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Resistance to Antibacterial Agents in *Escherichia coli* Isolated from Domestic Cats and Dogs in the Northern Region of the Republic of Kazakhstan

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Abstract: The widespread use of antimicrobial agents for pets in veterinary practice has led to the emergence and spread of drug resistance not only in pathogenic but also in opportunistic bacteria. The study aims to evaluate the profile of resistance to antibacterial agents in *Escherichia coli* strains isolated from domestic cats and dogs, as well as to look into the determinants of resistance responsible for the ability of microorganisms to resist the effects of antimicrobial agents. During 2021-2022, biological material from cats and dogs, taken in veterinary clinics of Kostanay, Republic of Kazakhstan, was analyzed. Identification of genes encoding antimicrobial resistance was carried out by Polymerase Chain Reaction (PCR). As a result, it was found that all isolated strains of microorganisms showed sensitivity to the action of meropenem included in the group of beta-lactam antibiotics and showed resistance to tetracycline, doxycycline, ofloxacin, ampicillin, and amoxicillin. Resistance genes were identified for all the studied groups of antibiotics in the DNA of *E. coli* isolated from cats and no genes encoding resistance to fluoroquinolones were detected in the DNA of strains isolated from dogs. The most common genes were genes encoding resistance to aminoglycosides (43.8%), tetracyclines (35%), beta-lactams (33.5%), sulfonamides (16.1%), and fluoroquinolones (1.24%). In general, domestic cats and dogs have a high prevalence of *E. coli* strains resistant to beta-lactam drugs (ampicillin: 40%, amoxicillin: 36%) and tetracyclines (tetracycline: 71%, doxycycline: 47%). Resistance in most cases was explained by the presence of such resistance genes as blaTEM, OXA, tetA, and tetB.

Keywords: Strain, Bacterium, Antibiotic, Antibiotic Sensitivity, Resistance Genes

Introduction

Previously, it was assumed that only animals consumed by humans were the main source of antibiotic-resistant strains of microorganisms, through the transmission of resistant bacteria or genes along the food chain (McEwen and Fedorka-Cray, 2002; Smith *et al*., 2002; Angulo *et al*., 2000). However, it was later found that the transmission of resistance by non-food route should be investigated equally (Barber *et al*., 2003).

Every year, an increasing number of antimicrobial agents are used to treat small pets, including medications used in human medicine (in particular cephalosporins and fluoroquinolones). There is evidence that, among the bacteria that cause infection in pets, the growth of antimicrobial resistance increases (Vasaikar *et al*., 2017; Karkaba *et al*., 2019; Umeda *et al*., 2019).

Studies conducted in the UK and Portugal show that resistance to the tetracycline group is widespread among *E. coli* microorganisms isolated from pets, reaching up to 40% (Costa *et al*., 2008; Wedley *et al*., 2017). Other recent clinical studies conducted in Belgium report that the resistance of *E. coli* strains was 12% higher to the beta-lactam group and 17% higher to tetracyclines, but a very low or undetectable level of resistance was detected to fluoroquinolones (De Graef *et al*., 2004). Studies

conducted in Australia also report increased resistance of *E. coli* isolates to β-lactams, as well as the absence of resistance to carbapenems (Saputra *et al*., 2017).

The transfer of antibiotic-resistant strains from animals to humans is still being studied globally (Guardabassi *et al*., 2004; Lloyd, 2007). To date, there is no data on the prevalence of both resistant strains and resistance genes among domestic cats and dogs living in the Republic of Kazakhstan.

The study of the determinants of resistance to antibacterial agents in small pets (cats and dogs) will allow us to assess the existing picture of the prevalence of resistance at the genetic level.

The study aims to evaluate the profile of resistance to antibacterial agents in *E. coli* strains isolated from domestic cats and dogs, as well as to look into the determinants of resistance responsible for the ability of microorganisms to resist the effects of antimicrobial agents.

Materials and Methods

No ethical permission was required to conduct this study.

