

High quantities of multidrug-resistant *Escherichia coli* are present in the Machángara urban river in Quito, Ecuador

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ABSTRACT

Urban river pollution by multidrug-resistant (MDR) bacteria constitutes an important public health concern. Epidemiologically important strains of MDR *Escherichia coli* transmissible at the human–animal–environment interfaces are especially worrying. Quantifying and characterizing MDR *E. coli* at a molecular level is thus imperative for understanding its epidemiology in natural environments and its role in the spread of resistance in precise geographical areas. Cefotaxime-resistant *E. coli* was characterized along the watercourse of the major urban river in Quito. Our results showed high quantities of cefotaxime-resistant *E. coli* (2.7×10^3 – 5.4×10^5 CFU/100 mL). The antimicrobial resistance index (ARI) revealed the exposure of the river to antibiotic contamination, and the multiple antibiotic resistance index indicated a high risk of contamination. The *bla*_{CTX-M-15} gene was the most prevalent in our samples. Isolates also had class 1 integrons carrying aminoglycoside-modifying enzymes and folate pathway inhibitors. The isolates belonged to phylogroups A, B1 and D. Clonal complex 10 was found to be the most prevalent (ST10, ST44 and ST 167), followed by ST162, ST394 and ST46. Our study provides a warning about the high potential of the major urban river in Quito for spreading the epidemiologically important MDR *E. coli*.

Key words | antimicrobial resistance genes, Ecuador, extended spectrum β -lactamase, *Escherichia coli*, Machángara, urban river

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INTRODUCTION

The rise of difficult-to-treat infections caused by Enterobacteriaceae resistance to third-generation cephalosporins (3GC) is a central health concern worldwide (Dolejska & Papagiannitsis 2018). Extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* are especially important due to their ability to cause intestinal and extraintestinal infections, and therapeutic responses to them are limited to last resort antibiotics (carbapenems, colistin and tigecycline) (Iovleva & Doi 2017; Srinivas & Rivard 2017; Talaga-ćwiertnia *et al.* 2017). Intriguingly, ESBL-producing *E. coli* can be a part of the intestinal microbiota of healthy

carriers for years before producing an infection. This particular characteristic leads to an underestimated impact of environmental and food contaminations with ESBL-producing *E. coli* on human health (Tellevik *et al.* 2016).

Currently, increasing evidence has revealed the spread of ESBL-producing *E. coli* through the human–animal–environment interfaces (Seni *et al.* 2018). Therefore, the potential dissemination of epidemiologically important strains of *E. coli* through these interfaces is particularly alarming. Evidence of environmental distribution of ESBL-producing *E. coli* (Seni *et al.* 2018) has forewarned about

the growing and complex problem of the dissemination of bacterial resistance that compromises environment integrity and public health (Chong *et al.* 2018). In a typical model, human activities produce high microbiological and chemical polluted water that is discharged into urban rivers. Subsequently, this contamination travels downstream to reach small towns, production fields and natural habitats, which has a negative impact on human and animal health, agricultural products and environment (Dolejska & Papagiannitsis 2018; Leonard *et al.* 2018; Manaia *et al.* 2018; Sanderson *et al.* 2018; Vounba *et al.* 2019). Nevertheless, there is limited information on the levels and characteristics of ESBL-producing *E. coli* in polluting rivers.

The characterization of *E. coli* from rivers at a molecular level (clonal groups/phylogroups and resistance profiles) could improve our understanding of their epidemiology on natural environments and the role of rivers in the spread of resistance in particular geographical areas (Bajaj *et al.* 2015). This topic has not been considered in our location. Consequently, the aim of our study was to quantify and characterize *E. coli* resistance to cefotaxime in the major urban river in the city of Quito, Ecuador.

METHODS

Study area

Quito, the capital city of Ecuador, is located in the Andean Region at an altitude that ranges from 1,800 meters above sea level (masl) to 4,794 masl. Most of the wastewater of the city is discharged directly towards the Machángara River through sewage drainage. This water flow originates in the Atacazo Highlands, located approximately 6.5 km from the south border of the city, and following a length of 40 km crosses the city from southwest to northeast until it reaches the Tumbaco valley.

