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1 **Revised version**

2 **Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia***
3 ***coli* isolates recovered from healthy pets**

4

5 Daniela Costa^{1,4}, Patricia Poeta^{1,2}, Yolanda Sáenz⁴, Ana Cláudia Coelho¹, Manuela
6 Matos^{1,3}, Laura Vinué⁴, Jorge Rodrigues^{1,2} and Carmen Torres^{4*}

7 ¹Universidade de Trás-os-Montes e Alto Douro; Departamento de Ciências
8 Veterinárias; Vila Real, Portugal; ²Centro de Estudos de Ciência Animal e Veterinária,
9 Vila Real, Portugal; ³Departamento de Genética e Biotecnología/Instituto de
10 Biotecnología e Bioingeniería, Vila Real, Portugal; ⁴Area de Bioquímica y Biología
11 Molecular, Universidad de La Rioja, Logroño, Spain

12

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15

16 **Corresponding author.**

17 Carmen Torres

18 Área de Bioquímica y Biología Molecular

19 Universidad de La Rioja

20 Madre de Dios, 51

21 26006 Logroño, Spain

22 FAX: 34-941299721

23 Phone: 34-941299750

24 e-mail: carmen.torres@unirioja.es

25 **Abstract**

26 Faecal samples of healthy dogs (n=39) and cats (n=36) obtained in Northern Portugal
27 were seeded on Levine agar plates, and two *Escherichia coli* isolates per sample were
28 recovered (78 of dogs and 66 of cats). The susceptibility to 16 antimicrobial agents was
29 tested in this series of 144 *E. coli* isolates. Almost 20% of them showed tetracycline
30 resistance and 12 and 15% presented ampicillin or streptomycin resistance, respectively.
31 The percentage of resistance to the other antimicrobial agents was in all cases below 4%
32 and no resistant isolates were detected for ceftazidime, imipenem, ceftiofur or amikacin.
33 Two isolates (from one dog) showed cefotaxime-resistance and harboured both the
34 CTX-M-1 and OXA-30 beta-lactamases. A *bla*_{TEM} gene was detected in 12 of 17
35 ampicillin-resistant isolates, the *aac*(3)-II gene in the three gentamicin-resistant isolates,
36 *aadA* in seven of 22 streptomycin-resistant isolates, and *tet*(A) and/or *tet*(B) gene in all
37 28 tetracycline-resistant isolates. The gene encoding class 1 integrase was detected in
38 six *E. coli* isolates, including the four trimethoprim-sulfamethoxazole-resistant isolates
39 and those two harbouring CTX-M-1 and OXA-30 beta-lactamases; different gene
40 cassette arrangements were identified: *dfrA1+aadA1* (2 isolates), *dfrA12+orfF+aadA2*
41 (2 isolates) and *bla*_{OXA30+} *aadA1* (2 isolates). One amino acid change in GyrA protein
42 (Ser83Leu or Asp87Tyr) was detected in four nalidixic-acid-resistant and ciprofloxacin-
43 susceptible isolates and two amino acid changes in GyrA (Ser83Leu + Asp87Asn) and
44 one in ParC (Ser80Ile) were identified in one nalidixic acid- and ciprofloxacin-resistant
45 isolate. Faecal *E. coli* isolates of healthy pets harbour could be a reservoir of
46 antimicrobial resistance genes.

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50 **1. Introduction**

51 In the last years there is a great concern about the problem of antimicrobial
52 resistance either in human and in animal medicine, that is associated with failures in the
53 treatment of infectious diseases. The high use of antimicrobial agents in humans and in
54 animals is probably the main cause of this situation (Authier et al., 2006). These agents
55 exert a selection pressure not only on pathogenic bacteria, but also on commensal
56 microorganisms of the intestinal tract of humans and animals, and resistant commensal
57 bacteria constitute a reservoir of resistant genes for potentially pathogenic bacteria (van
58 den Bogaard and Stobberingh, 2000; Guardabassi et al., 2004; Moyaert et al., 2006; de
59 Graef et al., 2004). *Escherichia coli* is commonly found in the intestinal tract of
60 animals and humans (Tannock, 1995; Sørnum and Sunde, 2001), and can also be
61 implicated in animal and human infectious diseases (Sáenz et al., 2001; Rosas et al.,
62 2006). For this reason faecal *E. coli* is considered as a very good indicator for selection
63 pressure by antimicrobial use and for resistance problems to be expected in pathogens
64 (van den Bogaard and Stobberingh, 2000).

