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RAPID COMMUNICATION

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International clones of extended-spectrum β-lactamase (CTX-M)-producing *Escherichia coli* in peri-urban wild animals, Brazil

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Abstract

CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* clones have been increasingly reported worldwide. In this regard, although discussions of transmission routes of these bacteria are in evidence, molecular data are lacking to elucidate the epidemiological impacts of ESBL producers in wild animals. In this study, we have screened 90 wild animals living in a surrounding area of São Paulo, the largest metropolitan city in South America, to monitor the presence of multidrug-resistant (MDR) Gram-negative bacteria. Using a genomic approach, we have analysed eight ceftriaxone-resistant *E. coli*. Resistome analyses revealed that all *E. coli* strains carried $bla_{CTX\text{-}M}$-type genes, prevalent in human infections, besides other clinically relevant resistance genes to aminoglycosides, β-lactams, phenicols, tetracyclines, sulphonamides, trimethoprim, fosfomycin and quinolones. Additionally, *E. coli* strains belonged to international sequence types (STs) ST38, ST58, ST212, ST744, ST1158 and ST1251, and carried several virulence-associated genes. Our findings suggest spread and adaptation of international clones of CTX-M-producing *E. coli* beyond urban settings, including wildlife from shared environments.

KEYWORDS

Enterobacterales, ESBL, MDR bacteria, resistome, wildlife

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1 | INTRODUCTION

The spread of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales has been broadly reported worldwide (Brolund, 2014; Fernandes et al., 2018; Pardon et al., 2015). In this respect, a number of interlinked factors, such as food animals, environmental sources, human migration and access to basic sanitation in highly populated cities, are contributing for the accelerated dissemination of these bacteria in urban and wild environments (Radhouani et al., 2014; Sacramento et al., 2018; Sellera, Fernandes, Moura, Carvalho, & Lincopan, 2018).

While the exposure to polluted environments constitutes a risk factor for humans to acquire multidrug-resistant (MDR) bacteria, recent studies have pointed out that it could also have implications for wildlife (Cerdà-Cuéllar et al., 2019; Sellera, 2019; Wang et al., 2017). In fact, although this matter remains poorly addressed under ecological perspectives, the scientific community and nature conservation authorities have begun to see wild animals as reservoirs and potential disseminators of ESBL-producing bacteria (Ardiles-Villegas, González-Acuña, Waldenström, Olsen, & Hernández, 2011; Cerdà-Cuéllar et al., 2019; Sellera, 2019; Wang et al., 2017). Nowadays, most ESBL-producing *Escherichia coli* circulating at the human–animal–environment interface belong to international sequence types (STs) such as ST10, ST38, ST58, ST131, ST212, ST648, ST744, ST1158 and ST1251 (Borges, Tarlton, & Riley, 2019; Cao et al., 2014; Castellanos et al., 2017; Haenni et al., 2018; Nüesch-Inderbinen et al., 2019; Pitout, 2012; Tacão et al., 2017; Tafoukt, Touati, Leangapichart, Bakour, & Rolain, 2017; Vignoli et al., 2016; Zurfluh et al., 2017), suggesting a broad host adaptation of these pathogens. In this study, we report the occurrence of pandemic clones of CTX-M-producing *E. coli* recovered from a diversity of peri-urban wild animals in Brazil, highlighting the transmission of this sort of bacteria in anthropogenic-shared environments.

2 | MATERIALS AND METHODS

Between June 2017 and July 2018, a local surveillance study was conducted to monitor the presence of MDR Gram-negative bacteria in urbanized wild animals, in São Paulo, Brazil, the largest metropolitan city in South America. For this purpose, we sampled rectal or cloacal swabs from 90 wild animals, including reptiles, birds and mammals' species rescued by authorities (firefighters and environmental police) and delivered to wildlife rehabilitation centres. The sampled species included *Alouatta guariba* (*n* = 4), *Asio clamator* (*n* = 7), *Asio stygius* (*n* = 1), *Caracara plancus* (*n* = 2), *Coragyps atratus* (*n* = 27), *Didelphis aurita* (*n* = 11), *Egretta thula* (*n* = 1), *Hydrochoerus hydrochaeris* (*n* = 14), *Hydromedusa tectifera* (*n* = 2), *Megascops choliba* (*n* = 2), *Nasua nasua* (*n* = 13), *Nycticorax nycticorax* (*n* = 1), *Sapajus apellla* (*n* = 1), *Tapirus terrestris* (*n* = 1), *Tupinambis merianae* (*n* = 2) and *Tyto furcata* (*n* = 1). Biological sample collections were authorized by the Authorization System and Information on Biodiversity (SISBIO licence number 55804–2).

