



Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2025, Volume 5, Issue 1, Page No: 1-10

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Available online at: www.esvpub.com/

Prevalence of Extended-Spectrum β -Lactamase-, AmpC β -Lactamase-, and Carbapenemase-Producing *Escherichia coli* in Fecal Samples of Stray Cats in Italy

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ABSTRACT

Carbapenem-resistant bacteria pose a serious worldwide public health problem, and growing evidence suggests that companion animals may contribute to the expansion of antimicrobial resistance. This research assessed the intestinal presence of *Escherichia coli* producing extended-spectrum β -lactamase (ESBL), AmpC, and carbapenemase (CP) enzymes, along with possible associated factors, in both healthy and diseased stray cats admitted to the Veterinary Teaching Hospital of Lodi, University of Milan, Italy. Fecal material gathered between 2020 and 2022 underwent both microbiological and molecular testing. Overall, 18 of 94 (19.1%) cats carried *E. coli* producing ESBL, AmpC, or CP. Specifically, ESBL-, AmpC-, and CP-type *E. coli* were identified in 12 (12.8%), 4 (4.3%), and 7 (7.4%) cats, respectively. Genetic screening confirmed blaCTX-M in all ESBL isolates, blaCMY-2 in every AmpC isolate, and either blaNDM (4/7; 57.1%) or blaOXA-48 (3/7; 42.9%) among the CP group. Some isolates showed overlapping gene and resistance patterns. Minimum inhibitory concentration (MIC) testing revealed all isolates were multidrug resistant. Significant predictors of ESBL-, AmpC-, and/or CP-positive *E. coli* carriage included hospitalization ($P < 0.0001$), antimicrobial administration during hospitalization ($P < 0.0001$), and poor clinical condition ($P < 0.0001$). The occurrence of CP-producing *E. coli* in stray cats is alarming and underscores the need for ongoing monitoring of CP-producing Enterobacteriaceae and for rational antibiotic use to limit resistant bacterial spread. Given the study's constraints, a One Health strategy is recommended to further explore whole-genome profiles and AMR epidemiology in stray cats, including other bacterial species and environmental sources.

Keywords: Stray cats, Antimicrobial resistance, Carbapenemase, ESBL, AmpC

Received: 17 October 2024

Revised: 09 January 2025

Accepted: 12 January 2025

How to Cite This Article: Coelho AM, Mourato P, Delgado S, Martins IS. Prevalence of Extended-Spectrum β -Lactamase-, AmpC β -Lactamase-, and Carbapenemase-Producing *Escherichia coli* in Fecal Samples of Stray Cats in Italy. *Int J Vet Res Allied Sci*. 2025;5(1):1-10. <https://doi.org/10.51847/sRf3TihzPy>

Introduction

Antimicrobial resistance (AMR) is now one of the leading global health concerns [1]. Since resistant organisms circulate among humans, animals, and the environment, integrated One Health strategies are essential to control their transmission [2]. Over the last ten years, Enterobacteriaceae capable of producing extended-spectrum β -lactamases (ESBL), AmpC β -lactamases, and carbapenemases (CP) have been increasingly reported in both humans and animals, indicating that animals might serve as a potential source of resistant bacteria for people [3].

Although the spread of resistant zoonotic bacteria through food-producing animals has been intensively examined [4], much less attention has been directed to pets within the One Health framework, despite their close contact with humans and potential to share resistant microbes in households [5, 6]. In cats, antibiotic use can select for resistant bacteria with implications for both veterinary and public health [7]. Pets have already been recognized as possible carriers of ESBL- and AmpC-producing Enterobacteriaceae, notably *E. coli*, a major pathogen responsible for both hospital-acquired and community infections in humans [8].

Effective AMR control depends on comprehensive, cross-sectoral monitoring based on the One Health approach [9]. Because of its clinical importance, ESBL-producing *E. coli* has been identified as a key indicator for AMR surveillance across human, animal, and environmental sectors [9, 10]. Research has largely focused on dogs rather than cats [11], and in dogs, resistant *E. coli* carriage has been correlated with factors such as earlier antimicrobial treatment, illness, hospitalization, and consumption of raw diets [12–15].

Additionally, companion animals have occasionally been found to harbor carbapenemase-producing Enterobacteriaceae (CPE), including *E. coli* isolates from cats [16], even though carbapenems are not authorized for routine veterinary use in the European Union [17, 18].