65 Cats and dogs are companion animals that are in close contact with humans
66 since ancient times, being possible the transference of bacteria between animals and
67 humans (Guardabassi et al., 2004). Various authors have studied antimicrobial
68 resistance in *E. coli* isolates recovered from pets and these studies have been performed
69 either in Europe (Nordman et al., 2000; Guardabassi et al., 2004; Carattoli et al., 2005;
70 Moyaert et al., 2006), as well as in other continents (Authier et al., 2006; Ogeer-Gyles
71 et al., 2006). In Portugal there are studies about antimicrobial resistance in *E. coli*
72 isolates recovered from human clinical samples (Mendonça et al., 2006; Machado et al.,
73 2006), healthy humans (Machado et al., 2004), pigs (Pena et al., 2004), and clinical
74 samples of pets (Féria et al., 2002); nevertheless, to our knowledge, there is only one

75 previous study carried out on healthy pets (Costa et al., 2004), but in that case the
76 unique objective was to detect the presence of *E. coli* isolates harbouring extended-
77 spectrum beta-lactamases. The aim of our present study is to investigate the prevalence
78 of antimicrobial resistances in faecal *E. coli* isolates recovered from healthy dogs and
79 cats in Portugal and the mechanisms of resistance implicated in order to assess the
80 possible role of the faecal *E. coli* isolates of pets as a reservoir of antimicrobial
81 resistance.

82

83 **2. Material and Methods**

84 **Samples and bacterial isolates.** Seventy-five faecal samples of healthy pets (39 of
85 dogs and 36 of cats) were included in this study. They were obtained from individually
86 owned animals in two cities of Northern Portugal in 2003, and they were collected
87 either during routine examination of the animals at two veterinary clinics (one located in
88 Porto and the other in Vila Real) or directly by their owners. None of the animals had
89 taken antimicrobials during the four months prior to sampling. All the samples were
90 seeded on Levine agar plates and incubated at 37 °C for 24 h. Two colonies per sample
91 with typical *E. coli* morphology were selected and identified by classical biochemical
92 methods (gram, catalase, oxidase, indol, Methyl-Red-Voges-Proskauer, citrate and
93 urease), and by the API 20E system (BioMérieux, La Balme Les Grottes, France).

94 **Antimicrobial susceptibility testing.** Antimicrobial susceptibility was performed by
95 the agar disk diffusion method as recommended by the Clinical and Laboratory Standards
96 Institute (CLSI, 2007), and a total of 16 antimicrobial agents were tested: ampicillin,
97 amoxicillin-clavulanic acid (AMC), cefotaxime, cefoxitin, ceftazidime, imipenem,
98 aztreonam, gentamicin, tobramycin, amikacin, streptomycin, tetracycline, trimethoprim-
99 sulfamethoxazole (SXT), nalidixic acid, ciprofloxacin and chloramphenicol. The

100 isolates with resistance to one or more antimicrobial agents were selected for the
101 characterization of antimicrobial resistance genes.

102 **Characterization of antimicrobial resistance genes.** The presence of genes encoding
103 TEM, SHV, OXA, CTX-M, and CMY beta-lactamases were studied by PCR in all
104 ampicillin-resistant isolates using primers and conditions previously reported (Table 1).
105 The obtained DNA amplicons were sequenced on both strands and sequences were
106 compared with those included in the GeneBank database in order to identify the specific
107 beta-lactamase gene. In addition, the presence of *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)* and *tet(E)*
108 genes were studied by PCR for the tetracycline-resistant isolates. The following genes
109 were also studied by PCR: *aadA1* and *aadA2* (in streptomycin-resistant isolates),
110 *aac(3)-I*, *aac(3)-II* and *aac(3)-IV* (in gentamicin-resistant isolates), and *sul1*, *sul2* and
111 *sul3* (in SXT-resistant isolates). The presence of the *intI1* and *intI2* genes, encoding
112 class 1 and 2 integrases, respectively, as well as *qacEΔ1* gene, part of the 3' conserved
113 segment of the class 1 integrons, were also analysed by PCR in SXT-resistant isolates.
114 The variable region of class I integrons was studied by PCR and sequencing. Primers
115 and conditions used for all PCRs are indicated in Table 1. Positive and negative controls
116 from the bacterial collection of the University of La Rioja, Spain, were used in all
117 assays.

