

Original Article

## Antibiotic resistance profile of commensal *Escherichia coli* isolated from healthy sheep in Qatar

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### Abstract

**Introduction:** The uncontrolled antibiotics use in livestock is a leading factor for the emergence and spread of resistant bacteria from food animals to humans through the food chain. This study aims at evaluating the magnitude of the antimicrobial resistance (AMR) in food animals, acknowledging the lack of information on the prevalence of resistance in the veterinary field in Qatar.

**Methodology:** Rectal samples were collected from 171 sheep across three localities in Qatar between December 2016 and July 2017. These rectal samples were suspended in Phosphate buffer solution (PBS). Then streaked onto a selective CHROMagar *E. coli* medium plates and incubated at 37°C for 18 to 24 h. Isolated *E. coli* were tested for antibiotic susceptibility against 16 clinically- relevant antibiotics using the E-test method. Statistical analyses were performed using SPSS statistics 24.

**Results:** *E. coli* was isolated from 144 samples (84.2%), of which 90% were resistant to at least one antibiotic and 44% were multi-drug resistant (MDR). The highest resistance was against ciprofloxacin 69.4% (100), followed by nitrofurantoin 47.2% (68), trimethoprim/sulfamethoxazole 45.8% (66), cephalothin 43% (62) and amoxicillin/clavulanic acid 18% (26). Low resistance was reported to fosfomycin, amikacin and tigecycline 1.4% (2), 0.7% (1), and 0.7% (1), respectively.

**Conclusions:** We reported high MDR *E. coli* in rectal swabs of sheep in Qatar. Such resistant bacteria can potentially be transmitted to humans, resulting in public health concerns. This requires a quick response to develop and implement a stewardship program for the monitoring of antibiotic use in the veterinary in Qatar.

**Key words:** Antibiotic resistance; Qatar; sheep; *E. coli*.

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### Introduction

Antibiotics are crucial remedies for the treatment of infectious diseases in humans and food-producing animals, however, the increasing resistance to antibiotics, which spans all classes, becomes a significant worldwide concern [1]. The uncontrolled use of antibiotics in livestock for treatment, disease prevention or growth promotion, is a leading factor for the emergence and spread of resistant bacteria [2-5]. Antibiotic resistant bacteria can spread from animals to humans through direct contact or consumption of animal products. This transmission, coupled with the increased prevalence of antimicrobial resistance (AMR) in the medical sector, pose a significant threat to public health [6-8]. It is estimated that almost 75 percent of all antibiotics given to animals are not used for treating infection but mainly for growth promotion or prophylaxis purposes [9]. Importantly, many of the

antibiotics used in animals are identical to- or closely resemble drugs prescribed to humans [4].

Limited data is available about antibiotic use and patterns of antibiotic resistant bacteria in animal sector in Qatar. Hence recommendations were made by World Health Organization (WHO) “Joint External Evaluation of Qatar: Mission Report” [10] to boost and support an active antimicrobial resistance surveillance program in animals. Besides, the report mandated a quick response to develop and implement a stewardship program for antibiotic use in agriculture, as well as humans.

Recently, we published the first report about AMR of commensal *E. coli* in broiler chicken in Qatar. Our data demonstrated the dissemination of multidrug resistant bacteria [11], including resistance to colistin. We therefore conducted this study targeting other animal species (sheep), to establish a base line data

among food animals for veterinary surveillance national program.

### Methodology

#### Sample collection

A total of 171 rectal swabs were collected randomly from sheep across three localities in Qatar (46 samples from one farm, Wakra municipality, 61 from courtyard farm that includes 7 small farms, Khor municipality, and 54 from the slaughterhouse, Doha municipality) during a period of 8 months between the beginning of December 2016 and the end of July 2017. 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

*E. coli* was isolated and identified as previously described [11]. Briefly, 1gm of sheep fecal sample was suspended in 3 ml of Phosphate buffer solution (PBS) and vortexed vigorously. Then 20 µL of each samples (n = 171) was streaked onto a selective CHROMagar *E. coli* medium plates (BD–Medysinal FZCO, Dubai,

UAE) which were then incubated at 37°C for 18 to 24 hours. Typical *E. coli* colonies (green color with a smooth surface) were randomly selected and subsequently streaked onto blood agar plates (BD–Medysinal FZCO, Dubai, UAE), incubated under similar conditions 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), before running indole spot test (Remel, Thermo Fisher Scientific, Lenexa, KS) for lactose fermenter isolates and biochemical reactions using Crystal™ Enteric/nonfermenter id KIT, BD. Results were interpreted by means of Biomic V3 (Giles scientific, Santa Barbara, USA). Isolates were then transferred to Cryovial tubes (Technical Service Consultant, Lancashire, UK), and stored at -80°C until further analysis.

