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Multidrug-resistant *Escherichia coli* isolated from canine faeces in a public park in Quito, Ecuador

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ABSTRACT

Objectives: This study focused on estimating the prevalence of extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases, carbapenemases and MCR-1-producing *Escherichia coli* in canine faeces from a public park in Quito, Ecuador.
Methods: Phenotypic and genotypic characterisation of *E. coli* isolated from 50 canine faecal samples recovered from a city park in Quito was performed. In addition, a multiple choice survey was conducted among 50 dog owners.
Results: Of the 50 faecal samples, 20 (40.0%) presented *E. coli* resistant to ceftriaxone. Moreover, 23 *E. coli* isolates were recovered for further analysis. All of the isolates showed as multidrug-resistant (MDR) phenotype (resistant to three or more antibiotic families). Resistance to carbapenems, tigecycline and amikacin was not observed. No major clonal relatedness was observed among the resistant isolates. The ESBL genes $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ were the most common. Two isolates harboured the $bla_{\text{CMY-2}}$ gene and one isolate harboured both *mcr-1* and $bla_{\text{CTX-M-65}}$. Statistical analysis showed that older people were more conscious of collecting and disposing of dog faeces than subjects aged <35 years ($P < 0.05$).
Conclusion: The finding of MDR *E. coli* in dog faeces in a city park in Ecuador illustrates the importance of analysing canine faeces in public settings (e.g. parks, playgrounds) as part of surveillance programmes for MDR *E. coli*. In addition, this research might be a sentinel sampling method to gain a better understanding of community sources of antibiotic-resistant Enterobacteriaceae at human–animal–environment interfaces.

1. Introduction

Worldwide, the presence of animal faeces in the ground of urban areas is a significant public-health problem owing to the presence of various micro-organisms that can be transmitted to humans [1]. This concern is increasing with the growing pet population and free-roaming animals in large cities like Quito in Ecuador [2,3]. This growth has increased the probability of contact between animals and humans and has heightened the exposure risk to different pathogens, including multidrug-resistant (MDR) *Escherichia coli* [1]. MDR *E. coli* have been isolated from humans, animals and the environment worldwide [4]. The most important resistance mechanisms in these strains are extended-spectrum β-lactamase (ESBLs), plasmid-mediated AmpC β-lactamases (pAmpCs), carbapenemases and lipid A phosphoethanolamine transferases (mobile colistin resistance, *mcr*), which may lead to therapy failure.

Despite studies on ESBLs, pAmpCs, carbapenemases and *mcr*-producing *E. coli* from different sources, the routes of transmission

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are unknown and the epidemiology of these strains is poorly understood. So far, the prevalence and potential for transmission of MDR *E. coli* between companion animals and humans are unknown. The aim of this study was to estimate the prevalence of ESBL, pAmpC, bla_{KPC} and *mcr-1* genes in ceftriaxone-resistant *E. coli* isolated from canine faeces in a city park in Quito, Ecuador.

2. Materials and methods

2.1. Study design

An exploratory observational study was performed to determine the prevalence of ceftriaxone-resistant *E. coli* in canine faeces from a city park in our Quito. Quito, the capital city of Ecuador, is located in the Andean Region of Ecuador (0°13′07″S and 78°30′35″W) at an altitude of 2810 m above sea level, with a population of 1 911 966 [5]. In this study, a transect of 1 km^2 was established in a linear park in the southern part of the city (−0.262678, −78.538390; −0.266270, −78.550295), where families walk their dogs, people and children play and do exercise, street food is sold, and where there are no facilities for the collection of canine faeces. Collection of faeces is regulated by two local ordinances [6,7].

2.2. Sampling and isolation

A total of 50 canine faecal samples were collected in August 2017. Samples were taken from the top of fresh faeces in order to avoid cross-contamination from environmental bacteria of the ground. Samples were collected in sterile plastic flasks and were sent for processing to a laboratory up to 1 h after collection. Samples were plated using a sterile swab on MacConkey agar (Becton Dickinson & Co., Le Pont-de-Claix, France) supplemented with 3 μg of ceftriaxone and were incubated at 35 °C for 18–20 h. Plates growing red/pink colonies were registered as positive. All different *E. coli*-like colonies were spiked and isolated in the same medium. A loopful of bacteria was mixed with trypticase soy broth (Becton Dickinson & Co.) plus 40% glycerol (HiMedia, Levallois-Perret, France) and was stored at −20 °C for further analysis.

