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Detection of multidrug resistance and extended-spectrum/plasmid-mediated AmpC beta-lactamase genes in Enterobacteriaceae isolates from diseased cats in Italy

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Abstract

Objectives The aim of this study was to determine the antimicrobial susceptibility of Enterobacteriaceae isolated from cats affected by diseases commonly encountered in practice, and to characterise the third-generation cephalosporin (3GC)-resistance molecular mechanisms involved.

Methods Clinical samples (n = 100) included 58 rectal swabs from cats with diarrhoea, 31 nasal swabs from cats with clinical signs of upper respiratory tract disease, four ear swabs from cats with otitis, three conjunctival swabs from cats with conjunctivitis, two oral swabs from cats with stomatitis, one swab from a skin abscess and one urine sample from a cat with cystitis. A total of 125 Enterobacteriaceae were isolated from 90 cats. *Escherichia coli* was the most frequently isolated species (n = 65), followed by *Enterobacter* species (n = 20), *Proteus* species (n = 13), *Citrobacter* species (n = 12) and others (n = 15). Bacterial susceptibility testing was performed with respect to eight antimicrobial classes. Beta (β)-lactamase genes were identified by PCR and nucleotide sequencing.

Results Overall, the higher frequency of resistance was to amoxicillin–clavulanate (61.3%), trimethoprim/sulfamethoxazole (33.6%) and cefotaxime (32.8%). Thirty-six percent of the isolates (n = 45) were resistant to 3GCs. Of these isolates, 34 were tested by PCR and nucleotide sequencing and 23 were confirmed as encoding β-lactamase genes. Fourteen 3GC-resistant isolates harboured extended-spectrum β-lactamases (ESBLs) belonging to groups CTX-M-1 (n = 12, two of which were CTX-M-79), CTX-M-2 (n = 1) and CTX-M-9 (n = 1), as well as SHV-12 (n = 1) and TEM-92 (n = 1). Nine isolates had CMY-2 plasmid-mediated AmpC β-lactamases (pAmpC). Thirty-one percent (n = 39) of the isolates were multidrug resistant (MDR) and were isolated from 34% (n = 31/90) of the cats.

Conclusions and relevance A high frequency of MDR and ESBL/pAmpC β-lactamase-producing Enterobacteriaceae were detected among bacteria isolated from a feline population in southern Italy with a variety of common clinical conditions, which poses limitations on therapeutic options for companion animals. We describe the first detection of CTX-M-79 and TEM-92 ESBL genes in isolates from cats.

Keywords: Multidrug resistance; Enterobacteriaceae; beta-lactamases; gastrointestinal disease; upper respiratory disease

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Introduction

Members of the family Enterobacteriaceae can be isolated from healthy and diseased companion animals suffering from several clinical conditions.[1] Resistance to antimicrobials in these microorganisms, namely to third-generation cephalosporins (3GCs), is an increasing global public health threat for both humans and animals.[2,3] Companion animals represent an important element in the ecology of antimicrobial resistance through close contact with humans.[4] In addition, a selection pressure is determined by mutual exposure of humans and pets to antimicrobial agents for the treatment and prophylaxis of disease.[5]

In Enterobacteriaceae, beta (β)-lactamases are the most frequent mechanism of resistance to 3GCs. These are considered the highest priority critically important antimicrobials for humans, and resistance is frequently associated with the production of β-lactamases.[6,7] The most important β-lactamases among human and animal Enterobacteriaceae are extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases (pAmpCs) and carbapenemases.[8] Plasmid-encoded β-lactamases often carry genes encoding resistance mechanisms to other antimicrobial classes, with the consequent phenomenon of multidrug resistance, which may lead to failure of antimicrobial treatment.[9] β-Lactamase-producing Enterobacteriaceae in companion animals have been reported worldwide, both in healthy[10–14] and diseased animals.[15–22]