Sampling

Using a collection of three satellite orthorectified images from the Pleiades-1A sensor (date: June 2013, spatial resolution: 0.5 m), multispectral remote data processing, spatial analysis techniques and supported fieldwork testing, a land use/cover map at a 1:15,000 scale was derived. Using this

input, from October to November 2015, we collected samples from nine sites along a segment of approximately 13 km near the Machángara River. Sites were selected based on their accessibility, altitude (a gradient of 2,778–2,785 masl) and types of land use (agricultural, residential, conservation and industrial) (Figure 1). Each site was sampled five times and characterized by its socioeconomic allocation, altitude range as well as water pH and temperature. At each site, we sampled water from the middle of the stream with sterile 100 mL plastic flasks. The samples were stored in a 4 °C cooling box until it reached the laboratory for analysis, which was performed within 6 h of collection.

Microbiological procedures

The quantitative evaluation of bacterial contamination was conducted using the filtration method. Briefly, in accordance with the estimate of coliform contamination, appropriate volumes of each sample were mixed with 100 mL of saline water (0.85%) and passed through 50 µm filters (Millipore, USA) using Microfil S Filtration Devices (Merck, USA) following the manufacturer's guidelines. The membranes were deposited on one of three kinds of plates, as follows: m-ColiBlue24® medium plates (Merck, USA) without antimicrobial selection for total *E. coli* count, plates supplemented with cefotaxime (3 µg/mL) for the quantification of 3GC-resistant *E. coli* and plates supplemented with imipenem (2 µg/mL) for carbapenem-resistant *E. coli*. The plates were incubated at 35 °C for 24 h. To determine *E. coli* quantities, we manually counted all blue colonies and expressed the data as the geometric means of *E. coli*/100 mL (Table 1).

Three resistant *E. coli* colonies from each positive sampling point were stored for further analysis at –80 °C using glycerol and Trypticase Soy Broth (TSB) (1:1, v/v). The species was confirmed using a Microflex LT Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) spectrometer (Bruker Daltonics, Inc., USA) and examined in the MALDI-TOF Biotyper v.3.1. ESBL production was identified using the double-disk test, and MIC profiles were performed using the VITEK®2 Compact with the AST-GN66 card (bioMérieux, USA), which is an automated microdilution system. Antimicrobial susceptibility data were interpreted following clinical breakpoints (CLSI 2019).