2.3. Bacterial identification and antimicrobial susceptibility testing

Species confirmation was performed using biochemical tests including triple sugar iron, Simmons' citrate test, urease production, tryptophan reduction (indole), methyl red test and Voges–Proskauer test and was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI/TOF-MS) analysis using a microflex™ LT MALDI-TOF spectrometer (Bruker Daltonics, Inc., Billerica, MA, USA).

To identify ESBL-, pAmpC- or carbapenemase-producing phenotypes, *E. coli* isolates were screened by the double-disk synergy test using cefotaxime (30 μg), cefotaxime/clavulanic acid (30/10 μg), ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg) as well as for susceptibility to cefepime (30 μg), imipenem (10 μg) and cefoxitin (30 μg). Antimicrobial resistance profiles were determined using the disk diffusion method with aztreonam (30 μg), imipenem (10 μg), tetracycline (30 μg), tigecycline (10 μg), doxycycline (30 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), norfloxacin (5 μg), levofloxacin (5 μg), gentamicin (10 μg), netilmicin (30 μg), amikacin (30 μg), fosfomycin (200 μg), nitrofurantoin (300 μg), chloramphenicol (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) and azithromycin (15 μg) (Becton Dickinson & Co.). A BD Phoenix™ Automated Microbiology System (Becton Dickinson & Co.) was used for colistin susceptibility testing. The results were interpreted following Clinical and Laboratory Standards Institute CLSI 2018 recommendations [8].

2.4. Genotypic characterisation

Antimicrobial resistance genes were identified using PCR protocols described elsewhere: $bla_{CTX\text{-}M}$ group 1 [9]; $bla_{CTX\text{-}M}$ group 2 [10]; $bla_{CTX\text{-}M}$ group 8 [11]; $bla_{CTX\text{-}M}$ group 9 [12]; bla_{KPC} [13]; and the colistin resistance gene *mcr-1* [14]. Positive and negative controls were included in each reaction. The phylogenetic group was also identified [15]. All amplicons were sequenced on both strands using the dideoxy sequencing method. Sequences were edited and analysed in Geneious R10 using the database of ResFinder 3.0 [16]. Clonal analysis was carried out using the BOX-PCR method described elsewhere [17], and cluster analysis was performed in GelCompar II v.6.6.11 (Applied Maths, Sint-Martens-Latem, Belgium). Fragments between 200 bp and 1500 bp in size were included in the analysis using the unweighted pair-group method with arithmetic mean (UPGMA) model with Dice coefficient.

2.5. Multiple choice survey

Multiple choice questionnaires were completed with face-to-face interviews conducted with 50 dog owners who were walking their dogs in the park (after observing whether or not they picked up their dog's faeces). Dog owners were asked several questions with the intention of assessing their dog owner habits, in particular their attitudes regarding the disposal of their dog's faeces. The questionnaire included three questions focused on establishing the participant's attitudes and behaviour with respect to dog fouling in the park and what factors (e.g. availability of bags and bins, fines and reasons) influenced the decision to clean up their dog's waste. In addition, participants were asked to rate the importance of stated factors that have influenced their behaviour in relation to clearing up their dog's faeces (e.g. claims from a member of the public). Qualitative information from the participants' points of view regarding waste disposal as well as personal opinions about the reasons for not collecting faeces in the park were also gathered. Information about whether the dogs present in the park lived in a home/dwelling with at least one other animal, the frequency of visiting the park and information regarding animal health care was not collected. The survey was adapted from the '*Dog park survey*' of the Parks Advisory Commission of the City of Ann Arbor, MI [18].

2.6. Statistical analysis

Absolute and relative frequencies for qualitative variables of the survey were calculated. Fisher's exact test was used to compare proportions, and a *P*-value of <0.05 was considered statistically significant. Confidence interval (CI) analysis was carried out using R v.3.2.5.