Cats are the most represented species among household pet animals in Europe.[23] Yet, most studies describing the occurrence of ESBL-producing Enterobacteriaceae in cats also included other domestic animals such as dogs, horses and farm animals.[12,13,18,24–32] Moreover, in these and other studies, isolates were obtained from tissue samples at necropsy,[28] faeces from healthy cats[12,13,33,34] or from urinary tract infections.[32,35,36] Susceptibility testing results from canine and feline bacterial isolates are frequently presented together.[13] Until now, only one study has been conducted that included samples obtained exclusively from cats.[36]

The present study aimed to investigate the antimicrobial susceptibility of Enterobacteriaceae isolates from a population of cats affected by diseases commonly encountered in practice. Furthermore, 3GC resistance was evaluated, and the molecular mechanisms of resistance were characterised.

Materials and methods

Study design and sampling

From November 2014 to February 2015, samples were collected from 100 European domestic shorthair cats admitted to the Veterinary Teaching Hospital of the Department of Veterinary Sciences of Messina (Italy) and to private veterinary practices in the cities of Palermo and Messina (Italy). Only one sample per animal was collected. Sampling included 58 rectal swabs from cats with diarrhoea (gastrointestinal disease [GID]), 31 nasal swabs from cats with clinical signs of upper respiratory tract disease (URTD), four ear swabs from cats with otitis, three conjunctival swabs from cats with conjunctivitis, two oral swabs from cats with stomatitis, one swab from a skin abscess and one urine sample from a cat with cystitis.

Enterobacteriaceae isolation and identification

Swabs were enriched in buffered peptone water and then subcultured onto MacConkey agar overnight at 37°C. All bacteria showing distinct morphology in MacConkey agar were isolated. The bacterial isolates were identified by matrix-assisted laser desorption/ionisation–time of flight (MALDI–TOF) mass spectrometry, designed to provide rapidly an accurate and reliable bacterial identification. The resulting spectra were analysed with a VITEK MS system (bioMérieux), using Axima (Shimadzu) software and the Spectral ARchive and Microbial Identification System (SARAMIS) database (AnagnosTec). The spectra were acquired in positive linear mode in the range of 2000–20,000 m/z. The isolated colonies were seeded in a 48-well metal plate with disposable loops, using as a reference strain *Escherichia coli* ATCC 8739 (Biomérieux Italia).

Antimicrobial susceptibility testing

Susceptibility testing and interpretation were performed using the disc diffusion method according to Clinical and Laboratory Standards Institute guidelines.[37,38]

The following antimicrobial discs were used: amikacin 30 µg; amoxicillin–clavulanate 30 µg; aztreonam 30 µg; cefotaxime 30 µg; ceftazidime 30 µg; ceftriaxone 30 µg; ciprofloxacin 5 µg; chloramphenicol 30 µg; meropenem 10 µg; and trimethoprim/sulfamethoxazole 25 µg.

Isolates displaying resistance to at least one antimicrobial in at least three different antimicrobial classes were considered as multidrug resistant (MDR).[39]

Detection of β-lactam resistance genes in Enterobacteriaceae and phylogenetic group determination of E coli

3GC-resistant isolates (n = 34) were screened by PCR for the presence of β-lactamase genes of groups CTX-M, and SHV and TEM.[40,41] The $bla_{\text{CTX-M-group1}}$, $bla_{\text{CTX-M-group9}}$, $bla_{\text{CTX-M-group2}}$, $bla_{\text{CTX-M-group8}}$ and $bla_{\text{CTX-M-group25}}$ genes were identified by PCR with specific primers, and positive amplicons were submitted for nucleotide sequencing.[42]

Furthermore, a multiplex PCR for the detection of pAmpC-coding genes of CIT, FOX, DHA, MIR, ACT and MOX groups was performed, using specific primers as previously described.[43] Isolates that were positive for the group CIT were submitted for nucleotide sequencing after a specific PCR targeting the entire bla_{CMY} gene.[18]

Escherichia coli harbouring β-lactamase genes were categorised into phylogroups (A, B1, B2 or D) by multiplex PCR.[44]

Statistical analysis

Results are expressed as percentages. A Fisher's exact test using an alpha level of 0.05 was performed to examine the relationship between antimicrobial treatment and isolation of MDR isolates in cats with GID and cats with URTD.

