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mcr-1 encoding colistin resistance in CTX-M-1/CTX-M-15- producing *Escherichia coli* isolates of bovine and caprine origins in Tunisia. First report of CTX-M-15-ST394/D *E. coli* from goats

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ABSTRACT

The objective of this study was to isolate and characterize ESBL-producing *Escherichia coli* (ESBL-EC) from raw bovine and caprine milk samples, as well as from bovine faeces in Tunisia. Therefore, 120 bovine faecal samples and 9 caprine raw milk samples were collected from 2 extensive dairy-cow-farms and 5 ovine farms, respectively. In addition, 94 raw bovine milk samples, from containers and holding tanks from 50 small public-markets in the North of Tunisia, were processed for the isolation of cefotaxime-resistant *E. coli* (CTX^R). Antimicrobial susceptibility testing was carried out by disc-diffusion/broth-microdilution methods. The presence of genes encoding ESBL, as well as those encoding colistin (*mcr*-1 to 5 genes)- sulfonamide-, tetracycline-, gentamicin-, quinolone and chloramphenicol-resistance and class 1 integrons were tested by PCR (and sequencing in some cases). ESBL-EC isolates were further characterized by phylogrouping and MLST/PFGE typing. Eight samples (3.6%) contained ESBL-EC isolates (3/2 from raw bovine/goat milk and 3 from cattle faeces) and one isolate/sample was characterized. Four ESBL-EC isolates, all of bovine origin (3 faeces/1 milk), were resistant to colistin (MIC: 8–16 µg/ml), harboured the *mcr*-1 gene and carried IncP- and IncFIB-type plasmids. The 8 ESBL-EC strains had the following characteristics: a) bovine faeces: *mcr*-1/CTX-M-1/D-ST1642 (3 strains); b) raw milk: *mcr*-1/CTX-M-1/A-ST10 (1 strain); CTX-M-15/B1-ST394 (3 strains), and CTX-M-15/A-ST46 (1 strain). Most of bovine ESBL-EC isolates were multidrug-resistant (4/5). Our results showed that ESBL-EC were detected in bovine and caprine samples (CTX-M-1/CTX-M-15 producers), being some of them colistin-resistant (associated with *mcr*-1 gene), and they belonged to international clonal lineages.

1. Introduction

Worldwide, there are increasing problems with multiresistant bacteria [1]. The use of a wide variety of antimicrobials in human medicine, veterinary clinics, livestock industries and aquaculture has resulted in the emergence of antimicrobial resistance across different bacterial groups and against all antibiotic classes [2]. The use of antimicrobial agents for therapeutic or prophylactic purposes in animals could end up in antimicrobial residues or the emergence of antimicrobial resistant bacteria in animal products (meat or milk) or residual products (as faeces) [3,4]. Nowadays, the emergence and wide spread of extended-spectrum beta-lactamases (ESBL)-*Enterobacteriaceae* especially *Escherichia coli* from animal origins or food products of animal origin are a cause of concern [5,6]. The limitation of the treatment options for infections caused by such ESBL-producing isolates was solved by the use of a former antibiotic employed in veterinary medicine since the 1960s in food-producing animals [7]. Actually, colistin is being used again in human medicine [8]. Recently, *mcr*-1 gene has designated the first plasmid-mediated colistin resistance gene to occur worldwide [9,10]. Newly, eight novel plasmid-mediated colistin resistance gene variants (*mcr*-2/9) have been reported [11]. In this study, we screened ESBL-producing *E. coli* (ESBL-EC) isolates collected from caprine and bovine samples for antimicrobial resistant phenotype and genotype characterization.

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2. Materials and methods

2.1. Sampling and isolation of bacteria

During the period 2017–2018, a total of 120 bovine faecal samples were collected from two extensive dairy farms (Mateur and Bousalem regions) and 9 raw goat milk samples from 5 medium-size caprine farms (10 to 20 lactating goats by farm) (Nabeul region) in the North of Tunisia. In addition, 94 raw bovine milk samples were collected from containers and holding tanks (Bulk tank milk) from 50 small public markets from various towns in the North of Tunisia. For bacteria enrichment, 25 g of faeces samples and 10 mL of milk samples were added to 225 mL and 90 mL of brain-heart infusion (BHI) broth (Oxoid Ltd., Basingstoke, UK), respectively, and incubated overnight at 37 °C. Then, 1 mL from each sample was inoculated on Trypton Bile X-glucuronide (TBX) Agar (Oxoid Ltd., Basingstoke, UK) supplemented with 2 mg/L cefotaxime (TBX-CTX), and was incubated overnight at 37 °C for recovery of cefotaxime-resistant *E. coli* (potential producers of ESBLs and acquired plasmid-mediated AmpC ß-lactamases). For all samples, one colony per sample showing typical morphology of *E. coli* was selected and isolated on TBX-CTX agar to obtain a pure culture. Isolates were identified using MALDI-TOF/MS (Bruker Daltonik, Bremen, Germany) [12].

