A SURVEY OF CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN COMPANION DOGS IN MADRID, SPAIN

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Abstract

We found a low prevalence (0.6%) of carbapenemase-producing Enterobacteriaceae (CPE) in fecal microbiota of companion dogs. A single VIM-1-producing *K. pneumoniae* isolate belonging to the ST2090 was detected. *bla*\textsubscript{VIM-1} was carried on a class 1 integron and an untypable ~ 48 kb plasmid. Emergence and spread of CPE in this group of animals may represent a threat to public health in human and veterinary medicine. This finding supports the need of active surveillance studies in companion animals that live close to humans as interspecies transmission may occur within the same household.

Text

The emergence and worldwide spread of carbapenemase-producing Enterobacteriaceae (CPE) have significantly increased in recent years mainly in *K. pneumoniae* (1-3). Most carbapenemases are encoded on mobile genetic elements (4) which usually contain other antimicrobial resistance genes. CPE and other carbapenemase-producing bacteria, which seemed limited to humans, have also been described in animals, particularly in livestock. The detection of *Pseudomonas aeruginosa* with VIM-2, and *Acinetobacter baumannii* with OXA-23 and OXA-58 in livestock animals from Lebanon (5) was recently reported. Several VIM-1 carbapenemase producing *E. coli* and *Salmonella* isolates were recovered from pigs in Germany (6, 7), *Acinetobacter spp* harboring OXA-23 was isolated from cattle in France (8), and *A. baumannii* and *Acinetobacter lwoffii* isolates producing NDM-1 were detected from porcine and chicken sources in China (9). In addition, NDM-1 producing *Salmonella enterica* subsp. *enterica* serovar Corvallis was reported in Germany from a...
wild bird (10). Only exceptionally CPE have been found in pets, specifically in three studies which detected OXA-48 producing *E. coli* and *K. pneumoniae* from dogs in Germany (11), NDM-1 producing *E. coli* from dogs and cats in the U.S.A (12), and OXA-23 producing *Acinetobacter* spp. from horses in Belgium (13).

Since there is very little information about CPE in companion animals, here we describe a study aimed to assess the prevalence of CPE in rectal swabs from companion dogs.

Between October 2014 and January 2015, 160 rectal swabs from different dogs who attended a veterinary hospital in Fuenlabrada (Madrid, Spain) were collected without any inclusion or exclusion criteria by inserting a swab into the rectum while rotating it. The swab was then transported to the laboratory in Amies medium. At the time of swab collection 54% of the animals were under antibiotic treatment. The drugs used as monotherapy were in order of frequency amoxicillin-clavulanic acid (18.6%), cephalexin (15.1%), metronidazole (9.3%), marbofloxacin (8.1%), sulfadoxine-trimethoprim (SxT) (7%), ampicillin (4.7%), amoxicillin (3.5%), cefazolin (1.2%), nitrofurantoin (1.2%) and azithromycin (1.2%). Antibiotic combinations included metronidazole + cephalexin (7%), amoxicillin-clavulanic acid + marbofloxacin (5.8%), cephalexin + marbofloxacin (3.5%), metronidazole + marbofloxacin (3.5%), metronidazole + marbofloxacin + cephalexin (3.5%), metronidazole + SxT (2.3%), amoxicillin-clavulanic acid + metronidazole (1.2%), amoxicillin-clavulanic acid + SxT (1.2%), marbofloxacin + azithromycin (1.2%) and ampicillin + marbofloxacin (1.2%).

Samples were inoculated on a selective chromogenic media bi-plate (chromID™ CARBA SMART, bioMérieux) which consists of an association of media to screen for OXA-48 on one side, and other carbapenemases on the other side. Plates were then incubated at...
37°C for 24 h in aerobic atmosphere. Susceptibility testing and identification were performed on every isolate with growth on this media using WIDER system (Francisco Soria Melguizo).

Phenotypic carbapenemase characterization was conducted, according to the susceptibility testing results, on those isolates that matched the EUCAST screening criteria (14) using the combination disk test (ROSCO®) which includes meropenem (10 µg) alone and combined with boronic acid (BO), cloxacillin (CX) and dipicolinic acid (DPA). A temocillin disk (30 µg) was used as a phenotypic indicator of OXA-48.

In addition, those isolates with growth on chromID™ CARBA SMART but with negative results for carbapenemase-production were further investigated for the presence of extended-spectrum β-lactamases (ESBL) and/or AmpC-type enzymes according to EUCAST guidelines (14). The phenotypic confirmation method used was the combination disk test which included a disk containing cefotaxime (30 µg) alone and in combination with clavulanic acid, cloxacillin, and cloxacillin-clavulanic acid (ROSCO®). Furthermore, the double-disk synergy test, which included a disk containing cefepime and another one containing clavulanic acid, was performed to confirm the presence of ESBL in those Enterobacteriaceae with inducible chromosomal AmpC.

The presence of genes encoding carbapenemases (blaOXA-48, blaKPC, blaVIM, blaIMP, and blaNDM) (15), ESBLs (blaTEM, blashv, and blaCTX-M) (16) and plasmid-mediated AmpC (blaCMY, blalox, blamox, bladha, bladha, blaci) (17) was determined using PCR and DNA sequencing assays. The sequences obtained were compared with those available in the public databases GenBank and Lahey Clinic (http://www.lahey.org/Studies/)
Multilocus sequence typing (MLST) was performed for all carbapenemase-producing isolates using the Institut Pasteur scheme [1].

