**Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant
Escherichia coli in Swiss Pets: Molecular Features, Risk Factors, and
Transmission with Owners**

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Running title: Swiss pets colonized with cephalosporin-resistant *E. coli*

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28 The overlap of molecular features of extended-spectrum cephalosporin-resistant
29 *Enterobacteriaceae* (ESC-R-Ent) recovered from both humans and animals indicates that a
30 possible interaction between these two niches exists. Frequently, these strains carry CTX-M-
31 1/-14/-15 and CMY-2 β -lactamases in IncF or IncII plasmids in different sequence types
32 (ST), including the high risk clones ST131, ST648, and those belonging to clonal complex
33 (CC) 10 [1].

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35 In this study, we aimed to assess the role of the healthy pets' intestine in shaping the
36 epidemiology of ESC-R-Ent, the prevalence of intestinal colonization by these organisms, and
37 potential lifestyle factors associated with this phenomenon. Thus, between July 2013 and May
38 2016, 81 pets (44 dogs and 37 cats) and their owners (n=72) from 66 different Swiss
39 households were enrolled in the analysis. Owners were instructed to collect stools into sterile
40 containers; a questionnaire about their pets was also filled in.

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42 Stools were enriched overnight in LB broth in different conditions to detect both ESC-R-Ent
43 and colistin-resistant (COL-R) strains [2, 3]. Species identification was obtained by using the
44 MALDI-TOF MS (Bruker). MICs were obtained using the microdilution SentitreTM GNX2F
45 plate (Trek Diagnostic Systems) and interpreted with the 2016 EUCAST breakpoints
46 (www.eucast.org/clinical_breakpoints/). β -lactamase genes (*bla*) were identified using the
47 CT103XL microarray (Check-Points) with subsequent PCR and sequencing. Clonality was
48 assessed with MLST (<http://mlst.ucc.ie/mlst/dbs/Ecoli>) and phylogenetic group (PhG)
49 determination [2, 3]. PCR based replicon typing (PBRT) kit (Diatheva) was used to type
50 plasmids' incompatibility groups. Statistical analysis was performed to compare colonized
51 and non-colonized pets' lifestyle factors using GraphPad Prism version 7.0. Continuous

variables were analyzed using unpaired t-test, whereas categorical variables with Fisher's exact test. Odds Ratios (ORs) and Confidence Intervals (CIs) 95% were computed for categorical variables.

Among the total pets analyzed, 4 cats and 3 dogs (overall prevalence of 8.6%; CI, 4.3%-16.9%) resulted colonized with ESC-R *E. coli*, whereas COL-R Enterobacteriaceae were not detected. Compared to previous reports, our data indicates that the prevalence is steadily increasing in Switzerland, whereas it is still lower compared to other countries [1]. Reports on cats are scarce, with 4% reported in Kenya, 21% in Tunisia, and 0% in the Netherlands [1].

We recovered eight ESC-R *E. coli* isolates from seven pets; one dog was co-colonized with two different strains (Table 1). None of the isolates was resistant to carbapenems and resistance to non- β -lactams was low, with $\leq 25\%$ of the isolates non-susceptible to trimethoprim-sulfamethoxazole, aminoglycosides, quinolones, and tetracyclines.

Interestingly, these observations are in contrast with previous Swiss and international studies where higher resistance rates were observed [1, 4].

ESC-R-Ent isolates from pets were CTX-M-type or CMY-2 producers and carried IncF and/or IncII plasmids (Table 1), as reported in other international studies [1, 4]. The majority of isolates belonged to PhG B1 (37.5%) that is frequently found in the gut of animals [2]. STs were highly diverse, including CC10, CC155 and CC73 which were also found in pets and wildlife in many geographic regions [1, 5]. Additionally, some of these STs (ST127, ST73, CC155, CC10) were previously reported causing extra-intestinal infections in humans [1, 5].