In this study, the following biological material (n = 944) was analyzed: Urine, feces, and swabs from the nasal and oral cavities, rectum, and vagina from cats (n = 582) and dogs (n = 362) (clinically healthy and with various pathologies) taken in veterinary clinics of Kostanay in the period from January 2021 to June 2022. The laboratory studies were conducted at the Department of microbiological analysis of the research institute of applied biotechnology of the Kostanay Regional University (KRU) named after A. Baitursynov.

When the owners brought their animals to the veterinary clinic, they were asked to provide data concerning biological information about the animal (age, gender), diseases, and antimicrobial treatment received before admission to the clinic using a questionnaire. An employee of the clinic took a smear (from the wound surface, rectal, oronasal, or vaginal). The smears were collected with dry sterile swabs, after which they were sent to the laboratory.

Isolation and Identification of Microorganisms

Isolation and identification of *E. coli* strains were carried out by seeding the test material on a non-selective chromogenic medium CHROMagar Orientation, followed by incubation at a temperature of +37°C for 18-20 h. The samples were seeded onto the Petri dishes with a streak immediately or after the enrichment stage. When distinct colonies appeared, characteristic of the growth of *E. coli* on this medium, smears were prepared and Gram-stained. When gram-negative straight bacilli typical in morphology with rounded ends were found in the smears, their biochemical properties were studied. The interpretation of the results of biochemical tests was carried out according to Bergey's manual (Garrity *et al*., 2005).

The ability of bacteria to ferment lactose and glucose, as well as to form gas and hydrogen sulfide was determined by the change in the color of the medium and the appearance of gas bubbles in the Kligler agar. The change in the medium was taken into account after 24 h incubation at a temperature of +37°C.

The enzymatic properties of bacteria were studied on Hiss media with lactose and mannitol. The utilization of sodium citrate during culture growth was studied by changing the color of the Simmons medium and the formation of indole was studied by the appearance of a red ring on the surface of the medium after the addition of Kovacs reagent (4-dimethylaminobenzaldehyde, amyl alcohol and hydrochloric acid). The mobility of the isolates in question was studied by their growth when seeded with an injection into semi-liquid agar. The change of media during growth was taken into account after 2 days of incubation.

The Voges-Proskauer test was performed based on the detection of acetoin by adding α-naphthol and potassium hydroxide (KOH) to a 2-day culture of microorganisms on Clark's medium.

The methyl red test was used for a certain concentration of ions (pH) in the medium of glucose-fermenting microorganisms by adding 5 drops of the methyl red indicator to the culture of the microorganism and observing the color change.

To detect indole, a reaction was carried out using Kovacs reagent, by adding it to the culture of the microorganism on Beef Extract Broth (BEB). With a positive reaction, the formation of a red ring was observed.

Antibiotic Sensitivity

The sensitivity to antibacterial agents in the obtained isolates was investigated by applying standard antibiotic discs to the freshly sown bacterial lawn using Mueller-Hinton agar. The results were considered by the presence of microbe growth inhibition zones around the discs, which, according to the instructions, indicates either the sensitivity of the pathogen to the medication or its resistance to this antibiotic.

All isolated cultures of microorganisms were tested to determine sensitivity to beta-lactams (ampicillin, amoxicillin, cefoperazone, cefoxitin, cefpodoxime, meropenem), aminoglycosides (streptomycin, kanamycin, gentamicin), amphenicols (levomycetin), tetracyclines (tetracycline and doxycycline), fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, gemifloxacin), quinolones (nalidixic acid), sulfonamides (trimethoprim/sulfamethoxazole) and nitrofurans (furazolidone and furadonin). Sensitivity was determined following the recommendations of the EUCAST version 11.0, CLSI and according to Methodical Instructions (MI) 4.2.1890-04 (CLSI, 2019; Kahlmeter *et al*., 2019).

Identification of Resistance Genes of Opportunistic Microorganisms

For molecular research, DNA material was obtained by bacterial lysis according to the recommendations of the European Union Reference Laboratory for Antimicrobial Resistance (SVA, 2021). Identification of genes encoding antimicrobial resistance was carried out by Polymerase Chain Reaction (PCR).

