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Prevalence of Multidrug Resistance in Canine and Human Uropathogenic *Escherichia Coli* Isolates

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Introduction and aim of the study - Treatment of urinary infections caused by *Escherichia coli* has become increasingly problematic due to the emergence and spread of multidrug-resistant (MDR) strains. This retrospective study was performed to evaluate patterns of antibiotic resistance among *E. coli* isolated from urine samples from dogs and humans.

Materials and methods - Fifty-five canine and 85 human isolates were processed. Bacteria were identified and susceptibility tests were performed using an automated system. A panel of 23 antibiotics was tested. Strains resistant to at least three classes of antibiotics were defined as MDR.

Results and discussion - In dogs and humans the highest rates of resistance were observed for tetracycline (100% both), norfloxacin (38% and 26%, respectively), piperacillin (35% and 42%) and ampicillin (31% and 45%). Susceptibility to phosphomycin, carbapenems and nitrofurantoin was between 98% and 100%. In both species about 35% of isolates were MDR. While in humans 76% of strains were resistant to three or four classes of antibiotics and only 3% to seven classes, in dogs 37% of strains were resistant to seven classes. A high rate of resistance against the antimicrobial agents more commonly prescribed in human and veterinary medicine (e.g. fluoroquinolones) was observed. Although carbapenems show high efficacy, the administration of last-line antimicrobials should be discouraged. Nitrofurantoin still represents a valid therapeutic option, especially in empirical treatment, but its use is off-label in veterinary medicine. Our findings highlight the need for a thorough diagnostic work-up before starting a targeted antimicrobial therapy, especially for canine infections. The overuse of antimicrobial agents is an important risk factor for the emergence of bacterial resistance.

Key words - *E. coli*, cystitis, antibiotic resistance, dog, human

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INTRODUCTION

Urinary tract infections (UTI) are very common in both dogs and humans. From among the various aetiological agents, *Escherichia coli* is one of the most common bacterial causes of clinical forms of cystitis. It is, in fact, the causal agent in 70-90% of human UTI and in up to 55% of canine infections.[1,2,3] These infections can be difficult to treat because of the micro-organism's capacity to colonise the mucosa of the bladder, the presence of virulence factors and the phenomenon of resistance to antibiotics. These factors contribute to the establishment of recurrent infections with obvious repercussions on the outcome and costs of treatment.[4] Furthermore, in recent years there has been an increase in antibiotic resistance in both human and veterinary medicine.[5] Some studies have shown a correlation between the use of antimicrobial agents and the level of resistance to antibiotics, drawing attention to the role of companion animals as a reservoir of bacteria that are resistant to antibiotics in humans.[6,7] This is one of the reasons for making prudent use of antibiotics in human and veterinary medicine a public health priority in both Italy and Europe.

Companion animals are potential reservoirs of MDR uropathogens for humans.

In Italy, limited data is available on the degree of antibiotic-resistant cystitis in companion animals.[8,9,10,11] Consequently, we considered it useful to analyse the prevalence of multidrug-resistant (MDR) strains of uropathogenic *E. coli* isolated from the urine of dogs with cystitis and to compare the results obtained with those of *E. coli* isolated from humans with UTI.

MATERIALS AND METHODS

Bacterial strains. This was a retrospective study based on data collected between March 2013 and March 2014. We studied 55 strains of *E. coli* of canine origin and 85 of human origin.

The bacteria were isolated from samples of urine taken in all cases from patients with a clinical diagnosis of uncomplicated cystitis.

All the samples came from patients (both dogs and humans) living in Piedmont, Italy.