Three out of 72 owners were colonized with ESC-R-*E. coli* (prevalence 4.2%, CI 95% 1.4%-11.6%) (Table S1), as recently observed in the overall Swiss population [2, 3]. Possible transmission among human and pet was observed in one household (1.5%) where the owner and one of the cats were colonized with an identical *E. coli* clone (B2₃-ST127-CTX-M-15). We are unable to clarify the directionality of transmission, but this finding reinforces the hypothesis that the pets' intestine can shape the epidemiology of ESC-R-Ent [1].

None of the studied pet factors was statistically associated with an increased risk of being colonized by ESC-R-Ent (Table S2). However, we note that the small sample size of this explorative study decreased the power of the statistical analysis. Moreover, owners were unable to provide stools from all their pets. Thus, some of the studied factors (i.e., previous antibiotic intake and contact with pets from other owners, both P=0.11 and OR=4.3) could potentially be correlated with increased ESC-R-Ent colonization and should be further investigated. Indeed, previous studies have associated the consumption of antibiotics, animal species, breed as factors associated with colonization by these organisms in healthy pets [1].

Our data indicates that the prevalence of ESC-R-Ent in Swiss pets may be lower than in other countries. The identification of similar *bla* and plasmid types stresses the importance of plasmids in the dissemination of these genes in the pet setting. Moreover, since we identified the same *E. coli* strain in both human and pet within the same household, we highlight the importance of pets as a potential source of pathogens responsible for human extra-intestinal infections. Further studies are needed to better understand the lifestyle factors associated with an increased colonization risk and characteristics that enable the zoonotic spread of clones and genetic platforms across the animal and the human ecological niches.

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Table 1

Table 1. Molecular and phenotypic features of the 3GC-R *E. coli* strains recovered from the stools of pets

Household	Pet	Main β -lactamase ^a	PhG-ST (Clonal complex, CC)	Plasmid replicon types	Non-susceptible phenotype (MICs, μ g/mL) ^b
5	Dog	CMY-2 CTX-M-1	D ₂ -ST963 B1-ST949	I1, FII, FIB I1, FII, FIB, X1	TIM ($\geq$ 128), P/T4 (32), FOT (64) TAZ (64), AZT ($\geq$ 16) FOT (16), DOX (16)
163	Dog	CTX-M-14	B1-ST162 (CC469)	FII, FIB	TIM (32), FOT (16), DOX ($\geq$ 32), MIN (16), GEN ($\geq$ 8)
168	Cat #1 Cat #2	CTX-M-15 CTX-M-15	B ₂₃ -ST127 ^c B ₂₃ -ST73 (CC73)	I1 N, X1	FOT (16), AZT (8), GEN ($\geq$ 8), TOB (8) FOT (64), TAZ (8), FEP (8), AZT (16)
195	Dog	SHV-2a	D ₁ -ST1421	FII, FIA, FIB	TIM (32), FOT (8), AZT (8), SXT ($\geq$ 8/152)
198	Cat	CMY-2	B1-ST56 (CC155)	I1, FII, FIA, X1, B/O	FOT (16), TAZ (16), AZT (8)
425	Cat	CTX-M-15	A ₁ -ST43 (CC10)	FII, I2, K, B/O	FOT (16), TAZ (4), AZT (8), CIP (2), LEV (2), SXT ($\geq$ 8/152)

Note. TIM -ticarcillin-clavulanate; P/T4 – piperacillin/tazobactam; FOT – cefotaxime; FEP – cefepime; TAZ – ceftazidime; AZT – aztreonam; DOX – doxycycline; MIN – minocycline; TOB - tobramycin; GEN – gentamicin; SXT - trimethoprim-sulfamethoxazole; CIP - ciprofloxacin; LEV –levofloxacin.

^a Only β -lactamases conferring resistance to extended-spectrum cephalosporins are shown

^b MIC values were interpreted according to the EUCAST criteria (Version 6.0; 2016), except for doxycycline and minocycline for which CLSI guidelines (M100-S26; 2016) were used. Only antibiotics with a non-susceptible phenotype were reported

^c This identical strain was also found in the owner