2.2. Antimicrobial susceptibility and detection of ESBLs production

All the isolates were processed for antimicrobial susceptibility testing by the disk-diffusion method on Mueller-Hinton agar (Oxoid Ltd.) plates, according to the recommendations of Clinical Laboratory Standard Institute [13]. The Minimal Inhibitory Concentration (MIC) for colistin was determined by broth microdilution method [13], and all isolates with an MIC value of ≥ 4 μg/mL were considered as colistin-resistant (COL^R).

Double Disc Synergy Test (DDST) was used to detect ESBL production, as recommended by CLSI [13]. *E. coli* ATCC 25922 was used as a control strain for the susceptibility tests and *K. pneumoniae* ATCC 700603 as control strain for ESBL production [14].

2.3. Detection of resistance genes

Genomic DNA was extracted by boiling method [15]. Genes encoding beta-lactamases (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) were amplified as previously reported [16]. Amplified DNA fragments were sequenced on both strands. To determine ESBLs, the nucleotide and their deduced amino acid sequences were compared with those included in the GenBank database to confirm the specific type of β-lactamase gene. The presence of *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 genes was tested by PCR in all ESBL-producing isolates, as previously described [17,18]. Genes encoding resistance to tetracycline [*tet*A and *tet*B], sulphonamide [*sul*1 and *sul*3], gentamicin [*aac* (3)-I, *aac*(3)-II, and *aac*(3)-IV], chloramphenicol [*cml*A], and ciprofloxacin [*qnr*A, *qnr*B, *qnr*S, and *aac*(6´)-*Ibcr*] were also investigated by PCR [19,20]. The *qnr*S variant was determined by sequencing of PCR products.

2.4. Detection and characterization of integrons

The presence of *int1* gene encoding class 1 integrase was examined by PCR for trimethoprim/sulfamethoxazole-resistant isolates. The variable region of class 1 integrons was studied by PCR and sequencing in all *int*1-positive isolates [21].

2.5. Phylogenetic groups, Pulsed-field gel electrophoresis (PFGE) and Multilocus-sequence typing (MLST)

Phylogenetic grouping was determined for the ESBL-producing isolates according to Clermont et al. [22]. Internal fragments of seven conserved housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*) were amplified by PCR and sequenced. A detailed scheme of the MLST procedure, including the primers, PCR conditions, allelic type and sequence type assignment methods, is available at MLST database website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). PFGE was performed according to Sáenz et al. [19].

2.6. Plasmid incompatibility groups

Determination of incompatibility groups (IncP, IncHI2, IncI1, and IncFIB), known as the most relevant carriers of ESBL-and *mcr*-1-encoding genes, was performed in all colistin-resistant isolates by PCR-based replicon typing, as previously described by Carattoli et al. [23].

3. Results

3.1. Cefotaxime-resistant E. coli isolates and antibiotic susceptibility

Out of the 223 samples tested, eight (3.6%) were positive for cefotaxime resistant (CTX^R) *E. coli* isolates. Three were from bovine faeces from the farm in Mateur region (2.5%; 3/120), 3 from bovine bulk tank milk samples collected from 3 different markets (3.2%; 3/94) and two from caprine raw milk from two goats reared in the same farm (22.2%; 2/9).

All CTX^R isolates were ESBL producers, according to DDST, and five of these isolates were considered multidrug-resistant (including at least 3 classes of antimicrobials), especially to trimethoprim/sulfamethoxazole, streptomycin, tetracycline, and fluoroquinolones (Table 1). In addition, four isolates were colistin resistant (three bovine faeces and one of bovine raw milk) showing MICs ranging from 8 to16 μg/ml.

