Major diseases, extensive misuse, and high antimicrobial resistance of *Escherichia coli* in large- and small-scale dairy cattle farms in Jordan

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ABSTRACT

This study aimed to determine the major diseases, antimicrobial use, and resistance in commensal *Escherichia coli* in dairy cattle in Jordan. Forty-three (large, $n = 21$; small, $n = 22$) farms were surveyed. A validated questionnaire was administered to the herdsmen to elicit information about disease prevalence, antimicrobial knowledge, and antimicrobial use. In addition, fecal samples were collected from 5 lactating animals on each farm. A total of 520 *E. coli* isolates were tested for resistance to 12 antimicrobials. From the herdsmen's perspective, the diseases that require use of veterinary services in large and small production systems were mastitis (51.2%), metritis (51.2%), and enteritis (39.5%), and the most commonly used antimicrobials were oxytetracycline and streptomycin. Dairy herdsmen (83.7%) reported that it is easy to purchase antimicrobials without a veterinary prescription and 97.7% of them more frequently changed the antimicrobial drug rather than increasing the dose when presented with nonresponse to treatment. *Escherichia coli* isolates exhibited high resistance to streptomycin (47.5%), tetracycline (45.4%), and ampicillin (34.2%). Less than 10% of isolates were resistant to chloramphenicol, kanamycin, gentamicin, ciprofloxacin, and ceftriaxone. Overall, 64.6 and 37.1% of the *E. coli* isolates exhibited resistance to ≥ 1 antimicrobial and multidrug resistance (resistance to ≥ 3 antimicrobial classes), respectively. The isolates exhibited 107 antimicrobial resistance profiles. This study indicates that antimicrobials are frequently misused in dairies in Jordan and that resistance among commensal *E. coli* toward antimicrobials of human and veterinary importance is high. Therefore, educational programs for herdsmen and enacting regulations and

guidelines are necessary to promote the judicious use of antimicrobials in dairy animals in Jordan.

Key words: antibiotics, ruminant, rural, developing countries

INTRODUCTION

The emergence of antimicrobial resistant pathogens compromises the effectiveness of clinical treatment of human and animal infectious diseases and has major global public health and negative economic impacts (File et al., 2014; Friedman et al., 2016). Inappropriate antimicrobial use in human and veterinary medicine selects for resistance in bacterial pathogens, and some of these pathogens are zoonotic and can be transmitted from animals to humans (Leverstein-van Hall et al., 2011; Landers et al., 2012). Antimicrobial resistance is a worldwide problem and exists in both resource-rich and resource-poor settings. This problem, however, might be more severe in developing countries due to less-restricted availability and use of antimicrobials in food animal production (WHO, 2014).

Studying antimicrobial resistance in commensal bacteria is valuable as these bacteria can serve as reservoirs for resistance genes and can transfer these genes to pathogenic bacteria (DeFrancesco et al., 2004; EFSA, 2008). Antimicrobial resistance in commensal bacteria is positively correlated with local antimicrobial selective pressure and may be a risk for developing antimicrobial resistance in pathogenic bacteria (Hao et al., 2014). Thus, frequent monitoring of antimicrobial resistance in bacterial isolates from animals can help detect emerging antimicrobial-resistant phenotypes in commensal and pathogenic bacteria within the food production chain.

Concerns about antimicrobial resistance led to the launching of surveillance and monitoring programs in resource-rich countries such as the United States, Canada, Denmark, France, Sweden, and Norway (NORM/NORM-VET, 2010; RESAPATH, 2012; CIPARS, 2013; DANMAP, 2014; SWEDRES/SWEDVARM,

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2014; ECDC, 2015; NARMS, 2015). However, data on nonprescription antimicrobial treatment of livestock in resource-poor countries such as Jordan are lacking. Although Jordan relies on beef importation to satisfy its domestic meat demand, dairy cattle are a major component of the Jordanian animal industry and 2 dairy production systems exist in Jordan: large and small scale. Large intensive production systems use modern management practices with zero-grazing and minimum separation between lactating and nonlactating cows in the same farm. These farms are predominantly located in the Al-Dulail area, which produces around 50% of the country's milk (DoS, 2015). Small-scale production systems are scattered in different regions of Jordan, mainly in the Highland area. In this system, cows are housed in small, traditional brick barns, with no separation among cows, and herdsmen use more traditional management practices than in the large systems. Both systems raise Holstein-Friesian dairy cows and together contribute US\$160 million annually to the national gross domestic product (DoS, 2015).

