogroup
	AMP	AMC	CTX	NEO	GEN	SXT	UB/FLM	ENR	TET	FUR	CHL	Colistin MIC (µg/mL)	bla_{CTX-M}	bla_{TEM}	*hylF*	*iroN*	*iss*	*ompT*	*iutA*	
S6	R	I	S	S	S	S	R	R	R	S	S	2	/	/	+	+	+	-	+	B1
S13	R	I	S	S	S	R	R	R	R	S	S	4	/	/	+	-	+	-	+	B1
S18	R	S	S	R	S	R	R	R	R	I	S	4	/	/	+	-	+	+	+	A
S26	R	S	S	R	S	S	R	R	R	S	S	4	/	/	+	+	+	-	+	C
S29	R	I	S	S	S	I	R	R	R	S	R	4	/	/	+	+	+	-	+	C
S31	R	S	S	R	S	I	S	I	R	S	S	1	/	/	+	-	-	+	-	B1
S72	R	S	S	R	S	R	S	R	R	S	S	0,25	/	/	-	-	-	+	-	C
S38	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	+	+	-	+	B1
S40	R	S	S	R	S	R	R	R	R	S	S	8	/	/	+	+	-	-	+	B1
S45	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	-	-	-	+	B1
S46	R	S	S	R	S	R	R	R	R	S	S	8	/	/	+	+	-	-	+	B1
S48	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	+	-	-	+	B1
S52	R	I	S	R	S	R	R	R	R	S	S	4	/	/	+	+	-	-	+	B1
S56	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	+	-	-	+	B1
S57	R	S	S	S	S	R	R	R	R	S	R	4	/	/	-	+	-	-	+	B1
S62	R	I	S	R	R	R	R	R	R	R	I	0,5	/	/	+	+	-	-	+	B1
S63	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	+	-	-	+	B1
S64	R	I	S	R	S	R	R	R	R	S	S	4	/	/	+	+	-	-	+	B1
S65	R	R	R	R	R	S	S	R	R	S	S	8	+	+	+	+	-	-	+	B1
S66	R	S	S	S	S	S	R	R	R	S	S	4	/	/	+	+	+	-	+	B1
S74	R	S	S	R	S	R	R	R	R	S	R	4	/	/	+	+	-	-	+	E
S75	R	I	S	R	S	R	R	R	R	S	R	4	/	/	+	+	-	-	+	B1
S78	R	S	S	R	S	R	R	R	R	S	R	8	/	/	+	+	-	+	+	E
S82	R	S	S	S	S	R	R	R	R	S	R	4	/	/	+	+	+	-	+	B1
S83	R	S	S	S	S	R	R	R	R	S	R	8	/	/	+	+	+	-	-	C
S90	R	S	S	R	S	R	R	R	R	S	S	4	/	/	+	+	+	-	+	B1
S95	R	R	S	R	S	R	R	R	R	I	R	4	/	/	+	+	+	-	+	B1
S98	R	S	S	S	S	R	R	R	R	S	R	4	/	/	+	+	+	-	+	B1
S104	R	S	S	R	S	R	R	R	R	S	R	0,25	/	/	+	+	+	-	+	B1
S105	R	S	S	R	S	R	R	R	S	S	R	4	/	/	+	-	+	-	+	B1
S106	R	R	S	R	S	R	R	R	S	S	R	4	/	/	+	+	+	-	+	B1
S109	R	S	S	R	S	R	R	R	R	S	S	8	/	/	+	+	+	-	+	B1
S119	R	R	S	R	S	R	R	R	S	S	R	8	/	/	+	+	+	-	+	B1
S128	R	S	S	R	S	R	R	R	R	S	R	1	/	/	+	+	+	-	-	C
S131	R	S	S	R	S	R	R	R	S	S	R	8	/	/	+	+	+	-	+	B1
S132	R	I	S	S	S	S	R	R	R	S	S	<0,03	/	/	+	+	+	-	+	B1
S140	R	I	S	S	S	R	R	R	R	S	R	8	/	/	+	+	+	-	+	B1
S141	R	I	S	R	S	R	R	R	R	S	R	4	/	/	+	+	+	-	+	Unknown
S142	R	S	S	S	S	R	I	S	R	S	S	8	/	/	+	-	-	-	+	F
S143	R	I	S	S	S	R	R	R	R	S	S	1	/	/	+	+	+	-	+	B1
S153	R	S	S	R	S	R	R	R	R	S	R	4	-	+	+	+	+	-	+	B1

Table II. Characterization of the 41 *mcr-1* positive APEC isolates. /: not realized.

Phylogrouping

Phylogrouping classified the 41 mcr-1 positive isolates into 5 phylogroups (Table II): phylogroup A (*arpA* +, *chuA* -, *yjaA*-, TSPE4.C2-), phylogroup B1 (*arpA*+, *chuA*-, *yjaA*-, TSPE4.C2+), phylogroup F (*arpA*-, *chuA*+, *yjaA*-, TSPE4.C2-), phylogroup C (*arpA*+, *chuA*-, *yjaA*+, TSPE4.C2-), and phylogroup E (*arpA*+, *chuA*+, *yjaA*-, TSPE4.C2-). The most common phylogroup was phylogroup B1 (76%; n = 31/41), followed by phylogroup C (13%; n = 5/41), phylogroup E (5%; n = 2/41), and phylogroups A and F (2%; n = 1/41 each) (Figure 5). In addition, one isolate had an unknown phylogroup.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

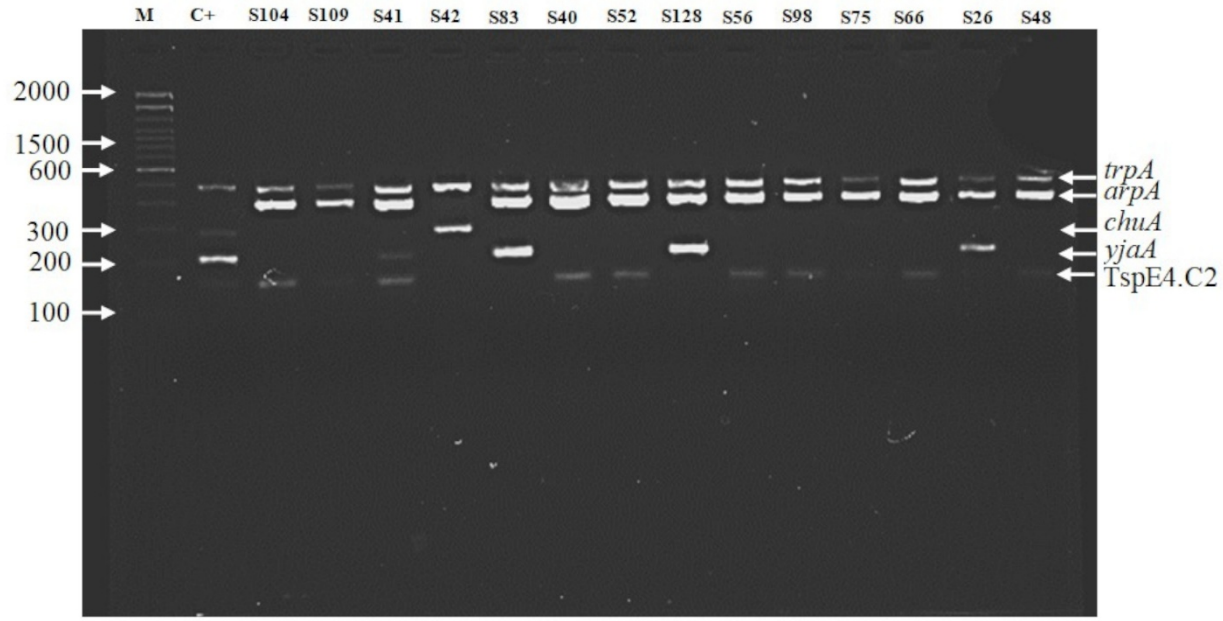

Figure 5. Representative quadriplex PCR results for the Clermont phylogrouping, arrows indicate the four Clermont markers, *arpA* (400 bp), *chuA* (288 bp), *yjaA* (211 bp), TspE4.C2 (152 bp). The *trpA* (489 bp) represent the internal control. Patterns of presence (+) and absence (-) correspond to phylogroups. M, molecular weight marker (100 bp DNA Ladder); C+, positive control of group B2 (-+++); isolates S104, S109, S40, S52, S56, S66 and S48, group B1 (+--+); isolate S41, unknwon (+-++); isolate S42, group F (-+--), isolates S83, S128 and S26, group C (+-+-).