To determine the bacterial resistance profiles, primers were used and 242 samples of *E. coli* DNA were tested by PCR for the presence of genes encoding resistance.

Data Analysis

Data analysis was carried out using the MS Excel 2017 package.

Results

In total, biological material from 944 animals was examined, including 362 (38.3%) dogs and 582 (61.7%) cats. In 25.6% of the subjects (242 animals), the presence of *E. coli* was isolated and identified, where 10.6% (100 isolates) were isolated from dogs and 15% (142 isolates) from cats (Fig. 1).

Morphological, tinctorial, and cultural properties of the obtained isolates were characteristic of their family and genus.

Biological information, data on admission to the clinic, and previous use of antibacterial agents of all animals participating in the study are shown in Table 1.

From the data presented in Table 1, it can be seen that the average age of dogs participating in the study was 3.6 years, the age of cats was 3.3 years, the predominant majority were females (54.9% of dogs and 59.1% of cats) and *E. coli* was isolated in 58% of female dogs and 59.8% of female cats. Among all the animals participating in the study, 79.5% (81.2% of dogs and 78.5% of cats) had diseases of various body systems and the rest of the animals were healthy. *E. coli* was detected in 24.9% of sick animals and 28.5% of healthy ones. Antimicrobial treatment before admission to the clinic had been received by 26.5% of all dogs and 17.7% of cats, of which *E. coli* was found in 40.6% of dogs and 18.4% of cats.

The ratio of the number of isolated *E. coli* isolates from cats and dogs, depending on the disease, is shown in Fig. 2.

Analysis of the frequency of isolation of *E. coli* microorganisms obtained from cats with various pathologies showed that 58.3% of *E. coli* was isolated from cats with surgical conditions, 13.8% from cats with orthopedic/traumatic diseases, 11.11% from cats with diseases of the gastrointestinal tract, 9.26% from cats with diseases of the genitourinary system, 3.7% from cats with infectious diseases, 1.85% from cats with neoplasms and the smallest number of microorganisms (0.95%) were isolated from cats with dermatological and invasive diseases.

Of 79 *E. coli* isolates obtained from dogs with various diseases, 26.6% were found in animals with surgical pathologies, 22.8% in dogs with infectious diseases, 12.6% in dogs with diseases of the genitourinary system, 10.13% in dogs with orthopedic/traumatic diseases and neoplasms, 8.9% in dogs with invasive diseases, 7.6% in dogs with gastrointestinal diseases and the smallest number of microorganisms (1.3%) was isolated from dogs with dermatological diseases.

Of the 142 studied *E. coli* strains isolated from cats, 70.4% (100 strains) were resistant to tetracycline, 47.2% (67 strains) to doxycycline, 42.3% (60 strains) to ofloxacin, 38.7% (55 strains) to ampicillin, 36.6% (52 strains) to amoxicillin, 33% (47 strains) to cefpodoxime, 30.3% (43 strains) to norfloxacin, 25.4% (36 strains) to ciprofloxacin, 24.6% (35 strains) to enrofloxacin, 22.5% (32 strains each) to trimethoprim/sulfamethoxazole and nalidixic acid, 18.3% (26 strains) to cefoperazone and levomycetin, 13.4% (19 strains) to gemifloxacin, 11.9% (17 strains) to cefoxitin, 10.6% (15 strains) to gentamicin, 8.5% (12 strains) to kanamycin, 2.8% (4 strains) to furadonin and 1.4% (2 strains each) to streptomycin and furazolidone. No strains resistant to meropenem were identified (Fig. 3)