To better define the use of antimicrobials in dairy production systems in Jordan, we surveyed dairy farms and sampled dairy cattle feces to investigate disease incidence and antimicrobial treatment practices and to estimate the prevalence of antimicrobial resistance among fecal *Escherichia coli*. In addition, we compared large and small dairy farms with respect to the above parameters.

MATERIALS AND METHODS

Farm and Animal Selection

Forty-three farms (21 large farms from intensive production systems in Al-Dulail area and 22 small farms from small-scale production systems in the Highlands) were selected and visited in 2015. Large production systems had >50 cows per farm, whereas the small-scale production systems had <50 cows per farm.

From each farm, 5 lactating cows that were 2 to 5 yr of age and had no antimicrobial treatment within the last 2 wk were eligible for inclusion and sampled. In each farm, the number of cows from a sampling frame of all cows meeting these inclusion criteria was divided by 5 (total number of cows/5 = k) and every k th cow was sampled. To prevent selection bias, neither individual disease history nor milk production performance was known for the sampled animals. From our pretest activities, we found that herdsmen were unwilling to allow researchers to sample more than about 5 cows because they did not want researchers to spend long periods of time on their farms.

Sampling Approach

In each area, designated and trained practicing veterinarians were recruited to assist with questionnaire data and sample collection because they have strong ties with the herdsmen (their clients), are from the same community, and speak the same dialect. This approach allowed access to the selected farms and helped ensure the accuracy of the questionnaire. To encourage voluntary participation from herdsmen, government veterinarians did not participate in the study. The herdsmen were briefed about the study objectives, assured that the study was only for research purposes, and that any generated data from their specific farm would be available to them upon request. Consent to conduct interviews and collect samples was obtained from the herdsman at each farm before beginning the study.

Questionnaire

A modified questionnaire based on Redding et al. (2014) was developed and used in this study to interview the herdsmen. The questionnaire was developed in Arabic, and then pretested with a convenience sample of herdsmen from 3 large and 3 small farms. Necessary revisions were made before the questionnaire was finalized. The repeatability of the questionnaire was examined by asking the same farmers the questions in 2 ways: during a face-to-face interview and by phone call. Then, repeatability was determined by calculating the agreement between answers to 4 questions: (1) What do you do for animals that receive antimicrobial treatment? (2) Do you obtain prescriptions before administering antimicrobials to your animals? (3) Does the veterinarian examine the diseased animals before prescribing antimicrobials? (4) Do you use antimicrobials for growth promotion? The kappa scores for each question were 0.94 (very good), 0.90 (very good), 0.86 (very good), and 0.83 (very good), respectively. The questionnaire was divided into 3 major sections to capture overall (1) farm management, (2) diseases of concern, and (3) antimicrobial use. The herdsmen were also asked to list the importance of several factors that they use before choosing an antimicrobial, including price, quality, brand, packaging, ease of acquisition, experience with the drug, and recommendation of the veterinarian or drug store vendor.

Commensal *E. coli* Isolation and Identification

Fecal samples (10 g) were collected with a clean rectal sleeve from the distal rectum of each individual cow

and placed into sterile containers that were labeled and kept on ice until delivered to the Food Hygiene Laboratories, Faculty of Veterinary Medicine, Jordan University of Science and Technology (Irbid) for analysis. Samples from 5 animals were taken from each farm and labeled with a unique identifier. A new rectal sleeve was used for each animal to prevent cross-contamination among the samples.

Escherichia coli was isolated from the fecal samples using a standard protocol as follows. Briefly, 10 g of each fecal sample was mixed with 225 mL of 0.1% peptone water. Then, 20 mL of the fecal mixture was mixed with 20 mL of double-strength MacConkey broth (Oxoid Ltd., Basingstoke, UK) and incubated at 35°C for 24 h. The broth culture was then streaked onto MacConkey agar (Oxoid) and incubated at 35°C for 24 h. Suspected *E. coli* (pink) colonies were streaked onto eosin methylene blue plates (Oxoid) and incubated at 35°C for 24 h. Metallic green colonies were considered presumptive *E. coli* and were streaked onto trypticase soy agar (Oxoid) and tested for indole and oxidase production. Suspect (indole-positive and oxidase-negative) isolates were confirmed by PCR targeting the *E. coli* translation elongation factor EF-Tu (*tuf*) gene using the same primers and cycling conditions as previously described (Maheux et al., 2009). Confirmed *E. coli* isolates were banked in trypticase soy broth with 20% buffered glycerol at -70°C for later antimicrobial resistance testing.

