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Overview of ESBL-producing *Escherichia coli* of Animal Origin in Tunisia: In the Way of the Global Spread of CTX-M β-Lactamases

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Abstract

Extended-Spectrum β-Lactamases (ESBL)-producing Escherichia coli isolates have emerged as a global threat to public human health, and have been isolated from human, animal and environment origins. Worldwide as well as in Tunisia, high prevalence of ESBL-producing Enterobacteriaceae, especially *E. coli*, of humans, animals and food-producing animal origin have been increasingly reported. Recent studies have suggested that these *E. coli* strains, and their antibiotic resistance genes, can spread from food-producing animals, via the food-chainfood chain, to humans. Our review will focus on the evolution and the emergence of ESBL-producing *E. coli*, coli of animal origin in Tunisia as well as the types of the blaESBL genes in relation with the predominant ESBL-genes circulating Tunisian's hospitals. Therefore, we have analysed the reported results from Tunisia during the last 10 years. This review showed the predominance of CTX-M group especially in chicken samples, heterogeneity of reported strains and the spread of particular plasmids belonging to the incompatibility group IncI1 and IncF. We conclude that the spread of CTX-M group in Tunisia reflects the global emergence of CTX-M β-lactamases in *E. coli* of human or animal origins.

Keywords: CTX-M; *Escherichia coli*; Animal origin; Tunisia

Introduction

E. coli is a common intestinal microorganism of humans and animals. It also represents an important pathogen causing urinary tract and gastrointestinal infections and septicaemia [1]. Over the past few years, resistance to antimicrobial agents has increased among *E. coli* from both human and animal sources [2]. Initially, resistance was described to particular agents, such as ampicillin, trimethoprim, sulphur-based antimicrobials or tetracyclines [3]. Recently, the resistance has broadened with the emergence of broad resistance to large families of antimicrobial agents among human and veterinary isolates. This resistance seems to be in relation to their wide clinical use of these agents. The mechanisms of resistance to oxyimino-cephalosporins in *E. coli* isolates from animals and food products by the production of extended-spectrum β-lactamases (ESBLs) have become prominent [4-6]. Recent reports describing the detection of ESBLs in bacterial isolates from animals have raised concern regarding the transmission of ESBL genes between human and animal isolates [2]. *E. coli* strains carrying AmpC-β-lactamases such as CMY-type lactamase have already been reported in healthy and sick animals and food-producing animals [7-9]. In addition to the resulting resistance to most β-lactam antibiotics, ESBL or plasmidic AmpC-β-lactamases producers are also frequently resistant to aminoglycosides and fluoroquinolones. The rate of resistance to quinolones and fluoroquinolones among *E. coli* isolates of animal origin has been increasingly reported [10,11]. The prevalence of antibiotic resistance in *E. coli* of animal origin has increased due to the inclusion of different antimicrobial resistance genes on mobile elements such as plasmids, transposons and integrons that facilitate the rapid dissemination of these genes among bacteria [12]. Commensal *E. coli* seems to be the major reservoir of resistance gene since they have been implicated in the transmission of genetic traits from one bacterium to another [13]. The impact of animal-derived broad-spectrum-β-lactamases

(ESBL)-producing *E. coli* on public health has drawn considerable attention worldwide [2,13]. The high level of increase of antibiotic resistance in *E. coli* of animal origin may be enhanced by the use of antimicrobials for preventive purposes. This practice could have been followed in Tunisia and have been probably applied to cover up the failures in farm management. Epidemiological studies on the antibiotic resistance in *E. coli* in Tunisia are relatively new; the first published paper appeared in 2007. Since then, more data have been made available and the real situation of antibiotic resistance in *E. coli* from animal origin is very alarming. In the present review, we focus on the analyses of ESBL-producing *E. coli* of animal origin in Tunisia and the genetic support of this resistance.

ESBL encoding genes in Tunisia

A chronologically listed summary of β-lactamases in *E. coli* from food-producing animals in Tunisia is given in **Table 1**. The detection of ESBL-producing Enterobacteriaceae that derivate from genes originally encoded for TEM-1, TEM-2 or SHV-1 has been increasingly reported in the last few years in Tunisia [14-16]. However, interestingly the epidemiology of ESBLs in *E. coli* has changed radically: novel ESBLs such as CTX enzymes are predominant, replacing the classical TEM and SHV-type ESBL in many countries as well as in Tunisia [16].