#### Antibiotic susceptibility testing

Antibiotic susceptibility test was performed as previously described [11] using the standard E-test strip technique in accordance with recommendations of the Clinical and Laboratory Standards Institute [12]. Briefly, *E. coli* isolates were recovered on blood agar (BD–Medysinal FZCO, Dubai, UAE), and single

**Table 1.** Minimum Inhibitory Concentration range for 16 antibiotics and interpretation of the results.

Antibiotic	Abbreviation	MIC range tested (µg/mL)	MIC Interpretive Standard (µg/mL)		
			S	I	R
<b>Penicillin and Penicillin β-lactamase inhibitor combination</b>					
Ampicillin	AM	0.016-256	≤ 8	16	≥ 32
Amoxicillin/Clavulanic acid	AMC	0.016-256	≤ 8/4	16/8	≥ 32/16
Piperacillin/Tazobactam	TZP	0.016-256	≤ 6/4	32/4-64/4	≥ 128/4
<b>Aminoglycosides</b>					
Amikacin	AK	0.016-256	≤ 16	32	≥ 64
<b>Quinolone</b>					
Ciprofloxacin	CIP	0.002-32	≤ 1	2	≥ 4
<b>Folate pathway inhibitors</b>					
Trimethoprim/Sulfamethoxazole	SXT	0.002-32	≤ 2/38	-	≥ 4
<b>Cephalosporin</b>					
Cephalothin	KF	0.016-256	≤ 8	16	≥ 32
Cefuroxime	CXM	0.016-256	≤ 8	16	≥ 32
Ceftriaxone	TX	0.016-256	≤ 1	2	≥ 4
Cefepime	FEP	0.016-256	≤ 2	-	≥ 16
<b>Polymyxin</b>					
Colistin*	CS	0.016-256	≤ 2	-	>2
Fosfomycin	FOS	0.064-1024	≤ 64	128	≥ 256
<b>Glycylcycline</b>					
Tigecycline*	TGC	0.016-256	≤ 1	2	>2
Nitrofurantoin	F	0.032-512	≤ 32	64	≥ 128
<b>Carbapenems</b>					
Ertapenem	ETP	0.002-32	≤ 0.5	1	≥ 2
Meropenem	MRP	0.002-32	≤ 1	2	≥ 4

\*No CLSI interpretive criteria are available; therefore, provisional breakpoints by the European committee on Antimicrobial Susceptibility Testing (EUCAST 2017) breakpoint tables were consulted.

colonies were suspended in 0.85% saline (BD-Medysinal FZCO, Dubai, UAE) 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, Dubai, UAE), then 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 hours. The zone of inhibition was examined to determine minimum inhibitory concentrations (MICs) that were interpreted according to the 2017 CLSI guidelines. *E. coli* strains ATCC 25922 (Sensitive) and ATCC 35218, a beta-lactamase-producing strain were used as quality controls (QC). Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI guidelines [12]. The 16 clinically relevant antibiotics used to screen the antibiotic susceptibility of *E. coli* are summarized in Table 1.