Class 1 integron structures, the integrase gene intI1 and the variable regions, were screened by PCR amplification and DNA sequencing [2]. Conjugation experiments were performed with carbapenemase-producing *K. pneumoniae* isolate using the kanamycin-azide resistant *E. coli* Hb101 as a recipient. Putative transconjugants were selected on Mueller-Hinton agar plates containing kanamycin (100 μg/ml), azide (160 μg/ml), and cefotaxime (4 μg/ml). Plasmids were classified according to their incompatibility group by a PCR-based replicon-typing scheme [3]. PFGE with S1 nuclease digestion of whole genomic DNA (S1-PFGE) was used to detect plasmids as previously described [4].

Three Enterobacteriaceae from three different dogs grew on the selective chromogenic media bi-plate: 2 *Klebsiella pneumoniae* (named “1” and “2”) and 1 *Enterobacter cloacae*. Only *K. pneumoniae*-1 produced carbapenemase and the remaining isolates were considered false-positives on the chromID™ CARBA SMART medium. Susceptibility testing results are listed on Table 1. The combination disk test showed a profile compatible with CPE in the *K. pneumoniae*-1 isolate confirmed by PCR and sequencing as a metallo-β-lactamase VIM-1, belonging to the new sequence type (ST) 2090, not related to any ST previously described. This isolate, also resistant to trimethoprim-sulfamethoxazole, tobramycin and fosfomycin, was detected in a dog from an animal shelter without any medication at the time of sampling neither in the
previous month. The presence of genes encoding ESBLs or plasmid-mediated AmpC were not detected in this isolate.

**bla**<sub>VIM-1</sub> gene was carried on a class 1 integron in the following cassette combination:

- **intI1** (integrase gene) - **bla**<sub>VIM-1</sub> - **aac(6')-Ib** (tobramycin-resistance gene, also called **aacA4** - **dfbB1** (trimethoprim-resistance gene) - **aadA1** (streptomycin-resistance gene) - **catB2** (chloramphenicol-resistance) - **qacEδ1/sul1** (quaternary ammonium compounds-resistance gene/sulphonamides-resistance gene). Carbapenem-non-susceptible *E. coli* transconjugant was obtained from VIM-1-producing *K. pneumoniae* isolate. Both, isolate from surveillance and transconjugant, carried a plasmid of ~48 kb that was untypeable by PCR. In addition, transconjugant was positive for class 1 integron and **bla**<sub>VIM</sub>. VIM-1-producing *E. coli* transconjugant presented also reduced susceptibility to tobramycin, and trimethoprim-sulfamethoxazole but not to fosfomycin.

According to the results, the *K. pneumoniae*-2 strain was further investigated as it seemed to harbor an ESBL and a plasmidic AmpC enzyme, confirmed as CTX-M-15 and DHA, respectively. It belonged to a dog that was being treated with marbofloxacin and amoxicillin-clavulanate.

The double-disk synergy test with cefepime and amoxicillin-clavulanic acid was positive for the *E. cloacae* strain, suggesting the ESBL production confirmed as SHV-12 by sequencing. No genes encoding carbapenemases were detected by PCR. The dog was receiving cephalexin and metronidazole at the time of sampling.
The isolation of multidrug resistant Enterobacteriaceae in companion animals is an emerging phenomenon. There are few studies describing colonization or infection of companion animals with these organisms, but the number seems to be increasing. According to a recent review (21) and similarly to humans (22), CTX-M type ESBL are the most prevalent, essentially CTX-M-1, 14 and 15, although SHV-12 and TEM-52 have also been described, mostly detected in E.coli and in K. pneumoniae strains from cats and dogs; CMY-2 and DHA are the most common AmpC β-lactamases described so far (21). Fortunately, carbapenemases are exceptionally rare in companion animals (11-13). In veterinary medicine, β-lactam antibiotics are the most widely used for bacterial infections(21). In dogs, first generation cephalosporins and amoxicillin/clavulanic acid are among the most commonly prescribed (23). Nevertheless, the use of cabapenems is not currently licenced although it has been reported exceptionally (24). Therefore resistance to carbapenems is not routinely evaluated in animal isolates, so it seems likely that its prevalence is underestimated (11).

However, although the use of carbapenems is not a common practice, the presence of genes encoding carbapenemases in mobile genetic elements, that can also include other antibiotic resistance genes, means that the use of other antimicrobials could contribute to co-selection (6). The detection of genes conferring resistance to aminoglycosides, trimethoprim and sulphonamides in the same class 1 integron with bla_{VIM-1} could facilitate that co-selection phenomenon.

The great increase and spread of CPE from human sources and its recent isolation from animals may reflect an emerging problem both in human and veterinary medicine as interspecies transmission may occur between humans and companion animals within
the same household (25). Our results support this hypothesis as the class 1 integron and plasmid carrying blavim-1 detected in this study are among the most frequent mobile genetic elements associated to blavim-1 dissemination in humans (26).

In Spain the isolation of CPE in humans has significantly increased in recent years. According to a recent multicenter Spanish study including 83 hospitals, OXA-48 carbapenemase is currently the most prevalent in our country followed by VIM-1 which appears much less frequently (27). The estimated overall prevalence of infection by carbapenemase-producing K. pneumoniae was 1.7% (range per hospital: 0–11.6%), and 23 (30.7%) hospitals had a prevalence >1% (27). In the present study we found a 0.6% CPE prevalence in companion dogs.

Emergence and spread of CPE in companion animals may represent an underestimated threat to public health. Our findings support probable human-animals transference of carbapenemases genes and reveal the need of active surveillance studies in companion animals.

Acknowledgements

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References


Table 1. MIC (mg/L) of β-lactam antibiotics.

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