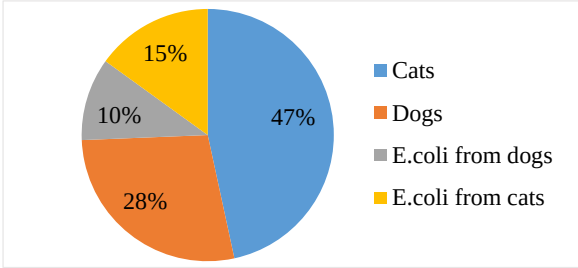

Fig. 1: Ratio of analyzed samples between cats and dogs

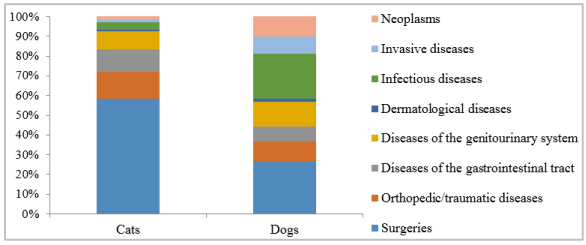

Fig. 2: The ratio of analyzed samples between cats and dogs depending on the disease

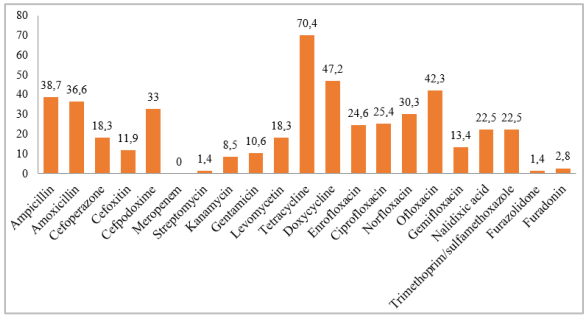

Fig. 3: Antibiotic resistance of E. coli isolates (%) obtained from cats

Table 1: Data on animals included in the study

	Dogs		Cats	
Parameters	Total (n = 362)	*E. coli* was found (n = 100)	Total (n = 582)	*E. coli* was found (n = 142)
Average age (years)	3.6	3.5	3.3	3.2
Gender				
Female	199.0	58.0	344.0	85.0
Male	163.0	42.0	238.0	57.0
Diagnosis				
Surgical operations	74.0	21.0	255.0	63.0
Orthopedic/traumatic diseases	41.0	8.0	64.0	15.0
Gastrointestinal diseases	23.0	6.0	30.0	12.0
Diseases of the genitourinary system	55.0	10.0	53.0	10.0
Dermatological diseases	7.0	1.0	6.0	1.0
Infectious diseases	54.0	18.0	12.0	4.0
Invasive diseases	20.0	7.0	3.0	1.0
Neoplasms	18.0	8.0	17.0	2.0
Other diseases	2.0	-	17.0	-
Healthy animals	68.0	21.0	125.0	34.0
Antimicrobial treatment before admission to the clinic	96.0	39.0	103.0	19.0
Antibiotic therapy				
Ceftriaxone	43.0	19.0	16.0	6.0
Lincomycin	8.0	5.0	14.0	6.0
Amoxicillin	38.0	14.0	33.0	2.0
Enrofloxacin	5.0	1.0	19.0	4.0
Trimethoprim	0.0	0.0	3.0	1.0
Penicillin	0.0	0.0	7.0	0.0
Gentamicin	0.0	0.0	3.0	0.0
Cefazolin	2.0	0.0	2.0	0.0
Levomycetin	0.0	0.0	3.0	0.0
Tetracycline	0.0	0.0	3.0	0.0

Table 2: Resistance genes of microorganisms

	Genes encoding *E. coli* resistance with the number of samples								
Group of antibiotics	From cats				From dogs				Total
Beta-lactams	BlaTEM	OXA	-	-	BlaTEM	OXA	-	-	81
	20	28			17	16			
Aminoglycosides	StrA	StrB	aadB	aphA1	StrA	StrB	aadB	aphA1	106
	25	27	1	5	21	21	1	5	
Tetracyclines	tetA	tetB	-	-	tetA	tetB	-	-	85
	39	13			21	12			
Sulfonamides	SUL1	SUL3	-	-	SUL1	SUL3	-	-	39
	12	11			5	11			
Fluoroquinolones	qepA	qnr	-	-	qepA	qnr	-	-	3
	2	1			0	0			

