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Antimicrobial resistant and extended-spectrum ß-lactamase (ESBL) producing *Escherichia coli* isolated from fecal samples of African dromedary camels

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ABSTRACT

This study was conducted to determine the distribution of antimicrobial resistance among *Escherichia coli* isolated from feces of healthy dromedary camels in Kenya. A total of 162 fecal samples were cultivated for *E. coli*. Samples were also subcultivated to detect *E. coli* with extended-spectrum ß-lactamases (ESBLs). Antimicrobial susceptibility testing (AST) was performed by disk diffusion using a panel of 16 antimicrobials. In addition, isolates were screened for the presence of the plasmid-mediated colistin resistance genes *mcr-1* to *mcr-5*.

Samples from 20 (12.4%) of the camels contained antimicrobial resistant (AMR) *E. coli*, and 85% of the AMR isolates were multidrug resistant (MDR). The highest frequency of resistance was observed to tetracycline (11.7%), followed by ampicillin and streptomycin (both 10.5%), and sulfamethoxazole/trimethoprim (9.9%). Two (1.2%) of the isolates showed intermediate resistance to cefazolin and streptomycin, respectively. All the isolates were susceptible to amoxycillin/clavulanic acid, ciprofloxacin, fosfomycin, aztreonam and kanamycin, and 86.4% of the isolates were susceptible to all 16 antimicrobials used in this study. The prevalence of fecal carriage of ESBL producing *E. coli* was 0.6%. PCR and amplicon sequencing showed that the ESBL producer belonged to *E. coli* phylogenetic group A, sequence type (ST) 48, and harbored $bla_{CTX-M-15}$. None of the isolates contained *mcr* genes. The results indicate that dromedary camels in Kenya may be reservoirs of AMR *E. coli,* including ESBL producers, that could potentially be transmitted to humans by direct contact or via the food chain.

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Introduction

Antimicrobial resistance (AMR) represents one of the major threats to public health, affecting the well-being of humans, animals, and the environment [1]. AMR is a challenge that requires international and multidisciplinary cooperation using a One Health approach [2]. Within this approach, targeted research is essential to identify reservoirs of AMR and demonstrate potential transmission routes within and between the human-animal-environmental interfaces.

Escherichia coli, natural inhabitants of the intestinal tracts of humans and mammals, potentially represent a major reservoir of AMR and play an important role in acquisition and the dissemination of AMR mechanisms [3]. Livestock and food producing animals have emerged as important hosts for AMR *E. coli*. Fecal carriage of AMR *E. coli* poses a potential health hazard, either through direct transmission of bacteria between animal hosts and humans, or indirectly through transmission of resistance genes by means of horizontal transfer [4]. The widely disseminated resistance to critically important third generation cephalosporins, and the recently emerged resistance to the last resort antimicrobial colistin are of particular concern to both human and animal health alike [5–7].

The number of reports of AMR *E. coli* isolated from healthy livestock, including strains harboring plasmid-mediated resistance genes such as extended-spectrum ß-lactamase (ESBL) genes belonging to the bla_{CTX-M} group, or colistin resistance *mcr* genes [4,8–10], is increasing worldwide. By contrast, for Africa, information on the prevalence of AMR in healthy husbandry animals is still scarce and there is a lack of a molecular studies of the clonal lineages of *E. coli* associated with the spread of bla_{ESBLs} and other resistance genes [11,12]. In East Africa, the one-humped Arabian camel (*Camelus dromedarius*) is one of the most important livestock species among nomadic pastoral communities [13,14]. Whilst camels are acknowledged as a reservoir for pathogens such as *Coxiella burnetii, Brucella* spp., the hepatitis E virus, and Middle East respiratory syndrome coronavirus [15–18], data on AMR in healthy camels in Africa are very restricted [19].

In response to this knowledge gap, this study aimed to evaluate the distribution of antimicrobial resistance among *Escherichia coli* isolated from camels in Eastern Africa by screening fecal samples of a herd of healthy animals kept under extensive husbandry systems on a ranch in Laikipia, Kenya. In addition, the prevalence of *E. coli* harboring bla_{ESBL} and *mcr* genes was investigated.

Material and methods

In a previous study, the occurrence of Shiga toxin-producing *Escherichia coli* (STEC) among grazing dromedaries from Kenya was investigated [20]. Fecal samples had been collected between during January and February 2017 from camels located in a ranch in the northern Laikipia county in Kenya. Fecal matter was collected by rectal palpation using one glove per animal. Although efforts were made to select both genders of camels in the herd, a truly random sampling was not possible due to logistical constraints, for example compliance of the animals. Samples were placed in sterile containers, cooled for transport and stored at $-80\,°C$ until analyses. For this study, 162 samples (from 116 females, 45 males and one of unknown gender) were analyzed.