Antimicrobial Susceptibility Testing

Pure frozen cultures were grown on trypticase soy agar overnight at 37°C and adjusted to a 0.5 McFarland turbidity in 5-mL sterile normal saline tubes. Isolates were screened for susceptibility to a panel of 12 antimicrobials on Mueller-Hinton agar (Oxoid) by the disk diffusion method, as described by the National Committee for Clinical and Laboratory Standards (CLSI, 2014) guidelines. The following disks (Oxoid) were used: β -lactams (ampicillin, 10 μ g; amoxicillin-clavulanic acid, 30 μ g; cephalothin, 30 μ g; and ceftriaxone, 30 μ g), aminoglycosides (gentamicin, 10 μ g; streptomycin, 10 μ g; and kanamycin, 50 μ g); quinolones (ciprofloxacin, 5 μ g; nalidixic acid, 30 μ g), phenicols (chloramphenicol, 30 μ g), tetracyclines (tetracycline, 30 μ g), and folate pathway inhibitors (sulfamethoxazole-trimethoprim, 25 μ g). Isolates with intermediate susceptibility to the tested antimicrobials were considered "susceptible" for analysis purposes. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as reference strains for the disk diffusion method quality control. An isolate was considered resistant when it was

resistant to ≥ 1 antimicrobials and multidrug resistant when it was resistant to ≥ 3 classes of antimicrobials (Magiorakos et al., 2012).

Data Analysis

Data were entered in Excel (Microsoft Corp., Redmond, WA) and analyzed using MedCalc for Windows (version 17.9; MedCalc Software, Ostend, Belgium). Chi-squared and Fisher's exact tests were used as appropriate to assess the differences in each variable of interest (antimicrobial resistance outcome or questionnaire variables) independently between large and small farms (Pagano and Gauvreau, 2000; Deeks and Higgins, 2010). Odds ratios were used to determine the association between farm-level antimicrobial use and resistance among fecal *E. coli* isolates.

RESULTS

Disease Incidence and Use of Veterinary Services

The response rate for this study was 98%, with only one farmer declining to participate. Overall, 51.2% of the dairy herdsmen reported clinical mastitis, and the same number reported metritis as frequent animal health concerns that require veterinary service, followed by dystocia (41.9%), enteritis (39.5%), and respiratory infections (27.9%; Table 1). However, herdsmen in large dairy farms reported mastitis and metritis more than herdsmen in small farms and this was statistically significant ($P = 0.01$). Lameness, left-displaced abomasum, and bloat were also reported by herdsmen in large dairy farms, and dystocia was reported by 81.1% of herdsmen in small dairy farms (Table 1). We detected no significant differences between large and small farms in reporting enteritis and pneumonia. All of the dairy herdsmen reported using veterinary services; 19% of the large dairy farms and none of the small farms reported having a resident veterinarian (Table 1).

Acquisition of Antimicrobials

Antimicrobials were obtained from 3 sources in Jordan: veterinary drug stores, Ministry of Agriculture (MOA) offices, and practicing veterinarians. Among the herdsmen ($n = 43$) interviewed, 79.1% reported obtaining antimicrobials from veterinary drug stores, 27.9% from field veterinarians, and 16.3% from MOA offices. However, one large farm reported obtaining antimicrobials directly from the manufacturers. In addition, 83.7% of herdsmen reported that it was easy to purchase antimicrobials without a veterinary

Table 1. Response (percentages) of dairy herdsmen to questions regarding veterinary services for specific diseases in Jordan by production system in 2015¹

Practice/response	Large system (n = 21)	Small system (n = 22)	All farms (n = 43)	P-value
Do you use veterinary services?	100	100	100	0.98
When do you use veterinary services?				
Mastitis	71.4	31.8	51.2	0.01
Metritis	71.4	31.8	51.2	0.01
Enteritis	38.1	40.9	39.5	0.65
Pneumonia	33.3	22.7	27.9	0.44
Left-displaced abomasum	38.1	0	18.6	0.03
Lameness	33.3	0	16.3	0.04
Bloat	28.6	0	14.0	0.05
Dystocia	0	81.8	41.9	0.00
Do you have a resident veterinarian on the farm?	19.0	0	9.3	0.11
Does the veterinarian examine the diseased animals before prescribing antimicrobials?				
Always	47.6	45.5	46.5	0.89
Sometimes	52.4	54.5	53.5	0.89
Do you obtain prescriptions before administering antimicrobials to your animals?				
Always	42.6	22.7	32.6	0.16
Usually	57.1	77.3	67.4	0.16

¹Percentages do not sum to 100% in a given column or row because farmers reported >1 disease.

prescription. Among herdsmen, 46.5% reported that the veterinarian always examined the animals before antimicrobial treatment, and 32.6% reported that they always obtained a prescription. However, 53.5% of the herdsmen reported that their veterinarian sometimes examined the animal before antimicrobial treatment and 67.4% reported that they usually obtained prescriptions before purchasing antimicrobials (Table 1).