Results

Feline population and Enterobacteriaceae isolates

Of the samples collected from 100 cats, only 90 were culture positive (56 cats with GID, 24 cats with URTD, four cats with otitis, two cats with conjunctivitis, two cats with stomatitis, one cat with a skin abscess and one cat with cystitis). Of these 90 cats, 49% (n = 44) were female and 51% (n = 46) were male, with a median age of 3.8 years (range 8 months to 12 years). Thirty-eight percent (n = 34) of cats were from households and 62% (n = 56) were from shelters.

GID (n = 56) and URTD (n = 24) cats were the most numerous groups within the studied population. GID cats had a median age of 3.4 years (range 8 months to 8 years), 46% (n = 26) were female, 54% (n = 30) were male, 48% (n = 27) were household cats, 52% (n = 29) were shelter cats and 9% (n = 5) were under antimicrobial treatment at the time of sample collection. URTD cats had a median age of 3.8 years (range 1–8 years), 54% (n = 13) were female, 46% (n = 11) were male, 17% (n = 4) were household cats, 83% (n = 20) were shelter cats and 42% (n = 10) were under antimicrobial treatment at the time of sample collection.

A total of 125 bacteria were isolated (Table 1). A single bacterial species was isolated in 79/90 samples (88%); two or more bacterial species were isolated in 11/90 samples (12%). Fifty-two percent (n = 65) of the isolates were *E coli*, 16% (n = 20) were *Enterobacter* species, 10% (n = 13) were *Proteus* species, 10% (n = 12) were *Citrobacter* species and 12% (n = 15) belonged to other bacterial species (Table 1). Isolates from cats with GID and URTD represented 66% (n = 83) and 24% (n = 29) of total isolates, respectively. Ten percent of isolates (n = 13, *E coli*) were from a cat with an abscess, two cats with conjunctivitis, one cat with cystitis, four cats with otitis and two cats with stomatitis (Table 1).

Antimicrobial susceptibility testing

The highest frequency of resistance among all isolates was observed against amoxicillin–clavulanic acid (61.3% [n = 57/93]; *Citrobacter* species and *Enterobacter* species are intrinsically resistant to amoxicillin–clavulanic acid and therefore were excluded), trimethoprim/sulfamethoxazole (33.6% [n = 42]) and cefotaxime (32.8% [n = 41]) (Table 2). Although lower, resistance to amikacin (31.2% [n = 39]), aztreonam (28.0% [n = 35]), ceftazidime (28.0% [n = 35]), ceftriaxone (24.0% [n = 30]), chloramphenicol (21.6% [n = 27]) and ciprofloxacin (20.0% [n = 25]) was also relevant (Table 2). Thirty-six percent of isolates (n = 45) were resistant to at least one 3GC. All isolates were susceptible to meropenem. Frequency of antimicrobial resistance of *E coli, Citrobacter* species, *Enterobacter* species and *Proteus* species is shown in Table 2.

Table 1 Enterobacteriaceae isolates recovered from diseased cats

Species	URTD	GID	Other*
Escherichia coli (n = 65)	12	40	13
Enterobacter species (n = 13)	3	10	0
Enterobacter cloacae (n = 7)	3	4	0
Proteus mirabilis (n = 12)	1	11	0
Proteus vulgaris (n = 1)	0	1	0
Citrobacter species (n = 12)	5	7	0
Providencia alcalifaciens (n = 3)	0	3	0
Providencia rustigianii (n = 2)	0	2	0
Providencia rettgeri (n = 1)	1	0	0
Buttiauxella agrestis (n = 2)	1	1	0
Kluyvera species (n = 2)	0	2	0
Serratia liquefaciens (n = 2)	2	0	0
Hafnia alvei (n = 1)	0	1	0
Klebsiella oxytoca (n = 1)	1	0	0
Leclercia adecarboxylata (n = 1)	0	1	0