Each fecal sample was enriched at a 1:10 ratio in *Enterobacteriaceae* enrichment (EE) broth (Becton, Dickinson, Heidelberg, Germany) for 24 h at 37 °C. For nonselective isolation of *E. coli*, the enrichment was spread on RAPID'*E. coli* agar (Biorad, Munich, Germany). One *E. coli* colony randomly selected from each agar plate was collected for further analysis. For selective isolation of ESBL producing *Enterobacteriaceae*, one loopful of each of the enrichment cultures was streaked onto Brilliance ESBL agar plates (Oxoid, Hampshire, UK). Plates were incubated under aerobic conditions for 24 h at 37 °C.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing was performed using the disk-diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute [21]. Antibiotics included ampicillin (AM), amoxicillin with clavulanic acid (AMC), azithromycin (AZM), cefazolin (CZ), cefepime (FEP), cefotaxime (CTX), chloramphenicol (C), ciprofloxacin (CIP), fosfomycin (FOS), gentamicin (G), kanamycin (K), nalidixic acid (NA), nitrofurantoin (F/M), streptomycin (S), sulfamethoxazole/trimethoprim (SXT), and tetracycline (TE) (Becton Dickinson, Allschwil, Switzerland). Results were interpreted according to CLSI performance standards [21]. For azithromycin, an inhibition zone of ≤ 12 mm was interpreted as resistant. Isolates displaying resistance to three or more classes of antimicrobials (counting ß-lactams as one class) were defined as multidrug-resistant (MDR).

Detection of antimicrobial resistance genes

DNA was extracted by a standard heat lysis protocol [22] and analysed by PCR for the presence of antimicrobial resistance genes. Synthesis of primers and DNA custom sequencing were carried out by Microsynth (Balgach, Switzerland). Purification of amplification products was performed using a PCR purification kit (Qiagen, Courtaboeuf, France).

The identification of bla_{ESBL} genes in ESBL producing *E. coli* was established by PCR and sequencing as described previously [23–25]. In brief, specific published primers were used to search for ß-lactamase encoding genes belonging to bla_{TEM}, bla_{SHV} [26], and the three bla_{CTX-M} alleles belonging to groups 1, 2 and 9, as described by Woodford et al. [24], using amplification conditions as described previously [24,26].
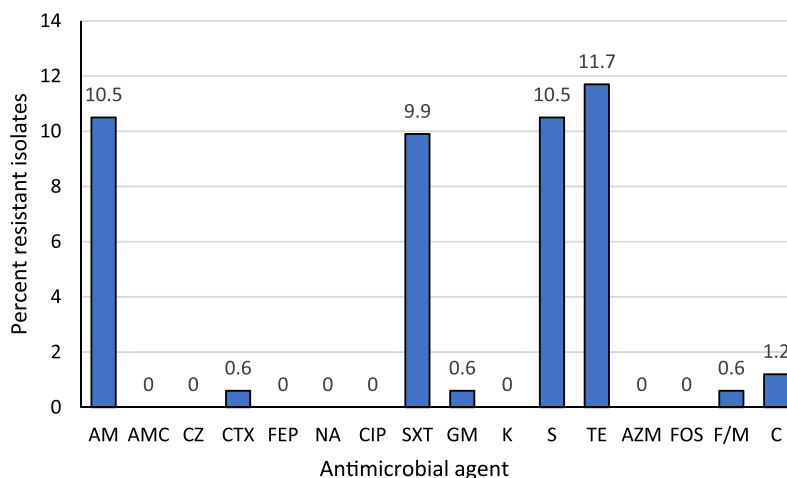
Fig. 1. Distribution of antimicrobial resistance among 162 *Escherichia coli* isolated from feces of healthy camels in Kenya. Numbers above columns indicate percentage of isolates.

AM, ampicillin; AMC, amoxicillin/clavulanic acid, AZM, azithromycin; C, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CZ, cefazolin; FEP, cefepime; F/M, nitrofurantoin; FOS, fosfomycin; GM, gentamicin; K, kanamycin; NA, nalidixic acid; S, streptomycin; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline.

Purified amplicons were custom sequenced using primers form up- and downstream bla_{CTX-M} flanking regions as noted by Geser at al. [23].

