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Research Paper

Antibiotic Resistance Profile of Commensal *Escherichia coli* Isolated from Broiler Chickens in Qatar

NAHLA O. ELTAI,[1] ELMOUBASHER A. ABDFARAG,[2] HAMAD AL-ROMAIHI,[2] EMAN WEHEDY,[1] MAHMOUD H. MAHMOUD,[3] OSAMA K. ALAWAD,[3] MOHAMMED M. AL-HAJRI,[2] ASMAA A. AL THANI,[1,4] AND HADI M. YASSINE[1,4*]

[1]*Biomedical Research Center and* [4]*College of Health Sciences, Qatar University, Doha, Qatar (ORCID: http://orcid.org/0000-0001-7592-2788 [H.M.Y.]);* [2]*Public Health Department, Ministry of Public Health, P.O. Box 42, Al Rumaila, Onaiza Street No. 222, Doha, Qatar; and* [3]*Department of Animal Resources, Ministry of Municipality and Environment, P.O. Box 22332, Doha, Qatar*

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ABSTRACT

Antibiotic resistance (AR) is a growing public health concern worldwide, and it is a top health challenge in the 21st century. AR among *Enterobacteriaceae* is rapidly increasing, especially in third-generation cephalosporins and carbapenems. Further, strains carrying mobilized colistin resistance (*mcr*) genes 1 and 2 have been isolated from humans, food-producing animals, and the environment. The uncontrolled use of antibiotics in food-producing animals is a major factor in the generation and spread of AR. No studies have been done to evaluate AR in the veterinary sector of Qatar. This study aimed at establishing primary baseline data for the prevalence of AR among food-producing animals in Qatar. Fecal samples (172) were obtained from two broiler farms and one live bird market in Qatar, and 90 commensal *Escherichia coli* bacteria were isolated and subjected to susceptibility testing against 16 clinically relevant antibiotics by using the E-test method. The results found that 81 (90%) of 90 isolates were resistant to at least one antibiotic, 14 (15.5%) of 90 isolates were colistin resistant, 2 (2.2%) of 90 isolates were extended-spectrum β-lactamase producers, and 2 (2.2%) of 90 isolates were multidrug resistant to four antibiotic classes. Extended-spectrum β-lactamase–producing *E. coli* and colistin-resistant isolates were confirmed by using double-disc susceptibility testing and PCR, respectively. Such a high prevalence of antibiotic-resistant *E. coli* could be the result of a long application of antibiotic treatment, and it is an indicator of the antibiotic load in food-producing animals in Qatar. Pathogens carrying AR can be easily transmitted to humans through consumption of undercooked food or noncompliance with hygiene practices, mandating prompt development and implementation of a stewardship program to control and monitor the use of antibiotics in the community and agriculture.

Key words: Antibiotics resistance; Colistin; *Escherichia coli*; Extended-spectrum β-lactamase; *mcr-1*; Poultry

Antibiotic resistance (AR) is a growing public health concern worldwide, and it is a top health challenge in the 21st century *(23, 30)*. Frequent and uncontrolled use of antibiotics in humans and animals is the primary driving force for the development and dissemination of antibiotic-resistant microorganisms *(25)*. There are remarkable differences in the use of antibiotics in humans and animals. Antibiotics are used in humans to treat individual patients suffering from infection, while they are widely used in food animals for therapeutic and prophylactic purposes, as well as growth promotion. Although the use of antibiotics as growth promoters has been banned in the European Union since 2006, this practice is still adopted in several countries: penicillin and tetracycline in the United States and chloramphenicol and fosfomycin in East and Southeast Asia *(14)*. Accordingly, multidrug-resistant (MDR) bacteria have been isolated from both humans and animals worldwide *(17, 29)*. Additionally, resistance to colistin, which is a last-resort antibiotic for treatment of MDR bacteria, has been reported on several occasions involving mainly chromosomal mutations. Plasmid-mediated colistin resistance through the *mcr-1* gene in *Enterobacteriaceae* was reported in the People's Republic of China for the first time in November 2015 *(16, 21)*. Strains carrying the *mcr-1* gene, especially *Escherichia coli,* have been isolated worldwide from humans *(4, 5, 15, 21, 27, 28),* food-producing animals *(12, 16, 19, 22, 27),* and the environment *(37)*. Recently, another plasmid carrying the colistin resistance gene (*mcr-2*) was detected in *E. coli* isolates from pigs in Belgium *(35)*. Further, a strain of bacteria resistant to all known antibiotics (pandrug-resistant organism) was reported for the first time in United States in 2016. These findings emphasize the urgent need for a coordinated global action to limit the dissemination of such bacteria though effective and sustainable stewardship programs *(9)*.

