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Prevalence of colistin resistance and *mcr-1*/*mcr-2* genes in extended-spectrum β-lactamase/AmpC-producing *Escherichia coli* isolated from chickens in Canada, Senegal and Vietnam

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ABSTRACT

Objectives: This study investigated the prevalence of *Escherichia coli* (*E. coli*) colistin resistance and *mcr-1* and *mcr-2* genes among extended-spectrum β-lactamase (ESBL)/AmpC-producing *E. coli* isolates recovered from chicken feces in Canada (Quebec), Senegal and Vietnam, and evaluated the susceptibility pattern of the colistin-resistant *E. coli* isolates to other clinically relevant antimicrobials.
Methods: A total of 327 potential ESBL/AmpC-producing *E. coli* isolates from chicken farms in Canada (Quebec), Senegal and Vietnam were analysed for colistin susceptibility by broth microdilution method and for the presence of *mcr* (*1-2*) genes by PCR. The *pmrA* and *pmrB* genes of colistin-resistant *E. coli* isolates, in the absence of *mcr* (*1-2*) genes, were sequenced. Antimicrobial resistance phenotypes of colistin-resistant *E. coli* isolates were determined by disk diffusion.
Results: None of the 108 potential ESBL/AmpC-producing *E. coli* isolates from seven farms in Canada were colistin-resistant or possessed *mcr-1* or *mcr-2* gene. A low prevalence of 2.2% of colistin resistance was observed in 93 Senegalese isolates from the 15 sampled farms, although neither *mcr-1* nor *mcr-2* gene was found. A prevalence of 8.7% of colistin resistance was observed among 126 Vietnamese isolates from two of the four sampled farms. The *mcr-1* gene was detected in 85% of the 13 phenotypically colistin-resistant isolates. Moreover, all colistin-resistant isolates presented a multidrug-resistant phenotype.
Conclusions: The co-existence of the *mcr-1* and ESBL/AmpC genes and the very high level of multiple drug resistance in all colistin-resistant *E. coli* isolates obtained from sampled chicken farms in Vietnam is a major concern.

1. Introduction

The poultry industry is one of the most important global animal food industries, with affordable selling prices, high-quality protein, and relatively low fat content in the meat [1]. Despite the progress that has been made in the control of bacterial diseases in poultry during the last decade, avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), remains a major cause of worldwide economic losses in this sector [2]. Colistin, also known as polymyxin E, is a cationic polypeptide antibiotic widely used for the prevention and the treatment of avian colibacillosis [3,4]. This antimicrobial is approved for use in poultry in most countries throughout the world, excluding North America (Canada and USA) [3,5]. The association between colistin use in food animals and the risk of colistin resistance in commensal bacteria has been described in several countries in Europe and in Vietnam [6,7]. Resistance to colistin in Gram-negative bacteria (GNB) can occur either through mutations in genes encoding for the two component systems – PhoP–PhoQ (PhoPQ) and PmrA–PmrB (PmrAB) – or through plasmid-mediated horizontal gene transfer (*mcr* genes) [4]. Both mechanisms lead to a modification in lipopolysaccharide molecules, by decreasing the net negative charge of its phosphate residues, and consequently reducing the electrostatic interaction between lipopolysaccharides and colistin positive charges [8]. In *Escherichia coli* (*E. coli*), mutation in the PmrAB two-component

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system has been identified as the main chromosomal target associated with resistance to colistin and polymyxin B [9].

Since the first identification, in 2015, of a plasmid-harboring *mcr-1* gene encoding for colistin resistance in *Enterobacteriaceae,* some authors have suggested that farm animals are mainly responsible for the global dissemination of plasmids carrying *mcr* genes [10,11]. The *mcr-1* gene has been detected in both retail chicken meat and poultry feces on several continents (Africa, Asia, Europe and South America) [10,12–14].

In Canada, although colistin is not approved in veterinary medicine, it is sometimes used in food animals, as a last-resort antimicrobial for the oral treatment of digestive infections caused by GNB [15]. Colistin use remains under veterinarian responsibility regarding justification, dose and setting of the withdrawal period [8]. On the other hand, in Senegal, colistin is approved for use in animal production and is widely used in chickens for either therapeutic, prophylactic or growth-promotion purposes [16]. To the best of our knowledge no study has been conducted in poultry to estimate the prevalence of *E. coli* colistin resistance or *mcr* genes in either Canada or Senegal. Interestingly, *mcr-1* gene has been identified in *E. coli* isolated from humans [17,18], as well as from lean ground beef in Canada [17]. In Vietnam, colistin is approved in veterinary medicine and widely used in poultry farms, mostly for disease prevention and to improve growth performance [19,20], and *mcr*-1-producing *E. coli* has been isolated with high prevalence in poultry [7,21].