Antibiotic sensitivity test. An automated system (Microscan Walkaway-96 system, Siemens, Germany) was used to identify the species of bacteria present and their sensitivity to antibiotics, determined by calculating the minimum inhibitory concentration (MIC). A panel of 23 antibiotics was tested, including the classes of drugs most frequently used in human and veterinary medicine. The antibiotics tested are listed in Table 1. Depending on the response of the bacteria to the sensitivity test, the isolates were classified as sensitive or resistant (the latter category also including intermediate strains) to the test drug. Isolates resistant to at least three classes of antibiotics were defined as MDR, in accordance with the recent proposal by Magiorakos *et al.*[12]

Statistical analysis. Differences in percentages of antibiotic resistance between strains isolated from dogs and humans were analysed using the χ^2 test (JavaStat). P values <0.05 were considered statistically significant.

Table 1 - Antibiotics, divided by class, investigated in the sensitivity tests (MIC) of 106 strains of uropathogenic *E. coli* isolated from dogs and humans

Antibiotic class	Active principle
Phosphonic acids	Phosphomycin
Aminoglycosides	Gentamicin
	Tobramycin
	Amikacin
Carbapenems	Ertapenem
	Imipenem
	Meropenem
Cephalosporins	Cefuroxime
	Cefotaxime
	Ceftazidime
	Cefepime
Fluoroquinolones	Ciprofloxacin
	Levofloxacin
	Norfloxacin
Glycyclines	Tige ne
Inhibitors of folate metabolism	Trimethoprim-sulfamethoxazole
Penicillins	Ampicillin
	Piperacillin
Penicillins + -lactam inhibitors	Amoxicillin-clavulanic acid
Piperacillin-tazobactam	Polymixins Colistin
Tetracyclines	Tetracycline
Nitrofurans	Nitrofurantoin

Approximately 30% of isolates from both dogs and humans were MDR: 7% of the MDR isolates from humans and 37% of those from dogs were resistant to seven classes of antibiotics.

RESULTS

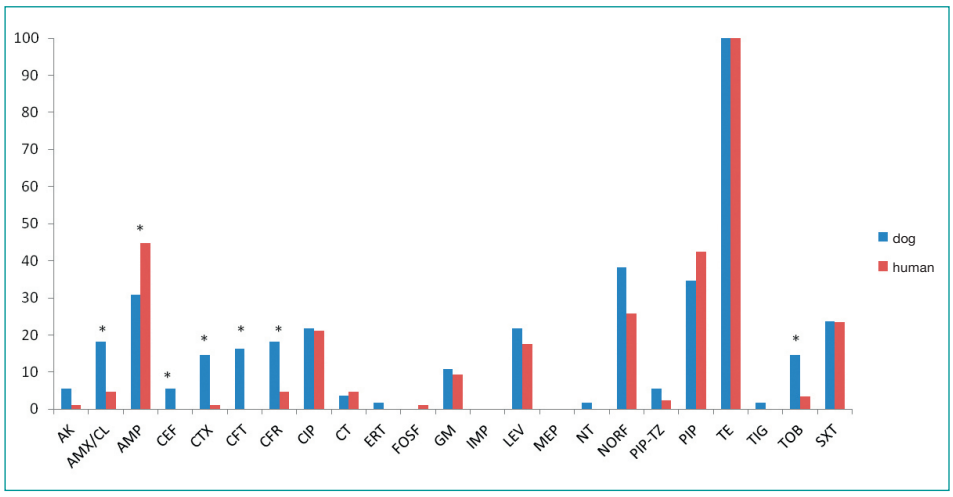
Figure 1 summarises the antibiotic resistance of *E. coli* strains isolated from dogs and humans. The bacteria isolated from both species showed 100% resistance to tetracycline.

The percentages of resistance were also high for norfloxacin (38% and 26% in dogs and humans, respectively), piperacillin (35% and 42%) and ampicillin (31% and 45%).

In both species the resistance to the other antibiotics was less than 30%. The sensitivity to phosphomycin, imipenem, meropemen and nitrofurantoin was between 98% and 100%.

As far as concerns the comparison of isolates of different origin (dogs and humans), the strains isolated from dogs were significantly more resistant than the strains from humans to cephalosporins, amoxicillin-clavulanic acid and tobramycin ($p<0.05$). In contrast, ampicillin had a weaker antibiotic activity against strains isolated from humans compared to those isolated from dogs ($p<0.01$).