3. Results

3.1. Bacterial isolation and phenotypic characterisation

Of the 50 canine faecal samples collected, 20 showed growth of *E. coli*-like colonies on ceftriaxone-supplemented MacConkey agar plates, giving a prevalence of 40.0% (95% CI 39.74–40.55%). From the 20 samples, 23 isolates were selected and confirmed as *E. coli*, comprising 1 isolate per sample, except for sample 26 in which colonies 26Apl, 26Bpl and 26Cpl were included and sample 49 in which colonies 49Apl and 49Bpl were included. All 23 isolates were submitted to screening for β-lactamase production, of which 21 were positive for ESBL production and 2 for pAmpC production; no isolates were suspicious of carbapenemase production. All isolates were resistant to cefotaxime and tetracycline. A high percentage of resistance to ceftazidime, aztreonam, cefepime,

ciprofloxacin, nalidixic acid, norfloxacin, levofloxacin and trimethoprim/sulfamethoxazole was observed, whereas <50% resistance to doxycycline, gentamicin, netilmicin, fosfomycin, nitrofurantoin, chloramphenicol, cefoxitin and azithromycin was observed; none of the isolates were resistant to imipenem, tigecycline or amikacin. All of the isolates were MDR (resistant to three or more antibiotic families). One isolate (4 pl) presented the *mcr-1* gene and was resistant to colistin with a minimum inhibitory concentration (MIC) of 4 μg/mL as well as being resistant to ampicillin (MIC > 16 μg/mL), ceftriaxone (MIC > 32 μg/mL), cefuroxime (MIC > 16 μg/mL), ciprofloxacin (MIC > 2 μg/mL) and levofloxacin (MIC > 4 μg/mL) (Figs. 1 and 2).

3.2. Genetic characterisation

No major clonal relatedness was observed among the resistant isolates. Phylogroup B1 was the most prevalent, followed by D, A and B2. $bla_{\text{CTX-M}}$ group 1 genes were the most prevalent (43.5%; 10 isolates), represented by the variants $bla_{\text{CTX-M-15}}$ (17.4%; 4 isolates), $bla_{\text{CTX-M-55}}$ (17.4%; 4 isolates) and $bla_{\text{CTX-M-3}}$ (8.7%, 2 isolates), followed by $bla_{\text{CTX-M}}$ group 9 (26.1%; 6 isolates), with the variants $bla_{\text{CTX-M-65}}$ (13.0%; 3 isolates), $bla_{\text{CTX-M-14}}$ (4.3%; 1 isolate), $bla_{\text{CTX-M-27}}$ (4.3%; 1 isolate) and $bla_{\text{CTX-M-106}}$ (4.3%; 1 isolate), and $bla_{\text{CTX-M}}$ group 2, with the variant $bla_{\text{CTX-M-2}}$ (8.7%; 2 isolates). In addition, one isolate (32 pl) presented the hybrid $bla_{\text{CTX-M-123}}$. $bla_{\text{CTX-M}}$ group 8 and bla_{KPC} genes were not detected. One isolate (19 pl) presented two $bla_{\text{CTX-M}}$ genes ($bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-3}}$). Two isolates (8.7%) harboured the $bla_{\text{CMY-2}}$ gene. Finally, isolate 4 pl harboured the $bla_{\text{CTX-M-65}}$ and *mcr-1* genes (Fig. 1).

3.3. Owners' survey results

The survey was carried out in Spanish; the questionnaire in English is given in the Supplementary material.

Answers were collected from 50 dog owners [mean ± standard deviation (S.D.) age 36.6 ± 13.6 years; 29 (58.0%) male]. Most of the owners (86%) collected the fresh faeces of their dogs, a tendency that was higher in men; however, there was no statistically significant difference between the habits of men and women in picking up dog faeces.

The mean ± S.D. age of owners aged <35 years was 25.6 ± 6.2 years, whilst older subjects had a mean ± S.D. age of 47.7 ± 8.2 years. There were statistically significant differences between these two groups regarding collecting and disposing of faeces ($P < 0.05$). Older people were more conscious about these tasks than subjects aged <35 years. Among those who collected the waste of their dog, approximately 60% always did it, whilst the rest sometimes did it ($P < 0.05$). There were no statistically significant differences in the other variables explored.