3.2. ESBL-encoding genes, resistance mechanisms to non-β-lactam antimicrobial agents and integrons

The *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$ genes were found, each, in four isolates, and the *bla*$_{TEM-1}$ gene was also detected in five of them (Table 1). The *mcr*-1 gene was detected in the four colistin-resistant isolates, being negative for the other *mcr* genes tested. The four colistin-resistant isolates harboured the *bla*$_{CTX-M-1}$ gene.

The *tet*(A) gene was detected in the four tetracycline-resistant isolates. Sulfonamide resistance was encoded by *sul1* in the five SXT-resistant isolates and in association to *sul*3 gene in one of them. The quinolone- resistant X236 isolate harboured the *qnr*S1 gene. Four isolates harboured class 1 integrons; however, only one of them (X243) was able to amplify a DNA fragment corresponding to their variable region and the gene cassette array detected was: *dfrA1* + *aadA1*. Interestingly, this strain from raw bovine milk was multidrug-resistant and contained in addition to the aforementioned gene cassettes, the *sul1*, *tetA*, *cmlA*, and *sul3* genes (Table 1).

3.3. Molecular typing and plasmid characterization

The four CTX-M-1-producing *E. coli* isolates, recovered from bovine samples, were typed as ST1642/phylogroup B1 (n = 3, from faecal samples) or ST10/A (n = 1; from raw milk sample). They carried the *mcr*-1 gene and were positive for IncP and IncFIB replicons. Three CTX-M-15-producing *E. coli* isolates of bovine (1 isolate) and caprine (2 isolates) raw milk samples were typed as ST394/phylogroup D and were colistin susceptible. An additional colistin-susceptible CTX-M-15-producing *E. coli* isolate of bovine raw milk was typed as ST46/phylogroup A. Six different PFGE profiles were detected among the 8 ESBL-producing *E. coli* isolates (assigned as E1-E6) (Table 1, supplementary Fig. S1).

Table 1
Characterization of ESBL-producing *E. coli* isolates recovered from bovine faeces and bovine/caprine raw milk samples.

Strain	Farm or Market/ region	Origin	Phylogroup/ST type	PFGE types	Resistance profiles for non-beta-lactam antibiotics [a]	Beta-lactamases	MIC of colistin (µg/mL)[b]	*mcr*-1	Plasmid (Inc)	Other genes detected
Bovine faecal samples										
X232	Farm/Mateur	Bovine	B1/ST1642	E1	COL, TET, SXT, S	CTX-M-1 +TEM-1b	16	+	P, FIB	*intI1, tetA, sul1*
X233	Farm/Mateur	Bovine	B1/ST1642	E2	COL, TET, SXT, S	CTX-M-1 +TEM-1b	8	+	P, FIB	*intI1, tetA, sul1*
X234	Farm/Mateur	Bovine	B1/ST1642	E2	COL, TET, SXT, S	CTX-M-1 +TEM-1b	8	+	P, FIB	*intI1, tetA, sul1*
Raw milk samples										
X243	Market/Intilaka	Bovine	A/ ST10	E3	COL, GEN, CHL, TET, SXT, S, NAL, CIP	CTX-M-1 +TEM-1b	16	+	P, FIB	*intI1, qacEΔ1-sul1, tetA, cmlA, sul3, dfrA1, aadA1*
X247	Market/Marsa	Bovine	A/ ST46	E4	TET, SXT, S	CTX-M-15 +TEM-1b	≤ 0.5	–	Nd[c]	*intI1, tetA, sul1*
X239	Market/Menzel Tmim	Bovine	D/ST394	E5	–	CTX-M-15	≤ 0.5	–	Nd	–
X237	Farm/Boumhel	Goat	D/ST394	E5	–	CTX-M-15	≤ 0.5	–	Nd	–
X236	Farm/Boumhel	Goat	D/ST394	E6	NAL, CIP	CTX-M-15	≤ 0,5	–	Nd	*qnrS1*

[a] NAL: nalidixic acid, CIP: ciprofloxacin, S: sulfonamide, SXT: trimethoprim-sulfamethoxazole; TET: tetracycline, GEN: gentamicin; CHL: chloramphenicol, COL: colistin.
[b] MIC: Minimum inhibitory concentration.
[c] Nd: Not done.

4. Discussion

ESBL-producing *E. coli* isolates have increasingly been reported in livestock, in the food chain and in companion animals worldwide, especially from pigs and poultry (intestinal microbiota) or their derived food products (retail meat, eggs) [5,24]. However, low rates of such isolates have been reported from bovine, ovine or caprine origins [6,25].