The prevalence of AR is rapidly increasing in humans in Qatar; thus, there are regular monitoring programs to survey for AR bacteria in hospitals and communities

* Author for correspondence. Tel: 00974-44036819; Fax: +974 4403 4801; E-mail: hyassine@qu.edu.qa.

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(annual antibiogram; A. Deshmukh, Hamad Medical Corporation, Doha, Qatar, personal communication). However, no work has been done to evaluate the level of AR in the veterinary sector. This has been cited as a deficiency by the World Health Organization "Joint External Evaluation of Qatar: Mission Report" *(34),* and recommendations were made to enhance and support an active antimicrobial resistance program in animals. Furthermore, the report mandated a quick response to develop and implement a stewardship program for antibiotic use in agriculture, as well as humans.

MATERIALS AND METHODS

Sample collection. A total of 172 cloacal swabs were collected randomly from two poultry farms and one live bird market in Qatar during a period of 5 months between the beginning of September 2016 and the end of January 2017. Individual swabs were collected from broiler chickens ranging in age from 32 to 40 days. All samples were collected under supervision of the Ministry of Public Health and Ministry of Municipality and Environment and were subsequently transferred to Qatar University, where they were kept at –20°C until further analysis within 15 days of collection.

***E. coli* isolation and identification.** Fecal samples ($n = 172$) were streaked by using sterile cotton-tipped swabs onto a selective medium CHROMagar E. coli plates (BD–Medysinal FZCO, Dubai, UAE) and incubated at 37°C for 18 to 24 h. Single typical *E. coli* colonies (green color with a smooth surface) were randomly selected and subsequently streaked onto blood agar plates and then incubated at 37°C for 18 to 24 h to obtain pure single colonies. For further confirmation, colonies were transferred onto MacConkey agar plates (BD-Medysinal FZCO) and then blood agar plates (BD-Medysinal FZCO), followed by an indole spot test (Remel, Thermo Fisher Scientific, Lenexa, KS) for lactose fermenter isolates. In total, 90 *E. coli* were isolated: 27 from farm 1, 33 from farm 2, and 30 from the live bird market. Isolates were then transferred to cryovial tubes (Technical Service Consultant, Lancashire, UK) and stored at –80°C until further analysis.

Antibiotic susceptibility testing. An antibiotic susceptibility test was performed by using the standard E-test strip technique in accordance with recommendations of the Clinical and Laboratory Standards Institute (CLSI) *(8). E. coli* isolates were recovered on blood agar (BD-Medysinal FZCO), and single colonies were suspended in 0.85% saline (BD-Medysinal FZCO) to achieve an inoculum equivalent to 0.5 McFarland standard as measured by DensiCHEK Plus (bioMérieux, Marcy l'Etoile, France). Suspensions were swabbed on Mueller-Hinton agar plates (BD-Medysinal FZCO). Antibiotic susceptibility test strips (E-test strip, Liofilchem, Roseto degli Abruzzi, Italy) were applied to the agar surface with sterile forceps, and plates were incubated at 37°C for 18 to 24 h. The zone of inhibition was examined to determine MICs that were interpreted according to the CLSI guidelines *(8). E. coli* strains ATCC 25922 and ATCC 35218 were used as controls. The 16 antibiotics used to screen the antibiotic susceptibility of *E. coli* are summarized in Table 1. As we acknowledge the pitfall of underestimating colistin resistance by an E-test, we included all isolates with MICs of 1 μg/mL (instead of 2 μg/mL; Table 1) in our confirmation tests by the agar dilution method *(6)* and PCR by using specific primers, as described in the following.

