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## Major article

# Occurrence of extended spectrum $\beta$ -lactamase-producing *Enterobacteriaceae* among pet dogs and cats: An emerging public health threat outside health care facilities

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## Key Words:

ES $\beta$ L  
Pets  
Hazard

We aimed to investigate the potential role of pet dogs and cats in the epidemiology of extended spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae*. Twenty bacterial isolates were recovered from rectal swabs obtained from 110 dogs and cats. The occurrence of extended spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae* in pets spotlights the emergence of a significant public health threat.

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Extended spectrum  $\beta$ -lactamases (ES $\beta$ LS) are plasmid-mediated enzymes that have the ability to hydrolyze  $\beta$ -lactams and thus inactivate a wide range of antibiotics.<sup>1</sup> During the 1980s, ES $\beta$ L-producing members of the *Enterobacteriaceae* family of bacteria were first introduced to the scientific community when ES $\beta$ L-producing *Klebsiella pneumoniae* emerged as the cause of dangerous nosocomial infections.<sup>2,3</sup> Since then, ES $\beta$ L-producing members of the *Enterobacteriaceae* family have become the focus of many clinicians, epidemiologists, and microbiologists. As time advances, these pathogens are becoming a critical worry because they constitute a great challenge in human medicine and public health and a major concern associated with nosocomial infections.<sup>4</sup> Meanwhile, ES $\beta$ L-producing members of the *Enterobacteriaceae* family have gone beyond the hospital boundaries and many cases of community-acquired ES $\beta$ L infections have been recorded worldwide.<sup>5</sup> The spread of infections caused by community-acquired ES $\beta$ L-producing members of the *Enterobacteriaceae* family convey a great clinical significance, especially among *Escherichia coli* and *K pneumoniae* strains, because these may be accompanied by severe clinical outcomes, including urinary tract infections, bacteremias, splenic abscesses, and sepsis.<sup>6-9</sup> Despite the great influence of community-acquired infections caused by ES $\beta$ L producers in human medicine, the epidemiology of such pathogens is largely unknown, as is the gate through which these organisms go outside hospitals to invade the community.<sup>10,11</sup> Recently, ES $\beta$ L pathogens were detected in food

animals, including pigs, cattle, and sheep, as well as chickens, which highlights the possible role of such animals in the epidemiology of these pathogens.<sup>12</sup> Therefore, our study was conducted to investigate the role of pet dogs and cats in the epidemiology of ES $\beta$ L-producing members of the *Enterobacteriaceae* family of bacteria and their public health implications.

## MATERIALS AND METHODS

### Specimens

Sixty-eight and 42 rectal swabs were obtained from dogs and cats, respectively. All swabs were selected from animals without any history of hospitalization during their visits to different private veterinary clinics in Cairo, Egypt, as well as the university hospital of the Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.

### Culture and identification of *Enterobacteriaceae*

Swabs were cultured on MacConkey agar plates (BD, Franklin Lakes, NJ) and incubated at 37°C for 24 hours. Colonies were identified as lactose-fermenting and nonlactose-fermenting colonies. Suspected colonies were then inoculated into Kligler iron agar (BD, Franklin Lakes, NJ), lysine iron agar (BD, Franklin Lakes, NJ), motility indole ornithine agar (BD, Franklin Lakes, NJ), citrate agar (BD, Franklin Lakes, NJ), and urea broth (BD, Franklin Lakes, NJ). API 20E (BioMerieux SA, Marcy l'Etoile, France) strips were used for confirmation of isolates.

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Conflicts of interest: None to report.

**Table 1**Occurrence of extended spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae* among pet dogs and cats

Animal	Positive	%
Dogs		
Puppies	6/27	22.2
Adult	9/41	21.9
Total	15/68	22
Cats		
Kittens	1/18	5.6
Adult	4/24	16.7
Total	5/42	11.9

### Initial screening of ES $\beta$ L by disk diffusion test

Disk diffusion testing<sup>13</sup> was applied to detect ES $\beta$ L-producing isolates. Mueller Hinton agar (BD, Franklin Lakes, NJ) was swabbed by a suspension of a pure culture and antibiotic disks were then loaded. Cefpodoxime (10  $\mu$ g), ceftazidime (30  $\mu$ g), aztreonam (30  $\mu$ g), cefotaxime (30  $\mu$ g), and ceftriaxone (30  $\mu$ g) were used for screening of ES $\beta$ L-producing *E coli* and *K pneumoniae*, whereas cefpodoxime (10  $\mu$ g), ceftazidime (30  $\mu$ g), and cefotaxime (30  $\mu$ g) were used for screening of ES $\beta$ L-producing *Proteus mirabilis*. Interpretation relied on the instructions of the Clinical and Laboratory Standards Institute.<sup>13</sup>

### Confirmation test for ES $\beta$ L

Confirmatory testing<sup>13</sup> was applied by using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (cefotaxime 30  $\mu$ g, cefotaxime/clavulanic acid 30/10  $\mu$ g) and (ceftazidime 30  $\mu$ g, ceftazidime/clavulanic acid 30/10  $\mu$ g). A  $\geq 5$  mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus the zone diameter of the agent when tested alone confirmed the presence of an ES $\beta$ L-producing organism.