In our study, 120 bovine faecal samples and 94 bovine and 9 caprine raw milk samples were collected to determine the frequency of ESBL-EC and to explore the colistin-resistant frequency. Three ESBL-EC isolates were collected from bovine faecal samples (2.5%), and 3 and 2 were isolated from raw bovine (3.1%) and goat (22.2%) milk samples, respectively. These low rates obtained of ESBL-EC from bovine samples are in accordance with previous ones reported from different countries including Tunisia [25,26]. However, the high frequency of ESBL-EC in raw goat milk samples, despite the small number of samples analysed, is surprising. Indeed, to the best of our knowledge, ESBL-producing *E. coli* has never been reported from raw goat milk. Interestingly, we have also recently reported the occurrence of CTX-M-15-producing *K. pneumoniae* from raw goat milk sample [25]. Interestingly, the goats belonged to medium-sized caprine farms containing no more than 20 goats per farm with any antibiotic use prior to the previous three months of the study. These findings highlight the plausible occurrence of antibiotic-resistance selector (s) other than antibiotic use such as direct contact of these goats with humans or other animals colonized with ESBL-EC isolates. As expected, according to the actual epidemiology of ESBL-genes in livestock and food products of animal origin in Tunisia, each of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ were found in four isolates, in association or not with $bla_{\text{TEM-1}}$ gene. Indeed, both genes were the predominant $bla_{\text{CTX-M}}$ genes reported from ESBL-EC isolates from animal origins in Tunisia [25,27–30]. Four out of the eight ESBL-EC isolates were resistant to colistin (MICs ranging from 8 to 16 µg/mL) and harboured the *mcr*-1 gene. This gene, firstly identified at the end of 2015, has been increasingly reported worldwide, especially in *E. coli* isolates from livestock or food products of animal origin [31,32]. In Tunisia, plasmid mediated colistin resistance has yet not been identified in human clinical isolates; however, recent studies have reported *mcr*-1 genes from CTX-M- and CMY-2-producing *E. coli* isolates collected from animal origins [29,30,33].

In order to assess the genetic relatedness of the ESBL-EC isolates, the eight isolates were typed by determining the phylogenetics groups, Inc groups, occurrence of integrons, genes encoding antibiotic resistance, PFGE patterns and STs types. Isolates belonged to four Sequence Types (ST) being ST1642 (3 bovine faeces), ST394 (1 from raw bovine milk, 2 from raw goat milk), ST10 (1 raw bovine milk), and ST46 (1 raw bovine milk). The three ST1642 isolates belonged to B1 phylogroup, co-produced CTX-M-1 and TEM-1, harboured IncP and IncFIB plasmids, class 1 integron, *sul* 1 and *tet*A genes; however, they belonged to two distinct PFGE patterns [E2 (two isolates), E1 (one isolate)]. These findings highlight the dissemination of this clone in the extensive bovine farm located in the region of Mateur (North of Tunisia). This clonal lineage exhibiting various antibiotic resistance traits seems to have a large spread in some regions around the world. Indeed, Tamang et al. [34] reported one $bla_{\text{CMY-2}}$-producing ST1642-B1 *E. coli* strain recovered from intestinal samples from dogs in South Korea. In addition, $bla_{\text{CTX-M-14}}$-producing *E. coli* ST1642-B1 isolates were detected in outpatients of a Portuguese hospital [35], and also in carbapenemase-producing isolates in South Korea [36,37]. However, *mcr*-1/$bla_{\text{CTX-M-1}}$ harbouring *E. coli* ST1642-B1 has not been reported previously. In Tunisia, the ST10-A lineage has been identified also among CTX-M-1-producing isolates from healthy chickens [29] and from CTX-M-15-producing isolates from raw cattle milk [38], faeces of sheep and from wastewater [25]. The ST10-A lineage has also been identified among CTX-M-1-producing *E. coli* isolates from bovine mastitis in Germany [39], as well as from healthy livestock, poultry meat and healthy humans [40]. *E. coli* ST46-A clone has been previously detected in pigs in Nigeria (CTX-M-15-producers), or in water samples in Tunisia and in Bangladesh (CTX-M-1/15 -producers) [41–43]. Similarly, ST394 *E. coli* isolates have been previously isolated from sewage in Pakistan [44] and was also associated with diarrhoeal disease and with healthy people in both industrialized and developing countries [45].

