ANTIMICROBIAL-RESISTANCE AMONG Escherichia coli ISOLATED FROM EITHER DIARRHEIC OR HEALTHY DOGS IN ITUVERAVA, SÃO PAULO STATE, BRAZIL

RESISTÊNCIA ANTIMICROBIANA EM CEPAS DE Escherichia coli ISOLADAS DE CÃES DIARREICOS OU SAUDÁVEIS EM ITUVERAVA, ESTADO DE SÃO PAULO, BRASIL

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SUMMARY

From January to December of 2006, 92 and 75 Escherichia coli isolates from diarrheic and healthy dogs, respectively, were examined for resistance to 11 antimicrobial agents. The predominantly observed resistance was to streptomycin (86.0%), cephalothin (71.0%), gentamicin (62.0%), amoxicillin (59.0%) among the isolates from diarrheic dogs and to nalidicxic acid (27.0%), cephalothin (25.0%), tetracycline (19.0%), ampicillin (19.0%) among the isolates from healthy dogs. Multidrug-resistance to four or more antimicrobial agents was found among 72.0% and 15.0% of the isolates from diarrheic and healthy dogs, respectively, what is a reason for concern.

KEY-WORDS: Escherichia coli. Antimicrobial agents. Multidrug-resistance. Diarrheic dog. Healthy dog.

RESUMO

De janeiro a dezembro de 2006, 92 e 75 cepas de *Escherichia coli* isoladas de cães diarréicos e cães saudáveis respectivamente, foram examinadas para a detecção de resistência a 11 agentes antimicrobianos. As resistências predominantemente observadas foram para a estreptomicina (86,0%), cefalotina (71,0%), gentamicina (62,0%) e amoxicilina (59,0%) entre as cepas isoladas de cães diarréicos e para o ácido nalidixico (27,0%), cefalotina (25,0%), tetraciclina (19,0%) e ampicilina (19,0%) entre as cepas isoladas de cães saudáveis. A resistência a quatro ou mais agente antimicrobianos foi encontrada em 72,0% e 15,0% das cepas isoladas de cães diarréicos e saudáveis respectivamente, o que representa um motivo de preocupação.

PALAVRAS-CHAVE: *Escherichia coli*. Agentes antimicrobianos. Resistência a múltiplas drogas. Cão diarréico. Cão saudável.

INTRODUCTION

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The crisis of antimicrobial drug resistance in human medicine has brought into question every aspect of use of these drugs in animals while there is considerable through often fragmented data on antimicrobial drug resistance in bacteria of food animal origin (WEGENER et al., 1999, VAN DEN BOGAARD et al., 2000), there is little useful data on antimicrobial resistance in companion animals. Antimicrobial resistance is a very complex problem involving various bacterial species, resistance mechanisms, transfer mechanisms and reservoir. Cats and dogs represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans (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 usually used in human beings. The most frequent causes of antimicrobial treatment in dogs are skin and wound infections, otitis, respiratory infections, and urinary tract infections. Gastrointestinal infections are also common but antimicrobial therapy is not warranted in this case, however are still used (PRESCOTT et al., 2002, GUARDABASSI et al., 2004). The etiology of acute, non viral diarrhea in dogs is poorly understood. Of the known groups of diarrheagenic *E. coli*: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), shiga toxin producing *E. coli* (STEC), diffuse adherent *E. coli* (DAEC) and enteroaggregative *E. coli* (EaggEC). EPEC, ETEC and STEC have been associated with enteric disease in dogs (HAMMERMUELLER et al., 1995, BEUTIN, 1999, STAATS et al., 2003, SANCAK et al., 2004).

The prevalence and degree of antimicrobial resistance found in bacteria of the fecal microbiota of animals are considered good indicators of the selective pressure of antimicrobial usage, they correlate with the amount and types of antimicrobial agents consumed by animal populations (VAN DEN BOGAARD & STOBBERINGH, 2000, VAN DEN BOGAARD et al., 2000). Surveillance is important because they can be reservoirs of resistance determinant and because they are more ubiquitous than pathogens. The aim of the present study was to verify the antimicrobial resistance detected among *E. coli* isolates from diarrheic or healthy dogs.

