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ANTIMICROBIAL RESISTANCE OF COMMENSAL ESCHERICHIA COLI

ISOLATED FROM FOOD ANIMALS IN QATAR

BY

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ABSTRACT

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Title: ANTIMICROBIAL RESISTANCE IN COMMENSAL ESCHERICHIA COLI ISOLATED FROM FOOD ANIMALS IN QATAR

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Background: The dissemination of antimicrobial resistance (AMR) bacteria is associated with the inappropriate use of antibiotics in both humans and animals. In particular, the use of antibiotics for prophylactic and growth promotion purposes in food-producing animals, in addition to animal-group medication for prolonged times, have rendered many of the antibiotics ineffective, returning us to the old times when a simple infection could be deadly. Objectives: This study aims at evaluating the incidence of AMR in Qatar under the one health approach; i.e., human-animal-environmental interface. Specifically, we aim to characterize the phenotypic and genotypic AMR pattern of 18 clinically relevant antibiotics in major food-producing animals. Ultimately, we aim to compare our findings to the data released by the monitoring programs in Hamad Medical Corporation (HMC) along with previously published reports from Qatar. Methods: Animal fecal samples from camels, cattle, and pigeons (100 samples from each species) were collected from slaughterhouses and farms distributed in different locations in Qatar. One gram of fecal sample was vigorously homogenized with 3 ml sterile Phosphate Buffered Saline (PBS), the suspension was streaked directly onto chromogenic Tryptone Bile X-glucuronide agar for E. coli detection. Single typical E. coli colonies (blue-green) were randomly selected and streaked onto MacConkey agar plates and then recovered on
nutrient agar plates for lactose fermentation testing by indole spot. The antibiotic susceptibility test was conducted using a Kirby-Bauer disk diffusion assay on Mueller-Hinton agar plates. Results: A total recovery rate of 88.7% (n=266) achieved from all samples. Overall, E. coli isolates had resistance to ten antibiotics in pigeon group, eight antibiotics in cattle group and only five antibiotics in camel group. Resistance to at least one antibiotic was recorded in 63 E. coli isolates (70.7%) from pigeons, 32 isolates (37.2%) from cattle and only in 19 isolates (20.8%) from camels. Multi-drug resistant (MDR) was highest in isolates from pigeons reaching 50% (n=44), followed by isolates from cattle (7%) and camels (2.2%). Highest resistance rate was observed against tetracycline, reaching a frequency of 64%, 27.9% and 15% of isolates from pigeons, cattle and camels, respectively (p <0.0001). Moreover, trimethoprim/sulfamethoxazole (an antibiotic used to treat a variety of bacterial infections) resistance was present in 22.2% (n=59) of all E. coli isolates, with the highest prevalence in pigeons (49.4%), followed by 11.6% in cattle and 5% in camels. Interestingly, one E. coli isolate from pigeon showed resistance to colistin, a drug of last resort in human medicine against gram-negative bacterial infection.

Conclusions: We previously reported high multi-drug resistance of commensal E. coli in chickens, with significant resistance to colistin. We observed lower AMR profile in ruminants, presumably due to the different antibiotics used in the two animal industries. Nonetheless, the high resistance profile observed in pigeons (70.7%), including high multidrug resistance (50%) is alarming, as these animals could rapidly disseminate resistant bacteria to various locations. Interestingly, no ESBL producing E. coli reported. Nonetheless, continuous monitoring of AMR in livestock animals in Qatar is necessary toward introducing antimicrobial stewardship program and control of antibiotics usage in
the veterinary sector.
DEDICATION

I dedicated my dissertation to my dear husband; Zayed, and my beloved son; Mohammed and my little angel Maryam. Your belief in me has kept me motivated & inspired. For my kids, may you never stop learning for any reason.
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I would like to express my deep gratitude to my supervisor Dr. Hadi Mohamed Yassine, for his continuous support and valuable guidance throughout this dissertation project. I am especially grateful to Dr. Nahla Eltai, who has been very supportive throughout all process and taught me the required lab and research skills. I would like to appreciate the involvement of my committee members; Dr. Gheyath Nasrallah and Dr. Asmaa Althani in this project. I wish to express my sincere thanks to the laboratory technician Sara Alhadidi for her timely help and technical support during all research steps.

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# TABLE OF CONTENTS

DEDICATION .................................................................................................................. vi
ACKNOWLEDGMENTS .................................................................................................. vii
LIST OF TABLES ............................................................................................................. ix
LIST OF FIGURES ........................................................................................................... x

I. INTRODUCTION ........................................................................................................ 1
   AIM: ............................................................................................................................ 9
   OBJECTIVES: ............................................................................................................. 9

II. LITERATURE REVIEW ................................................................................................ 10
   GENERAL ASPECTS OF ANTIMICROBIAL RESISTANCE: ........................................ 10
   PUBLIC HEALTH IMPACTS OF ANTIMICROBIAL USE IN FOOD ANIMAL PRODUCTION: ................................................................. 11
   COMMENSAL *E. coli* AS INDICATOR IN AMR SURVEILLANCE: ................................ 12
   AMR IN COMMENSAL *E. coli* FROM LIVESTOCK: .................................................. 13
      AMR IN COMMENSAL *E. coli* FROM CATTLE: ...................................................... 13
      AMR IN *E. coli* FROM CAMELS: ........................................................................ 14
   AMR IN COMMENSAL *E. coli* FROM PIGEONS: ....................................................... 15
   EFFECTS OF CONTROL USE OF ANTIBIOTICS IN FOOD ANIMALS: .................... 16

III. MATERIALS AND METHODS .................................................................................... 17
   SAMPLING SITES AND SAMPLES COLLECTION: ...................................................... 17
   *E. coli* ISOLATION AND IDENTIFICATION: ............................................................ 17
   ANTIBIOTIC SUSCEPTIBILITY TESTING OF *E. coli* ISOLATES: ................................ 18
   DNA EXTRACTION AND MULTIPLEX PCR DETECTION: ......................................... 21
   STATISTICAL ANALYSIS: ....................................................................................... 22

IV. RESULTS .................................................................................................................... 24

V. DISCUSSION ............................................................................................................... 35

REFERENCES ............................................................................................................... 40
LIST OF TABLES

Table 1: List of antibiotics tested, their concentrations and zone diameter interpretive results for *E. coli* .......................................................................................................................................................15

Table 2: Primers used for multiplex PCR panel to detect plasma-mediated colistin resistance genes (*mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5) .........................................................17

Table 3: Number of collected samples and *E. coli* isolates recovered, for each animal group and the corresponding percentages of isolates resistant to at least one antibiotic........19

Table 4: Phenotypic resistance profile of *E. coli* isolates from pigeons fecal samples (n=89) .................................................................................................................................................................................................25

Table 5: Phenotypic resistance profile of *E. coli* isolates from cattle fecal samples (n=86) .................................................................................................................................................................................................26

Table 6: Phenotypic resistance profile of *E. coli* isolates from camels fecal samples (n=91) .................................................................................................................................................................................................27
LIST OF FIGURES

Figure 1: Number of resistant *E. coli* isolates to at least one antibiotic across the animal groups ........................................................................................................................................20

Figure 2: Antibiotic resistance profile for resistant *E. coli* isolates (n=266) to different antibiotics per animal category. ...............................................................................................................................................21

Figure 3: Phenotypic antibiotic susceptibility tests used in this study: (A) disc diffusion, (B) E-test ........................................................................................................................................................................22

Figure 4: The mcr-1 gene detection in colistin-resistant *E. coli* isolated from pigeon .23

Figure 5: Frequency bar chart for the distribution of phenotypic antibiotic resistance among *E. coli* isolates from different animal groups ........................................................................................................24

Figure 6: Phenotypic profile of antibiotic resistance *E. coli* isolates from pigeons across three localities. ..................................................................................................................................................28

Figure 7: Phenotypic profile of antibiotic resistance *E. coli* isolates from Cattle across three localities ................................................................................................................................................29
I. INTRODUCTION

The introduction of antibiotics into the medical practice field was one of the major achievements contributing to the significant reduction of morbidity and mortality due to communicable diseases (Caruso, 2018). Antimicrobial agents include a wide variety of pharmaceutical and natural substances that exhibit antimicrobial activity against bacteria, protozoa, viruses, and fungi (Leekha, Terrell, & Edson, 2011). This thesis is exclusively focused on the antibacterial agent of clinical significance; thus, the term antibiotics will be used in this narrower context.

