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Non-canine Research

Spread of multidrug-resistant *Escherichia coli* within domestic aggregates (humans, pets, and household environment)

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ABSTRACT

Advances in veterinary medicine have been associated with recourse to antimicrobial therapies, which favors the emergence of resistant bacteria, potentially able to spread globally. The aim of the present study was to elucidate the manner in which shared environments between pets receiving antimicrobial treatments and their owners could contribute to the spread of antimicrobial drug-resistant *Escherichia coli*. Three domestic aggregates, including pets, owners, and household environment were studied. Each core pet had a history of previous antimicrobial therapies. Overall, 231 *E. coli* isolates were recovered from pets' feces, urine, oral secretion, skin, and fur; and owners' hands and stool swabs. Commonly touched household objects and surfaces (light switches, door knobs, TV remote control, mobile phones, banister, refrigerator door handle, kitchen floor, pet beds, leash, food, and water recipients) were also sampled. All strains were analyzed by antimicrobial susceptibility testing. Subsequently, some isolates were selected for Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction and Pulsed-Field Gel Electrophoresis analyses, to evaluate their genetic relatedness. Antimicrobial susceptibility tests displayed 20 different phenotypic patterns with an important representation of multidrug-resistant ones (75.0%). The 3 core dogs presented multidrug-resistant *E. coli* clones disseminated over various body sites. In 2 of 3 domestic aggregates, A and B, clonal disseminations among animals, owners, and household surfaces were also observed. Results confirmed the dissemination of multidrug-resistant *E. coli* within and through the household environment, highlighting the relevance of pets in the community spread of antimicrobial resistance.

Introduction

Antimicrobial resistance (AMR) is a major public health problem worldwide and will probably be the main global concern of the next decade (Carlet et al., 2012). The phenomenon of AMR is a complex problem involving many bacterial species, resistance mechanisms, transfer mechanisms, and reservoirs (Guardabassi et al., 2004). Although the major consequences of AMR are more noticeable in the clinical setting, emergence and dissemination of resistance happens primarily in the environmental microbiota including community settings, where studies aimed to fully understand the cycle of acquisition of resistance by human pathogens are needed (Martínez, 2012).

A notable improvement in companion animal health was accomplished by the appearance of veterinary hospitals and the adoption of clinical procedures similar to the ones used in human medicine, including the use of antibiotics and antimicrobials. Hospitalized pets under antibiotic treatment have provided a scenario that strongly favors the occurrence and dissemination of AMR (Hall et al., 2013) similar to what happens in human clinical

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settings. When these animals are discharged and go home, due to the close contact and contempt in hygiene practices between owners and their pets, they can easily transfer antimicrobial-resistant strains (Guardabassi et al., 2004; Lloyd, 2007; Murphy et al., 2009) directly (via contact with skin, saliva, or feces) or indirectly (via the household environment) to their animal or human cohabitants (Martins et al., 2013). The reverse (from humans to animals) can also happen, as demonstrated by Johnson et al. (2008).

Escherichia coli can be used to track the evolution of antibiotic resistance in different ecosystems not only due to its important role as acceptor and donor of transmissible drug resistance genes, from and to pathogenic bacteria (van den Bogaard and Stobberingh, 2000; Sáenz et al., 2004), but also because it is commonly found in the intestinal tract of humans and animals and widely spread in fecal contaminated water, soil, and food (Costa et al., 2008; Murphy et al., 2009; da Costa et al., 2013).

The above concerns led us to investigate 3 hypotheses: (1) that *E. coli* from dog feces can colonize other body sites of the animal; (2) that *E. coli* from dog feces can contaminate household surfaces and objects; and (3) that intraspecies and interspecies *E. coli* transmission can occur within the same domestic aggregate (DA).

Accordingly, we conducted a cross-sectional point prevalence survey of *E. coli* colonization patterns in 3 DAs. Cefotaxime supplemented media was used to facilitate the recovery of low-frequency clones and Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) and Pulsed-Field Gel Electrophoresis (PFGE) were the tools used to assess genetic diversity in *E. coli* from humans, pets, and household surfaces.

Materials and methods

Study design and compliance

The DAs integrating this study emerged from the universe of clients of the Veterinary Hospital of the University of Porto (UPVet). Eligibility criteria for this study required that the core pet (the animal visiting the hospital) from the applicant DA (including owners and other pets) had been submitted to at least one antimicrobial treatment over the previous 6 months. The owners were asked to sign in a term of acceptance; to complete a questionnaire about intrinsic and environmental variables of each one of the DA elements, including human and veterinary medical information regarding antibiotic exposure; to bring their own stool samples and to allow the collection of swabs from their hands; fecal, urinary, and oral secretions samples; skin and fur swabs from their pets; and commonly touched household objects and surfaces (light switches, door knobs, TV remote control, mobile phones, banister, refrigerator door handle, kitchen floor, pets beds, leash, food, and water recipients).