Double-disc synergy test. All isolates that showed resistance to third-generation cephalosporins (ceftriaxone) with a MIC ≥ 4 with E-test strips were further tested for extended-spectrum β-lactamase (ESBL) production by using the double-disc synergy test. The experiment was performed as previously described *(7)* by using the disc of β-lactamase inhibitor amoxicillin-clavulanate (20 and 10 μg; BD Sensi-Disc) along with cephalosporins ceftriaxone (30 μg; BD Sensi-Disc) and ceftazidime (30 μg; BD Sensi-Disc), and the cephamycin cefoxitin (30 μg; BD Sensi-Disc). Briefly, *E. coli* isolates were plated on a Mueller-Hinton agar plate (BD-Medysinal FZCO) as recommended by the CLSI *(7)* and a disc containing amoxicillin-clavulanate (20 and 10 μg) was placed in the center of the plate. Discs of cephalosporins were placed 15 mm apart from the edge of amoxicillin-clavulanate, and the cefoxitin disc was placed in any available space remaining on the plate that was then incubated at 37°C for 18 to 24 h *(8, 13).* Any augmentation or increase in the zone (by 5 mm) toward the disc of amoxicillin-clavulanate together with susceptibility to cefoxitin was considered positive for ESBL production. *E. coli* ATCC 51446 and ATCC 25922 were used as a control strain for a positive and negative ESBL production, respectively.

DNA extraction and PCR. Phenotypic colistin-resistant isolates that used the E-test were further evaluated for the presence of the *mcr-1* gene by targeting 114 base pair fragment (nucleotides 302 to 416) of the C2-007R phosphoethanolamine–lipid A transferase gene (*mcr-1*; accession no. KY01359700) by using standard PCR. DNA was extracted from colistin-resistant *E. coli* cultures by using a QIAamp UCP Pathogen Mini Kit following the manufacturer's instructions (Qiagen, Düsseldorf, Germany). Extracted DNA was subjected to PCR by using previously published primers *(33)*: MCR1_22697 F1 5′-cacttatggcacggtctatga-3′ and MCR1_22810 R1 5′-cccaaaccaatgatacgcat-3′ (Integrated DNA Technologies, Coralville, IA). The PCR mixture was made in a volume of 50 μL containing 0.5 μM of each primer, 3% dimethyl sulfoxide, 50 ng of DNA, 1× master mix (Phusion High Fidelity PCR Master Mix with HF Buffer, New England Biolabs, Hertfordshire, UK), and diethyl pyrocarbonate with H_2O up to 50 μL. The reaction was amplified in GeneAmp PCR System 9700 Thermocycler under the following conditions: initial denaturation at 96°C for 30 s; 32 cycles consisting of denaturation at 96°C for 10 s, annealing at 55°C for 30 s, and extension for 30 s at 72°C; and a final extension cycle at 72°C for 7 min. DNA was also screened for the presence of the *mcr-2* gene by using the previously described protocol and primers (MCR2-F 5′ tgttgcttgtgccgattgga 3′ and MCR2-R 5′ agatggtattgttggttgctg 3′) *(35)*. Amplified products were subjected to electrophoresis in 1.2% agarose (Agarose LE, Ambion, Paisley, UK), stained with ethidium bromide (Promega, Madison, WI) and visualized by using Bio-Rad gel doc system (Bio-Rad, Gel Doc XR System 170-8170, Quebec, Canada).

