ESBL- and mcr-1-producing Escherichia coli in veal calves in Tunisia

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Journal of Global Antimicrobial Resistance 19 (2019) 104–105

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

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Sir,

Antimicrobial resistance has become a major public-health concern since resistance to antibiotics such as extended-spectrum cephalosporins (ESCs), carbapenems and colistin have disseminated widely in humans, animals and the environment. In food-producing animals, particular attention has been paid in recent years to plasmid-mediated resistance to ESCs and colistin. Even resistance to carbapenems, which are prohibited antimicrobials in animals, can occur as exemplified by recurrent reports of VIM-1 in the agri-food sector in Germany. Numerous studies have also been performed in chicken and chicken meat, with high rates of ESC-resistant (ESC-R) *Escherichia coli* found worldwide. In contrast, antimicrobial resistance in veal calves is still under-reported, although French and Dutch studies showed a high proportion (20.4–39.0%) of healthy calves carrying ESC-R *E. coli* in slaughterhouses, sometimes associated with the *mcr-1* gene [1,2]. Thus, the aim of the current study was to characterise *E. coli* resistant to ESCs, carbapenems and colistin in faecal samples from veal calves in Tunisia, a sector that has never been explored in this country.

A total of 219 faecal samples were collected between January 2016 and September 2017 from diarrhoeic ($n = 160$) and healthy ($n = 59$) calves on 104 different dairy farms. A total of 182 *E. coli* isolates were recovered on MacConkey agar (dominant flora representing major clones), whilst 14 ESC-R *E. coli* (6.4%; 14/219) were recovered on MacConkey agar supplemented with cefotaxime (subdominant flora representing minor clones). In the dominant flora, isolates were frequently resistant to streptomycin (78.0%), tetracycline (72.0%) and amoxicillin (52.7%) but not to carbapenems (Supplementary Table S1). This is in line with the antibiotics most commonly used to treat veal calves in Tunisia and also with a study in France where these resistances were the most frequent [2]. No statistically significant differences were observed in the proportions of resistance in diarrhoeic versus healthy calves ($P \geq 0.05$).

All 14 ESC-R *E. coli* were confirmed as extended-spectrum β-lactamase (ESBL)-producers by the double-disk synergy test, among which 6 were also recovered from the dominant flora (Table 1). Only one ESBL-positive isolate was recovered from a healthy animal, highlighting the very low ESBL colonisation rate compared with France (20.4% in 2013) and the Netherlands (39.0% in 2010) [1,2]. Nevertheless, attention should be paid to high-density shedders (i.e. animals carrying ESBL-positive *E. coli* in the dominant flora) since they have been linked to a higher risk of contamination of the food chain after slaughter.

Several CTX-M enzymes were detected, with CTX-M-15 being the most frequent (7/14). Southern blotting on S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) gels using *Xba*I or *I-Ceu*I enzymes revealed that IncF-type plasmids were the most widespread plasmids carrying $bla_{\text{CTX-M}}$ genes (Table 1). Seven different sequence types (STs) were identified, and clonal *E. coli* isolates belonging to ST744, ST162 and ST1722 were identified in unrelated farms located approximately 40 km apart (Supplementary Fig. S1). Only ST1722, which was found in four animals from three different farms, presented a chromosomal insertion of $bla_{\text{CTX-M-15}}$. Such a chromosomal insertion in the same ST has already been found in migratory birds from Pakistan, suggesting that certain genetic backgrounds may be more prone to chromosomal integration [3]. Five isolates displaying different STs were collected from farm #7, a large state farm that was struggling with numerous cases of diarrhoea at the time of sampling. Of note, the human epidemic clone ST131 was identified in one isolate that carried the $bla_{\text{CTX-M-14}}$ gene on a widespread IncF1:A2:B20 plasmid. To date, ESBL-producing ST131 *E. coli* isolates outside the human sector remain sporadic and are rarely recovered from food-producing animals. However, whether this *E. coli* isolate was transferred from a human to calves is uncertain at this stage of investigation.

The *mcr-1* gene was detected in 3 ESBL-producing and 8 non-ESBL-producing *E. coli* isolates, meaning that 5.0% (11/219) of the samples harboured a colistin-resistant *E. coli* (Table 1). No other gene of the *mcr* family was found. The *mcr-1* gene was systematically carried on an IncHI2 plasmid and was co-localised with the $bla_{\text{CTX-M}}$ gene in ESBL-producing isolates. This $bla_{\text{CTX-M}}$/*mcr-1* IncHI2 plasmid has frequently been reported in animals, including in Tunisia where it has been described in poultry production and in dromedaries [4,5]. This plasmid has thus disseminated in the animal sector in Tunisia, although not in high proportions (4.6% in veal calves, 0.4% in dromedaries, 10.2% of ESBL-producing *E. coli* in chicken). Nine *mcr-1*-positive isolates originated again from farm #7 where colistin was used to treat diarrhoea, likely explaining the spread of the *mcr-1* gene in five genetic backgrounds (ST162, ST88, ST533, ST162 and ST744). Co-selection through other antibiotic treatments is also plausible given the number of associated antimicrobial resistances (Table 1).