The herdsmen considered different factors when purchasing antimicrobials but, in most cases (60.4%), price

was the most important factor, followed by veterinary recommendations, and then experience. Packaging was the least important factor considered.

Antimicrobial Use

The most commonly used antimicrobials on the dairy farms were oxytetracycline, penicillin G with streptomycin, gentamicin, and sulfamethoxazole-trimethoprim. Less commonly used antimicrobials were amoxicillin,

Table 2. Antimicrobials used (in descending percentages) for specific diseases in dairy farms in Jordan by production system in 2015¹

Disease	Used antimicrobial	Large system	Small system	All farms	P-value
Mastitis	Oxytetracycline	87.5	35.3	60.6	0.00
	Amoxicillin	81.3	58.8	69.7	0.17
	Gentamicin	37.5	47.1	42.4	0.58
	Penicillin-streptomycin	12.5	5.9	9.1	0.55
	Amoxicillin-clavulanic acid	12.5	0	6.1	0.26
	Spectinomycin	6.3	5.9	6.1	0.96
	Marbofloxacin	18.8	5.9	12.2	0.28
Metritis	Ceftiofur	81.8	50.0	70.5	0.18
	Tylosin	40.0	33.3	35.3	0.90
	Oxytetracycline	0	16.7	5.9	0.28
	Gentamicin	0	16.7	5.9	0.28
Enteritis	Marbofloxacin	0	16.7	5.9	0.28
	Sulfamethoxazole-trimethoprim	100	95.0	94.1	0.66
	Marbofloxacin	7.7	10.0	2.9	0.82
Pneumonia	Oxytetracycline	0	5.0	2.9	0.66
	Oxytetracycline with tylosin	37.5	19.0	27.0	0.22
	Tylosin	12.5	0	5.4	0.21
	Marbofloxacin	50.0	19.0	32.4	0.05
	Oxytetracycline	0	42.9	24.3	0.03
	Tilmicosin	6.25	0	2.7	0.39
	Tulathromycin	0	4.8	2.7	0.60

¹Percentages do not sum to 100% in a given column or row because some farmers might report >1 antimicrobial. Percentages equal the number of times the antimicrobial was used divided by the number of times the disease was reported.

Table 3. Percentages of specific antimicrobial uses in sampled Jordanian dairy farms by production system in 2015

Practice/response	Large system (n = 21)	Small system (n = 22)	All farms (n = 43)	P-value
Which antimicrobials do you use for growth promotion?	23.8	9.1	16.3	0.21
Oxytetracycline	14.3	0	3	0.17
Sulfamethoxazole-trimethoprim	14.3	0	3	0.17
Amoxicillin	4.7	0	1	0.47
Yeast	0	4.7	1	0.51
Oxytetracycline with multivitamin	0	4.7	1	0.51
Do you use antimicrobials for disease prevention?	81	100	90.7	0.11
Yes, for dry cow therapy	76.2	27.3	51.2	0.00
Yes, when other animals on the farm are sick	4.8	100	53.5	0.00

tylosin, marbofloxacin, and ceftiofur. Use of tilmicosin, alamycin, and lincomycin/spectinomycin was rarely reported (Table 2).

The dairy cattle diseases most commonly treated with antimicrobials on Jordanian dairy farms were mastitis, metritis, enteritis, and respiratory diseases. Mastitis was treated with intramammary infusions of amoxicillin (69.7%), followed in frequency by parenteral administration of oxytetracycline (60.6%) and gentamicin (42.4%). Oxytetracycline was used significantly more frequently ($P \leq 0.05$) on large than on small farms (Table 2). In general, metritis was primarily treated with ceftiofur (75% of the cases) and in some cases with tylosin (37.5%). Enteritis was treated with antimicrobials and in most cases by sulfamethoxazole-trimethoprim (94.1%). Respiratory diseases, including pneumonia, were treated with antimicrobials; the 3 major antimicrobials used were oxytetracycline (24.3%), oxytetracycline with tylosin (27.0%), and marbofloxacin (32.4%). Other antimicrobials were rarely used in treating the 4 major diseases outlined in Table 2.