Recently, resistant *E. coli* strains have also been detected among stray dogs and cats [19–21], highlighting the necessity of structured AMR surveillance programs for free-roaming animal populations [13, 14, 20]. Cats are currently the most common household pet in Europe [22], but data on stray cats' involvement in harboring ESBL-, AmpC-, or CP-producing *E. coli* remain scarce, warranting focused investigation.

In the province of Lodi in northern Italy, local records list about 1,770 unowned cats distributed among 221 colonies within the Veterinary District of the Regional Health Protection Agency (ATS Città Metropolitana di Milano – Distretto Alto Lodigiano; ATS-AL). This study was designed to (i) assess the occurrence of ESBL-, AmpC-, and CP-producing *E. coli* in feces from stray cats and describe their antimicrobial resistance patterns and gene profiles, and (ii) identify risk factors influencing the intestinal carriage of these resistant *E. coli* strains.

Materials and Methods

Sample collection

Fresh fecal material was obtained from stray cats admitted to the Veterinary Teaching Hospital (VTH) of Lodi, University of Milan, Italy, during the years 2020–2022. These cats were presented either for neutering procedures mandated by Italian Law No. 281/1991 for population management or due to health-related problems. A single veterinarian conducted all collections to maintain consistency. Each sample was placed in a sterile fecal container, stored at 4 °C, and delivered to the diagnostic laboratory within 24 h. All cats originated from colonies located in the Lodi province. For every individual, data regarding sex, age, colony location (if known), and clinical history—including health condition, hospitalization status, and antibiotic use during hospitalization—were documented. Age was categorized as < 2 years or ≥ 2 years, following Anpuanandam *et al.* (2021) [23]. Health condition was defined as either healthy or unhealthy based on physical examination. Cats receiving antimicrobial treatment while hospitalized were additionally grouped by the antibiotic class used. After surgery or medical care, cats were either released back to their original colony or adopted. The study protocol received approval from both the Institutional Animal Care and Use Committee and the Ethical Committee (approval no. OPBA_40_2020).

Detection of ESBL-, AmpC-, and CP-Producing E. coli

Approximately 1 g of each fecal sample was added to 9 mL of buffered peptone water (BPW) and incubated at 37 ± 1 °C for 18–22 h, with sterile BPW serving as the negative control. For selective isolation of ESBL-/AmpC-producing Enterobacteriaceae, 10 µL of the enrichment broth was streaked onto MacConkey agar containing 1 mg/L cefotaxime and incubated overnight at 37 ± 1 °C. One colony per cat was selected and identified using MALDI-TOF MS (MALDI Biotyper® microflex® LT/SH, Bruker Daltonics, GmbH & Co) by the direct transfer approach [24]. Each confirmed *E. coli* isolate was preserved in brain heart infusion (BHI) broth supplemented with 15 % glycerol at –80 °C for further testing.

Stored isolates were revived on MacConkey agar with 1 mg/L cefotaxime, incubated overnight at 37 ± 1 °C, and analyzed for ESBL, AmpC, and carbapenemase production. Phenotypic confirmation was performed using the combination disk test (CDT), AmpC detection set D69C, and Carba plus D73C (MAST Group Ltd., UK). Briefly, a 0.5 McFarland suspension in saline was uniformly spread over Mueller–Hinton agar plates, and disks were placed on the surface. After incubation at 35 ± 1 °C for 18 ± 2 h, ESBL, AmpC, and carbapenemase activities—

including metallo- β -lactamase (M β L), *Klebsiella pneumoniae* carbapenemase (KPC), and OXA-48—were interpreted according to manufacturer guidelines.

All isolates were additionally subjected to PCR testing and minimum inhibitory concentration (MIC) determination.

PCR detection of resistance genes

Genomic DNA was extracted using the boiling method (95 °C for 10 min) and used for amplification of ESBL-, AmpC-, and CP-associated genes. Multiplex PCR targeting blaCTX-M, blaTEM, and blaSHV was applied to all isolates for ESBL gene screening [25]. blaCTX-M-positive isolates were further analyzed to identify blaCTX-M-1 and blaCTX-M-9 groups [26, 27]. All samples were also screened for blaCMY-2 [28]. Isolates showing carbapenemase activity underwent additional PCR testing for blaNDM, blaKPC, blaVIM, and blaOXA-48 [29]. Each assay included previously characterized positive control strains from the laboratory collection and a DNA-free water sample as a negative control.