In Tunisia and in other African countries, the first CTX-M ESBL-type was detected in 2006 in samples of food origin plated onto selective medium with cefotaxime [17]. The ESBLs detected were blaCTX-M-1, blaCTX-M-14, blaCTX-M-8 and blaSHV-5, this was the first report of CTX-M-8 occurrence in Tunisia. In addition, the aforementioned report described the first detection of blaCTX-M-14 and blaSHV-5 genes in 2/11 and 1/11 *E. coli* isolates from faecal samples of chicken. In Europe, Danish and British ESBL-producing *E. coli* data from 2012 revealed the presence of blaCTX-M-14 gene respectively in pigs and in poultry; however blaSHV-5 gene was detected in Spain from pigs (Blanc et al. 2006) [18,19]. Chinese data demonstrated the occurrence of CTX-M-14 in combination with CTX-M-1 type ESBLs recovered from healthy and sick pets; SHV-5 was also isolated from livestock animals in Japan [1,20].

Since the number of food samples of animal origin used by Jouini et al. was small (38 samples), similar studies have been conducted by Ben Slama et al. in the same laboratory to characterize ESBL-producing *E. coli* recovered from 79 food samples of animal origin during 2007 [17,12]. Only the CTX-M-1 type ESBL was detected in this study. Since 2007, CTX-M-1 has been continuing to appear in Tunisia. Indeed, Mnif et al. have reported a high frequency of cefotaxime-resistant *E. coli* isolates from healthy chicken faecal samples (67 isolates, 42%, recovered from 24 of 36 Tunisian farms located in six different governorates of Tunisia) [21]. Again, all ESBLs (43 strains) belonged to CTX-M group 1: 39 CTX-M-1 and 4 CTX-M-15, and the AmpC phenotype (observed in 24 strains) harbored the blaCMY-2 gene **(Figure 1)**. This was the first report on CTX-M β-lactamases in live broiler chickens and the first report of blaCTX-M-15 gene in *E. coli* strains from animal origin in Tunisia. CTX-M-15 has been identified in food-producing animals for the first time in France in one isolate recovered from poultry, then in Denmark from pigs and in Argentina and Great Britain from poultry [18,19,22,23]. Recently, a novel investigation reported the detection of CTX-M-15 type ESBL in diseased cattle from milk sample for the first time in Tunisia and even Africa [24].

In the same period (2012), Ben Sallem et al. have reported the isolation of cefotaxime-resistant *E. coli* isolates from 11 out of 80 fecal samples of healthy food-producing animals analysed (13.8%) [25]. Samples where chicken (17 samples), sheep (9 samples), cow (6 samples), horse (1 sample) dromedary (1 sample), and rabbit (3 samples). Cefotaxime-resistant isolates were from chicken (10 isolates) and dromedary (1 isolate). Nine of these isolates harboured the blaCTX-M-1 gene, and two of them harbored the blaTEM-135 or blaTEM-1b genes. The remaining two *E. coli* isolates contained the blaCMY-2 gene (encoding the beta-lactamase CMY-2). In addition, the same investigators found the blaCTX-M-1 gene in 92.8% of cefotaxime-resistant isolates in

Table 1 The occurrence of β-lactamases reported in *E. coli* of animal origin in Tunisia.

References	Year of isolation	ESBL and AmpC detected	Origin
Jouini et al. 2007	2006	CTX-M-1	Beef, turkey
		CTX-M-1 + TEM-1	Beef
		CTX-M-14+ TEM-1	Chicken
		CTX-M-8	Chicken
		SHV-5	Chicken
		TEM-1	Turkey
Ben Slama et al.2010	2007	CTX-M-1	Chicken, sheep, beef
		CTX-M-1 + TEM-20	Sheep
		CTX-M-1 + TEM-1b	Chicken
		CMY-2	Chicken
Mnif et al. 2012	2010	CTX-M-1, TEM-1, CTX-M-15, CMY-2	fecal samples of healthy chickens
Ben Sallem et al.2012	2011	CTX-M-1, TEM-135, CMY-2, TEM-1b	fecal samples of healthy chickens
		CTX-M-1	fecal samples of healthy dromedary
Ben Sallem et al.2013	2010	CTX-M-1, TEM-1c, TEM-135, TEM-1b, CMY-2	fecal samples of healthy pets
Grami et al.2013	2011-2012	CTX-M-1	Fecal samples of chicken and healthy pets
		CTX-M-15	Fecal sample of dog
		CTX-M-9	Fecal sample of chicken
Grami et al. 2014	2010-2011	CTX-M-15	Diseased cattle milk
Kilani et al 2015	2013-2014	CTX-M-15, TEM-1	Healthy chicken

