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Antimicrobial use and resistance among commensal *Escherichia coli* and *Salmonella enterica* in rural Jordan small ruminant herds

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ABSTRACT

Small-scale small ruminants' farming is the major system in poor resources settings, yet their farming practices largely unknown. This paper assessed the husbandry practices, antimicrobial use and antimicrobial resistance of commensal *Escherichia coli* and *Salmonella enterica* in sheep and goat pastoralists in rural Jordan. Fifty-two sheep and goat farmers were interviewed concerning disease incidence, antimicrobial use and knowledge of antimicrobials. *E. coli* and *Salmonella* were isolated from freshly passed fecal pellets by standard methods, confirmed by molecular methods, and tested for resistance against 12 antimicrobial by the disc diffusion method. Interview results indicated that a limited variety of antimicrobial drugs (oxytetracycline, penicillin and tylosin) are used by small ruminant farmers in Jordan. Moreover, farmers store the antimicrobials at improper temperatures and frequently obtain antimicrobials without prescription; veterinary consultation prior to antimicrobial use is infrequent. Higher antimicrobial resistance than most worldwide similar studies was exhibited by the isolates: 67.7% and 76.9% of the *E. coli* and *Salmonella* isolates, respectively, exhibited resistance to at least one antimicrobial and 33.3% and 38.5% exhibited resistance to at least three classes of antimicrobials. Among all bacterial isolates, the most frequent resistance was to tetracycline and cephalothin; resistance to ceftriaxone, gentamicin, and ciprofloxacin was rare. In general, *E. coli* exhibited higher resistance percentages than *Salmonella* for the tested antimicrobials. This study shows that upgrading the role of veterinarian and improving antimicrobial use practices at the grassroots level through educating farmers on proper handling and judicious use of antimicrobials are essential, as many antimicrobials are critically important for treating human infections.

1. Introduction

Antimicrobial resistant pathogens significantly impact both human and animal health because they are difficult to treat and they have been associated with higher virulence than susceptible pathogens (Da Silva and Mendonca, 2012). Human infections with antimicrobial-resistant zoonotic pathogens have been attributed to use of antimicrobials in the animal reservoir (Smet et al., 2010; Landers et al., 2012; Robinson et al., 2016). Inappropriate or overuse of antimicrobials probably contributes to the emergence and dissemination of antimicrobial resistance in bacteria (Witte, 1998; McEwen and Fedorka-Cray, 2002). This risk might be augmented in the future due to the anticipated increase in antimicrobials use in livestock production (Garcia-Migura et al., 2014; Van Boeckel et al., 2015). This likely to happen as the level of antimicrobials used strongly and positively correlates to the resistance levels these antimicrobials agents in commensal *E. coli* (Chantziaras et al., 2014). Data on the epidemiology of antimicrobial resistance in humans and animals in Jordan are sparse. Data from the Antimicrobial Resistance Surveillance & Control in the Mediterranean Region (ARMed) Project collected between 2003 and 2008 revealed high levels of resistance to four classes of antimicrobials among invasive strains of *E. coli* collected from blood cultures and cerebrospinal fluid in five Jordanian hospitals (Borg et al., 2008). In addition, there is a scarcity of literature to document antimicrobial usage in small ruminant husbandry, particularly in pastoralist husbandry. Publications describing the prevalence of resistance in *E. coli* or other bacteria isolated from animals in Jordan, or describing the prevalence of non-prescription antimicrobial treatment of livestock, are also lacking.

A reported high burden of disease in humans from potentially zoonotic pathogens such as non-typhoid *Salmonella enterica*

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and *Brucella* spp. (Gargouri et al., 2009) suggests that zoonotic transmission of antimicrobial resistant bacteria may be important. Anecdotal evidence suggests that the existence of using antimicrobials without a prescription in livestock in rural Jordan (Dr. Dayyat, personal communication). Because of the potential zoonotic transmission of antimicrobial resistant pathogens, the extent of antimicrobial use in livestock, particularly in the absence of veterinary supervision, the development of antimicrobial resistance may be of concern.We interviewed small ruminant herd owners in rural Jordan to determine the prevalence of this practice, and obtained fecal samples to determine whether antimicrobial use without a veterinary prescription may be associated with antimicrobial resistance in small ruminant fecal *E. coli* and *Salmonella enterica* isolates.

2. Materials and methods

2.1. Farms selection

This study was conducted to cover all regions and governorates of Jordan by visiting herdsmen randomly while grazing their animals or at their farms. The studied farms included 15 farm in Southern Jordan (Tafela, Maan, and Karak), 18 farm in Northern Jordan (Irbid and Jarash), 13 farm in the Badia (Mafrqa) and 6 farms in the Jordan Valley. The approached herdsmen were informed that the purpose of the study is for scientific research, their participation is completely voluntary and their decision to participate or not will not affect their right of future veterinary services. All approached herdsmen agreed to participate in the study.