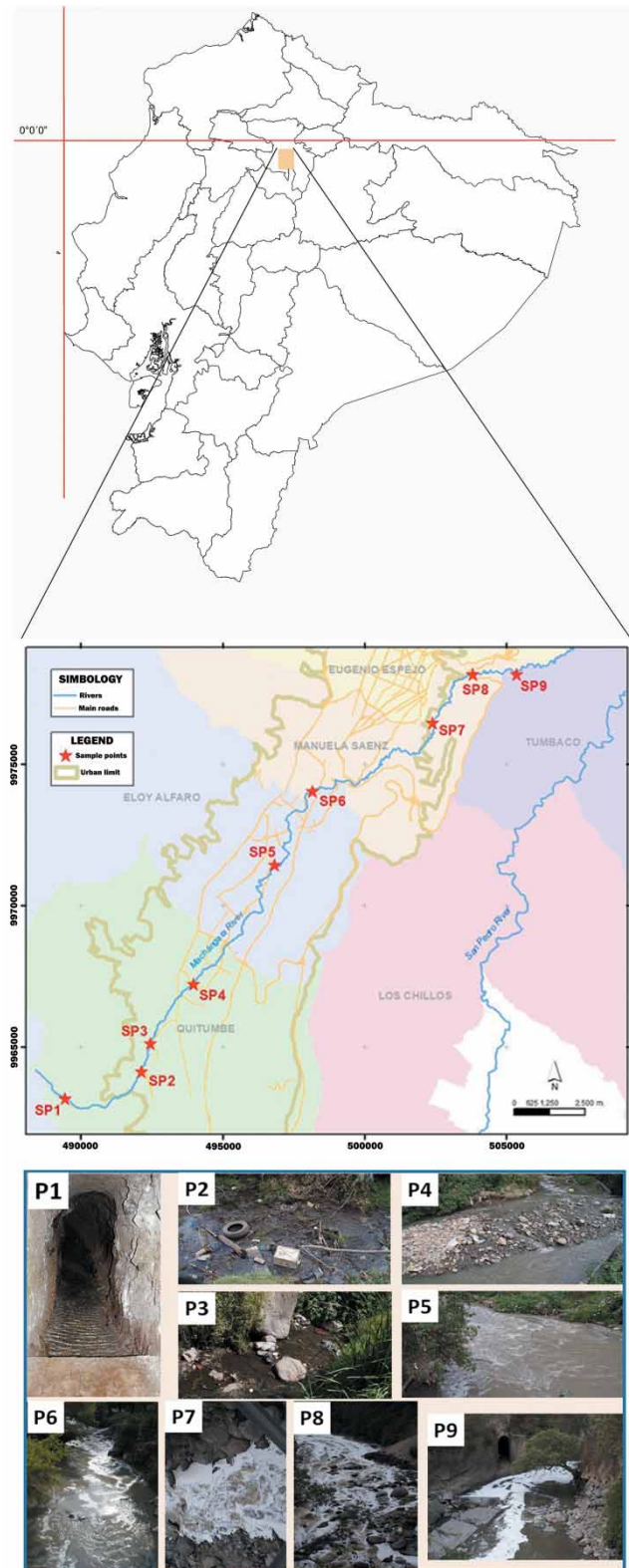


Figure 1 | Study area and sampling points.

Genetic analysis

ESBL genes were identified with polymerase chain reaction (PCR) protocols described by Carattoli *et al.* (2008) for *bla*_{CTX-M-1}, Jiang *et al.* (2006) for *bla*_{CTX-M-2}, Hopkins *et al.* (2006) for *bla*_{CTX-M-8}, Paauw *et al.* (2006) for *bla*_{CTX-M-9}, Liebert *et al.* (2004) for *bla*_{TEM}, Arlet *et al.* (1997) for *bla*_{SHV} and Zurita *et al.* (2013) for *bla*_{KPC} (Ortega-Paredes *et al.* (2016) for colistin-resistant *mcr-1* gene and Lévesque *et al.* (1995) for integron 1 variable region). Fingerprint characterization was performed in selected isolates by repetitive element palindromic PCR (REP-PCR) analysis (Araújo *et al.* 2017), and multilocus sequence typing (MLST) was achieved using a scheme proposed at the University of Warwick (Wirth *et al.* 2006). Phylogroups were assigned as described elsewhere (Clermont *et al.* 2000). Positive and negative controls were included in each experiment. The PCR products of resistance genes and MLST were purified and sequenced, both strands, at Macrogen Inc. (South Korea). Sequences were edited and analyzed in Geneious R10 using the database of ResFinder-3.0 (Zankari *et al.* 2012).

Statistical analysis

We used the equation described by Mohanta & Goel (2014) to calculate the antibacterial resistance index (ARI) for each cefotaxime-resistant *E. coli*-positive sampling point. Likewise, we calculated the multiple antibiotic resistance index (MARI) for each resistant *E. coli* isolate as outlined previously by Blasco *et al.* (2008).

REP-PCR bands were analyzed using BioNumerics software v.7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Fragments ranging from 200 to 1,500 bp were included in the analysis. The unweighted pair group method using the arithmetic averages algorithm (UPGMA) with a 1.5% of tolerance was used to construct a dendrogram.

