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Extended-Spectrum β-Lactamases in *Escherichia coli* Isolated from Dogs and Cats in Rome, Italy, from 2001 to 2003

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We report expanded-spectrum cephalosporin resistance in *Escherichia coli* from dogs and cats in Rome, Italy. Three major β-lactamases (CMY-2, SHV-12, and CTX-M-1) are reported for the first time in *E. coli* from sick and healthy dogs and cats. Molecular characterization suggests the presence of several combinations of β-lactamase genes in *E. coli* from companion animals.

Escherichia coli is a common microorganism found in the intestinal flora of humans and animals, although pathogenic strains cause serious diseases, including urinary and wound infections and septicemia. While antimicrobial use in production animals has been shown to lead to the emergence of resistant bacteria throughout the food chain (5), little is known about the development of resistance in companion animals (9). The objective of this study was to assess the presence of expanded-spectrum cephalosporin resistance in *E. coli* recovered from dead, sick, and healthy dogs and cats living in kennels or with private owners.

Over a 3-year period (2001 to 2003), 298 *E. coli* isolates obtained from specimens from 204 dogs and 61 cats submitted for routine diagnostic investigation were collected at the Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy. Of a total 226 canine isolates, 144 were obtained from necropsies (86 from gut contents and 58 from infected organs), 33 were from diagnostic samples, and 49 were from fecal samples from healthy animals submitted for parasite screening. A total of 72 *E. coli* isolates of feline origin were obtained, 51 of which were from necropsy specimens (29 from gut contents and 22 from infected organs), while 6 and 15 isolates were from diagnostic samples and fecal samples from healthy animals, respectively. Two-thirds (67%) of the dogs investigated were from private owners, and the rest were from five different municipal facilities for unclaimed stray or lost dogs and from authorized private animal shelters. The cats tested belonged mainly to private owners (57%) and colonies of abandoned cats (38%) that are cared for by volunteers. An additional *E. coli* isolate was obtained from the gut of a brown rat (*Rattus norvegicus*) found dead in a kennel in which dogs had also been tested.

All strains were screened by antimicrobial susceptibility testing performed by the agar diffusion method with 16 different antimicrobial drugs. Sensitivity testing for ampicillin, amikacin, amoxicillin-clavulanic acid, cefotaxime, cephazolin, chloramphenicol, enrofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfonamides, tetracycline, and trimethoprim-sulfamethoxazole were interpreted in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (7, 8). For colistin, breakpoint diameters of 8 mm for resistance and 11 mm for sensitivity were used.

Twenty-one strains (7%) from healthy, dead, and diseased dogs and cats, and from the rat, showed resistance to cefotaxime (20 [6.7%] of 298) and/or cefoxitin (13 [4.4%] of 298). Twelve strains (4%) also showed resistance to β-lactamase inhibitors (amoxicillin-clavulanic acid), and all strains showed resistance to several different antimicrobials, including nalidixic acid (20.1%), enrofloxacin (15.1%), aminoglycosides (gentamicin [8.1%], kanamycin [15.4%], streptomycin [37.2%], and amikacin [0.7%]), trimethoprim-sulfamethoxazole (33.9%), chloramphenicol (18.1%), and tetracyclines (45.0%). Resistance to extended-spectrum cephalosporins was defined on the basis of conventional NCCLS breakpoints; thus, the number of expanded-spectrum β-lactamase producers might have been underestimated in this collection.

The characteristics of the 21 *E. coli* strains showing resistance to expanded-spectrum cephalosporins are shown in Table 1.

This wide spectrum of antimicrobial resistance, especially toward extended-spectrum cephalosporins, prompted further characterization of the isolates. To investigate the genetic relationship among the isolates, we analyzed the chromosomal patterns obtained by pulsed-field gel electrophoresis (PFGE) after digestion with the XbaI restriction enzyme. Twelve different PFGE profiles were obtained (PFGE patterns differing for more than three DNA fragments were classified as different profiles and are designated A to N in Table 1), demonstrating that there was not a unique resistant *E. coli* clone spreading among the animals (12). However, five strains, four of them isolated from dogs from the same kennel, show similar chromosomal patterns (pattern G in Table 1), differing by one or two bands, indicating the diffusion of this strain among animals living in kennel C.