RESULTS

Of the 172 chicken cloacal samples collected from three different locations in Qatar, commensal *E. coli* were isolated from 90 samples, with a recovery rate of 52%. Positive samples were screened for resistance patterns against 16 clinically relevant antibiotics (Table 1), and 90% of the isolates were resistant to at least one antibiotic (Table 2). The highest percentage of resistance was recorded against ampicillin (72.2%; $n = 65$), followed by trimethoprim-sulfamethoxazole (63.3%; $n = 57$), ciprofloxacin (40%; $n = 36$), cephalothin and colistin (15.6%; $n = 14$ each), and then cefuroxime (4.4%; $n = 4$), fosfomycin (3.3%; $n = 3$),

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TABLE 1. *MIC range for 16 antibiotics and interpretation of the results*

Antibiotic	MIC (μg/mL)	MIC interpretive standard (μg/mL)[a]		
		S	I	R
Colistin[b]	0.016–256	≤2		>2
Piperacillin-tazobactam	0.016–256	≤6/4	32/4–64/4	≥128/4
Fosfomycin	0.064–1024	≤64	128	≥256
Ciprofloxacin	0.002–32	≤0.006	0.12–0.5	≥1
Nitrofurantoin	0.032–512	≤32	64	≥128
Amikacin	0.016–256	≤16	32	≥64
Ampicillin	0.016–256	≤8	16	≥32
Cephalothin	0.016–256	≤8	16	≥32
Cefuroxime	0.016–256	≤8	16	≥32
Ceftriaxone	0.016–256	≤1	2	≥4
Cefepime	0.016–256	≤2		≥16
Amoxcicillin–clavulanic acid	0.016–256	≤8/4	16/8	≥32/16
Ertapenem	0.002–32	≤0.5	1	≥2
Meropenem	0.002–32	≤1	2	≥4
Trimethoprim-sulfamethoxazole	0.002–32	≤2/38		≥4
Tigecycline[b]	0.016–256	≤1	2	>2

[a] S, sensitive; I, intermediate; R, resistant.
[b] No CLSI interpretive criteria are available; therefore, provisional breakpoints by the European Committee on Antimicrobial Susceptibility Testing *(11)* breakpoint tables were used.

ceftriaxone (2.2%; $n = 2$), and cefepime (1.1%; $n = 1$). Also, 45 (50%) of 90 isolates exhibited intermediate resistance to cephalothin (Fig. 1). Two isolates (2.2%) were ESBL producers with resistance to ampicillin, cephalothin, cefuroxime, and ceftriaxone, and another isolate exhibited resistance to ciprofloxacin, ampicillin, cefuroxime, cephalothin, ceftriaxone, cefepime, trimethoprim-sulfamethoxazole, as demonstrated by an E-test (Table 2) and confirmed with the double-disc synergy test. Further, 33% ($n = 30$) of the isolates were MDR to three classes of antibiotics (Table 2). Six isolates exhibited intermediate resistance to ciprofloxacin, with MICs ranging from 0.12 and 0.5 μg/mL. Resistance to colistin was detected in 14 isolates (15.6%), and molecular confirmation was done by PCR (Fig. 2), followed by sequence analysis of the *mcr-1* gene fragment. Sequence analysis of the PCR products showed 98% similarity to phosphoethanolamine–lipid A transferase (*mcr-1*) gene of *E. coli* strain C2-007R (sequence accession no. KY01359700; nucleotide sequence 302 to 363; data not shown). The *mcr-2* gene was not detected in any of the colistin-resistant isolates. No resistance was observed against five of the antibiotics, namely, amikacin, meropenem, ertapenem, tigecycline, and piperacillin-tazobactam.

DISCUSSION

To our knowledge, we report here for the first time about the *mcr-1* colistin resistance gene in *E. coli* from food-producing animals in the Middle East. Additionally, our results indicates the emergence of MDR *E. coli,* including ESBL-producing bacteria.

TABLE 2. *Combined resistance profile determined by an E-test of commensal* E. coli *isolated from broiler chicken fecal samples (*n = *90)*[a]