Antimicrobial susceptibility testing

MICs for each *E. coli* isolate were obtained using the broth microdilution technique with commercial COMPAGNIF Sensititre plates (Thermo Scientific®). Results were manually read using the Sensititre™ Manual Viewbox. Interpretations followed Clinical and Laboratory Standards Institute guidelines [30] in accordance with manufacturer instructions. Isolates were classified as multidrug-resistant (MDR) when resistance was observed to at least one agent in three or more antimicrobial categories [31].

Sample size and statistical analysis

To estimate the prevalence of ESBL-, AmpC-, and CP-producing *E. coli* among stray cats, a sample of 94 animals was calculated using the WinEpi tool (<http://www.winepi.net/uk/index.htm>, accessed 1 February 2020), assuming a total population of 1,170 cats, a 95 % confidence level, and a minimum expected prevalence of 3 %. Differences in the proportions of positive cats by sex, age group, hospitalization, health condition, antibiotic use during hospitalization, and antibiotic class were assessed using Fisher's exact test. Statistical analyses were performed with Epitools (<https://epitools.ausvet.com.au/>), and $p < 0.05$ was considered significant.

Results and Discussion

A total of 94 fecal specimens were examined, representing 5.3 % (94/1770) of the stray cat population residing in the Lodi province. The descriptive data and clinical features of these animals are outlined in **Table 1**. Origin details were available for 58 cats that participated in the neutering initiative, distributed among 11 feline colonies within the province (**Figure 1**).

Out of the 94 samples analyzed, 18 (19.1 %; 95 % CI: 11.2–27.1 %) carried *Escherichia coli* strains producing ESBL, AmpC, or carbapenemase. Isolates exhibiting an ESBL phenotype were recovered from 12 cats (12.8 %), with the bla_{CTX-M-1} group detected in 9/12 (75 %) and bla_{CTX-M-9} in 3/12 (25 %) of them. The combination of phenotypic and molecular results is summarized in **Figure 2**.

Six isolates harboring bla_{CTX-M-1}, bla_{TEM}, and/or bla_{SHV} genes lacked ESBL expression but showed AmpC and/or carbapenemase activity. ESBL-producing strains were identified in both clinically compromised/hospitalized cats and two apparently healthy, untreated cats participating in the sterilization program, both belonging to the same colony (**Figure 1**). These two isolates (28EC and 34EC) (**Figure 2**) were collected seven days apart and presented identical resistance profiles.

AmpC-producing *E. coli* were found in 4 cats (4.3 %), all of which were hospitalized and clinically unwell, and each carried the bla_{CMY-2} gene.

In addition, 7 of 94 samples (7.4 %) contained carbapenemase-producing isolates, again from unhealthy, hospitalized animals. Among them, 4/7 (57.1 %) displayed metallo- β -lactamase (M β L) activity associated with the bla_{NDM} gene, while 3/7 (42.9 %) exhibited OXA-48-type carbapenemase activity confirmed by the bla_{OXA-48} gene. Neither bla_{KPC} nor bla_{VIM} was identified in these isolates.

Multiple resistance mechanisms were observed in 3 of the 18 isolates (16.7 %), which showed combinations of phenotypic traits and resistance genes (**Figure 2**).

MIC evaluations are presented in **Figure 2**. Imipenem resistance occurred exclusively in isolates carrying bla_{NDM}. All *E. coli* strains were classified as multidrug resistant (MDR), since each showed resistance to at least one antimicrobial in three or more categories. CP-producing isolates were resistant to 15–16 of the drugs tested, while the remaining *E. coli* strains resisted 6–11 of the 19 antimicrobials assessed.

Further examination of CP-producing isolates (**Figure 2**) revealed that three out of four M β L-positive *E. coli* harboring bla_{NDM} shared identical phenotypic and genetic characteristics, while the fourth was nearly identical. These isolates were recovered within a 16-day period. Similarly, two OXA-48-producing isolates showed matching profiles despite being isolated seven months apart.

Statistical outcomes (**Table 1**) demonstrated significant associations between the detection of ESBL-, AmpC-, and CP-producing *E. coli* and three variables: disease status (OR = 22.4; 95 % CI: 4.72–106.18; $p < 0.0001$), hospitalization (OR = 24; 95 % CI: 5.05–114.09; $p < 0.0001$), and antibiotic use during hospitalization (OR = 13.12; 95 % CI: 3.8–45.38; $p < 0.0001$). The likelihood of detection was particularly elevated following treatment with β -lactam/ β -lactamase inhibitor combinations (OR = 11.46; 95 % CI: 2.87–45.65; $p = 0.0006$) and cephalosporins (OR = 8.5; 95 % CI: 2.61–27.64; $p = 0.0004$).

