Molecular characterization and antimicrobial resistance of faecal and urinary Escherichia coli *isolated from dogs and humans in Italy*

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Keywords
Antibiotic resistance, Dog, *Escherichia coli*, Human, Phylogenetic group, Virulence factors.

Summary
During this study, 109 faecal *Escherichia coli* samples isolated from 61 dogs and 48 humans were characterised according to phylogenetic group, extraintestinal virulence factors and antibiotic resistance. The isolates from dogs were predominantly distributed within phylogroup B1 (36%), while the majority of human strains belonged to phylogroup B2 (54%). The prevalence of *cnf1*, *hlyA*, *papC* and *sfa* virulence genes was significantly associated with the group B2. Canine isolates showed multidrug resistance (MDR) more frequently than human strains. Since group B2 contains most of the strains that cause extraintestinal infections, all 46 B2 faecal strains were confronted against an addition population of 57 urinary *E. coli* strains belonging to the same phylogroup. The comparison shows that there was no significant difference in the occurrence of virulence factors or in the distribution of antibiotic resistance between faecal and urinary *E. coli* isolates fromd dogs. At the same time, a highly significant association was detected between multiple resistence and the source of the strains and between MDR and E. coli isolated from urine in human. This study highlighted similar features of *E. coli* isolated across sources and hosts. The data suggest a high prevalence of antibiotic resistance in faecal strains, which may represent a serious health risk since these strains can function as a reservoir for uropathogenic *E. coli*.

Caratterizzazione molecolare e resistenza antimicrobica di Escherichia coli *fecali e urinari isolati in cane e uomo in Italia*

Parole chiave
Cane, *Escherichia coli,* Gruppo filogenetico, Fattore di virulenza, Resistenza antimicrobica, Uomo.

Riassunto
Lo studio ha coinvolto 109 campioni di *Escherichia coli* fecali isolati da 61 cani e 48 uomini. I campioni sono stati caratterizzati in base al gruppo filogenetico, alla presenza di fattori di virulenza extraintestinali e alla resistenza antimicrobica. Gli isolati provenienti da cani si sono distribuiti principalmente nel filogruppo B1 (36%), la maggior parte dei ceppi umani nel filogruppo B2 (54%). Lo studio ha mostrato come la prevalenza dei geni di virulenza *cnf1*, *hlyA*, *papC* e *sfa* fosse significativamente associata al gruppo B2 e gli isolati canini presentassero multi-resistenza (MDR) con frequenza maggiore rispetto ai ceppi umani. Poiché al gruppo B2 sono risultati appartenere ceppi responsabili di infezioni extraintestinali, i 46 ceppi fecali del filogruppo B2 sono stati comparati con 57 isolati urinari associati anch'essi al gruppo B2. Tra i ceppi fecali e urinari non sono state osservate differenze concernenti la distribuzione dei fattori di virulenza e la prevalenza dell'antibiotico-resistenza ma è stata osservata un'associazione altamente significativa sia tra multi-resistenza e origine dei campioni sia tra MDR ed *Escherichia coli* urinari nei ceppi umani. Gli isolati hanno mostrato caratteristiche simili rispetto alla provenienza e all'ospite. Lo studio ha permesso di evidenziare nei ceppi fecali un'alta prevalenza di resistenza antibiotica, aspetto che rappresenta un serio rischio, in quanto questi stessi ceppi possono comportarsi come serbatoi di *Escherichia coli* uropatogeni.