Several studies have reported a co-existence of *mcr* genes and extended-spectrum β-lactamase (ESBL) genes in the same bacterial strains in poultry meat, clinical isolates from sick birds, and fecal samples from healthy birds in Denmark and China [22,23]. A recent study suggested an increasing trend of co-existence and transmission of ESBL and *mcr-1* gene in both human and veterinary medicine [23]. ESBL/AmpC-producing *E. coli* have been frequently reported in poultry, and therefore this animal production was suggested as a reservoir for these resistant bacteria, posing a potential threat to human health [24]. Similarly, a high prevalence of farms carrying third-generation cephalosporin-resistant fecal *E. coli* isolates has been demonstrated in Senegal, Quebec and Vietnam (unpublished data). Third-generation cephalosporin resistance is a major issue, as these antimicrobials are critically important in animal and human health.

The current study aimed to: 1) investigate prevalence of colistin resistance, and of *mcr-1* and *mcr-2* genes among fecal potential ESBL/AmpC-producer *E. coli* isolated in a previous study in 2011–2012 from healthy chicken farms located in Canada (Quebec), Senegal and Vietnam; and 2) evaluate the susceptibility patterns to 14 clinically relevant antimicrobials in the colistin-resistant isolates.

2. Material and methods

2.1. Isolate collections

This project was conducted using chicken *E. coli* isolates from Quebec (Canada), Senegal and Vietnam collected as part of the activities of the OIE Reference Laboratory for *Escherichia coli* (EcL Laboratory) at the Université de Montréal. In Canada, fecal samples (n = 35) were originally collected, as part of a previous study [25], from 12 clinically healthy broiler farms practicing thinning (i.e. partial depopulation), from June 2011 to February 2012, in the region of Saint-Hyacinthe, Quebec. Each farm housed 20 000–30 000 birds aged between 35–40 days. Each sample consisted of a pool of five fecal samples taken from different places in the broiler house. Samples were then sent to the EcL Laboratory for *E. coli* isolation and identification. Samples were stored at −80 °C, at the EcL laboratory, before processing. A quantity of 10 g of each thawed sample was incubated in a filter bag with 90 mL of buffered peptone water for 12 h at 37 °C to allow bacterial growth. The filtrate was streaked on MacConkey agar, and incubated at 37 °C overnight to obtain primary cultures (initial *E. coli* culture obtained from fecal samples). Each primary culture was suspended in 15% glycerol and stored at −80 °C before further analyses.

In Senegal, fecal samples (n = 50) were collected from clinically healthy birds in 32 chicken farms randomly selected in the Dakar region, between May and July 2011, using the same sampling approach described above for Canadian farms. These samples were transported in a cooler at 4 °C to the microbiology laboratory of the École Inter-États des Sciences et Médecine Vétérinaires (EISMV) of Dakar. At the EISMV, each pooled fecal sample was homogenised 1/10 (weight/volume) in buffered peptone water, using a sterile filter sample bag (Whirl-pak®). The filtrate was streaked on MacConkey plates and incubated overnight at 37 °C to obtain primary cultures. All primary cultures were kept at 4 °C until their shipping to the EcL Laboratory and then handled as described above.

In Vietnam, fecal samples (n = 51) were taken from five healthy chicken farms, chosen by convenience, and located in the provinces of Hoa Binh, Thai Nguyen, and Bac Giang in the north east of Vietnam. Fecal sampling was conducted between July and September 2011. From each farm, fecal swabs were randomly collected from five points on each pen floor using sterile cotton swabs, and then pooled to constitute one sample per farm. All samples were transported to the National Veterinary Institute in Hanoi, Vietnam. After enriching overnight in peptone water at 37 °C, samples were cultured on MacConkey agar, and incubated overnight at 37 °C. All cultures were kept at 4 °C until shipping to the EcL laboratory in Canada and then handled as described above.

