frontiers in
MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 09 March 2012
doi: 10.3389/fmicb.2012.00083

Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy region)

Alain Hartmann[1,2]*, Aude Locatelli[1], Lucie Amoureux[3], Géraldine Depret[1,2], Claudy Jolivet[4], Eric Gueneau[5] and Catherine Neuwirth[3]

[1] INRA, UMR Microbiologie des Sols et de l'Environnement (MSE), The Institut National de la Recherche Agronomique, Dijon, France
[2] Université de Bourgogne, Dijon, France
[3] Laboratory of Bacteriology, University Hospital of Dijon, Dijon, France
[4] Unité InfoSol, The Institut National de la Recherche Agronomique, Orléans, France
[5] Laboratoire Départemental de la Côte d'Or, Dijon, France

Edited by:
Rustam I. Aminov, University of Aberdeen, UK

Reviewed by:
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***Correspondence:**
Alain Hartmann, UMR Agroécologie 1347, INRA, The Institut National de la Recherche Agronomique Centre, 17 rue Sully, BP 86510, Dijon 21065, France.
e-mail: alain.hartmann@dijon.inra.fr

CTX-M [a major type of extended-spectrum beta-lactamase (ESBL)] producing *Escherichia coli* are increasingly involved in human infections worldwide. The aim of this study was to investigate potential reservoirs for such strains: soils, cattle, and farm environment. The prevalence of $bla_{\text{CTX-M}}$ genes was determined directly from soil DNA extracts obtained from 120 sites in Burgundy (France) using real-time PCR. $bla_{\text{CTX-M}}$ targets were found in 20% of the DNA extracts tested. Samples of cattle feces ($n = 271$) were collected from 182 farms in Burgundy. Thirteen ESBL-producing isolates were obtained from 12 farms and further characterized for the presence of *bla* genes. Of the 13 strains, five and eight strains carried $bla_{\text{TEM-71}}$ genes and $bla_{\text{CTX-M-1}}$ genes respectively. Ten strains of CTX-M-1 producing *E. coli* were isolated from cultivated and pasture soils as well as from composted manure within two of these farms. The genotypic analysis revealed that environmental and animal strains were clonally related. Our study confirms the occurrence of CTX-M producing *E. coli* in cattle and reports for the first time the occurrence of such strains in cultivated soils. The environmental competence of such strains has to be determined and might explain their long term survival since CTX-M isolates were recovered from a soil that was last amended with manure 1 year before sampling.

Keywords: extended-spectrum beta-lactamase, CTX-M, cattle, soil, Burgundy, farm environment

INTRODUCTION

The production of extended-spectrum beta-lactamases (ESBLs) is one of the most significant mechanisms of resistance to oxyiminocephalosporin antibiotics in *E. coli* (Pitout and Laupland, 2008). Among these enzymes, the CTX-M type ESBLs have emerged worldwide, they have progressively replaced the TEM and SHV families (Bonnet, 2004). To date, 123 $bla_{\text{CTX-M}}$ genes have been reported[1], the corresponding CTX-M enzymes are clustered in five groups, group 1, 9, and 2 being predominant. The producing organisms are sometimes involved in nosocomial infections but are widely encountered in community settings (Arpin et al., 2009; Woerther et al., 2010). Their rate of dissemination might suggest the occurrence of environmental reservoirs potentially leading to human contamination through water, food consumption or direct contact with animals (Leverstein-Van Hall et al., 2011). There are many descriptions of fecal carriage of such organisms among food-producing animals especially poultry (broilers) and pigs (Costa et al., 2009; Bortolaia et al., 2010; Cortes et al., 2010). The reports concerning livestock cattle are much less abundant (Horton et al., 2011), and there is no published data about the prevalence of CTX-M producing *E. coli* in soils. Nevertheless cultivated soils are frequently fertilized with agricultural or urban organic residues that may contain antibiotic resistant microorganisms (Moodley and Guardabassi, 2009; Reinthaler et al., 2010) and thus might act as environmental reservoirs.