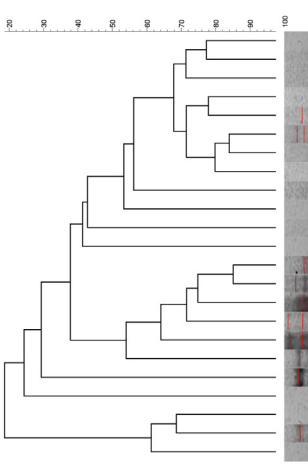
A high percentage of participants collected the faeces for hygiene reasons. The possibility of getting a fine was also stated by the majority as a good reason to collect their dog's faeces. The leading excuse for not properly disposing of the faeces was the lack of availability of bins and bags (Table 1).

Among those who did not collect their dog's faeces, 71.4% did not collect because of disgust or lack of bags or bins (Fig. 3). Moreover, 43% did not care whether or not they picked up the dog's faeces.

Regarding qualitative information, some participants expressed not having knowledge about ordinances [6,7] related to faeces disposal, whilst others think that there is a lack of personnel to enforce ordinances and they also expressed a need for more educational campaigns related to the management of animal faeces disposal.

4. Discussion

This is the first study performed to assess canine faecal contamination and antimicrobial resistance in an urban park and in dogs frequenting such a park in Ecuador. In most countries, the overall number of antimicrobial agents used for companion animals is not consistently measured. However, antimicrobials used in human and veterinary hospitals are almost identical. In Ecuador, there are no data for veterinary prescription of antibiotics. Nevertheless, in our experience the most commonly prescribed antimicrobials in small animals are amoxicillin, fluoroquinolones, third-generation cephalosporins and tetracyclines. These prescribing trends have been described elsewhere [19,20]. Antimicrobial prescription has received little attention in veterinary clinical settings regarding the increased bacterial resistance worldwide. The role of pets in the dissemination of antimicrobial resistance has been given little attention compared with that of food animals [21]. Companion animals are thought to be a reservoir of antimicrobial-resistant bacteria, and the risk of spread to humans is facilitated through their faeces. In a study performed in Portugal, Ferreira et al. found that almost all dog owners (94.1%) claimed to collect their dog's faeces [22], whereas this figure was lower in the current study (86%). This percentage may be overestimated since the survey was not anonymous.