Swab samples were streaked onto MacConkey agar plates supplemented with ceftriaxone (2 μg/ml), colistin (2 μg/ml) or meropenem (2 μg/ml), and the grown bacteria were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Antimicrobial susceptibility was determined by disc diffusion and/or E-test methods, using breakpoints approved by the Clinical and Laboratory Standards Institute (CLSI, 2015, 2017). Twenty-two antibiotics were tested including amikacin, amoxicillin/clavulanic acid, ampicillin, aztreonam, ceftazidime, cephalothin, ciprofloxacin, chloramphenicol, ceftriaxone, ceftiofur, cefotaxime, doxycycline, enrofloxacin, cefepime, gentamicin, nalidixic acid, sulphonamide, trimethoprim/sulphamethoxazole, tetracycline, kanamycin, tobramycin and streptomycin. Additionally, the presence of CTX-M-type ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-9}}$) groups, carbapenemase ($bla_{\text{KPC-2}}$) and mobilized colistin resistance (*mcr-1*) genes was evaluated by PCR analysis (Dropa et al., 2016; Liu et al., 2016; Minarini, Poirel, Trevisani, Darini, & Nordmann, 2009; Muzaheed et al., 2008; Poirel, Walsh, Cuvillier, & Nordmann, 2011; Saladin et al., 2002).

The isolates confirmed positive by PCR were whole-genome sequenced. Genomic DNA was extracted from overnight cultures using the PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Whole-genome sequencing (WGS) was performed using Illumina NextSeq500 platform (Illumina, San Diego, CA) (150 bp paired-end), and the reads were de novo assembled using Velvet 1.2.10 (Zerbino & Birney, 2008) or SPAdes 3.9 (Bankevich et al., 2012). Sequence types, serotypes, plasmid replicon types, antimicrobial resistance genes and virulence genes were identified using MLST 2.0, SerotypeFinder 2.0 (identity ≥ 85%; coverage ≥ 60%), PlasmidFinder 2.1 (identity ≥ 95%; coverage ≥ 60%), ResFinder 3.2 (identity ≥ 90%; coverage ≥ 60%) and VirulenceFinder 2.0 (identity ≥ 90%; coverage ≥ 60%) tools, respectively, available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/). Analysis of the genetic context of $bla_{\text{CTX-M}}$ genes was performed with BLASTn and ISFinder analyses (Siguier, Perochon, Lestrade, Mahillon, & Chandler, 2006) followed by manual curation using Geneious 10.2.6.

Plasmid transfer was performed by conjugation using streptomycin-resistant *E. coli* C600 or azide-resistant *E. coli* J53 recipient strains in LB broth assays, ratio 3:1 (recipient:donor). Transconjugants were selected using MacConkey agar supplemented with ceftriaxone (2 μg/ml) and streptomycin (2000 μg/ml), or ceftriaxone (2 μg/ml) and sodium azide (200 μg/ml). In transformation assays, plasmids were extracted by the alkaline lysis method (Sambrook & Russel, 2001), and ultra-competent *E. coli* TOP10 was heat shock transformed as previously described (Inoue, Nojima, & Okayama, 1990), increasing the thermal shock time at 42°C to 1.5 min. Transformants were selected using MacConkey agar supplemented with ceftriaxone (2 μg/ml). Positive transconjugants and transformants strains were confirmed by $bla_{\text{CTX-M}}$ genes using PCR.

3 | RESULTS AND DISCUSSION

In this study, eight ceftriaxone-resistant *E. coli* isolates (8/90; 8.88%) were recovered from five birds (one owl and four vultures) and three mammals (coatis). MDR profiles, defined as resistant to three or more classes of antibiotics (Magiorakos et al., 2012), were evidenced in six isolates (ECPET11, ECPET31, ECPET36, ICBUR6, ICBUR15 and ICBUR20). ECPET3 displayed resistance only to cephalosporins and aztreonam, whereas ECPET13 was resistant to cephalosporins, aztreonam and nalidixic acid. Additionally, ESBL production was confirmed by double-disc synergy test (DDST), and PCR analysis revealed the presence of $bla_{\text{CTX-M}}$-type genes in all eight bacterial isolates (Table 1). No MCR-1-positive or carbapenemase-producing bacteria were identified.

WGS analysis revealed six different serotypes (i.e. O18/O18ac:H49, O89/O162:H10, O78:H21, O130:H26, O17/O44/O77:H34, O86:H18). In this regard, the O86:H18 has been previously identified in diarrhoeagenic *E. coli* isolated from humans, in Brazil (Ghilardi, Gomes, & Trabulsi, 2001; Piva et al., 2003), and in Asian and African countries (Sonda et al., 2018; Suzuki et al., 2009). On the other hand, while *E. coli* O89/O162:H10 has been associated with hospital-acquired infections, in Asian countries (Lin, Kuroda, Suzuki, & Mu, 2019; Nguyen et al., 2019), *E. coli* O18:H49 and O78:H21 have been reported in wild animals from Europe and Asia (Bai et al., 2013; Eggert et al., 2013). *Escherichia coli* O130:H26 and O17/O44/O77:H34 have been identified in human and animal samples from Asia, Europe, Australia, Antarctica and South America (Bettelheim et al., 2003; Delgado-Blas, Ovejero, Abadia-Patino, & Gonzalez-Zorn, 2016; Ho, Tan, Ooi, Yeo, & Thong, 2013; Mora et al., 2018; Müller et al., 2007).