Distribution of VAGs in *mcr-1* positive isolates

Of the five VAGs detected in this study, *hylF* was the most prevalent among the mcr-1 positive isolates (95%; n = 39/41), followed by *iutA* (90%; n = 37/41), *iroN* (83%; n = 34/41), *iss* (59%; n = 24/41), and *ompT* (5%; n = 4/41). Overall, 88% (n = 39/41) of the isolates carried at least 3 VAGs. It should be noted that 51% (n = 24/41) of the isolatescarried 4 VAGs, 37% (n = 15/41) carried 3 genes, 10% (n = 4/41) carried 2 genes, and 2% (n = 1/41) carried only onegene (*ompT*).

The detection of VAGs revealed 10 genomic virulence profiles (P1-P10), reflecting the diversity of virulence traits. Indeed, 47% (n = 19/41) of the isolates carried profile P2, followed by 28% (n = 11/41) carrying profile P1. In contrast, 5% (n = 2/41) of isolates carried profiles P3, P5, and P9, while 2% (n = 1/41) carried profiles P4, P6, P7, P8, and P10 (Table III).

Profile	VAGs	Rates of isolates (n=number of isolates)
P1	*iutA*, *hylF*, and *iroN*	28% (n = 11)
P2	*iutA*, *hylF*, *iroN*, and *iss*	47% (n = 19)
P3	*iutA* and *hylF*	5% (n = 2)
P4	*iutA* and *iroN*	2% (n = 1)
P5	*hylF*, *iroN*, and *iss*	5% (n = 2)
P6	*hylF* and *ompT*	2% (n = 1)
P7	*iutA*, *hylF*, *iroN*, and *ompT*	2% (n = 1)
P8	*iutA*, *hylF*, *iss*, and *ompT*	2% (n = 1)
P9	*iutA*, *hylF*, and *iss*	5% (n = 2)
P10	*ompT*	2% (n = 1)

Table III . Virulence profile of the 41 *mcr-1* positive isolates.

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Genotyping

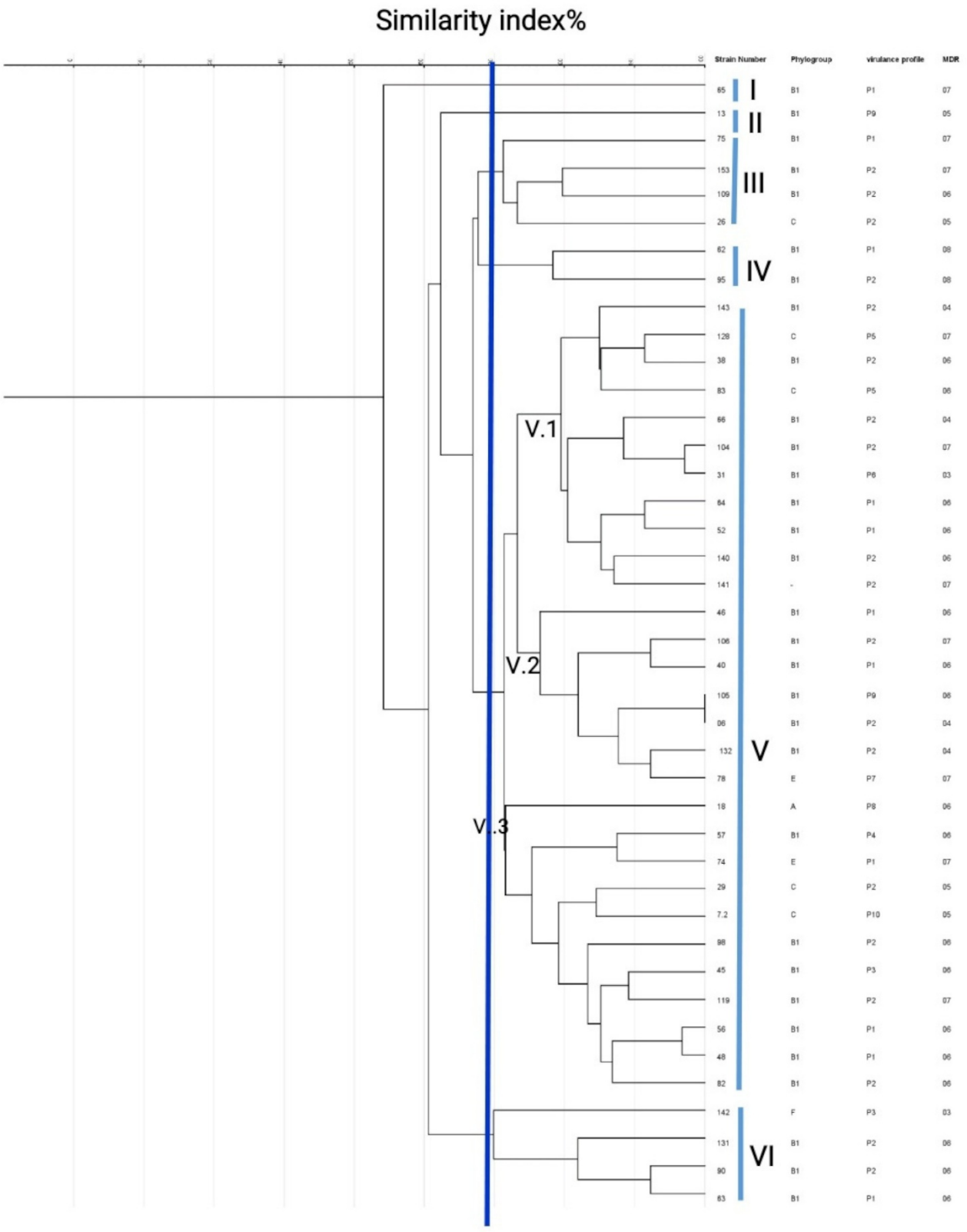

Figure 6. Phylogenetic tree constructed from of ERIC-PCR profiles using UPGMA method through Gel J v2-3 software.

The ERIC-PCR genotyping analysis grouped the *mcr-1* positive isolates into 6 clusters (Figure 6). Cluster V was the predominant cluster, representing 70% of the isolates (n = 29/41), with most isolates belonging to phylogroup B1 and displaying diverse virulence profiles, predominantly P6 and P9. Cluster IV included 2 isolates belonging to phylogroup B1, and exhibiting the highest MDR pattern, with resistance to 7 antibiotic families. The remaining clusters (I, II, III,

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

and VI) mainly comprised phylogroup B1 isolates, with a few isolates from phylogroups C (Cluster III) and F (Cluster VI). Overall, isolates within the same cluster showed highly similar antibiotic resistance profiles.

Discussion

Colibacillosis, caused mainly by APEC, is a significant poultry disease associated with high mortality rates and considerable economic losses worldwide (Nawaz et al., 2024). APEC infections are treated with various antibiotics, including colistin for both prophylactic and curative purposes (Anyanwu et al., 2020; Jansen et al., 2022; Ahmed and Daw, 2023). However, the widespread use of colistin in veterinary medicine has led to the emergence of colistin resistance (Anyanwu et al., 2024; Osisiogu et al., 2025; Saeed et al., 2025). This makes animals, including poultry, natural reservoirs of resistance to this antibiotic. From a One Health perspective, this poses a real threat to public health due to the transmission of resistance genes through the food chain (Dziri et al., 2021). The aim of this study was to investigate the patterns of antibiotic resistance among APEC in Algeria, with special emphasis on colistin-resistance APEC.

In our study, most of the isolates collected from different Algerian provinces were MDR (95%). Similar rates have been reported in previous studies on APEC isolated from poultry and turkeys in Algeria (Halfaoui et al., 2017; Halfaoui et al., 2024; Chenouf et al., 2025). It was also reported in other countries, such as Tunisia (88%) (Thabet et al., 2022), Spain (99%) (Monroy et al., 2025), and Bangladesh (100%) (Saha et al., 2020). These results can be attributed to the widespread and unregulated use of antibiotics in poultry and the misuse of antibiotics resulting from farmers selfmedication practices (Hackman et al., 2025).