RESULTS AND DISCUSSION

At the first sampling point (SP1), water was collected in a tunnel system that accumulates water filtered by the highlands ecosystem (Páramo). Part of this water is stored for future use and, after treatment, is distributed to the city; the rest feeds the tributaries of Machángara River. At this

Table 1 | Quantification of total *Escherichia coli* and cefotaxime-resistant *Escherichia coli* in the sampling points

Sampling points	GPS coordinates	Altitude	Activities	Mean pH	Mean T° (°C)	Geometric mean of <i>E. coli</i> /100 mL	Geometric mean of <i>E. coli</i> resistant to CTX/100 mL	Ratio <i>E. coli</i> resistant to CTX/total <i>E. coli</i>	ARI of <i>E. coli</i> resistant to CTX
SP1	17M 0767699 UTM 9963162	3,616 m	Natural reserve	6.9	14.1	0	0	–	–
SP2	17M 0770448 UTM 9964422	3,102 m	Animal rearing, irrigation, domestic and recreational purposes	6.5	21.2	1.14×10^4	0	–	–
SP3	17M 0770873 UTM 9965390	3,029 m	Domestic and industrial activities	6.6	21.7	6×10^5	0	–	–
SP4	17M 0770873 UTM 9965390	3,029 m	Domestic and industrial activities	6.8	21.4	9.8×10^5	2.7×10^5	$1/3.63 \times 10^2$	0.48
SP5	17M 0775475 UTM 9972248	2,808 m	Domestic and industrial activities	6.5	22	2.8×10^6	5.8×10^5	$1/4.83 \times 10^2$	0.52
SP6	17M 07754711 UTM 9972248	2,809 m	Domestic and industrial activities	6.8	20.1	1.3×10^7	3.3×10^5	1/39.3	0.56
SP7	17M 0780894 UTM 9976817	2,605 m	Domestic and industrial activities	6.5	20.9	1.1×10^7	4.6×10^5	1/23.9	0.60
SP8	17M 0784248 UTM 9978510	2,393 m	Domestic and industrial activities	6.6	20.8	1.4×10^6	2.7×10^4	1/51.8	0.35
SP9	17M 0780894 UTM 9976818	2,598 m	Domestic and industrial activities	6.9	22.8	9.4×10^6	5.4×10^5	1/18.1	0.38

location point, we did not detect *E. coli*. Downstream, in points SP2 and SP3, the water flows freely along open grasslands where some people and domestic and wild animals use it, which may be a possible source of water contamination. At these points, we identified *E. coli*, but cefotaxime- and imipenem-resistant *E. coli* strains were absent. From points SP4 to SP9, we registered increasing quantities of *E. coli* and cefotaxime-resistant *E. coli*, consistent with the intensification of anthropogenic disturbance. Interestingly, with the increasing levels of *E. coli* loads, the number of cefotaxime-resistant *E. coli* within the total *E. coli* counts also increased, from 1 cefotaxime-resistant *E. coli* per 363 *E. coli* in SP4 to 1 per every 18 in SP9 (Table 1).

In our study, we recorded the quantification of cefotaxime-resistant *E. coli* between 2.7×10^3 (SP4) and 5.4×10^5 (SP9) CFU/100 mL, which is similar to ESBL-producing *E. coli* counts previously recorded in hospital wastewater effluents (2.5×10^3 to 10^5 CFU/100 mL) (Jiang et al. 2006; Drieux et al. 2016). These data and the data of river flow reported by Aguilar Alegria (2010) and de Bievre et al. (2008) allow us a simple projection to suggest that at P9 discharge to the ecosystems could occur at approximately 10^{11} multidrug-resistant (MDR) *E. coli* per second. However, the data presented in this report are a snapshot of the Machángara River contamination. Further research programs are needed to establish seasonal variation of resistant bacteria in the river.

Despite imipenem-resistant *E. coli* not being detected in the samples, red colonies (other coliforms) were observed in points SP7, SP8 and SP9. Therefore, one colony for each of these points was characterized, resulting in *Citrobacter freundii*-producing *bla*_{KPC-2} in points SP7 and SP8 and *Klebsiella pneumoniae*-producing *bla*_{KPC-2} in SP9. However, no quantification data were registered. Consistent with these results, in Quito, resistance to carbapenems is frequently related to the *bla*_{KPC-2} gene, which is primarily identified in *K. pneumoniae* (Zurita et al. 2013), and is less frequently found in other species.