*Abscess, conjunctivitis, cystitis, otitis, stomatitis
URTD = upper respiratory tract disease; GID = gastrointestinal disease

Table 2 Antimicrobial susceptibility of Enterobacteriaceae associated with diseased cats

Antimicrobials	Resistant isolates				
	Escherichia coli (n = 65)	*Enterobacter* species (n = 20)	*Proteus* species (n = 13)	*Citrobacter* species (n = 12)	Enterobacteriaceae (n = 125)
Amoxicillin–clavulanate	36 (55.4)	NA	12 (92.3)	NA	57/93 (61.3)
Amikacin	25 (38.5)	4 (20.0)	2 (15.4)	5 (41.7)	39 (31.2)
Aztreonam	20 (30.8)	7 (35.0)	3 (23.1)	2 (16.7)	35 (28.0)
Cefotaxime	20 (30.8)	7 (35.0)	6 (46)	6 (50.0)	41 (32.8)
Ceftazidime	17 (26.2)	5 (25.0)	4 (30.8)	5 (41.7)	35 (28.0)
Ceftriaxone	20 (30.8)	2 (10.0)	3 (23.1)	4 (33.3)	30 (24.0)
Chloramphenicol	9 (13.8)	3 (15.0)	9 (69.2)	2 (16.7)	27 (21.6)
Ciprofloxacin	9 (13.8)	4 (20.0)	5 (38.5)	2 (16.7)	25 (20.0)
Trimethoprim/ sulfamethoxazole	23 (35.4)	5 (25.0)	9 (69.2)	3 (25.0)	42 (33.6)
Multidrug resistance	18 (27.7)	5 (25.0)	10 (76.9)	2 (16.7)	39 (31.2)

Data are n (%)
NA = intrinsic resistance

Multidrug resistance was displayed by 31.2% of total isolates (n = 39), which included 76.9% of *Proteus* species (n = 10/13), 27.7% of *E coli* (n = 18/65), 25.0% of *Enterobacter* species (n = 5/20) and 16.7% of *Citrobacter* species (n = 2/12) (Table 2).

Eighty percent of MDR isolates (n = 31/39) were resistant to 3GC. Among MDR isolates showing resistance to 3GC, 64% (n = 25/39), 46% (n = 18/39), 44% (n = 17/39) and 44% (n = 17/39) also showed resistance to trimethoprim/sulfamethoxazole, ciprofloxacin, amikacin and chloramphenicol, respectively.

MDR Enterobacteriaceae were detected in 34.4% of cats (n = 31/90). These were affected by GID (n = 20), URTD (n = 6), otitis (n = 2), abscess (n = 1), cystitis (n = 1) and stomatitis (n = 1). All GID cats receiving antimicrobial treatment (n = 5) harboured MDR isolates. Among GID cats not receiving antimicrobial treatment, 29.4% (n = 15/51) harboured MDR isolates. Among URTD cats under antimicrobial treatment, 40% (n = 4/10) harboured MDR isolates, whereas only 14% (n = 2/14) of URTD cats not under antimicrobial treatment harboured MDR bacteria. GID cats and URTD cats receiving an antimicrobial treatment were significantly more likely to carry MDR isolates than untreated GID and URTD cats ($P = 0.004$ and $P = 0.01$, respectively).

ESBL/pAmpC genes and E coli *phylogenetic groups*

Genes encoding ESBL/pAmpC enzymes, which are the main mechanisms of resistance to 3GC, could be identified in 67.6% (n = 23/34) of 3GC-resistant isolates studied (Table 3). The 23 ESBL/pAmpC-producing Enterobacteriaceae identified belonged to eight cats with GID, five cats with URTD, one cat with otitis, one cat with an abscess, one cat with stomatitis and one cat with cystitis (Table 3).