E. coli strains were analyzed by PCR for the presence of the bla_{SHV}-, bla_{TEM}-, $bla_{CTX\text{-}M}$-, bla_{AmpC}-type genes with previously described primer pairs (bla_{SHV} and bla_{AmpC} gene primers in reference 6, CTX-MA and CTX-MB primers in refer-

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TABLE 1. Characteristics of *E. coli* isolates recovered from sick and healthy dogs and cats in Rome, Italy from 2001 to 2003

Isolate	Species	Source[a]	Origin	Resistance pattern[b]	PFGE profile[c]	bla_{SHV-12}	bla_{CMY-2}	$bla_{CTX-M-1}$	bla_{TEM}[d]
18196	Dog	Organs	Kennel A	AMP AMC CTX FOX KAN SUL SXT TET	A	−	+	−	−
16117	Dog	Feces	Kennel B	AMP AMC CHL CTX FOX GEN	B	−	+	−	−
20432	Dog	Organs	Kennel B	AMP AMC CHL CTX ENO FOX KAN NAL STR SUL SXT TET	C	−	−	+	+
14083	Dog	Organs	Kennel B	AMP AMC CHL CTX ENO FOX KAN NAL STR SUL SXT TET	ND	−	−	+	+
331	Dog	Feces	Kennel B	AMP AMC CHL CTX ENO FOX GEN KAN NAL STR SUL SXT TET	D	−	−	+	+
1092	Dog	Infection	Kennel B	AMP AMC CHL CTX FOX GEN KAN NAL SPT STR SUL SXT TET	E	−	−	+	+
31038	Rat	Gut contents	Kennel B	AMP AMC CTX FOX GEN STR SUL SXT TET	F	−	+	+	−
24623	Dog	Feces	Kennel B	AMP AMC CHL CTX ENO FOX NAL SUL SXT TET	G	−	−	+	+
1599B	Dog	Gut contents	Kennel C	AMP CTX ENO GEN KAN NAL STR SUL SXT TET	G	+	−	+	+
1599C	Dog	Organs	Kennel C	AMP CTX ENO GEN NAL SUL SXT TET	G	+	−	+	+
1599D	Dog	Organs	Kennel C	AMP CTX ENO GEN NAL STR SUL SXT TET	G	+	−	−	+
17795	Dog	Gut contents	Kennel C	AMP CHL CTX ENO NAL SUL SXT TET	G	−	−	+	+
1599A	Dog	Gut contents	Kennel C	AMP CHL CTX ENO NAL STR SUL SXT TET	H	−	−	+	+
1599E	Dog	Organs	Kennel C	AMP CHL CTX ENO NAL STR SUL SXT TET	H	−	−	+	+
322	Dog	Organs	Kennel D	AMP CTX STR SUL SXT TET	I	−	−	+	+
11361	Dog	Organs	Private owner	AMP CHL CTX KAN SPT STR SUL SXT TET	L	+	−	−	+
362	Dog	Feces	Private owner	AMP AMC CHL CTX ENO FOX KAN NAL SUL SXT TET	ND	−	−	+	+
17419	Cat	Organs	Private owner	AMP AMC CHL CTX ENO FOX KAN NAL STR SUL SXT TET	C	−	−	+	+
3050	Dog	Organs	Private owner	AMP AMC CTX ENO FOX NAL STR SUL SXT TET	M	−	−	+	+
34430	Cat	Organs	Private owner	AMP CTX ENO FOX NAL SUL SXT TET	M	−	−	+	−
8113	Cat	Organs	Private owner	AMP AMC CHL ENO FOX KAN NAL SPT STR SUL SXT TET	N	−	−	−	+

[a] Gut contents and organs are from necropsy specimens.
[b] AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CHL, chloramphenicol; CTX, cefotaxime; ENO, enrofloxacin; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; SPT, spectinomycin; STR, streptomycin; SXT, sulfometoxazole-trimethoprim; SUL, sulfonamides; TET, tetracycline;
[c] ND, not determined. PFGE patterns differing for more than three DNA fragments were classified as different profiles.
[d] bla_{TEM} genes were identified by PCR, although several amplicons were sequenced identifying bla_{TEM-1a} and bla_{TEM-1b} gene variants.

ence 2, and bla_{TEM} gene primers in reference 4). The amplicons obtained for the bla_{SHV}-, bla_{CTX-M}-, and bla_{AmpC}-type genes were sequenced, and a comparative analysis of the nucleotide sequences was performed with advanced BLAST search program 2.0 within the QBLAST system at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/BLAST/).