2.2. Selection of potential ESBL/AmpC-producing isolates

Potential ESBL/AmpC-producing *E. coli* isolates were obtained using the method developed by Agersø et al. [26] with some minor modifications. Briefly, 50 μL of the primary cultures, following storage at −80 °C, was added to 5 mL of peptone water, and after 30 minutes of incubation at 37 °C, 20 μL of this broth was streaked on MacConkey agar supplemented with 1 mg/L ceftriaxone, and incubated overnight aerobically at 37 °C. Isolates demonstrating growth on this culture medium were considered as potential ESBL/AmpC producers. The potential ESBL/AmpC isolates were obtained from 15, 4 and 7 farms in Senegal, Vietnam and Canada (Quebec), respectively. Five morphologically different colonies lactose-positive on MacConkey agar supplemented with ceftriaxone, when available, were confirmed as *E. coli* by colony morphology, biochemical analysis, and by the presence of the β-glucuronidase (*uidA*) housekeeping gene, as previously described [27].

2.3. Colistin susceptibility testing

All potential ESBL/AmpC-producing *E. coli* isolates were inoculated on in-house-prepared Mueller Hinton agar supplemented with 2 mg/L colistin. Isolates recovered from this media were considered as potential colistin-resistant bacteria, as previously described [27]. The minimum inhibitory concentrations (MICs) of colistin were only determined for potential colistin-resistant isolates using the broth microdilution method in duplicate, according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) as previously described [15,27]. The *E. coli* ATCC 25922 strain was used, in each microplate, as a positive control. Phenotypically, *E. coli* isolates with colistin MICs > 2 mg/L were defined as resistant (R), and those with colistin MICs ≤ 2 mg/L as susceptible (S), according to the European Committee on

Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/clinical_breakpoints/).

2.4. DNA extraction and molecular detection of mcr (1-2) genes

Bacterial DNA extraction was performed by heat lysis, as previously described [28]. All potential ESBL/AmpC-producing *E. coli* isolates, resistant or not to colistin, were screened by PCR for the presence of the *mcr-1* and *mcr-2* genes, using primers and conditions previously described [29,30]. DNA from *E. coli* strains harboring either the *mcr-1* or *mcr-2* gene were used as positive control, as previously described [27].

2.5. Sequencing of pmrAB genes

The *pmrA* and *pmrB* genes of colistin-resistant *E. coli* isolates, in the absence of mcr (1-2) genes, were amplified by PCR, sequenced, and polymorphisms were identified by comparison with the reference sequences, AY725333.1 and KX652271.1 for *PmrA* and *PmrB*, respectively. These reference sequences are available in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/).

The coding sequences of the *pmrA* and *pmrB* genes were amplified using primers designed in this study for *E. coli pmrA* [forward 5′-CAAACTTGCAGGAGAGTGAG-3′ and reverse 5′-GCTGATCAGCTCAAACACCA-3′] and *E. coli pmrB* [forward 5′-GGCTTTGGCTATATGCTGGT-3′ and reverse 5′-TTAACTACCGTGTT-CAGCGT-3′]. PCR was performed using the following cycling conditions: 25 cycles of 94 °C × 30 s, 57.5 °C × 30 s, 72 °C × 60 s; and 25 cycles of 94 °C × 30 s, 57 °C × 30 s, 72 °C × 75 s, for *pmrA* and *pmrB* genes, respectively. DNA fragments were excised then purified from gels using PureLink® PCR Purification Kit (Invitrogen, Canada). Sequencing of the PCR products, was conducted by an external service provider (McGill University and Génome Québec Innovation Centre, Canada), using the Sanger sequencing method. Sequence alignment was performed using Clustal Omega (European Molecular Biology Laboratory–European Bioinformatics Institute, Hinxton, United Kingdom).

2.6. Detection of beta-lactamase genes and antimicrobial susceptibility testing

All colistin-resistant *E. coli* isolates were tested for the presence of β-lactamase genes and for their susceptibility to 14 clinically relevant antimicrobials belonging to nine classes, as used in the Canadian Integrated Program for Antimicrobial Resistance Surveillance [31]. The susceptibility of colistin-resistant *E. coli* isolates to the selected antimicrobials was examined using the disk diffusion (Kirby-Bauer) method, as previously described [31]. Isolates demonstrating intermediate susceptibility or resistance to three or more classes of antimicrobial agents, including colistin, were considered to be multidrug-resistant (MDR) and isolates demonstrating intermediate susceptibility or resistance to all but two classes of antimicrobial agents were considered to be extensively drug-resistant (XDR) [32]. The colistin-resistant isolates were screened by PCR for the presence of five β-lactamase genes (bla_{CTX-M}, bla_{TEM}, bla_{OXA-1}, bla_{SHV} and bla_{CMY-2}) using previously described primers and conditions [33].