Biofilm is a crucial factor in enhancing the survival and persistence of APEC in the environment and at sites of infection (Milanov et al., 2015). It acts as a physical barrier that protects the bacteria against antimicrobials and the immune system, which makes prevention, control, and treatment more difficult (Skyberg et al., 2007; Sivaranjani et al., 2022).

Our study demonstrated the significant ability of APEC to form biofilms. Indeed, biofilm formation was detected in 91% of isolates using the CVS test, with 55% classified as strong biofilm forming isolates. Several studies have described biofilm production in APEC isolates, reporting variable rates ranging from low to high production (Rodrigues et al., 2019; Ugwu et al., 2020; Dhaouadi et al., 2023; Dančová et al., 2024).

In our study, a low rate of haemolytic isolates was observed (4%). This is consistent with the findings of Shankar et al. (2010), who also found a low rate of haemolytic isolates (1.5%). However, other studies, such as those conducted by Ugwu et al. (2020) and Al-Saiedi and Al-Mayah (2014), reported higher rates of 19% and 37%, respectively.

The high rate of biofilm formation and low rate of haemolysis observed in our isolates may be attributed to their origin, as they were obtained from organs rather than blood. This ability to adhere to and invade various organs contributes to their pathogenicity and virulence (Ugwu et al., 2020).

In Algeria, colistin resistance has been poorly documented in the poultry sector, with a few studies addressing this issue in broilers suspected or not of colibacillosis. In our study, we performed a preliminary screening for the *mcr-1* gene by PCR and then we determined the MIC for colistin. This approach is explained by the fact that *mcr-1* gene is the main indicator of colistin resistance in poultry (Poirel et al., 2017).

Our results revealed the presence of the *mcr-1* gene in 41 isolates; of these, 20% were sensitive to colistin. Although the *mcr-1* gene is the primary indicator of colistin resistance in poultry (Poirel et al., 2017), its expression can be unpredictable. This can result in low phenotypic resistance that falls below the defined critical resistance thresholds (Smelikova et al., 2022). In addition, mutations in this gene could result in its inactivation (Terveer et al., 2017; Peng et al., 2021). Previous studies have also reported colistin sensitivity in *E. coli* isolates carrying the *mcr-1* gene (Terveer et al., 2017; Peng et al., 2021; Jalil et al., 2023).

The main source of *mcr-1* positive isolates was broilers, with one originating from turkeys. In Algeria, several studies have reported the presence of the *mcr-1* gene in poultry isolates, including those from chickens suspected of colibacillosis, as well as from chicken meat, faeces, and healthy chickens (Chaalal et al., 2021; Halfaoui et al., 2024; Akkari et al., 2026). However, our study is the first to report the presence of the *mcr-1* gene in APEC isolates from turkeys. Similar findings have been reported in Italy (Alba et al., 2018), Serbia (Mišić et al., 2021), and recently in the Gaza Strip (Palestine) (Thabet et al., 2023). The *mcr-1* gene has been reported in *E. coli* isolates from various sources in Algeria, including farmland, aquatic environments, humans, animals, and vegetables (Berrazeg et al., 2016; Bachiri et al., 2018; Drali et al., 2018; Nabti et al., 2019; Touati et al., 2020; Chelaghma et al., 2022; Cherak et al.,

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

2022; Boukli-Hacene et al., 2024). Several studies worldwide have also reported the widespread distribution of *mcr-1* in isolates of different origins (Igwaran et al., 2018; Saidani et al., 2019; Ngbede et al., 2020; Cordeiro-Moura et al., 2022; Srisrattakarn et al., 2025; Wang et al., 2025). This wide distribution of *mcr-1* explains its role in the global spread of colistin resistance and underlines the importance of the One Health concept, which recognises the close connection between humans, animals, and environments in the spread of antibiotic resistance.

Interestingly, we report for the first time in Algeria, the co-existence of the *mcr-1* and *bla* genes (bla_{CTX-M} and bla_{TEM}), in two isolates. The same observation has also been reported in France, Argentina, and Tunisia (Haenni et al., 2016; Dominguez et al., 2018; Dhaouadi et al., 2020). The co-occurrence of *mcr-1* and *bla* genes is worrying, as it implies simultaneous co-selective pressure exerted by one of the two antibiotics (Wu et al., 2018).

Phylogenetic grouping revealed that most of the *mcr-1* positive isolates (76%) belonged to phylogroup B1. The remaining isolates belonged to phylogroups C, E, A, and F, with one isolate having an unknown phylogroup. Our results were similar to those recently obtained by Boulbair et al. (2025), who also found that most Algerian APEC isolates (43.87%) belonged to phylogroup B1. Another Algerian study showed that phylogroup D was the most common in Algerian poultry farms (Chenouf et al., 2025).

While the B1 phylogroup is typically associated with commensal strains, the presence of the *mcr-1* gene in these isolates poses a significant risk of colistin resistance spreading to other intestinal bacteria. This observation suggests the uncontrolled and frequent use of colistin in poultry, which potentially contributes to the emergence and spread of resistance to this critical antibiotic. Similar findings have been reported in studies of *mcr-1* positive *E. coli*, predominantly belonging to the B1 phylogroup, in other countries, including Pakistan (Jalil et al., 2023), the Czech Republic (Kubelová et al., 2021), and Jordan (Gharaibeh et al., 2024). The predominance of phylogroup B1 which is considered commensal, may suggest that colibacillosis could result from secondary or superinfection caused by opportunistic strains. According to Ovi et al. (2023) distinguishing between APEC and commensal *E. coli* based only on phylogroup is confusing. To overcome this problem, research into virulence genes is recommended.

Analysis of virulence genes revealed predominance of *hylF* (95%), *iutA* (90%), and *iroN* (83%), followed by *iss*(59%) gene. However, only 10% of the isolates carried the *ompT* gene. Our findings were similar to those of Fancher et al. (2021), who also observed a higher occurrence of both the *hylF* and genes at rates of 94.3% and 90.8%, respectively. Mohamed et al. (2018), also found a high prevalence of the *iutA* (90.6%) and hylF (82.6%) genes in APEC isolates, in addition to a higher prevalence of the *iss* gene (85.9%) than was observed in our isolates. However, concerning the *ompT* gene, which was rarely encountered among our isolates (10%), other studies have reported higher rates of this gene in APEC isolates, ranging from 55% to 87% (Mohamed et al., 2018; Fancher et al., 2021; Halfaoui et al., 2024; Uddin et al., 2025).

The variations in the prevalence of virulence genes can be affected by several factors, such as fluctuations in environmental temperature, humidity, and housing conditions (Fancher et al., 2021). The high prevalence of the *hylF, iutA*, and *iroN* genes, which are involved in iron uptake, is considered to be characteristic of APEC, this explains their critical role in colibacillosis and the subsequent lesions observed in poultry (Johnson et al., 2008). The *iss* gene, which encodes serum resistance and protection against complement activation, could contribute to the increased virulence of APEC (Lounis et al., 2020).

In our study, 88% of the isolates had at least 3 VAGs. APEC strains are generally defined as isolates harbouring at least three of these genes (Johnson et al., 2008; Amer et al., 2020). However, it is important to emphasise that the presence of these genes alone is not enough to definitively classify an isolate as APEC. Pathogenicity is multifactorial and depends on a combination of genes and clinical context (Johnson et al., 2008; Schouler et al., 2012; Mellata, 2013).

In Algeria, few studies have investigated the clonality of APEC isolates (Meguenni et al., 2015). In the present study, ERIC-PCR was carried out to evaluate the clonal relatedness of our isolates. This method has been used previously in several studies on APEC isolates from different countries (Saha et al., 2020; Kimura et al., 2021; Rezatofighi et al., 2021; Jalil et al., 2023). The 41 *mcr-1* positive isolates were classified into 6 clusters, using a threshold value of 70% similarity (Rezatofighi et al., 2021). However, previous studies using the same technique have found more clusters, as reported by Rezatofighi et al. (2021),who reported 15 different clusters with the same similarity index (70%), and Jalil et al. (2023), who classified 147 APEC isolates into 40 clusters with an 80% similarity index.