Isolate n = 23	Resistance Profiles	ESBL/AmpC	*mcr*	Phylogroup
38pl	CTX, CAZ, FEP, TE, DO, CIP, NA, NOR, LEV, FF, C, SXT	ND	-	B1
45pl	CTX, CAZ, ATM, FEP, TE, DO, NA, GEN, FF	$bla_{\text{CTX-M-3}}$	-	A
43pl	CTX, CAZ, FEP, TE, CIP, NA, NOR, LEV, SXT, AZM	$bla_{\text{CTX-M-14}}$	-	D
4pl	CTX, FEP, TE, CIP, NA, FF, C, SXT, AZM	$bla_{\text{CTX-M-65}}$	*mcr*-1	A
10pl	CTX, CAZ, FEP, TE, CIP, NA, NOR, LEV, SXT, AZM	$bla_{\text{CTX-M-27}}$	-	B1
26Cpl	CTX, CAZ, ATM, FEP,TE, CIP, NA, NOR, LEV, GEN, NET, SXT, AZM	$bla_{\text{CTX-M-15}}$	-	B1
41pl	CTX, CAZ, FOX,TE, DO, CIP, NA, NOR, F	$bla_{\text{CMY-2}}$	-	B2
8pl	CTX, CAZ, ATM, FEP, TE, CIP, NA, NOR, LEV, F, FF, C	$bla_{\text{CTX-M-55}}$	-	B1
49Bpl	CTX, CAZ, ATM, FEP, TE, DO, CIP, NA, NOR, LEV, GEN, NET, FF, C, SXT	$bla_{\text{CTX-M-65}}$	-	B1
26Apl	CTX, CAZ, ATM, FEP, TE, DO, CIP, NA, NOR, LEV, GEN, NET, SXT, AZM	$bla_{\text{CTX-M-15}}$	-	B1
40pl	CTX, CAZ, ATM, FEP, TE, DO, CIP, NA, NOR, LEV, F, FF, SXT	$bla_{\text{CTX-M-65}}$	-	B2
29pl	CTX, CAZ, FEP,TE, CIP, NA, NOR, LEV, SXT, AZM	$bla_{\text{CTX-M-106}}$	-	A
36pl	CTX, CAZ, ATM, FEP, TE, DO, CIP, NA, NOR, LEV, FF, C	$bla_{\text{CTX-M-55}}$	-	B1
47pl	CTX, FEP, TE, DO, CIP, NA, NOR, SXT	$bla_{\text{CTX-M-2}}$	-	B1
49Apl	CTX, CAZ, ATM, FEP, TE, CIP, NA, NOR, LEV, F, C	$bla_{\text{CTX-M-55}}$	-	B1
17pl	CTX, CAZ, ATM, FEP, TE, CIP, NA, NOR, LEV, SXT	$bla_{\text{CTX-M-2}}$	-	D
50pl	CTX, CAZ, ATM, FEP, TE, CIP, NA, NOR, LEV, FF, C	$bla_{\text{CTX-M-3}}$	-	D
24pl	CTX, CAZ, FEP, TE, NOR	$bla_{\text{CTX-M-15}}$	-	B1
26Bpl	CTX, CAZ, ATM, FEP, TE, CIP, NA, NOR, LEV, SXT, AZM	$bla_{\text{CTX-M-15}}$	-	B2
19pl	CTX, CAZ, ATM, FEP, TE, DO, CIP, NA, LEV, GEN, NET, C, SXT	$bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-2}}$	-	A
13pl	CTX, CAZ, FOX, TE, DO, CIP, NA, NOR, LEV, FF, C	$bla_{\text{CMY-2}}$	-	D
21pl	CTX, FEP, TE, DO, NA, FF, SXT	$bla_{\text{CTX-M-55}}$	-	D
31pl	CTX, CAZ, FEP,TE, CIP, NA, NOR, LEV, F, SXT	$bla_{\text{CTX-M-123}}$	-	D

Fig. 1. Resistance patterns and genetic characterisation of *Escherichia coli* isolates from dog faeces. ATM, aztreonam; AZM, azithromycin; C, chloramphenicol; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; DO, doxycycline; ESBL, extended-spectrum β-lactamase; F, nitrofurantoin; FEP, cefepime; FF, fosfomycin; FOX, cefoxitin; GEN, gentamicin; LEV, levofloxacin; NA, nalidixic acid; ND, not determined; NET, netilmicin; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline.

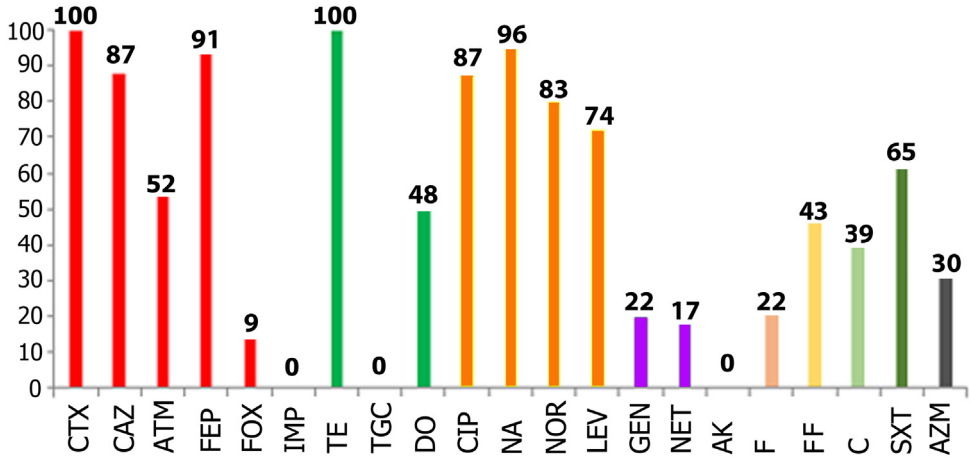


Fig. 2. Antimicrobial resistance rates of *Escherichia coli* isolates ($n = 23$) from dog faeces. AK, amikacin; ATM, aztreonam; AZM, azithromycin; C, chloramphenicol; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; DO, doxycycline; F, nitrofurantoin; FEP, cefepime; FF, fosfomycin; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; LEV, levofloxacin; NA, nalidixic acid; NET, netilmicin; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; TGC, tigecycline.