Approximately one-third of isolates from both dogs and humans were MDR. However, while most of the isolates from humans (76%) were resistant to three or four classes of antibiotics and only 3% were resistant to seven classes, 37% of the canine strains were resistant to seven classes of antibiotics (Table 2).



AK=amikacin; AMX/CL=amoxicillin/clavulanic acid; AMP=ampicillin; CEF=cefepime; CTX=cefotaxime; CFT=ceftazidime; CFR=cefuroxime; CIP=ciprofloxacin; CT=colistin; ERT=ertapenem; FOSF=phosphomycin; GM=gentamicin; IMP=imipenem; LEV=levofloxacin; MEP=meropenem; NT=nitrofurantoin; NORF=norfloxacin; PIP-TZ=piperacillin-tazobactam; PIP=piperacillin; TE=tetracycline; TIG=tigecycline; TOB=tobramycin; SXT=trimethoprim-sulfamethoxazole

Figure 1 - Comparison of antibiotic resistance (%) between uropathogenic *E. coli* isolated from dogs and humans (* $p<0.05$).

DISCUSSION

This study shows that, as in other countries,[13] in Italy, and more specifically in Piedmont, the phenomenon of antibiotic resistance is very widespread among uropathogenic species of *E.coli* isolated from dogs and humans. The prevalence of tetracylcine resistance is in line with that observed in previous studies performed in both dogs and humans and is probably due to the pharmacological pressure exerted by commensal strains of *E. coli* during the treatment of infections by other pathogens.[14,15] This class of antibiotics was, and still is, widely used in both human and veterinary medicine. Likewise, although the percentages differ slightly, the results concerning ampicillin, amoxicillin-clavulanic acid and fluoroquinolones are similar to those in the literature.[13] With regards to the strains isolated from dogs, it should be highlighted that there are currently no authorised drugs for dogs containing the fluoroquinolones tested in this study; the possibility of cross-resistance between various fluoroquinolones has, however, been demonstrated. As far as concerns *E. coli*, a correlation was observed between the use of enrofloxacin and flumequine and the subsequent appearance of resistance to ciprofloxacin.[16] Enrofloxacin and ciprofloxacin are, in fact, structurally and functionally similar and the latter is the active metabolite of the former.[17] It has now been well documented that there is correlation between the frequency of prescription of this group of antibiotics and an increase in drug-resistant strains of uropathogenic bacteria.[18]

Resistance to semi-synthetic penicillins can also be explained by the widespread availability and frequent prescription of these drugs in both human and veterinary medicine. According to Wagner *et al.*,[13] up to 100% of MDR strains of *E. coli* isolated from dogs with UTI may be resistant to amoxicillin-clavulanic acid. The marked sensitivity of isolates to phosphomycin and nitrofurantoin is consistent with the findings of epidemiological studies on *E. coli* responsible for UTI.[19,20]

In this study, one third of the strains of bacteria isolated from both dogs and humans were MDR. The findings regarding the human strains are consistent with those in a study by Baral *et al.*[3] in which the percentage of MDR was about 38%. In a recent study in dogs, the percentage of MDR *E. coli* was

Table 2 - Distribution of MDR strains of uropathogenic *E. coli* isolated from dogs and humans according to the number of different classes of antibiotics to which they are resistant

Origin of strains	Resistant to								
	3-4 classes		5-6 classes		7 classes		≥ 8 classes	Total strains	
	n. of isolates	%	n. of isolates	%	n. of isolates	%	n. of isolates	n.	%
Dogs	5	26	7	37	7	37	0	19/55	34.5
Humans	22	76	7	24	1	3	0	29/85	35.3

57% and the majority of the strains were actually resistant to seven antibiotics, with high percentages of resistance to beta-lactams and fluoroquinolones.[21] The problem of multidrug resistance of bacterial strains isolated from dogs is particularly evident among hospitalised subjects being treated with antibiotics.[22]