Virulome analysis revealed a diversity of virulence determinants, including *celb* (endonuclease colicin E2), *iha* (*irgA* homolog adhesin), *air* (enteroaggregative immunoglobulin repeat protein), *ireA* (siderophore receptor), *astA* (EAST1 toxin), *cma* (colicin M), *gad* (glutamate decarboxylase), *eilA* (*Salmonella HilA* homolog), *lpfA* (long polar fimbriae), *iroN* (enterobactin siderophore receptor protein) and *iss* (increased serum survival) (Table 1). Interestingly, *air*, *astA* and *eilA* genes have been found in enteroaggregative *E. coli* (EAEC) causing acute and chronic diarrhoea (Konno, Yatsuyanagi, & Saito, 2012; Nüesch-Inderbinen, Hofer, Hachler, Beutin, & Stephan, 2013; Sheikh et al., 2006). The *lpfA* gene has been identified in enteropathogenic *E. coli* (EPEC), the most important diarrhoeal pathogen in paediatric patients (Afset et al., 2006).

In addition to $bla_{\text{CTX-M}}$-type genes, resistome analysis confirmed that the *E. coli* strains carried other clinically relevant resistance genes to β-lactams [$bla_{\text{TEM-1B}}$], aminoglycosides [*aadA1*, *aadA2*, *aadA5*, *aac(3)-IId*, *aac(3)-IIa*, *aac(3)-IV*, *aac(6')Ib-cr*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(3)-Id*, *aph(4)-Ia* and *aph(6)-Id*], phenicols [*catA1*, *catB3* and *cmlA1*], tetracyclines [*tet(A)* and *tet(B)*], sulphonamides [*sul1* and *sul2*], trimethoprim [*dfrA1*, *dfrA7*, *dfrA14* and *dfrA17*], fosfomycin [*fosA3*], quinolones [*qnrB1*, *qnrB19* and *aac(6')Ib-cr*] and macrolides [*mdf(A)*]. Interestingly, in Brazil, there are only three studies regarding the plasmid-mediated quinolone resistance *qnrB1* gene, which has been harboured by *K. pneumoniae* and *Enterobacter cloacae* (Scavuzzi et al., 2017; Viana et al., 2013), and *Enterobacter hormaechei* (Pereira et al., 2015). On the other hand, *qnrB19*, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ genes have been carried by members of Enterobacterales genus isolated from human and non-human hosts (Goldberg et al., 2019; Monte et al., 2019; Rocha, Pinto, & Barbosa, 2016; Sartori et al., 2017; Silva et al., 2018).

Schematic representations of the genetic contexts surrounding $bla_{\text{CTX-M}}$-type genes in *E. coli* strains are presented in Figure 1. International genetic contexts of $bla_{\text{CTX-M-14}}$ (IS*Ecp1*-$bla_{\text{CTX-M-14}}$-IS*903D*) (Lartigue, Poirel, & Nordmann, 2004) and $bla_{\text{CTX-M-15}}$ (IS*Ecp1*-$bla_{\text{CTX-M-15}}$-*orf477*) (Dhanji et al., 2011) were identified in *E. coli* strains ST38 (ICBUR15 and ICBUR20) and ST1251 (ECPET36) isolated from *C. atratus* and *N. nasua*, respectively. In addition, two different contexts were found surrounding the $bla_{\text{CTX-M-55}}$. The typical structure IS*Ecp1*-$bla_{\text{CTX-M-55}}$-*orf477* (2,956 bp) was present in *E. coli* belonging to ST744 (ECPET11), whereas a similar array exhibiting a 243 bp with IS*Ecp1* truncated by an IS*26* upstream of the $bla_{\text{CTX-M-55}}$ gene was found in *E. coli* strains ECPET3 and ECPET13 (ST212). Similar genetic contexts of $bla_{\text{CTX-M-55}}$ have been reported in Enterobacteriaceae isolated from humans, animals and food animals (Hu et al., 2018; Lv et al., 2013). Furthermore, $bla_{\text{CTX-M-2}}$ gene from *E. coli* strains ST58 (ECPET31) and ST1158 (ICBUR6) was present into complex class 1 integrons (9,456 and 8,879 bp, respectively), sharing 99.7% and 99.9% nucleotide identity with partial integrons of *E. coli* (GenBank: AM040710) (8,133 bp) and *K. pneumoniae* (GenBank: KY286109) (7,824 bp) isolated from French and Chilean hospitals, respectively.