Antimicrobials were used primarily for therapeutic purposes; however, a few (16.3%) of the herdsmen reported using antimicrobials for growth promotion in calves, including oxytetracycline (7.0%) and sulfa-

methoxazole-trimethoprim (7.0%). The latter practice was significantly more frequent in the large dairy farms than on the small farms. On the other hand, 90.7% of the herdsmen reported the use of antimicrobials for prevention, specifically for dry cow therapy (51.2%), and prophylactically when other animals are sick in the farm (53.5%; Table 3). Large dairy farms in Al-Dulail practiced dry cow therapy (76.2%) significantly more frequently than the farms in small-scale production systems (27.3%). Interestingly, all farms in the small-scale production systems reported administering antimicrobials to healthy cows prophylactically when other cows were sick; however, very few herdsmen reported this practice in the large production systems (Table 3).

Management of Antimicrobial Treatments

Most herdsmen (74.4%) reported marking recently treated animals for identification, but this was significantly more frequent among large farms than small farms. In general, 40.9% of herdsmen reported milking treated animals last. Most herdsmen (97.7%) reported changing the antimicrobial, rather than increasing the dose, when it did not appear to be effective before completing the treatment course. Most (95.3%) herdsmen

Table 4. Percentage of antimicrobial administration practices in sampled dairy cattle farms in Jordan by production system in 2015

Practice/response	Large system (n = 21)	Small system (n = 22)	All farms (n = 43)	P-value ¹
What do you do for animals that receive antimicrobials?				
Visibly marked	90.5	59.1	74.4	0.03
Milked last	9.5	40.9	25.6	0.03
If an antimicrobial does not work, what do you do?				
Change the antimicrobial	95.2	100.0	97.7	0.47
Increase the dose	4.8	0	2.3	0.47
What do you do with milk of cows that receive antimicrobials?				
Feed to calves for 3 to 5 d	90.5	100.0	95.3	0.27
Throw away for 4 d	9.5	0	4.3	0.27

¹Fisher's exact test.

Table 5. Antimicrobial resistance percentage (in descending order) among *Escherichia coli* isolated from dairy cattle fecal samples in Jordan in 2015, by production system

Antimicrobial (breakpoint, mm)	Large system (n = 207)	Small system (n = 313)	All farms (n = 520)	P-value ¹
Streptomycin (≤ 11)	54.1	43.1	47.5	0.01
Tetracycline (≤ 11)	45.5	45.4	45.4	0.99
Ampicillin (≤ 13)	28.9	37.7	34.2	0.04
Cefalothin (≤ 14)	20.8	29.4	26.0	0.03
Amoxicillin-clavulanic acid (≤ 13)	14.0	21.7	18.7	0.03
Sulfamethoxazole-trimethoprim (≤ 10)	23.7	9.9	15.4	0.00
Nalidixic acid (≤ 13)	13.0	11.5	12.1	0.60
Chloramphenicol (≤ 12)	11.1	7.7	9.0	0.18
Kanamycin (≤ 13)	10.1	7.7	8.6	0.33
Gentamicin (≤ 12)	5.8	2.9	4.0	0.10
Ciprofloxacin (≤ 15)	4.8	3.4	3.8	0.46
Ceftriaxone (≤ 19)	2.4	1.9	2.1	0.70
Resistant to ≥ 1 antimicrobial	69.1	62.0	65.6	0.10
Resistant to ≥ 3 classes of antimicrobials	36.7	37.4	37.1	0.88

¹Chi-squared P-value.

also reported feeding the milk of treated cows to calves for 3 to 5 d rather than discarding it (Table 4).

Antimicrobial Resistance of Commensal *E. coli*

All farms, both large and small, had at least one animal carry antibiotic-resistant *E. coli*. However, 89.5 and 89.1% of individual animals in large and small farms; respectively, shed antibiotic-resistant *E. coli*. A total of 520 *E. coli* isolates were confirmed by PCR and tested for antimicrobial resistance. The most frequent resistance phenotypes among the *E. coli* isolates in this study included resistance to streptomycin (47.5%), tetracycline (45.4%), and ampicillin (34.2%). Fewer than 10% of isolates were resistant to chloramphenicol, kanamycin, gentamicin, ciprofloxacin, or ceftriaxone.

Overall, 65.6% of *E. coli* isolates exhibited resistance to at least one antimicrobial and 37.1% of the isolates exhibited multidrug resistance (Table 5).

