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Sequence Type 48 *Escherichia coli* Carrying the $bla_{CTX-M-1}$ IncI1/ST3 Plasmid in Drinking Water in France

Jean-Yves Madec,[a] Marisa Haenni,[a] Cécile Ponsin,[a] Nicolas Kieffer,[a] Emmanuelle Rion,[b] Benoit Gassilloud[b]

Unité Antibiorésistance et Virulence Bactériennes, ANSES Site de Lyon, Lyon, France[a]; Unité de Microbiologie des Eaux, Laboratoire d'Hydrologie de Nancy, ANSES Site de Nancy, Nancy, France[b]

Drinking water has rarely been recognized as a source of antimicrobial resistance for humans, and only in low-income countries. Here, a sequence type 48 *Escherichia coli* isolate carrying the $bla_{CTX-M-1}$ IncI1/ST3 plasmid was recovered from drinking water in France. This plasmid was similar to other $bla_{CTX-M-1}$ IncI1/ST3 plasmids found previously in animals and humans. Our findings highlight the possible human transfer of extended-spectrum β-lactamase (ESBL) genes through drinking water in high-income countries.

Extended-spectrum β-lactamases (ESBLs) are widespread enzymes that confer resistance to broad-spectrum cephalosporins. ESBL genes are mostly located on plasmids, which play key roles in horizontal transfer of the genes. Consumption of ESBL-contaminated foodstuff or contacts with ESBL-colonized/infected animals enhance the risk of human transfer of ESBL genes from nonhuman sources. ESBLs have also been found in soils and rivers polluted by human or animal waste, such as hospital sewage or runoff from land occupied by livestock. Drinking water is also a source of human contamination with waterborne pathogens and/or antimicrobial-resistant bacteria. However, reports of drinking water contaminated with ESBL-producing organisms are scarce (1–3).

Here, we describe an ESBL-positive *Escherichia coli* isolate (isolate 32420) recovered in 2011 from a 100-ml water sample collected after treatment from an old (>30-year-old) water supply located at the periphery of a city in northeastern France. Drinking water samples were collected from 28 water supplies throughout France between December 2011 and April 2012. Water supplies were selected on the basis of repeated quality failures, i.e., detection of coliforms, over the previous 3 years. Twenty-six supplies were small, serving fewer than 2,000 people, whereas two served around 10,000 people. Water treatment before distribution included disinfection with chlorine in the smallest water supplies or sand filtration, coagulation with aluminum, and a final chlorine dioxide treatment in larger supplies collecting water from rivers.

One-liter water samples were collected in sterile bottles containing sodium thiosulfate, at the point at which drinking water enters the distribution system or at a point of consumption. Samples were preserved in ice boxes and transported to the ANSES Nancy laboratory, where 100 ml was analyzed by membrane filtration and growth on selective media (4) to determine the presence of coliforms. *E. coli* isolates ($n = 67$) (confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) were detected in all samples, with counts ranging from 1 to 45 CFU/100 ml. None of the isolates was considered virulent, since stx_1, stx_2, and *eae* genes were absent. All isolates were tested for antimicrobial susceptibility to 32 antibiotics by disk diffusion, according to the Antibiogram Committee of the French Society for Microbiology guidelines (www.sfm-microbiologie.org), and 6 *E. coli* isolates from 6 different water supplies were determined to be resistant to at least one antimicrobial (Table 1). Among those isolates, one *E. coli* isolate that was resistant to ceftiofur, tetracyclines, and sulfonamides was studied further.

TABLE 1 Antimicrobial-resistant *E. coli* isolates from the 28 water supplies

Water supply system	Antibiotic resistance pattern[a]
WS1	AMX
WS3	STR, TET, SUL
WS6	STR, TET, NAL
WS7	AMX, AMC, CEF, TET, SUL
WS13	SUL
WS20	ESBL phenotype, TET, SUL

[a] AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CEF, cephalothin; STR, streptomycin; TET, tetracyclines; SUL, sulfonamides; NAL, nalidixic acid.

PCR and sequencing showed that *E. coli* isolate 32420 harbored the $bla_{CTX-M-1}$ gene. Transferability of the ESBL-carrying plasmid was proved, since CTX-M-1-positive transconjugants presenting no additional resistance to non-β-lactam antibiotics were obtained after broth mating on Mueller-Hinton agar supplemented with rifampin (250 μg/ml) and cefotaxime (4 μg/ml). The $bla_{CTX-M-1}$ gene was carried by a 100-kb IncI1/ST3 plasmid, as detected with S1 nuclease treatment of DNA followed by pulsed-field gel electrophoresis (PFGE), Southern blot hybridizations with adequate digoxigenin (DIG)-labeled probes (Roche Applied Science, Mannheim, Germany), PCR-based replicon typing (Diatheva, Fano, Italy), and plasmid multilocus sequence typing (MLST) (5). The restriction fragment length polymorphism (RFLP) profile of the IncI1/ST3 plasmid after digestion with PstI or EcoRI was comparable but nonidentical to those of previously published $bla_{CTX-M-1}$ IncI/

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Address correspondence to Marisa Haenni, marisa.haenni@anses.fr.