Fourteen 3GC-resistant isolates harboured CTX-M ESBLs belonging to groups CTX-M-1 (n = 12), CTX-M-2 (n = 1) and CTX-M-9 (n = 1). The rest of ESBL-producer isolates harboured $bla_{\text{SHV-12}}$ (n = 1) and $bla_{\text{TEM-92}}$ (n = 1) genes (Table 3). Nine isolates were pAmpC CMY-2-producers, with two isolates also harbouring $bla_{\text{CTX-M-79}}$ or $bla_{\text{CTX-M-9}}$ genes (Table 3). Overall, $bla_{\text{CTX-M-15}}$ genes were the most commonly detected, followed by $bla_{\text{CMY-2}}$.

A high percentage of ESBL/pAmpC-producing isolates were MDR (n = 17/23 [74%]), and 8/23 were fluoroquinolone resistant.

The majority of ESBLs and AmpC-producing *E coli* isolates belonged to the phylogenetic group B2 (n = 8), followed by group D (n = 5), group B1 (n = 4) and group A (n = 2) (Table 3).

ESBL/pAmpC β-lactamase-producing isolates were recovered from 17 cats. Feline clinical data are presented in Table 4.

Discussion

In Italy, only three studies have been conducted to detect β-lactamases in companion animals including cats.[28,36,45] This present study highlights that a large number of cats from Italy affected by a variety of clinical conditions commonly encountered in practice carry MDR Enterobacteriaceae (34%). Furthermore, a high percentage of cats carrying 3GC-resistant isolates were confirmed as carriers of Enterobacteriaceae harbouring MDR ESBL/pAmpC genes. Although the production of ESBL/pAmpC β-lactamases is the main mechanism of resistance to 3GCs, permeability changes in the outer bacterial membrane owing to the

Table 3 β-Lactam resistance mechanisms and antimicrobial resistance profile of 34 third-generation cephalosporin (3GC)-resistant Enterobacteriaceae strains

Species	Sample ID	Phylogenetic group	Resistance profile	β-Lactamase	Associated disease
E coli	It1	A	AUG-3GC-ATM-CIP-SXT	SHV-12	Abscess
E coli	It9	A	AUG-3GC-ATM-AK-SXT	CTX-M-15, OXA-1	Stomatitis
E coli	It13	B2	AUG-3GC-ATM-SXT	CTX-M-group-1	URTD
E coli	It15	B1	AUG-3GC-ATM-CIP-SXT	TEM-1, OXA-1, CMY-2	URTD
E coli	It18	B1	AUG-3GC-ATM-AK-SXT	CTX-M-2	URTD
E coli	It24	D	AUG-3GC-ATM-CIP-C-SXT	CTX-M-79, OXA-1	Otitis
E coli	It25	B1	AUG-3GC-SXT	CTX-M-9, CMY-2	URTD
E coli	It30	D	AUG-3GC-ATM-CIP-AK-C-SXT	CTX-M-79, CMY-2	GID
E coli	It32	D	AUG-3GC-ATM-CIP-AK-C-SXT	TEM-1	GID
E coli	It40	D	AUG-3GC-ATM-AK-C	CMY-2	GID
E coli	It41	B2	AUG-3GC-ATM-SXT	CTX-M-15	GID
E coli	It42	B2	AUG-3GC-ATM-SXT	CTX-M-15	GID
E coli	It47	B2	AUG-3GC-ATM-CIP-SXT	CTX-M-15	GID
E coli	It48	B1	AUG-3GC-ATM-AK-SXT	TEM, CTX-M-15	GID
E coli	It49	D	AUG-3GC-SXT	Neg	GID
E coli	It61	B2	AUG-3GC-ATM	CTX-M-15	GID
E coli	It65	B2	AUG-3GC-ATM-AK-C	CMY-2	Cystitis
E coli	It71	B2	AUG-3GC-ATM-CIP-AK-C-SXT	CMY-2, OXA-1	Cystitis
E coli	It72	B2	AUG-3GC-ATM-AK-C-SXT	CMY-2	Cystitis
Enterobacter species	It6		3GC-ATM-CIP-AK-SXT	Neg	GID
E cloacae	It27	–	3GC-ATM-CIP-C-SXT	CTX-M-15, OXA-1	GID
E cloacae	It28	–	3GC-ATM-C-SXT	CTX-M-15, OXA-1	GID
Enterobacter species	It29	–	3GC-ATM-CIP-C-SXT	CTX-M-15	GID
E cloacae	It34		3GC-ATM	Neg	GID
Enterobacter species	It54		3GC-ATM-CIP-SXT	Neg	GID
P mirabilis	It14	–	3GC	TEM-92	URTD
P mirabilis	It46	–	AUG-3GC-CIP-AK-C-SXT	TEM-1	GID
P mirabilis	It59		AUG-3GC-CIP-C-SXT	Neg	GID
P mirabilis	It66	–	AUG-3GC-ATM-C-SXT	TEM-1, CMY-2	GID
P mirabilis	It67	–	AUG-3GC-ATM-C-SXT	TEM, CMY-2	GID
Citrobacter species	It11		3GC-ATM-CIP-C-SXT	Neg	URTD
Citrobacter species	It37		3GC-CIP-AK	Neg	GID
Citrobacter species	It73		3GC-AK	Neg	GID
S liquefaciens	It12		AUG-3GC	Neg	URTD