Antibiotics are either a naturally occurring, semi-synthetic or synthetic substances (Demain, 2009). Initially, potential antibiotics are initially tested ‘in vitro’ for their ability to inhibit bacterial growth as bacteriostatic or bactericidal (Murray et al., 2011). Those antibiotics that are able to inhibit bacterial growth in vitro, are then tested in vivo as well as clinical trials. There are different mechanisms by which these antibiotics inhibit bacterial growth. Mainly, the action of antibiotics is mediated by their interactions with specific bacterial target sites leading to inhibition of bacterial cell wall synthesis, protein or other metabolites synthesis, as well as nucleic acid replication (Neu, 1992).

The development of antimicrobial resistance (AMR) phenomena has emerged in the post-antibiotic era, in which, different bacterial species showed resistance to the actions of different antibiotics as a response to selective antibiotic pressure (Mulvey & Simor, 2009). Thus, antimicrobial resistance is then defined by the ability of the bacterium to survive and grow after exposure to an antibiotic that initially used to inhibit or kill such bacterial population, under the effect of selective pressure (Fair & Tor, 2014).
As a matter of fact, the extensive and uncontrolled use of antibiotic is the main factor that selects for the development of resistant strains which later may become predominant (Mulvey & Simor, 2009). Therefore, antimicrobial resistance is a multifactorial phenomenon that compromised the antibiotic drug efficacy, in which bacteria have developed mechanisms to overcome the action of the antibiotics via different resistance mechanisms (Demain, 2009).

The bacteria could be intrinsically resistant to the antibiotic, or they can acquire the resistance by different means due to the bacterial genome flexibility (D'Costa, McGrann, Hughes, & Wright, 2006). Mainly, the intrinsic resistance is mediated by the inheritance of chromosomal genes. Virtually, all gram-negative bacilli have intrinsic resistance to vancomycin (Murray et al., 2011). On the other hand, resistance could be acquired either due to a mutation in the bacterial chromosomal DNA or by acquiring new resistance genes that present on transferable DNA segments including plasmids, integrons as well as transposons (Shehabi, Odeh, & Fayyad, 2006; Summers, 2002).

Together, resistance mediated genes encode proteins or ribosomal RNA that enable bacteria to have biochemical mechanisms to evade the actions of antibiotics. For instance, resistance determinants including the production of enzymes that hydrolyze the drug, alterations in the target site structure, and limit the access of adequate concentration of antibiotics via decrease the permeability or increase the expression of efflux pumps in the bacterial outer membrane (Neu, 1992).

The emergence of antimicrobial resistant bacteria has been considered globally as a growing public health threat in the 21st century. Clinically, the standard therapies for infections caused by antibiotic-resistant strains become less efficient leading to mortality
and burden financial cost (WHO, 2015). The AMR problem has been exaggerated considering the limited releasing of new antibiotics in the market (Freire-Moran et al., 2011).

Globally, the estimated mortality due to AMR infections reaches 700,000 deaths annually (O’Neill, 2016). It is expected that by the year 2050, the figure is amplified as more than 10 million deaths per year will be attributed to AMR infections among human worldwide (O’Neill, 2016). The emergence and dissemination of AMR bacteria are majorly associated with the inappropriate use of antibiotics in both human healthcare and veterinary sector (Anthony D. So, 2016). In particular, there is a growing evidence that the non-judicious use of antibiotics in animal agriculture majorly contributes to the development of antimicrobial resistance in animal-associated bacteria (Molbak, 2004). Moreover, there are accumulating reports from different regions around the globe about the emergence of multidrug-resistant bacteria in different livestock species. Subsequently, food animals are also shown to be responsible for accelerating the spread of AMR bacteria to humans and environment (Caruso, 2018); (Threlfall, Ward, Frost, & Willshaw, 2000).

In food animals, although the antibiotics are prescribed clinically to control infections, the crisis of antibiotic resistance is driven by inappropriate use. The group medication of animals for prolonged times and in low dosages, mostly through the feed for nontherapeutic purposes, have been demonstrated to be the main risk factors for the development of resistance (WHO, 2017b). Since the 1950s, antimicrobial growth promotants (AGPs) were promoted for the use in subtherapeutic concentrations. Examples of these antibiotics includes penicillin and tetracycline that are used in animals’ food to increase the weight ratio for poultry, cattle as well as swine (Pagel & Gautier, 2012).
Interestingly, it has then been reported that the intestinal commensal bacteria of both animals and workers in farms using those growth promoters have a high antimicrobial resistance to common antibiotics (Aarestrup, Wegener, & Collignon, 2008). Moreover, antibiotics have been prescribed for animals for either metaphylaxis and prophylaxis purposes. Metaphylaxis is a control treatment for the group of animals to prevent the occurrence of the highly probable outbreak when part of the group is diagnosed with an infection (FDA, 2012; Pagel & Gautier, 2012). On the other hand, prophylaxis is a preventive treatment to prevent the occurrence of an infection (FDA, 2012; WHO, 2017b). More importantly, it has been demonstrated that the chronic and continues administration of a single antibiotic would lead to development of resistance to other antibiotics through different mechanisms, noting that resistance genes could be carried on plamids and/or transposon (Marshall & Levy, 2011).

Of high importance, it is estimated that 75% to 90% of antibiotics consumed by food animals are not metabolized; therefore excreted largely into the surrounding environment (Marshall & Levy, 2011). Consequently, the activities of the excreted antibiotics are showed to be retained in the environment for considerable time (Manyi-Loh, Mamphweli, Meyer, & Okoh, 2018). Hence, the propagation of active antibiotics and metabolites represents another route of transmission from farms to the environment, where environment becomes a reservoir of circulating resistance genes in the community (Manyi-Loh et al., 2018). As a matter of fact, studies reported AMR bacteria in farm dust (Hamscher, Pawelzick, Sczesny, Nau, & Hartung, 2003), the groundwater within farms locations (Dolliver, Kumar, & Gupta, 2007), and the food crops where antibiotic-containing manure is used (Brooks & McLaughlin, 2009). The environmental organisms
that are exposed to the discharge of unmetabolized antibiotics will be effectively promoted to undergo selection with resistance mutations and thereafter exchange such genes among various bacterial species (Aarestrup et al., 2008).

In particular, the antimicrobial classes that ranked by World Health Organization (WHO) as highly important for human medicine (WHO, 2017a), become commonly administrated in livestock agriculture and production (OIE, 2017). Recent sales data released in 2015 by the Food and Drug Administration (FDA) reported that almost 10 million kilograms of medically important antibiotics were sold for food animals in the United States (FDA, 2016). Furthermore, in France only, 60% of total antibiotics consumption is recorded in the veterinary section (Moulin et al., 2008). As a consequence, there are direct and indirect pieces of evidence supporting the potential role posed by antimicrobial resistant bacteria from animal sources on human health (Marshall & Levy, 2011). Recent reports from WHO have further emphasized the burden food-borne and animal-origin antimicrobial resistant bacteria on animal health (Scott et al., 2018; WHO, 2017b).

