Screening for fecal presence of colistin-resistant *Escherichia coli* and *mcr-1* and *mcr-2* genes in camel-calves in southern Tunisia

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**Abstract**
Camels (*Camelus dromedarius*) are known to harbor multidrug resistant Gram-negative bacteria and to be involved in the transmission of various microorganisms to humans. Data on the occurrence of colistin resistant *Escherichia coli* as well as mobilized colistin resistance (*mcr*) genes in camels are lacking. We investigated the presence of colistin resistance and *mcr* (1–2) genes in *E. coli* from the feces of camels in Tunisia. Presumptive *E. coli* isolates from camel-calves in southern Tunisia were qualitatively screened for growth on Mueller–Hinton agar supplemented with 2 mg/L of colistin. The minimal inhibitory concentration of colistin was determined for isolates growing on this medium. All isolates were screened for the presence of the *mcr-1* and *mcr-2* genes by polymerase chain reaction without detecting any of these genes. However, one isolate was confirmed resistant to colistin and further testing of this isolate revealed it to be *Enterobacter cloacae*. Our study demonstrated absence of colistin resistance and of the *mcr-1* and *mcr-2* genes in *E. coli* isolated from camel feces in southern Tunisia. Thus, there is no evidence that camels represent a major source of *mcr* genes contamination for the local population or for tourists visiting southern Tunisia.

**Keywords:** Camel, Colistin resistance, *E. coli*, Human, MCR

**Findings**
One-humped camels (*Camelus dromedarius*) are raised extensively in southern Tunisia for the production of meat and milk as well as for activities related to Sahara tourism. One-humped camels are considered part of the cultural heritage and represents a national wealth for many countries [1]. Camel-calves have a high susceptibility to bacterial infections, particularly those caused by *Escherichia coli* [1–3]. In fact, colibacillosis in young camelids results in considerable economic losses in camel farms, being associated with mortality, growth retardation and medical treatment costs [1, 4]. The absence of antimicrobials approved to treat bacterial infections in dromedaries poses a major challenge for veterinarians working in this animal production [5]. The use of antimicrobials approved for ruminants, horses or other animal species in the treatment of sick camels has not been associated with satisfactory results because of the physiological particularities of this animal species [5]. Colistin sulfate (CS), a polypeptide antibiotic, is used off-label in Tunisia for the oral treatment of *Enterobacteriaceae* infections in camel-calves at the dose of 25,000 IU/kg twice a day for 3 consecutive days. Following the recent identification of a stable plasmid mediated mobilized colistin resistance (*mcr-1*) gene encoding for resistance to colistin in *Enterobacteriaceae*, this “novel” mechanism of colistin resistance has been widely studied in bacteria isolated from several origins such as farm animals, humans, food and the environment [6, 7]. In July and August 2017, we searched PubMed with the terms...
“colistin in dromedary or colistin in camels”, “colistin resistance in dromedary or in camels”, “MCR-1 in dromedary or in camels” and “mcr genes in dromedary or in camels”. However, no studies on fecal presence of plasmid-mediated colistin resistance genes (mcr-1 to -5) or E. coli colistin resistance in camels were found. On the other hand, it was recently reported that camels are a potential source of human contamination with multidrug-resistant (MDR) bacteria such as extended-spectrum beta-lactamase (ESBL) producing E. coli or Pseudomonas aeruginosa [5, 8]. Moreover, co-localisation of mcr-1 and ESBL genes on a unique plasmid has been reported [9].

The present study aimed (1) to determine the fecal prevalence of E. coli colistin resistance in camel-calves with and without diarrhea in Tunisia, and (2) to determine the prevalence of mcr-1 and mcr-2 genes among both colistin resistant and susceptible E. coli isolates.

In a previous study, the distribution of virulence genes, pathotypes, serogroups, phylogenetic groups, and antimicrobial resistance of presumptive E. coli isolated from camel-calves with and without diarrhea in Tunisia was investigated [2]. Fecal samples had been collected between January 2011 and April 2013 from 25 extensive camel farms located in three districts in southern Tunisia: Kebili (n = 13), Gabes (n = 2), and Medenine (n = 10) (Fig. 1) with an average flock size of 75 animals. Rectal swabs from 120 camel-calves aged between 1 and 3 months, with or without diarrhea, were suspended in buffered peptone water solution. Selected dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were plated on MacConkey agar. The plates were incubated aerobically for 24 h at 37 °C. Colonies growing on MacConkey agar with a morphology typical for E. coli (red or pink, non-mucoid colonies) were tested by polymerase chain reaction (PCR) for the β-glucuronidase (uidA) housekeeping gene, and PCR-positive colonies obtained (n = 52) were considered as presumptive E. coli isolates (Table 1) [10]. These isolates were stored at −80 °C until further analysis.

After thawing, the 52 isolates were inoculated on Mueller–Hinton agar supplemented with CS at the concentration of 2 mg/L. Isolates demonstrating growth were considered as possibly colistin-resistant and were examined for minimum inhibitory concentration (MIC) of colistin, using the broth microdilution method in technical duplicate according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) [11]. Enterobacteriaceae with colistin MICs > 2 mg/L were defined as resistant (R), and those with colistin MICs ≤ 2 mg/L as susceptible (S) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

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guidelines (http://www.eucast.org/clinical_breakpoints/). Isolates with colistin MICs > 2 mg/L were further identified using the API 20E system (bioMérieux, Quebec City, Canada).