An ARI is an excellent tool that allows one to analyze the dissemination of bacterial resistance in a given population at a specific location. Previous reports have identified that an ARI value of >0.2 is observed when isolates are exposed to antibiotic contamination (Mohanta & Goel 2014; Titilawo et al. 2015). In our study, the ARI value for cefotaxime-resistant *E. coli* in the sampling points

ranged from 0.35 to 0.60. Interestingly, the ARI changed along the length of the river that was studied – with higher values at sites in the middle of the study length – which could be related to higher amounts of pollutant (from point or nonpoint sources), including antibiotics, reaching the river at these middle sites. Indeed, points P5 and P6 receive discharge from the city center (including hospitals) and P7, which receives urban wastewater, exhibited the highest ARI value (0.60) at three times higher than the threshold. In contrast, the lowest ARI values were registered at P4, which receives discharge from agriculture and dairy farms (where antibiotic use is common), industry and domestic uses, and P8 and P9 at the end of the city, possibly due to the distance from the principal contamination sources and a dilution effect with water from the natural tributaries (Table 1).

The MARI indicates the level of multidrug resistance of a certain isolate. The isolation of strains with >0.2 in the MARI has been proposed as high-risk contamination (Titilawo et al. 2015). In our study, all cefotaxime-resistant *E. coli* isolates showed MARI values above this threshold (from 0.31 to 0.75). This might be a strong indication for a high-risk source of water contamination and evidence of the widespread antimicrobial resistance to the environment from Machángara River water. Even though none of the sampling sites presented *E. coli* isolates resistance to carbapenems, we did isolate two *C. freundii* isolates that were resistant to carbapenems from P7 and P8, with an MARI of 0.94; we also detected *K. pneumoniae* resistance to carbapenems from site P9, with an MARI of 0.50. These extremely resistant isolates suggest the existence of a genetically rich resistome in this water; however, more studies, including the analysis of other bacterial groups and metagenome analysis, are needed for more robust conclusions (Table 2).

The allele *bla*_{CTX-M-15} was the most prevalent of the ESBL genes identified in our study, followed by *bla*_{CTX-M-18}, *bla*_{CTX-M-3}, *bla*_{CTX-M-65} and *bla*_{CTX-M-20}. In Latin America, the variant *bla*_{CTX-M-15} is the most frequently reported in clinical settings. Moreover, to the best of our knowledge, this variant has been reported in *E. coli* isolated from bloodstream and urinary tract infections, chicken farms, dog feces and fresh vegetables in Quito (Garrido et al. 2017; Chiluisa-Guacho et al. 2018; Ortega-Paredes et al. 2018, 2019; Vinueza-Burgos et al. 2019; Zurita et al. 2019). These findings demonstrate the ubiquitous nature of *bla*_{CTX-M-15} in our city.

Table 2 | Susceptibility profiles of cefotaxime-resistant *E. coli* and imipenem-resistant *C. freundii* and *K. pneumoniae*