The predominant plasmid replicon types found in antibiotic resistant *Enterobacteriaceae* isolated from humans and animals include incompatibility (Inc) groups F, A/C, L/M, I1, HI2, and N [46,47]. The *mcr*-1 gene was so far associated with different plasmid replicon types such as IncI2, IncHI2, IncP, IncFIP and IncX4 [48,49]. In addition, different bla_{CTX-M} genes are associated with particular plasmid replicon types (IncN, I1, FII, and L/M) [47]. IncF replicons are widely distributed among *E. coli* strains and seem to be well adapted to this species [46]. In our ESBL-EC strains, two of the four incompatibility plasmid types investigated (IncP, IncHI2, IncI1, and IncFIB) were identified in the four ESBL-producing and colistin-resistance isolates, including IncP and IncFIB, in combination. Now, owing to many limitations, we are unable to localize the $bla_{CTX-M-1}$ and *mcr*-1 genes on these plasmids. However, previous Tunisian studies performed on avian *E. coli* isolates the *mcr*-1 gene was detected on a IncP plasmid [50] and on IncHI2 plasmids (co-harboured $bla_{CTX-M-1}$ gene) [29].

In conclusion, in this work we report a low prevalence of ESBL-EC from bovine faeces as well as from raw bovine and goat milk samples. The CTX-M-1 and CTX-M-15 remain the most important ESBL enzymes from animal origins in Tunisia. Interestingly, our isolates belonged to well-known international clones linked to ESBL/carbapenemase production with multidrug resistance phenotypes and harbouring plasmid replicons implicated in *mcr*-1 and CTX-M-1 spread worldwide. In addition, we report for the first time in Tunisia, and maybe in the world, of CTX-M-15- producing *E. coli* from raw goat milk samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cimid.2019.101366.

