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Article

Molecular characteristics of extraintestinal pathogenic *E. coli* (ExPEC), uropathogenic *E. coli* (UPEC), and multidrug resistant *E. coli* isolated from healthy dogs in Spain. Whole genome sequencing of canine ST372 isolates and comparison with human isolates causing extraintestinal infections.

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Abstract: Under one-health perspective and the worldwide antimicrobial resistance concern, we investigate extraintestinal pathogenic *Escherichia coli* (ExPEC), uropathogenic *E. coli* (UPEC), and multidrug resistant (MDR) *E. coli* from 197 isolates recovered from healthy dogs in Spain between 2013 and 2017. Ninety-one (46.2%) isolates were classified as ExPEC and/or UPEC including 50 clones, among which (i) four clones were dominant (B2-CH14-180-ST127, B2-CH52-14-ST141, B2-CH103-9-ST372 and F-CH4-58-ST64815) and (ii) 15 had been shown to be displayed by previously published isolates causing extraintestinal infections in humans. Twenty-eight (14.2%) isolates were classified as MDR, associated with B1, D and E phylogroups and included 24 clones, of which eight had also been identified among human isolates causing infections. We selected 23 ST372 strains, 21 healthy dogs faecal isolates and two human clinical isolates for whole genome sequencing and built a SNP-tree with these 23 genomes and 174 genomes (128 from canine strains and 46 from human strains) obtained from public databases. The analysis of these 197 genomes allowed to identify six clusters. Cluster 1 comprised 74.6% of the strain genomes that were mostly composed of canine strain genomes ($P < 0.00001$). Clusters 4 and 6 also included canine strain genomes, while clusters 2, 3 and 5 were significantly associated with human strain genomes. All these findings suggest that dogs are reservoirs of ExPEC, UPEC and MDR *E. coli* isolates with zoonotic potential.

Keywords: *Escherichia coli*; dogs; virulence genes; antibiotic resistance; WGS; ST372; clonal structure.

1. Introduction

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Escherichia coli is a common commensal of the gastrointestinal tract. However, *E. coli* is also the main bacterial pathogen responsible for extraintestinal infections in humans and dogs, including urinary tract infections (UTIs) [1–5]. Most UTIs are thought to result from ascending infections. The two theories for the origin of uropathogenic isolates are the "prevalence" and the "special pathogenicity". The prevalence hypothesis postulates that most UTIs are opportunistic infections caused by bacteria that predominate in the faecal microbiota, whereas the special pathogenicity hypothesis suggests that most UTI are caused by pathogenic strains that possess appropriate virulence factors (VFs) [5,6]. More than 50 *E. coli* genes associated with extraintestinal infections have been identified, encoding adhesins, toxins, siderophores, capsular antigens, and invasins [7,8]. Isolates are designed presumptively as extraintestinal pathogenic *E. coli* (ExPEC) if they contained ≥ 2 of 5 virulence genes *papAH* and/or *papC*, *sfa*/*focDE*, *afa*/*draBC*, *kpsM II*, and *iutA* [7], and as uropathogenic *E. coli* (UPEC) if they are positive for ≥ 3 of 4 virulence markers *chuA*, *fyuA*, *vat*, and *yfcV* [8].

The majority of ExPEC and UPEC isolates belong to B2 phylogenetic group. Although there is a notable diversity of phylogenetic groups among *E. coli* isolates causing human and animal extraintestinal infections, some epidemiological studies indicate that certain O:H serotypes, sequence types (STs) and clonotypes are more predominant and especially successful [3,9–14]. Two recent studies showed the dominance of some specific STs in dogs in the United States and France, such as ST372, assessed to be specifically associated with dogs, and ST12, ST73, ST127 and ST141, assessed to be specifically associated with humans [10,13]. On the other hand, within-household sharing of ExPEC ST73 and ST95 strains, those with same serotypes and VF-encoding genes have been documented in the United States among humans and dogs [15]. Furthermore, in Australia and the United States, human and canine *E. coli* ST127, ST131 and ST1193 that exhibited identical virulence genotypes and highly similar PFGE profiles have been identified [16–18]. These findings suggest that some *E. coli* infections may sometimes be a zoonosis in either direction (human to pet or pet to human).

The antimicrobial resistance of human ExPEC and UPEC isolates has increased dramatically due to the emergence of the pandemic clone ST131 and more especially to subclade C2 (also known as subclone *H*30Rx) [19–25]. This subclone has also been occasionally isolated from dogs in several countries [26–30]. The emergence of multidrug resistance (MDR) among *E. coli* causing infections in dogs is of great concern and increases the risk of treatment failure [31–41]. Additionally, exposure to dogs and/or dog faeces has been identified as a risk factor for the development of drug-resistant *E. coli* UTI in women [42].

As relatively little is known on the clonal structure of canine ExPEC, UPEC and MDR isolates, the present study was carried out (i) to establish which clones (defined by the association of phylogroup, clonotype and ST) dominate in dogs and (ii) to compare these clones with those causing extraintestinal infections in humans. To our knowledge, this is the first study that uses whole genome sequencing (WGS) to define the genetic relatedness between the ST372 *E. coli* lineage, which we found dominant among the Spanish dog faecal *E. coli* populations, and human *E. coli* ST372 that cause extra-intestinal infections.

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2. Materials and Methods

2.1. E. coli Isolates

A total of 197 non-duplicate *E. coli* isolated from faecal samples of 104 healthy dogs collected in Spain between 2013 and 2017 were characterized.

2.2. Phylogenetic Grouping

Assignment to the main phylogroups (A, B1, B2, C, D, E, F) was based on the protocol of Clermont et al. [43].

2.3. Serotyping

The determination of O and H antigens was carried out using the method previously described by Guinée et al. [44] with all available O (O1 to O181) and H (H1 to H56) antisera. Isolates that did not react with any antisera were classified as O non-typeable (ONT) or H non typeable (HNT) and those non motile were denoted as HNM.

2.4. Multilocus Sequence Typing (MLST)

The sequence types (STs) were established following the MLST scheme of Achtman by gene amplification and sequencing of the seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) according to the protocol and primers specified at the *E. coli* MLST web site (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) [45].

2.5. CH Typing

Clonotype identification was determined by *fumC* and *fimH* (CH) sequencing [46,47]

2.6. Virulence Genotyping

Virulence factor (VF)-encoding genes of *E. coli* causing extraintestinal infections were screened by PCR [4,48]. The virulence gene score was the number of extraintestinal virulence-associated genes detected. The isolates were designed presumptively as extraintestinal pathogenic *E. coli* (ExPEC) if positive for ≥ 2 of 5 markers, including *papAH* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA* [7], and as uropathogenic *E. coli* (UPEC) if positive for ≥ 3 of 4 markers, including *chuA*, *fyuA*, *vat*, and *yfcV* [8].