The aim of this study was to develop an integrated approach encompassing soils, livestock, and farm environment in a whole region (Burgundy, France). A systematic large scale study was conducted using a molecular detection approach to detect $bla_{\text{CTX-M}}$ directly from soil DNA extracts. Conventional bacteriological methods were used to isolate ESBL-producing *E. coli* from cattle feces from 182 farms. When positive animals were detected, the corresponding farm environment, i.e., cultivated and pasture soils as well as cattle manure was sampled and analyzed by bacteriological methods. All environmental and animal isolates recovered during the study have been subjected to genotypic characterization and $bla_{\text{CTX-M}}$ genes have been sequenced.

MATERIALS AND METHODS

SOIL SAMPLING

Soils were sampled in the "Réseau de Mesures de la Qualité des Sols" (RMQS = French Soil Quality Monitoring Network) which is a network based on the sampling of soil with a 16 × 16 km systematic grid covering the whole French territory (Arrouays et al., 2002). The RMQS consisted in 2200 monitoring sites, which are located close to the center of each 16 × 16 km cell. Corresponding

[1] http://www.lahey.org/studies/

land covers were recorded and categorized as: large scale crops, pastures, orchards, vineyards, natural vegetation such as forests or meadows. Each site is geo-positioned with a precision <0.5 m. Twenty five individual core samples were collected from the topsoil (0–30 cm) using a stratified random sampling design within a 20 m × 20 m area. Core samples were bulked to obtain a composite sample for each site. Soil samples were air-dried and sieved to 2 mm before analysis. For this study 120 soil samples corresponding to the Burgundy region (four departments Côte d'Or, Saône et Loire, Yonne, and Nièvre) were analysed.

BOVINE FECES SAMPLING

From April 2009 to June 2009, a total of 271 fecal swabs of cattle were collected from 182 farms located in three departments of the Burgundy region, namely Côte d'Or, Nièvre, and Saône et Loire. Three groups of animals were sampled: (i) healthy adults (218), (ii) enteritic diseased calves (35), and healthy adults linked with sick calves (18). Swabs (COPAN, CML, France) were immediately transferred into tubes containing Amies agar gel transport medium. Bacteriological analyses of the fecal swabs were performed within 3 days after sampling. In farm 2 where positive animals had been detected in 2009, feces samples of the whole cow herd were analyzed (90 animals) in July 2010 as described above.

FARM ENVIRONMENT SAMPLING

For three farms (farms 1, 2 and 3), where CTX-M producing *E. coli* occurred in animals, we conducted further analysis of several environmental samples during the autumn 2009. These farms were chosen on the basis of the willingness of the farmers to cooperate to the study. In each farm one cropped and one pasture soils were sampled by taking three individual cores in the top soil (using a 7 cm width disinfected auger) that were pooled to obtain a composite sample. Manure samples were collected in the three farms using the same equipment (disinfected auger). All samples were kept moist in single use plastic bags at room temperature. Bacteriological analyses were performed on these samples within 3 days after sampling.

SOIL MICROBIAL COMMUNITY DNA EXTRACTION

Microbial DNA was extracted from bulk RMQS soil samples according to the method described by Ranjard et al. (2003). Briefly, 1.5 g of each soil was mixed with 5 ml of a solution containing 100 mM Tris (pH 8.0), 100 mM EDTA (pH 8.0), 100 mM NaCl, and 2% (w/v) sodium dodecyl sulfate. Two grams of 106 µm diameter glass beads and eight glass beads of 2-mm diameter were added in a bead-beater tube. The samples were then homogenized for 30 s at 1,600 rpm in a mini bead-beater cell disruptor (Mikrodismembrator; S.B. Braun Biotech International) and centrifuged at 7,000 × g for 5 min at 4°C after 30 min incubation at 70°C. The collected supernatants were incubated for 10 min on ice after adding 1/10 volume of 3 M potassium acetate (pH 5.5) and centrifuged at 14,000 × g for 5 min. After precipitation with one volume of ice-cold isopropanol, the nucleic acids were washed with 70% ethanol. DNA extracts were purified on polyvinyl polypyrrolydone minicolumns (Bio-Rad, France) by centrifugation at 1,000 × *g* for 2 min at 10°C. Residual impurities from DNA extracts were finally removed by using a Geneclean Turbo kit as recommended by the manufacturer (Q Biogene®, Illkirch, France). DNA's were quantified by agarose gel electrophoresis (1% agarose in TBE buffer) using calf thymus DNA dilutions as standards and the ImageQuant software (Applied Biosytems). Five nanograms of DNA were used per PCR reaction.