2.7. Statistical analysis

Prevalence with 95% confidence interval was estimated at isolate level and farm level for each country, for colistin resistance among potential ESBL/AmpC-producing *E. coli* isolates, and for *mcr-1* or *mcr-2* gene presence in the isolates. At isolate level, estimates were adjusted for clustering of isolates within farms. For the estimation at farm level, a farm was considered as positive when at least one positive isolate was detected in the pool sample, and exact confidence intervals were estimated. All analyses were conducted in SAS version 9.4, using the freq or surveyfreq procedures.

3. Results

3.1. Prevalence of potential ESBL/AmpC-producing E. coli isolates

A total of 327 potential ESBL/AmpC-producing *E. coli* isolates were recovered from chicken fecal samples using MacConkey agar supplemented with 1 mg/L of ceftriaxone. These were considered as potential ESBL/AmpC-producing *E. coli* isolates until confirmation by subsequent molecular characterisation of the target ESBL/AmpC genes. The median number of potential ESBL/AmpC isolates per farm was: 12 (range 3–40) in Quebec; five (range 1–10) in Senegal; and 35 (range 2–55) in Vietnam.

3.2. Prevalence of E. coli colistin resistance, and mcr-1 and mcr-2 genes among potential ESBL/AmpC-producer E. coli isolates

From the 327 potential ESBL/AmpC-producing *E. coli* isolates, 62 (19%) were identified as potential colistin-resistant *E. coli*, using Mueller Hinton agar supplemented with 2 mg/L of colistin (Table 1). Thirteen isolates (4%) were confirmed resistant to colistin by determining the MIC (Table 1), comprising two (2.2%, 95% CI 0.0–5.4) of 93 Senegal-derived isolates, and 11 (8.7%, 95% CI 0.0–34.4) of 126 Vietnam-derived isolates. None of the 108 Canada-derived potential ESBL/AmpC-producing *E. coli* isolates from seven sampled farms were found to be resistant to colistin (Table 1). At farm level, colistin-resistant isolates were found in two of 15 sampled farms in Senegal and two of four sampled farms in Vietnam (Table 2). It should be stressed that the numbers of

Table 1
Prevalence of colistin resistance and *mcr-1* gene among potential ESBL/AmpC-producing *Escherichia coli* isolates recovered from chicken feces in Canada (Quebec), Senegal and Vietnam.

Country	Number of isolates	Number of potential colistin-resistant isolates	Resistance to colistin			Presence of *mcr-1* gene		
			Number of resistant isolates	Prevalence (%)		Number of *mcr-1*-positive isolates	Prevalence (%)	
				Estimate	95% CI		Estimate	95% CI
Senegal	93	33	2	2.2	0.0–5.4	0	0.0	–
Canada (Quebec)	108	2	0	0.0	–	0	0.0	–
Vietnam	126	27	11	8.7	0.0–34.4	11	8.7	0.0–34.4

Abbreviations: CI ; Confidence interval; ESBLextended-spectrum β-lactamase.

Table 2
Prevalence of colistin resistance and *mcr-1* gene among potential ESBL/AmpC-producing *Escherichia coli* isolates in chicken farms in Canada (Quebec), Senegal and Vietnam.

Country	Number of farms	Number of farms with potential colistin-resistant isolates	Resistance to colistin			Presence of *mcr-1* gene		
			Number of farms with resistant isolates	Prevalence (%)		Number of farms with *mcr-1*-positive isolates	Prevalence (%)	
				Estimate	95% CI		Estimate	95% CI
Senegal	15	12	2	13.3	1.7–40.5	0	0.0	0.0–0.1
Canada (Quebec)	7	2	0	0.0	0.0–40.9	0	0.0	0.0–40.9
Vietnam	4	4	2	50.0	6.8–93.2	2	50.0	6.8–93.2

Abbreviations: CI, Confidence interval; ESBL, extended-spectrum β-lactamase.

