COMPARISON OF ANTIBIOTIC RESISTANCE OF *Escherichia coli* STRAINS ISOLATED FROM DIARRHEIC OR HEALTHY CATS IN ITUVERAVA, SP, BRAZIL

COMPARAÇÃO DA RESISTÊNCIA ANTIBACTERIANA ENTRE CEPAS DE Escherichia coli ISOLADAS DE GATOS DIARREICOS OU SAUDÁVEIS EM ITUVERAVA, SÃO PAULO, BRASIL.

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SUMMARY

The number of pets has increased substantially in modern society and attention is increasingly devoted to pet welfare. The aim of this study is to investigate the occurrence of antimicrobial resistance in *Escherichia coli* strains present in cat intestinal microbiota. From January to December of 2009, 190 *E. coli* strain isolates from diarrheic (n=19) and healthy (n=21) cats from Ituverava, São Paulo state, Brazil were examined for resistance to 20 antimicrobial agents. The predominantly observed resistance was to cephalothin (42.1%), tetracycline (20.0%), and ampicillin (15.8%) among the isolates from diarrheic cats and to tetracycline (30.5%), cotrimoxazole (17.9%), and ampicillin (20.0%) among the isolates from healthy cats. Multidrug-resistance to three or more antimicrobial agents was found among 8.4% and 17.8% of the isolates from diarrheic and healthy cats, respectively. It is obvious that the *E. coli* strains from 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 antimicrobials used in veterinary medicine are needed.

KEY-WORDS: Pet. Companion animals. Antimicrobial agents. Multidrug resistance.

RESUMO

O número de animais de estimação tem aumentado substancialmente na sociedade moderna e uma atenção cada vez maior tem sido devotada ao bem estar destes animais. O objetivo deste estudo foi investigar a ocorrência de resistência antimicrobiana em cepas de *Escherichia coli* da microbiota intestinal de gatos. De Janeiro a dezembro de 2009, 190 cepas de *E. coli* isoladas de gatos diarréicos (n=19) e gatos saudáveis (n=21) provenientes de Ituverava, Estado de São Paulo, Brasil foram examinadas para a detecção de resistência a 20 agentes antimicrobianos. As resistências predominantemente observadas foram cefalotina (42,1%), tetraciclina (20,0%) e ampicilina (15,8%) entre as cepas isoladas de gatos diarréicos e tetraciclina (30,5%), cotrimoxazole (17,9%) e ampicilina (20,0%) entre as cepas isoladas de gatos saudáveis. A resistência a três ou mais agente antimicrobianos foi encontrada em 8,4% e 17,8% das cepas isoladas de gatos diarréicos e saudáveis respectivamente. É óbvio que as linhagens de *E. coli* provenientes dos gatos podem atuar como um reservatório de genes de resistência. Assim, para permitir o uso correto dos antimicrobianos é fundamental uma constante atualização do nível de resistência apresentado pelos antimicrobianos utilizados em medicina veterinária.

PALAVRAS-CHAVE: Pet. Animal de companhia. Agente antimicrobiano. Resistência a múltiplas drogas.

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INTRODUCTION

Throughout recent years, there has been a 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 resistance bacteria and/or their resistance genes. Most studies show that antibiotics and chemotherapeutics exert selection pressure either on pathogenic or commensal microbiota bacteria (NORMAND et al., 2000; MOYAERT et al., 2006). Emergence of resistance in pathogenic bacteria may reduce the effectiveness of previously successful antimicrobial regimens. Resistant commensal bacteria constitute a reservoir of resistance determinants for potentially pathogenic bacteria (van den BOGAARD & STOBBERING, 2000).

Escherichia coli is commonly found in the intestinal tract of animals and humans (SORUM & SUNDE, 2001), and can also be implicated in animal and human infectious diseases (ROSAS et al., 2006). For this reason fecal *E. coli* is considered a very good indicator for selection pressure by antimicrobial use and for resistance problems to be expected in pathogens (van den BOGAARD & STOBBERINGH, 2000).

Cats and dogs represent potential spreading sources of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans (GUARDABASSI et al., 2004). Already, several studies suggest a possible exchange of resistant organisms and/or their resistance genes between humans and their pets (SIMJEE et al., 2002; GUARDABASSI et al., 2004). Antimicrobial classes frequently used in small animal veterinary medicine include penicillins, cephalosporins, macrolides, lincosamides, tetracyclines, potentiated sulphonamides, aminoglycosides and fluoroquinolones, the same drugs commonly used to treat humans. The most frequent causes of antimicrobial treatments in cats are skin and wound infections, external otitis, respiratory infections, and urinary tract infections. Gastrointestinal infections are also common but antimicrobial therapy is not warranted in most of these syndromes (GUARDABASSI et al., 2004). The most common infections in cats are those affecting skin wounds, particularly inflicted by bites and scratches from other cats and dogs (LOVE et al., 2000).