Several unique multidrug resistance (MDR) profiles (n = 107) were exhibited by the *E. coli* isolates—78 and 68 profiles from large- and small-scale production systems, respectively. Thirty-nine profiles were shared between the 2 production systems and are presented in Supplemental Table S1 (<https://doi.org/10.3168/jds.2017-13665>). Overall, the most common resistance profiles were cephalothin (n = 39), streptomycin (n = 37), ampicillin-amoxicillin-clavulanic acid-tetracycline-streptomycin (n = 23), and ampicillin-tetracycline-streptomycin (n = 23). Diverse resistance profiles were detected within the same farm and by size of farm (supplementary table). Moreover, we found no clear

Table 6. Association between farm-level (n = 43) antimicrobial use and resistance among fecal *Escherichia coli* isolates (n = 520) in dairy farms in Jordan¹

Antibiotic	Farms (no.) with resistant isolates		Farms (no.) without a resistant isolate		P-value	Odds ratio (95% CI)
	Using antimicrobial	Not using antimicrobial	Using antimicrobial	Not using antimicrobial		
Ampicillin	0	39	0	4	0.29	0.11 (0.002–6.47)
Amoxicillin-clavulanic acid	2	30	0	11	0.69	1.89 (0.08–42.32)
Cefalothin	0	35	0	8	0.48	0.24 (0.004–12.95)
Ceftriaxone	1	9	3	30	0.93	1.11 (0.10–12.04)
Tetracycline	40	3	0	0	0.24	11.57 (0.20–677.08)
Sulfamethoxazole-trimethoprim	31	6	5	1	0.98	1.03 (0.10–10.50)
Streptomycin	22	20	1	0	0.55	0.37 (0.01–9.49)
Kanamycin	0	26	0	17	0.84	0.66 (0.01–34.86)
Gentamicin	11	6	17	9	0.96	0.97 (0.27–3.50)
Ciprofloxacin	0	17	0	26	0.84	1.51 (0.03–79.93)
Nalidixic acid	0	31	0	12	0.65	0.40 (0.01–21.12)
Chloramphenicol	0	29	0	14	0.73	0.50 (0.01–26.05)

¹Where zeros caused problems with computation of the odds ratio or its standard error, 0.5 was added to all cells (a, b, c, d in the 2 × 2 table) (Pagano and Gauvreau, 2000; Deeks and Higgins, 2010).

association between the use of and microbial resistance to a given antimicrobial (Table 6).

DISCUSSION

This study provides critical information regarding the antimicrobials used in Jordanian dairies. The most common diseases treated with antimicrobials in these dairies (in descending order) were mastitis, metritis, and enteritis. Interestingly, some of these diseases; specifically enteritis, can be caused by pathogens other than bacteria, such as parasites and viruses. Redding et al. (2014) reported the use of antiparasitics and antibiotics for treating diarrhea cases in small farms in Peru, but noted that the antiparasitics might be used to treat bacterial and viral diarrhea causes, which would be ineffective and exacerbate the drug-resistant fascioliasis in Peru. Mastitis in Jordanian dairies is treated with oxytetracycline, amoxicillin, and sometimes gentamicin, which is a critically important antimicrobial for human medicine (WHO, 2012) and not approved for use in dairy cattle in the United States (Smith et al., 2005). Metritis is mostly treated with ceftiofur, which is the same as in the United States (Galvão, 2012). The use of this antimicrobial is approved and has no milk withdrawal period and a short or no meat withdrawal period (Galvão, 2012). Although foot rot is a problem in US dairy cattle (Sawant et al., 2005), it was not reported to be a problem in Jordanian dairies, which is likely due to the drier climate.

This study showed that a limited number of antimicrobial classes are used in Jordanian dairies; and generally, oxytetracycline and streptomycin (critically important antimicrobials) are the most commonly used. These 2 antimicrobials are the most frequently used in the United States and Japan as well (Sawant et al., 2005; Harada et al., 2006; Parveen et al., 2006), and oxytetracycline is the most commonly used in small farms in Peru (Redding et al., 2014). Tetracyclines, sulfonamides, and β -lactams are the most commonly used in Europe (NORM/NORM-VET, 2010; DANMAP, 2014; Merle et al., 2014; SWEDRES/SWEDVARM, 2014). Other studies in resource-poor countries reported a similar limited number of antimicrobial classes for treating animals in Costa Rica, Sudan, and Kenya (Roderick et al., 2000; Luna-Tortos et al., 2006; Eltayb et al., 2012).

