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First Detection of CTX-M and SHV Extended-Spectrum β-Lactamases in *Escherichia coli* Urinary Tract Isolates from Dogs and Cats in the United States[▽]

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One hundred fifty canine and feline *Escherichia coli* isolates associated with urinary tract infections were screened for the presence of extended-spectrum β-lactamase (ESBL) genes. Out of 60 isolates suspected to be ESBL positive based on antimicrobial susceptibility testing, 11 ESBLs were identified, including one SHV-12 gene, one CTX-M-14 gene, and nine CTX-M-15 genes. This study provides the first report of CTX-M- and SHV-type ESBLs in dogs and cats in the United States.

The first detection of an extended-spectrum β-lactamase (ESBL) in an organism from an animal was reported in Japan in 1988 in an *Escherichia coli* isolate from a laboratory dog (13). Since that time, numerous reports of ESBL-positive isolates from dogs and cats, as well as from other animal species (5), have been made worldwide (4, 8, 11, 17, 25, 26). Only one study has identified the presence of an ESBL in isolates from animals in the United States, i.e., in *Salmonella enterica* serovar Newport from horses (21). We hypothesized that ESBL genes would be present in urinary *E. coli* isolates from companion animals in the United States. The purpose of this study was to screen a group of 150 *E. coli* isolates from canine and feline patients that had been diagnosed with a urinary tract infection (UTI) for the presence of ESBL genes.

A convenience sample of 150 *E. coli* isolates collected from canine and feline patients at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania with clinical signs or evidence on routine urinalysis of a UTI between 1 September 2004 and 31 December 2007 was used in this study. Isolates were frozen in Microbank tubes (ProLab Diagnostics, Austin, TX) and stored at −70°C prior to use.

MICs were determined using a Negative Combo 31 panel on a MicroScan Walkaway 40 (Dade Behring, Siemens Healthcare Diagnostics, Deerfield, IL). Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) breakpoints (7). Isolates collected from the same individual animal within a 45-day period were considered to be the same strain, and only the first isolate collected was analyzed (subsequent isolates were considered redundant). All isolates with a cefpodoxime MIC of ≥4 μg/ml and a ceftazidime MIC of ≥1 μg/ml were identified as an "ESBL alert" on the MicroScan Walkaway. ESBL confirmatory testing was performed via the Etest method using ceftazidime–ceftazidime-clavulanic acid and cefotaxime–cefotaxime-clavulanic acid strips in accordance with CLSI guidelines (7).

Due to the high prevalence of cefoxitin resistance in this population, PCR was performed to detect the presence of a bla_{AmpC} gene, the product of which can mask the effects of clavulanic acid on the ESBL confirmatory test (18, 27). Since the primers used in this study to identify the bla_{AmpC} gene (18) have since been shown to amplify the plasmid-mediated bla_{CMY} gene from *Citrobacter freundii* (19), it can be inferred that the genes detected were part of the bla_{CMY} lineage. *Salmonella* Newport strain 0007-33 was used as the positive control (21). PCR was performed for the genes bla_{TEM}, bla_{SHV}, and $bla_{\mathrm{CTX\text{-}M}}$ as published previously (9, 10, 27). *Salmonella* Newport strain 0007-33 was also used as the TEM and SHV positive control (21), and *E. coli* strain MISC 336 (CTX-M-1 positive) was used as the CTX-M positive control.

The bla_{TEM}, bla_{SHV}, and $bla_{\mathrm{CTX\text{-}M}}$ PCR products were sequenced using both strands of DNA for each PCR product. Protein sequences were aligned using Lasergene software (DNASTAR, Inc., Madison, WI) and included GenBank sequences (http://www.ncbi.nlm.nih.gov/GenBank/index.html) to confirm ESBL genotype. Mutations were evaluated with reference to the Lahey Clinic website (http://www.lahey.org/studies/). GenBank accession numbers used for alignment of protein sequences were AAR25033 for TEM-1 and ABF29674 for SHV-2. CTX-M accession numbers were derived from a list on the Lahey Clinic website. Specific primers for the CTX-M-1 group (M13U and M13L) were used to amplify the entire coding sequences of these $bla_{\mathrm{CTX\text{-}M}}$ genes (23). Sequencing and analysis were carried out as described above to identify the specific CTX-M subtype.

Seventy of the 150 *E. coli* isolates had an "ESBL alert" on the MicroScan Walkaway, and after removal of redundant isolates, 60 isolates were tested further. ESBL confirmatory testing was positive for six of these 60 isolates (Table 1, column 3).

Fifty-three of the 60 isolates were positive for the bla_{CMY} gene (43 canine and 10 feline samples) (Table 1, column 4). Of the seven negative isolates, six were those previously found to be positive for ESBL production via ESBL testing. The re-

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TABLE 1. Distribution of β-lactamase and extended-spectrum β-lactamase genes