**Data Analysis**

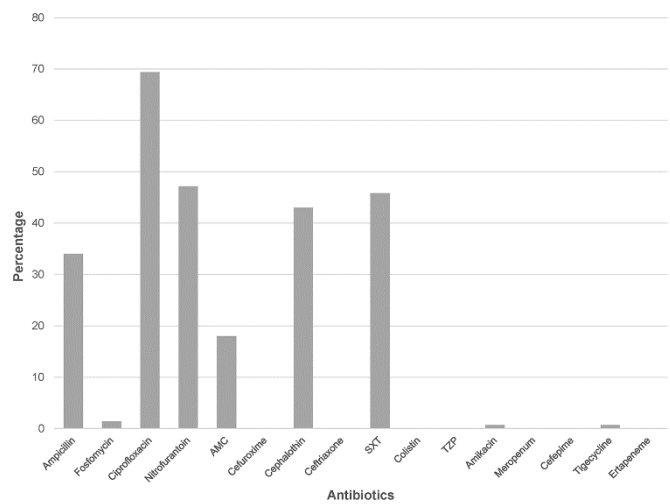
Data was introduced into Microsoft Excel 2010 (Microsoft Corporation, New York, USA) to generate figures and run initial analysis. Further statistical analyses were done using SPSS statistics version 24 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA). The difference in the percentage of individual antibiotic resistance between the samples of the three localities was calculated using Pearson chi-square test. Probability value (P-value) less than 0.05 was considered statistically significant.

**Results**

A total of 144, 84.2% (farm = 37; courtyard = 61 and slaughter house = 46) *E. coli* isolates were recovered from 171 samples, each isolate represented one sample. The prevalence of resistance against 16 antibiotics is shown in Figure 1. Overall, we reported resistance to nine antibiotics, with 129 isolates (90%) being resistant to at least one antibiotic. The prevalence of resistance varied according to the type of antibiotic examined and location. The most frequent resistance was observed to ciprofloxacin 69.4% (n = 100), followed by nitrofurantoin 47.2% (n = 68), trimethoprim/sulfamethoxazole 45.8% (n = 66), cephalothin 43% (n = 62), ampicillin 34% and amoxicillin/clavulanic acid 18% (n = 26). Low resistance was reported to fosfomycin, amikacin and tigecycline at 1.4% (n = 2), 0.7% (n = 1), and 0.7% (n = 1) prevalence, respectively. Resistance patterns,

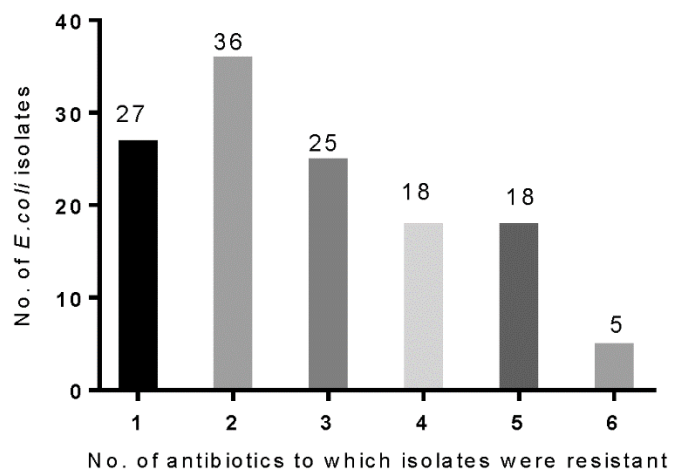
including multidrug resistance (MDR), varied considerably among different isolate. While only 3.5% (n = 5) of the isolates were resistant to a maximum of six antibiotics, 12.5% (n = 18) were resistant to five and four antibiotics, 17.4 % (n = 25) were resistant to three antibiotics, 25% (n = 36) were resistant to two antibiotics, and 18.8% (n = 27) were resistant to one antibiotic (Figure 2). Forty-four percent (n = 64) of the isolates were Multi drug resistant (MDR); i.e., resistant to at least one agent of three or more antimicrobial classes.

**Figure 1.** Antimicrobial percentage resistance profile *E. coli* (144) bacteria isolated from 171 rectal samples of sheep.



Isolates were tested for antibiotics resistance to 16 clinically relevant antibiotics using E-test. The figure depicts the percentage of isolates with resistance to each of the antibiotics. TZP: piperacillin/ tazobactam; SXT: trimethoprim/sulfamethoxazole; AMC: Amoxicillin/clavulanic acid.

**Figure 2.** Frequency bar chart exemplifying the distribution of phenotypic antibiotic resistance to up to six antibiotics among *E. coli* isolates (n = 144) from sheep’s rectal fecal samples in Qatar.