Commensal bacteria including Enterobacteriaceae are common enteric flora in mammals and are frequently studied for AMR purposes (van den Bogaard & Stobberingh, 2000). Experimentally, antimicrobial resistance genes have been reported to transmit horizontally between commensal and zoonotic members of the Enterobacteriaceae including both Escherichia coli and Salmonella species, respectively (Poppe et al., 2005). Moreover, E. coli can acquire and maintain resistance genes, and serve as a reservoir of resistant genes for zoonotic bacteria (Poppe et al., 2005). From the public health viewpoint, E. coli is a representative marker for the selection pressure created by antibiotic use, and is
the predictor for future development of antibiotic resistance in others pathogens in various environment (Caruso, 2018).

Considering all the above, the AMR is currently investigated and looked at under the “One Health” approach, which represents the interlink between three domains: human, animals, and environment. Principally, the One Health approach is defined as the multidisciplinary efforts/collaborations to achieve the optimal health of people through tackling the problem at animals and environment levels, in addition to humans (Robinson et al., 2016). In this regard, the contribution of antibiotics use in livestock production to the global AMR crises is determined by the ability of AMR genetic determinants to subsequently transmit to human pathogens as well as other commensal microbiota. This could happen either directly through contacts of animals, or indirectly through contact with contaminated environment or consumption of contaminated food (Chang, Wang, Regev-Yochay, Lipsitch, & Hanage, 2015).

As an action, countries such as United States, Canada, and Australia, have decided to limit the use of clinically-relevant antibiotics in food animals, supported by active surveillance and continuous monitoring of AMR (Singer, Shaw, Rhodes, & Hart, 2016). In Netherland, the government has regulated the use of antibiotics in food animals reaching a 50% reduction in usage in 2013 as compared to 2009 and a 70% decrease in 2015. AMR continues monitoring surveillance concluded significant reduction in AMR pattern within a period of six years (Havelaar et al., 2017). Officially, the FDA supported the conclusion that the nontherapeutic use of medically important antibiotics in food animal production contributes to a potential health risk for public health (FDA, 2016). A recommendation document was released in 2010 to ultimately limit the use of these drugs in animals (FDA,
In contrast, this is not the situation in Qatar, where only scanty short-term studies emerged to assess the level of AMR in the veterinary sector. Clinically, antimicrobial stewardship program (AMSP) has been implemented in Qatar general hospitals since March 2015 (Ribero Pombo MH & Thompson D, 2018). Accordingly, the appropriate use of antibiotic has been improved by promoting the selection of the optimal antibiotic regimen including dosing, duration of therapy, and route of administration (Pawluk, Black, & El-Awaisi, 2015).

AMSP program is structured to monitor antibiotic use and reduce the antimicrobial resistance at the hospital level through enforcement of the antibiotic restriction policy in Qatar (Pawluk et al., 2015). For instance, the effect of the implementation of an antimicrobial stewardship program in Hamad Medical Corporation was clear in the prevalence of MDR *Pseudomonas Aeruginosa* that declined from 8.1% in 2015 to 4.9% in 2017 (HMC, antibiogram report, 2017).

Nevertheless, there is a lack of AMR surveillance data in bacteria isolated from food-producing animals to understand AMR pattern in the veterinary sector. On 2016, WHO; International Health Regulations (IHR); released a mission report evaluating the health security system in Qatar with recommendations to urgently prioritize the development of integrated AMR surveillance including animal sector under the one health approach (WHO, 2017c). Therefore, there is an urgent need to develop an AMR monitoring program in the veterinary sector as a step to develop and implement the antimicrobial stewardship (AMS) in agriculture (WHO, 2017c).

Recently, antibiotic-resistant bacteria has been studied in food-producing animals
including broiler chickens (N. O. Eltai et al., 2018) and sheep (unpublished data) as baseline studies in Qatar. Both studies estimated the AMR level using commensal *E. coli* as an indicator. Data from chicken revealed a high prevalence of antimicrobial resistance in commensal *E. coli*, including MDR, with 90% of *E. coli* isolates being resistant to at least one antibiotic. Moreover, the study reported colistin resistance in about 15% of *E. coli* isolates, all being encoded by plasmid-mediated colistin resistance gene (mcr-1). That was the first report of its kind in the MENA region. Detection of colistin resistance is a worrying finding as colistin is the last resort antibiotic prescribed for MDR gram-negative bacterial infections. This is more worrying considering its mobility on plasmids (Liu et al., 2016). Furthermore, 2.2% of the isolates were extended-spectrum β-lactamase (ESBL) producers (N. O. Eltai et al., 2018). In another unpublished data from the same research group, commensal *E. coli* isolates from healthy sheep also had a high prevalence of resistance (90%), with MDR isolates frequency reaching 44% (Personal communication; Dr. Nahla Eltai). One of the significant findings on AMR pattern in sheep study is the high prevalence of resistance to critically important antibiotic ciprofloxacin, which is a member of fluoroquinolones class that has been banned on 2005 in the united states to be used in livestock agriculture (OIE, 2017).

Considering the recently previous studies on both broiler chickens and sheep in Qatar, such alarming findings necessitates a broader assessment of AMR in the animal sector locally. Accordingly, we initiated this study to evaluate AMR in two main food-producing animals (cattle and camels) as well as pigeons, which could be a vector for transporting resistant bacteria across long distances.
AIM:

Considering the recent findings in chicken, the lack of AMR studies in veterinary sector in Qatar, and the need to immediate comply with the antimicrobial stewardship (AMS) under the “one health approach, the aim of this study is to provide baseline information on the incidence of antimicrobial resistance (AMR) in several food-producing animals in Qatar.

OBJECTIVES:

The overall objective of this study is to characterize the phenotypic and genotypic AMR pattern of 18 clinically relevant antibiotics in commensal E. coli isolated from fecal samples of camels, cattle, and pigeons collected from slaughterhouses and farms in Qatar. Our ultimate goal is to compare our findings to the data released by the monitoring programs in Hamad Medical Corporation (HMC) along with previously published reports from Qatar and to provide evidence-based recommendations to guide and improve stewardship programs in clinical and veterinary medicine.
II. LITERATURE REVIEW

GENERAL ASPECTS OF ANTIMICROBIAL RESISTANCE:

Antibiotics are a group of synthetic, semisynthetic, or naturally occurring agents with bacteriostatic or bactericidal properties (WHO, 2017a). The introduction of antibiotics in human medicine was one of the greatest medical achievement in the 20\textsuperscript{th} century to reduce morbidity and mortality associated with infectious diseases (Fair & Tor, 2014). Afterward, antibiotics have been introduced to food animal agriculture for different purposes including treatment, prevention, and growth promotion (Pagel & Gautier, 2012). Nonetheless, the excessive and uncontrolled use of antibiotics in both human and veterinary medicine contributed to the development of resistant pathogens where simple bacterial infections become hard to be treated (Fair & Tor, 2014; WHO, 2015). Precisely, the antibiotic resistance has been defined as the ability of the bacterium to grow and survive in the presence of the antibiotic agent that previously showed an antibacterial effect via the acquisition of different antibiotic resistance mechanisms (Fair & Tor, 2014).

There are major antibiotic resistance mechanisms including production of enzymes that inactivate the antibiotic agent; modification in the antibiotic target site; reduction of bacterial cell wall permeability; and increasing export of antibiotics via active pumps (Tenover, 2006). Further, studies showed that resistance genes could be exchanged from resistant, to susceptible bacteria through conjugation, transformation, or transduction in environmental settings (Tenover, 2006).
PUBLIC HEALTH IMPACTS OF ANTIMICROBIAL USE IN FOOD ANIMAL PRODUCTION:

The antimicrobial use in food animals has been shown to have a negative impact on human health (WHO, 2015). In particular, resistant animal-associated commensals have been shown to directly impacts the health of people in close contact with farm animals, and indirectly to a wider population via the food chain (Marshall & Levy, 2011). Of great concern, antibiotic-resistant commensals from animal sources can colonize humans and transfer their resistance genes to human-associated commensals as well as to human foodborne pathogens including *Salmonella* and *Campylobacter* (Barlow et al., 2015; WHO, 2017b).