Antimicrobial use in lactating cows could lead to residues in milk, which can have adverse public health, dairy product manufacturing, and economic effects. This risk can be reduced by marking, milking last, and discarding the milk of treated cows following the recommended withdrawal period for each antimicrobial (Spiegel et al., 2013). In our study, 74.4% of herds-

men marked their treated animals and 25.6% milked those animals last. Although this finding suggests that herdsmen are following correct practices, they might do this because their milk cannot be used to make fermented dairy products (antimicrobial residues slow the fermentation process by inhibiting the starter culture). However, most herdsmen used a shorter withholding period of 3 to 5 d, which is less than the mandated withdrawal period for the widely used antimicrobial oxytetracycline. Herdsmen from small farms sell their milk to small dairy shops, which are numerous and scattered throughout the country. These dairy shop owners generally have little knowledge of the adverse effect of antimicrobial residues on human health; rather, they focus on whether the milk ferments or not. The large dairy farms, however, sell their milk to large dairy plants that check physical characteristics of the milk, including specific gravity, pH, fat percentage, and, sometimes, antibiotic residues for antibiotics that are important from the point of view of processing. Most herdsmen (95.3%) fed milk from treated cows to other calves on the farm for 3 to 5 d after treatment. A similarly high use of this practice was reported in England and Wales, where 65.4% of herdsmen fed mastitic milk to calves (Brunton et al., 2014). The same scenario was reported in Sweden, where 56 and 79% of herdsmen fed milk to calves during the course of antimicrobial treatment and during the milk withdrawal period, respectively (Duse et al., 2013). This practice is associated with shedding cefotaximase (*CTX_M*)-positive *E. coli* for a long period in calves (Brunton et al., 2014), as well as shedding quinolone-resistant *E. coli* (Duse et al., 2015).

The frequently used antimicrobials in dairy farms in Jordan are of veterinary importance (OIE, 2015); however, some herdsmen (6%) reported using spectinomycin, which is not recommended for dairy cattle older than 20 mo because the withdrawal period of this antimicrobial has not been established (FDA-CVM, 2015).

Jordanian dairy herdsmen reported changing an antimicrobial when an animal did not respond to it during the course of treatment. Switching antimicrobials has been described in Peru as well (Redding et al., 2014), where 64.6% of the herdsmen administered a different antimicrobial and 16.9% increased the dose when an animal did not respond to initial treatment. The practice of using antimicrobials below or above the recommended doses was also reported in dairies in Switzerland (Gonzalez et al., 2010) and in veal calves in Belgium (Pardon et al., 2012).

In our study, a high percentage (64.6%) of the *E. coli* isolates exhibited resistance to at least one antimicrobial. In contrast, lower percentages (<20%) were reported in dairy cattle of resource-rich countries including the United States, Germany, and Denmark (Lundin

et al., 2008; Kaesbohrer et al., 2012; DANMAP, 2014). In the current study, the MDR profiles included 2 common profiles; resistance to ampicillin, tetracycline, and streptomycin (23 isolates = 21.5% of the MDR isolates) and resistance to these 3 antimicrobials plus sulfamethoxazole-trimethoprim (12 isolates = 11.2% of the MDR isolates), amoxicillin-clavulanic acid (23 isolates = 21.5% of the MDR isolates), and amoxicillin-clavulanic acid and cephalothin (14 isolates = 13.1% of the MDR isolates). The ampicillin-tetracycline-streptomycin resistance pattern has been reported by other researchers in different locations (White et al., 2000; Lim et al., 2007; Alexander et al., 2010; Kaesbohrer et al., 2012). This pattern might be associated with frequent exposure to minimal levels of tetracycline, often in combination with chloramphenicol (Blake et al., 2003), and linked to transferable high-molecular-weight plasmids (Karczmarczyk et al., 2011).

Almost half of the isolates (45.4%) exhibited resistance to tetracycline in this study. Several studies in multiple worldwide locations reported frequent resistance to tetracycline among *E. coli* isolates (Teshager et al., 2000; Sayah et al., 2005; Lim et al., 2007; Lundin et al., 2008). The extensive use of tetracycline in human and veterinary medicine is attributed to their low cost, having few side effects and growth-promotion properties (Chopra and Roberts, 2001). Using tetracycline to promote growth is performed in Jordan dairies for treating calves and is proven to augment tetracycline resistance (Alexander et al., 2010; Love et al., 2011; Marshall and Levy, 2011; Mirzaagha et al., 2011). The use of antimicrobials for growth promotion is banned in European countries (Maron et al., 2013). Moreover, the reported use of ceftiofur in Jordanian dairies can contribute to the high resistance to tetracycline because the use of ceftiofur can co-select for and facilitate co-amplification of *E. coli* that exhibit co-resistance to tetracycline (Lowrance et al., 2007; Alali et al., 2009).