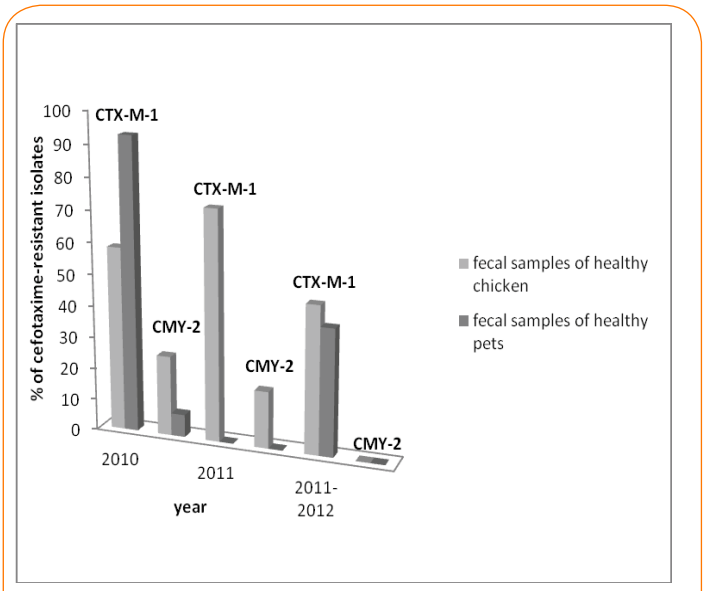

Figure 1 Prevalence of CTX-M-1 and CMY-2 in fecal *E. coli* isolates from chicken and pets.

fecal *E. coli* from healthy pets (cats and dogs) for the first time in Tunisia and in Africa **(Table 1)** [26]. In the period between 2011 and 2012, the blaCTX-M-1 gene has been also reported in faecal samples from healthy chickens and pets **(Figure 1)** [27]. In this study, they reported for the first time in Tunisia of CTX-M-15 enzyme in fecal swabs from pet dogs with low percentage (6.6% of detected ESBL). Moreover this study reported the first data on CTX-M-9 type ESBL in healthy chickens, despite the occurrence of this β-lactamase exclusively in a clinical strain of *Enterobacter cloacae* isolated from a stool culture in intensive care unit of the Military Hospital of Tunis in 2005 for the first time in Tunisia [28]. These findings are in concordance with the study of Mora and co-workers performed in Spain in 2010 describing the zoonotic potential of CTX-M-9-producing avian isolates [29]. It seems that the expansion of CTX-M enzymes remains in evolution in Tunisian's livestock. Indeed, more recently, in 2015, our team reported the occurrence of blaCTX-M-1 gene, one of them co-harbored the TEM-1 enzyme, in 16 out of 17 cefotaxime-resistant *E. coli* isolates recovered from feces of healthy poultry from two avian husbandries [30].

It is noteworthy, that all Tunisian reports showed the high percentage of faecal carriage of ESBL-positive *E. coli* isolates from healthy food-producing animals and from pets. On the other hand, these Tunisian reports confirmed the prevalence of CTX-M-1 β-lactamase in food-producing animals and indicated that the poultry food (particularly chicken samples) and pets play an important role as a reservoir of cefotaxime-resistance genes. This finding is in agreement with that of previous studies carried out in France, Great Britain, Belgium, Portugal, Spain and China showing increased raw poultry meat colonized by CTX-M-1-producing *E. coli* isolates [13,31-36].

Despite the predominance of CTX-M-15 enzyme in human patients in Tunisia during the last decade, it seems that the emergence of CTX-M-1 in poultry isolates has influenced the epidemiology of CTX-M type enzymes in healthy humans in Tunisia **(Table 2)** [37-50]. This emergence might alarm for possible increase of blaCTX-M-1 over blaCTX-M-15 in Tunisian human isolates. Indeed, a recent study demonstrated the occurrence of blaCTX-M-1 genes-producing *E. coli* in 7.3% of Tunisian healthy humans [51]. The authors have warned for possible dissemination of such poultry isolates to human. This hypothesis is in concordance with previous studies performed in the Netherlands showing clonal relationships of blaCTX-M-1-producing *E. coli* isolates from poultry and humans [52]. The dissemination of such strains has likely happened through the food chain. However, it seems that this phenomenon of poultry to human dissemination might slightly influence the occurrence of blaCTX-M genes in human isolates. This statement is consistent with a recently completed survey of *E. coli* carrying ESBL in broiler chickens in Great Britain indicating the predominance of CTX-M-1, despite the widespread prevalence of *E. coli* CTX-M-15 producers among human populations [35,53].