MATERIAL AND METHODS

Sample collection

In order to study the antimicrobial susceptibilities of *E. coli* isolates to 11 antimicrobial agents, twenty-five diarrheic and ten healthy dogs, all of them adult dogs, were examined after arriving for clinical consultation in a private clinic in the Ituverava City, State of São Paulo, between January to December of 2006. Unfortunately, information about previous treatment with antimicrobial drugs was rarely given by the owners and our data therefore does not allow any meaningful comparison of this data. The sample collection was performed under the supervision of a veterinarian doctor by means of rectal swabbing with a sterile cotton swab. Samples were placed in Stuart transport medium and taken to a laboratory for immediate processing.

Culture

The samples were transferred to MacConkey Agar (Difco, Detroit,MI, USA) and incubated for 24 h at 37oC . At least five (diarrheic dogs) or ten (healthy dogs) colonies with characteristics of *E. coli* from each plate inoculated with rectal swab from diarrheic or healthy dogs respectively were selected for analysis. Biochemical confirmation of the strains was performed and *E. coli* was defined as oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative (KONEMAN et al., 1997).

Susceptibility Testing

Antimicrobial disk susceptibility tests were performed using the disk diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2002). Drug-impregnated disks (CEFAR, São Paulo, BR) were placed on the surface of the agar using a disk dispenser. The following eleven antimicrobial agents were tested: ampicillin (AMP,10µg); amoxicillin (AMO,10µg); amikacin (AMK,30µg); cephalothin (CFL,30µg); ceftriaxone (CEF,30µg); gentamicin (GEN,5µg); streptomycin (STR,10µg); nalidixic acid (NAL,30µg); cotrimoxazole (SUT, 25µg); ciprofloxacin (CIP,5µg).

RESULTS

A total of 92 and 75 *E. coli* strains were isolated from 25 diarrheic and 10 healthy dogs respectively. Among the isolates from diarrheic dogs the highest resistance was observed against streptomycin (86.0%), followed by that to cephalothin (71.0%), gentamicin (62.0%) and amoxicillin (59.0%), while among the healthy dogs the highest frequencies were for nalidixic acid (27.0%), cephalothin (25.0%), ampicilin (19.0%) and tetracycline (19.0%) (Table 1). Among diarrheic dogs only 1 isolate showed susceptibility to all antimicrobial agents tested, while among healthy dogs 15 isolates (20.0%) showed susceptibility to all of them (data not shown).

Multidrug-resistance characterized as resistance to four or more antimicrobial agents was found among both groups, but among diarrheic isolates the frequency was extremely high (72.0%, Figure 1).

Table 1- Resistance percentages of 92 and 75 *Escherichia coli* strains isolated from diarrheic and healthy dogs respectively, from Ituverava City, State of São Paulo, January-December 2006.

Antimicrobial drugs	Diarrheic dogs	Healthy dogs	*P* value *
Ampicilin	53.0	19.0	< 0,0001
Amoxicillin	59.0	4.0	< 0,0001
Cephalothin	71.0	25.0	< 0,0001
Ceftriaxone	4.0	4.0	1,00
Tetracycline	51.0	19.0	< 0,0001
Gentamicin	62.0	5.0	< 0,0001
Streptomicin	86.0	3.0	< 0,0001
Amikacin	39.0	1.0	< 0,0001
Nalidixic acid	47.0	27.0	0,01
Ciprofloxacin	23.0	9.0	0,02
Cotrimoxazole	39.0	11.0	< 0,0001