Table 3
Antimicrobial resistance phenotypes and molecular analysis of colistin-resistant *Escherchia coli* in absence of *mcr (1-2)* genes, Senegal, 2011.

Isolates	Farm ID	Colistin MIC (mg/L)	ESBL gene	Antimicrobial resistance profile	PmrAB polymorphisms	Multidrug resistance profile
ECL23197	02	8	bla_{CTX-M}	STR, CRO, TIO, NAL, SSS, SXT, AMP, TET	S29G (PmrA) P94L (PmrB)	MDR (7)
ECL23181	07	16	bla_{CTX-M}	CRO, TIO, NAL, SSS, SXT, AMP, TET	S29G (PmrA) D283G (PmrB)	MDR (6)

Abbreviations: AMP, ampicillin; CRO, ceftriaxone; TIO, ceftiofur; NAL, nalidixic acid; STR, streptomycin; SSS, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; D, aspartic acid; G, glycine; L, leucine; S, serine; P, proline; MDR [N], multidrug resistance expressed against N classes of antimicrobials; ESBL, extended-spectrum β-lactamase.

Table 4
Antimicrobial susceptibility of *mcr-1*-positive *Escherichia coli* isolates from chicken, Vietnam, 2011.

Sample-Isolate ID	Farm ID	ESBL genes	AmpC genes	Antimicrobial resistance profile	Colistin MIC (mg/L)	Colistin-resistance gene	Multidrug resistance profile
ECL23357	01	bla_{TEM}, bla_{CTX-M}	–	KAN, STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (8)
ECL23223	01	bla_{TEM}, bla_{CTX-M}	–	STR, CRO, TIO, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (8)
ECL23358	01	bla_{TEM}, bla_{CTX-M}	–	STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (8)
ECL23216	01	bla_{TEM}, bla_{CTX-M}	–	KAN, STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, TET	4	*mcr-1*	MDR (7)
ECL23359	01	bla_{TEM}, bla_{CTX-M}	–	CRO, TIO, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (7)
ECL23360	01	bla_{TEM}, bla_{CTX-M}	–	CRO, TIO, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (7)
ECL23361	01	bla_{TEM}, bla_{CTX-M}	–	KAN, STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, TET	4	*mcr-1*	MDR (7)
ECL23362	01	bla_{TEM}, bla_{CTX-M}	–	KAN, STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, TET	8	*mcr-1*	MDR (7)
ECL23363	01	bla_{TEM}, bla_{CTX-M}	–	KAN, CRO, TIO, CIP, NAL, SSS, SXT, AMP, TET	4	*mcr-1*	MDR (7)
ECL23364	05	bla_{TEM}, bla_{CTX-M}	–	GEN, KAN, STR, CRO, TIO, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (8)
ECL23253	05	bla_{TEM}, bla_{OXA-1}	bla_{CMY-2}	GEN, KAN, STR, CRO, TIO, FOX, AMC, CIP, NAL, SSS, SXT, AMP, CHL, TET	4	*mcr-1*	MDR (10)(Possible XDR)

Abbreviations: ESBL, extended-spectrum β-lactamase; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; CRO, ceftriaxone; TIO, ceftiofur; FOX, cefoxitin; AMC, cmoxicillin-clavulanic acid; CIP, ciprofloxacin; NAL, nalidixic acid; SSS, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; AMP, ampicillin; CHL, chloramphenicol; TET, tetracycline; MDR [N], multidrug resistance expressed against N classes of antimicrobials; XDR, extensive drug resistance.
Antimicrobial with intermediate susceptibility is underlined.

sampled farms (seven, 15 and four) do not represent the total sampled farms but only those where potential ESBL/AmpC-producing *E. coli* isolates were found. The *mcr-1* gene was only detected in Vietnam, in 11 (85%) of the 13 colistin-resistant isolates found in this study; all were derived from two farms (Table 1 and Table 2). The *mcr-2* gene was not detected.

3.3. Molecular characterisation of colistin-resistant E. coli isolates in the absence of mcr (1-2) genes

Sequencing of the *pmrA* and *pmrB* genes of the two colistin-resistant isolates obtained from Senegalese farms demonstrated one polymorphism that produces a protein variant in PmrA (S29 G), and two polymorphisms that produce protein variants in PmrB (P94 L and D283 G) (Table 3). In addition, these two isolates were bla_{CTX-M} positive (Table 3).