Overall, our data confirm the need for prudent and carefully considered use of antibiotics. In particular, given the high prevalence of canine strains resistant to seven classes of antibiotics, it is important that the diagnostic work-up is thorough and that, when possible, the micro-organism responsible is isolated and *in vitro* sensitivity tests are performed. In this context, it is preferable to obtain MIC values, since the efficacy *in vivo* is influenced by the concentrations of the drug that can be reached at the site of action. In the case of empirical therapy, the treatment regimen should be established taking into account the trends in resistance of pathogens in the geographical area in question. The guidelines on the treatment of UTI in dogs currently recommend amoxicillin-clavulanic acid (11-15 mg/kg PO *tid*) or potentiated sulphonamides (15 mg/kg PO *bid*) as the first-line treatment of choice.[23] However, our research shows that the level of resistance to these drugs is not negligible.

The problem of multidrug resistance of bacterial strains isolated from dogs is particularly evident among hospitalised subjects being treated with antibiotics, suggesting the need for prudent and carefully considered use of antibiotics.

Other drugs used for the treatment of UTI in dogs are nitrofurantoin and phosphomycin. Although this latter drug has been in use since the 1960s, the sensitivity of the isolates that we tested, including the MDR strains, was very high, confirming the findings of other authors.[24,25] The marked sensitivity to this drug, together with its high therapeutic index and kinetic characteristics, justify its use in canine UTI caused by MDR strains of bacteria.[25] It is, however, essential to point out that this treatment is used in exemption of article 10 of Legislative Decree n. 193/2006 and must, therefore, only be prescribed in exceptional circumstances, on the basis of real need and/or laboratory evidence, and preferably following regulatory notification.

Nitrofurantoin, too, shows good efficacy against *E. coli*. In the case of MDR strains isolated from dogs, the MIC_{50} of nitrofurantoin (16 μg/ml) was lower than the concentrations that can be reached *in vivo* in the urine (60-100 μg/ml) following oral intake of the drug.[1] Like phosphomycin, nitrofurantoin is not recommended as the drug of first choice for the treatment of UTI in dogs. It is, however, a valid alternative in the case of infections caused by MDR bacteria when conventional treatment has been ineffective. Finally, despite the marked *in vitro* activity of carbapenems against *E. coli*, these drugs should not be used, or limited to extreme cases. They are last-line drugs in humans, to be prescribed only in cases of severe infections caused by MDR Gram-negative bacteria.[15]

KEY POINTS

- *E. coli* is the causal pathogen in the majority of uncomplicated urinary tract infections in both humans and dogs.
- In recent years, reports of multidrug-resistant *E. coli*, selected in part as a result of excessive and incorrect use of antibiotics, have increased throughout the world.
- In this *in vitro* study we tested antibiotics used in human and veterinary medicine. About one third of the strains of *E. coli* isolated from both humans and dogs were multidrug-resistant.
- Our findings highlight the need for a thorough diagnostic work-up and targeted treatment. Prudent use of antibiotics in human and veterinary medicine is a public health care priority in Italy and Europe.