2.7. Antimicrobial Susceptibility and ESBL and pAmpC Typing

Antimicrobial susceptibility was determined by the minimal inhibitory concentrations (MICs) and/ or the disc diffusion method. Resistance was interpreted based on the recommended breakpoints of the CLSI [49]. Fifteen classes of antimicrobial agents were analyzed: penicillins (ampicillin), penicillins and β-lactamase inhibitors (amoxicillin-clavulanic acid), 1st and 2nd generation of non-extended spectrum cephalosporins cephalosporins (cefazolin and cefuroxime), extended-spectrum cephalosporins (cefotaxime, ceftazidime and cefepime), cephamycins (cefoxitin), monobactams (aztreonam), carbapenems (imipenem), aminoglycosides (gentamicin, tobramycin, amikacin), tetracyclines (doxycycline), phenicols (chloramphenicol), nitrofurans (nitrofurantoin), quinolones (nalidixic acid and ciprofloxacin), folate pathway inhibitors (trimethoprim-sulphamethoxazole), phosphonic acids (fosfomycin) and polymyxins (colistin). *E. coli* MDR was defined as resistance to one or more agents in three or more classes of tested drugs [50]. Genetic identification of ESBL and pAmpC types was carried out by PCR followed by amplicon sequencing [51–53].

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2.8. Whole Genome Sequencing (WGS)

The WGS of 23 ST372 isolates from our LREC collection was performed under the protocol of the Genomics and Bioinformatics Core Facility (Centre for Biomedical Research of La Rioja) as it was described previously [54]. The assembly information of draft genomes, database sources and input parameters can be found in Table S1 (NCBI Bioproject accession PRJNA627579).

PLACNET webserver [55,56] was used for the genome reconstruction after what Prokka [57] was used to annotate the assembled genetic elements. Primary *in silico* analyses were carried out using the Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/) services with home-made databases, the CGE databases and other complementary databases to explore the resistance and virulence factors. Plasmid typing was complemented by subtyping relaxases with the method defined by Alvarado et al. [58]. EasyFig tool was used to explore and made comparisons of the genetic environment in our genomes [59].

Besides, we performed a single nucleotide position (SNP) tree analysis of the 23 ST372 genomes sequenced in this study plus 174 ST372 full-genome references retrieved from NCBI bioproject and EnteroBase. The SNP-tree was done using the CSI Phylogeny 1.4 server from the CGE with J22 strain as reference (ID: GCA_009497315). After analyzing the SNP matrix, we took all the ST372 genomes from human strains plus some representatives genomes from canine strains to made a tree visualization using EnteroBase [60]. The accession number of all the genomes included in this study can be found in Table S2.

2.9. Statistical analysis

All the *P* values were calculated using Fisher´s exact test, except for the comparison of the means that was performed using the one-way ANOVA test. *P* values <0.05 were considered statistically significant.

3. Results

3.1. Phylogenetic Groups of the 197 Canine Isolates

The most common phylogenetic group displayed by the 197 faecal canine *E. coli* isolates was B2 (42.6%), followed by A (16.2%), B1 (13.2%), F (9.1%), E (7.1%), C (5.1%), and D (3.0%) (Table S3).

3.2. Virulence Factor (VF)-Encoding Genes in the 197 Canine Isolates

Of the 28 VF-encoding genes analyzed, eight (*fimH*, *yfcV*, *vat*, *iroN*, *fyuA*, *chuA*, *malX*, *usp*) were detected in more than 40% of the 197 canine isolates and nine (*papAH*, *papC*, *sfa/focDE*, *cnf1*, *hlyA*, *kpsM II*, *kpsM II-K5*, *traT*, *ibeA*) in at least 20%. In contrast, six VF-encoding genes (*afa/draBC*, *sat*, *cdtB*, *neuC-K1*, *kpsM II-K2*, *kpsM III*) were found in less than 10% of these isolates (Table 1).

A higher mean of VF-encoding gene score was observed in the 84 canine isolates belonging to the dominant B2-phylogenetic group (mean of 12.79) ($P < 0.05$) compared with the isolates belonging to phylogroups A (2.31), B1 (2.96), C (6.60), D (5.67), E (4.14) and F (7.94) (Table 1).

Of the 197 canine isolates, 74 (37.6%) were presumptively classified as ExPEC and 82 (41.6%) as UPEC (Table 1) resulting in 91 ExPEC and/or UPEC isolates. The majority (85.7%; 78 of 91) of ExPEC and/or UPEC isolates belonged to phylogenetic group B2. In contrast, only 5.7% (6 of 106) of non-ExPEC and non-UPEC isolates were assigned to this phylogenetic group ($P < 0.00001$). The A, B1, C, and E phylogenetic groups were significantly associated with non-ExPEC and non-UPEC isolates (Table S4).

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Table 1. Virulence factor (VF)-encoding genes detected in the 197 canine *E. coli* isolates. Relationship with phylogenetic groups.

VF-encoding gene	Number of isolates (%)									
	Total (n=197)	**A (n=32)**	**B1 (n=26)**	**B2 (n=84)**	**C (n=10)**	**D (n=6)**	**E (n=14)**	**F (n=18)**	**Clade V (n=1)**	**NT[1] (n=6)**
Adhesins										
fimH	192 (97.5)	29	24	84	10	6	14	18	1	6
fimAvMT78	20 (10.2)	12	1	1	0	0	2	3	0	1
papAH	62 (31.5)	2	3	54	0	1	1	1	0	0
papC	62 (31.5)	2	3	54	0	1	1	1	0	0
sfa/focDE	66 (33.5)	0	2	63	0	0	0	1	0	0
afa/draBC	1 (0.5)	0	0	0	0	1	0	0	0	0
yfcV	96 (48.7)	4	0	80	0	0	0	11	0	1
Toxins										
sat	3 (1.5)	0	0	2	0	0	0	1	0	0
cnf1	54 (27.4)	0	0	53	0	0	0	1	0	0
hlyA	56 (28.4)	0	2	54	0	0	0	0	0	0
cdtB	8 (4.1)	0	0	6	0	0	0	2	0	0
tsh	21 (10.7)	2	2	0	8	0	2	6	0	1
vat	79 (40.1)	1	0	76	0	0	0	2	0	0
Iron uptake										
iutA	33 (16.8)	3	3	7	8	1	2	9	0	0
iroN	93 (47.2)	3	6	66	8	0	2	8	0	0
fyuA	106 (53.8)	7	2	77	8	5	1	5	0	1
chuA	122 (61.9)	0	0	84	0	6	14	18	0	0
Capsule										
kpsM II	64 (32.5)	1	0	41	0	4	4	10	1	3
neuC-K1	12 (6.1)	0	0	10	0	0	0	2	0	0
kpsM II-K2	7 (3.6)	0	0	2	0	3	0	1	0	1
kpsM II-K5	45 (22.8)	1	0	29	0	1	4	7	1	2
kpsM III	6 (3.0)	1	0	2	0	2	1	0	0	0
Miscellaneous										
cvaC	21 (10.7)	1	4	2	8	0	0	6	0	0
iss	31 (15.7)	2	5	6	8	0	2	8	0	0
traT	59 (29.9)	2	12	17	8	2	7	10	0	1
ibeA	54 (27.4)	0	0	49	0	0	0	3	0	2
malX	93 (47.2)	0	5	79	0	0	0	7	0	2
usp	86 (43.7)	1	3	77	0	1	1	2	0	1
ExPEC status	74 (37.6)	2	2	61	0	1	1	7	0	0
UPEC status	82 (41.6)	1	0	77	0	0	0	4	0	0
Range of VGs	0 to 18	0 to 10	1 to 10	2 to 17	1 to 8	5 to 7	2 to 7	3 to 18	3	1 to 9
Mean of VGs	7.87	2.31	2.96	12.79	6.60	5.67	4.14	7.94	3.00	3.67

[1]Using the revised protocol developed by Clermont et al. [43] six isolates were not typeable (NT). These six isolates belonged to phylogroup A using the first protocol developed by Clermont et al. [43] that classifies isolates into only four phylogenetic groups (A, B1, B2, D).