The low diversity of our isolates likely reflects their common origin. In contrast, although ERIC-PCR targets highly conserved sequences within *Enterobacteriaceae* (Hulton et al., 1991), its resolution for differentiating strains of the same species is limited (Casarez et al., 2007). Other methods, such as whole genome sequencing, provide higher resolution for typing bacterial isolates (Fratamico et al., 2016).

How to cite: Djouzi et al. Phylogrouping and genotyping of mcr-1 postives avian pathogenic Escherichia coli isolates in Algerian poultry farms. Veterinaria Italiana, Vol. 62 No. 2 (2026) DOI: 10.12834/VetIt.3929.40804.3

Conclusion

This study confirms the high rate and widespread dissemination of colistin resistance, encoded by the *mcr-1* gene, among APEC isolated from poultry farms across different provinces in central and eastern Algeria. The *mcr-1* gene was found in chickens, and interestingly, for the first time on Algerian turkey farms. It is important to note the co-presence of the *mcr-1* gene and ESBL genes in some isolates. This combination poses a significant threat to public health, given the importance of colistin and beta-lactam antibiotics in human and veterinary medicine. Within the framework of the One Health concept, urgent measures are needed to control the widespread dissemination of APEC on Algerian poultry farms in order to preserve human and animal health.

Acknowledgments

We would like to express our sincere thanks to the veterinarians who contributed to the sampling: Hicham Cherifi and Oussama Driss.

Ethical approval

No ethical approval was required for this study.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Conceptualisation: HR, AB; Methodology: AD, HR, MD, IL, HA; Formal analysis: AD, HR, MD, AB; Investigation: AD, HR, MD, BA; Writing original draft preparation: AD; Writing, review and editing: AD, HR, MD, AB; Visualisation: AD, AB, MD, HR, HA, IL, MS; Supervision: HR, AB, MD. Project administration: MS, AB; Funding acquisition: AD, AB, MD, HR, HA, IL, MS.

All authors have read and agreed to the published version of the manuscript.

Data availability

All data are available upon request to the corresponding author.

Fundings

This research received no external funding.

References

Aberkane, C., Messaï, A., Messaï, C. R., & Boussaada, T. (2023). Antimicrobial resistance pattern of avian pathogenic Escherichia coli with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. Veterinary World, 16 (3) 449-454. https://doi.org/10.14202/vetworld.2023.449-454.

Ahmed, M. O., & Daw, M. A. (2023). Is Colistin Essential for the treatment and metaphylaxis of infections in Food-Producing Animals; One Health Debate. Public Health and Healthcare. https://doi.org/10.20944/preprints202311.0872.v1.

Akkari, H., Heleili, N., Ozgumus, O. B., Akarsu, N., & Kiliç, A. O. (2026). 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. Microbial Pathogenesis, 210, 108180. https://doi.org/10.1016/j.micpath.2025.108180.

Alba, P., Leekitcharoenphon, P., Franco, A., Feltrin, F., Ianzano, A., Caprioli, A., Stravino, F., Hendriksen, R. S., Bortolaia, V., & Battisti, A. (2018). Molecular Epidemiology of mcr-Encoded Colistin Resistance in Enterobacteriaceae From Food-Producing Animals in Italy Revealed Through the EU Harmonized Antimicrobial Resistance Monitoring. Frontiers in Microbiology, 9, 1217. https://doi.org/10.3389/fmicb.2018.01217.

Al-Saiedi, R. L. R., & Al-Mayah, A. A. S. (2014). Pathogenicity testing of several APEC isolates obtained from naturally infected broiler birds reared in Basrah. International Journal of Poultry Science, 13(7), 374–378. https://doi.org/10.3923/ijps.2014.374.378.

Amer, M. M., Mekky, H. M., Fedawy, H. S., EL-Shemy, A., Bosila, M. A., & Elbayoumi, Kh. M. (2020). Molecular identification, genotyping of virulence-associated genes, and pathogenicity of cellulitis-derived Escherichia coli. Veterinary World, 13(12), 2703-2712. https://doi.org/10.14202/vetworld.2020.2703-2712.

Anyanwu, M. U., Ikenna-Ezeh, N. H., Okafor, S. C., Ezemuoka, C. F., Nwobi, O. C., Ogunniran, T. M., Obodoechi, L. O., Okorie-Kanu, O. J., Mgbeahuruike, A. C., Okosi, I. R., & Jaja, I. F. (2024). Commercial Day-Old Chicks in Nigeria Are Potential Reservoirs of Colistin- and Tigecycline-Resistant Potentially Pathogenic Escherichia coli. Antibiotics, 13(11), 1067. https://doi.org/10.3390/antibiotics13111067.

Anyanwu, M. U., Jaja, I. F., & Nwobi, O. C. (2020). Occurrence and Characteristics of Mobile Colistin Resistance (mcr) Gene-Containing Isolates from the Environment : A Review. International Journal of Environmental Research and Public Health, 17(3), 1028. https://doi.org/10.3390/ijerph17031028.

Bachiri, T., Lalaoui, R., Bakour, S., Allouache, M., Belkebla, N., Rolain, J. M., & Touati, A. (2018). First report of the plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli ST405 isolated from wildlife in Bejaia, Algeria. Microbial Drug Resistance, 24(7), 890-895. https://doi.org/10.1089/mdr.2017.0026.

Badr, H., Samir, A., El-Tokhi, E. I., Shahein, M. A., Rady, F. M., Hakim, A. S., Fouad, E. A., El-Sady, E. F., & Ali, S. F. (2022). Phenotypic and Genotypic Screening of Colistin Resistance Associated with Emerging Pathogenic Escherichia coli Isolated from Poultry. Veterinary Sciences, 9(6), 282. https://doi.org/10.3390/vetsci9060282
Basak, S., Singh, P., & Rajurkar, M. (2016). Multidrug Resistant and Extensively Drug Resistant Bacteria : A Study. Journal of Pathogens, 2016, 1 5. https://doi.org/10.1155/2016/4065603.

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. Antimicrobial agents and chemotherapy, 60(11), 6996 6997. https://doi.org/10.1128/aac.01117-16.

Biström, M., Vennerström, P., Lienemann, T., Kurittu, P., Heikinheimo, A., & Pohjanvirta, T. (2025). Genomic epidemiology of avian pathogenic Escherichia coli in two broiler colibacillosis outbreaks in Finland. Veterinary Microbiology, 308, 110617. https://doi.org/10.1016/j.vetmic.2025.110617.

Bougouizi, A., Chekroud, Z., Rahab, H., Boumegoura, A., & Touati, A. (2024). Prevalence and characterization of Carbapenem-Resistant Enterobacterales among inpatients and outpatients in Skikda, Algeria. The Journal of Infection in Developing Countries, 18(03), 383 390. https://doi.org/10.3855/jidc.18263.

Boukli-Hacene, F., Djouadi, L. N., Raddaoui, A., Hachem, Y., Boumerdassi, H., Achour, W., & Nateche, F. (2024). Sheep and goats as reservoirs of colistin-resistant E. coli : First detection of ETEC ST10 and E. coli ST6396 mcr-1 positive strains in North Africa. Journal of Applied Microbiology, 135(9), lxae227. https://doi.org/10.1093/jambio/lxae227.

Boulbair, I., Hu, J., Hammoudi, A., Zhang, B., Aissat, S., Wang, X., Foudil, M., & Wang, S. (2025). Molecular Characterization and Antibiotic Resistance of Avian Pathogenic Escherichia coli (APEC) Isolates from Broiler Chickens in Algeria. Animals, 15(22), 3324. https://doi.org/10.3390/ani15223324.

Casarez, E. A., Pillai, S. D., & Di Giovanni, G. D. (2007). Genotype diversity of Escherichia coli isolates in natural waters determined by PFGE and ERIC-PCR. Water Research, 41(16), 3643-3648.

https://doi.org/10.1016/j.watres.2007.03.020.