DEVELOPMENT AND USE OF A REAL-TIME PCR ASSAY FOR *bla*$_{\text{CTX-M}}$ DETECTION FROM SOIL EXTRACTED DNA

Twenty seven *bla*$_{\text{CTX-M}}$ genes sequences were aligned using the ClustalW program. Sequences were chosen to be representative from the five distinct groups of CTX-M enzymes described by Bonnet (2004). Seven sequences from CTX-M-1 group were used (GenBank accession numbers: DQ658221, X92506, EU921825, NC004464, Y10278, NC005327, and AY238472) corresponding to CTX-M-12, CTX-M-1, CTX-M-32, CTX-M-3, CTX-M-15, CTX-M-33, respectively. Four sequences from CTX-M-9 group (GenBank accession numbers: AF174129, EU916273, EU921824, and EU921826) corresponding to CTX-M-9, CTX-M-27, CTX-M-24, and CTX-M-65, respectively. Three sequences from CTX-M-2 group were used (GenBank accession numbers: X92504, EF374097, and AM982521) corresponding to CTX-M-2, CTX-M-56, and CTX-M-77 respectively. Three sequences from CTX-M-25 group were used (GenBank accession numbers: AF518567, DQ023162, and AM982522) corresponding to CTX-M-25, CTX-M-41, and CTX-M-78 respectively. Two sequences from CTX-M-8 group were used (GenBank accession number: AF189721 and AY750914) corresponding to CTX-M-8 and CTX-M-40. From these alignments, primers and Taqman type probes specific for either *bla*$_{\text{CTX-M}}$ genes encoding CTX-M-1 or CTX-M-9 groups were selected using Primer Express software (Applied Biosystems). For CTX-M-1 group, primers were F469 (5′-CAGCTGGGAGACGAAACGTT-3′) and R532 (5′-CCGGAATGGCGGTGTTTA-3′) and the probe was S490 (5′ -6FAM-CGTCTCGACCGTACCGAGCCGAC-TAMRA-3′). For the CTX-M-9 group, primers were F446 (5′-GAGGCGTGACGGCTTTTG-3′) and R513 (5′-CGTAGGTTCAG TGCGATCCA-3′), and the probe was S470 (5′-6FAM-CGATCGGCGATGAGACGTTTCGT-TAMRA-3′). Duplicate real-time PCRs were run with the ABI Prism 7900 sequence detection system (Applied Biosystems, France) and Absolute QPCR ROX master mix (Thermo Scientific, France) in 25-µl reaction mixtures and under reaction conditions of 95°C for 15 min (enzyme activation), and 40 two-step cycles consisting of 95°C for 15 s and 60°C for 1 min. Primers concentrations were 400 nM and Taqman type probe concentration was 200 nM, 1-µl of T4 GP32 (MP Biomedicals, France) was added per reaction. Five microliters of template DNA was added per reaction. PCR targets were cloned in pCR II-TOPO plasmid and recombinant linearized plasmids were used as standards. Gene copy numbers were calculated by amplifying six serial dilutions of the standard (10^1–10^5 copies per reaction mixture) in parallel with the samples. The specificity of the CTX-M detection systems has been checked *in silico* using the BLASTN algorithm across the GenBank nucleotide database (NCBI website). The specificity of the CTX-M detection systems has also been validated by

Table 1 | Human clinical strains used to validate the CTX-M-1 group and CTX-M-9 group detection systems (clinical strains were collected between 2006 and 2007 at CHU Dijon, France).