Table 1. Characteristics of the cats analyzed in the present work.

Characteristic	Category	Sample Size (%)	ESBL-, AmpC-, CP-Positive Cases (%)	P-value
Gender	Male	48 (51.1)	10 (20.8)	0.8715
	Female	46 (48.9)	8 (17.4)	
Age ^a	<2 years	41 (56.8)	4 (9.8)	0.0623
	≥ 2 years	52 (43.2)	14 (26.9)	
Health Status	Healthy	58 (61.7)	2 (3.4)	<0.0001
	Unhealthy	36 (38.3)	16 (44.4)	
Hospitalized	Yes	35 (37.2)	16 (45.7)	<0.0001
	No	59 (62.8)	2 (3.4)	
Antibiotic Use	Yes	30 (31.9)	14 (46.7)	<0.0001
	No	64 (68.1)	4 (6.3)	
β -lactam/ β -lactamase Inhibitor Use ^b	Yes	11 (11.7)	7 (63.6)	0.0006
	No	83 (88.3)	11 (13.3)	
Fluoroquinolone Use ^b	Yes	9 (9.6)	2 (22.2)	0.68
	No	85 (90.4)	16 (18.8)	
Cephalosporin Use ^b	Yes	17 (18.1)	9 (63.6)	0.0004
	No	77 (81.9)	9 (11.7)	

^a Age information was missing for one cat. Values in bold correspond to $p < 0.05$.

^b Both single and combined drug treatments were included.

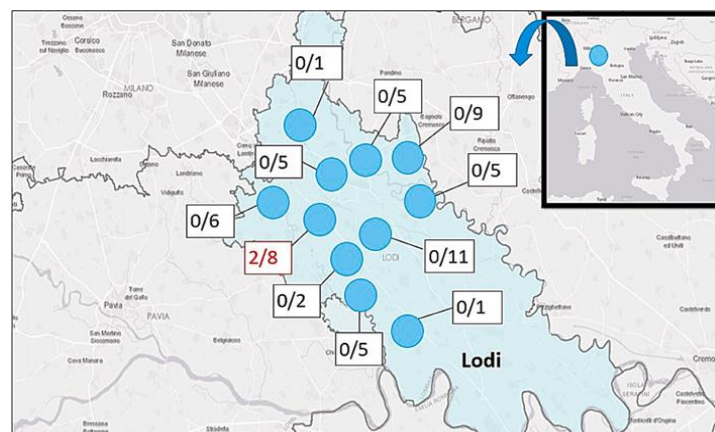


Figure 1. Map illustrating the geographic distribution of feline colonies sampled and the occurrence of ESBL-positive *E. coli* isolates. Each box reports the number of positive and total sampled cats per colony; red boxes mark positive colonies

Isolate	Phenotype and resistant genes											Resistance to antimicrobial agents based on MIC results																			
	ESBL phenotype	<i>bla</i> _{CTX-M-1}	<i>bla</i> _{CTX-M-9}	<i>bla</i> _{TEM}	<i>bla</i> _{SHV}	AmpC phenotype	<i>bla</i> _{CMY2}	MβL phenotype	<i>bla</i> _{NDM}	OXA-48 phenotype	<i>bla</i> _{OXA-48}	AMP	FAZ	LEX	FOV	POD	TAZ	AUG2	P/T4	AMI	GEN	CHL	ENRO	MAR	ORB	PRA	TET	DOX	IMI	SXT	
63EC	+	+	-	-	-	-	-	-	-	-	-	R	R	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S
87EC	+	+	-	-	-	-	-	-	-	-	-	R	R	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S
28EC	+	+	-	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
34EC	+	+	-	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
64EC	+	+	-	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
92EC	+	+	-	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	R	R	S	S	S	R	R	S	R
69EC	+	-	+	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	S	R	R	R	R	S	R	S	R
71EC	+	-	+	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S	S	R	R	S	R
88EC	+	-	+	+	-	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	R	R	S	R
80EC	+	+	-	+	+	-	-	-	-	-	-	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
67EC	-	-	-	+	+	+	+	-	-	-	-	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	R	S	R
72EC	-	+	-	+	-	-	-	+	+	-	-	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R
90EC	-	+	-	+	-	-	-	+	+	-	-	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	R	S
93EC	-	+	-	+	-	-	-	+	+	-	-	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	R	S
94EC	-	+	-	+	-	-	-	+	+	-	-	R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	S	R	R	S
91EC	-	+	-	-	-	+	+	-	-	+	+	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	S	R	S	S
73EC	+	+	-	+	-	+	+	-	-	+	+	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	S	S	R
78EC	+	+	-	+	-	+	+	-	-	+	+	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	S	R

