

Antimicrobial resistance profiles of *Escherichia Coli* isolated from diarrheic or healthy cats

Abstract

The number of pets has increased substantially in modern society and attention is increasingly devoted to pet welfare. The aim of the present study is to investigate the occurrence of antimicrobial resistance in *Escherichia coli* isolates present in cat intestinal micro biota. A hundred-ninety *E. coli* isolates from diarrheic (n=19) and healthy (n=21) cats from northwest of São Paulo State, Brazil were examined for resistance to 16 antimicrobial drugs. The predominantly observed resistance was to tetracycline (30.5%), ampicillin (20.0%) and cotrimoxazole (17.9%) among the isolates from healthy cats and to cephalothin (42.1%), tetracycline (20.0%) and ampicillin (15.8%) among the isolates from diarrheic cats. A moderate multidrug-resistance was found among 17.8% and 8.4% of the isolates from healthy and diarrheic cats, respectively. The *E. coli* isolates from healthy cats may act as a reservoir of resistance genes. To support the development of antimicrobial usage policies, regular updates on the status of resistance to antimicrobial agents used in veterinary medicine are needed.

Keywords: pet, companion animals, multidrug resistance, antibiotic

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Introduction

Throughout recent years, there has been growing concern about the increasing prevalence of antimicrobial resistance in human and veterinary medicine as well. It is generally accepted that the main cause of this problem is the increased use of antimicrobial agents, which has led to the emergence and spread of resistant bacteria and/or their resistance genes. The emergence of resistance in pathogenic bacteria may reduce the effectiveness of previously successful antimicrobials regimens.¹

Escherichia coli are commonly found in the intestinal tract of animals and humans, and can also be implicated in animal and human infectious diseases. For this reason fecal isolates of *E. coli* are considered a very good indicator for to investigated the selection pressure exerted by antimicrobial agents over the commensal flora as well as the pathogenic strains.^{1,2} In contrast to food animals, our knowledge about antimicrobial resistance of commensal *E. coli* in companion animals is much more limited and much of the available data about antimicrobial resistance come from retrospective studies of clinical isolates, with the inherent potential bias for overestimation of resistance. The relationship between companion animals and humans has radically changed, with cats increasingly being in close contact with humans by touching and kissing and living in the same environment using the same table and bed. This raises public health concerns, regarding the transmission of bacteria, including antimicrobial-resistant and/or pathogenic strains.^{3,4}

Only a few authors have reported the antimicrobial resistance in commensal *E. coli* isolates recovered from cats and most of them are from Europe^{5,6} and Canada,^{7,8} nevertheless, to our knowledge, there is no previous study carried out with cats in Brazil. The aim of the present study is to verify the antimicrobial resistance detected among *E. coli* isolates from diarrheic or healthy cats.

Materials and methods

A total of 190 *E. coli* isolates from nineteen diarrheic and twenty-one healthy cats, all of the adult cats, were provided by a

private veterinary diagnostic laboratory (VDL) to the Genetic and Microbiology laboratory (GML) of the Department of Morphology, Physiology and Basic Pathology of the São Paulo University. Samples had been submitted to the VDL from January to December 2009 by veterinary practitioners after collection of a rectal sterile cotton swab in Stuart carrier medium, in a routinely visit for clinical consultation in Ituverava City, northwest of the São Paulo State, Brazil. Samples collected were taken to the VDL for immediate processing, transferred to MacConkey agar (Mac-Difco Laboratories, Detroit, USA) and incubated at 37°C for 24h. At least 5 colonies per sample with typical *E. coli* morphology were selected and identified by standard methods for *E. coli* identification.⁹ Isolates were stored as pure cultures in 10% skimmed milk at -70°C until further use. Unfortunately, information about previous treatment with antimicrobial drugs was rarely given by the owners and these data were not used in the present study. Although susceptibility testing had been performed by the VDL prior to submission to the GML, this data was not available to the study investigators. On receipt in the GML, each isolated was confirmed as *E. coli* by the same method above mentioned.⁹