Various authors have studied antimicrobial resistance in *E. coli* isolates recovered from pets and these studies have been performed either in Europe (NORMAND et al., 2000; GUARDABASSI et al., 2004; MOYAERT et al., 2006) or in other countries as well (AUTHIER et al., 2006; FÉRIA et al., 2002), 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.

MATERIAL AND METHODS

Sample collection and culture

In order to study the antimicrobial susceptibilities of *E. coli* isolates to 20 antimicrobial agents, nineteen diarrheic and twenty-one healthy cats, all of them adult cats, were examined after arriving for clinical consultation in a private clinic in Ituverava city, São Paulo State, between January and December 2009. Unfortunately, information about previous treatment with antimicrobial drugs was rarely given by the owners and these data therefore does not allow any meaningful comparison to previous data. Samples collected under the supervision of a veterinarian using a rectal sterile cotton swab were placed in Stuart carrier medium, taken to the laboratory for immediate processing, transferred to MacConkey agar (Mac-Difco Laboratories, Detroit,USA) and incubated at 37^{o}C for 24 h. At least 5 colonies, isolated from each plate, were submitted to further analysis by standard methods for *E. coli* identification (KONEMAN et al., 1997).

Susceptibility Testing

Antimicrobial disk susceptibility tests were performed using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2007) and a total of 20 antimicrobial agents (Cefar, São Paulo, Brasil) were tested: amikacin (30µg), amoxicillin-clavulanic acid (30µg), ampicillin (10µg), aztreonam (30µg), cephalothin (30µg), cefepime (30µg), cefoxitin (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefuroxime (30µg), ciprofloxacin (5µg), cotrimoxazole (25µg) , gentamicin (10µg), imipenem (10µg), nalidixic acid, nitrofurantoin (300µg), norfloxacin (10µg), pipemid acid (20µg), tetracycline and tobramycin (10µg). *E. coli* reference strains ATCC 25922 and ATCC 35218 were used for strain quality control.

Statistical Analysis

Significant differences in the frequencies of resistance to the tested antimicrobial drugs were determined by the chi-square test at $P \leq 0.05$ significance level.

RESULTS

A total of 190 *E. coli* isolates were recovered from the 19 fecal samples of diarrheic cats (95 isolates) and 21 fecal samples of healthy cats (95 isolates). The susceptibility to 20 antimicrobial agents 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 agents was in almost all cases below 6.0%. A score test confirms that the difference between diarrheic and healthy cats is statistically significant for

Table 1 - Percentages of antimicrobial resistance among the 190 *Escherichia coli* isolates from fecal samples of diarrheic and healthy cats in Ituverava, SP, Brazil, 2009.

Antimicrobial agents	Antimimicrobial resistant *E. coli* isolated from			
	Diarrheic cat(n=95)		Healthy cat(n=95)	
	Number	Percentage	Number	Percentage
Amikacin	01	1.0	0	0
Ampicillin	15	15.8	19	20.0
Amoxicillin-clavulanic acid	0	0	0	0
Aztreonam	0	0	0	0
Cefepime	0	0	0	0
Cefoxitin	0	0	0	0
Ceftazidime	0	0	0	0
Ceftriaxone	0	0	0	0
Cefuroxime	01	1.0	01	1.0
Cephalothin	40	42.1	0	0
Ciprofloxacin	01	1.0	0	0
Cotrimoxazole	03	3.1	17	17.9
Gentamicin	0	0	02	2.0
Imipenem	0	0	0	0
Nalidixic acid	05	5.3	02	2.0
Nitrofurantoin	02	2.0	0	0
Norfloxacin	0	0	02	2.0
Pipemidic acid	0	0	03	3.1
Tetracycline	19	20.0	30	30.5
Tobramycin	0	0	0	0
Susceptible	42	44.2	62	65.2

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 ($x^2 = 7.67$; $\alpha = 0.01$).