Table 1
Statistical analysis of the survey on attitudes of dog owners regarding the disposal of dog faeces.

Characteristic	Collects faeces [*n* (%)]			*P*-value*
	Yes (*N* = 43)	No (*N* = 7)	Total *N* (%)	
Age group				
≤35 years	20 (46.5)	6 (85.7)	26 (52.0)	<0.05
>35 years	23 (53.5)	1 (14.3)	24 (48.0)	
Collection frequency				
Always	26 (60.5)	0 (0.0)	26 (52.0)	0.01
Sometimes	17 (39.5)	5 (71.4)	22 (44.0)	
Never	0 (0.0)	2 (28.6)	2 (4.0)	
Sex				
Male	24 (55.8)	5 (71.4)	29 (58.0)	0.48
Female	19 (44.2)	2 (28.6)	21 (42.0)	
Factors influencing their behaviour				
Claims from a member of the public				
Yes	9 (20.9)	3 (42.9)	12 (24.0)	0.20
No	34 (79.1)	4 (57.1)	38 (76.0)	
Hygienic reasons				
Yes	34 (79.1)	6 (85.7)	40 (80.0)	0.68
No	9 (20.9)	1 (14.3)	10 (20.0)	
Because of children				
Yes	14 (32.6)	4 (57.1)	18 (36.0)	0.20
No	29 (67.4)	3 (42.9)	32 (64.0)	
Availability of bins				
Yes	27 (62.8)	5 (71.4)	32 (64.0)	0.65
No	16 (37.2)	2 (28.6)	18 (36.0)	
Availability of bags				
Yes	22 (51.2)	3 (42.9)	25 (50.0)	0.68
No	21 (48.8)	4 (57.1)	25 (50.0)	
Fines				
Yes	22 (51.2)	3 (42.9)	25 (50.0)	0.68
No	21 (48.8)	4 (57.1)	25 (50.0)	

* Fisher's exact test.

Although there were dog faeces bag dispensers at the park, all of them were without plastic bags. Dog owners who picked up the faeces carried their own plastic bags. Collection of pet waste is regulated in Quito by two ordinances. The ordinance of the Metropolitan Council of Quito # 48 in articles 6 and 30 of the Municipal Code for the Metropolitan District Of Quito, Municipal Ordinance 1, specifically the article 357.2 [6,7], provides a penalty for not picking up dog faeces. Nevertheless, these ordinances are not well known by the owners of animals in the city and are poorly implemented by the local police. The fact that older people are more aware of hygiene than younger people (Table 1) suggests that more educational campaigns are needed. These educational campaigns should be related to the management of animal faeces disposal in order to avoid the spread of MDR *E. coli*.

To date, the prevalence of third-generation cephalosporin-resistant *E. coli* in dog faeces from public parks has been poorly described. In the current work, a 40% (20/50) prevalence was reported, which is much higher than the prevalence reported for other public spaces such as public gardens in Denmark (1.9%; 4/209) [23]. On the other hand, several studies on the prevalence *of* ESBL-producing *E. coli* in healthy dogs have reported prevalences ranging from 45% (9/20) in the Netherlands [24] to 17% (9/53) in Mexico [25] and 4.9% (5/102) in Algeria [26]. To the best of our knowledge, there are no reports for the Andean region in the indexed literature.

Despite using direct plating on selective MacConkey agar plates without an enrichment step, which could lead to the loss of positive samples with low counts as reported elsewhere [24], the prevalence of samples carrying *E. coli* resistant to third-generation cephalosporins in this study (40.0%) is worrisome for a public area.

Genes belonging to the $bla_{\text{CTX-M}}$ family are the most frequently reported in human, animal and environmental ESBL-producing *E coli* isolates [27], and $bla_{\text{CMY-2}}$ is the most frequently identified pAmpC gene worldwide [28]. The same pattern has been identified in canine faecal samples, where $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$ ($bla_{\text{CTX-M group 1}}$) and $bla_{\text{CMY-2}}$ have been frequently described [24–26].