References

[1] J.F. Prescott, J.D. Baggot, R.D. Walker, Antimicrobial Therapy in Veterinary Epidemiology, Iowa State University Press, Ames, 2000.
[2] H. Liu, H. Zhou, Q. Li, Q. Peng, Q. Zhao, J. Wang, X. Liu, Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from the rivers and lakes in Northwest China, BMC Microbiol. 18 (2018) 125.
[3] İ.H. Tekiner, H. Özpınar, Occurrence and characteristics of extended spectrum beta-lactamases-producing *Enterobacteriaceae* from foods of animal origin, Braz. J. Microbiol. 47 (2016) 444–451.
[4] K. Hille, M. Felski, I. Ruddat, J. Woydt, A. Schmid, A. Friese, J. Fischer, H. Sharp, L. Valentin, G.B. Michael, S. Hörmansdorfer, U. Messelhäußer, U. Seibt, W. Honscha, B. Guerra, S. Schwarz, U. Rösler, A. Käsbohrer, L. Kreienbrock, Association of farm-related factors with characteristic profiles of extended-spectrum β-lactamase-/plasmid-mediated AmpC β-lactamase-producing *Escherichia coli* isolates from German livestock farms, Vet. Microbiol. 223 (2018) 93–99.
[5] J.Y. Madec, M. Haenni, P. Nordmann, L. Poirel, Extended-spectrum β-lactamase/AmpC- and carbapenemase-producing *Enterobacteriaceae* in animals: a threat for humans? Clin. Microbiol. Infect. 23 (2017) 826–833.
[6] I. Dandachi, S. Chabou, Z. Daoud, J.M. Rolain, Prevalence and emergence of extended-spectrum cephalosporin-, carbapenem- and colistin-resistant Gram-negative bacteria of animal origin in the Mediterranean basin, Front. Microbiol. 9 (2018) 2299.
[7] I. Kempf, E. Jouy, C. Chauvin, Colistin use and colistin resistance in bacteria from animals, Int. J. Antimicrob. Agents 48 (2016) 598–606.
[8] E. Temkin, A. Adler, A. Lerner, Y. Carmeli, Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management, Ann. N. Y. Acad. Sci. 1323 (2014) 22–42.
[9] F. El Garch, M. Sauget, D. Hocquet, D. LeChaudee, F. Woehrle, X. Bertrand, mcr-1 is borne by highly diverse *Escherichia coli* isolates since 2004 in food-producing animals in Europe, Clin. Microbiol. Infect. 23 (2017) 1–4.
[10] L. Lei, Y. Wang, S. Schwarz, T.R. Walsh, Y. Ou, Y. Wu, M. Li, Z. Shen, mcr-1 in Enterobacteriaceae from companion animals, Beijing, China, 2012–2016, Emerg. Infect Dis. 23 (2017) 710–725.
[11] L.M. Carroll, A. Gaballa, C. Guldimann, G. Sullivan, L.O. Henderson, M. Wiedmann, Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate using a combination of high-throughput, in silico screening and functional analysis, MBio 539 (2019) 361.
[12] A. Bizzini, G. Greub, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification, Clin. Microbiol. Infect. 16 (2010) 1614–1619.
[13] CLSI, Performance standards for antimicrobial susceptibility testing, CLSI Supplement M100, 27th ed., Clinical and Laboratory Standards Institute, Wayne, PA, 2017.
[14] J. Kaur, S. Chopra, G.M. Sheevani, G. Mahajan, Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*, J. Clin. Diagn. Res. 7 (2013) 229–233.
[15] M.H. Abdelhai, H.A. Hassanin, X. Sun, Comparative study of rapid DNA extraction methods of pathogenic bacteria, Am. J. Biosci. Bioeng. 4 (2016) 1–8.
[16] A. Jouini, L. Vinué, K.B. Slama, Y. Saenz, N. Klibi, S. Hammami, C. Torres, Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia, J. Antimicrob. Chemother. 60 (2007) 1137–1141.
[17] Y.Y. Liu, Y. Wang, T.R. Walsh, L.X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L.F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J.H. Liu, J. Shen, Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, Lancet Infect. Dis. 16 (2016) 161–168.
[18] A.R. Rebelo, V. Bortolaia, J.S. Kjeldgaard, S.K. Pedersen, P. Leekitcharoenphon, I.M. Hansen, B. Guerra, B. Malorny, M. Borowiak, J.A. Hammerl, A. Battisti, A. Franco, P. Alba, A. Perrin-Guyomard, S.A. Granier, C. De Frutos Escobar, S. Malhotra-Kumar, L. Villa, A. Carattoli, R.S. Hendriksen, Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 for surveillance purposes, Euro Surveill. 23 (2018), https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672 pii = 17-00672.
[19] Y. Sáenz, L. Briñas, E. Dominguez, J. Ruiz, M. Zarazaga, J. Vila, C. Torres, Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins, Antimicrob. Agents Chemother. 48 (2004) 3996–4001.
[20] E. Ruiz, Y. Sáenz, M. Zarazaga, R. Rocha-Gracia, L. Martínez-Martínez, G. Arlet, C. Torres, *Qnr*, *aac (6′)-Ib*-cr and *qep*A genes in *Escherichia coli* and *Klebsiella* spp.: genetic environments and plasmid and chromosomal location, J. Antimicrob. Chemother. 67 (2012) 886–897.
[21] K.B. Slama, R.B. Sallem, A. Jouini, S. Rachid, L. Moussa, Y. Sáenz, V. Estepa, S. Somalo, A. Boudabous, C. Torres, Diversity of genetic lineages among CTX-M-15 and CTX-M-14 producing *Escherichia coli* strains in a Tunisian hospital, Curr. Microbiol. 62 (2011) 1794–1801.
[22] O. Clermont, S. Bonacorsi, E. Bingen, Rapid and simple determination of the *Escherichia coli* phylogenetic group, Appl. Environ. Microbiol. 66 (2000) 4555–4558.
[23] A. Carattoli, A. Bertini, L. Villa, V. Falbo, K.L. Hopkins, E.J. Threlfall, Identification of plasmids by PCR-based replicon typing, J. Microbiol. Methods 63 (2005) 219–228.
[24] A. Dorado-García, J.H. Smid, W. van Pelt, M.J.M. Bonten, A.C. Fluit, G. van den Bunt, J.A. Wagenaar, J. Hordijk, C.M. Dierikx, K.T. Veldman, A. de Koeijer, W. Dohmen, H. Schmitt, A. Liakopoulos, E. Pacholewicz, T.J.G.M. Lam, A.G. Velthuis, A. Heuvelink, M.A. Gonggrijp, E. van Duijkeren, A.H.A.M. van Hoek, A.M. de Roda Husman, H. Blaak, A.H. Havelaar, D.J. Mevius, D.J.J. Heederik, Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis, J. Antimicrob. Chemother. 73 (2018) 339–347.
[25] S. Sghaier, M.S. Abbassi, A. Pascual, L. Serrano, P. Díaz-De-Alba, M.B. Said, B. Hassen, C. Ibrahim, A. Hassen, L. López-Cerero, Extended-spectrum β-lactamase-producing Enterobacteriaceae from animal origin and wastewater in Tunisia: first detection of O25b-B23-CTX-M-27-ST131 *Escherichia coli* and CTX-M-15/OXA-204-producing *Citrobacter freundii* from wastewater, J. Glob. Antimicrob. Resist. 17 (2019) 189–194.
[26] D. Eisenberger, A. Carl, J. Balsliemke, P. Kämpf, S. Nickel, G. Schulze, G. Valenza, Molecular Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from milk samples of dairy cows with mastitis in Bavaria, Germany, Microb. Drug Resist. 24 (2018) 505–510.