118 **Characterization of the mechanisms of quinolone resistance.** The quinolone-
119 resistance-determining region (QRDR) of the *gyrA* gene, as well as the analogous
120 region of the *parC* gene, were amplified by PCR in all quinolone-resistant *E. coli*
121 isolates (Sáenz et al., 2003). Amplified fragments were purified (Qiagen), and both
122 strands were automatically sequenced by the Applied Biosystem 3730 sequencer
123 (Genome Express, France), using the same set of primers as for the PCR reactions.
124 Sequences obtained were compared with those previously reported for *gyrA* (GenBank

125 accession number X06373) and *parC* genes (M58408 with the modification included in
126 L22025).

127

128 **3. Results**

129 A total of 144 *E. coli* isolates were recovered from the 75 faecal samples of dogs
130 and cats analysed in this study (78 isolates of dogs and 66 of cats). No *E. coli* isolates
131 were recovered in three of the faecal samples obtained of cats. The susceptibility to 16
132 antimicrobial agents for these isolates is shown in Table 2. Almost 20% of the isolates
133 showed tetracycline resistance and 12-15% of the isolates exhibited ampicillin or
134 streptomycin resistance. The percentage of resistance to the other antimicrobial agents
135 was in all cases below 4% and no resistant isolates were detected to ceftazidime,
136 imipenem, cefoxitin or amikacin. It is interesting to indicate that two isolates (from the
137 same animal) showed cefotaxime and aztreonam resistance. Table 2 shows the
138 percentages of antimicrobial resistance detected depending on the canine or feline origin
139 of the isolates.

140 The phenotypes of resistance exhibited by the 144 *E. coli* isolates are presented in
141 Table 3. The most frequent detected phenotype was tetracycline-resistance, that was
142 found among 6.3% of the isolates, followed by ampicillin-tetracycline-streptomycin-
143 resistance and streptomycin-resistance (3.5% each one). Seventy-two per cent of the
144 *E. coli* isolates showed a susceptible phenotype to the 16 antimicrobial agents tested.

145 The phenotype of antimicrobial resistance exhibited by the two isolates recovered
146 from the same animal were compared in order to know the degree of diversity among
147 them. In 83% of the animals, the two recovered *E. coli* isolates presented similar
148 phenotype of resistance, differing in the remaining 17% of the cases.

149

150 The presence of β -lactamase genes was investigated in all 17 ampicillin-resistant
151 isolates and a *bla*_{TEM} gene was detected in 12 of them. Other two of the ampicillin-
152 resistant isolates (recovered from one dog) showed cefotaxime and aztreonam resistance
153 and they harboured the genes encoding CTX-M-1 and OXA-30 beta-lactamases. No
154 beta-lactamase genes were identified in the remaining three ampicillin-resistant isolates,
155 which were recovered from samples of cat origin (Table 4). The *aac*(3)-II gene was
156 identified in the three gentamicin-resistant isolates of cat origin detected in this study,
157 and the *aadA* gene was detected in 7 of 22 streptomycin-resistant isolates (all of them
158 recovered from dogs). In addition, *tet*(A) and/or *tet*(B) genes were found in all 28
159 tetracycline-resistant isolates (Table 4). It is interesting to underline that *tet*(A) was
160 more frequently detected among *E. coli* isolates of dogs and *tet*(B) gene among those of
161 cats.

162 The *intI1* gene encoding class 1 integrase was detected in all four SXT-resistant
163 isolates, but only two of them showed the *qacE Δ 1* and *sul1* genes and amplified the
164 class 1 integron variable region that included in both cases the *dfrA1* plus *aadA1* gene
165 cassettes arrangement. The remaining two SXT-resistant isolates were studied in detail
166 by PCR mapping and a 1,650 bp amplicon was obtained using the primers Int-F and
167 *aadA*-R. The sequencing of this fragment revealed the presence of the *dfrA12* plus *orfF*
168 plus *aadA2* gene cassette arrangement. The *sul1* + *sul2* or *sul3* genes were detected in
169 these SXT-resistant isolates (Table 4).