A study of 100 *E. coli* microorganisms isolated from dogs for antibiotic resistance showed that 71% of the strains were resistant to tetracycline, 47% to doxycycline, 46% to ofloxacin, 40% to ampicillin, 36% to amoxicillin, 32% to norfloxacin, 30% to cefpodoxime, 29% to ciprofloxacin, 27% to nalidixic acid, 23% to enrofloxacin, 22% to trimethoprim/sulfamethoxazole, 18% were resistant to cefoxitin, 15% to cefoperazone and levomycetin, 14% to gemifloxacin, 6% to gentamicin and 3% to kanamycin. The smallest number of microorganisms (2%) were resistant to furazolidone and furadonin and (1%) to streptomycin. No resistant strains were identified for meropenem (Fig. 4).

All *E. coli* isolates showing phenotypic resistance to antibacterial agents were tested by PCR for the presence of genes encoding resistance. The results are presented in Table 2.

Based on the data presented in Table 2, it can be seen that resistance genes were observed for all the studied groups of antibiotics in the DNA of *E. coli* isolated from cats and no genes encoding resistance to fluoroquinolones were detected in the DNA of strains isolated from dogs. The genes encoding resistance to aminoglycosides were most often isolated in 43.8% of strains (StrA: 46 samples, strB: 48 samples, aadB: 2 samples, aphA1: 10 samples).

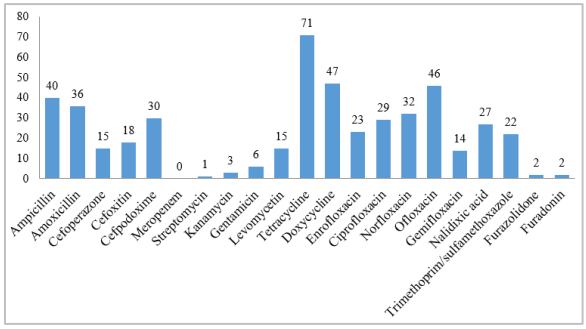

Fig. 4: Antibiotic resistance of *E. coli* isolates (%) isolated from dogs

Genes encoding resistance to tetracyclines were detected in 35% of cases (tetA: 60 samples, tetB: 25 samples). Beta-lactam resistance genes were found in 33.5% of isolates (blaTEM: 37 samples, OXA: 44 samples). Genes encoding resistance to sulfonamides were also identified in 16.1% of cases (SUL1: 17 samples, SUL3: 22 samples). The number of samples having genes causing resistance to fluoroquinolones (1.24%) was the smallest of all obtained values (qepA: 2 samples, qnr: 1 sample).

Discussion

As a result of the conducted studies, 25.6% (242 animals) of them were found to carry the *E. coli* strains, including 41.3% (100 isolates) obtained from dogs and 58.7% (142 isolates) from cats. These data correlate with the results of several studies conducted in the Netherlands, where the prevalence of colonization by enterobacteria in dogs and cats (including healthy and sick animals) ranged from 3.1-55% (Baede *et al*., 2015; Van den Bunt *et al*., 2020). In studies conducted in Thailand and the US, a higher percentage of *E. coli* excretion was observed, where it was 66 and 52%, respectively (Thungrat *et al*., 2015; Soonthornsit *et al*., 2022).

The average age of dogs participating in the study was 3.6 years, the age of cats was 3.3 years, the majority of them were females (dogs: 54.9%, cats: 59.1%) and *E. coli* was isolated in 58% of female dogs and 59.8% of female cats. *E. coli* was more often isolated in female dogs than in males, which is consistent with previous studies. Perhaps this is due to the anatomical difference between the urinary tract in females and males: In particular, the female's urethra is shorter and wider than that of the male, and *E. coli*, as the causative agent of urinary tract infection, will be detected more often in females than in males (Hall *et al*., 2013).

Among all the animals participating in the study, 79.5% (81.2% of dogs and 78.5% of cats) had diseases of various body systems and the rest of the animals were healthy. *E. coli* was detected in 24.9% of sick animals and 28.5% of healthy ones. The data obtained by us have average indicators since in studies conducted in the Netherlands intestinal carrier state was detected in 45% of healthy dogs and in Korea, *E. coli* was detected in 23.8% of isolates from admitted dogs (So *et al*., 2012; Hordijk *et al.*, 2013).

