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Resistance patterns, ESBL genes, and genetic relatedness of *Escherichia coli* from dogs and owners

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ABSTRACT

Antimicrobial resistance in *Escherichia coli* isolated from pet dogs can be considered a potential threat of infection for the human population. Our objective was to characterize the resistance pattern, extended spectrum beta-lactamase production and genetic relatedness of multiresistant *E. coli* strains isolated from dogs ($n = 134$), their owners ($n = 134$), and humans who claim to have no contact with dogs ($n = 44$, control), searching for sharing of strains. The strains were assessed for their genetic relatedness by phylogenetic grouping and pulsed-field gel electrophoresis. Multiresistant *E. coli* strains were isolated from 42 (31.3%) fecal samples from pairs of dogs and owners, totaling 84 isolates, and from 19 (43.1%) control group subjects. The strains showed high levels of resistance to ampicillin, streptomycin, tetracycline, trimethoprim and sulfamethoxazole regardless of host species or group of origin. The bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes were detected in similar proportions in all groups. All isolates positive for *bla* genes were ESBL producers. The phylogenetic group A was the most prevalent, irrespective of the host species. None of the strains belonging to the B2 group contained *bla* genes. Similar resistance patterns were found for strains from dogs, owners and controls; furthermore, identical PFGE profiles were detected in four (9.5%) isolate pairs from dogs and owners, denoting the sharing of strains. Pet dogs were shown to be a potential household source of multiresistant *E. coli* strains.

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Introduction

The implications of microbial resistance for public health have increased the interest of the scientific community in the presence and circulation of resistant organisms between pets and the human population. Among pets, dogs share a close proximity with humans, and proximity is known to increase the possibility of transmitting resistant bacteria between these host species. It is important to note that the bacterial ability to transfer genetic cassettes, which confer resistance to several classes of drugs, and observations of the spread of resistance have increased in recent years.[1,2] The molecular characterization of antimicrobial resistance could be very useful not only in surveillance studies and monitoring and tracking of multidrug-resistant strains but also in obtaining information about commonality among human and animal bacterial isolates.

Escherichia coli is characterized by a substantial genetic diversity, broad host range, versatility in pathogenic potential, and distribution between hosts in the environment.[3] *E. coli* isolates, commensal or pathogenic, obtained from humans and other animals, have been extensively studied and characterized in terms of their drug resistance profiles based on their phenotypic sensitivity to various antimicrobial drugs as well as by their genetic resistance to major classes of antibiotics detected by molecular assays of genetic similarities among isolates.[4–6] Similarities in resistance profiles among isolates have been described, and the molecular characterization of these isolates has demonstrated substantial differences depending on the population and the geographic region of origin. In general, comparisons of resistance profiles are performed on samples of individuals who are not necessarily cohabiting and are therefore not epidemiologically related. Further studies are needed to characterize related human and dog isolates in Brazil as well as to investigate the possibility of humans and canines sharing multiresistant strains. The aim of this study was to compare resistance profiles of isolates of *E. coli* from the intestinal tracts of dogs and their owners and to assess the presence of extended-spectrum beta-lactamase (ESBL) genes in *E. coli* isolates recovered from dogs and humans living in the same household. In addition, we intended to characterize the genetic relatedness among antimicrobial-resistant isolates.

Materials and methods

Study design

A total of 134 fecal sample pairs from dogs and their owners of households located in the city of Rio das Ostras (Rio de Janeiro, Brazil) were studied. Every owner (>18 years old) chosen for the study had only one dog. A control group of 44 fecal samples from individuals who claimed have no contact with dogs was also included in the study. The samples were collected for one year (2010), and the human and dog participants had not been using antimicrobials for at least three months prior to the study. Bacterial strains were selected using a protocol for growth in selective media containing antibiotics. The protocol of this study was submitted to the Research Ethics Committee of HUAP/UFF (CAAE0146.0.258.000-09) and the Animal Ethics Committee of the NAL/UFF (NAL00126-09), and it was approved and certified by both committees.