Legend

AMP

ampicillin

FAZ

cefazolin

LEX

cephalexin

FOV

cefovecin

POD

cefpodoxime

TAZ

ceftazidime

AUG2

amoxicillin/clavulanic acid 2:1

P/T4

piperacillin/tazobactam contant 4

AMI

amikacin

GEN

: gentamicin

CHL

chloramphenicol

ENRO

enrofloxacin

MAR

marbofloxacin

ORB

orbifloxacin

PRA

pradofloxacin

TET

tetracycline

DOX

doxycycline

IMI

imipenem

SXT

trimethoprim/sulfamethoxazole 1:19

R

Penicillins

R

Cephalosporin I

S

R

Cephalosporin III

S

R

β-lactam combination agent

S

R

Aminoglycosides

S

R

Phenicol

S

R

Fluoroquinolones

S

R

Tetracycline

S

R

Carbapenems

S

R

Folate pathway antagonists

Figure 2. Overview of the phenotypic behavior, resistance genes, and MIC values of *E. coli* isolates producing ESBL, AmpC, or carbapenemase enzymes obtained from stray cats.

Numerous investigations have examined antimicrobial-resistant (AMR) *Escherichia coli* in livestock, wild species, and domestic pets [5]. Recent publications have pointed out that free-roaming cats and dogs can act as vectors of AMR *E. coli*, emphasizing the importance of dedicated monitoring programs for these animal populations [19, 20]. Despite this, the specific contribution of cats as carriers has been poorly addressed in the literature [8, 11].

In the present research, the 12.8 % rate of ESBL-producing *E. coli* corresponds with earlier reports [11], reinforcing the evidence that cats can harbor isolates bearing bla_{CTX-M-1} or bla_{CTX-M-9}, which represent the most prevalent CTX-M variants worldwide [32]. The lack of phenotypic ESBL expression in six *E. coli* isolates containing ESBL genes might be explained by coexisting resistance mechanisms such as AmpC or carbapenemase production, or by the presence of narrow-spectrum β-lactamase genes, including bla_{TEM} or bla_{SHV} [33, 34]. Complex combinations of genes and resistance phenotypes similar to those identified here have been documented in *E. coli* from dogs [11, 35].

Most ESBL-positive isolates were recovered from sick or hospitalized cats, which aligns with expectations. Nonetheless, this work also identified ESBL-producing *E. coli* in clinically healthy stray cats, suggesting that intra-colony spread can occur even in the absence of clinical illness. The discovery of genetically related isolates in two cats belonging to the same colony indicates possible transmission of a single clone. Because detailed information on the animals' previous exposure history was unavailable, the source of infection remains uncertain. Given that long-term fecal shedding of resistant *E. coli* has been observed [36], extended surveillance will be essential to clarify how these strains are acquired and maintained within feline populations.

The detection of AmpC-producing *E. coli* in this study aligns with prior findings [8, 37]. Identification of bla_{CMY-2} further confirms its dominant occurrence among AmpC-positive *E. coli* isolated from pets [37–39].

Although infrequent, the presence of carbapenemase-producing (CP) *E. coli* in stray cats is a significant concern. Comparable carriage rates, ranging from 0 % to 2.5 %, have been described in privately owned companion animals [15, 16, 40–42]. However, this study might underreport CPE occurrence, since MacConkey agar with

cefotaxime—used here for screening—is not highly selective for carbapenemase producers, and no specialized CPE-detection protocol was applied [43, 44].

Because CPE pose major human health risks, it is essential to understand how carbapenem-resistant *E. coli* arise in stray cats. Their appearance is unlikely connected to therapeutic carbapenem exposure, as such drugs are not licensed for veterinary application. It is plausible that co-selection pressures from other antibiotics used in animal practice favor the persistence of carbapenemase-encoding genes [45]. In addition, cross-species transfer—either through human-to-animal transmission or horizontal plasmid exchange (particularly pOXA-48-like elements)—could play a role [42, 45].