Antimicrobial treatment before admission to the clinic had been received by 26.5% of all dogs and 17.7% of cats, of which *E. coli* was found in 40.6% of dogs and 18.4% of cats. These data are consistent with previous observations carried out in different countries, in which bacterial carrier was observed after a certain period after the use of antibiotics and prove the spread of antibiotic-resistant strains among humans and pets (Espinosa-Gongora *et al*., 2015; Zhao *et al*., 2016; Karkaba *et al*., 2019).

In general, all isolated *E. coli* strains showed the highest percentage of resistance (71%) to the action of tetracycline, which is consistent with studies conducted in several countries (Great Britain, Portugal), where, of all the selected antibiotics, the maximum resistance was found to the action of tetracycline (Costa *et al*., 2008; Wedley *et al*., 2017). Besides, in studies of previous years (2007-2014), a low level of sensitivity to the action of antimicrobial agents of the tetracycline group was demonstrated (Pedersen *et al*., 2007; Ball *et al*., 2008; Kroemer *et al*., 2014).

The study found a low percentage of isolates resistant to fluoroquinolones. These antimicrobial agents are often used to treat dogs and cats (Lloyd, 2007; Pedersen *et al*., 2007; Murphy *et al*., 2012). We evaluated the sensitivity of bacteria to second-generation fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, and gemifloxacin). The percentage of strains resistant to ofloxacin and norfloxacin was slightly higher, by 22.7% and 10.3%, respectively than to other antibiotics of this group. In studies conducted in Australia and Poland, a decrease in the effectiveness of fluoroquinolones against enterobacteria was also observed (Gibson *et al*., 2010; Rzewuska *et al*., 2015).

Other recent studies conducted in Australia confirm our data that *E. coli* isolates have increased resistance to the beta-lactam group (ampicillin, amoxicillin, cefpodoxime), as well as a lack of resistance to carbapenems (meropenem) (Saputra *et al*., 2017). Probably, this may be due to the prolonged and more intensive use of ampicillin and amoxicillin in pets, compared with meropenem. In a study conducted in Spain, beta-lactams were the antimicrobial agent most commonly prescribed to dogs (Gómez-Poveda and Moreno, 2018). This antimicrobial agent should be used with caution for the treatment of infections caused by *E. coli* due to the rapid development of resistance caused by the production of beta-lactamases (Boehmer *et al*., 2018).

Resistance to other groups of antimicrobial agents (aminoglycosides and nitrofurans) tested against *E. coli* isolates from dogs and cats was absent or at a very low level. However, studies conducted in European countries show the opposite results, speaking of high resistance to

these antibiotics (Bywater *et al*., 2004; Hendriksen *et al*., 2008). In general, there are differences in the prevalence of resistance in isolates of opportunistic enterobacteria in different countries (Harada *et al*., 2012). Perhaps the reason for this is the differences in the strains and resistance genes circulating in different territories.

As a result of the conducted studies, it was found that in 88 *E. coli* DNA samples that had phenotypic resistance to antibacterial agents, there was a link with genotypic resistance. However, in 21 samples, resistance genes to antibacterial agents of the aminoglycoside group (StrA, strB, aadB, aphA1) were found and in 8 samples we found resistance genes to the beta-lactam group (blaTEM), while phenotypic resistance in these microorganisms to these groups of medications was not detected. Probably, we are dealing with the so-called "silent" genes, which have been found in studies conducted in the UK and China but have been little studied so far (Xu *et al*., 2014; Wang *et al*., 2017; Zhang *et al*., 2018).

In 124 *E. coli* strains, phenotypic resistance to the group of fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, gemifloxacin) was detected, but genotypic resistance, i.e., the presence of qepA and qnrA genes was not observed. This is probably due to differences in the mechanisms of resistance, such as a decrease in membrane permeability and the overactivity of the efflux pump, a similar situation occurs in studies by other scientists (Bardasheva *et al*., 2021; Galal *et al*., 2019).