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3.3. Antimicrobial Resistance in the 197 Canine Isolates

Twenty-eight (14.2%) of the 197 analyzed faecal canine *E. coli* isolates were classified as MDR. Multidrug resistance was significantly associated with isolates belonging to B1, D and E phylogenetic groups (Table S5). Furthermore, only 8 (28.6%) of MDR isolates showed the ExPEC and/or the UPEC status (Table S6).

Ten of the 28 MDR isolates produced an ESBL enzyme: CTX-M-1 (4 isolates), CTX-M-14 (4 isolates), CTX-M-55 (1 isolate) and SHV12 (1 isolate). Besides, 10 other isolates produced a plasmid-mediated AmpC β-lactamase of CMY-2 type.

3.4. Sequence Types, Clones and Serotypes Displayed by the 91 Canine ExPEC and/or UPEC Isolates and 28 MDR isolates

Sequences types (STs), clones (defined by the association of phylogroup, clonotype and ST) and O:H serotypes were established only for the 91 canine isolates classified as ExPEC and/or UPEC and the 28 canine MDR isolates

Thirty-four STs were identified in the canine ExPEC and/or UPEC isolates and 22 in the MDR isolates. Among these STs, eighteen were previously undescribed (Table S7). Each of these eighteen new STs was displayed by one isolate. Seven dominant STs (ST12, ST38, ST73, ST127, ST141, ST372 and ST648) were observed among the 91 canine ExPEC and/or UPEC and the 28 canine MDR isolates. There was a strong correlation between VF-encoding gene profiles and the dominant STs (Table 2)

Table 2. Virulence factor (VF)-encoding genes detected in the 65 canine *E. coli* isolates included in the 7 most frequent sequence types identified in ExPEC, UPEC and MDR isolates.

VF-encoding gene	Number of isolates						
	B2-ST12 (n = 9)	D-ST38 (n = 4)	B2-ST73 (n = 4)	B2-ST127 (n = 8)	B2-ST141 (n = 5)	B2-ST372 (n = 29)	F-ST648 (n = 6)
Adhesins							
fimH	9	4	4	8	5	29	6
fimAv$_{MT78}$	0	0	0	0	0	0	0
papAH	9	0	4	7	1	21	0
papC	9	0	4	7	1	21	0
sfa/focDE	9	0	3	8	4	26	1
afa/draBC	0	0	0	0	0	0	0
yfcV	9	0	4	8	5	29	6
Toxins							
sat	0	0	0	0	0	0	0
cnf1	8	0	4	7	0	23	0
hlyA	9	0	4	7	1	23	0
cdtB	0	0	2	0	0	0	1
tsh	0	0	0	0	0	0	5
vat	8	0	4	8	5	29	2
***Iron* uptake**							
iutA	1	0	0	1	0	0	6
iroN	9	0	2	7	4	26	5
fyuA	8	4	4	8	5	29	3
chuA	9	4	4	8	5	29	6
Caps*u*le							
kpsM II	6	4	4	7	5	2	6
neuC-K1	0	0	0	0	5	0	1
kpsM II-K2	0	3	0	0	0	0	1
kpsM II-K5	6	1	4	7	0	2	4
kpsM III	2	0	0	0	0	0	0
Miscellaneous							
cvaC	0	0	0	0	0	0	5
iss	0	0	0	1	0	0	5
traT	1	1	2	1	0	4	6
ibeA	0	0	0	0	4	29	1
malX	9	0	4	8	5	29	2
usp	9	1	4	7	5	29	1
ExPEC status	9	0	3	8	4	20	6
UPEC status	8	0	3	8	5	29	3
Range of VGs	12 to 16	5 to 7	14 to 16	12 to 17	10 to 14	8 to 16	9 to 18
Mean of VGs	14.40	5.50	15.30	14.40	12.00	13.10	12.17

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A total of 50 clones were identified among the 91 canine isolates classified as ExPEC and/or UPEC, with 11 of them including at least two isolates and only four, at least four isolates *i.e.* B2-CH14-180-ST127 (4 isolates), B2-CH52-14-ST141 (4 isolates), B2-CH103-9-ST372 (25 isolates) and F-CH4-58-ST648 (5 isolates) (Table 3). In a recent study conducted by our research group [61], we had identified, as indicated in Table 3, 15 of the 50 canine ExPEC/UPEC clones comprising 49 isolates among the 261 human ExPEC and/or UPEC isolates included in a collection of 394 *E. coli* isolates causing extraintestinal infections. However, only 31 of the 49 human ExPEC and/or UPEC isolates presented the same clone-related O:H serotype as the canine isolates (Table 3).

Table 3. Clones and clonal-related serotypes of 91 canine ExPEC and/or UPEC isolates. Prevalence of the canine clones and clonal-related serotypes among ExPEC and/or UPEC isolates causing extraintestinal infections in humans [61].

Clone of canine ExPEC and/or UPEC isolates	Clone-related serotype of canine ExPEC and/or UPEC isolates (number of isolates)	Number of human ExPEC and/or UPEC isolates with same clone of canine isolates (49 of 261)	Number of human ExPEC and/or UPEC isolates with same clone and serotype of canine isolates (31 of 261)
A-CH11-NEG-ST93	O5:H4 (1)	3	0
A-CH11-27-ST new 1	O4:H27 (1)	0	0
B1-CH4-27-ST58	O8:H25 (1), O9:H25 (1)	0	0
B2-CH13-5-ST12	O4:HNM (1), O18:H5 (1)	0	0
B2-CH13-7-ST12	O4:H1 (1), O4:HNM (1)	0	0
B2-CH13-130-ST12	O18:H5 (1)	0	0
B2-CH13-223-ST12	O18:H5 (2)	1	0
B2-CH13-430-ST12	O4:H5 (1)	0	0
B2-CH13-431-ST12	O4:H5 (1)	0	0
B2-CH24-9-ST73	O120:H31 (1)	0	0
B2-CH24-27-ST73	**O6:H1** (1)	1	1
B2-CH24-30-ST73	**O6:H1** (1)	4	3
B2-CH24-103-ST73	**O6:H1** (1)	6	4
B2-CH24-1-ST80	O75:H7 (1)	0	0
B2-CH38-30-ST95	O1:H7 (1)	1	0
B2-CH14-2-ST127	**O6:HNM** (2)	4	3
B2-CH14-180-ST127	**O6:HNM** (3), O6:H11 (1)	1	1
B2-CH14-fimH$_{TR}$new 1-ST127	O6:H31 (1), O6:HNM (1)	0	0
B2-CH40-NEG-ST131	O25:H4 (1)	0	0
B2-CH52-5-ST141	**O2:H6** (1)	13	9
B2-CH52-14-ST141	**O2:H6** (4)	2	2
B2-CH103-9-ST372	O4:H31 (9), O15:H31 (1), O21:H31 (3), O25:H31 (4), **O83:H31** (7), O117:H28 (1)	2	2
B2-CH103-10-ST372	O15:H31 (1)	0	0
B2-CH103-17-ST372	O15:H31 (1)	0	0
B2-CH103-240-ST372	O83:H31 (1)	0	0
B2-CH103-706-ST372	O83:H31 (1)	0	0
B2-CH96-433-ST646	O22:HNM (1)	0	0
B2-CH43-fimH$_{TR}$new 2-ST929	O138:H14 (1)	0	0
B2-CH13-175-ST961	O4:HNM (1)	0	0
B2-CH52-428-ST998	O2:H6 (1)	0	0
B2-CH14-64-ST1193	**O75:HNM** (2)	4	3
B2-CH363-75-ST2622	O83:H6 (1)	0	0
B2-CH195-2-ST5644	O175:H5 (1)	0	0
B2-CH13-fimH$_{TR}$new 3-ST new 2	O18:HNM (1)	0	0
B2-CH13-429-ST new 3	O4:H5 (1)	0	0
B2-CH103-9-ST new 4	O4:H31 (1)	0	0
B2-CH103-12-ST new 5	O6:HNM (1)	0	0
B2-CH11-34-ST new 6	O5:H11 (1)	0	0
B2-CH40-20-ST new 7	O1:H4 (1)	0	0
B2-CH363-75-ST new 8	O83:H4 (1)	0	0
B2-CH24-2-ST new 9	ONT:H1 (1)	0	0
B2-CH24-1473-ST new 10	O120:H5 (1)	0	0
B2-CH23-31-ST new 11	O103:H4 (1)	0	0
B2-CH40-20-ST new 12	O1:H4 (1)	0	0
B2-CH40-21-ST new 13	O13:H4 (1)	0	0
D-CH35-27-ST new 14	O77:H18 (1)	0	0
E-CH132-65-ST501	ONT:H1 (1)	0	0
F-CH32-41-ST59	**O1:H7** (1)	4	3
F-CH4-27-ST648	O4:H6 (1)	1	0
F-CH4-58-ST648	O153:H42 (5)	2	0

