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Short communication

Quantitative assessment of faecal shedding of β-lactam-resistant *Escherichia coli* and enterococci in dogs

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ABSTRACT

Quantitative data on faecal shedding of antimicrobial resistant bacteria are crucial to assess the risk of transmission from dogs to other animals as well as humans. In this study we investigated prevalence and concentrations of β-lactam-resistant *Escherichia coli* and enterococci in the faeces of 108 dogs presenting at a veterinary hospital in Denmark. The dogs had not been treated with antimicrobials for 4 weeks prior to the study. Total *E. coli* and enterococci were quantified by counts on MacConkey and Slanetz–Bartley, respectively. Resistant *E. coli* and enterococci were counted on the same media containing relevant antibiotic concentrations, followed by species identification using MALDI-TOF. Ampicillin- and cefotaxime-resistant *E. coli* were detected in 40% and 8% of the dogs, respectively, whereas approximately 15% carried ampicillin-resistant enterococci, mainly *Enterococcus faecium*. In the faeces of the carriers, the proportion of resistant strains in the total bacterial species population was on average 15% for both ampicillin-resistant *E. coli* (median faecal load 3.2×10^4 cfu/g) and *E. faecium* (5.8×10^2 cfu/g), and 4.6% for cefotaxime-resistant *E. coli* (8.6×10^3 cfu/g). Cefotaxime resistance was associated with the presence of $bla_{CTX\text{-}M\text{-}1}$ ($n=4$), $bla_{CMY\text{-}2}$ ($n=4$) or multiple mutations in the promoter and coding region of chromosomal *ampC* ($n=1$). Altogether the results indicate that the risks of zoonotic transmission of β-lactam-resistant bacteria via human exposure to canine faeces greatly vary amongst individual dogs and are influenced by unidentified factors other than recent antimicrobial use.

1. Introduction

Escherichia coli and enterococci are widespread commensal organisms with the potential to cause infections in animals and humans worldwide. Resistance to β-lactams is of high clinical relevance for both bacterial groups since aminopenicillins (alone or in combination with clavulanic acid) and cephalosporins are widely used for treatment of *E. coli* infections, and penicillins (alone or in combination with aminoglycosides) are first choice antibiotics for treatment of enterococcal infections. Cephalosporin resistance mediated by extended spectrum (ESBL) and AmpC β-lactamases is increasingly reported among *E. coli* isolated from human infections and healthy carriers in the community (Woerther et al., 2013). Similarly, a rise in the prevalence of ampicillin resistance has been observed amongst clinical *Enterococcus faecium* isolates in Europe over the last two decades (Arias and Murray, 2012). While food animals are generally considered an important reservoir of these resistant organisms, possible non-foodborne reservoirs have been generally neglected (Barber et al., 2003). For example, dogs are a possible source for transmission of these β-lactam-resistant bacteria due to their close interaction with humans, the high consumption of β-lactams in small animal veterinary practice (De Briyne et al., 2014), the frequent occurrence of ESBL/AmpC-producing *E. coli* (Haenni et al., 2014) and ampicillin-resistant *E. faecium* (Damborg et al., 2009) as well as the similar genetic backgrounds and virulence profiles of canine and human strains (Damborg et al., 2009; Johnson et al., 2001). However, quantitative data on faecal shedding of these bacteria in dogs, which are essential to assess the risk of zoonotic transmission, are very limited. In order to fill this knowledge gap, the objective of this study was to estimate the prevalence and faecal

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concentrations of β-lactam-resistant *E. coli* and *Enterococcus* in a population of dogs presenting to a veterinary referral hospital in Denmark.

2. Methods

Between November 2014 and January 2015, 108 dogs presenting to The University Hospital for Companion Animals in Copenhagen were recruited after obtaining informed consent from the owners. Dogs were included if they had not received systemic or local antimicrobial or other chemotherapeutic treatment during the previous four weeks, as assessed by interview with the owner and medical record. Breed, age, sex and health status information was recorded for each dog. Health status was recorded as either (i) healthy (dogs presenting for prophylactic therapy such as vaccinations or elective surgery) or (ii) diseased. Diseased dogs were categorized according to the organ system affected.

Stool samples were collected directly from the rectum and processed in the laboratory on the same day. Faeces were suspended in 0.9% sterile saline to a 1:10 dilution (10^{-1}) and homogenized using a stomacher. Two technical replicates of tenfold dilutions were prepared up to 10^{-6}. Three 20 μl-drops of each dilution were inoculated onto different selective agar media (Oxoid, UK): MacConkey not supplemented with antimicrobials for counts of total *E. coli*; MacConkey supplemented with ampicillin (AMP 32 μg/ml) or cefotaxime (CTX 1 μg/ml) for counts of *E. coli* resistant to these antibiotics; Slanetz–Bartley without antimicrobials for counts of total enterococci; and Slanetz–Bartley supplemented with ampicillin (16 μg/ml) for counts of ampicillin-resistant enterococci. All batches of media supplemented with antibiotics were tested with positive/resistant and negative/susceptible control strains. MacConkey agar plates were incubated 24 h at 37 °C and Slanetz–Bartley agar plates at 42 °C for 48 h. Lactose-positive colonies growing on MacConkey agar and redish presumptive enterococcal colonies on Slanetz–Bartley agar were quantified and one colony was picked from the highest dilution for species identification using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectophotometry (MALDI-TOF MS) (BioMerieux, France). If more than one colony morphology was observed, quantification and species confirmation were performed separately for each colony type.

