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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-Dulial 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 kth 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