Although different plasmid replicon types were found among CTX-M-producing *E. coli* strains, $bla_{\text{CTX-M}}$ genes were carried on IncF (FIA, FIB, and FII) plasmids, except $bla_{\text{CTX-M-14}}$, which were carried on IncI2 plasmids. Most plasmids harbouring $bla_{\text{CTX-M}}$ genes were successfully transferred by conjugation (from *E. coli* donors ECPET3, ECPET13, ECPET36 and ICBUR6), or by transformation assays using plasmids from ICBUR15 and ICBUR20 strains. As previously reported, IncF plasmids have been widely associated with the spread of $bla_{\text{CTX-M-15}}$, whereas IncF, IncK and IncI are commonly associated with $bla_{\text{CTX-M-14}}$ and other $bla_{\text{CTX-M}}$-type genes (Zhao & Hu, 2013). Regarding other plasmids identified in this study, IncN and IncHI2 have been related to the spread of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$, respectively, and IncQ1 or IncX plasmid has been responsible by dissemination of carbapenemase encoding genes (Cerdeira et al., 2019; Mollenkopf et al., 2017; Paul et al., 2017; Zhao & Hu, 2013).

In this study, genomic analysis identified *E. coli* strains belonging to international ST38, ST58, ST212, ST744, ST1158 and ST1251 (Table 1). The global distribution of these *E. coli* clones is presented in Figures 2 and 3. The broadly distributed *E. coli* ST38 and ST744 have been reported in wildlife, farm animals and human samples from Europe, Africa, Asia, Australia and America, in general associated with the production of clinically significant beta-lactamases (i.e. carbapenemases or ESBL) (Abraham et al., 2015; Belmahdi, Bakour, Al Bayssari, Touati, & Rolain, 2016; Guenther et al., 2017; Hasan et al., 2012; Ho et al., 2016; Mshana et al., 2011; Pitout, 2012; Poirel, Bernabeu, et al., 2011; Sellera et al., 2018; Stoesser

TABLE 1 Phenotypic and genotypic features of ESBL-producing *E. coli* strains isolated from peri-urban wild animals, Brazil

ID strains	Animal sources	ST/CC	Serotype	Virulence genes	Resistance phenotype	Resistance genotype	Plasmid type	Accession number
ECPET3	Black Vulture (*Coragyps atratus*)	212/–	O18/ O18ac:H49	*gad*, *iss*, *lpfA*	CRO, CTX, CAZ, CPM, ATM	$bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1B}$, *mdf(A)*	FII, N	PQET00000000
ECPET11	South American Coati (*Nasua nasua*)	744/10	O89/ O162:H10	*gad*, *iss*, *cma*, *iroN*	CRO, CTX, CAZ, CPM, ATM, CIP, NAL, GEN, KAN, TOB, STR, CLO, SUT, TET	$bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1B}$, *aadA1*, *aadA2*, *aadA5*, *aac(3)-IId*, *aph(3′)-Ia*, *aph(3″)-Ib*, *aph(6)-Id*, *fosA3*, *catA1*, *cmlA1*, *sul1*, *sul2*, *dfrA17*, *tet(B)*, *mdf(A)*	Q1, FIB, FII, N, X1	PQEU00000000
ECPET13	Striped Owl (*Asio clamator*)	212/-	O18/ O18ac:H49	*iss*, *lpfA*	AMC, CRO, CTX, CAZ, CPM, ATM, NAL	$bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1B}$, *mdf(A)*	FII, N	PQEV00000000
ECPET31	South American Coati (*Nasua nasua*)	58/155	O78:H21	*gad*, *iss*, *lpfA*	CRO, CTX, CAZ, CPM, ATM, CIP, GEN, KAN, TOB, STR, CLO, SUT, TET	$bla_{CTX\text{-}M\text{-}2}$, *aadA1*, *aac(3)-IV*, *aph(3″)-Ib*, *aph(3′)-Ia*, *aph(4)-Ia*, *aph(6)-Id*, *sul1*, *sul2*, *dfrA7*, *tet(A)*, *mdf(A)*	FIA, HI2, HI2A, Q1, FII	PQEW00000000
ECPET36	South American Coati (*Nasua nasua*)	1251/-	O130:H26	*gad*	AMC, CRO, CTX, CAZ, CPM, ATM, CIP, NAL, GEN, KAN, TOB, STR, CLO, SUT	$bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1B}$, *qnrB1*, *aac(3)-IIa*, *aac(6′)Ib-cr*, *aadA1*, *aph(3″)-Ib*, *aph(6)-Id*, *catB3*, *catA1*, *sul2*, *dfrA1*, *dfrA14*, *mdf(A)*	HI2, HI2A, p0111	PQEX00000000
ICBUR6	Black Vulture (*Coragyps atratus*)	1158/31	O17/O44/ O77:H34	*iss*, *astA*, *eilA*, *celb*, *iha*, *air*, *ireA*	CRO, CTX, CPM, ATM, KAN, TOB, CLO, SUL, TET	$bla_{CTX\text{-}M\text{-}2}$, *qnrB19*, *aadA1*, *aph(3′)-Ia*, *aph(6)-Id*, *aph(3″`)-Ib*, *catA1*, *sul1*, *sul2*, *tet(B)*, *mdf(A)*	FIB, FII, Col156	PPCS00000000
ICBUR15	Black Vulture (*Coragyps atratus*)	38/38	O86:H18	*gad*, *iss*, *astA*, *eilA*	CRO, CTX, CPM, ATM, CIP, NAL, TOB, STR, SUL	$bla_{CTX\text{-}M\text{-}14}$, *aph(6)-Id*, *aph(3″)-Ib*, *sul2*, *mdf(A)*	I2	PPCU00000000
ICBUR20	Black Vulture (*Coragyps atratus*)	38/38	O86:H18	*iss*, *astA*, *eilA*, *air*	CRO, CTX, CPM, ATM, CIP, NAL, AMK, STR, SUL	$bla_{CTX\text{-}M\text{-}14}$, *aph(6)-Id*, *aph(3″)-Ib*, *sul2*, *mdf(A)*	I2	PPCT00000000

