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Major article

Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in healthy companion animals living in nursing homes and in the community

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Background: Animals could be reservoirs of extended-spectrum β-lactamases (ESBL) strains, but epidemiologic data on ESBL-producing bacteria in healthy pets are missing. We determined the prevalence of ESBL-producing *Enterobacteriaceae* in pets living in nursing homes and in households to investigate the potential role of companion animals as carriers of ESBL.

Methods: Three hundred seventy-six rectal swabs were taken from cats and dogs visiting or living in 68 randomly selected nursing homes or brought to 26 veterinary practices in Switzerland for routine mandatory vaccination. Isolates were identified by matrix-assisted laser desorption ionization time of flight mass spectrometry. Confirmatory tests were performed on the isolated *Enterobacteriaceae*. Phenotypic ESBL isolates were investigated for genetic determinants of resistance.

Results: The overall prevalence of ESBL isolates, adjusted for clustering, was 2.5% (95% confidence interval: 1.3-4.6). Pets that received an antibiotic treatment in the 3 months prior to the study had a higher risk to be carriers of these microorganisms (Adjusted odds ratio, 7.8; 95% confidence interval: 2.2-26.9).

Conclusion: ESBL-producing *Enterobacteriaceae* were present in healthy cats and dogs, particularly from those with a history of antibiotic treatment. These animals could become ESBL reservoirs. Investigations are needed to assess the possible transmission of these microorganisms between pets and humans.

The introduction, in the early 1980s, of third-generation cephalosporins was followed rapidly by the emergence of extended-spectrum-β-lactamases (ESBL) production in bacteria belonging to the *Enterobacteriaceae*.[1] ESBL-producing bacteria are mainly resistant to β-lactam antibiotics (eg, penicillins and first-, second-, third-, and, occasionally, also fourth-generation cephalosporins) but also to other classes of antibiotics such as aminoglycosides and tetracyclines.[2] Resistance genes in ESBL-producing bacteria are mainly located on plasmids.[3] The presence of ESBL determinants on these genetic elements, which can be easily exchanged among isolates of the same or different bacterial species, can enhance the spread of resistance.

ESBL-producing bacteria were first identified as nosocomial pathogens of humans, with up to 30% of nosocomial *Klebsiella pneumoniae* isolates presenting this phenotypic resistance profile.[4] Recently, ESBL emerged also in pathogens in the community as a cause of urinary tract infections,[5] and, presently, ESBL-producing strains have a worldwide distribution. Focal outbreaks are not uncommon, and a coordinated approach to control ESBL spread is required.[6] It has been suggested that *Escherichia coli* producing CTX-M β-lactamases are true "community ESBL-producers," and these strains have been most probably imported into the hospital setting.[7] The number of new ESBL types continues to grow. For instance, a *K pneumoniae* carrying the new gene *bla*NDM-1 (New Delhi Metallo-β-lactamase), which confers resistance to carbapenems, was reported for the first time in 2009.[8]

Recent studies have shown that ESBL-producing *E coli* are present in farm animals (eg, poultry, pigs, rabbits, cows)[9,10] and wild animals (eg, birds of prey).[11] An increasing proportion of ESBL has been reported in *Enterobacteriaceae* isolated from companion animals,[12] but only little is known on the presence of these bacteria in healthy cats and dogs in the community or in nursing homes[13] and even less on the potential role of pets as ESBL carriers. To date, the highest rate of ESBL-producing *E coli* was reported in an

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observational study in healthy dogs (7.8%) and healthy cats (12.1%) in Portugal.[14] No epidemiologic study, however, has assessed the prevalence in pets of ESBL-producing *Enterobacteriaceae* other than *E coli*.

Reports on the occurrence of probable exchange of antibiotic-resistant microorganisms between human and animals have raised questions on the role of pets as reservoir of multiresistant bacteria.[15,16] Companion animals may represent potential sources of spread of antimicrobial resistance, also owing to the extensive use of antimicrobial agents in veterinary practices dealing with small animals[13,17] and their close contact with humans. The direct contact among cats, dogs, and their owners in European households and nursing homes has intensified in the last decades.[18,19] The high morbidity rate and the frequent use of antibiotics in nursing homes could favor the development of resistant strains in nursing homes more than in the community, for example in pets used to assist therapy. This study aimed at determining the prevalence of colonization by ESBL-producing *Enterobacteriaceae* in pets in nursing homes and in the community in Switzerland and at determining risk factors of colonization.

METHODS

Study design and setting

We carried out a cross-sectional study in nursing homes and in the community of 4 different Swiss cantons (Berne, Ticino, Vaud, and Zurich) using a 2-stage random cluster sampling. We investigated all cats and dogs that lived permanently in each nursing home as well as pets that visited the homes for pet-assisted therapy, whereas the community sample included healthy cats and dogs that were present in randomly selected veterinary practices on the day of sampling for routine vaccinations. Exclusion criteria for all pets were any urinary tract infection, intestinal disease, or participation in a clinical trial. Pets attending a veterinary practice, but spending at least 2 hours per day in a nursing home or being known as *active pet-therapy animals*, were not included in the community sample. Pet owners had to give their informed consent before pets could be included in the study. The study received ethical clearance from the corresponding ethic committees and authorization for animal experimentation from the Cantonal and Federal Veterinary Offices.