**Table 2.** Phenotypic resistant profiles of *E. coli* isolates from sheep rectal swabs (n = 144).

Resistance phenotype	frequency	Percentage (%)
*AM; AMC; KF; SXT	1	0.7
*AM; FOS; F; AMC; KF; SXT	1	0.7
F; KF	1	0.7
*AM; CIP; AMC; KF; SXT	2	1.4
*AM; CIP; SXT	2	1.4
*F; KF; SXT	2	1.4
*AM; F; KF	2	1.4
AM; KF	2	1.4
AM; KF	2	1.4
*AM; KF; AMC; SXT	3	2.0
*CIP; F; SXT	3	2.0
*AM; CIP; F; AMC; KF; SXT	4	2.8
*AM; CIP; F; KF; SXT	4	2.8
*AM; CIP; F; SXT	4	2.8
F; SXT	4	2.8
*CIP; F; KF; SXT	5	3.5
*CIP; KF; SXT	6	4.2
CIP; KF	6	4.2
CIP; SXT	7	4.9
*CIP; F; SXT	9	6.3
*AMC; CIP; F; AMC; SXT	10	7.0
CIP; F	13	9.0
No resistance	15	10.4
Resistant to only one antibiotic	27	18.8
*Ampicillin; Nitrofurantoin; Amoxicillin-Clavulanic acid; Cephalothin	1	0.7
*AMC; F; AMC; KF; SXT	1	0.7
AM; AMC; SXT	1	0.7
*AM; FOS; CIP; F; AMC; SXT	1	0.7
*AM; CIP; KF; SXT; AK	1	0.7
*AM; CIP; KF; SXT	1	0.7
*AM; F; KF; TGC	1	0.7
AMC; CIP; AMC	1	0.7

\*MDR multidrug -resistant. MDR (44.4%, n = 64); AM: Ampicillin; FOS: Fosfomycin; CIP: Ciprofloxacin; F: Nitrofurantoin; AMC: Amoxicillin/Clavulanic acid; KF: Cephalothin; SXT: Trimethoprim/Sulfamethoxazole; AK: Amikacin; TGC: Tigecycline.

**Table 3.** Phenotypic profile of antibiotic resistant *E. coli* isolated from sheep rectal samples collected from three different localities.

Antibiotics	Fa	Courtyard farm	Slaughter house	Pearson Chi- square significance value
	*n = 37	*n = 61	*n = 46	
Percentage /no. of resistant isolates				
Ampicillin (AMP)	(10.8) 4	(56.7) 34	(23.4) 11	0.000
Fosfomycin (FOS)	(0) 0	(1.7) 1	(2.1) 1	0.422
Ciprofloxacin (CIP)	(51.4) 19	(75) 45	(76.6) 36	0.050
Nitrofurantoin (F)	(0) 0	(75) 45	(49) 23	0.000
Amoxicillin/Clavulanic (AMC)	(5.4) 2	(28.3) 17	(14.9) 7	0.014
Cephalothin (KF)	(37.8) 14	(56.7) 34	(29.8) 14	0.016
Trimethoprim/Sulfamethoxazole (SXT)	(35.1) 13	(68.3) 41	(25.5) 12	0.000
Amikacin	(0) 0	(1.7) 1	(0) 0	0.494
Tigecycline	(0) 0	(1.7) 1	(0) 0	0.494

\*n: Total number of isolates.

Distribution of MDR pattern is summarized in Table 2. The most frequently occurring MDR pattern was recorded to ampicillin; ciprofloxacin; nitrofurantoin; amoxicillin /clavulanic acid; trimethoprim /sulfamethoxazole in 7% of the isolates. Other MDR patterns were recorded between 0.7%, for a combination of ampicillin; fosfomycin; nitrofurantoin; amoxicillin-clavulanic acid or a combination of ampicillin; amoxicillin-clavulanic acid; cephalothin; trimethoprim /sulfamethoxazole, and up to 6.3% for a combination of ciprofloxacin; nitrofurantoin; trimethoprim /sulfamethoxazole. Frequency of resistance to the nine antibiotics in three localities were cross-tabulated using Chi-square test and results are summarized in Table 3. Six antibiotics were significantly different between localities (p < 0.05), namely, ampicillin, ciprofloxacin, nitrofurantoin, amoxicillin /clavulanic, cephalothin and trimethoprim /sulfamethoxazole. The highest resistance was significantly observed among isolates obtained from the courtyard farm compared to isolates from other sites, with exception of ciprofloxacin that showed insignificant difference (p > 0.05) between isolates collected from the courtyard and slaughterhouse.