Abbreviations: CC, clonal complex; ST, sequence type. Resistance phenotype: AMC, amoxicillin/clavulanic acid; AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CLO, chloramphenicol; CPM, cefepime; CRO, ceftriaxone; CTX, cefotaxime; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfonamide; SUT, trimethoprim-sulfamethoxazole; TET, tetracycline; TOB, Tobramycin.

et al., 2012; Yamamoto, Takano, Iwao, & Hishinuma, 2011). *Escherichia coli* ST38 has been frequently reported causing extraintestinal diseases, mainly bloodstream and urinary tract infections (Cao et al., 2014; Mendes, Jones, Woosley, Cattoir, & Castanheira, 2019; Pitout, 2012). In some cases, *E. coli* ST744 has been associated with plasmid-mediated colistin resistance genes (*mcr-1* and *mcr-3*) (Haenni et al., 2018; Tacão et al., 2017). Furthermore, ESBL or CMY-2-producing *E. coli* ST212 and ST1158 were previously isolated from farm animals, animal production chain and humans (Cadona, Bustamante, Gonzalez, & Sanso, 2016; Castellanos et al., 2017; Maamar et al., 2016; Mo, Slettemeas, Berg, Norstrom, & Sunde, 2016; Steinsland, Lacher, Sommerfelt, & Whittam, 2010; Vignoli et al., 2016; Zurfluh et al., 2014). Carbapenemase or CMY-2-producing *E. coli* ST212 was also recovered from diseased companion animal and water environments (Tafoukt et al., 2017; Vingopoulou et al., 2014), whereas *E. coli* ST1158 carrying $bla_{CTX\text{-}M}$