Other ESBL-encoding genes have also been reported in *E. coli* of animal origin. Indeed, TEM-1, TEM-20 and TEM-135 β-lactamases were also present in association with CTX-M-type ESBLs [12,17,25]. In the world, few studies reported the detection of TEM-20 ESBL type of animal origin [52].

The CMY type is the most frequently reported plasmidic AmpC beta-lactamases (pAmpC-BL) in *E. coli* [32]. In Tunisia, *E. coli* strains carrying the plasmid-borne blaCMY-2 gene have been sources of concern in animal production and public health. In fact, previous studies demonstrated high occurrence of CMY-2 AmpC-BL from different poultry farms, healthy pet animals and food-producing animals with percentages of 25.3%, 7.1% and 18% of cefotaxime-resistant strains, respectively **(Figure 1)** [21,25,26]. In the last few years, different reports alerted about the dissemination of ESBL/CMY-producing *E. coli* from food animals and healthy companion animals in different countries [9,13,32,34,54].

Clonality of ESBL-producing *E. coli* isolates and incompatibility groups of plasmid-borne ESBL-encoding genes

All cefotaxime-resistant *E. coli* isolates (including ESBL-and/or pAmpC-BL-producing strains) showed unrelated PFGE patterns, in the majority of Tunisian reports. This high genetic diversity detected indicated that the spread of blaCTX-M-1 and blaCMY-2 genes is not due to the dissemination of clonal strains but might reflect the spread of transferable plasmids. Indeed, Mnif et al. have reported the occurrence of blaCTX-M-1 and blaCMY-2 genes on IncI1 plasmids in the majority of studied strains, and also on IncK plasmids [21]. Moreover, Jouini et al. have detected the presence of IncI1 plasmids in blaCTX-M-1-containing isolates and IncK plasmids in blaCTX-M-14 and blaCTX-M-1-producing isolates [55]. These plasmids might carry the aforementioned genes, despite molecular confirmation has not been carried out. In the meantime, another study performed in Tunisia highlighted the dissemination of blaCTX-M-1 and blaCTX-M-9 genes located on IncI1 conjugative plasmids, whereas the blaCTX-M-15 gene was carried on IncFII plasmid [27]. More Recently in 2014, Ben Sallem et al. showed the detection of IncI1 and IncF replicon

Table 2 The incidence of reported ESBL enzymes in Enterobacteriaceae isolates from patients and healthy human in Tunisia since (2000-2015).

References	Species concerned	Detected ESBL enzymes (number of isolates, or frequencies, remakes)
Rhimi et al. 2002	6 *Klebsiella pneumoniae* 1 *Proteus mirabilis* 3 *Salmonella* Livingstone	ACC-1
Makanera et al. 2003	31 *Salmonella* Mbandaka	TEM-4 (13 isolates); SHV-2a (16 isolates); ACC-1a (18 isolates)
Ben-Hamouda et al. 2004	49 *K. pneumoniae*	SHV-12; SHV-2a
Bouallegue et al. 2005	16 *Salmonella* Livingstone	CTX-M-27 (all isolates)
Ktari et al.2006	11 *K. pneumoniae*	VIM-4; CTX-15; CMY-4 (Clonal isolates harbored the three genes)
Mamlouk et al.2006	35 *E. coli* 27 *K. pneumoniae*	CTX-M-15 (31 *E. coli* and 24 *K. pneumoniae* isolates, the majority of isolates of both species were clonal) ; CTX-M-16 (4 *E. coli* and 3 *K. pneumoniae* , clonal isolates)
Abbassi et al. 2008	9 *K. pneumoniae* 2 *E. coli*	SHV-11 (1 *K. pneumoniae*) ; SHV-27 (one *K. pneumoniae*); CTX-M-15 (all isolates)
Ben Achour et al. 2009	1 *K. pneumoniae*	TEM-164
Ben Achour et al. 2009	1 *K. pneumoniae*	CTX-M-28
Bourouis et al. 2009	1 *Enterobacter cloacae*	CTX-M-9
Ktari et al. 2009	112 *Salmonella* Livingstone	SHV-2a (2 isolates) ; ACC-1 (111 isolates ; clonal isolates)
Ben Slama et al. 2011	14 *E. coli*	CTX-M-15 (12 isolates) ; CTX-M-14a (1 isolate) ; CTX-M-14b (1 isolate)
Dahmen et al. 2010	45 *K. pneumoniae* 1 *Citrobacter freundii* 3 *Morganella morgani* *2 Providencia rettgeri* 31 *E. coli* *16 E. cloacae* 2 *K. oxytoca*	CTX-M-15 (45 *K.* pneumoniae, 31 *E. coli*, 7 *E. cloacae*, 3 *M. morgani*) ; SHV-2a (2 *E. cloacae*) ; SHV-12 (9 *E. cloacae*, 2 *K. oxytoca*, 2 *P. rettgeri*)
Elhani et al. 2010	103 *K. pneumoniae*	CTX-M-15 (43 isolates) ; CTX-M-14 (2 isolates) ; CTX-27 (2 isolates) ; SHV-12 (58 isolates) ; SHV-2a (3 isolates)
Ben Sallem et al. 2012	11 *E. coli*	CTX-M-1 (11 isolates) ; TEM-52c (1 isolate)
Hammami et al. 2012	44 *E. cloacae*	CTX-M-15 (39 isolates) ; SHV-12 (6 isolates) ; SHV-27 (1 isolate)
Hammami et al. 2013	15 *E. coli*	CTX-M-15 (13 isolates, not clonal isolates by PFGE); SHV-12 (2 isolates)
Alibi et al. 2015	118 *K. pneumoniae*	SHV (89%), CTX-M (81.35%) TEM (56.78%), (*bla* genes were not sequenced in all isolates)