Strain	Strain origin	*bla* gene (CTX-M Group)	Detection by CTX-M-1 group PCR	Detection by CTX-M-9 group PCR
E. coli 913SE	CHU Dijon, bacteriology	CTX-M-1 (1)	+++	–
E. coli 882SE	CHU Dijon, bacteriology	CTX-M-2 (2)	–	–
E. coli 886SE	CHU Dijon, bacteriology	CTX-M-3 (1)	+++	–
E. coli 803SE	CHU Dijon, bacteriology	CTX-M-9 (9)	–	+++
E. coli 912SE	CHU Dijon, bacteriology	CTX-M-14 (9)		+++
E. coli 902SE	CHU Dijon, bacteriology	CTX-M-15 (1)	+++	–
K. ascorbata 268SL	CHU Dijon, bacteriology	KLUA (2)	–	–
K. cryocrescens 254SL	CHU Dijon, bacteriology	KLUG-1 (8)	–	–

E. coli, Escherichia col; K. ascorbata, Kluyvera ascorbata; K. cryocrescens, Kluyvera cryocrescens.

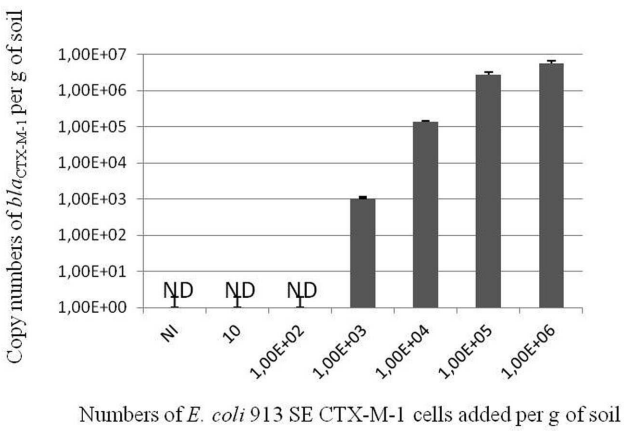

FIGURE 1 | Detection levels of $bla_{CTX-M-1}$ genes by real-time PCR (CTX-M-1 group detection system) in soil microcosms spiked with increasing amounts of *E. coli* strain 913SE CTX-M-1. ND, not detected. Results are the means of two replicates and bars represent standard deviations.

using human clinical isolates harboring known bla_{CTX-M} genes (**Table 1**). In order to determine the efficiency of these detection systems in soil, soil samples (2 *g*) were spiked with known titers of *E. coli* strain EC 913 SE carrying $bla_{CTX-M-1}$, soil DNA was extracted immediately as described above and subjected to real-time PCR using the primers and probe specific for CTX-M-1 group (**Figure 1**).

BACTERIOLOGICAL METHODS AND ANTIBIOTIC RESISTANCE TESTING

All fecal samples from the cows and all environmental samples have been inoculated on ESBL screening agar plates containing Drigalsky medium supplemented with either cefotaxime (4 mg/l) or ceftazidime (4 mg/l). The antibiotic susceptibility testings have been performed by the disk diffusion method. A range of antibiotics including penicillins, cephalosporins, carbapenem (imipenem), aminoglycosides (kanamycin, tobramycin, gentamycin, streptomycin, amikacin, netilmicin), chloramphenicol, quinolones (ciprofloxacin, ofloxacin), doxycycline, cotrimoxazol, and colistin was used to determine antibiotic susceptibility patterns of the isolates and the production of ESBL was assessed by the double-disk synergy test (Jarlier et al., 1988). Guidelines for the interpretation of antibiotic susceptibility testing were from the Clinical and Laboratory Standards Institute (CLSI, 2010).

IDENTIFICATION OF ESBL TYPES

The characterization of the *bla* gene was performed by PCR on isolates with a positive double-disk synergy test by using primers specific for the genes encoding ESBL from TEM, SHV, and CTX-M families (Chanal et al., 1992; Neuwirth et al., 1995; Sabate et al., 2002). PCR products were sequenced on both strands using ABI PRISM 3100 (Applied Biosystems, France).

GENOTYPING BY THE DIVERSILAB SYSTEM (BIOMÉRIEUX)

The Diversilab system is a typing technique which is based on the repetitive-sequence-based PCR (rep-PCR) and proved to be a useful method for genotyping of *E. coli* (Fluit et al., 2010). All reagents, automates and software used for this study were provided by bioMérieux, France. DNA was extracted from *E. coli* colonies using an UltraClean Microbial DNA isolation kit and following the manufacturer's instructions. The extracted DNA was amplified using a DiversiLab *Escherichia* DNA fingerprinting kit. Electrophoresis of the amplified fragments using a microfluidics LabChip with an Agilent 2100 Bioanalyzer and analysis were performed according to the protocol of the manufacturer. Isolates with a similarity of <95% were considered different, and isolates with a similarity of >98% were considered indistinguishable.