Bacterial DNA extraction was performed using the Chelex® method (Bio-Rad, ON, Canada). All isolates were screened by PCR for the presence of *mcr*-1 and *mcr*-2 genes, using primers and conditions as previously described [12, 13]. DNA from *E. coli* strains harbouring either *mcr*-1 gene [14] or *mcr*-2 gene [13] were used as positive control.

More than half of the presumptive *E. coli* isolates (55%) (n = 28) in both groups of animals were identified as possible colistin resistant (Fig. 2). However, only one of these isolates was confirmed to be resistant to colistin with an MIC value of 4 mg/L (Table 1). This resistant isolate, recovered from a camel-calf with diarrhea, was identified as *Enterobacter cloacae*.

Neither the *mcr*-1 nor *mcr*-2 gene were detected in any of the colistin resistant or susceptible isolates.

It is important to monitor colistin resistance in dromedary herds in Tunisia as the *mcr*-1 gene is highly prevalent in food-producing animals (such as chickens) in this country [15]. In addition, Tunisia being an important tourist destination [16], close contact between

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**Table 1 Distribution of possibly colistin resistant isolates according to their origin; camel-calves with or without diarrhea**

| Health status of camel-calves | Number of isolates | Species (number) | Colistin resistant isolates (number) | MIC of colistin value (mg/L) |
|------------------------------|--------------------|-----------------|-------------------------------------|-----------------------------|
| No diarrhea                  | 23                 | *E. coli* (n = 23) | 0                                  | –                           |
| Diarrhea                     | 29                 | *E. coli* (n = 28) | 0                                  | –                           |
|                              |                    | *E. cloacae* (n = 1) |                                    | 4                           |

*Escherichia coli, Enterobacter cloacae*

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**Fig. 2** Distribution of minimum inhibitory concentrations (MICs) in *E. coli* isolates possibly resistant to colistin (n = 28) recovered from camel-calves with and without diarrhea
camels and tourists visiting southern Tunisia may result in spread of resistant bacteria internationally.

Our finding that colistin-resistance and the mcr genes were absent in E. coli isolated from camel-calves in southern Tunisia suggest that dromedaries are not a major source of contamination with these genes for inhabitants as well as for tourists visiting this region. It should be noted that colistin-susceptible isolates were included in the current study, as previous studies have reported the identification of mcr-1 gene among colistin-resistant and colistin-susceptible isolates [6, 17].

We report here for the first time the isolation of E. cloacae resistant to colistin from a diarrheic camel. However, this isolate did not harbor mcr-1 or mcr-2 genes. Similarly, colistin-resistant E. cloacae isolates with or without the mcr-1 gene have been found in healthy people or primary care patients [18–20]. Currently, the mechanism of colistin resistance in E. cloacae strains in the absence of plasmid-borne colistin resistance genes (mcr-1 to -5) remains poorly understood. Zhong et al. [21] recently identified 17 genes in the chromosome and one gene in pASM1 encoding efflux pump proteins in a highly colistin-resistant E. cloacae strain. Nevertheless, mechanisms of colistin resistance not associated with plasmids in Enterobacter spp. require further investigation.

It should be emphasized that we had no information regarding the medical treatment history of the sampled camel-calves, specifically, whether or not colistin has been used in these dromedary farms. Additional studies are needed in order to assess the spread of colistin-resistant bacteria and mcr genes among enteric bacteria of camel origin worldwide. Future surveys should take into consideration other areas where dromedary density is very high, such as the Arabian Peninsula where the mcr-1 gene has been identified in E. coli isolates [22].

In the present study, the number of isolates obtained was limited because of the difficulty of handling camel-calves in extensive farms. However, we believe that our sampling was representative of dromedary production in Tunisia, as fecal samples were obtained from districts containing the highest density of dromedary farms.

As expected, the use of Mueller–Hinton agar supplemented with 2 mg/L of CS overestimated the frequency of presumed colistin-resistant bacteria. But the culturing on this medium was only used as a screening method for reducing the number of isolates to be tested for colistin MIC. In addition, because of the poor diffusion of colistin in agar, it was always recommended to confirm presumed colistin-resistant isolates, growing on colistin-containing agar, by colistin CMI determination [23, 24].

The results suggest that camel-calves do not represent a major source of mcr gene contamination for the local population or for tourists visiting southern Tunisia. Nevertheless, it highlights the need to widen the scope of monitoring colistin resistance to other Enterobacteriaceae species than those conventionally identified in veterinary diagnostic laboratories, such as E. cloacae. Moreover, with the global initiatives for the establishment of a One Health antimicrobial resistance surveillance approach, camels should be also considered as an important animal species in the tracking of resistant bacteria at the human, animal and environment interface.

Abbreviations
CLSI: Clinical and Laboratory Standards Institute; CS: colistin sulfate; ESBL: extended-spectrum beta-lactamase; EUCAST: European Committee on Antimicrobial Susceptibility Testing; DNA: deoxyribonucleic acid; MDR: multidrug resistant; MICS: minimal inhibitory concentrations; PCR: polymerase chain reaction.

Authors’ contributions
MR, SB, IS and PF planned and designed the study. MR and WT carried out the microbiological and molecular biological tests. MR, PF, and JMF analyzed and interpreted the data. MR drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All data generated or analysed during this study are included in this published article. The colistin resistant bacterial strain is available from the authors upon reasonable request.

Ethics approval and consent to participate
Not applicable.

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