Introduction

Escherichia coli is a normal inhabitant of the mammalian intestine, including the human intestine. The gut also constitutes an important reservoir of strains that cause extraintestinal infections, such as urinary tract infection (UTI). Uropathogenic *E. coli* (UPEC) is often marked by the presence of special virulence factors, such as P and S pili, afimbrial adhesins, siderophores and toxins (Féria *et al.* 2006), and all of these traits can be encoded on mobile genetic elements, such as plasmids, bacteriophages and pathogenicity-associated islands (PAIs) (Sabate *et al.* 2006). Most pathogenic *E. coli* strains belong to group B2 or, to a lesser extent, to group D (Zhang *et al.* 2002), while commensal *E. coli* belong mostly to phylogenetic groups A and B1 and generally lack virulence factors (Duriez *et al.* 2001, Moreno *et al.* 2006). Urinary tract infections (UTIs) in humans and dogs present an important clinical problem, i.e. the presence of virulence factors and the microorganisms' abilities to colonize tissues, which frequently proves difficult to resolve due to antimicrobial resistance. In some instances, these characteristics contribute to the chronicity and persistence of the pathology (Idress *et al.* 2010). Although antimicrobial therapy is often able to provide an efficient treatment for UTI infections, resistance to antimicrobials is widespread and is an aspect of growing concern, in human as well as in veterinary medicine (Harada *et al.* 2012, Johnson *et al.* 2003). The transmission of *E. coli* between pet animals and humans was recently documented; specifically, extraintestinal pathogenic *E. coli* (ExPEC) isolated from dog were shown to infect humans causing urinary diseases (Johnson *et al.* 2008, Stenske *et al.* 2009). No report of *E. coli* transmission between pet animals and humans has yet been reported in Italy, nor have investigations been conducted so far to characterise *E. coli* isolated from human and pet hosts living in the same geographical area.

The aims of this study were: i) to characterise canine and human faecal *E. coli* populations according to phylogenetic group, virulence profile and antibiotic resistance status; ii) to analyse the differences between B2 strains isolated from faecal *vs.* urinary samples.

Material and methods

Samples and *E. coli* strains

In 2011, 109 faecal samples were collected from humans and dogs living in the same geographical area (North-Eastern Piedmont, Italy): 61 from dogs (of various breed, 35 females and 26 males; mean age 7 years within the range of 2 - 13 years). Forty-eight samples were collected from human volunteers (28 females and 20 males; mean age 43 years, within the range of 30 - 55 years). Neither dogs nor humans had undergone antibiotic therapy in the 6 months prior the sampling and all the sampled individuals were seemingly healthy, presenting no clinical signs of gastro-intestinal tract disorders at the time of sample collection.

Each faecal specimen was streaked onto MacConkey Agar (Oxoid, Basingstoke, UK) and incubated overnight at 37°C. One putative *E. coli* colony per sample was arbitrarily selected from the solid medium and subjected to the BBL Crystal test (Becton Dickinson, Franklin Lakes, NJ, USA) in order to identify *E. coli* isolates. All identified *E. coli* strains were stored at -80 °C in Luria-Bertani broth (Oxoid, Basingstoke, UK) containing 15% glycerol until further testing.

For comparative purposes, a collection of 57 urinary *E. coli* belonging to phylogroup B2 was also included in this study: 27 samples isolated from dogs (21 females and 6 males; mean age 7 years, within the range of 2 - 13 years) with uncomplicated cystitis (Salvarani *et al.* 2011, Tramuta *et al.* 2011); and 30 samples isolated from hospitalised humans with UTI (all women; mean age 47 years, within the range of 29 - 75 years). No subject had received any antimicrobial therapy in the preceding 6 months. The 30 human urine samples had been collected from patients living in the North-Eastern area of Piedmont, between January 2008 - April 2011 (unpublished data).

DNA extraction

All *E. coli* strains were cultured on Tryptic Soy Agar (Oxoid, Basingstoke, UK) for 20 hrs at 37°C. The bacterial genomic DNA was extracted using a commercially available kit (InstaGene DNA, BioRad, Philadelphia, PA, USA), following the manufacturer's instructions.

Detection of phylogenetic groups and virulence genes

Faecal strains were assigned to 1 of the 4 major phylogenetic groups (A, B1, B2 and D) by detecting the presence of specific marker genes (*chuA, yjaA* and *tspE*4.C2) using a triplex polymerase chain reaction (PCR) method. Virulence genes were also detected by PCR, using primers targeting 7 extraintestinal putative virulence factors, which included adhesion genes (*afa, papC*, and *sfa*), toxin genes (*cdt, cnf1*, and *hlyA*) and the aerobactin receptor gene (*iutA*) (Table I).