170 In addition, the *intI1* and *qacE Δ 1* plus *sul1* genes were detected in the two *E. coli*
171 isolates which harbored both CTX-M-1 and OXA-30 beta-lactamases. Their variable
172 regions of class 1 integron were analyzed and the *bla*_{OXA30} plus *aadA1* gene cassettes
173 were found in both isolates.

174 The *gyrA* and *parC* genes were amplified and sequenced in all five quinolone-
175 resistant isolates and the deduced amino acid changes detected in GyrA and ParC
176 proteins are shown in Table 5. Two amino acid changes in GyrA (Ser83Leu +
177 Asp87Asn) and one in ParC (Ser80Ile) were identified in the nalidixic acid- and
178 ciprofloxacin-resistant isolate found in this study and only one amino acid change in
179 GyrA (Ser83Leu or Asp87Tyr) was found in the four nalidixic acid-resistant and
180 ciprofloxacin-susceptible isolates.

181

182 **4. Discussion**

183 The moderate percentages of resistance of the faecal *E. coli* isolates of
184 healthy pets for ampicillin, streptomycin, and tetracycline (12-19%) identified in our
185 study, are similar to those previously detected in faecal *E. coli* isolates of cats in
186 Belgium (Moyaert et al., 2006), or in clinical isolates of pets in Switzerland (Lanz et
187 al., 2003). Nevertheless, higher percentages of resistance for these antimicrobial
188 agents (43-50%) were also reported in clinical *E. coli* isolates of pets in UK by other
189 authors (Normand et al., 2000). In addition, very high percentages of resistance to
190 tetracycline and ampicillin have been detected in faecal isolates of healthy food-
191 producing animals (Sáenz et al., 2001). The high use of antimicrobial agents in
192 food-producing animals in relation with healthy pets might explain these
193 differences.

194 A TEM beta-lactamase is the most frequent mechanism of ampicillin
195 resistance among our isolates (71%), as it has also been previously detected in
196 ampicillin-resistant *E. coli* isolates recovered from food, animals and humans
197 (Briñas et al., 2002). It is important to point out the detection in our study of two *E.*
198 *coli* isolates, obtained from the same animal (a seven month-old dog), which

199 harboured the genes encoding for CTX-M-1 and OXA-30 beta-lactamases. An *E.*
200 *coli* isolate harbouring CTX-M-1 beta-lactamase was previously detected from the
201 same animal when its faecal sample was seeded on a Levine agar plate
202 supplemented with 2 mg/L of cefotaxime for the study of faecal colonization by
203 ESBL-containing *E. coli* (Costa et al., 2004). It seems that the level of colonization
204 by ESBL-containing *E. coli* isolates was high in this animal because this resistant
205 isolate was also detected in this study when non-supplemented media was used for
206 *E. coli* detection. As far as we know, this dog had not received antimicrobial agents
207 in the previous four months of sampling, and considering the short life of the animal
208 (seven month old), it might have not received any antimicrobial agent in its whole
209 life. This is the first report, to our knowledge, of an *E. coli* isolate harbouring both
210 CTX-M-1 and OXA-30 beta-lactamases in animals, and probably also in humans.
211 There are previous reports about *E. coli* isolates harbouring a CTX-M in addition to
212 an OXA-30 beta-lactamase, but they were obtained in hospitals and the extended-
213 spectrum beta-lactamase was CTX-M-15 (Pai et al., 2006; Mendonça et al., 2006;
214 Kim et al., 2005). On the other hand, the presence of the *bla*_{OXA-30} plus *aadA1* gene
215 cassettes combination inside a class 1 integron variable region has been previously
216 described in *E. coli* of different origins (Dubois et al., 2003; Sunde, 2005), but this
217 is the first report of *E. coli* isolates from pets.

218 The detection of *tet(A)* and/or *tet(B)* genes in all our tetracycline-resistant
219 isolates indicates that the main mechanism of tetracycline resistance in pet *E. coli*
220 isolates is by active efflux. To date, eight different *tet* genes for efflux proteins have
221 been sequenced in gram-negative bacteria [*tet(A-E)*, (G), (H) and (J)] (Schwarz et
222 al., 2001). A predominance of *tet(A)* gene has been observed among tetracycline-
223 resistant *E. coli* isolates of dogs, and *tet(B)* gene among isolates of cats.