E coli = *Escherichia coli; E cloacae* = *Enterobacter cloacae; P mirabilis* = *Proteus mirabilis; S liquefaciens* = *Serratia liquefaciens;* AUG = amoxicillin–clavulanate; ATM = aztreonam; AK = amikacin; CIP = ciprofloxacin; C = chloramphenicol; SXT = trimethoprim/sulfamethoxazole; URTD = upper respiratory tract disease; GID = gastrointestinal disease; (–) = not applicable; Neg = negative

presence of efflux proteins or to alteration or loss of porins are a possible cause for 3GC resistance in isolates lacking all the ESBL/pAmpC β-lactamases tested.[6]

The very high frequency of resistance to antimicrobials that are categorised as critically important in human medicine (ie, amoxicillin–clavulanic acid, 3GCs and fluoroquinolones), and that are commonly used compounds in small animal and human practice is an alarming finding.[7]

Amoxicillin–clavulanic acid is the most prescribed antimicrobial worldwide for companion animal treatment.[4,46,47] The results of this study are in line with the high frequency of resistance to amoxicillin–clavulanic acid reported for clinical *E coli* isolates of different origins worldwide.[48,49]

Table 4 Clinical data of 17 cats harbouring extended-spectrum β-lactamase/plasmid-mediated AmpC β-lactamase-producing Enterobacteriaceae

Cat ID	Disease	Isolated bacteria (ID number of the strain[s])	Age (years)	Sex	Habitat	Antimicrobial therapy
C2588	GID	*E coli* (It30)	4	M	H	–
C2600	Otitis	*E coli* (It24)	12	F	H	–
C2608	URTD	*E coli* (It25)	5	F	S	Amoxicillin–clavulanate
C2610	Abscess	*E coli* (It1)	8	M	S	–
C2613	GID	*E coli* (It40)	2	F	S	–
C2616	GID	*E coli* (It41, It42)	3	F	S	–
C2618	URTD	*E coli* (It15)	4	F	S	–
C2621	Stomatitis	*E coli* (It9)	3	M	S	Cefovecin
C2637	URTD	*E coli* (It18)	5	F	S	–
C2639	GID	*E coli* (It47)	6	M	H	Spiramycin and metronidazole
C2642	URTD	*E coli* (It13)	6	F	S	–
C2644	GID	*E coli* (It48)	5	F	S	Cefovecin
C2657	GID	*P mirabilis* (It66, It67)	5	M	S	–
C2670	GID	*E coli* (It61)	4	F	S	–
C2761	URTD	*P mirabilis* (It14)	3	M	H	
C2855	GID	*E cloacae* (It27, It28, It29)	3	F	H	–
C2977	Cystitis	*E coli* (It65, It71, It72)	4	M	H	–