Antimicrobial susceptibility test

All *E. coli* strains were tested for antimicrobial susceptibility using the agar disk diffusion

Table I. *Polymerase chain reaction primers used to detect phylogenetic groups and extraintestinal virulence factors genes of* E. coli *in samples collected in 2011 in the North-Eastern Piedimont, Italy.*

Target gene	Primer sequence (5′ to 3′)	Product size (bp)	Annealing temp (°C)	Reference
chuA	GACGAACCAACGGTCAGGAT	279	55	Clermont *et al.* 2000
	TGCCGCCAGTACCAAAGACA			
yjaA	TGAAGTGTCAGGAGACGCTG	211	55	Clermont *et al.* 2000
	ATGGAGAATGCGTTCCTCAAC			
tspE4.C2	GAGTAATGTCGGGGCATTCA	152	55	Clermont *et al.* 2000
	CGCGCCAACAAAGTATTACG			
afa	GCTGGGCAGCAAACTGATAACTCTC	750	65	Le Bouguenec *et al.* 1992
	CATCAAGCTGTTTTGTTCGTCCGCCG			
papC	GACGGCTGTACTGCAGGGTGTGGCG	328	61	Le Bouguenec *et al.* 1992
	ATATCCTTTCTGCAGGGATGCAATA			
sfa	CTCCGGAGAACTGGGTGCATCTTAC	410	64	Le Bouguenec *et al.* 1992
	CGGAGGAGTAATTACAAACCTGGCA			
cdt	GAAAGTAAATGGAATATAAATGTCCG	466	55	Tòth *et al.* 2003
	AAATCACCAAGAATCATCCAGTTA			
	GAAAATAAATGGAACACACATGTCCG	466	55	
	AAATCTCCTGCAATCATCCAGTTA			
cnf1	GGCGACAAATGCAGTATTGCTTGG	522	60	Pass *et al.* 2000
	GACGTTGGTTGCGGTAATTTTGGG			
hlyA	AACAAGGATAAGCACTGTTCTGGCT	1177	62	Yamamoto *et al.* 1995
	ACCATATAAGCGGTCATTCCCGTCA			
iutA	ATGAGCATATCTCCGGACG	587	58	Moulin-Schouleur *et al.* 2006
	CAGGTCGAAGAACATCTGG			

method, according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI)[1]. The following antibiotics (Oxoid, Basingstoke, UK) were used: cephalothin 30 µg (KF), cefotaxime 30 µg (CTX), gentamicin 10 µg (CN), imipenem 10 µg (IPM), piperacillin 100 µg (PRL), sulfamethoxazole-trimethoprim 25 µg (SXT) and a fluoroquinolone: ciprofloxacin 5 µg (CIP) for human strains and enrofloxacin 5 µg (ENR) for canine strains. Strains showing intermediate susceptibility were defined as resistant; and multidrug-resistant (MDR) strains were defined as those resistant to antimicrobials belonging to at least 3 of the following classes of antibiotics: aminoglycosides, carbapenems, cephalosporines (cefotaxime), fluoroquinolones, penicillins, sulfamethoxazole-trimethoprim.

Statistical analyses

Data were organized into 2x2 tables considering the presence/absence of the virulence factor/antibiotic resistance as the *outcome variable* and the source of the strain (fecal/urinary) as the *exposure variable*. Tables were analyzed using EPIINFO v. 6 by performing a stratified analysis, considering the species as the *stratum variable*. The same stratified analysis was performed for faecal strains, considering the association between the presence of virulence traits/antibiotic resistance and strain phylogroup (B2 versus non B2). The χ^2 test and the Fisher's Exact probability test (FEP) were used to assess the significance of the associations in single *strata*, and the Mantel–Haenszel χ^2 was calculated for the summary analysis. In addition, for B2 strains, in order to evaluate the association between MDR and the source of strain, the same stratified analysis was performed considering the species as the *stratum variable*. *P* values <0.05 were considered statistically significant, and *P* values <0.01 were considered highly significant. Within each phylogroup, faecal strains were compared between species with respect to aggregate virulence factors scores (calculated by adding up the number of virulence genes possessed by each strain). A similar analysis was performed for urinary strains (all belonging to phylogroup B2). All comparisons were made using the Mann-Whitney U test, considering $P<0.05$ to be significant.

[1] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational Supplement. Document M100-S20. Wayne, PA, CLSI, 2010.