Bacterial samples

Fecal samples on swabs from dogs and humans were obtained and processed for testing. Typical *E. coli* colonies were selected and tested for antimicrobial susceptibility. After enrichment growth in *E. coli* broth containing gentamicin (8 μg/mL) and cephalothin (32 μg/mL), the samples were cultured on MacConkey agar (Himedia, Mumbai, India) supplemented with gentamicin (8 μg/mL) and cephalothin (32 μg/mL) to promote the growth of potentially multidrug-resistant *E. coli* strains.[7] Samples that did not grow were discarded. For quality control purposes, each sample was also inoculated into medium without antibiotics. Three colonies, believed to be *E. coli*, were chosen from the MacConkey agar containing antibiotics and identified based on standard biochemical tests.[8]

The characterization portion of this study included only isolates that displayed multidrug resistance, which was defined as resistance to two or more classes of drugs. With our protocol of selective isolation and susceptibility testing, one hundred and three multidrug-resistant *E. coli* isolates were identified from dogs ($n = 42$), their respective owners ($n = 42$) and control subjects ($n = 19$).

Antimicrobial susceptibility testing

E. coli isolates were tested for antimicrobial susceptibility to the drugs ampicillin (AMP), amoxicillin + clavulanate (AMC), cephalexin (CEF), chloramphenicol (CLO), trimethoprim-sulfamethoxazole (SUT), streptomycin (EST) + gentamicin (GEN), doxycycline (DOX) + tetracycline (TET), ciprofloxacin (CIP), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (CPM), cefotaxime (CTX) and aztreonam (ATM) using Clinical Laboratory Standards Institute (CLSI) methodologies and interpretive criteria.[9,10]

Disk approximation tests using the drugs AMC, CAZ, ATM, CTX and CPM were performed on all isolates to screen for ESBL producers as previously described[11] and according to CLSI recommendations (2012 S22). The bacterial strains *E. coli* ATCC 25922 and *Klebsiella pneumoniae* 700603 were used as control strains.

Characterization and identification of beta-lactamase genes

The presence of the beta-lactamase (*bla*) genes bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{SHV} was detected by polymerase chain reactions (PCR)in third- and fourth-generation cephalosporin- and ATM-resistant strains classified as those exceeding the breakpoints recommended by the CLSI (2012) and those that tested positive in disk approximation experiments.[11] The primers and amplification conditions used in PCR assays are listed in Table 1. The bacterial strains used as positive controls in PCR reactions for the detection of *bla* genes were: *E. coli* H21, *E. coli* A41 (previously characterized[12]), and *K. pneumonia* (ATCC 700603) for genes bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{SHV}, respectively. *E. coli* DH5α was used as a negative control.

Table 1 – Primers and amplification conditions used in PCR reactions for the detection of ESBL genes and phylogenetic characterization.

Gene or target region	Primers	Amplicon size (bp)	Annealing temperature (°C)	Reference
bla_{TEM}	**T1** ATTCTTGAAGACGAAAGGGCCT **T3** TTGGTCTGACAGTTACCAATGC	1100	55	Wiegand et al.[14]
bla_{SHV}	**S1** ATGAGTTATATTAGAATGGT **S2** GTTAGCGTTGCCAGTGCTCG	860	58	Fu et al.[15]
bla_{CTX}	**CTM-MA** CGCTTTGCGATGTGCAG **CTX-MB** ACCGCGATATCGTTGGT	550	60	Bonnet et al.[16]
chu A	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	55	Clermont et al.[13]
yja A	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211		
tspE4.C2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152		

Determination of phylogenetic groups

The *E. coli* isolates were subjected to phylogenetic classification according to the methodology described by Clermont et al.[13] based on standard PCR amplification of the genes *yja*A, *chu*A and a DNA fragment TspE4.C2. The primers and amplification conditions used are shown in Table 1.

PFGE assays

Paired isolates of *E. coli* from dogs and owners presenting similar profiles of resistance and ESBL resistance genes were selected for pulsed field gel electrophoresis (PFGE) of XbaI digested total DNA according to a standardized methodology of the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/pulsenet/pathogens/index.html). The generated PFGE profiles of within-household isolates were compared according criteria proposed by Tenover[17]: indistinguishable (I) – nodistinct bands; closely related (CR)-2-3 distinct bands; possibly related (PR) – 4–6 distinct bands; different (D) – more than seven distinct bands. Restriction fragment profiles were also compared by using the Bio Numerics® software version6.6 (Applied Maths, Austin, TX), using the Dice similarity index and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method to construct the dendrograms. *E. coli* isolates with ≥94% PFGE similarly were considered to be clonal.[5]

Statistical analysis

The prevalence of resistance to antimicrobials, the presence of ESBL genes and the distribution of phylogenetic groups among canine, human and control group samples were compared using the Chi-squared test with $p \leq 0.05$ used as the criterion for statistical significance.