3.4. Resistance phenotypes of colistin-resistant E. coli isolates

Antimicrobial susceptibilities of the eleven *mcr-1*-positive *E. coli* isolates derived from two chicken farms in Vietnam, and the two colistin-resistant isolates derived from two chicken farms in Senegal are shown in Tables 3 and 4. All these isolates were confirmed to be phenotypically colistin-resistant with an MIC $\geq$ 4 mg/L (Tables 3 and 4). These isolates were also confirmed as ESBL/AmpC-producing *E. coli* by identifying the targeted genes

using PCR (Tables 3 and 4). A co-existence of *mcr-1* and the targeted ESBL genes bla_{CTX-M}, and bla_{TEM} was identified in 10 isolates and of *mcr-1*, the targeted AmpC genes bla_{CMY-2}, and bla_{OXA-1} were identified in one isolate from sampled chicken farms in Vietnam (Table 4). Susceptibility testing to other antimicrobials in colistin-resistant ESBL-producing isolates in Vietnam revealed MDR against seven or eight classes of antimicrobials (MDR(7) or MDR (8)), whereas the AmpC-producing isolate was MDR (10) or possibly XDR, being non-susceptible to all tested classes of antimicrobials (Table 4). The two colistin-resistant ESBL-producer isolates from Senegal were each positive to the bla_{CTX-M} gene and MDR (6) and MDR (7), respectively (Table 3).

4. Discussion

This study provides some insight into the co-existence of the *mcr-1* gene and colistin resistance in ESBL/AmpC-producing *E. coli* isolates obtained from chicken farms in three different countries: Canada (Quebec), Senegal and Vietnam. The lack of colistin resistance and *mcr* (*1-2*) genes in ESBL/AmpC-producing *E. coli* in samples from Canadian farms is coherent with the limited use of colistin in food animals in Canada, considering that this antimicrobial is not approved for use in animals in this country [15]. Nevertheless, it has been reported that two *mcr-1*-positive *E. coli* isolates were recovered from lean ground beef in Ontario, although the origin of this meat (domestic or imported) was unknown [17].

The current study reported 8.7% (95% CI 0.0–34.4) of colistin-resistant isolates, combined with the presence of *mcr-1*, among the ESBL/AmpC-producing *E. coli* in the north east of Vietnam. These results corroborate those of Nguyen et al. [21], who estimated a prevalence of 22.2% (95% CI 17–32) of colistin-resistant-*mcr-1*-positive fecal *E. coli* isolated from chickens in Vietnam. In addition, a much higher prevalence of 59.4% (95% CI 47.9–71.0) of colistin-resistant *mcr-1*-positive fecal *E. coli* [7], and of *mcr-1* gene (33.3%) in CTX-M-producing fecal *E. coli* isolates [34] was observed in chicken farms in the Red River Delta region of Vietnam. Yamaguchi et al. [35] also reported a high prevalence of *mcr-1* gene (39%) in ESBL or AmpC-producing *E. coli* isolates obtained from chicken meat in Ho Chi Minh City in Vietnam. On the other hand, neither the *mcr-1* gene nor phenotypic colistin resistance were previously identified in a collection of bla_{CTX-M}-producing *E. coli* strains (n = 11) derived from chicken fecal samples in the Van Lam district of Hung Yen province, Vietnam [36]. Thus, the Delta region of Vietnam, where colistin is extensively used in chickens as a growth promoter as well as for prophylactic and therapeutic purposes, has been associated with a higher prevalence of *E. coli* colistin resistance in this animal production [20,34]. In the current study, the proportion of ESBL/AmpC-producing *E. coli* isolates positive to *mcr-1* was not homogeneous between farms; the gene was not detected in two farms, while 40.9% and 4.3% of ESBL/AmpC-producing *E. coli* isolates were *mcr-1* positive in the two other farms, respectively. It is worth noting that no information was collected regarding the history of antimicrobial use, specifically whether or not colistin has been used in these sampled chicken farms. The current study did not identify the *mcr-2* gene in any of the colistin-resistant *E. coli* isolates from Vietnamese farms, supporting results of previous studies conducted in chicken farms in this country [35]. In contrast, it did observe the presence of resistance to colistin, but not of the *mcr-1* or *mcr-2* genes, in ESBL-producing *E. coli* from Senegalese farms.