Results

Distribution of phylogenetic groups, virulence factors and antibiotic resistance in faecal strains

The distribution of the phylogenetic groups differed depending on the species. In dogs (n = 61 strains), 36% of isolates belonged to group B1, 33% to group B2, 26% to group A, and 5% to group D. In humans (n = 48 strains), phylogroup B2 was predominant (54%) followed by phylogroups A (25%) and D (21%) – no human strains belonged to group B1.

The detection of virulence factors using PCR revealed that 52 (48%) of all strains (n = 109) were positive for at least 1 of the virulence genes tested; of these strains, 24 (46%) were canine and 28 (54%) were human. In the strains isolated from dogs, *sfa* and *hlyA* were the most common virulence genes identified (20% and 18%, respectively), followed by *iut* (15%), *cnf* and *pap* (13% each), while *afa* and *cdt* were never detected. In the human strains, *iutA, hlyA* and *papC* were the most common virulence genes detected, present in 38%, 31% and 31% of cases, respectively, followed by *cnf* and *sfa* (21% each), whereas *afa* and *cdt* genes were never detected (Table II). In strains belonging to phylogroup B2,

Table II. *Frequency of virulence genes (%) in relation to phylogenetic group (FG) among faecal and urinary* E. coli *isolated from dog and human samples collected in 2011 in the North-Eastern Piedimont, Italy.*

Source	FG	No. strains	Virulence genes (%)						
			afa	*cdt*	*cnf1*	*hlyA*	*iutA*	*papC*	*sfa*
Canine faeces	Total	61	0	0	8 (13)	11 (18)	9 (15)	8 (13)	12 (20)
	A	16 (26)	0	0	0	0	0	0	2 (13)
	B1	22 (36)	0	0	0	3 (14)	5 (23)	0	0
	B2	20 (33)	0	0	8 (40)	8 (40)	2 (10)	8 (40)	10 (50)
	D	3 (5)	0	0	0	0	2 (67)	0	0
Human faeces	Total	48	0	0	10 (21)	15 (31)	18 (38)	15 (31)	10 (21)
	A	12 (25)	0	0	0	0	4 (33)	2 (17)	0
	B1	0	-	-	-	-	-	-	-
	B2	26 (54)	0	0	10 (38)	11 (42)	10 (38)	11 (42)	10 (38)
	D	10 (21)	0	0	0	4 (40)	4 (40)	2 (20)	0
Canine urine	B2	27	1 (4)	2 (7)	21 (78)	8 (30)	6 (22)	19 (70)	22 (81)
Human urine	B2	30	0	0	7 (23)	10 (33)	17 (57)	12 (40)	6 (20)

Table III. *Frequency of antibiotic resistance (%) in relation to phylogenetic group among faecal and urinary* E. coli *isolated from dog and human samples collected in 2011 in the North-Eastern Piedimont, Italy.*

Source	FG	No. strains	Antibiotic resistance* (%)						
			CN	CTX	ENR/CIP	IPM	KF	PRL	SXT
Canine faeces	Total	61	16 (26)	10 (16)	7 (11)	0	55 (90)	21 (34)	13 (21)
	A	16 (26)	6 (38)	2 (13)	0	0	13 (81)	6 (38)	2 (13)
	B1	22 (36)	2 (9)	3 (14)	2 (9)	0	20 (91)	6 (27)	6 (27)
	B2	20 (33)	7 (35)	3 (15)	3 (15)	0	19 (95)	6 (30)	4 (20)
	D	3 (5)	1 (33)	2 (67)	2 (67)	0	3 (100)	3 (100)	1 (33)
Human faeces	Total	48	29 (60)	0	3 (6)	0	19 (40)	19 (40)	4 (8)
	A	12 (25)	12 (100)	0	0	0	8 (67)	6 (50)	2 (17)
	B1	0	-	-	-	-	-	-	-
	B2	26 (54)	16 (62)	0	3 (12)	0	11 (42)	10 (38)	2 (8)
	D	10 (21)	1 (10)	0	0	0	0	3 (30)	0
Canine urine	B2	27	6 (22)	2 (7)	5 (19)	0	10 (37)	7 (26)	4 (15)
Human urine	B2	30	5 (17)	12 (40)	10 (33)	0	16 (53)	17 (57)	7 (23)

* As determined by the disk diffusion method.
FG = phylogenetic group; CN = gentamicin; CTX = cefotaxime; ENR = enrofloxacin (used for canine strains); CIP = ciprofloxacin (used for human strains); IPM = imipenem; KF = cephalothin; PRL = piperacillin; SXT = sulfamethoxazole-trimethoprim.