Every CP-producing isolate detected carried either bla_{NDM} or bla_{OXA-48}, which are recognized as the most frequent carbapenemase determinants in *E. coli* from pets [44]. Both genes have been recently identified in Italian canine and feline isolates [46, 47], while bla_{OXA-48} appears especially common among hospitalized animals [45]. In the current study, CP-producing strains were found exclusively in hospitalized stray cats, and several showed identical resistance profiles and gene patterns, strongly implying nosocomial acquisition [35, 45, 48, 49].

The observed correlation between hospital stays, illness, and antimicrobial therapy with carriage of ESBL-, AmpC-, and CP-producing *E. coli* indicates that veterinary hospitals may act as focal points for AMR dissemination, confirming previous reports involving dogs [15]. Nevertheless, in stray populations, these factors could be interrelated, since severe disease often necessitates both hospitalization and antibiotic use. The strong link between β -lactam/ β -lactamase inhibitor or cephalosporin treatment and the presence of resistant *E. coli* mirrors earlier findings [15, 50, 51]. These drug classes likely exert selection pressure on the intestinal microbiota, promoting the growth of resistant strains [50].

Finally, the detection of multidrug resistance (MDR) across all isolates, combined with high resistance to key antimicrobial classes commonly prescribed for small animals in Northern Italy—penicillins, cephalosporins, fluoroquinolones, and tetracyclines [52]—illustrates the serious risk of therapeutic failure. These outcomes emphasize the urgent requirement for rational antimicrobial use and comprehensive stewardship policies within veterinary practice.

Our findings underscore the importance of implementing targeted monitoring systems for carbapenemase-producing Enterobacterales (CPE) in companion animals, particularly within veterinary clinical environments. Traditionally, carbapenem resistance detection has relied on elevated minimum inhibitory concentrations (MICs) for carbapenems to identify CP-producing isolates [44]. Nevertheless, results from our MIC testing revealed that OXA-48-like producers can exhibit in vitro susceptibility to imipenem or meropenem, leading to false-negative outcomes. Although our data suggest that *E. coli* colonies grown on MacConkey agar supplemented with cefotaxime could also be screened for carbapenemase activity, it remains crucial to employ dedicated confirmatory assays in standard diagnostic workflows. Doing so will prevent underestimation of CPE prevalence in companion animals and ensure accurate detection of CP-producing bacteria [44].

In a broader context, this study's results carry significant implications for both veterinary and public health sectors. Within the One Health paradigm, further investigations are encouraged to evaluate horizontal gene exchange and environmental factors influencing AMR spread, since the environment itself functions as a reservoir and transmission medium for antimicrobial resistance genes [53]. This aligns with recent recommendations advocating that environmental AMR monitoring should complement ongoing clinical, food chain, and veterinary surveillance programs [54].

However, several limitations must be acknowledged. The lack of detailed demographic information on the sampled cats restricted the interpretability of epidemiological associations. Moreover, the unknown histories of the stray animals limited risk factor assessment and reduced overall epidemiological precision. As the research design was primarily descriptive, it did not permit an in-depth analysis of bacterial transmission mechanisms. Future studies should aim to trace routes of AMR dissemination among stray cats, considering potential environmental reservoirs and cross-species transmission pathways under the One Health approach. Additionally, the decision to analyze only one isolate per fecal sample might have underrepresented intra-sample genetic variation. Upcoming research should therefore include multiple colonies per specimen to capture this diversity.

Given the comparable phenotypic and genotypic patterns observed in certain *E. coli* isolates, whole-genome sequencing (WGS) is recommended for a more detailed molecular and epidemiological understanding of ESBL-, AmpC-, and CP-producing *E. coli* in cats. Expanding future AMR surveillance to incorporate other bacterial taxa would further enhance the comprehensiveness of resistance mapping efforts.

Conclusion

The detection of ESBL-, AmpC-, and carbapenemase-producing *E. coli* in stray cats, particularly in those hospitalized due to illness, demonstrates a pressing need for focused surveillance programs addressing CPE in felines. Such initiatives, combined with responsible antimicrobial stewardship in veterinary medicine, are essential to curb potential transmission of resistant organisms to humans, animals, and the broader environment.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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