Screening by PCR for *mcr-1* through *mcr-5* was undertaken as described by Rebelo et al. [27], using a multiplex PCR protocol that specifically amplifies the fragments of the five *mcr* genes, which range in size from 320 bp (*mcr-1*) to 1644 bp (*mcr-5*) [27].

Nucleotide sequences were analysed with CLC Main Workbench 6.6.1. Database searches were performed using the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/).

Phylogenetic characterization of ESBL producing E. coli

E. coli can be classified into one of the eight phylogroups A, B1, B2, C, D, E, F (*E. coli sensu stricto*), or *Escherichia* clade I, whereby commensal strains mainly belong to phylogroups A and B1, and virulent extraintestinal *E. coli* strains to group B2 and, to a lesser extent, to group D [28]. Phylogenetic grouping was performed by using quadruplex PCR as described by Clermont et al. [28]. In brief, the presence or absence of the four gene targets *arpA, chuA, yjaA* and *TspE4.C2* was established, using primers and amplification conditions described previously [28]. Phylogroup assignment was carried out according to the scheme described by Clermont et al. [28].

Multilocus sequence typing (MLST) of ESBL producing E. coli

For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven housekeeping genes *adk, fumC, gyrB, icd, mdh, purA,* and *recA* were amplified by PCR, using primers and conditions described by Wirth et al. [29]. Amplicons were sequenced and allele numbers were obtained by comparing with corresponding alleles available in the *E. coli* MLST database website (https://enterobase.warwick.ac.uk), to determine MLST types and clonal complexes (CCs).

Results and discussion

By nonselective cultivation, 162 *E. coli* isolate were obtained for AST. Thereof, 140 (86.4%) were fully susceptible to all 16 antimicrobials used in this study, and two (1.2%) showed intermediate resistance to cefazolin and streptomycin, respectively (supplementary Table 1). All isolates were susceptible to amoxycillin/clavulanic acid, ciprofloxacin, fosfomycin, aztreonam and kanamycin (supplementary Table 1).

AMR *E. coli* were isolated from 20 (12.4%) of the samples. Resistance to tetracycline was detected most frequently (11.7%), followed by ampicillin and streptomycin (both 10.5%), and sulfamethoxazole/trimethoprim (9.9%). Resistance to all other antimicrobial agents were detected at rates between 0% and 1.2%. MDR was identified in 17 (85%) of the 20 resistant *E. coli* isolates, corresponding to 10.5% of the total of 162 *E. coli*. The distribution of antimicrobial resistance among the 162 isolates is shown in Fig. 1, and the resistance profiles of the AMR *E. coli* are summarized in Table 1.

There is a scarcity of previous data on AMR in camels in Africa to compare our findings with. However, a previous study from Tunisia showed that among *E. coli* isolated from fecal samples of camel calves, the ampicillin/tetracycline resistance phenotype was most prevalent, corresponding to the most commonly used antibiotics in that country [30]. For Kenya,

Table 1

Antimicrobial resistance profiles of 20 *Escherichia coli* isolates with AMR isolated from 162 fecal samples of dromedary camels in Kenya.

No. (%) of AMR isolates ($n = 20$)	Resistance profile	Prevalence (%) among fecal samples ($n = 162$)
14 (70)	AM, SXT, S, TE	8.6
2 (10)	AM, SXT, S, C, TE	1.2
1 (5)	AM, S, TE	0.6
1 (5)	CTX	0.6
1 (5)	F/M, TE	0.6
1 (5)	GM, TE	0.6

AM, ampicillin; CTX, cefotaxime; F/M, nitrofurantoin; C, chloramphenicol; GM, gentamicin; S, streptomycin; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline.

recent reports on the consumption of antimicrobials in animals are not available, however, the tetracycline and sulfamethoxazole/trimethoprim resistance phenotypes observed in this study correlate with data reported from 1997 to 2001 that show that tetracyclines, sulfonamides and trimethoprim are the most frequently used antimicrobials in food-producing animals in Kenya [31]. Notably, the AST results for the antimicrobials were interpreted using breakpoints relevant for the administration in humans and thus may not be reflective of susceptibility when applied to camels. Nevertheless, the results presented in this study provide the possibility to monitor trends in antimicrobial susceptibility and to identify emerging resistance. Accordingly, quantitative data such as the zone diameter values provided in supplementary Table 1 could be helpful.