[1]bold highlights those canine clones and clone-related serotypes also detected among ExPEC and/or UPEC isolates causing extraintestinal infections in humans.

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Among the 28 canine MDR isolates, we observed 24 different clones, of which eight had also been identified among the above cited 394 isolates causing infections in humans (Table 4) [61].

Table 4. Clones and clonal-related serotypes of 28 canine multidrug resistant (MDR) *E. coli* isolates. Prevalence of the canine clones and clone-related serotypes among *E. coli* isolates causing extraintestinal infections in humans [61].

Clone of canine MDR isolates	Clone-related serotype of canine MDR isolates (number of isolates)	Type of ESBL and pAmpC enzymes produced by canine MDR isolates	Number of human *E. coli* isolates with same clone of canine MDR isolates (Number and type ESBL produced by human isolates) (35 of 394)	Number of human *E. coli* isolates with same clone and serotype of canine MDR isolates (1 of 394)
A-CH11-54-ST10	O128:HNM (1)	SHV12	10 (3 SHV12)	0
A-CH11-NEG-ST93	O5:H4 (1)	none	4 (1 CTX-M-14)	0
A-CH11-54-ST8953	O101:HNM (1)	CMY-2	0	0
A-CH11-27-ST new 1	O4:H27 (1)	none	0	0
B1-CH4-27-ST58	O8:H25 (1), **O9:H25** (1)	none	4 (1 CTX-M-14 and 1 CTX-M-32)	1
B1-CH4-121-ST155	O5:H11 (1)	none	0	0
B1-CH4-366-ST155	O9:H10 (1)	CMY-2	1 (1 CTX-M-1)	0
B1-CH4-425-ST new 15	O123:H11 (1)	CTX-M-1	0	0
B1-CH4-31-ST new 16	O8:H7 (1)	CTX-M-1	0	0
B1-CH29-38-ST new 17	O8:H49 (1)	CTX-M-1	0	0
B1-CH30-38-ST new 18	O12:H8 (1)	CMY-2	0	0
B2-CH13-223-ST12	O18:H5 (1)	CMY-2	1	0
B2-CH13-429-ST new 3	O4:H5 (1)	CMY-2	0	0
C-CH4-39-ST88	O45:HNM (1)	CTX-M-1	11 (1 CTX-M-14)	0
D-CH26-5-ST38	O86:H18 (3)	CTX-M-14	0	0
D-CH26-65-ST38	O1:H34 (1)	CMY-2	2 (1 CTX-M-15)	0
D-CH35-27-ST new 14	O77:H18 (1)	none	0	0
E-CH31-54-ST57	O27:H40 (1)	CMY-2	1	0
E-CH11-167-ST695	O99:H38 (1)	none	0	0
E-CH4-31-ST1011	O166:H45 (1)	CTX-M-55	0	0
E-CH23-221-ST1140	O44:H39 (1)	none	0	0
E-CH485-426-ST3774	O9:H31 (1)	CMY-2	0	0
F-CH88-145-ST457	O11:H25 (2)	CMY-2	0	0
F-CH4-27-ST648	O4:H6 (1)	CTX-M-14	1 (1 CTX-M-15)	0

[1]bold highlights those canine clones and serotypes also detected among *E. coli* isolates causing extraintestinal infections in humans.

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3.5. Whole Genome Sequencing (WGS) and Molecular Characterisation of ST372 Isolates

For WGS, we selected 23 of the above studied ST372 isolates. They comprised 21 of the 29 Spanish healthy dog faeces ST372 strains that were isolated in 2013 (n=9) and 2017 (n=12) and two previously published human ST372 strains isolated in 2016 [3, 61]: strains LREC_341 isolated in Spain from an abscess and LREC_342 isolated in France from a bone infection. Both human strains showed serotype O18:H31 and clonotype CH103-9 whereas the 21 canine strains showed six different serotypes [O4:H31 (7 isolates), O83:H31 (4 isolates), O25:H31 (4 isolates), O15:H31 (3 isolates), O21:H31 (2 isolates) and O117:H28 (1 isolate)] and four clonotypes [CH103-9 (18 isolates), CH103-10, CH103-17 and CH103-240].

The main objectives were to get more insights into the *E. coli* ST372 lineage that appears as one of the most prevalent *E coli* lineages among the canine faeces *E. coli* populations and to elucidate if there is any relation between dog and human ST372 strains.

To infer the phylogeny, we performed a SNP-tree with 197 genomes of ST372 strains [23 from this study (labeled LREC strains) and 174 obtained from public databases] corresponding to 151 genomes from canine strains and 46 genomes from human strains. Seventy percent of these genomes corresponded to strains collected between 2017 and 2019 while the remaining 30% corresponded to strains isolated between 1995 and 2016. Regarding geographical distribution, 46 genomes (23.4%) were from strains collected in Europe and 143 (72.6%) from strains collected in North America.

The SNP analysis of the *E. coli* ST372 lineage of revealed a wide and heterogeneous population allowing us to described six clusters. Figure 1 only includes 97 representative genomes (including the 23 LREC genomes sequenced in this study and the 46 genomes from human strains) of the 197 analyzed so that it is possible to visualize all the information.