The mutations in both PmrA and PmrB found in chicken isolates obtained from Senegalese farms have been previously described in colistin-resistant *E. coli* isolates from other species, for example in humans in China and South Korea [37,38]. However, it would be important for a future study to determine if these isolates harbor any of the other six *mcr* genes discovered to date. In fact, some *E. coli* isolates of animal origin were confirmed resistant to colistin by identifying other *mcr* genes such as *mcr-3* or *mcr-4* [39]. Moreover, mutations in the PmrAB systems and *mcr* genes have been identified in the same colistin-resistant isolate [39]. It is believed that this is the first report of these mutations in the PmrAB two-component system in colistin-resistant *E. coli* strains isolated from healthy chickens. Moreover, the *pmrB* P94 L mutation has not previously been described in isolates of animal origin.

The current study overestimated, as expected, the number of presumed colistin-resistant bacteria in Mueller–Hinton agar supplemented with 2 mg/L of colistin, which was probably due to the poor diffusion of colistin in solid growth medium [15]. However, culture on this medium was only used as a screening method for reducing the number of isolates to be tested for colistin MIC determination. In the current study, the MIC values of the colistin-resistant *E. coli* derived from the Senegalese farms were four to eight times higher than the EUCAST clinical breakpoint (2 mg/L). This finding is concordant with previous studies showing that the chromosomally encoded mechanisms of colistin resistance, contrary to the *mcr* gene mechanisms, are generally associated with high levels of colistin resistance in *Enterobacteriaceae*s trains [4]. Although it could not obtain information regarding colistin use in sampled farms, a substantial use of this antimicrobial has been reported in chickens in Senegal [16], which could contribute to the colistin resistance observed in the current study. A relatively low prevalence of *E. coli* colistin-resistant isolates (2.2%) was observed; however, as sampling was carried out in 2011, a more recent study is needed to document the evolution of *E. coli* colistin resistance in chicken since this date.

It should be noted that a larger number of isolates was used to determine the status of farms in Vietnam (average of 31.5) and in Quebec (median of 34.5) as compared with Senegal (average of 5.0). Therefore, the ability to detect resistance on a farm in Vietnam was higher than in Senegal, which may partly explain the higher prevalence of farms with colistin resistance in Vietnam. Moreover, differences in the enrichment protocol between countries may partly contribute to the observed differences. In addition, isolates within a sample were selected based on their morphology, while sampling weights were not considered for prevalence estimation, which could bias the estimates at the isolate level. This approach, however, maximized the probability of detecting the presence of isolates with less frequent profiles or genes at farm level. Also, a convenience sample of farms was used in Vietnam and thus results should be considered as preliminary, as the representativeness of these farms could not be assessed. Likewise, in Canada, only farms practicing thinning from a small geographical area were selected, limiting the ability to infer the results.

There is major concern over the finding of co-existence of the *mcr-1* gene and ESBL/AmpC genes, and the very high level of MDR and even possible XDR in all *E. coli* strains phenotypically resistant to colistin (n = 11) obtained from sampled chicken farms in Vietnam. This may favour the selection of colistin-resistant strains under the selective pressure imposed by this antimicrobial or even by the use of a wide range of other antimicrobials of less critical importance for human health. Hence, it is important at farm level to apply an overall strategy that includes a reduction, not only in colistin use but also in the use of all antimicrobials, including those of critical importance for human health [5]. It is worth noting that the transmission of *mcr-1* gene between chickens and humans has been confirmed in Vietnam [7], and this country is a potential source of worldwide dissemination of *mcr-1* gene among international travelers [40].

5. Conclusions

This study demonstrated the presence of colistin resistance in ESBL/AmpC-producing *E. coli* isolates in chicken farms located

in Senegal and Vietnam, but not in Canada, which is coherent with reported portraits of colistin use in these countries. The co-existence of the *mcr-1* and ESBL/AmpC genes and the very high level of multiple drug resistance in all colistin-resistant *E. coli* strains obtained from sampled chicken farms in Vietnam is a major concern for both human and veterinary medicine. This co-existence could contribute to selecting and enhancing the worldwide dissemination of colistin resistance with pressure by antimicrobials other than colistin.

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Competing interest

None.

Ethical approval

Not required.

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