Studies suggested that antibiotic-resistant bacteria and resistance genes can enter into the community settings via environmental spread from farms, slaughterhouses as well as occupational workers and their families (Marshall & Levy, 2011). Katsunuma et al. reported a significantly high prevalence of AMR in commensal bacteria among poultry, pigs and cattle farm workers around Japan, suggesting occupational exposure risk factor for spreading AMR (Katsunuma et al., 2007). In another study, Aubry-Damon et al. compared the AMR profile of commensal enterobacteria between farmers and non-farmers who reported no recent exposure to antibiotics. Results showed a significantly higher prevalence of AMR resistance in farmers against cotrimoxazole, tetracycline, streptomycin, and nalidixic acid compared to non-farmers (Aubry-Damon et al., 2004). Another supportive study reported that risk the for having multidrug-resistant *E. coli* was 32 times higher in farm workers compared with community referents (Price et al., 2007). Bertrand et al. documented chronologically the transfer of extended-spectrum beta-
lactamase (ESBL) gene (CTX-M-2) in *Salmonella enterica* from poultry species in farms to poultry meat products, and then to humans in the same region in Belgium (Bertrand et al., 2006).

On the other hand, supplementary studies reported the direct link between reducing the antimicrobial use in food animals and the decrease in resistance to those antimicrobials in these animals. A randomized controlled trial (RCT) by Kaneene et al. studied the impact of continues feeding of dairy calves with oxytetracycline and neomycin antibiotics in milk versus a discontinuing feeding group. Results showed that discontinuation of antimicrobial consumption leads to an increase in susceptibility to tetracyclines in both *E. coli* and *Salmonella* species in treated animals (Kaneene et al., 2008).

**COMMENSAL *E. COLI* AS AN INDICATOR FOR AMR SURVEILLANCE:**

Commensal bacteria are considered to be the reservoir of AMR genes that could be transferred to zoonotic pathogenic bacteria (Tenover, 2006). Therefore, AMR level in commensal bacteria can be measured as a good indicator to predict the prevalence of AMR in other pathogens within a particular environment. In particular, commensal intestinal *Escherichia coli* (*E. coli*) strains are considered as good reliable indicators for antimicrobial resistance surveillance in both human and animals (Szmolka & Nagy, 2013; van den Bogaard, Willems, London, Top, & Stobberingh, 2002). *E. coli* is characterized to have genetic flexibility to adapt to environmental changes; thus, it easily acquires different resistance mechanisms during the lifetime of their host (Szmolka, Anjum, La Ragione, Kaszanyitzky, & Nagy, 2012). Importantly, *E. coli* could be isolated and recovered with cost-effective laboratory standards (van den Bogaard et al. 2000).
AMR IN COMMENSAL E. COLI FROM LIVESTOCK:

Here, we focus only on selected major livestock species, especially cattle, camels; and poultry including pigeons, which considered globally as food-producing animals.

AMR IN COMMENSAL E. COLI FROM CATTLE:

AMR bacteria have been investigated and characterized widely in cattle species. A recent study reported a high prevalence of resistance to streptomycin (47.5%), tetracycline (45.4%), and ampicillin (34.2%) in commensal E. coli isolated from different dairy cattle farms in Jordan (Obaidat, Bani Salman, Davis, & Roess, 2018). The same study showed that 83.7% of herdsmen purchased antibiotics without a veterinary prescription. Further, antibiotics use and their doses were frequently changed if no treatment response was recorded (Obaidat et al., 2018). Likewise, Pereira et al. studied the phenotypic antibiotic resistance of 1,423 E. coli isolates from rectal swabs of dairy calves, from eight farms in New York. They reported significant phenotypic resistance to both ciprofloxacin and ceftriaxone, where fluoroquinolones and third-generation cephalosporins were used in food animals (Pereira et al., 2014). Moreover, a cross-sectional study of 1,736 fecal samples from pre-weaned calves and cows cattle on 38 farms in California, Oregon, and Washington showed that 55% of E. coli isolates were resistant to tetracycline, 28% to ampicillin and 31% were multi-drug resistant (Berge, Hancock, Sischo, & Besser, 2010).

In another study, Bosman et al. investigated the resistance of commensal E. coli isolated from white veal calves and reported highest phenotypical resistance against amoxicillin-tetracycline (31.7%), followed by tetracycline only (23.2%) and amoxicillin-tetracycline-ciprofloxacin-TMP/SMX (10.5%) (Bosman, Wagenaar, Stegeman, Vernooij, & Mevius,
On the other side of the world, researchers from South Korea reported that around 94% of *E. coli* isolates from beef cattle fecal samples were phenotypically resistant to tetracycline, associated with presence of tetracycline resistance genes including *tet(A)* (46.5%), *tet(B)* (45.1%) and *tet(C)* (5.8%). These data suggested the exchange of genetic materials encoding for resistance in the studied population (Shin, Shin, Jung, Belaynehe, & Yoo, 2015). Interestingly, Duse et al. reported that antimicrobials treatments of cows during lactation resulted in significantly more antibiotic resistant *E. coli* strains in the feces of pre-weaned dairy calves. In this particular study, they reported the resistance of *E. coli* to streptomycin (90%), nalidixic acid (49%), or cefotaxime (11%) (Duse et al., 2015). The authors concluded that minimizing the feeding of milk from cows treated with antimicrobials during lactation should lower the prevalence of resistant *E. coli* in the gastrointestinal tract of the calves.

In contrast, a study performed on fecal samples from beef cattle, dairy cattle and veal calf from slaughterhouses in Australia revealed a low level of resistant *E. coli* to antibiotics of low importance in human medicine (Barlow et al., 2015). This finding positively reflected the strict governmental regulations of antimicrobials usage in food animals in Australia (Barlow et al., 2015; Pagel & Gautier, 2012).

**AMR IN E. COLI FROM CAMELS:**

Few studies have been published on AMR incidence in camel’s species. A recent study from Nigeria showed that multidrug-resistant Shiga toxin-producing *E. coli* (STEC) isolated from the fecal samples is higher in cattle compared to camels (Adamu et al., 2018). In another study from Tunisia, *E. coli* strains isolated from diarrheic and healthy camel
fecal samples showed a high frequency of resistance to tetracycline (52.8%), and ampicillin (37.1%) (Bessalah et al., 2016). In another study from southern Tunisia demonstrated the absence of plasmid-mediated mobilized colistin resistance genes (mcr-1 and mcr-2) in E. coli isolated from camel feces (Rhouma et al., 2018). In an interesting study from Kingdom of Saudi Arabia (KSA), Fadlelmula et al. compared AMR level in commensal E. coli isolated from camels with those of pathogenic E. coli isolated from human urinary tract infections (UTIs) (Fadlelmula, Al-Hamam, & Al-Dughaym, 2016). Molecular testing revealed the presence of ESBL producer E. coli strains in 26.9% of camel samples and 36.4% of human samples, suggesting that camels could be a potential reservoir for AMR E. coli strains in Saudi Arabian community (Fadlelmula et al., 2016).