URTD = upper respiratory tract disease; GID = gastrointestinal disease; *E coli* = *Escherichia coli; P mirabilis* = *Proteus mirabilis; E cloacae* = *Enterobacter cloacae;* F = female; M = male; H = household; S = shelter

An association between ESBL/pAmpC β-lactamase genes and resistance to fluoroquinolones was found in our study and has also been shown in a previous study,[50] which identified new mechanisms of resistance and plasmid-mediated fluoroquinolone resistance determinants in ESBL-producing *E coli* of canine and feline origin. In the present study, we did not characterise the mechanisms of resistance to fluoroquinolones. Yet, the high prevalence of ESBL/pAmpC β-lactamase-producing Enterobacteriaceae showing resistance tofluoroquinolones should stimulate a more focused monitoring of fluoroquinolone resistance.

MDR was displayed by a high percentage of isolates (31%), and a high percentage of isolates carrying ESBLs/pAmpC β-lactamase genes were MDR (74%). These results constitute additional evidence for an association between MDR and ESBL/pAmpC production, which allows a co-selection process, because ESBL/pAmpC genes are usually encoded on plasmids that frequently carry aminoglycoside-, tetracycline-, sulfonamide- and/or fluoroquinolone-resistant genes.[51]

GID and URTD cats receiving antimicrobial treatment were more likely to host MDR isolates than cats not receiving antimicrobials. These results underline the importance of rational use of antimicrobials to prevent the selection of resistant bacteria. Although *E coli* are part of the normal intestinal microflora, they can be associated with gastroenteritis in the presence of bacterial virulence factors and impaired local or systemic immunity. Many *E coli* strains and other Enterobacteriaceae have been isolated from small animals with and without diarrhoea, and the role of many of these strains in disease causation in dogs and cats is poorly defined.[52] In our study, we characterised phylogenetic groups of some *E coli* isolates and found that two isolates belonged to phylogenetic group B1, which usually includes commensal strains exhibiting an increased drug resistance pattern but possessing few virulence genes.[53] Eight isolates belonged either to groups B2 or D, which usually include pathogenic *E coli* strains that cause extra-intestinal infections, possess several pathogenicity-associated islands and express multiple virulence factors such as adherence factors including biofilm production and high surface hydrophobicity, toxins and siderophore production, but are more susceptible to antibiotics.[54] Moreover, six *E coli* isolates (from two cats with URTD, one cat with otitis and one cat with cystitis) belonged either to phylogenetic groups B2 or D. Another five *E coli* isolates (from one cat with an abscess, one cat with stomatitis, three cats with URTD) belonged either to phylogenetic groups A or B1. For cats with URTD, otitis, stomatitis, abscess and cystitis, *E coli* isolates could

have been directly associated with the disease condition, but we cannot exclude completely their role in a secondary infection complicating a primary non-infectious disease condition or their commensal state. Characterisation of virulence factors of *E coli* and other Enterobacteriaceae isolates in cats with diarrhoea warrants further studies.

Feline URTD, frequently encountered in clinical practice, represented the second-most common disease of cats included in the study. Detection of Enterobacteriaceae strains harbouring MDR ESBL/pAmpC genes in cats with URTD is described for the first time in the literature. One of the *E coli* isolates from cats with URTD harboured a $bla_{\text{CTX-M-15}}$ gene and belonged to phylogenetic group B2, while the others belonged to phylogenetic group B1. These MDR ESBL/pAmpC-producing *E coli* may possibly complicate the feline URTD by causing infections that are difficult to treat.