Resistant phenotype	Resistance (%)
No resistance	3.3
Intermediate[b]	6.7
Resistant to only one antibiotic	15.6
Ampicillin, trimethoprim-sulfamethoxazole	15.6
Ampicillin, colistin	2.2
Ampicillin, cephalothin	1.1
Ciprofloxacin, trimethoprim-sulfamethoxazole	3.3
Ampicillin, ciprofloxacin	8.9
Ciprofloxacin, ampicillin, cephalothin	1.1
Colistin, ampicillin, trimethoprim-sulfamethoxazole[c]	7.8
Ampicillin, cephalothin, trimethoprim-sulfamethoxazole	4.4
Ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole[c]	16.7
Trimethoprim-sulfamethoxazole, colistin, ciprofloxacin[c]	1.1
Fosfomycin, ampicillin, trimethoprim-sulfamethoxazole[c]	2.2
Ampicillin, cephalothin, cefouroxime	1.1
Colistin, cephalothin, ampicillin, trimethoprim-sulfamethoxazole[c]	1.1
Ciprofloxacin, ampicillin, fosfomycin, trimethoprim-sulfamethoxazole[c]	2.2
Ampicillin, ciprofloxacin, cephalothin, trimethoprim-sulfamethoxazole[c]	2.2
Ampicillin, cefuroxime, cephalothin, trimethoprim-sulfamethoxazole	1.1
Ampicillin, cephalothin, cefuroxime, ceftriaxone[d]	1.1
Ciprofloxacin, ampicillin, cefuroxime, cephalothin, ceftriaxone, cefepime, trimethoprim-sulfamethoxazole[d]	1.1

[a] The table shows the number of isolates resistant to one, two, or multiple specific antibiotics.
[b] High MIC near the cut-off point of resistance.
[c] MDR.
[d] ESBL.

Although antibiotics are widely used in the food animal sector, relatively little attention has been paid to how antibiotic use in farm animals contributes to the overall problem of AR, especially in developing countries *(4)*. In Qatar, recent reports from Hamad Medical Corporation indicate the rapid rise of antibiotics resistance among different bacterial species *(2, 3, 18)*. Nonetheless, there have been no studies describing the profile of AR in the animal sector, despite the World Health Organization recommendation in its "Joint External Evaluation of Qatar: Mission Report" *(34)*. Here, we report on resistance patterns of commensal *E. coli* isolated from broiler chickens as an indicator for the presence of resistance determinants in bacterial flora because it is considered the main element of surveillance programs in food-producing animals *(31, 32)*. We selected 16 antibiotics representing the antibiotics used in health care facilities in Qatar for treatment of *Enterobacteriaceae* infections (A. Deshmukh, personal communication). Nonetheless, it is difficult to compare the profile of AR

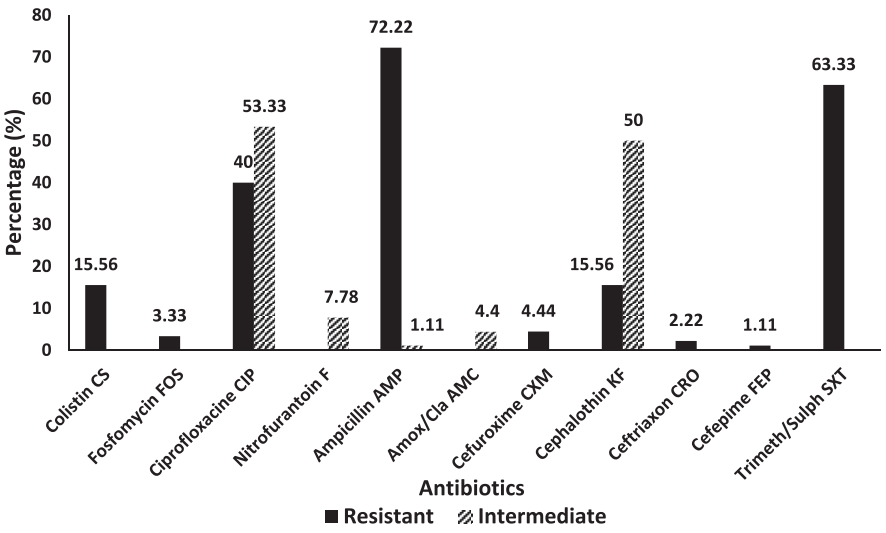

FIGURE 1. *AR profile of* E. coli *isolates (*n *= 90) obtained from broiler chickens. Ninety commensal* E. coli *were isolated from 172 fecal samples and were tested for antibiotics resistance against 16 clinically relevant antibiotics by using an E-test. The figure depicts the percentage of isolates with resistance (black bars) or intermediate resistance (striped bars) to 11 antibiotics.*