Considerable resistance to chloramphenicol was found in this study, despite no reported use of this antimicrobial in dairy farms. Similar findings were reported in Japan (Lim et al., 2007) and Korea (Harada and Asai, 2010). This might be attributed to co-selection of resistant determinants from using other antimicrobials (Bischoff et al., 2002; Harada and Asai, 2010) and to co-resistance with quinolone and nalidixic acid (Bialek et al., 2010). In addition, resistance to tetracycline can be transferred in *E. coli* due to the genetic linkage among their resistance genes (Bischoff et al., 2002).

A moderate proportion (26%) of the isolates exhibited resistance to cephalothin (a first-generation cephalosporin) but fewer (3.8%) exhibited resistance to ceftriaxone (a third-generation cephalosporin). The resistance to ceftriaxone, despite no reports of its use

in dairy farms in this study, might be attributed to the wide use of ceftiofur—using ceftiofur can co-select for resistance to ceftriaxone (Tragesser et al., 2006). This led the US Food and Drug Administration to limit extra-label use of cephalosporin antimicrobials in cattle, chickens, swine, and turkeys (FDA-DHHS, 2012). Therefore, continuous monitoring for resistance to third- and fourth-generation cephalosporins is warranted in Jordan because these agents are critically important antimicrobials in human medicine (WHO, 2012) and their resistance can be disseminated through the transfer of mobile genetic elements from animals to humans.

Resistance to fluoroquinolones was relatively infrequent (ciprofloxacin = 3.8%) among the *E. coli* dairy cattle isolates in this study but high enough to cause public health concern as this class of antimicrobials is of medical importance. Similarly low percentages (less than 2%) of fluoroquinolone resistance were reported in Korea, Italy, Finland, and Sweden (Harada and Asai, 2010) and none were reported in Canada, France, Denmark, Germany, and Australia (Harada and Asai, 2010). The reported resistance to fluoroquinolones in the current study might be attributed to the extensive use of tetracycline because resistance to fluoroquinolones in multi-antimicrobial resistance isolate can be co-selected by the use of tetracyclines (Asai et al., 2007). Moreover, due to their critical importance in human medicine (WHO, 2012), the use of fluoroquinolones in veterinary medicine is decreasing in several countries and banned in other countries (Cheng et al., 2012; Garcia-Migura et al., 2014).

This study showed no clear association between the use of and resistance to a given antimicrobial. However, our sample size was relatively small, we were unable to determine recent antimicrobial use, and it was not possible to have a control farm because there are no farms in Jordan with no or minimal antimicrobial use. Nonetheless, our findings corroborate previous studies that did not find significant associations between antimicrobial use and resistance toward a named antimicrobial. Rather, antimicrobial use can be a contributing factor but probably not the main or single factor causing antimicrobial resistance (Bergman et al., 2009; Oliver et al., 2011). In our study, the reason why resistance did not correlate with use of an antibiotic could be that the animals selected had not received treatment in the previous 2 wk. Several other studies have found that the resistance induced by use is transient (Jiang et al., 2006; Lowrance et al., 2007; Checkley et al., 2008; Platt et al., 2008; Mann et al., 2011; Morley et al., 2011; Boyer and Singer, 2012). Moreover, because herdsmen choose antimicrobials based on experience, we assume that they would refrain from using antimicrobials with

which they previously experienced a perceived treatment failure. Thus, an association between antimicrobial use and resistance might be difficult to detect.

CONCLUSIONS

Our research indicated that antimicrobials are misused in Jordanian dairies; specifically, antimicrobial agents are administered without a veterinary prescription and recommended withdrawal times are not adhered to. Furthermore, resistance among commensal *E. coli* toward antimicrobials of human and veterinary importance was high compared with that in other countries. To address this misuse and emerging resistance, it is essential to promote judicious use of antimicrobials in livestock operations, through educational programs for herdsmen and veterinarians conducted by the Ministry of Agriculture, veterinary association committees, and academic institutions. Immediate interventions that should be considered in Jordan include the use of cost-effective and simple antimicrobial sensitivity testing before treating animals. Banning the use of critically important antibiotics for human health has been successfully implemented in several countries and should be considered in Jordan.

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