Isolate	Species	ESBL test	bla_{CMY}	bla_{SHV}	bla_{TEM}	bla_{CTX-M}
1	Canine	+	−	SHV-12	TEM-1	−
3	Canine	−	+	−	−	−
6	Canine	−	+	−	TEM-1	−
11	Canine	−	+	−	TEM-1	−
17	Canine	−	+	−	TEM-1	−
19	Canine	−	+	−	−	−
21	Feline	+	−	−	−	CTX-M-15
26	Canine	−	+	−	TEM-1	−
27	Canine	−	+	−	TEM-1	−
31	Canine	−	+	−	TEM-1	−
32	Canine	−	+	−	TEM-1	−
33	Canine	−	+	−	−	−
41	Canine	−	+	−	TEM-1	−
42	Canine	−	+	−	−	−
44	Canine	−	+	−	TEM-1	−
53	Canine	−	+	−	−	−
57	Feline	−	+	−	−	−
62	Canine	−	+	−	TEM-1	−
67	Canine	−	+	−	−	−
74	Canine	−	+	−	TEM-1	−
75	Feline	+	−	−	TEM-1	CTX-M-15
82	Feline	−	+	−	TEM-1	−
85	Canine	−	+	−	TEM-1	−
86	Canine	−	+	−	TEM-1	−
87	Canine	−	+	−	−	−
88	Feline	−	+	−	−	−
91	Canine	−	+	−	−	−
98	Canine	−	+	−	TEM-1	−
102	Canine	−	+	−	−	−
104	Canine	−	+	−	−	−
112	Canine	−	+	−	TEM-1	−
119	Canine	−	+	−	−	−
131	Canine	−	−	Not done	Not done	Not done
133	Feline	−	+	−	−	−
138	Feline	−	+	−	TEM-1	−
147	Canine	−	+	−	TEM-1	−
149	Feline	−	+	−	TEM-1	−
157	Canine	+	−	−	−	CTX-M-15
165	Canine	−	+	−	TEM-1	−
166	Canine	−	+	−	TEM-1	−
168	Canine	−	+	−	−	−
182	Canine	−	+	−	−	−
183	Feline	−	+	−	TEM-1	−
190	Feline	−	+	−	TEM-1	−
205	Canine	−	+	−	TEM-1	−
209	Canine	−	+	−	TEM-1	−
210	Canine	−	+	−	−	−
219	Feline	−	+	−	TEM-1	CTX-M-15
220	Canine	+	−	−	TEM-1	CTX-M-15
230	Canine	−	+	−	TEM-1	−
234	Canine	−	+	−	TEM-1	−
236	Canine	+	−	−	TEM-1	CTX-M-14
240	Canine	−	+	−	TEM-1	−
242	Canine	−	+	−	TEM-1	CTX-M-15
246	Feline	−	+	−	−	CTX-M-15
265	Canine	−	+	−	TEM-1	CTX-M-15
266	Canine	−	+	−	TEM-1	−
267	Canine	−	+	−	−	CTX-M-15
268	Canine	−	+	−	−	−
269	Canine	−	+	−	−	−

maining canine isolate (isolate 131, negative for both bla_{CMY} PCR and ESBL testing) was not analyzed further.

A total of 40 *E. coli* isolates were found to carry one or more β-lactamase genes. PCR detected a bla_{SHV} gene in one canine isolate, a bla_{TEM} gene in 29 canine and seven feline isolates, and a $bla_{CTX\text{-}M}$ gene in six canine and four feline isolates. The remaining 19 isolates were confirmed as negative for bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes (Table 1, columns 5 to 7).

Based on sequence analysis (Table 1, column 5), the one SHV-positive strain (isolate 1) was determined to carry SHV-12. All 36 strains positive for a TEM gene were identified as carrying TEM-1 (Table 1, column 6). Nine of 10 strains positive for a CTX-M gene carried genes of the CTX-M-1 group. The final strain (isolate 236) was concluded to carry CTX-M-14, given the presence of mutations in the amplified consensus sequence unique to CTX-M-14. DNA from each of the nine CTX-M-1 group strains was amplified using the CTX-M-1 group-specific primer set (23). Sequence analysis identified all nine strains as carrying CTX-M-15 (Table 1, column 7).

The CTX-M-type ESBLs identified in this study provide evidence for the dissemination of these genes in the United States. The CTX-M-1 group has frequently been reported in animals in countries other than the United States (2, 4, 8, 11, 17). The CTX-M-15 gene has not been identified in any bacterial isolate from animals in the United States. Animal sources of this gene have been identified only in *E. coli* isolated from the cloacae of Belgian poultry and an *E. coli* isolate from the urine of a cow in France (14, 24). The presence of CTX-M-14 genes in the *Enterobacteriaceae* has also been documented across the globe (22), including in six *E. coli* strains isolated from the feces of dogs in Chile (17). The CTX-M-14 gene identified in this study is the first identified from an animal in the United States and the first linked to a clinical case of UTI in a dog. The SHV-12 gene has been detected in bacterial isolates from animals in several countries (1, 4, 6, 26), including, in 2005, a *Salmonella enterica* serovar Newport strain from a horse in the United States (21).

Interestingly, of the 11 ESBL-positive isolates identified by sequence analysis, only six were positive by ESBL confirmatory testing, likely due to the concurrent presence of a bla_{CMY} gene in the other five isolates. The product of this gene is known to mask the effects of clavulanic acid on the ESBL confirmatory test (28). By relying on ESBL confirmatory testing alone, it is likely that the prevalence of ESBLs is being underestimated, particularly in populations with a high frequency of bla_{AmpC}, such as in the current study. During the study period, *E. coli* was isolated from samples submitted to our laboratory from 1,318 individual animals, and of these, 257 met the criteria for ESBL confirmatory testing. Of the 257 isolates tested, 14 (5%) were identified as being ESBL producers based on the Etest method, including the six reported in this study. The overall prevalence of *E. coli* isolates that were positive for an ESBL during this period was 1% (S. Rankin, unpublished observations). It is possible that this is an underestimation, based on the high frequency of bla_{CMY} detected in this study.

Though, historically, the most common ESBLs in the United States have been TEM and SHV types (16, 20), more recent studies have identified CTX-M genes, first reported in 2003 (15). CTX-M ESBLs now predominate in some U.S. health care systems (12). The findings from the current study are in agreement with current trends in the United States and other parts of the world (3). This study is the first report of *E. coli* strains that encode SHV-12, CTX-M-15, and CTX-M-14 ESBL genes in companion animals in the United States.

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