Results and discussion

Multiresistant *E. coli* strains were selectively isolated from 42 (31.3%) fecal sample pairs from dogs and their owners, totaling 84 isolates, and from 19 (43.1%) control group subjects. The frequency of multiresistant *E. coli* obtained from fecal samples can reach 89%, according to some studies, with frequencies that vary according to the selection criteria.[18–21] To recover strains that exhibited potential resistance to multiple antimicrobials, we used cephalothin. Cephalothin resistance is clinically significant because it is frequently found in situations without strong selectivity (water reservoirs and non-hospital locations). Cephalothin resistance is also associated with other resistance markers, including markers for chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole, which are considered common markers in our categorization. Gentamicin was also used in our identification of multidrug resistance, due to the high correlation between gentamicin resistance and multiresistance profiles in nosocomial strains.[7] Because of this selective isolation protocol, some samples did not grow in the presence of the antimicrobials mentioned, and thus we may have lost some multiresistant strains sensitive to the drugs used in the selection. All of the isolates included in our study, however, were resistant to at least two drug classes, presumably because they were all subjected to a selective isolation procedure. When the same resistance profile was observed in a dog and owner pair, a different method of selection was used to enhance the detection of lower prevalence clones. This method, described by Damborg and colleagues,[22] entails selecting colonies that grow in the inhibition zones formed by the drugs amikacin, gentamicin, ampicillin, ciprofloxacin, cefotaxime, and sulfamethoxazole-trimethoprim on MacConkey agar, and submitting these for identification. This was done to enhance the identification of multidrug-resistant strains common to both dogs and owners of subject pairs.

Among the resistant *E. coli* fecal isolates, 74 patterns were observed, each with resistance to at least two drug classes (data not shown). Across all groups, the most frequent resistance patterns were AMP/EST (13.6%) followed by AMP/EST/SUT (3.9%). There were no significant differences in the resistance frequencies among the tested drugs except for sulfamethoxazole-trimethoprim for which the human isolates from owners and control group subjects had statistically higher resistance frequencies than the canine isolates ($p \leq 0.05$). Other studies showed few patterns comparable to our data.[21–23] In a similar study, Skurnik et al.[24] compared fecal *E. coli* from different animal species and noted that the resistance prevalence of strains was similar, but dogs showed

Table 2 – Numbers of resistant *E. coli* isolates of fecal origin (humans and dogs) for the drugs tested.

Antimicrobials	Number of resistant isolates (%)		
	Dogs $n=42$	Humans (owners) $n=42$	Humans (control) $n=19$
AMP	36 (85.7)	31 (73.8)	17 (89.4)
AMC	15 (35.7)	10 (23.8)	4 (21.1)
CFE	14 (33.3)	13 (30.9)	8 (42.1)
CAZ	7 (16.6)	3 (7.1)	4 (21.1)
CPM	4 (9.5)	3 (7.1)	3 (15.8)
CTX	8 (19.4)	6 (14.3)	2 (10.5)
CRO	9 (21.4)	6 (14.3)	5 (26.3)
ATM	7 (16.6)	6 (14.3)	5 (26.3)
GEN	13 (30.9)	17 (40.5)	11 (57.8)
EST	28 (66.6)	27 (64.3)	11 (57.8)
CLO	10 (23.8)	14 (33.3)	5 (26.3)
CIP	5 (11.9)	9 (21.4)	2 (10.5)
SUT	13 (30.9)	23 (54.7)	12 (63.2)
TET	21 (50.0)	22 (52.3)	11 (57.8)
DOX	14 (33.3)	11 (26.2)	5 (26.3)

AMP, ampicillin; AMC, amoxicillin + clavulanic acid; CFE, cephalexin; CAZ, ceftazidime; CPM, cefepime; CTX, cefotaxime; CRO, ceftriaxone; ATM, aztreonam; GEN, gentamicin; EST, streptomycin; CLO, chloramphenicol; CIP, ciprofloxacin; SUT, sulfamethoxazole + trimethoprim; TET, tetracycline; DOX, doxycycline.

the highest levels of multiresistant strains like those found in humans. These data are similar to those obtained in our study.