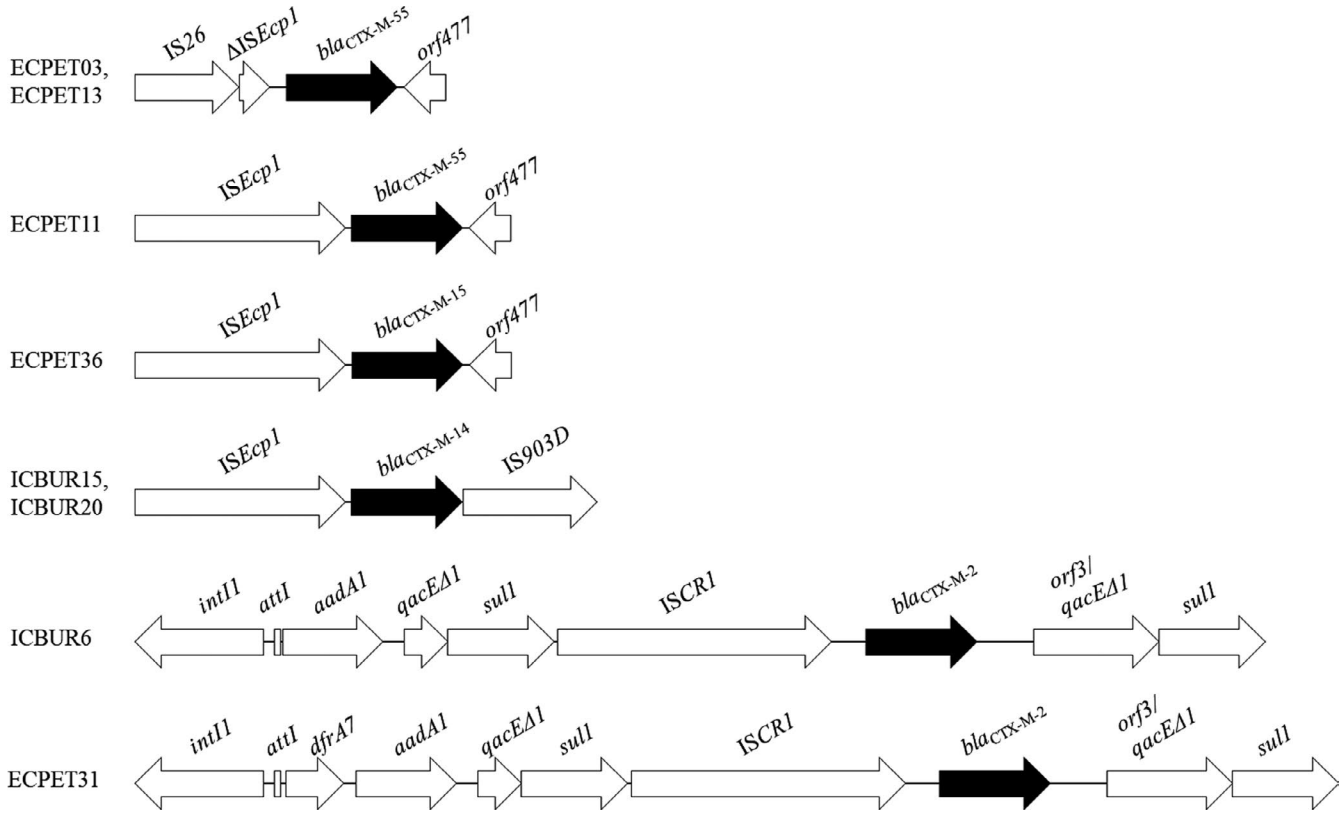

FIGURE 1 Schematic representation of the genetic context surrounding bla_{CTX-M} genes in *Escherichia coli* strains from peri-urban wild animals in Brazil. For ECPET03 and ECPET13 *E. coli* strains, the genetic array IS*26*-ΔIS*Ecp1*-$bla_{CTX-M-55}$-*orf477* was identified. In this regard, IS*26* belongs to IS*6* family, whereas *orf477* encodes a cupin fold metalloprotein of WbuC family. For ECPET11 and ECPET36 *E. coli* strains, carrying $bla_{CTX-M-55}$ or $bla_{CTX-M-15}$, respectively, IS*Ecp1* was upstream, whereas *orf477* was downstream of bla_{CTX-M} genes. *E. coli* strains ICBUR15 and ICBUR20, and ECPET36 displayed international genetic arrays IS*Ecp1*-$bla_{CTX-M-14}$-IS*903D* and IS*Ecp1*-$bla_{CTX-M-15}$-*orf477*, respectively

was recovered from food animals (Vogt et al., 2014). Regarding *E. coli* ST1251, fluoroquinolone-resistant strains have been reported in animal faeces and wastewater (Jamborova et al., 2015; Varela, Macedo, Nunes, & Manaia, 2015), as well as *mcr-1*-harbouring strains from food animals (Zurfluh et al., 2017). *Escherichia coli* belonging to ST58 has been globally reported from a variety of sources including food (Ben Said et al., 2015), polluted mangrove (Sacramento et al., 2018), poultry, hospital- and community-acquired infections (Borges et al., 2019; McKinnon, Chowdhury, & Djordjevic, 2018) and bovine mastitis (Nüesch-Inderbinen et al., 2019). Interestingly, ST58/CC155 frequently shares identical antimicrobial resistance patterns in both animal and human populations. Such evidence may significantly explain the successful establishment of this international lineage (Borges et al., 2019).

ESBL-producing *E. coli* in wild animals begun to be documented in 2006, in Portugal (Costa et al., 2006), and then were rapidly observed in other countries from Europe, Africa, Asia, South America, North America and Australia (Allen et al., 2010; Wang et al., 2017). Predominantly, *E. coli*- and *K. pneumoniae*-producing CTX-M seem to be the most adapted to these hosts; however, the identification of ESBL genes in other different species of Enterobacterales has already been reported (Wang et al., 2017). In most of cases, animals became colonized in gastrointestinal tract without any evidences of infection, contributing for the silent dissemination of these critically important pathogens in natural environments.

A widely debated example is the occurrence of ESBL-producing bacteria in migratory birds, which are probably involved in the spread of these pathogens through long distances, including natural reserves and pelagic areas with low anthropogenic impact (Ardiles-Villegas et al., 2011; Cerdà-Cuéllar et al., 2019). Otherwise, the role of peri-urban wild animals as disseminators of bacterial pathogens has been so far neglected. In this study, all animals sampled lived in the transboundary area of São Paulo city, the most populated metropolitan region of Brazil, with about 21.5 million inhabitants, and one of the ten most populous metropolitan regions in the world. Even though the source of these bacterial isolates remains uncertain, wildlife is not directly exposed to antibiotics in most cases and other anthropogenic pathways of transmission, such as contact to contaminated water and predation of infected animals, should be considered (Wang et al., 2017). Yet, it is important to take in account that some highly polluted rivers cross this area, where KPC-2- and ESBL-producing *K. pneumoniae* isolates from water samples were previously reported (Cerdeira et al., 2017; Oliveira et al., 2014).