Among the 20 camels with AMR *E. coli*, 17 (85%) were female, and three (15%) were male, amounting to 14.6% of the 116 female and 6.7% of the 45 male camels included in this study, respectively. Accordingly, fecal carriage of MDR *E. coli* was confirmed for 12.9% of the female and 6.7% of the male camels, respectively. The relatively high prevalence of AMR isolates among female camels is noteworthy. The frequency of ampicillin, streptomycin and sulfamethoxazole/trimethoprim resistance may be reflective of the usage of antimicrobials commonly used to treat mastitis in camels. In contrast to cattle, mastitis in camels is treated systemically with penicillin/aminoglycoside or trimethoprim–sulfamethoxazole [32]. However, for this study, no data on the use of antimicrobials was available, and further studies would be required in order to correlate the use of antimicrobials with the fecal carriage of AMR in camels.

By selective cultivation, one ESBL producing *E. coli* was detected, amounting to 0.6% of the 162 samples. Molecular characterization assigned the isolate to phylogenetic group A and sequence type (ST) 48 and identified the ESBL-encoding gene $bla_{CTX-M-15}$.

The ESBL producer was identified in a fecal sample of a female camel, corresponding to a fecal carriage rate of 0.6% among the 162 camels and 0.9% of the female camels.

ESBL producing *E. coli* from feces of healthy camels have very rarely been described in Africa. So far, only one CTX-M-1 producing *E. coli* isolated from a food producing dromedary camel in Tunisia has been described [19]. While CTX-M-1 is a livestock associated ESBL, CTX-M-15, which was identified in *E. coli* ST48 in this study, has emerged worldwide as one of the most important ESBLs in human medicine [10]. Although the phylogenetic group A and the sequence type of *E.coli* ST48 does not indicate high pathogenicity [28,29], it is to be noted that CTX-M-15 producing *E. coli* ST48 have been reported to cause nosocomial urinary tract infections in Belgium [33] and have been detected among human isolates in hospitals in Nigeria [34]. Furthermore, *E. coli* ST48 belongs to clonal complex 10 which is associated globally with the dispersion of CTX-M type ESBLs [35].

Although the prevalence of ESBL producing *E. coli* among the camels analyzed in this study is low, this observation is important, since such isolates could be transmitted to humans. Indeed, ESBL producing *Enterobacteriaceae* have been detected previously in raw and in fermented camel milk in Kenya [36]. Fecal carriage of ESBL producers in camels may contribute to such contaminations in non-industrialized milk production.

The emergence of plasmid-mediated colistin resistance is most probably related to the use of colistin in animal husbandry [6]. Monitoring colistin resistance is important, since the *mcr* gene has been detected in food producing animals worldwide, including Africa [37]. In our study, screening for plasmid-mediated colistin resistance genes *mcr-1* through *mcr-5* remained negative for all isolates (data not shown). The absence of plasmid-mediated colistin resistance confirms previous reports from Tunisia and suggests that camels do not play a major role as a reservoir for *mcr* harboring *E. coli* [38]. However, although we screened for all known variants at the time of analysis, novel *mcr* genes are emerging globally, and it cannot be excluded that variants including *mcr-6* through *mcr-8* may have been missed in this study. A further limitation of this study is the sampling scheme which may have led to bias associated with operational circumstances, e.g., sampling of more manageable animals. This may have resulted in an overrepresentation of samples originating from female camels.

In conclusion, our results indicate that dromedary camels in Kenya may be reservoirs of *E. coli* exhibiting resistance to antimicrobials commonly used in humans and animal medicine. Furthermore, camels may carry ESBL producing *E. coli* that could potentially be transmitted to humans by direct contact or via the food chain.

Declaration of Competing Interest

None to declare.

CRediT authorship contribution statement

Magdalena Nüesch-Inderbinen: Formal analysis, Writing - original draft. **Melinda Baschera:** Data curation, Formal analysis, Investigation. **Anne Liljander:** Data curation, Investigation. **Jörg Jores:** Project administration, Writing - review & editing. **Victor Max Corman:** Project administration, Writing - review & editing. **Roger Stephan:** Supervision, Visualization, Writing - review & editing.

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Ethics statement

The sample collection was performed in accordance with the United Kingdom's Animals (Scientific Procedures) Act 1986 that contains guidelines and codes of practice for the housing and care of animals used in scientific procedures, and with the Kenyan legislation for animal experimentation. The procedure was approved of by the institutional animal care and use committee (IACUC reference number 2014.08).

Disclosures

The authors report no conflict of interest. All authors have read and approved the final article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2020.e00274.

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