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Major article

Occurrence of extended spectrum β—lactamase-producing *Enterobacteriaceae* among pet dogs and cats: An emerging public health threat outside health care facilities

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

Extended spectrum β-lactamases (ESβLs) are plasmid-mediated enzymes that have the ability to hydrolyze β-lactams and thus inactivate a wide range of antibiotics.[1] During the 1980s, ESβL-producing members of the *Enterobacteriaceae* family of bacteria were first introduced to the scientific community when ESβL-producing *Klebsiella pneumoniae* emerged as the cause of dangerous nosocomial infections.[2,3] Since then, ESβL-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.[4] Meanwhile, ESβL-producing members of the *Enterobacteriaceae* family have gone beyond the hospital boundaries and many cases of community-acquired ESβL infections have been recorded worldwide.[5] The spread of infections caused by community-acquired ESβL-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.[6-9] Despite the great influence of community-acquired infections caused by ESβL 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.[10,11] Recently, ESβL 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.[12] Therefore, our study was conducted to investigate the role of pet dogs and cats in the epidemiology of ESβL-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.

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Table 1
Occurrence of extended spectrum β—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 ESβL by disk diffusion test

Disk diffusion testing[13] was applied to detect ESβL-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 μg), ceftazidime (30 μg), aztreonam (30 μg), cefotaxime (30 μg), and ceftriaxone (30 μg) were used for screening of ESβL-producing *E coli* and *K pneumoniae*, whereas cefpodoxime (10 μg), ceftazidime (30 μg), and cefotaxime (30 μg) were used for screening of ESβL-producing *Proteus mirabilis*. Interpretation relied on the instructions of the Clinical and Laboratory Standards Institute.[13]

Confirmation test for ESβL

Confirmatory testing[13] was applied by using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (cefotaxime 30 μg, cefotaxime/clavulanic acid 30/10 μg) and (ceftazidime 30 μg, ceftazidime/clavulanic acid 30/10 μg). A ≥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 ESβL-producing organism.

Genotyping of ESβL isolates and detection of bla_{CTx} *and* bla_{TEM} *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_{CTx} *and* bla_{TEM} *genes in all* Enterobacteriaceae *isolates*

The bla_{CTx} 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′.[14] 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_{TEM} 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 ESβL-producing *Enterobacteriaceae* in the examined animal species was 18.2%, whereas that for dogs and cats was 22% and 11.9%, respectively (Table 1). Moreover, apparently healthy animals revealed higher rates of ESβL (21.3%) than animals with diarrhea (14.2%) (Table 2). The isolated members of the *Enterobacteriaceae* family identified as ESβL-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 ESβL, either bla_{CTx} or bla_{TEM} (Table 3).

Table 2
Occurrence of extended spectrum β—lactamase (ESβL)-producing *Enterobacteriaceae* among apparently healthy pets and pets with diarrhea

Health condition of pet	Positive ESβL/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 β—lactamase (ESβL)-producing *Enterobacteriaceae* in pet dogs and cats

	Dogs (n = 68)		Cats (n = 42)		Genotyping of ESβL isolates	
Bacteria species	Positive	%	Positive	%	bla_{CTx}	bla_{TEM}
Klebsiella pneumoniae	3	4.4	0	0	3	0
Proteus mirabilis	1	1.5	0	0	1	0
Escherichia coli	11	16.2	5	11.9	9	7
Total	15	22	5	11.9	13	7

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.[15] The results of our study revealed the emergence of ESβL-producing *Enterobacteriaceae* among the examined pets (18.2%). Although dogs showed higher occurrence than cats, ESβL-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 ESβL-producing *Enterobacteriaceae* found its way toward pet animals and was transmitted easily among them. Notably, different members of ESβL-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 ESβL-producing *Enterobacteriaceae* among dogs and the wide variety of dangerous species point out the potential role of dogs in the epidemiology of community-acquired ESβL bacterial infections with special reference to ESβL-producing *K pneumoniae* and *E coli*, which are the main causes of many cases of hospital- and community-acquired ESβL bacterial infections in humans.[16] Interestingly, a study carried out in 2013[17] showed a high prevalence of ESβL-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 ESβL-producing *Enterobacteriaceae*, a subject that is largely