Sample size was computed considering clustering of pets within nursing homes and veterinary practices.[20] The intraclass correlation coefficient was estimated to be 0.2 in nursing homes and 0.15 in veterinary practices. Based on the results of a pilot study, we estimated the prevalence of pets carrying at least 1 ESBL strain to be 3.5%. To estimate the prevalence with a precision of $\pm$3.5%, 124 pets from 62 different nursing homes and 250 pets from 25 veterinary practices were to be included in the study. Each pet owner had to complete a questionnaire on demographic information, health status, and previous antibiotic treatments of its animals.

Sample collection and isolation

Sample collection was carried out between March and August 2010. Cotton swabs (Amies agar gel 114C and 116C; Copan, Italy) were introduced for few seconds 1 to 2 cm in the rectum of the animals, conserved in the transport medium at room temperature, and analyzed for the presence of ESBL bacteria within 24 hours of collection. Swabs were plated on chromID ESBL agar medium (Reference No. 43481; bioMérieux SA, Craponne, France), incubated under aerobic conditions during 48 hours at 37°C and enriched contemporaneously in trypticase soy broth during 24 hours at 37°C to increase the likelihood of detecting low bacterial numbers on the chromID.[21] The enriched samples were subsequently inoculated on chromID ESBL agar medium and incubated under aerobic conditions for 48 hours at 37°C.

All morphologically different colonies isolated using both direct and enrichment methods were transferred onto blood agar and incubated for 24 hours at 37°C under aerobic conditions. Identification was carried out using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) on an Axima Confidence spectrometer (Shimadzu-Biotech Corp, Kyoto, Japan) in positive linear mode (m/z = 2,000 to 20,000) as previously described.[22] Bacterial colonies were then frozen and stored at −80°C in 7% skimmed milk until further analysis.

Enterobacteriaceae isolates were considered to produce ESBL if they were positive in the double disc diffusion test carried out with discs of cefpodoxime (10 μg) and a combination of cefpodoxime and clavulanic acid (10 μg + 1 μg) (Reference numbers 2363212 and 2380912: Labolife, Pully, Switzerland).[23] A difference of $\geq$5 mm in the diameters of the inhibition zones surrounding the 2 discs confirmed the ESBL character of the strain. If the difference was <5 mm and the sensitivity to cefpodoxime reduced, the presence of ESBL with β-lactamase *AmpC* could not be excluded and a confirmatory test on cloxacillin agar (Rosco Diagnostica Neo-Sensitabs, Taastrup, Denmark) was carried out.

Susceptibility testing

We assessed phenotypic antibiotic resistance against ampicillin, amoxicillin, clavulanic acid, piperacillin-tazobactam, cefazolin, cefuroxime, ceftriaxone, ceftazidime, cefpodoxime, imipenem, ertapenem, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin, all currently used in clinical settings to handle gram-negative bacteria, by the Kirby-Bauer method. Strains were classified as susceptible, intermediately resistant, or resistant to the drug according to Clinical and Laboratory Standards Institute guidelines.[24] We considered isolates from the same animal as being different strains if they belonged to different species or had different phenotypic antibiotic resistance profiles.

Molecular characterization of isolates

We extracted genomic DNA using the InstaGene kit (Bio-Rad, Cressier, Switzerland; catalog No. 732-6030). Primers used for the amplification of the different resistance genes ($bla_{\text{CTx-M}}$, bla_{OxA}, bla_{SHV}, and bla_{TEM}) as well as polymerase chain reaction (PCR) conditions have been previously described.[25-27]

Statistical analysis

Age was categorized according to the quartiles (for dogs: <3, $\geq$3-7, $\geq$7-10, and $\geq$10 years; and for cats: <3.4, $\geq$3.4-7.5; $\geq$7.5-11; $\geq$11 years). Animals were considered to be positive if they carried at least 1 ESBL-producing or cefpodoxime-resistant strain. Univariable generalized estimating equation models with a logit link function and a random error on the institution identification (for both nursing homes and veterinary clinics) were used to test associations between explanatory variables and the binary outcomes ESBL-producing and cefpodoxime-resistant *Enterobacteriaceae* carriage in pets. We computed crude odds ratios (OR) and their 95% confidence intervals (95% CI). In addition, adjusted ORs (AOR) were calculated with the same model but adjusting for sex, age category, and species (cat or dog). The prevalence was calculated with the generalized estimating equation model as well, considering clustering within the institutions, which increases the confidence interval. All statistical analyses were performed with STATA 10.1 (Stata Corporation, College Station, TX).