Isolate	Species	AMP	SAM	TPZ	CZ	FOX	CAZ	CRO	FEP	ERT	IMP	GEN	TOB	CIP	LEV	NIT	SXT	No. of antibiotics	MARI
M4-1	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	≤1	16	2	≤0.5	≤0.25	≤1	≤1	≤0.25	≤0.12	≤16	≤20	4	0.25
M4-2	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	16	16	≥ 64	≥ 64	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	≤16	≤ 320	12	0.75
M4-3	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	8	4	16	2	≤0.5	≤0.25	≤1	≤1	≥ 4	4	≤16	≤ 320	7	0.44
M5-1	<i>E. coli</i>	≥ 32	≥ 32	≤4	≥ 64	≤4	16	≥ 64	2	≤0.5	≤0.25	≤1	8	≥ 4	≥ 8	≤16	≤ 320	9	0.56
M5-2	<i>E. coli</i>	≥ 32	≥ 32	8	≥ 64	≤4	16	≥ 64	2	≤0.5	≤0.25	≥ 16	≥ 16	≥ 4	≥ 8	64	≤ 320	11	0.69
M5-3	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	≤1	32	2	≤0.5	≤0.25	≤1	≤1	≤0.25	≤0.5	≤16	≤ 320	5	0.31
M6-1	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	8	16	≥ 64	16	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	≤16	≤ 320	11	0.69
M6-2	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	≤1	32	2	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	32	≤ 320	9	0.56
M6-3	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	4	16	2	≤0.5	≤0.25	≤1	≤1	≥ 4	≥ 8	≤16	≤ 320	7	0.44
M7-1	<i>E. coli</i>	≥ 32	8	≤4	≥ 64	16	4	≥ 64	≥ 64	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	≤16	≤ 320	10	0.63
M7-2	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	8	16	≥ 64	2	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	≤16	≤ 320	10	0.63
M7-3	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	≤1	32	≤1	≤0.5	≤0.25	≥ 16	8	≥ 4	≥ 8	≤16	≤ 320	9	0.56
M7-IMP	<i>C. freundii</i>	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 8	≥ 16	≥ 16	≥ 16	≥ 4	≥ 8	≤16	≤ 320	15	0.94
M8-1	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	16	≥ 64	2	≤0.5	≤0.25	≤1	≤1	≤0.25	1	32	≤ 320	6	0.38
M8-2	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	16	≥ 64	2	≤0.5	≤0.25	≤1	≤1	≤0.25	1	32	≤ 320	6	0.38
M8-3	<i>E. coli</i>	≥ 32	4	≤4	≥ 64	≤4	4	≥ 64	2	≤0.5	≤0.25	≤1	≤1	≥ 4	4	≤16	≤20	5	0.31
M8-IMP	<i>C. freundii</i>	≥ 32	≥ 32	64	≥ 64	≥ 64	16	≥ 64	16	≥ 8	≥ 16	≥ 16	≥ 16	≥ 4	≥ 8	≤16	≤ 320	15	0.94
M9-1	<i>E. coli</i>	≥ 32	≥ 32	≤4	≥ 64	8	≤1	32	2	≤0.5	≤0.25	≤1	≤1	≥ 4	≥ 8	≤16	≤ 320	7	0.44
M9-2	<i>E. coli</i>	≥ 32	≥ 32	≤4	≥ 64	≤4	≤1	≥ 64	2	≤0.5	≤0.25	≥ 16	2	≤0.5	1	≤16	≤ 320	6	0.38
M9-3	<i>E. coli</i>	≥ 32	16	≤4	≥ 64	≤4	≥1	16	2	≤0.5	≤0.25	≤1	≤1	≤0.25	≤0.12	≤16	≤ 320	5	0.31
M9-IMP	<i>K. pneumoniae</i>	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	4	16	8	≥ 8	≥ 16	≤1	≤1	1	1	≤16	≤20	8	0.50

Data in bold are interpreted as resistant. The MARI above 0.2 are related with MDR status.

AMP, ampicillin; SAM, ampicillin/sulbactam; TPZ, piperacillin/tazobactam; CZ, cephalothin; FOX, ceftioxitin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ERT, ertapenem; IMP, imipenem; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; NIT, nitrofurantoin; SXT, trimethoprim/sulfamethoxazole.