In our study, the highest frequency of resistance was recorded for ampicillin, tetracycline, streptomycin, and trimethoprim-sulfamethoxazole (Table 2). Resistance to streptomycin, ampicillin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole has been observed in other studies of dog fecal isolates[18–21,23,25] and human fecal isolates.[26,27] Aminopenicillins and aminoglycosides appear most often among the three classes of drugs with high frequencies of resistance in studies of samples of animal origin.[6,21,23,28–34] Strains resistant to the carbapenems tested in this study were not detected.

Importantly, the strains analyzed in this study were selected using media containing antibiotics, and although resistance rates may have been higher than reported elsewhere, the prevalence data, especially for ampicillin and streptomycin, were similar to those observed in other studies. Stenske et al.[5] also compared the resistance profiles of fecal strains from dogs and their owners, obtaining high frequencies of resistance to cephalothin, ampicillin, and streptomycin in both groups, a result comparable to our current findings, although our frequencies were higher and we used cephalexin rather than cephalothin. Harada et al.[35] observed similarities among isolates from dogs and humans; however, they showed significant differences between the groups analyzed in terms of which drugs corresponded to the highest rates of resistance.

Comparisons of strains of clinical origin has also been a focus of study of the epidemiology of antimicrobial resistance and has shown that the drugs ampicillin, streptomycin and sulfamethoxazole-trimethoprim exhibit high frequencies of resistance in strains of both human origin[36–38] and dog origin.[6,28–33] With regard to isolates from dogs, resistance to the first-generation cephalosporins, including cephalothin and cephalexin, was also identified in the studies cited. Differences in the proportions of resistance have been noted in relation to the site of infection, for example, in urinary infection, pyometra and osteomyelitis, in which different levels of resistance to the same drugs were encountered.[18,39–41] Some studies characterizing resistance in strains originating from human patients have analyzed the prevalence of resistant infections in samples from hospital patients and outpatients.[42] These studies show many differences in resistance prevalence, related not only to the origin of the samples but also to the site of infection, the continent and country where the study took place as well as the profiles of the patients from which the samples originated. Differences are also attributed to the repertoire of antibiotics used in the study.

In the current study, 28.5% of the *E. coli* isolates from dogs were resistant to at least one antibiotic belonging to the third- or fourth-generation cephalosporins and were positive for ESBL by disk-approximation test. This profile occurred in 16.6% and 36.8% of isolates from dog owners and control subjects, respectively. These differences, however, were not significant. Resistance to third-and fourth-generation cephalosporins is suggestive of beta-lactamase production by the isolates. In our tests, we detected beta-lactam resistance genes of the bla_{TEM}, $bla_{\text{CTX-M}}$ and bla_{SHV} families in isolates of both human and dog origin (Table 3).

We detected at least one ESBL encoding gene in 26.2% of the *E. coli* isolates and all of them were also positive in the phenotypic tests. However, five isolates (8H, 9H, 57H, 22F, 38F), despite presenting phenotypes suggestive of ESBL production, did not test positive for any of the three *bla* genes surveyed in our study. Other ESBL genes may be present in these strains because the OXA and CMY gene families are also common in *E. coli*.[43]

The $bla_{\text{CTX-M}}$ gene was significantly more prevalent in strains from the control group than in the other two groups; furthermore, all ESBL positive isolates in this group were positive for CTX-M. In dog isolates, the bla_{TEM} gene was present in 66.7% of the strains that tested positive for ESBL genes, and

Table 3 – Phylogenetic groups and ESBL genes among fecal *E. coli* isolates from dogs, their owners, and control human subjects.