Chabou, S., Leulmi, H., & Rolain, J.-M. (2019). Emergence of mcr-1-mediated colistin resistance in Escherichia coli isolates from poultry in Algeria. Journal of Global Antimicrobial Resistance, 16, 115-116. https://doi.org/10.1016/j.jgar.2018.12.012.

Chaalal, N., Touati, A., Bakour, S., Aissa, M. A., Sotto, A., Lavigne, J.-P., & Pantel, A. (2021). Spread of OXA-48 and NDM-1-Producing Klebsiella pneumoniae ST48 and ST101 in Chicken Meat in Western Algeria. Microbial Drug Resistance, 27(4), 492-500. https://doi.org/10.1089/mdr.2019.0419Chelaghma, W., Loucif, L., Bendjama, E., Cherak, Z., Bendahou, M., & Rolain, J.-M. (2022). Occurrence of extended spectrum cephalosporin-, carbapenem-and colistin-resistant Gram-negative bacteria in fresh vegetables, an increasing human health concern in Algeria. Antibiotics, 11(8), 988. https://doi.org/10.3390/antibiotics11080988.

Chenouf, N. S., Messaï, C. R., Carvalho, I., Álvarez-Gómez, T., Silva, V., Zitouni, A., Hakem, A., Poeta, P., & Torres, C. (2025). Serogrouping and Molecular Characterization of ESBL-Producing Avian Pathogenic Escherichia coli from Broilers and Turkeys with Colibacillosis in Algeria. Antibiotics, 14(4), 356. https://doi.org/10.3390/antibiotics14040356.

Cherak, Z., Loucif, L., Bendjama, E., Moussi, A., Benbouza, A., Grainat, N., & Rolain, J.-M. (2022). Dissemination of carbapenemases and MCR-1 producing gram-negative bacteria in aquatic environments in Batna, Algeria. Antibiotics, 11(10), 1314. https://doi.org/10.3390/antibiotics11101314.

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2012). The Clermont Escherichia coli phylotyping method revisited : Improvement of specificity and detection of new phylogroups. Environmental Microbiology Reports. 5(1), 58-65. https://doi.org/10.1111/1758-2229.12019.

CLSI (2012). Methods for dilution antimicrobialsusceptibility tests for bacteria that grow aerobically. Approved Standard, 9th Edn., CLSI document M07-A9.

Cordeiro-Moura, J. R., Kraychete, G. B., de Araujo Longo, L. G., Corrêa, L. L., da Silva, N. M. V., Campana, E. H., Oliveira, C. J. B., & Picão, R. C. (2022). Description and comparative genomic analysis of a mcr-1-carrying Escherichia coli ST683/CC155 recovered from touristic coastal water in Northeastern Brazil. Infection, Genetics and Evolution, 97, 105196. https://doi.org/10.1016/j.meegid.2021.105196.

Dančová, N., Gregová, G., Szabóová, T., Regecová, I., Király, J., Hajdučková, V., & Hudecová, P. (2024). Quinolone and Tetracycline-Resistant Biofilm-Forming Escherichia coli Isolates from Slovak Broiler Chicken Farms and Chicken Meat. Applied Sciences, 14(20), 9514. https://doi.org/10.3390/app14209514.

Decré, D., Burghoffer, B., Gautier, V., Petit, J.-C., & Arlet, G. (2004). Outbreak of multi-resistant Klebsiella oxytoca involving strains with extended-spectrum β-lactamases and strains with extended-spectrum activity of the chromosomal β-lactamase. Journal of Antimicrobial Chemotherapy, 54(5), 881-888. https://doi.org/10.1093/jac/dkh440.

Dhaouadi, S., Romdhani, A., Bouglita, W., Chedli, S., Chaari, S., Soufi, L., Cherif, A., Mnif, W., Abbassi, M. S., & Elandoulsi, R. B. (2023). High Biofilm-Forming Ability and Clonal Dissemination among Colistin-Resistant Escherichia coli Isolates Recovered from Cows with Mastitis, Diarrheic Calves, and Chickens with Colibacillosis in Tunisia. Life, 13(2), 299. https://doi.org/10.3390/life13020299.

Dhaouadi, S., Soufi, L., Hamza, A., Fedida, D., Zied, C., Awadhi, E., Mtibaa, M., Hassen, B., Cherif, A., Torres, C., Abbassi, M. S., & Landolsi, R. B. (2020). Co-occurrence of mcr-1 mediated colistin resistance and β-lactamase-encoding genes in multidrug-resistant Escherichia coli from broiler chickens with colibacillosis in Tunisia. Journal of Global Antimicrobial Resistance, 22, 538-545. https://doi.org/10.1016/j.jgar.2020.03.017.

Dho-Moulin, M., & Fairbrother, J. M. (1999.). Avian pathogenic Escherichia coli (APEC). Veterinary research, 30(2-3), 299-316.

Dominguez, J. E., Redondo, L. M., Figueroa Espinosa, R. A., Cejas, D., Gutkind, G. O., Chacana, P. A., Di Conza, J. A., & Fernández Miyakawa, M. E. (2018). Simultaneous Carriage of mcr-1 and Other Antimicrobial Resistance Determinants in Escherichia coli From Poultry. Frontiers in Microbiology, 9, 1679.

https://doi.org/10.3389/fmicb.2018.01679.

Drali, R., Berrazeg, M., Zidouni, L. L., Hamitouche, F., Abbas, A. A., Deriet, A., & Mouffok, F. (2018). Emergence of mcr-1 plasmid-mediated colistin-resistant Escherichia coli isolates from seawater. Science of the total environment, 642, 90 94. https://doi.org/10.1016/j.scitotenv.2018.05.387.

Dziri, O., Dziri, R., El Salabi, A. A., Alawami, A. A., Ksouri, R., & Chouchani, C. (2021). Polymyxin E–Resistant Gram-Negative Bacteria in Tunisia and Neighboring Countries : Are There Commonalities?. Infection and Drug Resistance, 14, 4821 4832. https://doi.org/10.2147/IDR.S327718.

EUCAST (2022). The European Committee onAntimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version12.0: 1-110.

Fancher, C. A., Thames, H. T., Colvin, M. G., Smith, M., Easterling, A., Nuthalapati, N., Zhang, L., Kiess, A., Dinh, T. T. N., & Theradiyil Sukumaran, A. (2021). Prevalence and Molecular Characteristics of Avian Pathogenic Escherichia coli in “No Antibiotics Ever” Broiler Farms. Microbiology Spectrum, 9(3), e00834-21. https://doi.org/10.1128/Spectrum.00834-21.

Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., & Feng, P. (2016). Advances in Molecular Serotyping and Subtyping of Escherichia coli. Frontiers in Microbiology, 7, 644. https://doi.org/10.3389/fmicb.2016.00644.

Ghanbarpour, R., Sami, M., Salehi, M., & Ouromiei, M. (2011). Phylogenetic background and virulence genes of Escherichia coli isolates from colisepticemic and healthy broiler chickens in Iran. Tropical Animal Health and Production, 43(1), 153-157. https://doi.org/10.1007/s11250-010-9667-2.

Gharaibeh, M. H., Al Sheyab, S. Y., Malkawi, I. M., & Al Qudsi, F. R. (2024). Phenotypic and genotypic characterization of Escherichia coli isolated from the chicken liver in relation to slaughterhouse conditions. Heliyon, 10(6), e27759. https://doi.org/10.1016/j.heliyon.2024.e27759.

Guabiraba, R., & Schouler, C. (2015). Avian colibacillosis : Still many black holes. FEMS Microbiology Letters, 362(15), fnv118. https://doi.org/10.1093/femsle/fnv118.

Hackman, H. K., Annison, L., Arhin, R. E., Aidoo, E. K., Wilberforce, E. A., Mensah, A. C., Appiah, L., Annison, S., & Ayim-Akonor, M. (2025). Awareness of antimicrobial resistance and antibiotic use among poultry farmers in Accra, Ghana : A cross-sectional survey. PLOS One, 20(12), e0337531. https://doi.org/10.1371/journal.pone.0337531.