Table 3 | Genetic profiles of ESBL-producing *E. coli* isolates

	Isolate	ST complex	ST	Phylogroup	<i>bla</i> _{CTX-M} G1	<i>bla</i> _{CTX-M} G2	<i>bla</i> _{CTX-M} G9	<i>bla</i> _{TEM}	<i>bla</i> _{SHV}	Integron 1 variable region
	M6-2	NA	New	D	<i>bla</i> _{CTX-M-3}	-	-	<i>bla</i> _{TEM-1B}	-	-
	M6-3	162	162	B1	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	-
	M7-3	10	10	A	-	-	<i>bla</i> _{CTX-M-65}	<i>bla</i> _{TEM-1B}	-	<i>dftA17, aadA5</i>
	M5-3	394	394	D	-	-	<i>bla</i> _{CTX-M-18}	-	-	<i>dftA1, aadA1</i>
	M5-2	46	46	A	<i>bla</i> _{CTX-M-15}	-	-	-	-	<i>dftA17, aadA5</i>
	M7-1	NA	457	D	<i>bla</i> _{CTX-M-15}	-	-	-	-	-
	M9-2	NA	1,711	A	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	<i>dftA12, orfF, aadA2</i>
	M4-2	NA	1,140	D	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	-
	M4-3	NA	457	D	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1C}	<i>bla</i> _{SHV-2}	-
	M6-1	10	167	A	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	<i>dftA12, orfF, aadA2</i>
	M7-2	10	167	A	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	<i>dftA12, orfF, aadA2</i>
	M8-1	10	10	A	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	<i>dftA1, aadA1</i>
	M8-2	10	10	A	<i>bla</i> _{CTX-M-15}	-	-	<i>bla</i> _{TEM-1B}	-	<i>dftA1, aadA1</i>
	M5-1	10	44	A	<i>bla</i> _{CTX-M-15}	-	-	-	-	<i>dftA17, aadA5</i>
	M4-1	10	10	A	<i>bla</i> _{CTX-M-3}	-	-	-	-	-
	M9-1	ND	ND	B1	-	-	<i>bla</i> _{CTX-M-18}	<i>bla</i> _{TEM-1B}	-	-
	M9-3	NA	362	D	-	<i>bla</i> _{CTX-M-29}	-	-	-	<i>aadA1</i>
	M8-3	23	New	A	<i>bla</i> _{CTX-M-55}	-	-	-	-	-

E. coli phylogroups A and B1 have been identified as animal commensals and having higher levels of resistance than B2 and D (Sarayaya et al. 2015). In our study, the isolates belonged to groups A, B1 and D, which probably result from multisource pollution and imply a possible role of polluted rivers as facilitators of resistance and virulence transference between *E. coli* strains. Previous studies in Quito reported these phylogroups in fresh vegetables and dog feces (Ortega-Paredes et al. 2018, 2019).

Some strains also had class 1 integrons carrying aminoglycoside-modifying enzymes and folate pathway inhibitors. The gene cassette related to aminoglycoside resistance has been identified previously in *E. coli* isolated from fresh vegetables in Quito (Ortega-Paredes et al. 2018). Additionally, the presence of these genetic recombination systems allows these strains to acquire more gene cassettes and increase their profiles of resistance in the river environment.

REP-PCR analysis showed low relatedness between isolates, and MLST was identified in most of our isolates which belonged to the clonal complex 10 (ST10, ST44 and ST 167). This clonal complex, particularly ST10, has been identified as predominant in MDR *E. coli* (Liu et al. 2018), which is related to extremely resistant phenotypes (Caltagirone et al. 2017) and with extraintestinal pathogenic potential (Gomi et al. 2017) in samples isolated from bodies of water. In Quito, we found *bla*_{CTX-M-15}-producing *E. coli* ST44 in fresh commercialized vegetables (Ortega-Paredes et al. 2018), which suggests a relationship between contaminated water and vegetable products. In this study, two MDR *E. coli* that are belonged to ST167 were identified. This clone has been recently identified as clinically important, potentially zoonotic and in possession of high levels of antimicrobial resistance (ESBL and carbapenemases) (Grönthal et al. 2018; Peterhans et al. 2018; Sun et al. 2018). However, to the best of our knowledge, *E. coli* ST167 has not been previously reported in the Ecuadorian environment (Table 3). The rest of our samples belonged to ST162, ST394, ST46, all of which have been isolated previously from poultry and sewage (Hayashi et al. 2018; Zahra et al. 2018).

Finally, in confirmation of our concern, the sequence types described in this study show the typical genetic backgrounds of the MDR *E. coli* capable of causing infections that are difficult to treat in humans and animals.

CONCLUSION

Our study reveals the high potential of polluted urban rivers as sites of emergence and means of spread of MDR *E. coli* and highlights the need to characterize their resistant determinants. The predominance of *bla*_{CTX-M-15} in the isolates suggests the establishment of this variant in the city and its aquatic dissemination through the sewage to the environment.

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CONFLICTS OF INTEREST

None declared.

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