Table 4 displayed cumulative MIC distribution for the 16 studied antibiotics against *E. coli*, expressed as the 50% MIC (MIC<sub>50</sub>), 90% MIC (MIC<sub>90</sub>). Overall,

carbapenemes (meropenem, ertapenem), tigacycline, ceftriaxon and cefepime with MIC<sub>50/90</sub> ≤ 0.75/ ≤ 0.75 µg/mL; and colistin with MIC<sub>50/90</sub> ≤ 0.75/1.5 µg/mL were most effective antibiotics tested against *E. coli* isolates with very low MIC<sub>50/90</sub>. Conversely, high MIC<sub>50/90</sub> distributions were recorded for ciprofloxacin, cephalothin, nitrofurantoin, trimethoprim /sulfamethoxazole and ampicillin with MICs <sub>50/90</sub> 8/32, 4/32, 32/256, ≤ 0.75/32 and 4/256 µg/mL respectively.

### Discussion

Misuse of antibiotics that lead to AMR is prevalent worldwide both in the veterinary and human sector [13,14]. Stewardship program to monitor and control the use of antibiotics have been implemented in the human medicine in Qatar starting from 2015 in all governmental health facilities. Although the Ministry of Public Health developed a draft of National Action Plan (NAP) to combat AMR both in human and veterinary sector [15], still, there are limited data on antibiotics use and resistance of bacteria in environment and veterinary section. Consequently, we piloted this study on AMR among sheep as a continuation of our preceding work on broiler chicken [11]. Sheep are considered the second source of meat in the nation [16]. This is the second study from our group that describes the phenotypic prevalence of AMR in commensal *E. coli*

**Table 4.** MIC distribution for the 16 antibiotics investigated against *E. coli* (n = 144) isolates from healthy sheep, Qatar.

ANTIBIOTI CS	No. (cumulative %) of isolates inhibited at an MIC (µg/mL)																	MIC 50	MIC 90		
	≤0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192			256	512
AM	3 (2.08)	3 (4.17)	11 (12.5)	16 (22.91)	21 (37.5)	19 (50.69)	15 (61.11)	7 (65.97)				10 (72.92)	0 (72.92)	0 (72.92)	0 (72.92)	0 (72.92)	0 (72.92)	39 (100)	NA	4	256
FOS	49 (34.03)	36 (59.03)	25 (76.39)	11 (84.03)	7 (88.89)	1 (93.5)	5 (94.44)	2 (95.83)	2 (95.83)	0 (95.83)	0 (95.83)	2 (97.22)	0 (97.22)	2 (98.61)				2 (100)	0 (100)	1	6
CIP	44 (30.6)	0 (30.6)	0 (30.6)	0 (30.6)	0 (30.6)	29 (50.7)	3 (52.7)	0 (52.7)	0 (52.7)	0 (52.7)	0 (52.7)	68 (100)	NA	NA	NA	NA	NA	NA	NA	4	32
F	0	0	0	0	0	0	1 (0.69)	8 (6.25)	14 (15.97)	18 (28.47)	17 (40.28)	18 (52.78)				53 (89.58)	0 (89.58)	2 (90.97)	13 (100)	32	256
AMC	6 (4.17)	5 (7.64)	13 (16.67)	26 (34.72)	36 (59.72)	18 (72.22)	11 (79.86)	3 (81.94)				0 (81.94)	0 (81.94)	0 (81.94)	0 (81.94)	0 (81.94)	0 (81.94)	26 (100)	NA	3	256
CXM	4 (2.78)	8 (8.33)	21 (22.92)	38 (49.31)	36 (74.31)	28 (93.75)	6 (97.91)	2 (99.31)				0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	3	4
KF	4 (2.78)	0 (2.78)	0 (2.78)	2 (4.17)	5 (7.64)	9 (13.89)	27 (32.64)	35 (56.94)				58 (97.22)	0 (97.22)	0 (97.22)	0 (97.22)	0 (97.22)	0 (97.22)	4 (100)	NA	8	32
TX	144 (100)	0 (100)				0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	≤ 0.75	≤ 0.75
SXT	78 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	0 (54.17)	66 (100)	NA	NA	NA	NA	NA	NA	NA	≤ 0.75	32
CS	75 (52.08)	38 (78.47)	21 (93.05)	10 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	≤ 0.75	1.5
TZP	47 (32.64)	48 (65.97)	31 (87.5)	11 (95.14)	7 (100)	0 (100)												0 (100)	0 (100)	1	2
AK	12 (8.33)	21 (22.92)	30 (43.75)	19 (56.94)	18 (69.44)	27 (88.19)	12 (96.53)	3 (98.61)	0 (98.61)	0 (98.61)	1 (99.31)		0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	1 (100)	NA	2	6
MRP	144 (100)	0 (100)				0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	≤ 0.75	≤ 0.75
FEP	144 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	NA	NA	NA	NA	NA	NA	≤ 0.75	≤ 0.75
TGC	143 (99.3)	0 (99.31)	0 (99.31)		0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	0 (99.31)	1 (100)	NA	≤ 0.75	≤ 0.75
ETP	144 (100)			0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	NA	NA	NA	NA	NA	NA	NA	≤ 0.75	≤ 0.75