Antimicrobial susceptibility was performed by the agar disk diffusion method as recommended by the Clinical and Laboratory Standards Institute-CLSI,¹⁰ and a total of 16 antimicrobial agents (Cefar, São Paulo, Brazil) were tested: amikacin (AMK, 30µg), amoxicillin-clavulanic acid (AMC, 30µg), ampicillin (AMP, 10µg), aztreonam (ATM, 30µg), cephalothin (CEP, 30µg), cefoxitin (FOX, 30µg), ceftazidime (CAZ, 30µg), cefuroxime (CEF, 30µg), ciprofloxacin (CIP, 5µg), cotrimoxazole (SUT, sulfamethoxazole 23.75µg+ trimethopim 1.75µg), gentamicin (GEN, 10µg), imipenem (IMP, 10µg), nalidixic-acid (NAL, 30µg), norfloxacin (NOR, 10µg), tetracycline (TET, 30µg), tobramycin (TOB, 10µg). *E. coli* reference strains ATCC 25922 and ATCC 35218 were used for disk quality control. Multidrug resistance analysis was carried according to the definition proposed by Magiorakos et al.¹¹ the isolate resistant to at least one antimicrobial in at least three different drug categories was identified as multidrug-resistant (MDR). Significant differences in the frequencies of resistance to the tested antimicrobial drugs were determined by the chi-square test at P<0.05 significance level.

Results and discussion

A total of 190 *E. coli* isolates were recovered from the fecal samples of diarrheic cats (95 isolates) and 21 fecal samples of healthy cats (95 isolates). Due to the great number of different clones of *E. coli* that can be found among the commensal strains in the micro biota of the intestinal tract, at least 5 isolates from each cat were selected to be tested against antimicrobial drugs.^{2,12} The susceptibility to 16 antimicrobial drugs for these isolates is shown in Table 1. Among the isolates from diarrheic cats the highest resistance was observed against cephalothin (42.1%), followed by tetracycline (20.0%) and ampicillin (15.8%), while among healthy cats the highest frequencies were for tetracycline (30.5%), cotrimoxazole (17.9%) and ampicillin (20.0%). The percentage of resistance to the other antimicrobial drugs was in almost all cases below 5.0%. A score test confirms that the difference between diarrheic and healthy cats is statistically significant for both cotrimoxazole ($x^2=9.44$; $\alpha=0.01$) and tetracycline ($x^2=2.75$; $\alpha=0.05$) in relation to antimicrobial resistance. Table 1 also shows that the number of isolates susceptible to all the antimicrobial drugs tested was statistically significant for the diarrheic cats ($x^2=7.67$; $\alpha=0.01$).

Table 1 Percentages of antimicrobial resistance among the 190 *Escherichia coli* isolates from fecal samples of diarrheic and healthy cats in northwest of São Paulo State, Brazil

Antimicrobial Resistant <i>E. coli</i> Isolated from		
Antimicrobial Agents	Diarrheic Cats (N=95) Number Isolates (%)	Healthy Cats (N=95) Number Isolates (%)
Ampicillin	15 (15.8)	00 (0.0)
Amoxicillin-clavulanic acid	00 (0.0)	00 (0.0)
Aztreonam	00 (0.0)	00 (0.0)
Cefoxitin	00 (0.0)	00 (0.0)
Ceftazidime	00 (0.0)	00 (0.0)
Cefuroxime	01 (1.0)	01 (1.0)
Cephalothin	40 (42.1)	00 (0.0)
Cotrimoxazole	03 (3.1)	17 (17.9)
Amikacin	01 (1.0)	00 (0.0)
Gentamicin	00 (0.0)	02 (2.0)
Tobramycin	00 (0.0)	00 (0.0)
Imipenem	00 (0.0)	00 (0.0)
Nalidixic acid	05 (5.3)	02 (2.0)
Ciprofloxacin	01 (1.0)	00 (0.0)
Norfloxacin	00 (0.0)	02 (2.0)
Tetracycline	19 (20.0)	30 (30.5)
Susceptible	42 (44.2)	62 (65.2)