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Table IV. *Main resistance pattern phenotypes detected among the 77 antibiotic resistant isolates of* E. coli *strains recovered from dogs and humans in phylogroup B2 of the sample collected in 2011 in the North-Eastern Piedimont, Italy.*

Number of resistance patterns per isolate	Phenotype of resistance pattern	Faecal isolates B2 (n=42)	Urine isolates B2 (n=35)	
6	CN, CTX, ENR/CIP, KF, PRL, SXT	2	2	
5	CN, CTX, KF, PRL, SXT	1	0	
	CTX, ENR/CIP, KF, PRL, SXT	0	3	
4	CN, KF, PRL, SXT	1	0	
	ENR/CIP, CN, KF, PRL	2	0	
	CTX, KF, PRL, SXT	0	2	
	CTX, ENR/CIP, KF, PRL	0	4	
	CN, CTX, KF, PRL	0	1	
	CN, ENR/CIP, PRL, SXT	0	1	
3	ENR/CIP, KF, PRL	1	2	
	ENR/CIP, KF, SXT	0	1	
	CTX, CN, KF	0	1	
	CN, KF, PRL	3	2	
	CN, PRL, SXT	0	1	
	ENR/CIP, PRL, SXT	0	1	
2	CN, PRL	4	0	
	ENR/CIP, KF	1	0	
	CTX, KF	0	1	
	CN, SXT	2	0	
	CN, KF	1	2	
	PRL, KF	3	1	
MDR		6	15	P-value 0.0306
NON MDR		25	16	

CN = gentamicin; CTX = cefotaxime; ENR = enrofloxacin (used for canine strains); CIP = ciprofloxacin (used for human strains); IPM = imipenem; KF = cephalothin; PRL = piperacillin; SXT = sulfamethoxazole-trimethoprim.

cnf1, *hlyA*, *papC*, and *sfa* were often present together (38% of isolates).

The aggregate virulence scores attributed to canine strains ranged from 0 to 4, the median being 0 (due to the prevalence of null scores); while in human strains, it ranged from 0 to 5, with a median score of 1.5.

The results of antibiotic susceptibility tests are summarised in Table III. The large majority of strains (96/109: 58 canine and 38 human) was resistent to at least one antimicrobic, while 13 strains (3 canine and 10 human) were sensitive to all antibiotics tested. Imipenem was the only antibiotic effective on all the strains. The highest frequencies of resistance were observed for cephalothin in canine strains (90%) and for gentamicin in human strains (60%). Fourteen strains (13%) were classified as MDR: 10 (16%) from dogs and 4 (8%) from humans. Among the 46 faecal *E. coli* strains belonging to phylogroup B2, 42 (91%) were resistant to at least 1 antimicrobic, 21 of these 42 stains were resistant only to 1 antimicrobial molecule. The most frequent resistance pattern phenotypes are reported in Table IV. In particular, 2 antimicrobial combinations were detected in 11 strains, 3 and 4 antimicrobial combinations were observed in 7 strains, and only 3 strains showed resistance to 5 or 6 antimicrobials.

Prevalence comparison in faecal strains

The only significant difference in phylogroup distribution for the 2 hosts of origin was observed for phylogroup B1, which included 36% of canine strains and none from humans ($P<0.001$).

The stratified analyses on the prevalence of virulence factors across phylogroup, stratifying by species, highlighted that *cnf1*, *papC*, *hlyA* and *sfa* genes were highly associated with phylogroup B2 ($P<0.001$).

The results of the stratified analyses on antibiotic resistance across phylogroups only revealed a significant association for resistance to cephalothin, which was higher in strains belonging to phylogenetic group B2 than in other groups ($P<0.001$).