Class	Type	Number of Isolates (%)			Total (%)
		Dogs ($n=42$)	Owners ($n=42$)	Controls ($n=19$)	
Phylogenetic group	A	21 (50)	24 (57.1)	10 (52.6)	55 (53.4)
	B1	09 (21.4)	08 (19.1)	02 (10.5)	19 (18.4)
	B2	03 (7.2)	04 (9.5)	00 (0)	07 (6.9)
	D	09 (21.4)	06 (14.3)	7 (36.9)	22 (21.3)
ESBL gene[a]	*bla*TEM	3	4	0	
	*bla*TEM/*bla*CTX-M	4	1	3	
	*bla*CTX-M	4	2	4	
	*bla*TEM/*bla*CTX-M/*bla*SHV	1	1	0	
	Total	12 (28.6)	8 (19.0)	7 (36.8)	27 (26.2)

[a] All isolates carrying an ESBL gene were also positive in phenotypic tests.

the bla_{CTX-M} gene was detected in 75% of these isolates. Only 8.4% of the dog isolates were positive for the bla_{SHV} gene. In human strains, the prevalent ESBL gene family was the bla_{TEM} family. The bla_{CTX-M} gene was present in similar proportions in strains of human and dog origin from the same household. The bla_{SHV} gene was detected in 8.4% of the human isolates.

The detection of ESBL genes in isolates of pet dogs was first published in 1988.[43] Other studies have also reported the presence of the *bla* genes in isolates from dogs, especially isolates of clinical origin. Different subtypes of the *bla* gene CTX-M are often detected in extraintestinal clinical isolates[44,45] and in fecal isolates.[22,29,46] The bla_{TEM} gene, detected in clinical strains from dogs since 2002,[6,28,44,45,47] was also found in intestinal isolates.[19,21,29,46] For the gene bla_{SHV}, data are similar, with detection in both clinical[29,44] and fecal isolates.[6,29,48] The prevalence of these genes in dog *E. coli* strains, however, is not well established.

The prevalence of ESBL genes in human clinical specimens varies considerably, depending on the origin of the strains, whether the infection originated in a hospital or in a community setting, and on the site of infection. Generally, isolates from infections acquired in hospitals exhibit more complex profiles with broader repertoires of resistance compared to isolates of community-acquired infections. Recent data indicate a prevalence of ESBL bla_{CTX-M} genes in the bloodstream[49,50] and in other infections in hospital settings.[51] Less than a decade ago, TEM and SHV ESBL isolates were thought to be limited to the hospital environment, and CTX-MESBL isolates were detected in urinary tract infections in community settings.[52] Currently, the most prevalent ESBL gene identified in isolates across many countries is the CTX-M gene, particularly the CTX-M-15 gene, which is associated with IncF variant plasmids.[53] The epidemiological profile of highly mutable enterobacteria can be considered an additional factor in this variability, which greatly influenced the spread of plasmids and other transposable elements acting as mobile carriers of multiple resistance genes against drugs, including aminoglycosides, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole.[54]

In our study, the distribution of phylogenetic groups in the host species examined was similar in all groups, with the phylogenetic group A being the most prevalent (Table 3), occurring in 50% of the canine strains, 57.2% of the human strains and 52.6% of the control group. For fecal strains, this finding is consistent with literature that describes the majority of isolates from commensal strains, regardless of their repertoire of resistance, belonging to groups A or B1.[55]

The distribution of phylogroups among dog isolates, however, has been shown to differ in terms of prevalence in extraintestinal, commensal and clinical samples. In the case of extraintestinal isolates, where the predominant phylogroups were expected to be groups B2 and D, Gibson et al.[32] found no isolates belonging to group B2, whereas Maynard et al.[6] reported a 63% frequency of the B2 phylogroup in extraintestinal samples. Moreover, commensal strains from animals in general predominantly included the groups A and B1. Fecal strains from dogs in a study by Harada et al.[35] showed a high prevalence of group B2. Davis et al.[25] also observed a predominance of B2 (and D) in their isolates from different anatomical regions of healthy dogs, including the rectal area.

Studies comparing human and canine isolates in terms of antimicrobial resistance profiles used samples of clinical origin, which had a higher prevalence of groups B2 and D, as expected. However, Harada et al.[35] analyzed fecal samples from dogs and their owners without using antimicrobial agents for selective isolation, and they observed a higher prevalence of group B2, whereas Damborg et al.[22] reported that in their experiments, human fecal isolates more often belonged to group A and the dog isolates to group B1. A study by Clermont et al.[4] showed that extraintestinal infection is mostly caused by *E. coli* strains of group B2. An assessment of extraintestinal clinical isolates from dogs revealed that the two main groups of *E. coli* resistant to fluoroquinolones are A and D.[56] Data reported in a study by Skurnik et al.,[57] however, suggested that clinical isolates from group B2 are less resistant to antimicrobials than non-B2 isolates.