In this study, $bla_{\text{CTX-M}}$ genes belonging to groups 1, 2 and 9 were identified. The $bla_{\text{CTX-M group 1}}$ variants $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ were the most prevalent. $bla_{\text{CTX-M-15}}$ is the most prevalent worldwide, and $bla_{\text{CTX-M-55}}$ is increasing in prevalence in several locations [29,30]. The most prevalent $bla_{\text{CTX-M-group 9}}$ gene was $bla_{\text{CTX-M-65}}$, previously described in the study location [31].

Screening for carbapenemase production was based on susceptibility to imipenem. However, there have been reports of Enterobacteriaceae strains harbouring bla_{KPC} genes that are susceptible to imipenem. In addition, in Ecuador bla_{KPC} is the most prevalent gene associated with carbapenem-resistant Enterobacteriaceae [13]. Therefore, we screened all isolates for this gene [32]. However, isolates harbouring bla_{KPC} were not observed. Nevertheless, carbapenemase-producing *E. coli* isolated from dog faeces have been reported previously [33].

Colistin is the last-resort antibiotic against extensively drug-resistant Gram-negative bacteria. The plasmid-mediated colistin resistance gene *mcr-1* was described in *E. coli* isolates from food animals and humans in November 2015 [34]. In Ecuador, the *mcr-1* gene was reported in 2016 in a human *E. coli* isolate from peritoneal fluid [14]. To date, the *mcr-1* gene has been reported worldwide from

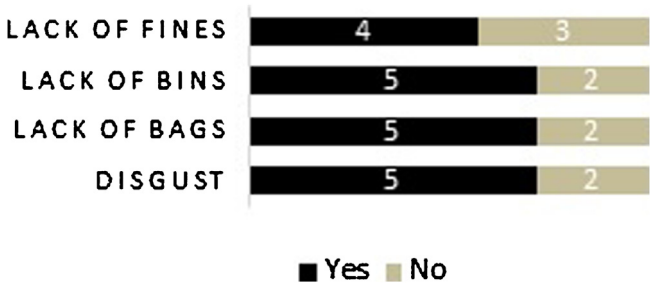


Fig. 3. Reasons for owners not collecting their dog's faeces.

a variety of sources [35]. However, there are few reports of *E. coli* harbouring *mcr-1* isolated from dogs. In this study, isolation of *E. coli* harbouring *mcr-1*/$bla_{CTX\text{-}M\text{-}65}$ from canine faeces in a city park opens the possibility of further studies to determine whether there is potential transmission of these strains to humans, as reported previously [36,37]. In addition, the high genotypic diversity shown by the clonal analysis suggests a variety of genetic backgrounds of resistance determinants present in MDR *E. coli* in dogs.

A variety of reports support the transmission of *E. coli* between dog faeces, dogs and humans. Schaufler et al. reported dog faeces around veterinary facilities as a non-point infection source of ESBL-producing *E. coli* both for animals and humans [38]. Dog faeces has been identified as a vector for dissemination of ESBL-producing *E. coli* within urban areas [23]. As MCR-1-producing *E. coli* in dogs can be transferred between companion animals and humans [36,39], we wanted to know whether dog faeces in a Ecuadorian park could be a source of MDR bacteria for humans. Finally, cross-transmission of resistant E. coli can easily occur between owners and non-owners [40]. Based on this evidence, dissemination of these bacteria from dog faeces in the park to humans is plausible. This hypothesis must be examined more thoroughly to establish the real implication of dog faeces in city parks on human and pet heath.

5. Conclusion

Although people consider public parks excellent recreational areas for their family and dogs, the results of this study highlight the potential of urban parks as a reservoir and source of transmission of MDR bacteria. In addition, actions such as improving the availability of disposal bags, dog owner education and fines could help to limit the dissemination of MDR bacteria. Further studies are required to evaluate the role of dog faeces contamination in public environments as a dissemination pathway of bacterial resistance. Characterisation of isolates from canine faeces in public parks might be a sentinel sampling method to gain a better understanding of community sources of antibiotic-resistant Enterobacteriaceae at human–animal–environment interfaces.

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Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.04.002.

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