between humans and animals in Qatar for several reasons: (i) the majority of the Qatari population (>80%) are expatriates that are frequent travelers and many of them do not reside in the country for a long period; (ii) there is no stewardship program to monitor and control the use of antibiotics in animals, and thus, there is a lack of information about antibiotic use in animal sector; (iii) most food animals in Qatar are imported from abroad; and (iv) there are no previous reports that describe the patterns of AR in animals sector. This is, therefore, a preliminary study to establish baseline data aimed at assessing the profile of AR in the food animal sector in Qatar.

The low recovery rate of commensal *E. coli* from fecal samples (~52%) was the first alarming evidence about the detrimental impact of antibiotics on the commensal bacteria present in the gut of food-producing animals. This collateral damage caused by antibiotics and its impact on animal health has not been thoroughly studied and requires further investigation.

We report here a high resistance rate to antibiotics among 90 *E. coli* isolates from three different locations in Qatar, with around 90% being resistant to at least one antibiotic. The highest rate of resistance was observed with ampicillin (72.2%), followed by trimethoprim-sulfamethoxazole (63.3%), and ciprofloxacin (40%). These outcomes correlate with recent findings of Nguyen et al. *(26)* who reported a high prevalence of resistance to ampicillin (97.8%), ciprofloxacin (73.3%), and colistin (22.2%) among *E. coli* isolated from chickens in southern Vietnam. Of greatest concern is that we report for what we think is the first time colistin resistance (15.6% of the isolates) in food animals in the Middle East and North Africa where it is used as a last resort for antibiotic treatment in humans. This resistance was mediated solely by the *mcr-1* gene, as confirmed by PCR and sequence analysis. Further, we detected ESBL-producing bacteria in about 2.2% of the samples, and a similar percentage was MDR, thus raising a huge concern about antibiotic misuse in agriculture settings in Qatar.

This study confirms other reports about the alarming spread of plasmid-mediated resistance to colistin through the *mcr-1* gene *(12, 19, 22, 26, 36)*. Interestingly, almost all

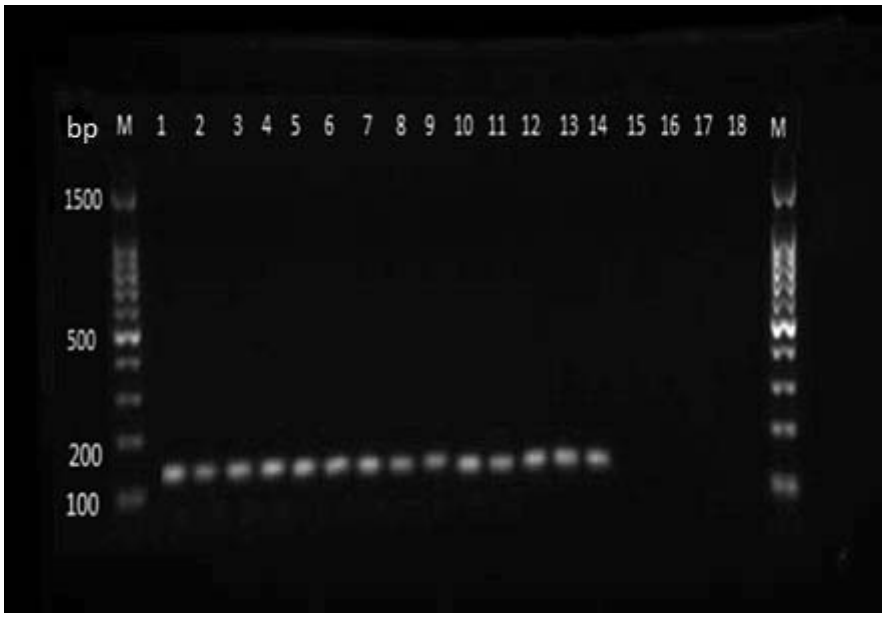

FIGURE 2. *The* mcr-1 *gene detection in colistin-resistant* E. coli *isolated from chickens. Phenotypic colistin-resistant isolates (*n *= 14) were tested for the presence of* mcr-1 *and* mcr-2 *genes by using standard PCR. Lanes 1 to 14, PCR products of* mcr-1 *gene fragment from the isolated stains (114 bp); lane 15, ATCC 25922* E. coli *(sensitive strain); lanes 16 to 18, negative isolated* E. coli *samples. M, molecular size (weight) standard marker; bp, base pairs.*