plasmids in combination with other replicons in CTX-M-1 and CMY-2- producing *E. coli* isolates obtained from healthy humans and animals [56]. These findings agreed with those of previous studies that showed the high prevalence of IncI1 plasmid carrying blaCTX-M and blaCMY-2 genes in ESBL producing- *E. coli* strains from animal origin [7,13,33,35,36,52].

Genetic environment of blaCTX-M genes

The investigation of genetic environments of blaCTX-M genes showed the presence of the ISEcp1-blaCTX-M-1-orf477 structure in the majority of *E. coli* isolates recovered both from food-producing animals and faecal samples of healthy pets [12,17,25,26]. Nevertheless, the ISEcp1 sequence was found truncated by the IS26 and IS5 elements, located in complementary orientation [17,27]. ISEcp1-blaCTX-M-14-IS903 structure was presented in two isolates recovered from chicken samples [17]. As regards the blaCMY-2 gene, the ISEcp1-blaCMY-2 structure was identified in faecal samples of healthy food-producing animals [25]. However, a novel sequence has been reported where the IS10 sequence was demonstrated in one isolate recovered from chicken sample, truncating the ISEcp1 sequence (Accession number JX440359). Several previous studies have reported the presence of the ISEcp1 element upstream of blaCTX-M-1 and blaCMY-2 genes in Enterobacteriaceae and indicated that ISEcp1 plays an important

role in the capture, expression, and continuous mobilization of these genes [33,57,58].

All these reported findings are undoubtedly of great interest to the scientific community in Tunisia and in other parts of the world. However, the main shortcomings in the studies conducted are the small number of samples analysed, therefore, further studies based on representative sample sizes for each animal species are needed to determine the true prevalence and the precise epidemiology of ESBL production in Tunisia. It will be certainly of great interest to realize a large epidemiological study on ESBL production in industrial husbandry, especially poultry industry, in human population, in agriculture (irrigation water, soil, and vegetables) as well as in wastewater or aquatic environments in order to assess the genetic epidemiology of circulating ESBL-producer isolates and the mobile genetic vehicles of blaSHV genes.

Conclusion

In Tunisia, cephalosporin-resistance by production of ESBLs enzymes of CTX-M group and CMY-type are prevalent especially from poultry. Chicken husbandry was identified as the main food sector contributing to the ESBL with other antibiotic resistance genes reservoir in animals that may be acquired by humans through handling or consumption of contaminated meat. In addition, the dissemination of blaCTX-M gene may spread differently in the future owing to increasing plasmid exchanges between the two hosts. Taken together, this situation is therefore alarming and needs establishment of continuous monitoring program of antimicrobial resistance in Tunisia.

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Conflict of Interest

Mahrouki Sihem and Abbassi Mohamed Salah have contributed equally to the realization of this work. None of the contributing authors has any conflict of interests relevant to the subject matter or materials discussed in the manuscript. No funding or other financial support was received.

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