Conclusion

In the Northern region of Kazakhstan, domestic cats and dogs have a high prevalence of *E. coli* strains. The study of antibiotic resistance of the isolated strains showed high resistance to medications of the beta-lactam group and tetracyclines. Resistance in most cases was explained by the presence of such resistance genes as blaTEM, OXA, tetA, and tetB.

Uncontrolled and frequent use of antibacterial agents of the beta-lactam group and tetracyclines leads to the spread of not only phenotypic but also genotypic resistance among microorganisms.

Further studies of antimicrobial resistance mechanisms in the *E. coli* population of dogs and cats in Kazakhstan are needed. Given the growing resistance of bacteria to medications widely used in the veterinary practice of small animals, it seems extremely important that the treatment of bacterial infections should take into account the testing of sensitivity to antibacterial agents. Thus, it is necessary to constantly monitor the sensitivity to *E. coli* antimicrobial agents in dogs and cats and develop recommendations for the use of antibiotics in the practice of small animals. Also, further research is needed on zoonotic strains of bacteria in other parts of the country, as the close bond between humans and their companion animals provides opportunities for exchange of microorganisms, including multidrug resistance pathogens.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

References

Angulo, F. J., Johnson, K. R., Tauxe, R. V., & Cohen, M. L. (2000). Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: Implications for the use of fluoroquinolones in food animals. *Microbial Drug Resistance*, *6*(1), 77-83. http://dx.doi.org/10.1089/mdr.2000.6.77

Baede, V. O., Wagenaar, J. A., Broens, E. M., Duim, B., Dohmen, W., Nijsse, R., ... & Hordijk, J. (2015). A longitudinal study of extended-spectrum-β-lactamase-and AmpC-producing Enterobacteriaceae in household dogs. *Antimicrobial Agents and Chemotherapy*, *59*(6), 3117-3124. https://doi.org/10.1128/aac.04576-14

Ball, K. R., Rubin, J. E., Chirino-Trejo, M., & Dowling, P. M. (2008). Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002–2007. *The Canadian Veterinary Journal*, *49*(10), 985.

Bardasheva, A. V., Fomenko, N. V., Kalymbetova, T. V., Babkin, I. V., Kreten, S. O., Zhirakovskaya, E. V., Tikunova, N. V., & Morozova, V. V. (2021). Geneticheskaya kharakteristika rosta izolyatov klebsiell tsirkuliruyushchikh v Novosibirske [Genetic characteristics of the growth of Klebsiella isolates circulating in Novosibirsk]. *Vavilovskii Zhurnal Genet Selektsii*, *25*(2), 234-245 (in Russian). http://dx.doi.org/10.18699/VJ21.49-o

Barber, D. A., Miller, G. Y., & Mcnamara, P. E. (2003). Models of antimicrobial resistance and foodborne illness: Examining assumptions and practical applications. *Journal of Food Protection*, *66*(4), 700-709. https://doi.org/10.4315/0362-028x-66.4.700

Boehmer, T., Vogler, A. J., Thomas, A., Sauer, S., Hergenroether, M., Straubinger, R. K., ... & Riehm, J. M. (2018). Phenotypic characterization and whole genome analysis of extended-spectrum beta-lactamase-producing bacteria isolated from dogs in Germany. *PLoS One*, *13*(10), e0206252. http://dx.doi.org/10.1371/journal.pone.0206252

Bywater, R., Deluyker, H., Deroover, E., De Jong, A., Marion, H., McConville, M., ... & Walters, J. (2004). A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *Journal of Antimicrobial Chemotherapy*, *54*(4), 744-754. https://doi.org/10.1093/jac/dkh422

CLSI. (2019). *M100 Performance standards for antimicrobial susceptibility testing*. 29th Ed. Clinical and Laboratory Standards Institute, Wayne, PA. https://clsi.org/media/2663/m100ed29_sample.pdf