Haenni, M., Poirel, L., Kieffer, N., Châtre, P., Saras, E., Métayer, V., Dumoulin, R., Nordmann, P., & Madec, J.-Y. (2016). Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids. The Lancet Infectious Diseases, 16(3), 281-282. https://doi.org/10.1016/S1473-3099(16)00007-4.

Halfaoui, Z., Rahab, H., Achek, R., & Menoueri, M. N. (2024). First report of detection of mcr-1 and virulence genes in avian pathogenic Escherichia coli in the center of Algeria. Iranian Journal of Veterinary Research, 25(1). https://doi.org/10.22099/ijvr.2024.47413.6840.

Halfaoui, Z., Menoueri, N. M., & Bendali, L. M. (2017). Serogrouping and antibiotic resistance of Escherichia coli isolated from broiler chicken with colibacillosis in center of Algeria. Veterinary World, 10(7), 830-835. https://doi.org/10.14202/vetworld.2017.830-835.

Hamoudi, A., & Aggad, H. (2008). Antibioresistance of Escherichia coli Strains Isolated from Chicken Colibacillosis in Western Algeria. Turkish Journal of Veterinary & Animal Sciences, 32(2), 123-126.

Hulton, C. S., Higgins, C. F., & Sharp, P. M. (1991). ERIC sequences : A novel family of repetitive elements in the genomes of Escherichia coli, Salmonella typhimurium and other enterobacteria. Molecular Microbiology, 5(4), 825-834. https://doi.org/10.1111/j.1365-2958.1991.tb00755.x.

Igwaran, A., Iweriebor, B. C., & Okoh, A. I. (2018). Molecular characterization and antimicrobial resistance pattern of Escherichia coli recovered from wastewater treatment plants in Eastern Cape South Africa. International journal of

environmental research and public health, 15(6), 1237. https://doi.org/10.3390/ijerph15061237.

Jalil, A., Masood, S., Ain, Q., Andleeb, S., Dudley, E. G., & Adnan, F. (2023). High resistance of fluoroquinolone and macrolide reported in avian pathogenic Escherichia coli isolates from the humid subtropical regions of Pakistan. Journal of Global Antimicrobial Resistance, 33, 5-17. https://doi.org/10.1016/j.jgar.2023.01.009.

Jansen, W., Van Hout, J., Wiegel, J., Iatridou, D., Chantziaras, I., & De Briyne, N. (2022). Colistin Use in European Livestock : Veterinary Field Data on Trends and Perspectives for Further Reduction. Veterinary Sciences, 9(11), 650. https://doi.org/10.3390/vetsci9110650.

Jhandai, P., Mittal, D., Gupta, R., & Kumar, M. (2024). Phylogrouping of Escherichia coli strains from colibacillosis affected chicken. International Journal of Advanced Biochemistry Research, 8(3S), 182-186. https://doi.org/10.33545/26174693.2024.v8.i3Sc.718.

Johnson, T. J., Miller, E. A., Flores-Figueroa, C., Munoz-Aguayo, J., Cardona, C., Fransen, K., Lighty, M., Gonder, E., Nezworski, J., Haag, A., Behl, M., Kromm, M., Wileman, B., Studniski, M., & Singer, R. S. (2022). Refining the definition of the avian pathogenic Escherichia coli (APEC) pathotype through inclusion of high-risk clonal groups. Poultry Science, 101(10), 102009. https://doi.org/10.1016/j.psj.2022.102009.

Johnson, T. J., Wannemuehler, Y., Doetkott, C., Johnson, S. J., Rosenberger, S. C., & Nolan, L. K. (2008). Identification of Minimal Predictors of Avian Pathogenic Escherichia coli Virulence for Use as a Rapid Diagnostic Tool. Journal of Clinical Microbiology, 46(12), 3987-3996. https://doi.org/10.1128/JCM.00816-08.

Johnson, T. J., Siek, K. E., Johnson, S. J., & Nolan, L. K. (2006). DNA Sequence of a ColV Plasmid and Prevalence of Selected Plasmid-Encoded Virulence Genes among Avian Escherichia coli Strains. Journal of Bacteriology, 188(2), 745-758. https://doi.org/10.1128/JB.188.2.745-758.2006.

Johnson, J. R., & Stell, A. L. (2000). Extended Virulence Genotypes of Escherichia coli Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. The Journal of Infectious Diseases, 181(1), 261-272. https://doi.org/10.1086/315217.

Kathayat, D., Lokesh, D., Ranjit, S., & Rajashekara, G. (2021). Avian Pathogenic Escherichia coli (APEC) : An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. Pathogens, 10(4), 467. https://doi.org/10.3390/pathogens10040467.

Kathayat, D., Helmy, Y. A., Deblais, L., & Rajashekara, G. (2018). Novel small molecules affecting cell membrane as potential therapeutics for avian pathogenic Escherichia coli. Scientific Reports, 8(1), 15329. https://doi.org/10.1038/s41598-018-33587-5.

Kiersten J., M., Zhang, L., Foxman, B., Bauer, R. J., & Marrs, C. F. (2003). Evaluation of Genotyping Large Numbers of Escherichia coli Isolates by Enterobacterial Repetitive Intergenic Consensus-PCR. Journal of Clinical Microbiology, 41(11), 5224-5226. https://doi.org/10.1128/jcm.41.11.5224-5226.2003.

Kiiru, J., Kariuki, S., Goddeeris, B. M., & Butaye, P. (2012). Analysis of β-lactamase phenotypes and carriage of selected β-lactamase genes among Escherichia coli strains obtained from Kenyan patients during an 18-year period. BMC Microbiology, 12, 155. https://doi.org/10.1186/1471-2180-12-155.

Kimura, A. H., Koga, V. L., De Souza Gazal, L. E., De Brito, B. G., De Brito, K. C. T., Navarro-Ocaña, A., Nakazato, G., & Kobayashi, R. K. T. (2021). Characterization of multidrug-resistant avian pathogenic Escherichia coli : An outbreak in canaries. Brazilian Journal of Microbiology, 52(2), 1005-1012. https://doi.org/10.1007/s42770-021-00443-0.

Kromann, S., Baig, S., Stegger, M., Olsen, R. H., Bojesen, A. M., Jensen, H. E., & Thøfner, I. (2022). Longitudinal study on background lesions in broiler breeder flocks and their progeny, and genomic characterisation of Escherichia coli. Veterinary Research, 53(1), 52. https://doi.org/10.1186/s13567-022-01064-7.

Kubelová, M., Koláčková, I., Gelbíčová, T., Florianová, M., Kalová, A., & Karpíšková, R. (2021). Virulence Properties of mcr-1-Positive Escherichia coli Isolated from Retail Poultry Meat. Microorganisms, 9(2), 308. https://doi.org/10.3390/microorganisms9020308.

Lima Barbieri, N., Nielsen, D. W., Wannemuehler, Y., Cavender, T., Hussein, A., Yan, S., Nolan, L. K., & Logue, C. M. (2017). Mcr-1 identified in Avian Pathogenic Escherichia coli (APEC). PLOS ONE, 12(3), e0172997. https://doi.org/10.1371/journal.pone.0172997.

Lounis, M., Zhao, G., Li, Y., Gao, Y., Wang, J., Oumouna, M., & Oumouna, K. (2020). Molecular profile of avian pathogenic Escherichia coli (APEC) from poultry associated with colibacillosis in Algeria. Journal of the Hellenic Veterinary Medical Society, 71(2), 2113. https://doi.org/10.12681/jhvms.23666.

Mageiros, L., Méric, G., Bayliss, S. C., Pensar, J., Pascoe, B., Mourkas, E., Calland, J. K., Yahara, K., Murray, S., Wilkinson, T. S., Williams, L. K., Hitchings, M. D., Porter, J., Kemmett, K., Feil, E. J., Jolley, K. A., Williams, N. J., Corander, J., & Sheppard, S. K. (2021). Genome evolution and the emergence of pathogenicity in avian Escherichia coli. Nature Communications, 12(1), 765. https://doi.org/10.1038/s41467-021-20988-w.

Mbanga, J., & Nyararai, Y. O. (2015). Virulence gene profiles of avian pathogenic Escherichia coli isolated from chickens with colibacillosis in Bulawayo, Zimbabwe. The Onderstepoort Journal of Veterinary Research, 82(1), e1-e8. https://doi.org/10.4102/ojvr.v82i1.850.