MLST TYPING OF *E. COLI* STRAINS

MLST typing of *E. coli* strains was done according to recommendations found at http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primersColi_html. The *E. coli* MLST scheme uses internal fragments of the following seven house-keeping genes: *adk* (adenylate kinase), *fum*C (fumarate hydratase), *gyr*B (DNA gyrase), *icd* (isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *pur*A (adenylosuccinate dehydrogenase), *rec*A (ATP/GTP binding motif). MLST typing was done at the Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany, by Dr. Sebastian Guenther.

RESULTS

bla_{CTX-M} DETECTION IN SOILS

The method of real-time PCR detection of bla_{CTX-M} has been validated on the basis of amplification of DNA from pure strains or from inoculated soil DNA. The two detection systems proved to be sensitive and specific of bla_{CTX-M} genes encoding group 1 and group 9 enzymes. Detection threshold was estimated to be one copy per PCR reaction. Detection limit in soil samples was estimated to be 10^3 copies per gram of soil (**Figure 1**). Out of the 120 soil extracted DNA's from Burgundy, 22 were found to be positive using one or two detection systems (**Figure 2**). The proportion of positive soil for occurrence of $bla_{CTX-M\ 1\ or\ 9}$ was of 3.3% in Yonne, 6.2% in Côte d'Or, 16.0% in Nièvre, and 24.2% in Saône et Loire. These results demonstrate the wide distribution of some bla_{CTX-M} genes in soils from Burgundy. No obvious correlation was found between soil physical and chemical properties and the occurrence of bla_{CTX-M} genes [analysis by principal component analysis (PCA), data not shown]. The causes of the higher prevalence of bla_{CTX-M} genes in soils from Saône et Loire remain to be deciphered.

DETECTION OF ESBL-PRODUCING *E. COLI* FROM LIVESTOCK

Out of the 271 feces samples analyzed in 2009, 13 proved to contain ESBL-producing *E. coli* (**Table 2**). Five isolates harbored the TEM-71-ESBL. Interestingly all these isolates were originated from "Nièvre." Four isolates were genotypically indistinguishable. Among them isolates 32 and 101 were originated from two farms located in the same village. The others (92 and 107) were recovered in animals from farms located 16 km apart. The eight remaining isolates harbored CTX-M-1. The CTX-M-1 producing isolates have been isolated in three departments (Nièvre, Saône et Loire, and Côte d'Or). The genotypical analysis performed by rep-PCR with Diversilab revealed a wide diversity among the strains isolated from different farms. However, the strains 234 and 235 originated from two animals sampled in a same farm (farm 2) were clonally related (not distinguishable).

One year later (July 2010), feces samples of the whole cow herd from farm 2 were analyzed (90 animals), and CTX-M-1 producing *E.coli* isolates were detected from two animals. Strains V71 and V9 were genotyped and were different from the strains 234 and 235 isolated in June 2009 (**Table 2**).