**AMR IN COMMENSAL E. COLI FROM PIGEONS:**

Similar to camels, few studies reported the antimicrobial resistance profile in pigeons. In a low-income setting in Nicaragua, Hasan et al. reported the existence of ESBL-producing E. coli in about 13% poultry pigeons compared to 8% in other domestic and wild birds, and 27% in healthy humans (Hasan et al., 2016). Interestingly, another study from South Africa addressed the risk of AMR E. coli spread due to pigeon fecal samples contamination of the roof-harvested rainwater (Chidamba & Korsten, 2015). In particular, they reported resistance to ampicillin (22.7.9%), gentamicin (23.6%), amikacin (24%), tetracycline (17.4) and amoxicillin (16.9%) (Chidamba & Korsten, 2015). In another study from Iran that evaluated AMR of commensal E. coli isolated from fecal samples from pigeons, high resistance to tetracycline (88.4%) and doxycycline (74.4%) was reported (Askari Badouei, Zahraei Salehi, Koochakzadeh, Kalantari, & Tabatabaei, 2014). In
Bangladesh, Hasan et al. examined resistance profile of *E. coli* from household pigeon fecal samples and showed that 89% of *E. coli* isolates were resistant to one or more critically important human antibiotics including ampicillin, ciprofloxacin, gentamicin, and tigecycline, with low ESBLs prevalence (Hasan et al., 2014). Another study from Poland investigated AMR profile of three bacterial species isolated from pigeons: *E. coli*, *Salmonella typhimurium*, and beta-hemolytic coagulase-positive *staphylococci*. It was found that *E. coli* isolates had higher resistance against Amoxicillin (63%), Oxytetracycline (75%) and Sulfamethoxazole/trimethoprim (53%) as compared to *Staphylococci* and *Salmonella typhimurium* (Stenzel et al., 2014).

**EFFECTS OF CONTROL USE OF ANTIBIOTICS IN FOOD ANIMALS:**

Unlike the situation in humans, control of antibiotics use in animals has not been strictly implemented in many nations. In the few countries that regulate the use of antimicrobials in agriculture, studies have shown a reduction in the prevalence of resistant bacteria. In a six-year period (2009-2015) of antibiotic control use in Netherlands for example, a significant reduction of resistant bacteria, reaching 70% in some cases, was reported in multiple animals including chickens, pigs, calves and cows (Havelaar et al., 2017). A similar observation was published in Australia cattle herds (Barlow et al., 2015). In Belgium, governmental restrictions for antimicrobial consumption in the veterinary section during the period between 2010 and 2013 also resulted in a reduction of resistance in commensal *E. coli* strains from veal calves, young beef cattle, broiler chickens and slaughter pigs against 11 antibiotics (Hanon et al., 2015). Interestingly, the overall prevalence of resistance and multi-resistance was the lowest in the beef cattle livestock category compared to other animal groups (Hanon et al., 2015).
III. MATERIALS AND METHODS

SAMPLING SITES AND SAMPLES COLLECTION:

Animal fecal samples from pigeons, dairy cattle and camels (100 samples from each species) were collected from slaughterhouses and farms distributed in different locations in Qatar. Sampling process was performed during the period between end of December 2018 to February 2019 by two qualified veterinarians in collaboration with the Ministry of Public Health (MOPH). Samples were subsequently transported to Qatar University; Biomedical Research Center; Microbiology Laboratory for immediate processing.

E. COLI ISOLATION AND IDENTIFICATION:

Research approval to process samples were obtained from Qatar University's Institutional Biohazard Committee under approval number QU (QU-IBC-2018/034). One gram of fecal sample was vigorously homogenized with 3 ml sterile Phosphate Buffered Saline (PBS). The suspended samples were streaked using sterile cotton swab onto chromogenic Tryptone Bile X-glucuronide agar (TBX; HiMedia Laboratories, India) for E. coli detection and incubated at 37°C for 18-24 hours. Subsequently, single typical E. coli colonies (blue-green colonies) were selected randomly and streaked onto MacConkey agar plates (HiMedia Laboratories, India) then incubated at 37°C for 18-24 hours to differentiate lactose fermenter isolates. Next, pink colonies from MacConkey agar were randomly selected to recovered on nutrient agar plates (HiMedia Laboratories, India) to obtain pure single colonies. Lactose fermenter isolates were further tested by indole spot test (Thermo Fisher Scientific, KS). For further confirmation, biochemical reactions tested
by Crystal™ Enteric/ nonfermenter id KIT, BD via Biomic V3 software (Giles scientific, USA). *E. coli* isolates were then directly tested for antibiotic susceptibility testing. Pure *E. coli* isolates were preserved in cryovial tubes (Technical Service Consultant, UK) and stored at -80°C until further testing.

**ANTIBIOTIC SUSCEPTIBILITY TESTING OF *E. COLI* ISOLATES:**

The antibiotic susceptibility test was conducted using a Kirby-Bauer disk diffusion assay on Mueller-Hinton agar plates (Medysinal FZCO, Dubai). *E. coli* isolates were recovered on nutrient agar, and single colonies were suspended in 0.85% saline (Medysinal FZCO, Dubai) to achieve an inoculum equivalent to 0.5 McFarland standard as measured by DensiCHEK Plus (bioMerieux, France). The suspension was streaked along the surface of Mueller-Hinton agar plates. Antibiotic-impregnated discs (Liofilchem, Italy) were applied to the inoculated plate surface with sterile forceps, and plates were incubated at 36±1°C for 18 to 24 hours. Maximum of six antibiotic discs were placed on a 100-mm plate using the antibiotic disc dispenser. The diameter of the inhibition zone around each disc was measured in millimeters and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2018). As the *E. coli* QC isolate ATCC 25922 is reported in CLSI to show a susceptibility to colistin disc diffusion in a range from 11 to 17 mm, therefore in our study, we considered any *E. coli* isolate with growth inhibition diameter ≤ 10 mm to be resistant to colistin. For subsequent confirmation, colistin-resistant *E. coli* isolates were tested for colistin resistance using susceptibility test strips (E-test strip, Liofilchem, Italy) on Mueller-Hinton agar plates. The zone of inhibition was examined, and the Minimum Inhibitory Concentrations (MICs) were determined. Alternatively; as no
CLSI interpretive breakpoints have been established to interrupt colistin resistance results; the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (EUCAST, 2019) were used. Thus, we included any *E. coli* isolate with MIC of $\leq 2 \, \mu g/mL$ according to EUCAST interpretive criteria. *E. coli* ATCC 25922 was used as a control strain in all steps. The eighteen clinically relevant antibiotics used to screen the antibiotic susceptibility of *E. coli* are listed in Table 1.
Table 1: List of antibiotics tested, their concentrations and zone diameter interpretive results for *E. coli*

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Code</th>
<th>Disk content (µg)</th>
<th>Zone diameter breakpoints and CLSI interpretive criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PENICILLINS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AMP</td>
<td>10</td>
<td>≥ 17</td>
</tr>
<tr>
<td><strong>β-LACTAM COMBINATION AGENTS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>AUG</td>
<td>30</td>
<td>≥ 18</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>TZP</td>
<td>110</td>
<td>≥ 21</td>
</tr>
<tr>
<td><strong>CEPHEMS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>KF</td>
<td>30</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>CXM</td>
<td>30</td>
<td>≥ 18</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>CRO</td>
<td>30</td>
<td>≥ 23</td>
</tr>
<tr>
<td>Cefepime</td>
<td>FEP</td>
<td>30</td>
<td>≥ 25</td>
</tr>
<tr>
<td><strong>CARBAPENEMS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>ETP</td>
<td>10</td>
<td>≥ 22</td>
</tr>
<tr>
<td>Meropenem</td>
<td>MRP</td>
<td>10</td>
<td>≥ 23</td>
</tr>
<tr>
<td><strong>LIPOPEPTIDES</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Colistin Sulfate</td>
<td>CS</td>
<td>30</td>
<td>≥ 11</td>
</tr>
<tr>
<td><strong>AMINOGLYCOSIDES</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AK</td>
<td>30</td>
<td>≥ 17</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GN</td>
<td>10</td>
<td>≥ 15</td>
</tr>
<tr>
<td><strong>TETRACYCLINES</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
<td>30</td>
<td>≥ 15</td>
</tr>
<tr>
<td><strong>QUINOLONES AND FLUOROQUINOLONES</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>5</td>
<td>≥ 21</td>
</tr>
<tr>
<td><strong>FOLATE PATHWAY ANTAGONISTS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>SXT</td>
<td>25</td>
<td>≥ 16</td>
</tr>
<tr>
<td><strong>PHENICOLS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>C</td>
<td>30</td>
<td>≥ 18</td>
</tr>
<tr>
<td><strong>FOSFOMYCINS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>FOS</td>
<td>200</td>
<td>≥ 16</td>
</tr>
<tr>
<td><strong>NITROFURANS</strong></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>F</td>
<td>300</td>
<td>≥ 17</td>
</tr>
</tbody>
</table>