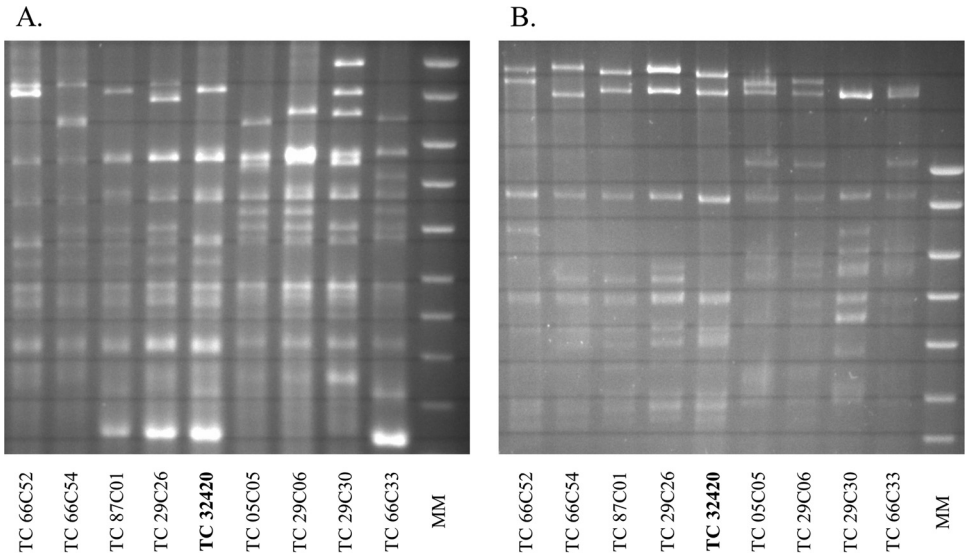

FIG 1 RFLP analysis of *E. coli* plasmid DNA digested with PstI (A) or EcoRI (B). The *E. coli* isolated from drinking water (isolate 32420) was compared with *E. coli* plasmids originating from humans (6). MM, molecular mass markers.

ST3 plasmids of animal and human origin (Fig. 1) (6). According to the protocol described on the MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli), *E. coli* isolate 32420 was of sequence type 48 (ST48) (clonal complex 10 [CC10]), an ST that has been observed previously in both humans and animals (7, 8).

Drinking water is a well-recognized source of direct human contamination with waterborne pathogens, but the risk of human transfer of resistance genes has rarely been documented. ESBL producers were detected in packaged drinking water bags sold by street vendors in Africa (9), in a public water supply in Bangladesh (3), and in drinking water in urban Nepal (1). In addition, NDM-1 carbapenemases were identified in tap water samples in India (2). However, these reports all came from countries with poor water supply systems and/or sanitation facilities, coupled to limited controls of antibiotic usage and residues. To the best of our knowledge, this is the first report of an ESBL producer in drinking water in a high-income country. Interestingly, isolate 32420 harbored a $bla_{CTX-M-1}$ IncI1/ST3 plasmid, which was widely found in *E. coli* in food animals in France and neighboring countries (10, 11). Cattle farms are known to be located upstream of the sampling point for the old water supply, which collected water directly from the river. No absolute signature of the origin of ESBL plasmids exists, since such $bla_{CTX-M-1}$ IncI1/ST3 plasmids are also sporadically found in humans (6), but an animal/environmental origin of this $bla_{CTX-M-1}$ IncI1/ST3 plasmid is highly plausible.

E. coli and enterococci are used as indicators of fecal contamination in drinking water and should be totally absent from water, according to European legislation (12). In France, almost 3.3% of the population was served at least once in 2012 with drinking water that did not meet this criterion; 94% of the unsuitable drinking water involved small water supplies serving fewer than 2,000 people (13). Efforts are made continuously to improve water treatment and, as an example, the water supply from which the ESBL-producing *E. coli* strain was isolated has recently been fully renovated. Nevertheless, further investigations along the water distribution pipelines, including the use of ESBL-selective media, would make sense, as distribution systems exhibit favorable conditions for the formation of biofilms, which may hide internal reservoirs of ESBL producers. Consequently, although the isolate described here would probably not have been pathogenic to humans, our findings highlight the extent to which the growing enrichment of the environmental reservoir with ESBL genes may allow their colonization of remote niches, including in high-income countries.

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