Distribution of virulence factors and antibiotic resistance in urinary strains belonging to phylogroup B2

The results on the prevalence of virulence factors and antibiotic resistance *status* for the 57 urinary *E. coli* strains belonging to phylogroup B2 are reported in Table II and Table III. It is worthwhile noticing that 26 (96%) canine and 24 (80%) human strains had at least 1 virulence factor. The aggregated median virulence score was 4.0 (*range* 0-5) for canine strains and 1.0 (*range* 0-5) for human strains, respectively. Thirty-five strains (61%) were resistant to at least 1 of the antimicrobics tested and 10 (29%) isolates showed resistance to just a single antimicrobic. The resistance patterns are reported in Table IV.

Prevalence comparisons in urinary strains

When comparing virulence factor prevalences according to host of origin, *cnf1* and *sfa* were detected in a significantly higher proportion in canine strains over human strains ($P<0.001$). Conversely, *iutA* was significantly more predominant in *E. coli* isolated from humans than in strains isolated from dogs ($P<0.05$). Moreover, the virulence score was significantly higher for canine than for human strains (Mann Whitney $Z = 3.1$, $P<0.05$).

Comparisons between faecal and urinary *E. coli* strains with respect to virulence factors and antibiotic resistance

Strains belonging to phylogroup B2 were chosen for the comparisons because of their potential pathogenicity. Faecal *E. coli* strains were compared with 2 collections of strains belonging to phylogroup B2 isolated from urine (Table II). In the faecal strains, neither *cdt* nor *afa* were detected in the hosts. However, a very low prevalence was detected when analysing urinary strains isolated from dogs. The results of the stratified analyses did not show any significant differences between the prevalence of virulence factors in urinary *vs*. faecal *E. coli*. A similar result was obtained when analysing the frequency of the antibiotic resistances (Table III). Conversely, the analysis of multidrug resistance and strain source indicated the presence of a highly significant association between MDR and urinary *E. coli* in humans ($P<0.01$), as shown in Table IV. In particular, in *E. coli* isolated from human urinary specimens the frequency of B2 MDR strains was higher (40%) than the one isolated in human faecal strains (8%). The analysis of the MDR frequency in dog specimens did not reveal any significant differences with regard to faecal *vs*. urine specimens, with an MDR frequency of 8% in faecal strains and 11% in urinary tract retrieved strains. Similarly, the stratified analysis did not reveal any significant difference for humans *vs*. dogs.

When we assessed the association between virulence factors and antibiotic resistance phenotypes, we observed a significant association between the presence of *iut* and resistance to enrofloxacin/ciprofloxacin ($P<0.01$) in urinary strains, and between *iut* and resistance to sulfamethoxazole-trimethoprim ($P<0.05$) in faecal strains. Moreover, the frequency of *iut* was significantly higher ($P<0.05$) in MDR *E. coli* isolated from the urinary tract than in those isolated from faeces.

Discussion

A total of 109 *E. coli* isolates collected from the faeces of healthy dogs (56%) and humans (44%) was tested to determine the phylogenetic group of each strain, the presence of the 7 ExPEC-virulence genes and susceptibility to 8 antibiotics. Finally, the faecal isolates belonging to phylogroup B2 were compared to (previously collected) human urinary *E. coli* strains of the same phylogroup to investigate the differences in virulence factor and antibiotic resistance prevalence between the 2 sources.

The results show that group B1 is the most prevalent phylogenetic group in canine faecal strains, while B2 is the most frequent group for human faecal strains. These results are consistent with various epidemiological studies, as reported by Tenaillon (Tenaillon *et al.* 2010). We did not observe human isolates belonging to group B1, the study would need to be repeated using a much larger sample population in order to explore this aspect in more depth. The 48% of B1 strains harboured extraintestinal virulence factors; a figure that is much higher than the prevalences of 22% and 35% previously reported in the literature (Stenske *et al.* 2009, Usein *et al.* 2003). The results highlight the presence of isolates harbouring a combination of *cnf1*, *hlyA*, *papC*, and *sfa* genes, which could implicate the presence of PAIs – typical chromosomal traits of the UPEC strains (Blum *et al.* 1995). Furthermore, the prevalence of strains showing multiple virulence factor patterns was slightly higher than reported in previous studies (Chen *et al.* 2003, Kuhar *et al.* 1998, Yuri *et al.* 1998) and, as expected, this prevalence was strongly associated with strains belonging to phylogroup B2 (Johnson *et al.* 2003, Zhang *et al.* 2002). As expected, the majority of urinary strains (88%) harboured extraintestinal virulence factor-encoding genes and many strains carried PAIs. Regarding the prevalence of *iutA* (22% in canine and 57% in human strains), our results are consistent with those of other studies and support the hypothesis that the low prevalence of