Since, in this study, ESBL-positive isolates were recovered from predators with different ecological behaviours [i.e. vultures are

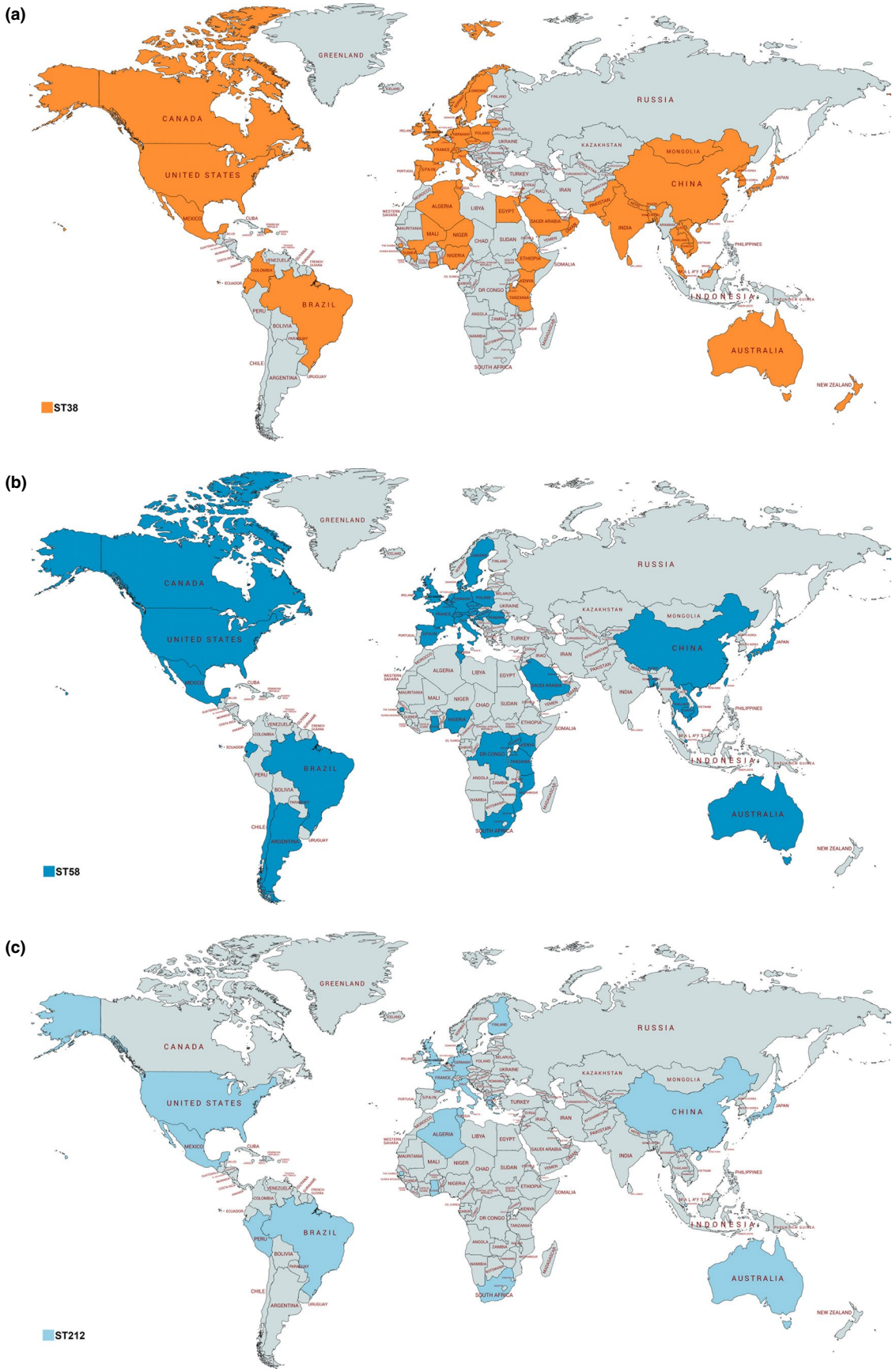

FIGURE 2 Global distribution of *Escherichia coli* belonging to sequence types (a) ST38, (b) ST58 and (c) ST212. This map was created using an online service (https://mapchart.net/) [Colour figure can be viewed at wileyonlinelibrary.com]