S, sensitive; I, intermediate; R, resistant
DNA EXTRACTION AND MULTIPLEX PCR DETECTION:

For any phenotypically confirmed colistin resistant, *E. coli* isolate, further molecular testing was performed to detect the presence of plasmid-mediated colistin resistance *mcr* genes. DNA was extracted using QIAamp UCP Pathogen Mini Kit (Qiagen, Germany) following the provided manufacturer’s instructions from overnight nutrient agar culture. Extracted DNA was subjected to a multiplex PCR protocol as recently described (Rebelo et al., 2018). The primers used in this protocol as summarized in Table 2; targeting *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 genes in all *Enterobacteriaceae*. The PCR was performed in a total volume of 25 µl containing 12.5 µL 1x Master Mix (New England Biolabs, UK), 3.5 µl of nuclease-free water, 0.5µl of each forward and reverse primers designed for each single gene, and 2µl of DNA template. The reaction mixture was amplified using GeneAmp PCR System 9700 Thermocycler under the following conditions: 1 cycle of denaturation at 94°C for 15 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 90 s and elongation at 72°C for 60 s, and a final cycle of elongation at 72°C for 10 min. The amplified products were visualized by electrophoresis using 1.2% agarose gel (Agarose LE, Paisley, UK), stained with ethidium bromide (Promega, WI) using iBright™ CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, KS). The DNA marker used was 1Kbp plus ladder (Invitrogen, Thermo Fisher Scientific, KS). *E. coli* isolate run in parallel with one positive control “colistin resistant” and one negative control “*E. coli* ATCC 25922”.
Table 2: Primers used for multiplex PCR panel to detect plasma-mediated colistin resistance genes (mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5)

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences</th>
<th>Expected band size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mcr1_320bp_fw</td>
<td>AGTCCGTTTGTCTTTGCGC</td>
<td>320bp</td>
</tr>
<tr>
<td>mcr1_320bp_rv</td>
<td>AGATCCTTGTCTTCTCGGCTT</td>
<td></td>
</tr>
<tr>
<td>Mcr2_700bp_fw</td>
<td>CAAGTGTGTTGCTGCTGAGTT</td>
<td>715bp</td>
</tr>
<tr>
<td>Mcr2_700bp_rev</td>
<td>TCTAGCCCGACAAGCATAACC</td>
<td></td>
</tr>
<tr>
<td>mcr3_900bp_fw</td>
<td>AAATAAAAATTGTTCGGCTTATG</td>
<td>929bp</td>
</tr>
<tr>
<td>mcr3_900bp_rev</td>
<td>AATGGAGATCCCCGTTTTT</td>
<td></td>
</tr>
<tr>
<td>mcr4_1100bp_fw</td>
<td>TCACCTTTTCATCATGCTGGT</td>
<td>1,116bp</td>
</tr>
<tr>
<td>mcr4_1100bp_rev</td>
<td>TTGGTCTCATGACTACCAATG</td>
<td></td>
</tr>
<tr>
<td>MCR5_fw</td>
<td>ATGCCGTTGTCTGCTTTATAC</td>
<td>1,644bp</td>
</tr>
<tr>
<td>MCR5_rev</td>
<td>TCATTGTGTTGTCTTTTCTG</td>
<td></td>
</tr>
</tbody>
</table>

STATISTICAL ANALYSIS:

Data were entered into Microsoft Excel 2016 spreadsheet (Microsoft Corporation, New York, USA) for initial analysis. Data were reviewed, and values were color-coded as green “sensitive”; yellow “intermediate”; and red “resistant.” Graphs were plotted, and statistical analysis was performed with GraphPad Prism v8.0.2. The resistance percentage was calculated for each antibiotic in each animal group separately by dividing the total number of resistant isolates on the total number of tested isolates. An E. coli isolate was considered as multi-drug resistant if it was resistant to at least three antibiotic classes as previously defined (Hanon et al., 2015). Correlations among resistances to a particular antibiotic across the three animal groups and in each animal group across three localities were done using Chi-square test. A $p$-value $\leq 0.05$ on a two-sided level was considered to be statistically significant. In this study, isolates with intermediate susceptibility to the
tested antibiotics were considered as susceptible for analysis.
IV. RESULTS

A total of 266 (88.7%) E. coli isolates were recovered, were each isolate represented one fecal sample (Table 3). We found no significant difference in the recovery rate from the three animal categories (p=0.5325). The frequency of antimicrobial resistance of those isolates for 18 clinically relevant antibiotics is shown in Figure1. Overall, E. coli isolates had resistance to ten antibiotics in pigeon group, eight antibiotics in dairy cattle and only five antibiotics in camel group. The distribution of the number of E. coli isolates per livestock category with the corresponding prevalence of isolates resistant to at least one antibiotic are shown in Table 3 and figure1. About 71% (n=63 of 89) of pigeon E. coli isolates were resistant to at least one antibiotic. On the other hand, 36.5% (n=31 of 86) and 20.8% (n=19) of E. coli isolates from dairy cattle group and camels, respectively, were resistant to at least one antibiotic.

Table 3: Number of collected samples and E. coli isolates recovered, for each animal group and the corresponding percentages of isolates resistant to at least one antibiotic

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Animal groups</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pigeons</td>
<td>Cattle</td>
<td>Camels</td>
</tr>
<tr>
<td>Number of collected samples</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Number of recovered E. coli isolates</td>
<td>89</td>
<td>86</td>
<td>91</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>89</td>
<td>86</td>
<td>91</td>
</tr>
<tr>
<td>Resistance to at least one antibiotic</td>
<td>63</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>(70.7%)</td>
<td>(36.5%)</td>
<td>(20.8%)</td>
</tr>
</tbody>
</table>
Figure 1. Number of resistant *E. coli* isolates to at least one antibiotic across the animal groups. **** indicates *P* value is statistically significant by Fisher’s exact test (<0.0001); while ns for non-significance.

As shown in Figure 2, The prevalence of resistance varied according to the type of antibiotics across the three animal categories examined. Resistance to tetracycline was the most common, observed in 64, 27.9 and 15% of isolates from pigeons, cattle and camels, respectively. Similarly, resistance to ampicillin was also observed across the three animal groups with a prevalence rate of 55.1, 14 and 7% in isolates from pigeons, cattle and camels, respectively. Moreover, trimethoprim/sulfamethoxazole resistance was present in 59 (22.2%) of all *E. coli* isolates, with higher prevalence in 49.4% pigeons, followed by 11.6% and 5% prevalence in cattle and camels, respectively. Similar resistance was observed for chloramphenicol which was detected in 15.7, 7 and 2% of isolates from pigeons, cattle and camels, respectively. Susceptibility (resistance was absent among *E. coli* from any animal group) for nine antibiotics were observed in all livestock categories.
for the following antibiotics: amoxicillin-clavulanic acid, piperacillin-tazobactam, second, third and fourth generation cephalosporins including cefuroxime, ceftriaxone, cefepime along with fosfomycin, amikacin and carbapenems ertapenem, and meropenem. Resistance to cephalothin was observed in pigeons (11.2%) and only one isolate from camels (1%). Resistance to gentamicin, and ciprofloxacin was observed in pigeon and cattle isolates. The resistance of *E. coli* isolates to nitrofurantoin was recorded only in pigeons (4.5%).