224 Four classes of AAC(3) acetyltransferases have been reported associated
225 with gentamicin resistance in *E. coli*. In our three gentamicin-resistant isolates
226 recovered from cats, the gene encoding AAC(3)-II enzyme was identified. This
227 mechanism of resistance has been also detected in a gentamicin-resistant *E. coli*
228 recovered from a broiler (Sáenz et al., 2004), although the AAC(3)-IV enzyme
229 seems to be more frequent in animal isolates (Guerra et al., 2003).

230 It is interesting to point out that the *E. coli* isolates recovered from healthy
231 pets in this study showed in general low percentages of resistance to
232 aminoglycosides (with the exception of streptomycin), quinolones, chloramphenicol
233 and trimethoprim-sulfamethoxazole, and these values were lower than those
234 previously reported for *E. coli* from food-producing animals or sick animals (Sáenz
235 et al., 2001; Normand et al., 2000; Lanz et al., 2003; Carattoli et al., 2005).

236 The detection of class 1 integrons in some of our *E. coli* isolates indicates
237 that this genetic mechanism for gene acquisition is present not only among clinical
238 isolates but also in *E. coli* isolates of the normal microbiota of pets. The
239 combination of two gene cassettes (*dfrA1+aadA1*) encoding resistance to
240 streptomycin and trimethoprim was identified in two integron-positive isolates. This
241 gene combination has been frequently detected among resistant *E. coli* isolates of
242 healthy animals and food products (Sáenz et al., 2004; Sunde, 2005). Two additional
243 *intI1*-positive isolates contained the combination *dfrA12* plus *orfF* plus *aadA2* gene
244 cassettes inside the class 1 integron variable region, and lacked the *qacEΔ1* and *sul1*
245 genes on the integron 3'-conserved region which is a non-expected result because
246 the *intI1*, *sul1* and *qacEΔ1* genes are usually included in class 1 integrons (Mazel et
247 al., 2000). Nevertheless, this phenomenon has been previously reported and in

248 addition, it has been associated with this gene cassette arrangement (*dfrA12* + *orfF* +
249 *aadA2*) (Guerra et al., 2003; Sáenz et al., 2004; Sunde, 2005).

250 It has been observed a correlation in the type and number of amino acid
251 changes in GyrA and ParC proteins with the level of resistance to nalidixic acid and
252 ciprofloxacin. This observation has been previously detected either in human *E. coli*
253 isolates and also in animal isolates (Vila et al., 1996; Sáenz et al., 2003).

254 As a conclusion, moderate percentages of resistance to ampicillin,
255 streptomycin, and tetracycline and low percentages for the other antimicrobial
256 agents have been detected in faecal *E. coli* isolates of healthy pets in Portugal. These
257 percentages are in general lower than those previously reported for food-producing
258 animals or from sick pets and could reflect a low antimicrobial pressure in this type
259 of animals in comparison with the other ones. Nevertheless, it is of interest the
260 detection of ESBL-producing *E. coli* isolates in pets, even in this case in which no
261 supplemented antimicrobial media was used for *E. coli* selection. More studies
262 should be carried out in the future in order to track the evolution of this type of
263 resistance among the faecal *E. coli* isolates of different ecosystems.

264

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269

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271

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Table 1. Primers and annealing temperatures used in the PCR reactions carried out in this study for detection of antimicrobial resistant mechanisms^a