REFERENCES

1. Maaland M, Guardabassi L. In vitro antimicrobial activity of nitrofurantoin against Escherichia coli and Staphylococcus pseudintermedius isolated from dogs and cats. Veterinary Microbiology 151:396-399, 2011.
2. Thompson MF, Litster AL, Platell JL *et al.* Canine bacterial urinary tract infections: new developments in old pathogens. Veterinary Journal 190:22-27, 2011.
3. Baral P, Neupane S, Marasini BP *et al.* High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Research Notes 19:38, 2012.
4. Hershberger E, Oprea SF, Donabedian SM *et al.* Epidemiology of antimicrobial resistance in enterococci of animal origin. Antimicrobial Chemotherapy 55:127-130, 2005.
5. Pitout JD. Extraintestinal pathogenic Escherichia coli: an update on antimicrobial resistance, laboratory diagnosis and treatment. Expert Review of Anti-Infective Therapy 10:1165-1176, 2012.
6. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. Journal Antimicrobial Chemotherapy 54:321-332, 2004.
7. Ball KR, Rubin JE, Chirino-Trejo M *et al.* Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002-2007. Canadian Veterinary Journal 49:985-990, 2008.
8. Ossiprandi MC, Cattabiani F, De Risio L *et al.* Antibiotic resistance in bacteria isolated from urinary tracts of cats and dogs with neurologic impairment. Igiene Moderna 125: 27-39, 2006.
9. Papini R. Ebani VV, Cerri D *et al.* Survey on bacterial isolates from dogs with urinary tract infections and their in vitro sensitivity. Revue de Medicine Veterinaire 157:35-41, 2006.
10. Nebbia P, Tramuta C, Odore R *et al.* Genetic and phenotypic characterisation of Escherichia coli producing cefotaximase-type extended-spectrum -lactamases: first evidence of the ST131 clone in cats with urinary infections in Italy. Journal of Feline Medicine and Surgery. 2014 [Epub ahead of print].
11. Tramuta C, Robino P, Nucera D *et al.* Molecular characterization and antimicrobial resistance of faecal and urinary Escherichia coli isolated from dogs and humans in Italy. Veterinaria Italiana 50:23-30, 2014.
12. Magiorakos AP, Srinivasan A, Carey RB *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18:268-81, 2012.
13. Wagner S, Gally DL, Argyle SA. Multidrug-resistant Escherichia coli from canine urinary tract infections tend to have commensal phylotypes, lower prevalence of virulence determinants and ampC-replicons. Veterinary Microbiology 169:171-178, 2014.
14. Karami N, Nowrouzian F, Adlerberth I *et al.* Tetracycline resistance in Escherichia coli and persistence in the infantile colonic microbiota. Antimicrobial Agents And Chemotherapy 50:156-161, 2006.
15. Gibson JS, Cobbold RN, Trott DJ. Characterization of multidrug-resistant Escherichia coli isolated from extraintestinal clinical infections in animals. Journal of Medical Microbiology 59:5928, 2012.
16. Moniri R, Dastehgoli K. Fluoroquinolone-resistant *Escherichia coli* isolated from healthy broilers with previous exposure to fluoroquinolones: Is there a link? Microbial Ecology in Health and Disease 17:69-74, 2005.
17. Küng K, Riond JL, Wanner M. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and oral administration of enrofloxacin in dogs. Journal of Veterinary Pharmacology And Therapeutics 16:462-468, 1993.
18. Goettsch W, van Pelt W, Nagelkerke N *et al.* Increasing resistance to fluoroquinolones in escherichia coli from urinary tract infections in the netherlands. Journal of Antimicrobial Chemotherapy 46:223-8, 2000.
19. Nickel JC. Urinary tract infections and resistant bacteria. Reviews in Urology 9:78-80, 2007.
20. Aboderin OA, Abdu AR, Odetoyin BW *et al.* Antimicrobial resistance in Escherichia coli strains from urinary tract infections. Journal of the National Medical Association 101:1268-1273, 2009.
21. Shaheen BW, Boothe DM, Oyarzabal OA *et al.* Antimicrobial resistance profiles and clonal relatedness of canine and feline Escherichia coli pathogens expressing multidrug resistance in the United States. Journal of Veterinary Internal Medicine 24:323-30, 2010.
22. Nam HM, Lee HS, Byun JW *et al.* Prevalence of antimicrobial resistance in fecal Escherichia coli isolates from stray pet dogs and hospitalized pet dogs in Korea. Microbial Drug Resistance 16:75-79, 2010.
23. Weese JS, Blondeau JM, Boothe D *et al.* Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases. Veterinary Medicine International 263768, 2011.
24. Falagas ME, Kastoris AC, Kapaskelis AM *et al.* Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. Lancet Infectious Diseases 10:43-50, 2010.
25. Hubka P, Boothe DM. In vitro susceptibility of canine and feline Escherichia coli to fosfomycin. Veterinary Microbiology 149:277-82, 2011.