Meguenni, N., Chanteloup, N., Tourtereau, A., Ahmed, C. A., Bounar-Kechih, S., & Schouler, C. (2019). Virulence and antibiotic resistance profile of avian Escherichia coli strains isolated from colibacillosis lesions in central of Algeria. Veterinary World, 12(11), 1840-1848. https://doi.org/10.14202/vetworld.2019.1840-1848.

Meguenni, N., Le Devendec, L., Jouy, E., Le Corvec, M., Bounar-Kechih, S., Bakour, R., & Kempf, I. (2015). First Description of an Extended-Spectrum Cephalosporin- and Fluoroquinolone-Resistant Avian Pathogenic Escherichia coli Clone in Algeria. Avian Diseases, 59(1), 20-23. https://doi.org/10.1637/10804-022414-Reg.1.

Mehat, J. W., Van Vliet, A. H. M., & La Ragione, R. M. (2021). The Avian Pathogenic Escherichia coli (APEC) pathotype is comprised of multiple distinct, independent genotypes. Avian Pathology, 50(5), 402-416. https://doi.org/10.1080/03079457.2021.1915960.

Mellata, M. (2013). Human and Avian Extraintestinal Pathogenic Escherichia coli : Infections, Zoonotic Risks, and Antibiotic Resistance Trends. Foodborne Pathogens and Disease, 10(11), 916-932. https://doi.org/10.1089/fpd.2013.1533.

Milanov, D., Prunic, B., Velhner, M., Pajic, M., & Cabarkapa, I. (2015). RDAR morphotype : A resting stage of some Enteroba cteriaceae. Food and Feed Research, 42(1), 43-50. https://doi.org/10.5937/FFR1501043.

Mišić, D., Kiskaroly, F., Szostak, M. P., Cabal, A., Ruppitsch, W., Bernreiter-Hofer, T., Milovanovic, V., Feßler, A. T., Allerberger, F., Spergser, J., Müller, E., Schwarz, S., Braun, S. D., Monecke, S., Ehricht, R., Korus, M., Benković, D., Korzeniowska, M., & Loncaric, I. (2021). The First Report of mcr-1-Carrying Escherichia coli Originating from Animals in Serbia. Antibiotics, 10(9), 1063. https://doi.org/10.3390/antibiotics10091063.

Mohamed, L., Ge, Z., Yuehua, L., Yubin, G., Rachid, K., Mustapha, O., Junwei, W., & Karine, O. (2018). Virulence traits of avian pathogenic (APEC) and fecal (AFEC) E. coli isolated from broiler chickens in Algeria. Tropical Animal Health and Production, 50(3), 547-553. https://doi.org/10.1007/s11250-017-1467-5.

Monroy, I., Catalá-Gregori, P., & Sevilla-Navarro, S. (2025). Assessment of antibiotic resistance and virulence in Escherichia coli strains isolated from poultry in Spain. Poultry Science, 104(2), 104838. https://doi.org/10.1016/j.psj.2025.104838.

Nabti, L. Z., Sahli, F., Hadjadj, L., Ngaiganam, E. P., Lupande-Mwenebitu, D., Rolain, J.-M., & Diene, S. M. (2019). Autochthonous case of mobile colistin resistance gene mcr-1 from a uropathogenic Escherichia coli isolate in Sétif Hospital, Algeria. Journal of global antimicrobial resistance, 19, 356 357. https://doi.org/10.1016/j.jgar.2019.10.006.

Nawaz, S., Wang, Z., Zhang, Y., Jia, Y., Jiang, W., Chen, Z., Yin, H., Huang, C., & Han, X. (2024). Avian pathogenic Escherichia coli (APEC) : Current insights and future challenges. Poultry Science, 103(12), 104359. https://doi.org/10.1016/j.psj.2024.104359.

Ngbede, E. O., Poudel, A., Kalalah, A., Yang, Y., Adekanmbi, F., Adikwu, A. A., Adamu, A. M., Mamfe, L. M., Daniel, S. T., & Useh, N. M. (2020). Identification of mobile colistin resistance genes (mcr-1.1, mcr-5 and mcr-8.1) in Enterobacteriaceae and Alcaligenes faecalis of human and animal origin, Nigeria. International Journal of Antimicrobial Agents, 56(3), 106108. https://doi.org/10.1016/j.ijantimicag.2020.106108.

Osisiogu, E. U., Mahmoud, F. C., Waqas, F. B., Singh, B., Feglo, P. K., & Duedu, K. O. (2025). Environmental mediation of colistin resistance in the African context. A systematic scoping review. Journal of Global Antimicrobial Resistance, 41, 39 43. https://doi.org/10.1016/j.jgar.2024.12.002.

Osman, K., Mustafa, A., Elhariri, M., & AbdElhamed, G. (2012). Identification of serotypes and virulence markers of Escherichia coli isolated from human stool and urine samples in Egypt. Indian Journal of Medical Microbiology, 30(3), 308-313. https://doi.org/10.4103/0255-0857.99492.

Ovi, F., Zhang, L., Nabors, H., Jia, L., & Adhikari, P. (2023). A compilation of virulence-associated genes that are frequently reported in avian pathogenic Escherichia coli (APEC) compared to other E. coli. Journal of Applied Microbiology, 134(3), lxad014. https://doi.org/10.1093/jambio/lxad014.

Peng, Z., Zhang, X., Li, X., Hu, Z., Li, Z., Jia, C., Dai, M., Tan, C., Chen, H., & Wang, X. (2021). Characteristics of colistin-resistant Escherichia coli from pig farms in Central China. Animal Diseases, 1(1), 9. https://doi.org/10.1186/s44149-021-00009-5.

Poirel, L., Madec, J.-Y., Lupo, A., Schink, A.-K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial Resistance in Escherichia coli. Microbiology Spectrum, 6(4), 6.4.14. https://doi.org/10.1128/microbiolspec.ARBA-0026-2017.

Poirel, L., Jayol, A., & Nordmann, P. (2017). Polymyxins : Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clinical Microbiology Reviews, 30(2), 557-596. https://doi.org/10.1128/CMR.00064-16.

Puterflam, J., Souillard, R., Balaine, L., Galliot, P., Lucas, P., Bougeard, S., Kempf, I., Keita, A., Delannoy, S., Schouler, C., & Le Bouquin, S. (2022). Colisée—Controlling colibacillosis in chicken. Agronomic innovations, 85, pp.83-92. https://dx.doi.org/10.17180/ciag-2022-vol85-art07.

Rebelo, A. R., Bortolaia, V., Kjeldgaard, J. S., Pedersen, S. K., Leekitcharoenphon, P., Hansen, I. M., Guerra, B., Malorny, B., Borowiak, M., Hammerl, J. A., Battisti, A., Franco, A., Alba, P., Perrin-Guyomard, A., Granier, S. A., De Frutos Escobar, C., Malhotra-Kumar, S., Villa, L., Carattoli, A., & Hendriksen, R. S. (2018). Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Eurosurveillance, 23(6). https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672.

Rezatofighi, S. E., Najafifar, A., Askari Badouei, M., Peighambari, S. M., & Soltani, M. (2021). An Integrated Perspective on Virulence-Associated Genes (VAGs), Antimicrobial Resistance (AMR), and Phylogenetic Clusters of Pathogenic and Non-pathogenic Avian Escherichia coli. Frontiers in Veterinary Science, 8, 758124. https://doi.org/10.3389/fvets.2021.758124.

Rodrigues, S. V., Laviniki, V., Borges, K. A., Furian, T. Q., Moraes, H. L. S., Nascimento, V. P., & Salle, C. T. P. (2019). Biofilm Formation by Avian Pathogenic Escherichia coli is Not Related to In Vivo Pathogenicity. Current Microbiology, 76(2), 194 199. https://doi.org/10.1007/s00284-018-1608-8.