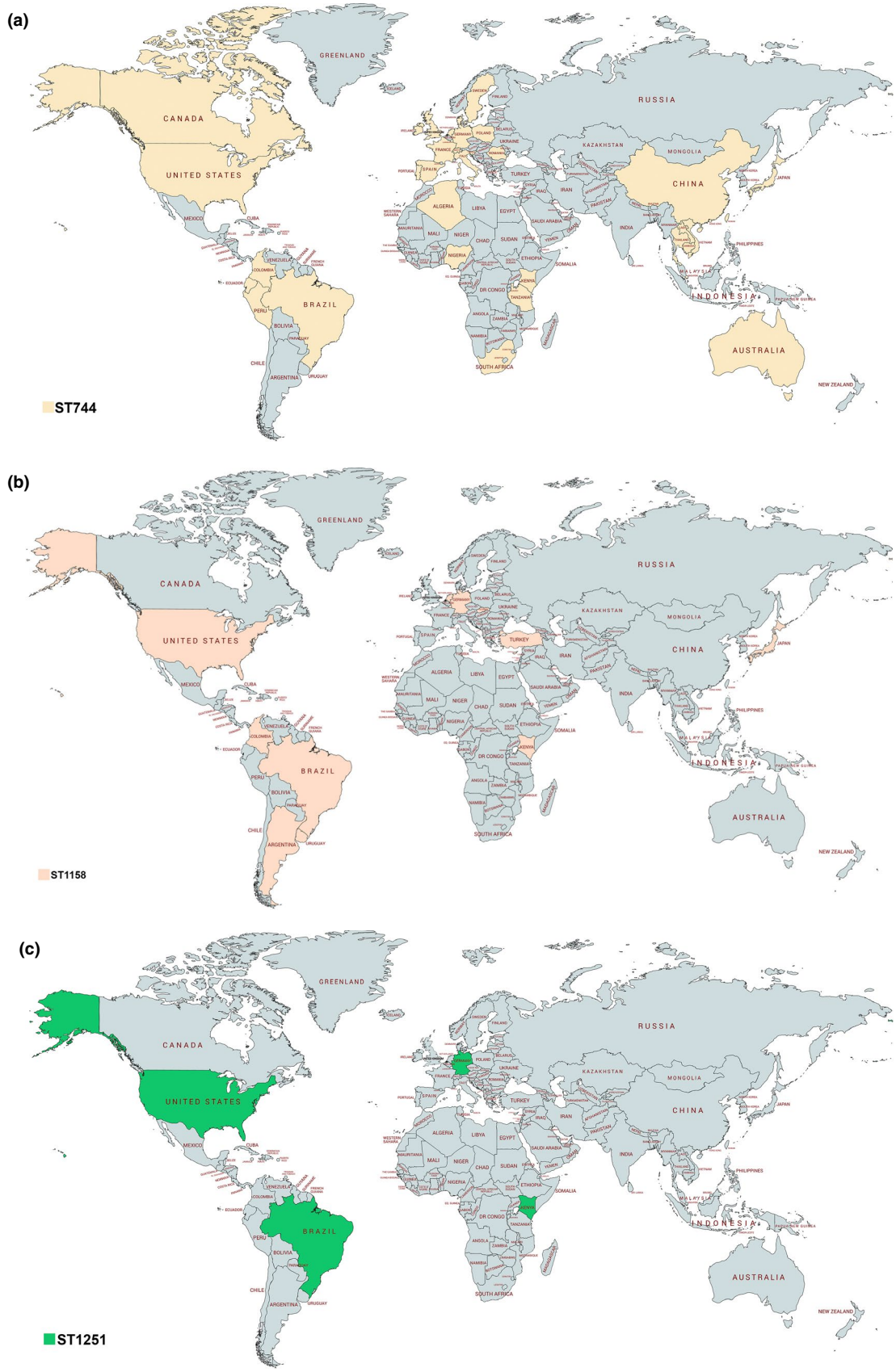

FIGURE 3 Global distribution of *Escherichia coli* belonging to sequence types (a) ST744, (b) ST1158 and (c) ST1251. This map was created using an online service (https://mapchart.net/) [Colour figure can be viewed at wileyonlinelibrary.com]

scavengers and diurnal predators; owls are nocturnal hunters of small rodents; and coatis are remarkably well adapted predators, feeding on fruits, insects and small vertebrates], in order to investigate in more detail the genetic relatedness among these *E. coli* strains, core genome multilocus sequence typing (cgMLST) was performed by uploading the sequencing reads of the eight strains into cgMLSTFinder 1.1 (https://cge.cbs.dtu.dk/services/cgMLSTFinder/). Interestingly, two *E. coli* ST212 strains (ECPET3 and ECPET13) isolated from different hosts (black vulture and striped owl) were nested together (Figure S1). Remarkably, these strains also shared identical serotype, resistome and plasmidome. These findings suggest an adaptation of CTX-M-producing *E. coli* into the wildlife food chain and the versatility of these bacteria to colonize different hosts. Indeed, interspecific interactions among wild animals colonized by ESBL producers represent an incommensurable threat to ecosystem maintenance, since Enterobacteriaceae constitutes the gut microbiota of most endothermic animals (Madoshi et al., 2016). Thus, antimicrobial resistance must also be viewed as an ecological problem (Fuentes-Castillo et al., 2019).

In conclusion, anthropogenic activities have been contributing for the dissemination of ESBL-producing bacteria in wildlife. The occurrence of ESBL-producing bacteria in peri-urban wild animals from highly populated cities is a critical issue and deserves special attention. Therefore, continuous epidemiological and genomic surveillance studies are urgently required to determine routes of transmission of these bacteria in wildlife. Finally, while humans can negatively affect nature environments for contributing to the spread of MDR bacteria, animals could also disseminate these pathogens to humans in a continuous cycle.

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ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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