White: Sensitive; Black: Intermediate; Grey: Resistant; MIC: Minimum Inhibitory Concentration; NA: not applicable; AM: Ampicillin; FOS: Fosfomycin; CIP: Ciprofloxacin; F: Nitrofurantoin; AMC: Amoxicillin/Clavulanic acid; CXM: Cefuroxime; KF: Cephalothin; TX: Ceftriaxone; SXT: Trimethoprim/Sulfamethoxazole; CS: Colistin; TZP: Piperacillin/Tazobactam AK: Amikacin; MRP: Meropenem; FEP: Cefepime; TGC: Tigecycline; ETP: Ertapenem. The figure depicts the cumulative percentage of isolates inhibited at different MICs.

among food animals, as an indicator for resistance pattern, in the veterinary sector. Our first notable observation in comparing the two studies was the higher recovery rate of commensal *E. coli* (84.2%; 144/172) in sheep compared to chicken, where we could only recover *E. coli* from 52% of the chicken samples. Further, we recorded resistance to colistin in 15.6% of chicken samples, compared to absence of this resistance among sheep. These results indicate differential use of antibiotics in both animals in Qatar, where chickens are known to receive antibiotics more often compared to other food animals for treatment, prophylaxis and growth promotion (personal communication). This excess use of antibiotics in chicken could explain their detrimental impact on the commensal bacteria, present in the gut of chicken which reflected by low recovery rate of *E. coli* from chicken compared to sheep. It is worth noting that both studies analyzed similar number of samples that were collected from same number of localities around the same time period. Nevertheless, we reported noteworthy high percentages of resistant *E. coli* reaching 90% to at least one antibiotic. The highest rate of resistance were reported to ciprofloxacin (69.4%), followed by nitrofurantoin (47.2%) then trimethoprim /sulfamethoxazole 45.8% and cephalothin (43%). This is slightly different from our previous study among broiler chicken, which illustrated high trimethoprim-sulfamethoxazole (63.3%), and ciprofloxacin (40%) resistance of isolated *E. coli*. In addition and of great importance, we reported remarkably high percentage (44.4%) of MDR, which was also high in broiler chicken, reaching 33% [11]. This might suggest high use of these antibiotics in food-producing animals. Since there are no reference studies for AMR among animals in Qatar, we compared our results to a recent study from Saudi Arabia in 2017 [17]. The study reported a high incidence of AMR among Shiga toxin-producing *E. coli* isolated from diarrheic sheep in Al-Madinah Al-Munawarah. The resistance involved different classes of antibiotics including Trimethoprim-Sulphamethoxazole (72.1%) and ampicillin (24.1%), which were investigated in our study as well and showed 45.4% and 34% resistant, respectively.