In small animal practices, the choice of an antimicrobial treatment is often made empirically when a treatment needs to be initiated before the test results are known. For the veterinarian, knowing the bacterial species possibly involved in the most frequently encountered infectious conditions and their possible resistance to antimicrobials is important. Various longitudinal retrospective studies in Europe^{5,6} and North America,^{7,8} have reported an increase in the prevalence of antimicrobial resistance among the commensal *E. coli* strains isolated from pets. All of them reported a resistance percentage of *E. coli* isolates from healthy cats quite similar to those reported in the present study, especially Costa et al.⁵. However some exceptions could be found among the quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin) with percentages of 24.7% and 10.1% respectively reported by Leite-Martins et al.,⁶ and also among the cephalosporin

(3th generation) ceftazidime 10.1%.⁶. Use of antimicrobial drugs in companion animals is subject to less stringent regulation, and there is likely more “off-label” use in companion animals what can promote a misuse of the drugs and also a more intensive use of antimicrobial drugs essentials for human therapeutics in pets.¹³

The present study also permits to identify a high frequency of *E. coli* isolates showing susceptibility to all drugs tested 44.2% and 65.2% among respectively diarrheic and healthy cats respectively what are good news for the veterinarian working in the area analyzed, and is much higher than reported by others.⁶⁻⁸ It is especially interesting for old aminoglycosides, quinolones and first and second generation cephalosporins that can be effectively used safely in pet empirical treatments.

Multidrug resistance (MDR) was found in both cat groups but among healthy isolates it was highest. The phenotypes of resistance exhibited by 190 *E. coli* isolates are presented in Table 2. The most frequent MDR phenotype was nalidixic acid-tetracycline-ampicillin-resistance that was found in 5.3% of the isolates from diarrheic cats and was tetracycline-cotrimoxazole-ampicillin-resistance found in 15.8% of the isolates from healthy cats Costa et al.⁵ reported a frequency of 10.5% of MDR *E. coli* isolates among healthy pets what is quite similar to our results in Brazil.

Table 2 Phenotypes of resistance detected among the 190 *E. coli* isolates recovered from diarrheic and healthy cats in northwest São Paulo State, Brazil

Resistance phenotype	Diarrheic cats Number of isolates (%)	Healthy cats Number of isolates (%)
AMK*	01 (1.0)	-----**
CEF	01 (1.0)	01 (1.0)
CEP	29 (30.5)	-----
TET	01 (1.0)	11 (11.5)
AMP	-----	01 (1.0)
SUT	-----	01 (1.0)
TET-AMP	05 (5.3)	01 (1.0)
TET-SUT	-----	01 (1.0)
CEP-TET	05 (5.3)	-----
CEP-CIP	01 (1.0)	-----
CEP-AMP	02 (2.0)	-----
NAL-TET-AMP	05 (5.3)	-----
TET-SUT-AMP	-----	15 (15.8)
CEP-TET-SUT-AMP	03 (3.1)	-----
NAL-NOR-GEN-TET-AMP	-----	02 (2.0)
Susceptible	42 (44.2)	62 (65.2)

In recent years cases of shared fecal *E. coli* isolates between dogs and their owners have been reported.¹² Thus, the significance of feline feces as a reservoir of antimicrobial-resistant *E. coli* for their owners should be carefully examined and effective monitoring of resistant organism and tracking the movement of the resistance genes is essential if resistance and resistant organisms want to be controlled.

Conclusion

As conclusion moderate percentages of resistance to ampicillin and tetracycline and low percentages for the other antimicrobial agents have been detected in fecal *E. coli* isolates from diarrheic or healthy cats in Brazil. Multidrug resistance among *E. coli* isolates was detected in both, diarrheic and healthy cats in a moderate percentage.

More studies should be carried out in the future in order to tract the evolution of this type of resistance among the fecal *E. coli* isolates from pets.

Conflicts of interest

There is no conflict of interest.

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