Figure 2. Antibiotic resistance profile for resistant *E. coli* isolates (n=266) to different antibiotics per animal category. TZP: piperacillin/tazobactam; SXT: Trimethoprim/ sulfamethoxazole. **** indicates that *P* value is statistically significant by Fisher’s exact test (<0.0001); while ns for non-significance.
One *E. coli* isolate from pigeon showed resistance to colistin. Antibiotic resistance for colistin was confirmed phenotypically using E-test strips (Figure 3; B). Furthermore, plasmid-mediated colistin resistance was confirmed using multiplex PCR for the detection for *mcr* genes (*mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4, and *mcr*-5), where the presence of *mcr*-1 gene was recorded (Figure 4).

Figure 3. Phenotypic antibiotic susceptibility tests used in this study: (A) disc diffusion, (B) E-test. (A) *In the disk-diffusion assay, the diameter of the growth inhibition zone is measured in mm around the antibiotic disc.* (B) *In antibiotic susceptibility test strips (E-test strip), zone of inhibition is examined to determine the minimal inhibitory concentration (MICs).*
Figure 4. The mcr-1 gene detection in colistin-resistant *E. coli* isolated from pigeon; lane 1, susceptible *E. coli* isolate; lane 2, *E. coli* isolate from pigeon; lane 3, positive control *E. coli* isolate. Lane 4, negative control corresponds to *E. coli* isolate (ATCC 25922). M, molecular size marker and the size of each amplicon is indicated at the side; bp, base pairs.

Phenotypic resistance pattern, including multidrug resistance, varied considerably among different isolates from the three animal groups. The antibiotic resistance pattern observed in isolates from pigeons was generally different from that of cattle and camels. Distribution of multidrug resistance in pigeon isolates is recorded in figure 5. Only 4.49% (n=4) of the isolates were resistant to a maximum of six antibiotics in pigeon group (figure 5), 8.99% (n=8) were resistant to five antibiotics, 12.4% (n=11) were resistant to four
antibiotics, 24.7% (n=22) were resistant to three antibiotics, 5.62% (n=5) were resistant to two antibiotics, and 14.6% (n=13) were resistant to one antibiotic.

![Bar chart](image)

**Figure 5.** Frequency bar chart for the distribution of phenotypic antibiotic resistance among *E. coli* isolates from different animal groups

In pigeons, fifty percent (n=44) of the isolates were Multi-drug resistant (MDR), with distribution pattern summarized in Table 4. The highest multidrug resistance was recorded to ampicillin; trimethoprim/sulfamethoxazole; tetracycline in 20.2 % (n=18) of the isolates. Other multidrug resistance patterns were recorded between 1.1% for a
combination of ampicillin; cephalothin; ciprofloxacin, and up to 6.7% for a combination of ampicillin; trimethoprim/sulfamethoxazole; chloramphenicol; tetracycline.

Table 4: Phenotypic resistance profile of *E. coli* isolates from pigeons fecal samples (n=89)

<table>
<thead>
<tr>
<th>Resistant phenotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resistance</td>
<td>26</td>
<td>29.2</td>
</tr>
<tr>
<td>Resistant to only one antibiotic</td>
<td>13</td>
<td>14.6</td>
</tr>
<tr>
<td>Ampicillin, Tetracycline</td>
<td>4</td>
<td>4.5</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin, Ciprofloxacin</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>19</td>
<td>21.3</td>
</tr>
<tr>
<td>Gentamicin, Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ampicillin, Gentamicin, Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Ciprofloxacin, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Chloramphenicol, Tetracycline</td>
<td>6</td>
<td>6.7</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin, Gentamicin, Ciprofloxacin, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Chloramphenicol, Ciprofloxacin, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin, Trimethoprim/sulfamethoxazole, Chloramphenicol, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Nitrofurantoin, Chloramphenicol, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Gentamicin, Trimethoprim/sulfamethoxazole, Nitrofurantoin, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin, Trimethoprim/sulfamethoxazole, Nitrofurantoin, Chloramphenicol, Tetracycline</td>
<td>2</td>
<td>2.2</td>
</tr>
</tbody>
</table>
In dairy cattle group, only 1.2% (n=1) of the isolates were resistant to a maximum of five antibiotics, 2.3% (n=2) were resistant to four and three antibiotics, 11.6% (n=10) were resistant to two antibiotics, and 17.4% (n=15) were resistant to only one antibiotic. Around 7% (n=6) of isolates from cattle group were Multi-drug resistant (MDR), with distribution pattern summarized in Table 5. All observed multidrug resistance patterns were recorded in a low frequency of 1.2% (n=1) for different antibiotic combinations.

Table 5: Phenotypic resistance profile of E. coli isolates from cattle fecal samples (n=86)

<table>
<thead>
<tr>
<th>Resistant phenotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resistance</td>
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<td>64</td>
</tr>
<tr>
<td>Resistant to only one antibiotic</td>
<td>15</td>
<td>17.4</td>
</tr>
<tr>
<td>Ampicillin, Tetracycline</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole</td>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>3</td>
<td>3.5</td>
</tr>
<tr>
<td>Chloramphenicol, Tetracycline</td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>Ampicillin, Ciprofloxacin, Tetracycline</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Chloramphenicol, Ciprofloxacin, Tetracycline</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Gentamicin, Trimethoprim/sulfamethoxazole, Chloramphenicol, Tetracycline</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Ciprofloxacin, Tetracycline</td>
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<td>1.2</td>
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<tr>
<td>Ampicillin, Gentamicin, Chloramphenicol, Ciprofloxacin, Tetracycline</td>
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<td>1.2</td>
</tr>
</tbody>
</table>
Ampicillin, Trimethoprim/sulfamethoxazole, Chloramphenicol, Ciprofloxacin, Tetracycline

On the other hand, the phenotypic antibiotic resistance profile in isolates from camels had a different pattern as illustrated in Figure 5. Only 2.2% (n=2) were resistant to three antibiotics, 7.7% (n=7) were resistant to two antibiotics, and 10.9% (n=10) were resistant to only one antibiotic. Antibiotic resistance to four, five and six antibiotics were not observed in camel isolates. As illustrated in Table 6, only two isolates (2.2%) showed multidrug resistance patterns for a combination of ampicillin; chloramphenicol; tetracycline, and a combination of ampicillin; trimethoprim/sulfamethoxazole; tetracycline.

Table 6: Phenotypic resistance profile of E. coli isolates from camels fecal samples (n=91)

<table>
<thead>
<tr>
<th>Resistant phenotype</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resistance</td>
<td>72</td>
<td>79.1</td>
</tr>
<tr>
<td>Resistant to only one antibiotic</td>
<td>10</td>
<td>11.0</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>Ampicillin, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Cephalothin</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Chloramphenicol, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Chloramphenicol, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>Ampicillin, Trimethoprim/sulfamethoxazole, Tetracycline</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
The frequency of resistance to different antibiotics in different localities for pigeons and cattle groups were cross-tabulated using Chi-square test. In pigeon group, as shown in Figure 6, only frequency in resistance to ampicillin, trimethoprim/sulfamethoxazole, and tetracycline was significantly different among the three localities (p<0.0001, <0.0001 and 0.0004, respectively).

![Figure 6. Phenotypic profile of antibiotic resistance E. coli isolates from pigeons across three localities.](image)

In dairy cattle group, a significant difference in resistance was reported only against ampicillin and trimethoprim/sulfamethoxazole, with p values (0.036, 0.009; respectively)
(Figure 7). Comparison between different localities for AMR resistance in *E. coli* isolates from camels have not been studied as all fecal samples obtained from the central slaughterhouse in Qatar.