*: Fisher's exact test

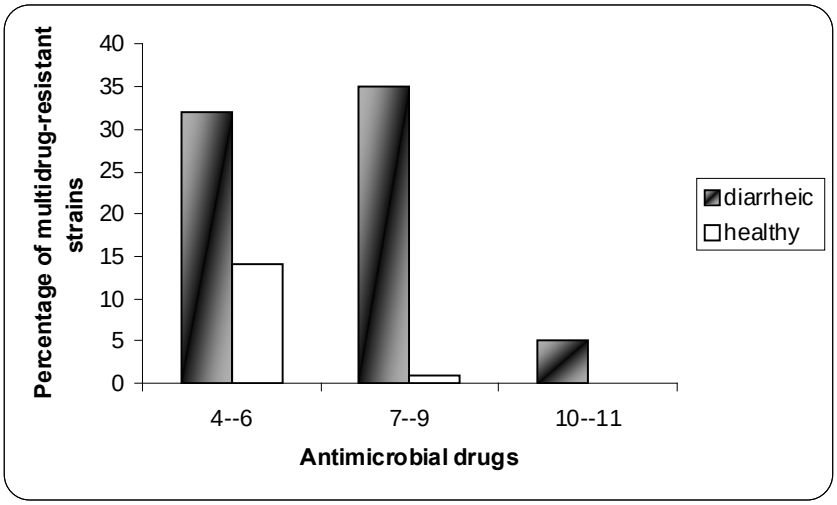

Figure 1- Distribution of multidrug resistance to 11 antimicrobial drugs among 92 and 75 strains of *Escherichia coli* isolated from diarrheic or healthy dogs, from Ituverava City, State of São Paulo, January-December 2006

DISCUSSION

Some authors advocated for a systematic surveillance of antimicrobial resistance among both pathogenic and indicator bacteria from companion animals (RANTALA et al., 2004, PEDERSEN et al., 2007). Various longitudinal retrospective studies in Europe and the United States have reported an increase in the prevalence of antimicrobial resistance in different bacterial species isolated from pet animals. Normand et al (2000) reporting from the United Kingdom, found significant rising trends for amoxicillin, amoxicillin/ clavulanic acid and streptomycin resistance in *E. coli*. Authier et al (2006) examined *E. coli* isolates from Canada in two periods 1992-1993 and 2002-2003, they found a diminution on the antimicrobial susceptibility of some antimicrobial agents after a 10 years period: amoxicillin/clavulanic acid 100% to 84%, ampicillin 85% to 67%, cephalothin 86% to 61%, enrofloxacin 100% to 97%, trimethoprin/sulfadiazine 100% to 92%, these values of susceptibility still were higher than those reported in the present study (Table 1).

Some studies indicate a possible association between antimicrobial use and emergence of antimicrobial resistance in pets. For example, the increased use of lincosamide observed in Sweden during the period 1990-1998 corresponded with a parallel increase in lincosamide resistance among staphylococcal isolates from canine pyoderma (HOLM et al., 2002). The high values of antimicrobial resistance found in the present study could indicate an inadequate use of these drugs in Brazil.

Although the authorization of fluoroquinolones for use in small animal veterinary practice is quite recent in

Europe (mid 1990s), resistance to this antimicrobial class is emerging in pet animal bacteria (COHN et al., 2003). In Brazil the use is more recent (end 1990s) but the resistance seems to be higher (Table 1) what could indicate an intense and maybe unspecific utilization.

Recent reports of extraintestinal infections in dogs due to multidrug-resistant *E. coli* (MDREC) with resistance to third-generation cephalosporins and fluoroquinolones are a potential public health concern (WARREN et al, 2001, SANCHEZ et al., 2002). Canine MDREC have been shown to possess class 1 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).

Carattoli et al (2005) analyzed 298 *E. coli* isolates obtained from specimens from 204 dogs submitted for routine diagnostic investigation in Italy between 2001 and 2003. The percentage of resistance reported as quite similar to the present study for the tetracycline, nalidixic acid, cotrimoxazole and fluoroquinolones but very different to gentamicin (8.1%) and amikacin (0.7%) in the isolates from healthy dogs but less than reported here for the diarrheic dogs. However, the frequency of multidrug-resistant strains was only 7.0% of the isolates what is much less than reported in the present study for the diarrheic dogs (72.0%, Figure 1).

Although, in general, it appears that widespread antimicrobial drug use has led to the on-going development and refinement of mechanism for spreading resistance, resistant bacteria isolated from companion animals are conspicuous by their absence as key organisms that have been studied to understand resistance gene organization. In other words, no sufficiently dramatic resistance event in bacteria isolated from dogs has attracted anyone's attention (PRESCOTT et al., 2002).

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. To conclude, the present study shows high frequencies of antimicrobial resistance among the *E. coli* isolates examined as well as an extremely high number of isolates showing multidrug-resistance, constituting a reason for concern.

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