Escherichia coli isolation

Fecal samples were immediately diluted 1:10 in saline buffer and stored at room temperature for 30 minutes. From the initial suspension, an aliquot of 5 μL was streaked on Tryptone Bile X-glucuronide agar (TBX; Biokar Diagnostics, Allonne, Beauvais, France), and 100 μL were spread on the same culture media containing 2 μg/mL of cefotaxime (Sigma-Aldrich, St. Louis, MO). The urine was cultured by directly streaking 5 μL on TBX agar and 100 μL on TBX containing cefotaxime. The swabs were immersed on Buffered Peptone Water (BPW; Oxoid, Basingstoke, Hampshire, England) for 30 minutes at room temperature, and subsequently, 100 μL were spread on nonsupplemented and cefotaxime-supplemented TBX agar plates.

Plates were incubated overnight at 37°C. A maximum of 5 colonies with typical appearance of *E. coli* were selected from each nonsupplemented TBX agar plate, and all colonies presenting different morphologies were additionally picked from the cefotaxime supplemented TBX agar plates. Standard biochemical methods were used for the confirmation of *E. coli* isolates (Berge et al., 2006). The described procedure was adapted from standard protocols used in related studies aiming to achieve the most reliable and accurate *E. coli* detection (Costa et al., 2008; Martins et al., 2013).

Antimicrobial susceptibility characterization

Disk diffusion assay, following CLSI (2012) guidelines, was performed to assess the antimicrobial susceptibility of each isolate. Selected antimicrobial drugs included those regularly used in both human and veterinary medicine and were representative of different antimicrobial classes. A total of 19 antimicrobial agents (Oxoid) were tested: ampicillin (AMP, 10 μg), amoxicillin-clavulanic acid (AMC, 30 μg), aztreonam (ATM, 30 μg), cephalothin (CEF, 30 μg), ceftazidime (CAZ, 30 μg), cefotaxime (CTX, 30 μg), cefoxitin (FOX, 30 μg), imipenem (IPM, 10 μg), gentamicin (GEN, 10 μg), amikacin (AMK, 30 μg), streptomycin (STR, 10 μg), tobramycin (TOB, 10 μg), kanamycin (KAN, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NAL, 30 μg), tetracycline (TET, 30 μg), chloramphenicol (CHL, 30 μg), trimethoprim-sulfamethoxazol (SXT, 25 μg), and nitrofurantoin (NIT, 300 μg). Bacteria were considered as being multidrug resistant according to previous reported definition (Magiorakos et al., 2011).

DNA extraction and E. coli phylogenetic group determination

The assortment of all phenotypically characterized isolates, from each DA, was examined for the number of AMR determinants and for repetitive resistance patterns.

Criteria designed for selecting the group of isolates, from each DA, eligible for genetic analysis were as follows: (1) multidrug-resistant *E. coli* with different AMR patterns and (2) multidrug-resistant strains that had similar AMR phenotypes but isolated from different sources.

The isolates were cultured in Müeller-Hinton agar (MH; Biokar Diagnostics) at 37°C and harvested at late exponential phase to perform DNA extraction by using the InstaGene Matrix (Bio-Rad Laboratories, California) as described by the manufacturer. A simple and rapid phylogenetic grouping technique based in a triplex PCR was applied, according to Clermont et al. (2000).

ERIC-PCR fingerprinting

A 25-μL ERIC-PCR reaction was carried out using the primers ERIC-1R (5′-ATG TAA GCT CCT GGG GAT TCA C) and ERIC 2 (5′-AAG TAA GTG ACT GGG GTG AGC G) as previously described (Versalovic et al., 1991; Meacham et al., 2003). The PCR amplifications were performed in a DNA thermal cycler MyCycler (Bio-Rad Laboratories), with an initial incubation at 94°C for 3 minutes, followed by 30 cycles consisting of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 3 minutes. A final extension at 72°C for 5 minutes was programmed to complete the amplification (Leung et al., 2004).

The ERIC-PCR patterns of each isolate were visualized after electrophoresis for 45 minutes at 150 V using a 1.5% agarose gel containing 1 × TBE buffer (National Diagnostics, Atlanta, GA) and 0.5 μg/mL ethidium bromide. Gels were photographed using a Molecular Imager Gel Doc XR (Bio-Rad Laboratories).