aerobactin in canine *E. coli* could indicate that these strains utilise other systems for iron acquisition (Chen *et al.* 2003, Yamamoto *et al.* 1995).

When we compared strains belonging to phylogroup B2 collected from faeces with those collected from urine, similar frequencies of virulence genes and antibiotic resistance patterns were observed. This observation may support the hypothesis that *E. coli* residing in the intestinal tract may act as a reservoir for UTIs.

This study also revealed an association between the presence of *iutA* (encoding the aerobactin receptor) and resistance to fluoroquinolones and sulfamethoxazole-trimethoprim. Indeed, previous studies have hypothesized that aerobactin-producing strains have a greater chance of survival in a habitat with low iron concentrations than strains that do not produce aerobactin. As a consequence, aerobactin-positive strains are characterised by a greater likelihood of antibiotic resistance acquisition when compared with other strains (Harada *et al.* 2012).

Another interesting result obtained in this research was the low prevalence of MDR strains in both faecal and urinary strains isolated from dogs, in contrast to the high prevalence observed in human urinary strains. This data suggest that in the geographical area considered, the treatment of human *E. coli* urinary infections represents a problem, which should be emphasized and addressed.

The antibiotics showing high resistance frequencies, such as gentamicin and cephalosporins, should not be selected as first choice therapies by clinicians. The use of these antibiotics should be dependent upon the prior execution of *in vitro* susceptibility testing. Of all the antibiotics tested, imipenem was the only effective drug against all isolated strains, probably due to its infrequent use in both veterinary and human medicine owing to its high cost.

In conclusion, the results show that enteric *E. coli* revealed no significant difference in dogs and humans: both populations commonly belonged to group B2 and were characterised by a similar distribution of virulence factors and antibiotic resistances. However, a large proportion of canine strains also belonged to group B1, whereas no human strains belonged to this phylogenetic group. Considering that in Italy reports on the characterization of faecal *E. coli* strains are scanty, this research may offer useful insights for both human and veterinary clinicians operating in this country. Further studies should be designed to assess the transmission of extraintestinal strains of faecal origin in pets, with the additional scope of increasing pet owners' awareness of their potential role in the control of the transmission of pathogenic strains of *E. coli*.

References

Blum G., Falbo V., Caprioli A. & Hacker J. 1995. Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and alpha-hemolysin form the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96. *FEMS Microbiol Lett*, **126**, 189-195.

Clermont O., Bonacorsi S. & Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol*, **66**, 4555-4558.

Chen Y.M., Wright P.J., Lee C.S. & Browning G.F. 2003. Uropathogenic virulence factors in isolates of *Escherichia coli* from clinical cases of canine pyometra and faeces of healthy bitches. *Vet Microbiol*, **94**, 57-69.

Duriez P., Clermont O., Bonacorsi S., Bingen E., Chaventre A., Elion J., Picard B. & Denamur E. 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. *Microbiology*, **147**, 1671-1676.

Féria C.P., Correia J.D., Gonçalves J. & Machado J. 2000. Detection of virulence factors in uropathogenic *Escherichia coli* isolated from humans, dogs and cats in Portugal. *Adv Exp Med Biol*, **485**, 305-308.

Harada K., Niina A., Nakai Y., Kataoka Y. & Takahashi T. 2012. Prevalence of antimicrobial resistance in relation to virulence genes and phylogenetic origins among urogenital *Escherichia coli* isolates from dogs and cats in Japan. *Am J Vet Res*, **73**, 409-417.