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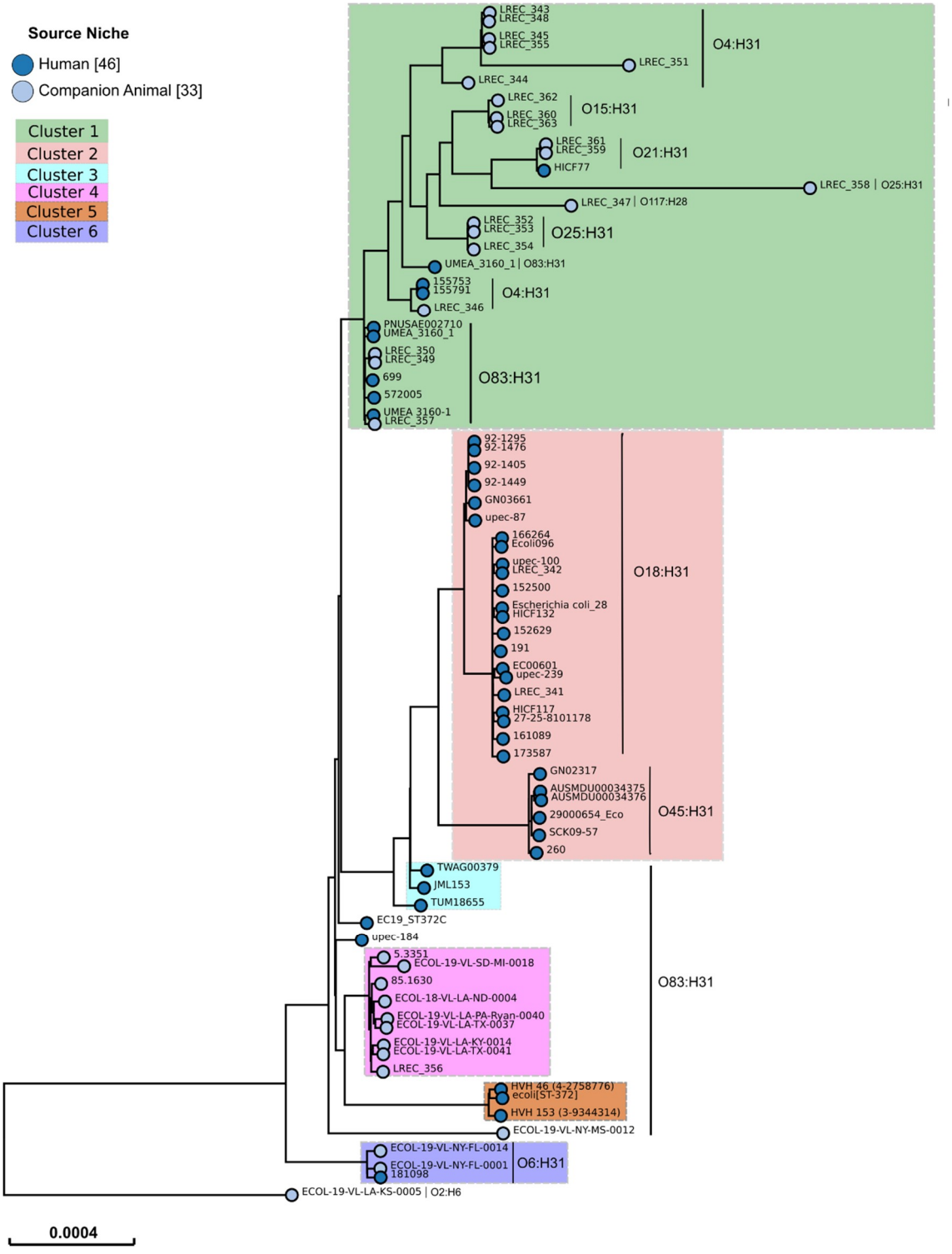
Figure 1. SNP-tree of 79 representative ST372 *E. coli* genomes from 46 human strains and 33 canine strains.



[1]Tree visualization by EnteroBase [60]. The 33 genomes from canine strains are representatives of the different clusters identified in the SNP matrix of a previous SNP-tree performed with the 197 genomes analysed in this study. The identified serotypes are listed beside the vertical line.

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The criterion established to define a cluster was that it should include genomes with less than 200 SNPs distance between them. An exception to this rule was the inclusion of the genome ECOL-19-VL-SD-MI-0018, with a maximum of 391 SNP distance, in cluster 4. Five genomes did not reach this criterion having more than 400 SNP distance between them and could form five other clusters. However, we have included those genomes in only one category (undefined) to simplify the following analysis.

According to the phylogenetic tree built from the genome of the 197 strains, cluster 1 comprised 147 (74.6%) of the 197 analyzed genomes. This cluster was mostly composed of genomes of canine strains (138 genomes; 93.9%). Genomes of canine strains were also included in clusters 4 (9 genomes) and 6 (2 genomes) while only human strain genomes were included in clusters 2 (28 genomes), 3 (3 genomes) and 5 (3 genomes) (Table 5). Thus, cluster 1 comprised significantly more canine strain genomes ($P < 0.00001$) while clusters 2 ($P < 0.00001$), 3 ($P = 0.01209$) and 5 ($P = 0.01209$) comprised significantly more human strain genomes. Twenty of the 21 genomes of the Spanish canine strains belonged to cluster 1 whereas, the genome of the remaining Spanish canine strain (LREC_356) belonged to cluster 4. The genomes of the Spanish and French human strains (LREC_341 and LREC_342) belonged to cluster 2.

Table 5. Distribution into the phylogenic clusters of the 197 canine and human ST372 strains.

Cluster	Number of strains (%)		P-value[1]
	Canine (n=151)	Human (n=46)	
1	138 (91.4)	9 (19.6)	<0.00001
2	0	28 (60.9)	<0.00001
3	0	3 (6.5)	0.01209
4	9 (6.0)	0	
5	0	3 (6.5)	0.01209
6	2 (1.3)	1 (2.2)	
Undefined	2 (1.3)	2 (4.3)	

[1]Two-tailed *P* values by Fisher´s exact probability test are shown where $P < 0.05$.

Both clusters 1 and 2 were the most frequent clusters observed among the studied *E. coli* ST372 strains (canine and human) isolated in Europe and North America. However, cluster 1 was significantly associated with North America strains ($P = 0.02476$), while cluster 2 was especially associated with Europe strains ($P = 0.01233$) (Table 6).

Table 6. Cluster distribution of the 197 studied ST372 strains according to countries.

Cluster (Number of strains)	Number of strains (%)		P-value[1] Europe *vs* North America	Countries (Number of strains)
	Europe (n = 46)	North America (n = 143)		
1 (n = 147)	30 (65.2)	117 (81.8)	0.02476	USA (109), Spain (20), Canada (6), UK (4), Sweden (3), France (2), Germany (1), unknown (2)
2 (n = 24)	11 (23.9)	13 (9.1)	0.01233	USA (13), UK (8), Spain (1), France (1), The Netherlands (1)
3 (n = 3)	0	0		Japan (1), Kenya (1), unknown (1)
4 (n = 9)	1 (2.2)	8 (5.6)		USA (7), Spain (1), unknown (1)
5 (n = 3)	3 (6.5)	0	0.01371	Denmark (2), France (1)
6 (n = 3)	1 (2.2)	2 (1.4)		USA (2), UK (1)

[1]Two-tailed *P* values by Fisher´s exact probability test are shown where $P < 0.05$.