In this study, 6.4% (14/219) of the samples presented an ESBL-producing *E. coli* and 5.0% (11/219) presented a colistin-resistant *E. coli*. Epidemic plasmids and *E. coli* clones were found, highlighting the importance both of prudent use of antibiotics and appropriate hygiene and biosecurity measures to control the spread of antimicrobial resistance in the agri-food sector in Tunisia.

Funding

This study was supported by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the

http://dx.doi.org/10.1016/j.jgar.2019.08.009

Table 1
Characteristics of extended-spectrum β-lactamase (ESBL)- and MCR-1-producing *Escherichia coli* isolates collected from veal calves in Tunisia.

Strain[a]	Health status	Farm	Phylogroup	ST	ESBL gene	Associated resistances	Colistin resistance gene (MIC in mg/L)	ESBL-carrying plasmid	FAB formula	Plasmid size (kb)
BV47	DC	1	B2	131	$bla_{CTX-M-14}$	NAL, ENR, TET, SXT	–	IncF	F1:A2:B20	150
BV82	HC	2	A	167	$bla_{CTX-M-15}$	CHL, GEN, NAL, ENR, TET, SXT	–	IncF	F1:A1:B49	130
V55	DC	3	A	744	$bla_{CTX-M-55}$	STR, TET, CHL, FFC, SXT, NAL, ENR	–	IncF	F18:A-:B1	170
V56	DC	4	A	744	$bla_{CTX-M-55}$	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR	–	IncF	F18:A-:B1	170
V61	DC	5	B1	162	$bla_{CTX-M-1}$	STR, TET, FFC, SXT, NAL, ENR, COL	*mcr-1* (6)	IncHI2	–	260
V62	DC	6	B1	162	$bla_{CTX-M-1}$	STR, TET, CHL, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	260
V66	DC	7	A	744	$bla_{CTX-M-55}$	STR, TET, CHL, FFC, SXT, NAL, ENR	–	IncF	F18:A-:B1	170
V73	DC	7	B1	162	$bla_{CTX-M-1}$	STR, TET, CHL, FFC, SXT, NAL, COL	*mcr-1* (8)	IncHI2	–	260
V75	DC	7	A	10	$bla_{CTX-M-15}$	STR, TET, CHL, FFC, SXT, NAL	–	IncX1	–	70
V76	DC	7	D	8149	$bla_{CTX-M-15}$	GEN, STR, TET, SXT, NAL, ENR	–	IncF	F18:A-:B30	170
V85	DC	7	D	1722	$bla_{CTX-M-15}$	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR	–	Chromosome	–	–
V89	DC	8	D	1722	$bla_{CTX-M-15}$	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR	–	Chromosome	–	–
V90	DC	8	D	1722	$bla_{CTX-M-15}$	STR, TET, CHL, FFC, SXT, NAL, ENR	–	Chromosome	–	–
V92	DC	9	D	1722	$bla_{CTX-M-15}$	STR, TET, CHL, SXT, NAL, ENR	–	Chromosome	–	–
BV6	DC	7	B1	88	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
BV15	DC	7	B1	533	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
BV87	HC	7	B1	533	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
BV95	DC	7	B1	533	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
BV96	DC	7	B1	533	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
BV118	HC	7	A	744	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
V47	DC	7	A	88	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280
V52	DC	7	A	88	None	GEN, STR, TET, CHL, FFC, SXT, NAL, ENR, COL	*mcr-1* (4)	IncHI2	–	280

ST, sequence type; MIC, minimum inhibitory concentration; DC, diseased calf; HC, healthy calf; NAL, nalidixic acid; ENR, enrofloxacin; TET, tetracycline; SXT, sulfonamides/trimethoprim; CHL, chloramphenicol; GEN, gentamicin; STR, streptomycin; FFC, florfenicol; COL, colistin.
[a] *Escherichia coli* isolates found both in the dominant and subdominant flora are underlined.

research project 'ResAntibioVet 06.680' funded by the Ministry of Agriculture of Tunisia. MS received a fellowship from the Tunisian Ministry of Higher Education and Scientific Research.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

The authors especially thank all of the veterinarians for their help during the sampling process. The authors also thank Estelle Saras, Pierre Châtre, Antoine Drapeau, Véronique Métayer and Sihem Chebil for their helpful assistance.

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.jgar.2019.08.009.

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Received 3 June 2019

Available online 19 August 2019