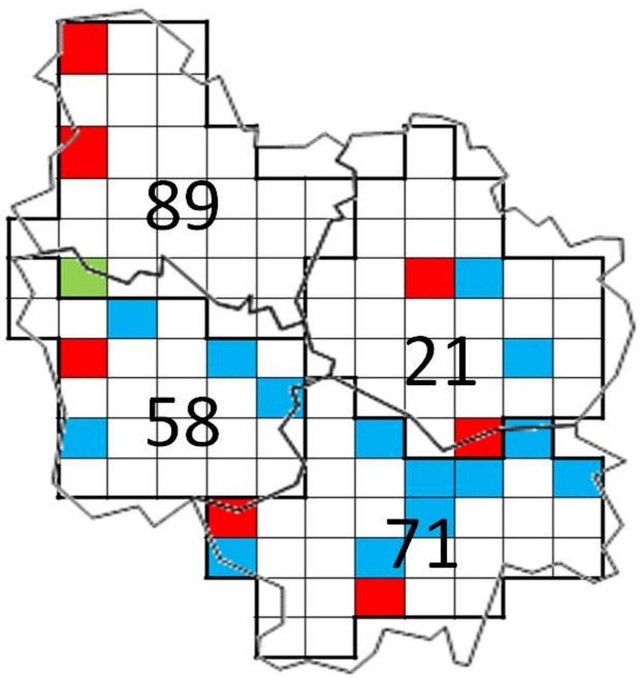

FIGURE 2 | Distribution of RMQS soils where bla_{CTX-M} genes were detected (colored squares) in the four departments of Burgundy (21: Côte d'Or, 58: Nièvre, 89: Yonne, and 71: Saône et Loire). Red square: detection of $bla_{CTX-M-1}$, blue square: detection of $bla_{CTX-M-9}$, green square: detection of both gene types.

DETECTION OF ESBL *E. COLI* ISOLATES IN ENVIRONMENTAL SAMPLES

The environments of three farms where positive animals have been detected were chosen for further investigations. In the farm 1, no ESBL-producing *E. coli* were found in the farm environment. On the contrary, 4 and 6 CTX-M-1 producing *E. coli* were detected in farms 2 and 3 respectively (**Table 2**). In farm 2, a cultivated soil amended one year before with liquid cow manure from the farm was found to contain CTX-M harboring *E. coli* strains. These isolates were genotypically indistinguishable from the animal strains F2/CO/234 and F2/CO/235. Interestingly the soil sampling site was located 3 km away from the cattle barn. In farm 3 the positive samples were: pasture soil, and composted manure. In that case, composted manure isolate was identical to the animal isolate, whereas one soil isolate had a different genotype.

DISCUSSION

The worldwide emergence of CTX-M producing *E. coli* in human clinical samples is a public health concern (Pitout and Laupland, 2008) and raises several interrogations regarding their high dissemination rate. These strains are also widely described in animals (pets, farm animals; Costa et al., 2009; Bortolaia et al., 2010; Cortes et al., 2010). It can be hypothesized that there is a cross-transmission between the human being and the animals (Leverstein-Van Hall et al., 2011), or that there are common environmental sources leading to human and animal contaminations. The treatment regimes for eradication of infections caused by such strains are sometimes very limited, i.e., when the CTX-M production is associated with the production of aminoglycosides modifying enzymes and gyrase mutations (fluoroquinolones resistance). We report in this study our findings concerning the prevalence of fecal carriage of *E. coli* CTX-M producing in cattle as well as the contamination of the farm environment.

DETECTION OF ESBL-PRODUCING *E. COLI* IN LIVESTOCK

We have detected ESBL-producing *E. coli* in livestock (5% of the animals tested). In Europe such strains have been only sporadically described in cattle: in Germany (Guerra et al., 2007), England (Horton et al., 2011), and Spain (Brinas et al., 2005). To our best knowledge this is the second report of ESBL-producing *E. coli* in cattle in France where Meunier et al. (2006) reported three isolates (carrying either CTX-M-1 or CTX-M-15 ESBL) responsible for infections in cow. Our results demonstrate that ESBL-producing *E.*

Table 2 | Characteristics of the *E. coli* strains isolated from animals, manure and soils.