In our tests, 12 ESBL isolates belonged to group A and 10 to group D, and no ESBL isolates carrying the genes bla_{TEM}, bla_{SHV} and bla_{CTX-M} were included in group B2. Our data suggest that resistant commensal *E. coli* strains do not necessarily belong to the phylogroups expected from resistant pathogenic strains. The explanation for this observation requires further investigation.

Table 4 – Resistance, ESBL genes and genetic relatedness of pairs of *E. coli* isolates of fecal origin (dogs and humans within a household) with similar profiles.

Isolate[a]	Host species	Resistance phenotype[b]	ESBL genes			Phylogroup	PFGE[c]
			bla_{TEM}	bla_{CTX-M}	bla_{SHV}		
15F	Dog	AMP AMC CFE CAZ CTX ATM	–	+	–	D	–
15H	Human	AMP AMC CFE CTX CRO	–	+	–	A	–
23F	Dog	AMP EST CLO SUT TET	–	–	–	D	I
23H	Human	AMP EST CLO SUT TET	–	–	–	A	I
27F	Dog	AMP CFE EST	–	–	–	B1	D
27H	Human	AMP CFE EST	–	–	–	A	D
48F	Dog	AMP CFE CAZ CTX CRO GEN EST CLO SUT	+	+	–	A	I
48H	Human	AMP CFE CTX CRO GEN EST CLO SUT TET	+	+	–	B1	I
49F	Dog	AMP AMC CFE CPM CTX CRO GEN EST CLO CIP SUT TET	–	+	–	B1	D
49H	Human	AMP CFE CPM CTX CRO GEN EST CLO SUT TET	+	–	–	B1	D
59F	Dog	AMP EST	–	–	–	B1	D
59H	Human	AMP EST	–	–	–	B1	D
61F	Dog	AMP AMC EST CLO SUT TET DOX	–	–	–	B2	I
61H	Human	AMP AMC EST CLO SUT TET DOX	–	–	–	B2	I
58F	Dog	AMP AMC EST CLO	+	–	–	B1	–
58H	Human	AMP EST CLO	–	–	–	B1	–
92F	Dog	AMP CAZ ATM GEN EST CIP SUT TET DOX	+	+	+	B1	I
92H	Human	AMP ATM GEN EST CIP SUT TET DOX	+	–	+	B1	I

[a] The letter F designates canine fecal isolates; H, human fecal isolates (dog owners).
[b] AMP, ampicilin; AMC, amoxicillin + clavulanic acid; CFE, cephalexin; CAZ, ceftazidime; CPM, cefepime; CTX, cefotaxime; CRO, ceftriaxone; ATM, aztreonam; GEN, gentamicin; EST, streptomycin; CLO, chloramphenicol; CIP, ciprofloxacin; SUT, sulfametoxazol + trimethoprim; TET, tetracycline; DOX, doxycycline.
[c] Tenover criteria applied for each isolate pair from the same household: I, indistinguishable; D, different; – not possible to compare due the DNA degradation of one isolate during the PFGE restriction.

The presence of ESBL genes was similar in *E. coli* isolates from dogs and from their owners, especially in some human and dog isolates from the same household. Thus, 18 *E. coli* isolates comprising nine isolate pairs from dogs and owners that presented similar antimicrobial resistance, ESBL genes and phylogenetic group profiles were selected for PFGE assays (Table 4). By applying the Tenover criteria to each isolate pair from the same household, we detected four isolate pairs with identical patterns. The dendrogram constructed with the PFGE profiles of these isolates reinforces the relatedness between these four strain pairs, and it shows a strong similarity to an isolate pair with another dog isolate (Fig. 1).

Some strains recovered from dogs and owners showed phenotypic and genotypic similarities suggesting a clonal relationship. Indeed, we demonstrated in this study the sharing of multiresistant *E. coli* strains in 9.5% (4/42) of the pairs of isolates from the dogs and their owners that we studied.