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To compare the virulence profile of the 197 canine and human ST372 strains, we *in silico* investigated the presence of 32 VF-encoding genes in the 197 strains and defined their ExPEC and UPEC status. We also investigated the distribution of those VF-encoding genes according to the classification of the strains into the six defined clusters. Table 7 summarized the results obtained from the mentioned analysis. Microbiological, geographical and genomic data of each of the 197 studied strains are available in Table S2.

The canine ST372 strains showed a higher VF-encoding gene score (mean 16.79) compared with the human ST372 strains (mean 13.76). However, three human stains belonging to cluster 5 were those with the highest number of VF-encoding genes (mean 21.67). Eight VF-encoding genes (*papAH*, *papC*, *papEF*, *focCD*, *focG*, *cnf1*, *hlyA*, *iroN*) were significantly associated with canine ST372 isolates, whereas that five (*hlyF*, *iutA*, *kpsM II*, *kpsM II-K5*, *iss1*) were significantly associated with human ST372 isolates. Interestingly, the ExPEC status was found more frequently among canine ST372 strains (74.8%) than human strains (21.7%) (P < 0.00001) (Table 7).

Table 7. Distribution of the VF-encoding genes detected among the 197 ST372 *E. coli* genomes according to strain origins (canine/human) and cluster types.

VF-encoding gene	Number of strains (%)									P-value[1] Canine *vs* Human
	Canine (n =151)	Human (n = 46)	Cluster 1 (n = 147)	Cluster 2 (n = 28)	Cluster 3 (n = 3)	Cluster 4 (n = 9)	Cluster 5 (n=3)	Cluster 6 (n = 3)	Undefined (n = 4)	
Adhesins										
fimH	151 (100)	46 (100)	147	28	3	9	3	3	4	
papAH	88 (58)	5 (11)	89	0	0	0	0	3	1	< 0.00001
papC	113 (75)	9 (20)	113	0	0	0	3	3	3	< 0.00001
papEF	112 (74)	9 (20)	113	0	0	0	3	2	3	< 0.00001
sfaDE	3 (2)	0 (0)	1	0	0	0	0	2	0	
sfaS	6 (4)	0 (0)	6	0	0	0	0	0	0	
focCD	136 (90)	12 (26)	144	0	0	0	1	1	2	< 0.00001
focG	138 (91)	12 (26)	144	0	0	0	1	3	2	< 0.00001
afaBCD/draP	0	0 (0)	0	0	0	0	0	0	0	
yfcV	150 (99)	46 (100)	146	28	3	9	3	3	4	
Toxins										
sat	0	0 (0)	0	0	0	0	0	0	0	
cnf1	117 (77)	11 (24)	120	0	0	0	3	3	2	< 0.00001
hlyA	117 (77)	11 (24)	119	0	0	0	3	3	3	< 0.00001
hlyF	1 (1)	3 (7)	1	0	0	0	3	0	0	0.04034
cdtB	0	0 (0)	0	0	0	0	0	0	0	
vat	148 (98)	45 (98)	144	27	3	9	3	3	4	
Iron uptake										
iutA	1 (1)	3 (7)	1	0	0	0	3	0	0	0.04034
iroN	139 (92)	14 (30)	144	0	0	0	3	3	3	< 0.00001
fyuA	151 (100)	46 (100)	147	28	3	9	3	3	4	
chuA	151 (100)	46 (100)	147	28	3	9	3	3	4	
ireA	5 (3)	1 (2)	5	0	0	0	0	0	1	
Capsule										
kpsM II	16 (11)	36 (78)	5	28	3	9	3	0	4	< 0.00001
kpsM II-K1	0 (0)	0 (0)	0	0	0	0	0	0	0	
kpsM II-K2	0 (0)	0 (0)	0	0	0	0	0	0	0	
kpsM II-K5	16 (11)	36 (78)	5	28	3	9	3	0	4	< 0.00001
Miscellaneous										
iss1	1 (1)	3 (7)	1	0	0	0	3	0	0	0.04034
iss2	140 (93)	45 (98)	136	27	3	9	3	3	4	
traT	34 (23)	10 (22)	25	5	0	9	3	1	1	
ibeA	149 (99)	46 (100)	146	28	3	9	3	3	3	
malX-PAI	150 (99)	46 (100)	146	28	3	9	3	3	4	
usp	151 (100)	46 (100)	147	28	3	9	3	3	4	
ompT	151 (100)	46 (100)	147	28	3	9	3	3	4	
ExPEC status	113 (75)	10 (22)	113	0	1	0	3	3	3	< 0.00001
UPEC status	151 (100)	46 (100)	147	28	3	9	3	3	4	
Mean of VFEGs	16.79	13.76	16.93	12.11	12.00	13.00	21.67	18.00	17.00	

[1]Two-tailed *P* values by Fisher's exact probability test are shown where $P < 0.05$.

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The more prevalent serotype was O83:H31 which represents 36.0 % of the 197 ST372 strains followed by O4:H31 (17.8%), O15:H31 (15.2%), O18:H31 (10.2%), O45:H31 (3.0%), O117:H28 (3.0%), O21:H14 (2.5%), O21:H31 (2.5%), O75:H31 (2.5%), O-unknown:H31 (2.5%), O25:H31 (2.0%), O2:H6 (0.5%) and O-unknown:H28 (0.5%). The serotypes O4:H31 (P = 0.02631) and O15:H31 (P = 0.00062) were significantly associated with canine ST372 strains, whereas the serotypes O18:H31 (P < 0.00001) and O45:H31 (P = 0.00012) were significantly more frequent among human ST372 strains. The 65 canine strains of serotypes O4:H31 and O15:H31 belonged to cluster 1 and the 26 human strains of serotypes O18:H31 and O45:H31 belonged to cluster 2 (Table 8). In contrast, the dominant serotype O83:H31 was frequently identified among canine (38.4%) and human (28.3%) strains, and although the majority of the strains with this serotype belonged to cluster 1, O83:H31 strains were also found in the clusters 3, 4 and 5.

Table 8. Distribution of serotypes among the 197 ST372 strains according to origins (canine and human) and cluster types.

Serotype *in silico*	Number of strains (%)		P- value[1] Canine *vs* Human	Number of isolates belonging to cluster						
	Canine (n = 151)	Human (n = 46)		1	2	3	4	5	6	Undefined
O2:H6	1 (0.7)	0								1
O4:H31	32 (21.2)	3 (6.5)	0.02631	35						
O6:H31	2 (1.3)	1 (2.2)							3	
O15:H31	30 (19.9)	0	0.00062	30						
O18:H31	0	20 (43.5)	<0.00001		20					
O21:H14	5 (3.3)	0		5						
O21:H31	4 (2.6)	1 (2.2)		5						
O25:H31	4 (2.6)	0		4						
O45:H31	0	6 (13.0)	0.00012		6					
O75:H31	5 (3.3)	0		5						
O83:H31	58 (38.4)	13 (28.3)		53		3	9	3		3
O117:H28	6 (4.0)	0		6						
O-unknown:H31	3 (2.0)	2 (4.3)		3	2					
O-unknown:H28	1 (0.7)	0		1						

[1]Two-tailed *P* values by Fisher´s exact probability test are shown where $P < 0.05$.