Strain designation[a], farm number/department/ isolate number	Origin[b]	Type of ESBL	Rep-PCR genotype (Diversilab)	Associated non-beta-lactam antibiotic resistance[c]	ST[d]
F1/SL/250	Animal EDC	CTX-M-1	E	CIP-G	
F2/CO/234	Animal H	CTX-M-1	A	SXT	
F2/CO/235	Animal H	CTX-M-1	A	SXT	2497
F2/CO/RET12	Cultivated soil	CTX-M-1	A	SXT	155
F2/CO/RET15	Cultivated soil	CTX-M-1	A	SXT	
F2/CO/RET20	Cultivated soil	CTX-M-1	A	SXT	155
F2/CO/RET21	Cultivated soil	CTX-M-1	A	SXT	155
F2/CO/V9 (2010)	Animal H	CTX-M-1	F	SXT	
F2/CO/V71 (2010)	Animal H	CTX-M-1	G	SXT	
F3/CO/241	Animal H	CTX-M-1	B	SXT	2498
F3/CO/RET23	Pasture soil	CTX-M-1	H	CIP-Cm-SXT	58
F3/CO/RET24	Pasture soil	CTX-M-1	B	SXT	58
F3/CO/RET25	Pasture soil	CTX-M-1	B	SXT	58
F3/CO/RET26	Pasture soil	CTX-M-1	B	SXT	58
F3/CO/RET27	Composted manure	CTX-M-1	B	SXT	2498
F3/CO/RET28	Composted manure	CTX-M-1	B	SXT	
F3/CO/RET29	Pasture soil	CTX-M-1	B	Cm-SXT	2499
F4/N/32	Animal HLEDC	TEM-71	C	CIP-G-Cm-SXT	
F5/N/87	Animal H	CTX-M-1	I	CIP-G-Cm-SXT	
F6/N/92	Animal EDC	TEM-71	C	CIP-G-Cm-SXT	
F7/N/101	Animal HLEDC	TEM-71	C	CIP-G-Cm-SXT	178
F8/N/105	Animal H	TEM-71	J	CIP-G-Cm-SXT	
F9/N/107	Animal H	TEM-71	C	CIP-G-Cm-SXT	
F10/SL/190	Animal EDC	CTX-M-1	K	CIP-Cm-SXT	
F11/CO/240	Animal H	CTX-M-1	D	SXT	
F12/CO/245	Animal H	CTX-M-1	D	SXT	

[a] *SL, department of Saône et Loire; CO, department of Côte d'Or; N, department of Nièvre. For farms 2 and 3, isolates from cattle and from environment were listed together to allow strain comparison. In farm 2, the two isolates isolated in 2010 are indicated.*

[b] *Strains were isolated from animal feces, manure or soil. H, healthy animal; EDC, enteritic disease calf; HLEDC, healthy animal linked with an enteritic disease calf.*

[c] *Associated antibiotic resistances: CIP, ciprofloxacin; G, gentamicin; Cm, chloramphenicol; SXT, Cotrimoxazol.*

[d] *ST, sequence type from MLST analysis as defined at University College Cork (UCC, Ireland) http://mlst.ucc.ie/mlst/dbs/Ecoli/GetTableInfo_html*

coli strain are carried indifferently by sick and healthy animals. Our results are in agreement with previously reported data: CTX-M-1 is the ESBL which is the most frequently encountered in animals (Girlich et al., 2007; Bonnedahl et al., 2009; Bortolaia et al., 2010).

Nevertheless we have detected for the first time *E. coli* TEM-71-producing in five animals whereas they have been exceptionally reported in human infection (Wong-Beringer et al., 2001; Rasheed et al., 2002; De Champs et al., 2004). This finding is interesting from the epidemiological point of view because four isolates are genotypically related whereas they were originated from different farms (F4, F6, F7, and F9) This indicates that some clonal isolates are widespread or might circulate (through animal transfer) at a regional scale or that there is a common reservoir in the region. At the farm level, this might suggest a potential cross-contamination among cattle or the presence of a reservoir within the farm. Finally, this is the first description of TEM-71 producing *E. coli* in animals. It is noteworthy that the TEM-71 producing isolates are multiresistant to ciprofloxacin, gentamicin, chloramphenicol, and cotrimoxazol. On the opposite, most of the CTX-M-1 producing isolates harbor a single associated resistance to cotrimoxazol (**Table 2**). Therefore, cefotaxim resistant *E. coli* recovered from animals originated from different farms show significantly different antibiotic resistance patterns. This might reflect different exposition of the animals to antimicrobial agents or different sources of contamination.

In farm 2, strains isolated from cattle in 2010 have a different genotype compared to those isolated in 2009. Several hypothesis might explain this result: (i) horizontal gene transfer may occur between *E. coli* genotypes carried by cattle, since $bla_{\text{CTX-M}}$ genes are carried by plasmids, (ii) new exogenous *E. coli* strains may have been disseminated in the farm environment.