![Phenotypic profile of antibiotic resistance *E. coli* isolates from Cattle across three localities.](image)

Figure 7. Phenotypic profile of antibiotic resistance *E. coli* isolates from Cattle across three localities.
V. DISCUSSION

The main idea of the “One Health” approach is that the health of humans and animals are closely linked and form one unity in a particular environment. Accordingly, knowledge of the AMR patterns in bacteria present in food-producing animals is essential to estimate the potential health risk on humans. In Qatar, many efforts have been pursued to control the antibiotics clinical use by implementing the antimicrobial stewardship program (AMSP) in general hospitals since April 2015 (Ribero Pombo MH & Thompson D, 2018). However, there is minimal control of antibiotics use in the veterinary sector. Further, there is a lack of data about the prevalence of antibiotic-resistant bacteria in food-producing animals. To fill this gap, we have recently started a surveillance study to estimate the prevalence of antibiotic-resistant bacteria in food-producing animals using commensal E. coli from fecal samples as an indicator. Globally, E. coli strains have become a common component of all AMR surveillance programs for their ability to acquire antimicrobial resistance genes and subsequently transfer them horizontally to other bacteria (Szmolka & Nagy, 2013).

In a recent study, we demonstrated a high level of antibiotics resistance of E. coli isolates from broiler chicken, including multidrug and colistin resistance (Nahla O. Eltai et al., 2018). This high level of resistance was associated with a low recovery rate of commensal E. coli from fecal samples (52%; 90/172). Using the same methodology, we achieved higher overall commensal E. coli recovery rate reaching 88.7% (266/300) from the three animal groups in this study: Cattle, camels, and pigeons.
In the chicken study, the overall resistance rate in commensal *E. coli* reached 90% for at least one antibiotic, with significant resistance to colistin (15.5%). In contrast, we observed a lower AMR profile in all livestock groups (70.7% in pigeons, 37.2% in cattle, and 20.8% in camels), presumably due to the different antibiotics use in different animal species industries.

Nonetheless, the high phenotypic AMR profile observed in pigeons for at least one antibiotic (70.7%) is a major finding. Further, we reported a remarkably high percentage (50%) of MDR, which is in agreement with the former study in broiler chickens (Nahla O. Eltai et al., 2018). MDR pattern in pigeons reached up to six antibiotic classes, mostly for a combination of ampicillin, trimethoprim/sulfamethoxazole, and tetracycline (20.2%). In part, the phenotypic AMR pattern in pigeons for the most prevalent antibiotics was similar to that reported in chickens; that is illustrated by high resistance to ampicillin (72.2%), trimethoprim-sulfamethoxazole (63.3%), and cephalothin (15.5%). Cumulatively, these findings suggest high use of the former three antibiotics in the poultry industry in Qatar. Unfortunately, we could not obtain information about the antibiotic practice in the three farms where pigeon samples were collected. Nonetheless, the high multidrug resistance of pigeon *E. coli* isolates is alarming as such birds can rapidly disseminate and spread the antibiotic-resistant bacteria species to various locations, affecting other animals and human health.

Furthermore, this study is the second report for colistin resistance in poultry birds in Qatar, where colistin is considered a drug of last resort in human medicine to treat infections with gram-negative bacteria. Although only one *E. coli* isolate from pigeon was found to have colistin resistance, such resistance could rapidly spread to other
environments considering it is plasmid mediated. Using PCR, we confirmed that this resistance was encoded by \textit{mcr-1} gene. Similarly, all colistin resistance in chicken were also \textit{mcr-1} encoded and were detected in about 15.5\% (n=14) of \textit{E. coli} isolates. It is worth noting that \textit{mcr-1} gene is the only gene reported in Qatar out of a total of eight genes reported globally. The prevalence of colistin resistance in the poultry industry, in particular, suggest the use of colistin as a prophylaxis agent in Qatar poultry industry. Interestingly, colistin resistance encoded by \textit{mcr-1} gene in addition to 15 other antibiotic resistance genes were detected in a virulent clone of \textit{E. coli} strain (MS8345), serotype O2:K1:H4, in Qatar (Forde et al., 2018).

On the other hand, the fecal samples collected from cattle were solely obtained from dairy cattle, where enough information about antibiotic usage was available, and thus, the correlation between antibiotic exposure and resistance development can be concluded. Considering the routine use of oxytetracycline and amoxicillin for treatment and control of multiple diseases, resistance frequency to tetracycline (27.9\%) and ampicillin (14\%) was expected. However, although gentamicin has also been reported to be used in the three cattle farms, the resistance against this antibiotic was only recorded in seven isolates (2.3\%).

Interestingly, the lowest AMR frequency was observed in \textit{E. coli} isolated from camel samples. Only 19 (20.8\%) isolates from camels showed resistance to at least one antibiotic, with overall resistance pattern recorded for only five antibiotics. Highest resistance was reported to tetracycline (15\%) and ampicillin (7\%). In contrast, a similar study from Tunisia higher frequency of resistance to tetracycline and ampicillin in \textit{E. coli} isolates from camels, reaching 52.8\% and 37.1\% (Bessalah et al., 2016). Moreover, a study
from KSA reported that 26.9% of *E. coli* isolates from camel fecal samples are ESBL producer (Fadlelmula et al., 2016). In agreement with our findings, another research group from Tunisia reported the absence of colistin resistance genes *mcr*-1 and *mcr*-2 in *E. coli* isolated from camel fecal samples in southern Tunisia territory (Rhouma et al., 2018).

Considering that Qatar is a relatively small county, we did not expect major changes in AMR profile between different localities. Expectedly, out of 18 clinically-relevant antibiotics used in this study, only the frequency of resistance to ampicillin and trimethoprim/sulfamethoxazole was significantly different among the three localities in pigeons (<0.0001, <0.0001; respectively) and cattle groups (0.036,0.009; respectively). Further, the resistance profile to tetracycline was also significantly different in pigeon samples collected from the three farms (0.0004). Although minor, the differences of AMR patterns between different localities reflects different antibiotic practices, and therefore, impose the need to assess and educate the herdsmen, veterinarians and farms owners about the judicious use of antibiotics in animals.

In contrast, all camel samples were collected from the central slaughterhouse in Qatar and hence, we could not compare the resistance profiles among different localities. On the other hand, having samples collected from the central slaughterhouse suggests different origins of these samples. Nonetheless, low AMR level observed.

This study revealed an overall low resistance pattern of *E. coli* isolates from large food animals. The *E. coli* isolates that did exhibit AMR for the panel we tested (18 clinically relevant antibiotics) are most likely resistant to the older set of antibiotics such as tetracycline, ampicillin, and trimethoprim-sulfamethoxazole (antibiotic used to treat a
variety of bacterial infections). The susceptibility of \textit{E. coli} isolates to all tested antibiotics, regardless of the animal origin, reached 57.5\% (153/266). Additionally, the absence of resistance to critical and highly important antibiotics including second, third and fourth generation, carbapenems, fosfomycin and no ESBL producing \textit{E. coli} was reported. These data suggest that control of antibiotics use in large food animals could be easily achieved, and hence, will help in reducing the burden of this globally important issue. On the other hand, control of antibiotics use in the poultry industry could be more difficult and requires prompt actions.

This study provides a snapshot and benchmark assessment of AMR patterns in livestock animals in Qatar using commensal \textit{E. coli} as an indicator. A further larger spatiotemporal analysis, including a higher number of farms, is needed. This study majorly focused on phenotypic profiling of AMR. Further characterization at the molecular level, including full genome/plasmid sequencing, is required. Such studies are now more important considering the rapid growth of livestock population in Qatar to overcome the blockade and reach self-sustenance. This should be also be paralleled with studying resistance pattern along the food chain, including farm workers and food handlers. Most importantly, this shall be accompanied with introducing antimicrobial stewardship program to control and monitor antibiotics usage.
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