### Genotyping of ES $\beta$ L isolates and detection of bla<sub>CTX</sub> and bla<sub>TEM</sub> genes

DNA was extracted from all obtained isolates by using QIAamp DNA mini kit (Qiagen, Hilden, Germany) and the test was carried out according to the manufacturer's instructions.

### Detection of bla<sub>CTX</sub> and bla<sub>TEM</sub> genes in all Enterobacteriaceae isolates

The bla<sub>CTX</sub> gene was amplified by using the following primer pairs: (F) 5'ATG TGC AGY ACC AGT AAR GTK ATG GC 3' and (R) 5' TGG GTR AAR TAR GTS ACC AGA AYC AGC GG 3'.<sup>14</sup> The polymerase chain reaction (PCR) amplification reaction was conducted according to the following thermal profile: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 45°C, and 45 seconds at 72°C, followed by an extension at 72°C for 10 minutes. PCR products were visualized at 593 bp. The bla<sub>TEM</sub> gene was amplified by using the following primer pairs: (F) 5' ATG AGT ATT CAA CAT TTC CG 3' and (R) 5' ACC AAT GCT TAA TCA GTG AG 3'. The PCR amplification reaction was conducted as follows: initial denaturation at 94°C for 3 minutes, followed by 25 cycles of 1 minute at 94°C, 1 minute at 50°C, and 1 minute at 72°C, followed by an extension at 72°C for 10 minutes. PCR products were visualized at 964 bp.

## RESULTS

The overall occurrence of ES $\beta$ L-producing *Enterobacteriaceae* in the examined animal species was 18.2%, whereas that for dogs and

**Table 2**Occurrence of extended spectrum  $\beta$ -lactamase (ES $\beta$ L)-producing *Enterobacteriaceae* among apparently healthy pets and pets with diarrhea

Health condition of pet	Positive ES $\beta$ L/total	%
Diarrhea	7/49	14.2
Apparently healthy	13/61	21.3
Total	20/110	18.2

**Table 3**Occurrence and genotyping of the different members of extended  $\beta$ -lactamase (ES $\beta$ L)-producing *Enterobacteriaceae* in pet dogs and cats

Bacteria species	Dogs (n = 68)		Cats (n = 42)		Genotyping of ES $\beta$ L isolates	
	Positive	%	Positive	%	bla <sub>CTX</sub>	bla <sub>TEM</sub>
<i>Klebsiella pneumoniae</i>	3	4.4	0	0	3	0
<i>Proteus mirabilis</i>	1	1.5	0	0	1	0
<i>Escherichia coli</i>	11	16.2	5	11.9	9	7
Total	15	22	5	11.9	13	7

cats was 22% and 11.9%, respectively (Table 1). Moreover, apparently healthy animals revealed higher rates of ES $\beta$ L (21.3%) than animals with diarrhea (14.2%) (Table 2). The isolated members of the *Enterobacteriaceae* family identified as ES $\beta$ L-producing organisms were *K pneumoniae*, *P mirabilis*, and *E coli* in the following rates: 2.7%, 0.9%, and 14.5%, respectively (Table 3). All isolates were positive for genes responsible for ES $\beta$ L, either bla<sub>CTX</sub> or bla<sub>TEM</sub> (Table 3).

## DISCUSSION

Dogs and cats as pet animals are companions that share human life in varying ways. They may share houses and workplaces with their humans and unfortunately this becomes a potential zoonotic pathway for disease transmission. During the past few years, pet dogs and cats have been found to be infected with some emerging serious human pathogens such as methicillin-resistant *Staphylococcus aureus*, a matter that has a great effect on public health.<sup>15</sup> The results of our study revealed the emergence of ES $\beta$ L-producing *Enterobacteriaceae* among the examined pets (18.2%). Although dogs showed higher occurrence than cats, ES $\beta$ L-producing *Enterobacteriaceae* were isolated from different ages (kittens, puppies, adults). Moreover, both apparently healthy animals and animals with diarrhea showed positive results. This may lead us to conclude that ES $\beta$ L-producing *Enterobacteriaceae* found its way toward pet animals and was transmitted easily among them. Notably, different members of ES $\beta$ L-producing *Enterobacteriaceae* were isolated from dogs (ie, *K pneumoniae*, *P mirabilis*, and *E coli*), whereas the only species isolated from cats was *E coli*. The high rates of ES $\beta$ L-producing *Enterobacteriaceae* among dogs and the wide variety of dangerous species point out the potential role of dogs in the epidemiology of community-acquired ES $\beta$ L bacterial infections with special reference to ES $\beta$ L-producing *K pneumoniae* and *E coli*, which are the main causes of many cases of hospital- and community-acquired ES $\beta$ L bacterial infections in humans.<sup>16</sup> Interestingly, a study carried out in 2013<sup>17</sup> showed a high prevalence of ES $\beta$ L-producing *E coli* among human patients with gastroenteritis in the Netherlands. This study underlines the risk of transmission of such infections through the fecal-oral route, which may easily happen after handling infected pets or using contaminated items within households.

## CONCLUSIONS

Our study provides novel information about the epidemiology of ES $\beta$ L-producing *Enterobacteriaceae*, a subject that is largely

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