DETECTION OF ESBL-PRODUCING *E. COLI* IN FARM ENVIRONMENT

Among the 12 farms where ESBL-producing *E. coli* were detected from animals, we chose three farms to further investigate the dissemination of these strains in the farm environment. Farms were chosen on the basis of which farmers will decide to cooperate to our study.

In two of the three farms where positive animals were reported, ESBL *E. coli* isolates were detected in environmental samples.

Interestingly, in farm 2 one of the soil sampling sites was a crop field that has been amended 1 year before (autumn 2008) with liquid manure collected in the barn. This crop field was located 3 km away from the cattle barn. In autumn 2009, we were able to isolate several CTX-M producing *E. coli* strains from this soil, indicating that such strains have the ability to survive at least 1 year after soil amendment under environmental conditions. In farm 3, the positive samples were: pasture soil, and composted manure. In that case, composted manure isolate was identical to the animal isolate, whereas one soil isolate had a different genotype.

FURTHER GENOTYPIC COMPARISON OF *E. COLI* STRAINS

MLST typing of *E. coli* strains isolated from this study has been partially done. Preliminary results indicate that some *E. coli* strains isolated from animal and soil belong to previously described ST58, ST155, and ST178 as defined in the MLST database hosted at UCC[2]. Four strains appear to belong to new genotypes namely ST2497, ST2498, and ST2499. $bla_{\text{CTX-M-1}}$ genes were found in *E. coli* isolates with variable ST. ST8 and ST155 grouped strains from animal and human origin, some strains belonging to these ST are pathogenic for human or animals. Plasmids of four strains were sequenced (454 pyrosequencing), $bla_{\text{CTX-M}}$ genes were found on plasmids and located close to one copy of ISEcp1 in each strain (data not shown).

MOLECULAR DETECTION OF $bla_{\text{CTX-M}}$ GENES IN SOILS FROM BURGUNDY

The origin of the $bla_{\text{CTX-M}}$ genes detected in soils remains to be elucidated, several hypotheses might be investigated. *E. coli* strains harboring $bla_{\text{CTX-M}}$ genes might have been disseminated in soils through manure application on cultivated soils (animal origin), sewage sludge application (human origin), or through irrigation with treated or untreated waste-water. As the progenitor $bla_{\text{CTX-M}}$ gene is the chromosomal *bla* genes of different species of *Kluyvera* (Decousser et al., 2001; Poirel et al., 2002) we can also hypothesize that *bla* genes detected in the studied soils might be harbored by bacterial species other than *E. coli*. These bacteria might thus act as potential environmental *bla* genes reservoirs. To our best knowledge, this is the first report about occurrence of $bla_{\text{CTX-M}}$ genes in soil.

The detection of CTX-M producing *E. coli* in soil was the major finding of this study. Our work demonstrated the survival of CTX-M producing *E. coli* in soil at least for one year. Future work will aim at linking $bla_{\text{CTX-M}}$ occurrence with the capacity of *E. coli* strains to survive under environmental conditions. Finally, potential risks for public health through water or vegetables contamination will have to be determined.

[2]http://mlst.ucc.ie/mlst/dbs/Ecoli/GetTableInfo_html

ACKNOWLEDGMENTS

This work was co-funded by the Conseil Régional de Bourgogne (Regional Council of Burgundy), and by MERIAL SA. We wish to thank the Groupement Technique Vétérinaire (GTV) of Saône et Loire for animal feces sampling. Finally, we wish to thank Dr. Sebastian Guenther (Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany) for his invaluable help in MLST typing of the *E. coli* strains. The authors wish to thank the GenoSol Platform (particularly M. Lelièvre and S. Dequiedt) of UMR Agroecology, for supplying soil DNA extracts from Burgundy and for data-mining.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 November 2011; accepted: 19 February 2012; published online: 09 March 2012.

Citation: Hartmann A, Locatelli A, Amoureux L, Depret G, Jolivet C, Gueneau E and Neuwirth C (2012) Occurrence of CTX-M producing Escherichia coli in soils, cattle, and farm environment in France (Burgundy region). Front. Microbio. ***3****:83. doi: 10.3389/fmicb.2012.00083*

This article was submitted to Frontiers in Antimicrobials, Resistance and Chemotherapy, a specialty of Frontiers in Microbiology.