In comparisons between *E. coli* isolates of human and dog origin, many similarities have been identified in the various traits studied, especially with regard to the repertoire of

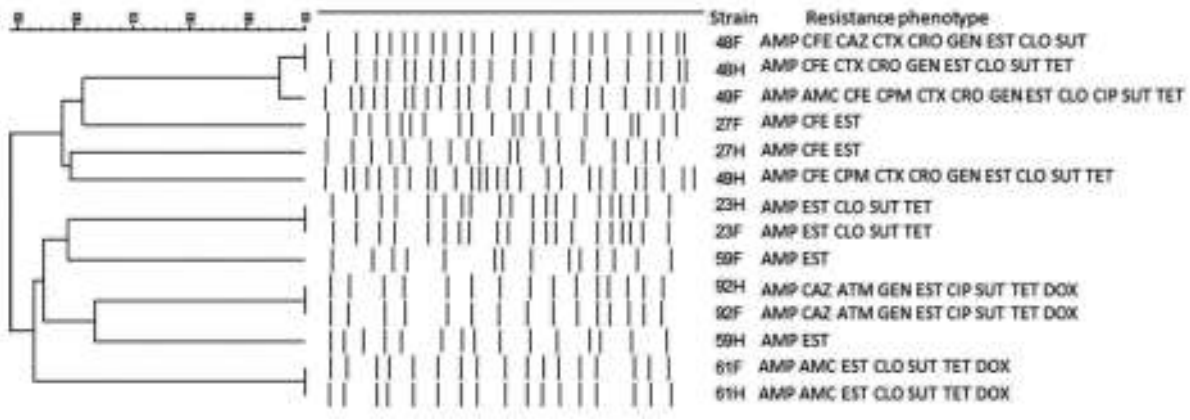

Fig. 1 – Dendrogram showing genomic PFGE fingerprint patterns of fecal *E. coli* isolates from dogs (F) and owners (H) with similar resistance profiles. The dendrogram was constructed using the Dice similarity coefficient and UPGMA clustering methods. The degree of similarity (%) is shown on the scale bar.

virulence of the strains.[5,6,35,58,59] It has been suggested that the host specificity of *E. coli* strains is determined by specific adhesions.[4] Studies have shown a similar occurrence of virulence factors associated with extraintestinal pathogenic *E. coli* in strains isolated from dogs and humans.[6,58,60] Some studies suggest that uropathogenic *E. coli* strains are shared between dogs and humans,[58] whereas others show a significant diversity in the virulence repertoires of strains isolated from distinct species or even within the same species.[6,35]

Regarding the genetics of resistance, the data are somewhat heterogeneous and differ depending on the population and the geographic region of origin as well as on whether the strains are commensal or pathogenic.[5,35,48] Despite the fact that a positive correlation between the presence of plasmids and integrons with a multiresistant phenotype is described in several studies,[24,61] additional factors that shape the genetic structure of these strains are important.[55,62] Comparisons of resistance profiles are not always performed on strains from cohabiting individuals, and samples are therefore not always epidemiologically related. The characterization, therefore, of related human and canine isolates, as well as the possibility of sharing multiresistant strains between humans and dogs, requires further study in Brazil.

Conclusion

We detected the simultaneous presence of multidrug-resistant *E. coli* in dogs and their owners, albeit with different phenotypic profiles of antibiotic resistance, especially for ESBLs. Furthermore, some strains from dogs and humans of the same household had similar resistance patterns, ESBL genes, identical phylogenetic groups and identical or closely related PFGE profiles. These data demonstrate high degrees of homology and therefore the possibility that resistant *E. coli* clones are circulating between individuals and animals in the same environment. Indeed some strains showed clonal relationships indicating within-household sharing.

In addition, our data suggest that the prevalence of antimicrobial-resistant commensal *E. coli* is very similar in dogs and humans and that there is no difference among the resistant *E. coli* isolated from dog-owners and humans without pet dogs.

This area needs more accurate investigations and many uncertainties remain regarding how resistance arises and spreads in the environment and among cohabiting humans and domestic animals.

Conflicts of interest

The authors declare no conflicts of interest.

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