[27] R. Ben Sallem, K. Ben Slama, Y. Sáenz, B. Rojo-Bezares, V. Estepa, A. Jouini, C. Torres, Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)-and CMY-2-producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia, Foodborne Pathog. Dis. 9 (2012) 1137–1142.

[28] C.A. Alonso, M. Zarazaga, R. Ben Sallem, A. Jouini, K. Ben Slama, C. Torres, Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective, Lett. Appl. Microbiol. 64 (2017) 318–334.

[29] M. Saidani, L. Messadi, A. Chaouechi, I. Tabib, E. Saras, A. Soudani, M. Daaloul-Jedidi, A. Mamlouk, F. Ben Chehida, C. Chakroun, J.Y. Madec, M. Haenni, High Genetic diversity of *Enterobacteriaceae* clones and plasmids disseminating resistance to extended-spectrum cephalosporins and colistin in healthy chicken in Tunisia, Microb. Drug Resist. (2019), https://doi.org/10.1089/mdr.2019.0138.

[30] M. Saidani, L. Messadi, J. Mefteh, A. Chaouechi, A. Soudani, R. Selmi, M. Dâaloul-Jedidi, F. Ben Chehida, A. Mamlouk, M.H. Jemli, J.Y. Madec, M. Haenni, Various Inc-types plasmids and lineages of *Escherichia coli* and *Klebsiella pneumoniae* spreading $bla_{CTX\text{-}M\text{-}15}$,$bla_{CTX\text{-}M\text{-}1}$ and *mcr*-1 genes in camels in Tunisia, J. Glob. Antimicrob. Resist. (2019), https://doi.org/10.1016/j.jgar.2019.05.007.

[31] A. Barlaam, A. Parisi, E. Spinelli, M. Caruso, P.D. Taranto, G. Normanno, Global emergence of colistin-resistant *Escherichia coli* in food chains and associated food safety implications: a review, J. Food Prot. 82 (2019) 1440–1448.

[32] S.C. Nang, J. Li, T. Velkov, The rise and spread of *mcr* plasmid-mediated polymyxin resistance, Crit. Rev. Microbiol. 45 (2019) 131–161.

[33] E. Maamar, S. Ferjani, A. Jendoubi, S. Hammami, Z. Hamzaoui, L. Mayonnve-Coulange, M. Houissa, I. Boutiba-Ben Boubaker, A. Slim, V. Dubois, High prevalence of gut microbiota colonization with broad-spectrum cephalosporin resistant *Enterobacteriaceae* in a Tunisian intensive care unit, Front. Microbiol. 7 (2016) 1859.

[34] M.D. Tamang, H.M. Nam, G.C. Jang, S.R. Kim, M.H. Chae, S.C. Jung, J.W. Byun, Y.H. Park, S.K. Lim, Molecular characterization of extended-spectrum β-lactamase and plasmid mediated AmpC β-lactamase-producing *Escherichia coli* isolated from stray dogs from Korea, Antimicrob. Agents Chemother. 56 (2012) 2705–2712.

[35] C. Rodrigues, E. Machado, J. Pires, H. Ramos, Â. Novais, L. Peixe, Increase of widespread A, B1 and D *Escherichia coli* clones producing a high diversity of CTX-M-types in a Portuguese hospital, Future Microbiol. 10 (2015) 1125–1131.