One of the conspicuous findings in our study is the worrying high resistance for fluoroquinolones (69.4%), which dictates a root-cause analysis and transparent review of antibiotics use in agriculture in Qatar. Further, the high percentage of AMR to ciprofloxacin in the three studied localities clearly demonstrate the high exposure of sheep to fluoroquinolones. However, it was not possible to obtain information on antibiotic

usage in the studied localities, as owners refused to reveal information about their practice in rearing sheep. Historically, fluoroquinolones are approved only for treatment of certain infections in poultry in United States of America (e.g., *E. coli*) to control mortality [3]. In 2005, United States Food and Drug Administration (FDA) banned the use of fluoroquinolone due to high resistance to this class of antibiotics in *Campylobacter*. As a result of this ban, resistance to ciprofloxacin declined to 13.5% from 30% in 2010 [18]. Recently, WHO [19], listed fluoroquinolones among the critically important restricted antibiotics for the use in food-producing animals. Compliance with this WHO recommendation may require introduction of legislation to monitor fluoroquinolone use in animal sector to help in preserving the effectiveness of this antibiotic to treat infection in human.

Of greatest concern was the high percentage (44.4%) of MDR. This emerging MDR in livestock will facilitate the subsequent transfer of resistant genes and bacteria along the food chain to humans. In fact, we have also reported a high multidrug resistant *E. coli* in multiple food-chain studies, reaching 27% in healthy food handlers [20] and 33% in broiler chickens [11]. Despite the high MDR, we did not identify any extended-spectrum beta-lactamase (ESBL) producing bacteria. This is in agreement with our previous study among broiler chicken where only 2.2% were found to be ESBL producing *E. coli*. This could be explained by the rare use of third-generation cephalosporin in the veterinary sector in Qatar. Similarly, we observed no resistance to meropenem, ertapenem, piperacillin-tazobactam, third generation cephalosporines and hardly to amikacin and tigecycline, as these antibiotics are used parenteral for therapeutic purposes and not used as growth promotor. From our data on MIC distribution (Table 4), we can predict 'MIC creep' over a period of time or drift towards a high MIC values resulting in escalation of resistance among most common medically prescribed antibiotics namely, ciprofloxacin (MIC<sub>50/90</sub>, 8/32), cephalothin (MIC<sub>50/90</sub>, 4/32), nitrofurantoin (MIC<sub>50/90</sub>, 32/256) and ampicillin (MIC<sub>50/90</sub> 4/256).

Regardless of the limited study localities number (n = 3), we recorded a significantly higher resistance among Courtyard farm (p < 0.05) against most of the antibiotics, designating a possible higher exposure of sheep to antibiotics without supervision and monitoring from professional veterinarian.

The results of this study augmented our proposed serial studies to establish a baseline data on the level and profile of AMR across different niches by adopting

a ‘One Health’ approach. Following the characterization of antibiotic resistance in humans and different food-animal species, we plan to conduct a comprehensive sequencing analysis on selected strain to decipher the mechanisms of resistance transfer amongst various systems.

In addition to *E. coli*, multidrug resistance has also, been reported among other bacteria in Qatar community including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Neisseria gonorrhoeae* [21-24]. The spread of these MDR bacteria is of huge public health concern, as they are often significantly difficult and more expensive to treat [25].

In summary, recent data from our groups and others indicate an increasing problem of antibiotic resistance among humans and animals in Qatar, probably due to the indigenous and exogenous uncontrolled use of antibiotics. Accordingly, national programs that contribute towards controlling antibiotic use in livestock in Qatar is needed.

## Conclusions

In conclusion, we reported high individual and MDR *E. coli* in rectal swaps of sheep in Qatar. Sheep that are apparently healthy could harbor and shed AMR enteric *E. coli*, resulting in a significant public health concern through the transmission to humans along the food chain. Despite the potential influence encountered by the spread of the resistant bacteria along the food chain or environment, this is the first pilot study to screen the AMR *E. coli* among apparently healthy sheep. More research studies are needed to longitudinally follow AMR commensal and pathogenic bacteria in sheep and other food animals and the relationship with unique and common strains found in hospital settings.

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## Authors’ contributions

NOE & HMY, designed the study. NOE & SH, performed the experimental work. MHM, OK& HA, management of sample collection. NOE, drafted the manuscript. HMY, review and editing of the manuscript. AA& EA, project administration. All authors read and approved the final manuscript.

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