We found different combinations of β-lactamase genes in the *E. coli* strains in our collection (Table 1). Sixteen isolates were positive by PCR, and confirmed by DNA sequencing, for the $bla_{CTX-M-1}$ gene. *E. coli* isolates with the $bla_{CTX-M-1}$ gene also frequently possessed the TEM β-lactamase. *E. coli* producing plasmid-mediated CTX-M β-lactamase have been reported in cattle from Japan (11), but to our knowledge, *E. coli* isolates carrying the $bla_{CTX-M-1}$ gene have never been described from healthy or diseased companion animals.

Three epidemiologically and genetically unrelated strains (identification no. 18196, 16117, and 31038) were positive to a bla_{CMY}-like gene, and the DNA sequence of the amplicons revealed the presence of the bla_{CMY-2} gene (1). Two of the bla_{CMY-2}-positive strains were isolated from dogs coming from different kennels (A and B): one was from infected organs of a necropsied animal, while the other was from the feces of a healthy animal. Interestingly, the third bla_{CMY-2}-positive isolate, also showing the presence of the $bla_{CTX-M-1}$ gene, was from the rat found dead in kennel B, showing a PFGE profile different from that of the *E. coli* isolates from the dogs that were tested in the same facility, thus suggesting that diffusion of the bla_{CMY-2} gene may have occurred in this kennel. This is the first evidence of community-acquired *E. coli* isolates carrying genes encoding CMY-2 from pets, although bla_{CMY-2}-positive *E. coli* strains were previously reported to be associated with nosocomial infections in dogs (10).

A significant extended-spectrum β-lactamase was also found in animals coming from kennel C. In this case, *E. coli* strains showed only two PFGE profiles (patterns G and H) and three of the strains isolated from necropsy specimens (identification no. 1599B, 1599C, and 1599D), from gut contents and from diseased organs, were positive for the same bla_{SHV} amplicon that, after sequencing, was identified as the bla_{SHV-12} gene (6) (Table 1). A fourth isolate (identification no. 11361) from a private owner's necropsied dog, was also positive for the bla_{SHV-12} gene (Table 1). The SHV-12 β-lactamase has previously been described in clinical *E. coli* isolates from humans, healthy production animals, and a dog with recurrent urinary tract infections (3, 13). In our study, the isolation of SHV-12-positive *E. coli* strains from lesions of dead animals from the same municipal facility suggests a community-acquired infection, probably favored by the high animal density in the kennel. However, it is of concern that the same bla_{SHV-12} gene was also

found in the dog of a private owner, indicating the possible future appearance of this resistance gene in other companion animals.

In several strains, the observed phenotype of resistance to cefoxitin or amoxicillin-clavulanic acid cannot be completely explained by the identified β-lactamase genes, suggesting the presence of additional mechanisms of resistance in these strains, such as inhibitor-resistant bla_{TEM} or bla_{OXA-1} genes or overproduction of non-inhibitor-resistant bla_{TEM} TEM-type enzymes that need further investigation.

With respect to the possible origin of CMY-2, SHV-12, and CTX-M in pets, Italian companion animal practitioners admit to rather diffuse off-label use of expanded-spectrum cephalosporins registered for human use in pet therapy that began in the early 1990s, even earlier than in farm animal practice, where their administration is still limited to selected cases, for obvious economic reasons. The results of this study are of public health concern because nonjudicious use or misuse of highly valuable antimicrobial drugs can result in selective pressure on bacterial populations of companion animals. This may lead to the spread of pathogens carrying resistance to newer antimicrobials by vertical and horizontal transmission of genes, with the subsequent risk of transfer to humans.

In this respect, further population-based epidemiological surveys may provide valuable information about the diffusion of multiresistant *E. coli* in companion animals.

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