Saeed, M. A., Asif, H., Ehtisham-ul-Haque, S., Khan, A. U., Rehman, A. U., Rehman, A., Rafique, M. K., Ahmed, I., Qamar, M. F., Tomaso, H., & El-Adawy, H. (2025). Detection and risk factor analysis of avian colibacillosis associated with colistin-resistant Escherichia coli and Klebsiella pneumoniae. Frontiers in Veterinary Science, 12, 1612542. https://doi.org/10.3389/fvets.2025.1612542.

Saha, O., Hoque, M. N., Islam, O. K., Rahaman, Md. M., Sultana, M., & Hossain, M. A. (2020). Multidrug-Resistant Avian Pathogenic Escherichia coli Strains and Association of Their Virulence Genes in Bangladesh. Microorganisms, 8(8), 1135. https://doi.org/10.3390/microorganisms8081135.

Saidani, M., Messadi, L., Mefteh, J., Chaouechi, A., Soudani, A., Selmi, R., Dâaloul-Jedidi, M., Chehida, F. B.,

Mamlouk, A., & Jemli, M. H. (2019). Various Inc-type plasmids and lineages of Escherichia coli and Klebsiella pneumoniae spreading blaCTX-M-15, blaCTX-M-1 and mcr-1 genes in camels in Tunisia. Journal of Global Antimicrobial Resistance, 19, 280-283. https://doi.org/10.1016/j.jgar.2019.05.007.

Schouler, C., Schaeffer, B., Brée, A., Mora, A., Dahbi, G., Biet, F., Oswald, E., Mainil, J., Blanco, J., & Moulin-Schouleur, M. (2012). Diagnostic strategy for identifying avian pathogenic Escherichia coli based on four patterns of virulence genes. Journal of Clinical Microbiology, 50(5), 1673-1678. https://doi.org/10.1128/JCM.05057-11.

Shankar, T. V. S., Sharma, A., & Grover, Y. P. (2010). Studies on different virulence factors of avian pathogenic Escherichia coli. Haryana Vet, 49, 45-47.

Sivaranjani, M., McCarthy, M. C., Sniatynski, M. K., Wu, L., Dillon, J.-A. R., Rubin, J. E., & White, A. P. (2022). Biofilm Formation and Antimicrobial Susceptibility of E. coli Associated With Colibacillosis Outbreaks in Broiler Chickens From Saskatchewan. Frontiers in Microbiology, 13, 841516. https://doi.org/10.3389/fmicb.2022.841516.

Skyberg, J. A., Siek, K. E., Doetkott, C., & Nolan, L. K. (2007). Biofilm formation by avian Escherichia coli in relation to media, source and phylogeny. Journal of Applied Microbiology, 102(2). https://doi.org/10.1111/j.1365-2672.2006.03076.

Smelikova, E., Tkadlec, J., & Krutova, M. (2022). How to : Screening for mcr-mediated resistance to colistin. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 28(1), 43-50. https://doi.org/10.1016/j.cmi.2021.09.009.

Srisrattakarn, A., Saiboonjan, B., Tippayawat, P., Angkititrakul, S., Chanawong, A., Pornchoo, C., Smithkittipol, C., & Lulitanond, A. (2025). CTX-M, SHV, TEM and VEB β-lactamases, and MCR-1 among multidrug-resistant Escherichia coli and Klebsiella isolates from environment near animal farms in Thailand. Journal of Infection and Public Health, 18(2), 102624. https://doi.org/10.1016/j.jiph.2024.102624.

Terveer, E. M., Nijhuis, R. H. T., Crobach, M. J. T., Knetsch, C. W., Veldkamp, K. E., Gooskens, J., Kuijper, E. J., & Claas, E. C. J. (2017). Prevalence of colistin resistance gene (mcr-1) containing Enterobacteriaceae in feces of patients attending a tertiary care hospital and detection of a mcr-1 containing, colistin susceptible E. coli. PloS One, 12(6), e0178598. https://doi.org/10.1371/journal.pone.0178598.

Thabet, A. M., Alzuheir, I. M., Al Laham, N. A., Helal, B. Y. A., Fayyad, A. F., Jalboush, N. H., & Gharaibeh, M. H. (2023). First report of mobile colistin resistance gene mcr-1 in avian pathogenic Escherichia coli isolated from turkeys in the Gaza Strip, Palestine. Veterinary World, 1260-1265. https://doi.org/10.14202/vetworld.2023.1260-1265.

Thabet, S., Souissi, N., & Khazri, I. (2022). Assessment of antimicrobial resistance in avian pathogenic Escherichia coli Strains isolated over four years in Tunisian poultry. Afr. J. Bacteriol. Res, 14(1), 1-7. https://doi.org/10.5897/JBR2016.0212.

Touati, M., Hadjadj, L., Berrazeg, M., Baron, S. A., & Rolain, J. M. (2020). Emergence of Escherichia coli harbouring mcr-1 and mcr-3 genes in North West Algerian farmlands. Journal of global antimicrobial resistance, 21, 132-137. https://doi.org/10.1016/j.jgar.2019.10.001.

Uddin, M. S., Masum, Md. H. U., Hosen, Md. R., Roy, S. C., Rahman, A. B. Z. N., Siddiquee, N. H., Siddiqua, A., Hossain, I., & Peas, T. (2025). Multidrug Resistance and Virulence Gene Profiles of E. coli in Broiler Chickens : A Study From Noakhali, Bangladesh. Veterinary Medicine International, 2025(1), 1157843. https://doi.org/10.1155/vmi/1157843.

Ugwu, I. C., Lee-Ching, L., Ugwu, C. C., Okoye, J. O. A., & Chah, K. F. (2020). In vitro assessment of pathogenicity and virulence encoding gene profiles of avian pathogenic Escherichia coli strains associated with colibacillosis in chickens. Iranian journal of veterinary research, 21(3), 180.

Valiakos, G., & Kapna, I. (2021). Colistin Resistant mcr Genes Prevalence in Livestock Animals (Swine, Bovine, Poultry) from a Multinational Perspective. A Systematic Review. Veterinary Sciences, 8(11), 265. https://doi.org/10.3390/vetsci8110265.

van der Westhuizen, W. A., & Bragg, R. R. (2012). Multiplex polymerase chain reaction for screening avian pathogenic Escherichia coli for virulence genes. Avian Pathology: Journal of the W.V.P.A, 41(1), 33-40. https://doi.org/10.1080/03079457.2011.631982.

Vougat Ngom, R., Ferreira, H. C. D. C., Ayissi, G., Tanyienow, A., & Piccirillo, A. (2025). A systematic review and meta-analysis on the efficacy of antibiotic treatment in controlling colibacillosis in broiler production. PLOS One, 20(7), e0326535. https://doi.org/10.1371/journal.pone.0326535.

Wang, S., Liu, L., Du, L., Gao, S., Yu, X., Cheng, L., Chen, Y., Kou, Z., & Sun, W. (2025). Emergence of coexisting bla NDM and mcr-1 genes in Escherichia coli isolates from the guts of healthy individuals. Microbiology Spectrum, e02014-25. https://journals.asm.org/doi/pdf/10.1128/spectrum.02014-25.

Wu, C., Wang, Y., Shi, X., Wang, S., Ren, H., Shen, Z., Wang, Y., Lin, J., & Wang, S. (2018). Rapid rise of the ESBL and mcr-1 genes in Escherichia coli of chicken origin in China, 2008–2014. Emerging Microbes & Infections, 7(1), 1-10. https://doi.org/10.1038/s41426-018-0033-1.

Zhu, Z., Cao, M., Wang, W., Zhu, Q., Wang, C., Zhang, Y., Shi, Y., Zhou, X., Cui, G., &Zhang, J. (2021). Virulence-Associated Genes and Genetic Diversity of Avian Pathogenic (APEC) and Fecal (AFEC) E. Coli Isolates From Chickens. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-644399/v2.

Zhu Ge, X., Jiang, J., Pan, Z., Hu, L., Wang, S., Wang, H., Leung, F. C., Dai, J., & Fan, H. (2014). Comparative Genomic Analysis Shows That Avian Pathogenic Escherichia coli Isolate IMT5155 (O2:K1:H5; ST Complex 95, ST140) Shares Close Relationship with ST95 APEC O1:K1 and Human ExPEC O18:K1 Strains. PLoS ONE, 9(11), e112048. https://doi.org/10.1371/journal.pone.0112048.