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colistin-resistant isolates (13 of 14) were detected in one farm that used colistin as a prophylaxis and the occurrence of resistance to colistin in this particular farm reached 13 (48%) of 27 isolates. Although we do not have enough information about the course and duration of colistin use in this farm, our results indicate that such resistance might easily develop and transmit to local settings. There was no significant variation in terms of an AR profile among isolates from three locations, except for colistin. In addition to colistin, this farm uses amoxicillin and enrofloxacin, to which resistance has been detected in 17 (63%) of 27 and 27 (100%) of 27 samples, respectively, indicating a correlation between antibiotic exposure and development of resistance. It was not feasible to obtain information about antibiotic usage from the live bird market, and owners of the second farm refused to disclose information about their practice. Nonetheless, our results implicate the high use amoxicillin and enrofloxacin in Qatar.

Several reports have linked the introduction of certain antibiotics to agriculture and the development of resistance. For example, the introduction of enrofloxacin in veterinary medicine has been related to the emergence of fluoroquinolone resistance among *Campylobacter* isolates from broilers, as well as humans shortly thereafter *(1)*. The same group *(1)* also reported that resistance to fluoroquinolones in human and animal populations remained rare in countries that had not used fluoroquinolones in food animals. Tetracycline, penicillin, and erythromycin are examples of important antibiotics in human medicine that are being exploited extensively in livestock production, and similar resistance profiles have been detected in animal and human isolates on several occasions around the globe *(20, 24)*.

The detection of borderline intermediate resistance to cephalothin and ciprofloxacin in about 50 and 53.3% of the isolates (Fig. 1), respectively, is another warning sign for continuous AR development in livestock in Qatar. The continuous use of these antibiotics will certainly result in the development of fully resistant bacteria in short period of time. These findings support the conclusion that agricultural antibiotics are associated with the selection and proliferation of antibiotic-resistant strains that can be disseminated to humans and the environment.

On the other hand, the occurrence of ESBL-producing bacteria was relatively low (2.2%) compared with other antibiotics. This could be partially explained by the rare use of third-generation cephalosporin in the veterinary sector. Not surprisingly, there was no resistance exhibited to amikacin, meropenem, ertapenem, tigecycline, and piperacillin-tazobactam, as we confirmed that these classes of antibiotics are not used, at least in the farm that we could obtain its antibiotic use information. In fact, these antibiotics are only used intravenously for management of patients with serious infection in health care facilities and are not used in the poultry industry worldwide.

Our data provide strong evidence to support curtailing antibiotic use in agriculture in Qatar. Evidently, the reduction of antibiotic use in agricultural settings has been associated with the reduction of resistance in farm animals. In Canada, a voluntary withdrawal of the extra-label use of ceftiofur in Québec chicken hatcheries resulted in a significant decrease in the ceftiofur resistance of *Salmonella* Heidelberg isolates from retail chicken and humans, as well as in *E. coli* from retail chickens *(10)*.

In summary, the level of AR we recorded in three different locations in Qatar is alarming, and it is a sign of the improper use of antibiotics in the veterinary sector. The continuous development and dissemination of such bacteria, which could spread to humans through improper hygiene practices and consumption of contaminated food, could have a negative impact on the management of human infection with pathogenic bacteria that might emerge. Generated data from this study may contribute toward the development of a stewardship program in the veterinary sector in Qatar. It will also serve as the baseline for future larger studies that evaluate AR of different bacterial species in various food animals for a prolonged period.

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