Primer name	Sequence (5' → 3')	Target gene(s) or region	Amplicon size (bp)	Annealing temp (°C)
TEM-F	ATTCTTGAAGACGAAAGGGC	<i>bla</i> _{TEM}	1,150	60
TEM-R	ACGCTCAGTGGAACGAAAAC			
SHV-F	CACTCAAGGATGTATTGTG	<i>bla</i> _{SHV}	885	52
SHV-R	TTAGCGTTGCCAGTGCTCG			
OXA-1 F	ACACAATACATATCAACTTCGC	<i>bla</i> _{OXA}	813	61
OXA-1 R	AGTGTGTTTAGAATGGTGATC			
CTX-M-10 F	CCGCGCTACACTTTGTGGC	<i>bla</i> _{CTX-M-10}	944	52
CTX-M-10 R	TTACAAACCGTTGGTGACG			
CTX-M-1 group F	GTTACAATGTGTGAGAAGCAG	<i>bla</i> _{CTX-M group 1}	1,049	50
CTX-M-1 group R	CCGTTTCCGCTATTACAAAC			
CMY-F	GATTCCTTGGACTCTTCAG	<i>bla</i> _{CMY}	1,800	53
CMY-R	TAAAACCAGGTTCCCAGATAGC			
Tet A-F	GTAATTCTGAGCACTGTTCGC	<i>tetA</i>	937	62
Tet A-R	CTGTCCTGGACAACATTGCTT			

Tet B-F	CTCAGTATTCCAAGCCTTTG	<i>tetB</i>	416	57
Tet B-R	CTAAGCACTTGTCTCCTGTT			
Tet C-F	TCTAACAATGCGCTCATCGT	<i>tetC</i>	570	62
Tet C-R	GGTTGAAGGCTCTCAAGGGC			
Tet D-F	ATTACACTGCTGGACGCGAT	<i>tetD</i>	1,104	57
Tet D-R	CTGATCAGCAGACAGATTGC			
Tet E-F	GTGATGATGGCACTGGTCAT	<i>tetE</i>	1,179	62
Tet E-R	CTCTGCTGTACATCGCTCTT			
AadA-F	GCAGCGCAATGACATTCTTG	<i>aadA1</i> or <i>aadA2</i>	282	60
AadA-R	ATCCTTCGGCGCGATTTTG			
AacC1-F	ACCTACTCCCAACATCAGCC	<i>aac(3)-I</i>	169	60
AacC1-R	ATATAGATCTCACTACGCGC			
AacC2-F	ACTGTGATGGGATACGCGTC	<i>aac(3)-II</i>	237	60
AacC2-R	CTCCGTCAGCGTTTCAGCTA			
AacC4-F	CTTCAGGATGGCAAGTTGGT	<i>aac(3)-IV</i>	286	60
AacC4-R	TCATCTCGTTCTCCGCTCAT			
Sul1-F	TGGTGACGGTGTTCGGCATTG	<i>sul1</i>	789	63
Sul1-R	GCGAGGGTTTCCGAGAAGGTG			
Sul2-F	CGGCATCGTCAACATAACC	<i>sul2</i>	722	50
Sul2-R	GTGTGCGGATGAAGTCAG			

Sul3-F	GAGCAAGATTTTTGGAATCG	<i>sul3</i>	792	51
Sul3-R	CATCTGCAGCTAACCTAGGGCTTTGGA			
IntI1-F	GGGTCAAGGATCTGGATTTTCG	<i>intI1</i>	483	62
IntI1-R	ACATGGGTGTAAATCATCGTC			
IntI2-F	CACGGATATGCGACAAAAAGGT	<i>intI2</i>	788	62
IntI2-R	GTAGCAAACGAGTGACGAAATG			
Int-F	GGCATCCAAGCAGCAAG	Class 1 integron variable region	variable	55
Int-R	AAGCAGACTTGACCTGA			
Qac-F	GGCTGGCTTTTTCTTGTTATCG	<i>qacEΔ1</i>	287	62
Qac-R	TGAGCCCCATACCTACAAAGC			
GyrA-F	TACACCGGTCAACATTGAGG	<i>gyrA</i>	648	64
GyrA-R	TTAATGATTGCCGCGTCGG			
ParC-F	AAACCTGTTTCAGCGCCGCATT	<i>parC</i>	395	55
ParC-R	GTGGTGCCGTTAAGCAAA			

^a All these primers have been previously included in the following references: Briñas et al., 2003; Coque et al., 2002; Mazel et al., 2000; Pagani et al., 2003; Sáenz et al., 2004; Sáenz et al., 2003.