The three most prevalent serotypes in Europe were O4:H31 (23.9%), O83:H31 (21.7%) and O18:H31 (19.6%), while in North America they were O83:H31 (39.9%), O15:H31 (18.2%) and O4:H31 (16.8%). The serotypes O18:H31 (P = 0.02203) and O25:H31 (P = 0.00317) were more frequently observed in Europe, whereas the serotype O83:H31 (P = 0.03291) was more prevalent in North America (Table 9).

Table 9. Serotypes of the 197 ST372 strains according to countries.

Serotype *in silico*	Number of strains (%)		P- value[1] Europe *vs* North America	Countries (Number of strains)
	Europe (n = 46)	North America (n = 143)		
O2:H6	0	1 (0.7)		USA (1)
O4:H31	11 (23.9)	24 (16.8)		USA (23), Spain (7), UK (2), France (1), Sweden (1), Canada (1)
O6:H31	1 (2.2)	2 (1.4)		USA (2), UK (1)
O15:H31	4 (8.7)	26 (18.2)		USA (25), Spain (3), Canada (1), France (1)
O18:H31	9 (19.6)	10 (7.0)	0.02203	USA (10), UK (7), Spain (1), France (1)
O21:H14	1 (2.2)	4 (2.8)		USA (4), Sweden (1)
O21:H31	3 (6.5)	2 (1.4)		Spain (2), UK (1), USA (1), Canada (1)
O25:H31	4 (8.7)	0	0.00317	Spain (4)
O45:H31	1 (2.2)	2 (1.4)		USA (2), Australia (2), Netherlands (1)
O75:H31	0	5 (3.5)		USA (5)
O83:H31	10 (21.7)	57 (39.9)	0.03291	USA (52), Spain (4), Canada (2), Denmark (2), UK (1), France (1), Sweden (1), Germany (1)
O117:H28	1 (2.2)	5 (3.5)		USA (5), Spain (1)
O-unknown:H31	1 (2.2)	4 (2.8)		USA (3), Canada (1), UK (1)
O-unknown:H28	0	1 (0.7)		USA (1)

1Two-tailed P values by Fisher´s exact probability test are shown where $P < 0.05$.

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The 23 LRCE genomes sequenced in this study were investigated in greater depth. These genomes were reconstructed to analyze the chromosome and plasmidome separately. The size of the chromosomes had an average of 5,043,308 pb and was encompassed in 55 to 178 contigs. We found an integrative conjugative element (ICE) with relaxase type MOB_Q in all the genomes except for LRCE_347. Those ICEs belong to the ICEKp1 family, a yersiniabactin synthesis-associated ICE type (similar to ICEEcoUMN026-1). The contigs that harbored the ICE region were revised allowing us to detect the presence of a pathogenic island (PAI) and some VF-encoding genes. Contig retrieves from LREC_356 was the longest (2,540,863 pb) and showed a high percentage of homology with the other contigs harboring the ICE region (Figure S1). Interestingly, the contig from LREC_356 harbored the *ompT*, *iss*, *vat*, *fyvA* and *yfcV* virulence genes, the last three mentioned genes being those used (in addition to *chuA*) to define UPEC status. We also identified the secretion system effector homolog type T6SS and the PAI_AET37190. We conclude that the presence of this type of ICE was a common feature in our ST372 genomes and may be involved in the acquisition of UPEC status.

We also described 11 plasmids (four conjugative plasmids, six mobilizable plasmids and one plasmid with no relaxase suggesting that it is not mobilizable) which belonged to the following relaxase families (MOB) and incompatibility groups (Inc.): MOB_{P3}/IncX1 (n=3); MOB_{P1}/nd (n=2); MOB_{F12}/IncFII-pCD1 (n=2); MOB_{F12}/IncFII-IncFIB (n=1); MOB_{H11}/IncHI2 (n=1); MOB_{Qu}/ColRNAI (n=1); nd/p0111 (n=1). To predict plasmid transferability, we investigated the presence of mating pair formation (Mpf) system proteins. These proteins were present in all the previously described MOB_{F12} and MOB_{H11} conjugative plasmids. Furthermore, *in silico* analysis showed that these plasmids did not carry resistance or virulence encoding genes except for the *cba* and *cma* genes that were found in plasmid pLREC354_1 and a bla_{TEM} gene found in pLREC346_1. Table 10 summarizes the MGE content of the 23 ST372 genomes.

Table 10. Description of mobile genetic elements (MGE)s found in the 23 ST372 strain genomes sequenced in this study.

Genome	MGEs		MOB/Inc typing; size (kb)	
	ICEs (kb of contig)	Number of plasmids	plasmid_1	plasmid_2
LREC_341	MOB_Q (308)	2	MOB_{P1}/nd; (164)	nd/p0111; (92)
LREC_342	MOB_Q (90)	2	MOB_{P1}/nd; (720)	MOB_{Qu}/ColRNAI; (4)
LREC_343	MOB_Q (474)	1	MOB_{F12}/IncFII [F-:A-:B-],pCD1; (66)	
LREC_344	MOB_Q (1208)	0		
LREC_345	MOB_Q (158)	1	MOB_{F12}/IncFII [F2:A-:B-],pCD1; (75)	
LREC_346	MOB_Q (653)	1	MOB_{P3}/IncX1; (47)	
LREC_347		1	MOB_{P3}/IncX1; (38)	
LREC_348	MOB_Q (472)	0		
LREC_349	MOB_Q (707)	0		
LREC_350	MOB_Q (707)	0		
LREC_351	MOB_Q (653)	0		
LREC_352	MOB_Q (658)	0		
LREC_353	MOB_Q (658)	0		
LREC_354	MOB_Q (658)	0		
LREC_355	MOB_Q (157)	0		
LREC_356	MOB_Q (2541)	2	MOB_{F12}/IncFIB, IncFII [F-:A-:B52]; (162)	MOB_{P3}/IncX1; (36)
LREC_357	MOB_Q (1678)	0		
LREC_358	MOB_Q (660)	0		
LREC_359	MOB_Q (1317)	0		
LREC_360	MOB_Q (86)	0		
LREC_361	MOB_Q (1317)	0		
LREC_362	MOB_Q (641)	0		
LREC_363	MOB_Q (172)	1	MOB_{H11}/IncHI2; (204)	

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We *in silico* investigated the presence of 189 VF-encoding genes, 87 antibiotic-resistance encoding genes (ARGs) and 18 types of point mutations (Table S8). Through this analysis, the 23 ST372 strains were shown with an UPEC status and harboring a wide variety of VF-encoding genes, reaching an average number of 80. In contrast, these 23 ST372 strains were shown as carrying very few ARGs. However, genes encoding drug efflux were detected but only in the two human strain genomes (LREC_341 and LREC342) that also harbored antibiotic-resistance encoding genes: *bla*$_{TEM-1A}$, *sul1*, *aadA1*, *dfrA1* and *mdf(A)*. These results were in agreement with those previously obtained by conventional methods.