Interviews were performed in fifty-two farms and fecal sampling was obtained from fourteen farm. The questionnaires were administered in Arabic by a trained veterinarian whose original dialect is Bedouin. The questionnaire was divided into four sections: 1) information on the farms and animals, 2) farm management and animal care, and 3) disease incidence, antimicrobial use and knowledge of antimicrobials. Questions included numbers of animals, animal feeding and housing management systems, frequency of diseases (diarrhea, respiratory diseases, abortion, abscesses, mastitis, and mortalities), whether veterinary care was ever used and reasons for seeking veterinary care, sources and access to antimicrobials and when they used antimicrobials. When possible, photographs of antimicrobial drug vials, including the expiration date, were obtained to supplement the interview information.

2.2. Bacterial isolation and identification

Freshly passed fecal samples (approximately 10 fecal pellets/sample) were randomly collected off the ground and placed into sterile containers which were labeled and stored on ice until return to the laboratory at the Jordan University of Science and Technology (JUST) for bacterial isolation, identification and antimicrobial agent susceptibility testing.

Fecal samples were cultured for *E. coli* and *Salmonella enterica* using standard protocols as follows (Wang et al., 2011). All incubations were conducted at 35 °C for 24 h and all media were manufactured by Oxoid Ltd. (Hampshire, England) and purchased through a local supplier (Al-Sami Tech Supplies Company, Amman, Jordan). Feces (5 g) were mixed with 45 ml of 0.1% buffered peptone water and mashed completely using sterile wooden spatulas. Fecal suspension (20 ml) was added to 20 ml of double-strength MacConkey broth and incubated. The resulting broth culture (100 μl) was streaked onto MacConkey agar. After incubation, pink to red colonies (one per sample) were transferred to eosin methylene blue plates and incubated. Presumptive *E. coli* colonies (dark center with a green metallic sheen; five per sample) on eosin methylene blue agar were subcultured on Trypticase soy agar (TSA Indole-positive and oxidase-negative isolates were maintained in Trypticase soy broth (TSB) with 20% glycerol at − 20 °C. Suspect *E. coli* isolates were confirmed by PCR targeting the *E. coli* translation elongation factor EF-Tu (*tuf*) gene. Genomic DNA was extracted by the boiling method (Kawasaki et al., 2005). The chosen *E. coli*-specific PCR primers were TEcol553 (5′-TGG GAG CGA AAA TCC TG-3′) and TEcol754 (5′-CAG TAC AGG TAG ACT TCT G-3′) (Integrated DNA Technologies, Coralville, IA, USA) (Maheux et al., 2009). Confirmed *E. coli* isolates were stored in TSB with 20% glycerol at − 20 °C.

Salmonella was isolated following a previously described method (Lestari et al., 2009) with some modifications. All incubations were conducted at 35 °C for 24 h unless otherwise specified. Briefly, 20 ml of the fecal suspension described above was pre-enriched for 6 h under shaking at 100 rpm. Enriched broth culture (10 ml) was transferred to 100 ml of tetrathionate broth and incubated at 42 °C. The tetrathionate broth culture was then streaked onto xylose lysine Tergitol 4 (XLT4) plates. Suspect *Salmonella* colonies (entirely black or pink to red with black centers; 3 per sample) were transferred to lysine iron agar, triple sugar iron and urea agar slants. Isolates with typical *Salmonella* phenotypes on the slants were confirmed by PCR to test for the *invA* gene using *inv*A-139 5′-GTG AAA TTA TCG CCA CGT TCG GGC AA-3′ and *inv*A-1341 5′-TCA TCG CAC CGT CAA AGG AAC C-3′ (Rahn et al., 1992). Confirmed *Salmonella* isolates were stored in TSB with 20% glycerol at − 20 °C.

2.3. Antimicrobial agent susceptibility testing

Isolates were screened for susceptibility to a panel of 12 antimicrobials on Mueller-Hinton agar (Oxoid Ltd.) by the disk diffusion method (Clinical and Laboratory Standards Institute, 2012). The following disks (Oxoid) were used: Am (ampicillin; 10 μg), Amc (amoxicillin–clavulanic acid; 30 μg), Cef (cephalothin; 30 μg), Cro (ceftriaxone, 30 μg), C (chloramphenicol; 30 μg), Cip (ciprofloxacin; 5 μg), Nal (nalidixic acid; 30 μg), Gm (gentamicin; 10 μg), S (streptomycin; 10 μg), Sxt (sulfamethoxazole–trimethoprim; 25 μg), Te (tetracycline; 30 μg), and K (kanamycin; 30 μg). To avoid zones overlap, four antimicrobials were tested for each Muller Hinton agar plate. The zones of inhibition around each disc was measured after 18 h of incubation at 35 ± 2 °C. 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 antimicrobial disk control. An isolate was defined as resistant if it was resistant to one or more of the agents tested, whereas isolates resistant to three or more antimicrobial classes were classified as MDR (Magiorakos et al., 2012).

2.4. Data analysis

Data were entered, stored and analyzed using Microsoft Excel (Redmond, WA, USA). Proportion of antimicrobial resistant among *E. coli* and *Salmonella enterica* isolates were compared using the Chi-square test or Fisher's exact test when appropriate. These statistics were calculated using WINPEPI (Abramson, 2011).

3. Results

3.1. Antimicrobial use

The majority of farmers reported that they understood the definition and use of antimicrobials. The majority of farmers also