The CTX-M enzymes were the most common ESBL and different types of these enzymes were detected, indicating some diversity of CTX-M-encoding genes in Enterobacteriaceae from diseased cats in Italy. By contrast, CMY-2 was the only pAmpC found in cats from this study. The high prevalence of CMY-2 is of concern as this β-lactamase confers resistance to a wide range of extended-spectrum cephalosporins used for the treatment of serious infections in humans and animals, because they are not inhibited by β-lactamase inhibitors and because they can easily be transferred between different bacterial species leading to its rapid dissemination.[4,22,55]

Of the ESBL genes, $bla_{\text{CTX-M-79}}$ was harboured by an *E coli* isolate from one cat with GID and one cat with otitis, both of which were from households and had not received any previous antimicrobial treatment. To the best of our knowledge, we describe for the first time the detection of an *E coli* isolate harbouring the $bla_{\text{CTX-M-79}}$ gene in cats. Previous reports described the $bla_{\text{CTX-M-79}}$ gene from Enterobacteriaceae isolated from wild birds (corvids), hospital waste, human patients and farmed fish in Asia,[56–58] and from the faeces of cattle in the USA.[59]

Furthermore, we also report here for the first time the isolation of *Proteus* species encoding a $bla_{\text{TEM-92}}$ gene from a nasal swab of a cat with URTD. The TEM-92 ESBL was first described in two bacterial strains, *Proteus mirabilis mirabilis* and *Providencia stuartii*, isolated in 1998 from the urinary tracts of two human patients.[60] Since then, TEM-92 has been reported only in isolates from human patients;[61] where therapeutic failure and mortality may occur when associated with bloodstream infections.[62]

In our study, *Buttiauxella* species, *Serratia liquefaciens*, *Kluyvera* species and *Providencia* species were MDR. Although the above-mentioned bacteria have been rarely reported to cause clinical infections in companion animals, the detection of MDR isolates highlights that these rarely pathogenic bacteria may pose important therapeutic limitations when causing opportunistic infections.

All isolates from this study were susceptible to meropenem. Production of carbapenemases, which represents an additional hazard to public health and veterinary medicine, has so far remained a rare phenomenon among Gram-negative bacteria isolated from companion animals.[63–66] Nevertheless, based on the advancing spread of this type of resistance in human isolates, this hazard could arise in the future in companion animals.

Conclusions

The prevalence of Enterobacteriaceae carrying ESBL or pAmpC genes identified in this study was higher than that documented in previous reports in Italy, which indicates that ESBL- and pAmpC-producing Enterobacteriaceae might be widespread. The spread of these enzymes can be attributed to the transfer of plasmids and other mobile genetic elements between bacterial species. The findings indicate that diseased cats may be reservoirs of 3GC-resistant Enterobacteriaceae and/or act as shedders of bacteria resistant to critically important antimicrobials in humans.

The problem posed by emerging resistance, underlined in this study by the discovery of β-lactamase genes not previously described in companion animals, the prospect of losing important antimicrobial efficacy and the possibility of an already attested interspecies spread emphasise the urgent need to define the epidemiology of β-lactamase-producing bacteria in companion animals.[66–68] The emergence of ESBL-/pAmpC-producing MDR Enterobacteriaceae could pose major limitations in therapeutic options for companion animals. Awareness should be raised among companion animal general practitioners about the threat they may encounter in their daily clinical activities.

Furthermore, these results highlight once more the need for bacteriological examination and susceptibility testing before instituting empirical antimicrobial therapy if a bacterial infection is suspected, in order to establish accurate treatment and avoid the antimicrobial selection pressure on the animal microbiota. This could reduce costs by avoiding resorting to ineffective compounds, and play a role in the control and monitoring of antimicrobial resistance in companion animal medicine.

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Ethical approval This work involved the use of non-experimental animals only (owned or unowned), and followed established internationally recognised high standards ('best practice') of individual veterinary clinical patient care. Ethical approval from a committee was not necessarily required.

Informed consent Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work for the procedure(s) undertaken. No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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