4. Discussion

The present study found that the intestinal tract of Spanish healthy dogs is an important reservoir of ExPEC, UPEC isolates and in a lesser way of MDR *E. coli* isolates. Indeed, of the 197 studied canine faecal isolates, 37.6% were classified as ExPEC, 41.6% as UPEC and 14.2% as MDR. Similar results were reported in numerous previous studies that, however, focused their analysis mostly on antibiotic-resistant dog isolates to assess the potential role of dogs as a reservoir for antibiotic-resistant strains [62–80]. Although we found a low prevalence of MDR isolates among the 197 studied isolates, we found, similarly to previous studies, that most of the here studied MDR isolates produced ESBLs or CMY-2 [63,65,66,69,71]. MLST assigned the 28 Spanish canine MDR isolates to 15 different established sequence types (ST10, ST12, ST38, ST57, ST58, ST88, ST93, ST155, ST457, ST648, ST695, ST1011, ST1140, ST3774, ST8953) and in seven new STs. The first ten established STs that we found have been identified in several studies carried out in different countries [1,10,13,14,27–29,32,33,36–41,65,67,70-72,74-76,79,80-85]. In contrast to other studies, we have not detected canine MDR isolates displaying the five important emerging STs: ST69, ST127, ST131, ST410 and ST1193 [1,2,10,13,14,16,26–35,37–40,69,70,73,77]. We have also not detected any isolate resistant to colistin with the *mcr*-1 gene or isolates producing carbapenemases while such isolates have been identified in China (ST93 isolates with the *mcr*-1 gene) [86] and in Germany [87], France [88], and United States [37] (ST12, ST58, ST88, ST131, ST372, ST410, ST648 and ST1196 isolates producing the OXA-48-carbapenemase). Finally, we found that none of the 29 ST372 isolates of our collection was MDR while previous studies have found ST372 isolates producing different types of ESBLs and CMY-2 [1,10,13,29,32,37,39-41,78].

The clonal structure of canine ExPEC and UPEC is poorly understood since only two studies have been carried out to date. LeCuyer *et al*. [10] analyzed 295 *E. coli* isolates from canine UTI in the United States. They found that ST372, which is uncommon among the human *E. coli* pathogens [3,11,61,89,90], was the predominant ST in canine UTI isolates (21.7%), and this, well ahead the five other most frequent STs: ST12 (6.4%), ST73 (6.4%), ST127 (4.1%), ST131 (4.1%) and ST297 (3.7%). A total of 170 (57.4%) of these isolates met the criterion to be classified as ExPEC, and, except for ST297, the most prevalent STs were associated with ExPEC status. In France, Valat *et al*. [13] analyzed 618 canine *E. coli* isolates collected from diagnostic laboratories, including 403 (65.2%) from UTIs. B2 phylogroup was over-represented (79.6%) and positively associated with the presence of numerous VFs, including those defining the ExPEC status. MLST of a randomly chosen subset of 89 isolates belonging to B2 phylogroup revealed five dominant STs: ST372 (17.9%), ST73 (17.9%), ST12 (10.1%), ST141 (7.9%) and ST961 (5.6%). In our study, 34 STs were found among the 91 Spanish canine ExPEC and/or UPEC faecal isolates, but 67.0% of them belonged to one of the six following STs: ST372 (31.9%), ST12 (9.9%), ST127 (8.8%), ST648 (6.6%), ST141 (5.5%) and ST73 (4.4%). The comparison of the ST-based population structure of *E. coli* isolates causing UTI in dogs with that we established for canine faecal *E. coli* isolates shows that three STs (ST12, ST73 and ST372) belong to the dominant STs observed in the USA, France and Spain and two other STs belonged to the dominant STs of two countries: ST127 in the USA and Spain and ST141 in France and Spain regardless of the dog niches (urine and faeces). Of note, ST372 was the most prevalent ST in the United States and Spain, while ST372 and ST73 was the two equally most prevalent STs in France. All these findings seem to support

both theories "prevalence" and the "special pathogenicity" for the origin of uropathogenic isolates, at least in dogs [5,6].

Interestingly, fifteen clones identified among 52 (57.1%) of the 91 Spanish canine ExPEC and/or UPEC isolates in the current study had also been found in a previous study conducted by our research group among 49 (18.8%) of 261 ExPEC and/or UPEC isolates causing human extraintestinal infections [61], suggesting a zoonotic potential of these clones. However, only 31 of the 49 human ExPEC and/or UPEC isolates presented the same O:H serotype as the canine ones suggesting possible niche-related strain modifications.

The SNP analysis of the ST372 lineage revealed a wide and heterogeneous population allowing us to described six clusters. However, cluster 1 comprised 91.4% of the genomes from canine strains and cluster 2 comprised 60.9% of the genomes from human strains. Cluster 2 was human genome specific and was associated with serotypes O18:H31 and O45:H31 that were exclusively found in human ST372 strains. Other three serotypes were the most prevalent serotype among strains belonging to cluster 1, including O4:H31 and O15:H31 associated with canine strains, and O83:H31 identified in similar proportion among canine and human strains. Thus, the WGS analysis suggests that canine strains of clone B2-CH103-9-ST372, identified in cluster 1 and having serotype O83:H31 might cause extraintestinal infections in humans and dogs, whereas strains of this clone belonging to cluster 2 and serotypes O18:H31 and O45:H31 might cause only human extraintestinal infections.

5. Conclusions

The intestinal tract of healthy dogs is an important reservoir of ExPEC, UPEC and MDR *E. coli* isolates. The most canine MDR isolates produced ESBLs or CMY-2. Among the canine isolates displaying an ExPEC and/or UPEC status, clone B2-CH103-9-ST372 was dominant. This canine clone and 14 others also displaying an ExPEC and/or UPEC status had been identified in isolates previously published as causing extraintestinal infections in human suggesting a zoonotic potential of these clones. WGS analysis suggests that canine strains of clone B2-CH103-9-ST372, belonging to cluster 1 and having serotype O83:H31 might cause extraintestinal infections in both humans and dogs, whereas those strains of this clone belonging to cluster 2 and serotypes O18:H31 and O45:H31 might cause only human infections. Further studies on the 14 other clones identified in canine and human ExPEC and/or UPEC isolates are necessary to establish the genetic relationship between canine and human isolates of each clone and if these isolates are membership to common clusters sustaining their potential involvement in infections in both humans and dogs.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Comparison of contigs harbouring integrative conjugative elements (ICEs) from 22 ST372 *E. coli* genomes, Table S1: Bioproject accession (PRJNA627579) and assembly genome information, Table S2: SNP matrix and VF-encoding genes of 197 ST372 genomes, Table S3: Prevalence of the phylogenetic groups in the 197 canine *E. coli* isolates, Table S4: Comparison of the distribution of the phylogenetic groups among the 197 canine isolates according to the strain ExPEC and UPEC status, Table S5: Comparison of the distribution of the phylogenetic groups among the 197 canine isolates according to the strain multidrug resistant (MDR) status, Table S6: Comparison of the strain ExPEC and UPEC status among canine multidrug resistant (MDR) and non-MDR isolates, Table S7: New sequence types observed in 18 canine *E. coli* isolates.

Author Contributions: SCFS, MT, VG, JEB, MB, MPA, AG, JDG, MHNC and JB undertook the laboratory work and the genome analysis. SCFS and JB conceived the concept for the paper and designed the experiments. All authors provided critical input and contributed to the writing of the manuscript and have approved the final version.

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