Table 2. Percentages of antimicrobial resistance in the series of 144 *E. coli* isolates from faecal samples of pets

Antimicrobial agent ^a	Antimicrobial resistant <i>E. coli</i> isolated from:					
	Dogs (n=78)		Cats (n=66)		Total pets (n=144)	
	Number	Percentage	Number	Percentage	Number	Percentage
Ampicillin	6	7.7	11	16.7	17	11.8
AMC ^b	2	2.6	3	4.5	5	3.5
Cefotaxime ^b	2	2.6	0	0	2	1.4
Aztreonam ^b	2	2.6	0	0	2	1.4
Ceftazidime	0	0	0	0	0	0
Cefoxitine	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0
Gentamicin	0	0	3	4.5	3	2.1
Tobramycin	0	0	2	3.0	2	1.4
Amikacin	0	0	0	0	0	0
Streptomycin	14	17.9	8	12.1	22	15.2
Tetracycline	16	20.5	12	18.2	28	19.4
SXT	4	5.1	0	0	4	2.8
Nalidixic acid	3	3.8	2	3.0	5	3.5
Ciprofloxacin	1	1.3	0	0	1	0.7
Chloramphenicol	4	5.1	0	0	4	2.8

^aAMC, amoxicillin-clavulanic acid; SXT, trimethoprim-sulfamethoxazole.

^bIsolates in the resistant and intermediate category are included in this section

Table 3. Phenotypes of resistance detected among the 144 *E. coli* isolates recovered from pets.

Phenotype of resistance ^a	Number of isolates	Percentage of isolates
TET	9	6.3
STR	5	3.5
NAL	2	1.4
TET-STR	3	2.1
TET-NAL	2	1.4
AMP-TET	1	0.7
AMP-GEN	1	0.7
AMP-STR-SXT	2	1.4
AMP-GEN-TOB	2	1.4
AMP-TET-STR	5	3.5
TET-STR-SXT-CHL	2	1.4
AMP-AMC ^b -STR-TET	3	2.1
AMP-TET-NAL-CIP	1	0.7
AMP-AMC ^b -CTX ^b -ATM ^b -STR-TET-CHL	2	1.4
Susceptible	104	72.2

^aAMP, ampicillin; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; ATM, aztreonam; GEN, gentamicin; TOB, tobramycin; STR, streptomycin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; CIP, ciprofloxacin; CHL, chloramphenicol.

^bResistance to the drug indicated is in the intermediate or resistance category according to CLSI standards.

Table 4. Genes of resistance detected among our antimicrobial resistant *E. coli* isolates of dog and cat origins.

Phenotype of resistance	Dogs			Cats		
	Number of isolates with this phenotype	Genes detected		No. of isolates with this phenotype	Genes detected	
		Genes	Number of isolates		Genes	Number of isolates
Ampicillin	6	<i>bla</i> _{TEM}	4	11	<i>bla</i> _{TEM}	8
		<i>bla</i> _{CTX-M-1} + <i>bla</i> _{OXA-30}	2 ^a			
Gentamicin	0	-	-	3 ^b	<i>aac(3)-II</i>	3
Streptomycin	14	<i>aadA</i>	7	8	-	-
Tetracycline	16	<i>tet(A)</i>	10	12	<i>tet(A)</i>	1
		<i>tet(B)</i>	5		<i>tet(B)</i>	11
		<i>tet(A)</i> + <i>tet(B)</i>	1			
SXT ^c	4	<i>dfrA1</i> ^d + <i>sul1</i> + <i>sul2</i>	2	0	-	-
		<i>dfrA12</i> ^d + <i>sul3</i>	2			

^a These isolates showed also resistance to cefotaxime and aztreonam

^b Two of these three isolates showed also tobramycin resistance

^cSXT: Trimethoprim-sulfamethoxazole

^dThis gene was found inside a class 1 integron

Table 5. Amino acid changes in GyrA and ParC proteins deduced from the sequences of the corresponding genes in our quinolone-resistant *E. coli* isolates^a

Phenotype of resistance to quinolones ^a	Number of <i>E. coli</i> isolates	Amino acid changes in:	
		GyrA	ParC
Nalidixic acid-ciprofloxacin	1	Ser83Leu + Asp87Asn	Ser80Ile
Nalidixic acid	2	Asp87Tyr	wild
Nalidixic acid	2	Ser83Leu	wild

^aSequences were compared with *gyrA* and *parC* genes included in the GenBank database with the accession numbers X06373 for *gyrA* and M58408 with the modification in L22025 for *parC*.