Multidrug resistance (MDR) characterized as resistance to three or more antimicrobial agents was found in both groups, but among healthy isolates it was highest. The phenotypes of resistance exhibited by the 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 diarrheic isolates and for the healthy isolates was tetracycline-cotrimoxazole-ampicillin-resistance found in 15.8% of the bacteria. Table 2 shows that the proportion of multidrug resistant isolates was significantly small among the diarrheic cats ($x^2 = 2.95$; $\alpha = 0.05$). Among both types of isolates from diarrheic and health cats more than forty per cent of the *E. coli* isolates showed a susceptible phenotype to the 20 antimicrobial agents tested.

DISCUSSION

In small animal practice, 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.

The relationship between companion animals and humans has changed radically throughout the years, with cats and dogs being more and more in close contact with humans. Close physical contact by touching, petting and licking occurs at high frequency on the basis of the current perception of household pets as actual family members what tremendously increase

Table 2 Phenotypes of resistance detected among the 190 *E. coli* isolates recovered from diarrheic and healthy cats in Ituverava, SP, Brazil, 2009.

Phenotype of resistance	Diarrheic cats		Healthy cats	
	Number of isolates	Percentage of isolates	Number of isolates	Percentage of isolates
AMI*	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
TET-AMP	05	5.3	01	1.0
TET-COT	---	---	01	1.0
CEP-TET	05	5.3	---	---
CEP-CIP	01	1.0	---	---
CEP-AMP	02	2.0	---	---
PIP-COT	---	---	01	1.0
NAL-TET-AMP	05	5.3	---	---
TET-COT-AMP	---	---	15	15.8
CEP-TET-AMP-COT	01	1.0	---	---
NIT-TET-AMI-COT-CEP-AMP	2	2.0	---	---
NAL-PIP-NOR-GEN-TET-AMP	---	---	02	2.0
Susceptible	42	44.2	62	65.2

*AMI-Amikacin; AMP-ampicillin; CEF-Cefuroxime; CEP-Cephalothin; CIP-Ciprofloxacin; COT-Cotrimoxazole; GEN-Gentamicin; NAL-Nalidixic acid; NIT-Nitrofurantoin; NOR-Norfloxacin; PIP- Pipemid acid; TET-Tetracycline.

the risk of a transmission of antimicrobial resistant bacteria from pets to humans (van den BOGAARD et al., 2000; GUARDABASSI et al., 2004).

Some authors advocated a systematic surveillance of antimicrobial resistance among both pathogenic and normal microbiota bacteria from companion animals (RANTALA et al., 2004; PEDERSEN et al., 2007). Various longitudinal retrospective studies in Europe and North America have reported an increase in the prevalence of antimicrobial resistance in different bacterial species isolated from pets. Authier et al (2006) and Costa et al (2008) from Canada and Moyaert et al (2006) from Belgium reported a resistance percentage of *E. coli* isolates for healthy cats quite similar to those reported in the present study especially for ampicillin, cephalothin and tetracycline. The only exception was to cotrimoxazole resistance that showed an unexpected high percentage of resistance (17.9%) among the isolates from healthy cats that was completely different from the data reported by the authors mentioned, what could indicate an inappropriate use of this drug in Brazil.

It is interesting to point out that the *E coli* isolates recovered from both diarrheic and healthy cats in this study showed in general low percentage of resistance to aminoglycosides, cephalosporins and quinolones, which could determine that these drugs in Brazil can be used safely in pet empirical treatments.

To support the development of antimicrobial usage policies regular updates on the status of resistance to antimicrobial drugs used in veterinary medicine are needed. Recent reports of extraintestinal infections in dogs due to multidrug- resistant *E. coli* with resistance to third-generation cephalosporins and fluoroquinolones are a potential public health concern (WARREN et al., 2001; SANCHEZ et al., 2002). Canine MDR have been shown to possess class I integron-associated resistance genes that have previously been identified in bacterial isolates from clinical infections in humans (KANG et al., 2005). This suggests the spread of common resistance mechanisms between canine and human isolates,

possibly through the co-selection and transfer of multidrug-resistance plasmids (TROTT et al., 2004). The class I integron-associated resistance genes were already reported in *E. coli* isolates from healthy cats (COSTA et al., 2008).

Little is known about the possible exchange of commensal bacteria between pets and humans living in contact. Routine and effective monitoring of resistant organisms and tracking the movement of resistance genes through the operation of nationwide and international epidemiological networks is absolutely essential if resistance and resistant organisms are to be controlled. As a 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 the *E. coli* isolates was detected in both, diarrheic and health cats in a moderate percentage that should be considered before an empirical treatment of cats with antimicrobial drugs. More studies should be carried out in the future in order to track the evolution of this type of resistance among the fecal *E. coli* isolates from pets.

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