Total *E. coli* and enterococci (cfu/g) and relative (%) abundance of each resistant population were calculated as the mean of the two technical replicates. The sensitivity of carriage detection (Se) by each replicate was compared to that obtained by both replicates for each bacterial species and antimicrobial resistance phenotype. Possible associations between sex, age (as a continuous variable), health status and carriage of AMP^R *E. coli*, CTX^R *E. coli*, total enterococci, AMP^R enterococci and total AMP^R resistance (i.e. AMP^R *E. coli* and AMP^R enterococci) were analysed by logistic regression models. The models were built including each bacterial indicator as the outcome and analysed by the *glm* function in R version 3.2.2 using the argument *binomial*. Statistical significance was set at p-values < 0.05.

CTX^R *E. coli* isolates were further investigated by PCR and sequencing using previously described primers for amplification of bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ and bla_{CMY} (Dierikx et al., 2010). The exact $bla_{CTX\text{-}M}$ and bla_{CMY} variant were determined by PCR and sequencing (Bortolaia et al., 2014; Carattoli et al., 2008). Isolates not yielding any PCR product were tested for mutations in the chromosomal *ampC* promoter and coding region (Caroff et al., 1999).

3. Results

A total of 108 dogs of age ranging from 3 months to 15 years (median age 5 years) was included in the study, including 45 breeds. The female:male ratio was 1.04. Thirty-four dogs (31%) were healthy and the remaining 74 were classified as diseased according to the organ or system affected, including skin/ears ($n = 15$), musculoskeletal ($n = 12$), gastrointestinal system and liver ($n = 9$), teeth and mouth ($n = 8$), neurological system ($n = 7$), urinary tract ($n = 5$), genital system ($n = 4$), cardiovascular system and airways ($n = 3$), haematological organs ($n = 1$) and miscellaneous ($n = 10$). Sixty-two dogs (57%) were admitted to the University primary practice, and 46 dogs (43%) to one of the University Hospital Specialty Services. Of these 46 dogs 28% were external referrals and 72% were internally referred from the University primary practice.

High variability was observed in the prevalence of dogs carrying different types of β-lactam-resistant bacteria (Fig. 1) as well as in the faecal concentrations (cfu/g) of total and resistant bacteria (Fig. 2) and in the relative abundance (%) of resistant bacteria amongst dog carriers (Fig. 3). The prevalence of dogs carrying *E. coli* and enterococci (*E. faecium*, *Enterococcus faecalis*, *Enterococcus hirae* and *Enterococcus avium/raffinosus*) was 98% and 90%, respectively. Approximately, 40% of the dogs carried AMP^R *E. coli* with faecal concentrations ranging between 8×10^1 and 6×10^8 cfu/g (<1–100% of total *E. coli*). Approximately 8% of the dogs carried CTX^R *E. coli* at concentrations between 8×10^1 and

Table 1
Median counts (cfu/g) of total and resistant *Escherichia coli* and enterococci in faecal samples of 108 dogs without recent antimicrobial treatment. Minimum and maximum values are reported in brackets. ND, not detected.

	Total	AMP^R	CTX^R
Escherichia coli	1.4×10^6 ($1.7 \times 10^2 - 4.2 \times 10^{11}$)	3.2×10^4 ($8.3 \times 10^1 - 6 \times 10^8$)	8.6×10^3 ($8.3 \times 10^1 - 2 \times 10^5$)
Enterococci	1.3×10^5 ($8.3 \times 10^1 - 9.4 \times 10^8$)	7×10^2 ($8.3 \times 10^1 - 6.2 \times 10^3$)	–
E. faecium	5.6×10^4 ($8.3 \times 10^1 - 5.5 \times 10^8$)	5.8×10^2 ($8.3 \times 10^1 - 2.1 \times 10^3$)	–
E. faecalis	5.7×10^4 ($1.6 \times 10^3 - 5.9 \times 10^8$)	4.4×10^3 ($1.3 \times 10^3 - 6.2 \times 10^3$)	–
E. hirae	3.8×10^6 ($8.3 \times 10^1 - 6 \times 10^8$)	ND	–
E. avium/raffinosus	3.2×10^4 ($4.2 \times 10^2 - 7.1 \times 10^7$)	ND	–