[36] S. Jeong, J.O. Kim, E.J. Yoon, I.K. Bae, W. Lee, H. Lee, S.H. Jeong, Extensively Drug-Resistant *Escherichia coli* Sequence Type 1642 carrying an IncX3 plasmid containing the $bla_{KPC\text{-}2}$ gene associated with transposon *Tn4401a*, Ann. Lab. Med. 38 (2018) 17–22.

[37] E.J. Yoon, J.W. Yang, J.O. Kim, H. Lee, K.J. Lee, S.H. Jeong, Carbapenemase-producing *Enterobacteriaceae* in South Korea: a report from the national laboratory surveillance system, Future Microbiol. (2018).

[38] R. Grami, S. Dahmen, W. Mansour, W. Mehri, M. Haenni, M. Aouni, J.Y. Madec, $bla_{CTX\text{-}M\text{-}15}$-carrying F2: a-: B-plasmid in *Escherichia coli* from cattle milk in Tunisia, Microb. Drug Resist. 20 (2014) 344–349.

[39] C. Freitag, G.B. Michael, K. Kadlec, M. Hassel, S. Schwarz, Detection of plasmid-borne extended-spectrum β-lactamase (ESBL) genes in *Escherichia coli* isolates from bovine mastitis, Vet. Microbiol. 200 (2017) 151–156.

[40] A. Müller, R. Stephan, M. Nüesch-Inderbinen, Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans, Sci. Total Environ. 541 (2016) 667–672.

[41] M. Rashid, M.M. Rakib, B. Hasan, Antimicrobial-resistant and ESBL-producing *Escherichia coli* in different ecological niches in Bangladesh, Infect. Ecol. Epidemiol. 5 (2015) 26–712.

[42] L.B. Said, A. Jouini, C.A. Alonso, N. Klibi, R. Dziri, A. Boudabous, K. Ben Slama, C. Torres, Characteristics of extended-spectrum β-lactamase (ESBL)-and pAmpC beta-lactamase-producing *Enterobacteriaceae* of water samples in Tunisia, Sci. Total Environ. 550 (2016) 1103–1109.

[43] K.F. Chah, I.C. Ugwu, A. Okpala, K.Y. Adamu, C.A. Alonso, S. Ceballos, C. Torres, Detection and molecular characterization of extended-spectrum beta-lactamase producing enteric bacteria from pigs and chickens in Nsukka, Nigeria, J. Glob. Antimicrob. Resist. 15 (2018) 36–40.

[44] R. Zahra, S. Javeed, B. Malala, D. Babenko, M.A. Toleman, Analysis of *Escherichia coli* STs and resistance mechanisms in sewage from Islamabad, Pakistan indicates a difference in *E. coli* carriage types between south Asia and Europe, J. Antimicrob. Chemother. 73 (2018) 1781–1785.

[45] I.N. Okeke, F. Wallace-Gadsden, H.R. Simons, Multi-locus sequence typing of enteroaggregative *Escherichia coli* isolates from Nigerian children uncovers multiple lineages, PLoS One 5 (2010) e14093.

[46] A. Carattoli, Resistance plasmid families in Enterobacteriaceae, Antimicrob. Agents Chemother. 53 (2009) 2227–2238.

[47] J.Y. Madec, M. Haenni, Antimicrobial resistance plasmid reservoir in food and food-producing animals, Plasmid 99 (2018) 72–81.

[48] K. Zurfluh, J. Klumpp, M. Nüesch-Inderbinen, R. Stephan, Full-length nucleotide sequences of mcr-1-harboring plasmids isolated from extended-spectrum-β-lactamase-producing *Escherichia coli* isolates of different origins, Antimicrob. Agents Chemother. 60 (2016) 5589–5591.

[49] M. Rozwandowicz, M.S.M. Brouwer, J. Fischer, J.A. Wagenaar, B. Gonzalez-Zorn, B. Guerra, D.J. Mevius, J. Hordijk, Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*, J. Antimicrob. Chemother. 73 (2018) 1121–1137.

[50] E. Maamar, C.A. Alonso, Z. Hamzaoui, N. Dakhli, M.S. Abbassi, S. Ferjani, M. Saidani, I. Boutiba-Ben Boubaker, C. Torres, Emergence of plasmid-mediated colistin-resistance in CMY-2-producing *Escherichia coli* of lineage ST2197 in a Tunisian poultry farm, Int. J. Food Microbiol. 269 (2018) 60–63.