Idress M., Mussarat U., Badshah Y., Qamar R. & Bokhari H. 2010. Virulence factors profile of drug-resistant *Escherichia coli* isolates from urinary tract infections in Punjab, Pakistan. *Eur J Clin Microbiol Infect Dis*, **29**, 1533–1537.

Johnson J.R., Kuskowski M.A., Owens K., Gajewski A. & Winokur P.L. 2003. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. *J Infect Dis*, **188**, 759-768.

Johnson J.R., Owens K., Gajewski A. & Clabots C. 2008. *Escherichia coli* colonization patterns among human household members and pets, with attention to acute urinary tract infection. *J Infect Dis*, **197**, 218-224.

Kuhar I., Grabnar M. & Zgur-Bertok D. 1998. Virulence determinants of uropathogenic *Escherichia coli* in faecal strains from intestinal infections and healthy individuals. *FEMS Microbiol Lett*, **164**, 243-248.

Le Bouguenec C., Archambaud M. & Labigne A. 1992. Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J Clin Microbiol*, **30**, 1189-1193.

Moreno E., Prats G., Planells I,. Planes A.M., Perez T. & Andreu A. 2006. Characterization of *Escherichia coli* isolates derived from phylogenetic groups A and B1 causing extraintestinal infection. *Enferm Infect Microbiol Clin*, **24**, 483-489.

Moulin-Schouleur M., Schouler C., Tailliez P., Kao M.R., Brée A, Germon P., Oswald E., Mainil J., Blanco M. & Blanco J. 2006. Common virulence factors and genetic relationships between O18:K1:H7 *Escherichia coli* isolates of human and avian origin. *J Clin Microbiol*, **44**, 3484-3492.

Pass M.A., Odedra R. & Batt R.M. 2000. Multiplex PCRs for identification of *Escherichia coli* virulence genes. *J Clin Microbiol*, **38**, 2001-2004.

Sabate M., Moreno E., Perez T., Andreu A. & Prats G. 2006. Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. *Clin Microbiol Infect*, **12**, 880-886.

Salvarani S., Tramuta C., Nebbia P. & Robino P. 2011. Identificazione e caratterizzazione di ceppi di *Escherichia coli* produttori di beta-lattamasi isolati da cani e gatti con cistite nella provincia di Torino. XIII Congresso Nazionale SIDiLV, 12-14 ottobre, Trani, 366-367.

Stenske K.A., Bemis D.A., Gillespie B.E., Oliver S.P., Draughon F.A., Matteson K. & Bartges J.W. 2009. Prevalence of urovirulence genes *cnf, hlyD, sfa/foc*, and *pap*GIII in faecal *Escherichia coli* from healthy dogs and their owners. *Am J Vet Res*, **70**, 1401-1406.

Tenaillon O., Skurnik D., Picard B. & Denamur E. 2010. The population genetics of commensal *Escherichia coli*. *Nat Rev Microbiol*, **8**, 207-217.

Tóth I., Hérault F., Beutin L. & Oswald E. 2003. Production of cytolethal distending toxins by pathogenic *Escherichia coli* strains isolated from human and animal sources: establishment of the existence of a new cdt variant (Type IV). *J Clin Microbiol*, **41**,4285-4291.

Tramuta C., Nucera D., Robino P., Salvarani S. & Nebbia P. 2011. Virulence factors and genetic variability of uropathogenic *Escherichia coli* isolated from dogs and cats in Italy. *J Vet Sci*, **12**, 49-55.

Usein C.R., Damian M., Tatu-Chițoiu D., Căpuşă C., Făgăraş R. & Mircescu G. 2003. Comparison of genomic profiles of *Escherichia coli* isolates from urinary tract infections. *Roum Arch Microbiol Immunol*, **62**, 137-154.

Yamamoto S., Terai A., Yuri K., Kurazono H., Takeda Y. & Yoshida O. 1995. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immunol Med Microbiol*, **12**, 85-90.

Yuri K., Nakata K., Katae H., Yamamoto S. & Hasegawa A. 1998. Distribution of uropathogenic virulence factors among *Escherichia coli* strains isolated from dogs and cats. *J Vet Med Sci*, **60**, 287-290.

Zhang L., Foxman B. & Marrs C. 2002. Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. *J Clin Microbiol*, **40**, 3951-3955.