Costa, D., Poeta, P., Sáenz, Y., Coelho, A. C., Matos, M., Vinué, L., ... & Torres, C. (2008). Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Veterinary Microbiology*, *127*(1-2), 97-105. https://doi.org/10.1016/j.vetmic.2007.08.004

De Graef, E. M., Decostere, A., Devriese, L. A., & Haesebrouck, F. (2004). Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. *Microbial Drug Resistance*, *10*(1), 65-69. http://dx.doi.org/10.1089/107662904323047826

Espinosa-Gongora, C., Shah, S. Q. A., Jessen, L. R., Bortolaia, V., Langebæk, R., Bjørnvad, C. R., & Guardabassi, L. (2015). Quantitative assessment of faecal shedding of β-lactam-resistant *Escherichia coli* and enterococci in dogs. *Veterinary Microbiology*, *181*(3-4), 298-302. https://doi.org/10.1016/j.vetmic.2015.10.004

Galal, L., Abdel Aziz, N. A., & Hassan, W. M. (2019). Defining the relationship between phenotypic and genotypic resistance profiles of multidrug-resistant enterobacterial clinical isolates. *Advances in Microbiology, Infectious Diseases and Public Health*: Volume *13*, 9-21. http://dx.doi.org/10.1007/5584_2018_208

Garrity, G. M., Brenner, D. J., Krieg, N. R., Staley, J. R., & Manual, B. S. (2005). Systematic bacteriology. The Proteobacteria, Part C: *The Alpha-, Beta-, Delta-, and Epsilonproteobacteria, Bergey's Manual Trust, Department of Microbiology and Molecular Genetics*, 2. https://doi.org/10.1007/0-387-29298-5

Gibson, J. S., Cobbold, R. N., Kyaw-Tanner, M. T., Heisig, P., & Trott, D. J. (2010). Fluoroquinolone resistance mechanisms in multidrug-resistant *Escherichia coli* isolated from extraintestinal infections in dogs. *Veterinary Microbiology*, *146*(1-2), 161-166. https://doi.org/10.1016/j.vetmic.2010.04.012

Gómez-Poveda, B., & Moreno, M. A. (2018). Antimicrobial prescriptions for dogs in the capital of Spain. *Frontiers in Veterinary Science*, *5*, 309. http://dx.doi.org/10.3389/fvets.2018.00309

Guardabassi, L., Schwarz, S., & Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria. *Journal of Antimicrobial Chemotherapy*, *54*(2), 321-332. https://doi.org/10.1093/jac/dkh332

Hall, J. L., Holmes, M. A., & Baines, S. J. (2013). Prevalence and antimicrobial resistance of canine urinary tract pathogens. *Veterinary Record*, *173*(22), 549-549. https://doi.org/10.1136/vr.101482

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. *American Journal of Veterinary Research*, *73*(3), 409-417. https://doi.org/10.2460/ajvr.73.3.409

Hendriksen, R. S., Mevius, D. J., Schroeter, A., Teale, C., Meunier, D., Butaye, P., ... & Aarestrup, F. M. (2008). Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002–2004. *Acta Veterinaria Scandinavica*, *50*, 1-10. https://doi.org/10.1186/1751-0147-50-28

Hordijk, J., Schoormans, A., Kwakernaak, M., Duim, B., Broens, E., Dierikx, C., Mevius, D., & Wagenaar, J. A. (2013). High prevalence of fecal carriage of extended-spectrum beta-lactamase/AmpC-producing Enterobacteriaceae in cats and dogs. *Frontiers in Microbiology*, *4*, 242. https://doi.org/10.3389/fmicb.2013.00242

Kahlmeter, G., Giske, C. G., Kirn, T. J., & Sharp, S. E. (2019). Point-counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute recommendations for reporting antimicrobial susceptibility results. *Journal of Clinical Microbiology*, *57*(9), 10-1128. https://doi.org/10.1128/jcm.01129-19

Karkaba, A., Hill, K., Benschop, J., Pleydell, E., & Grinberg, A. (2019). Carriage and population genetics of extended spectrum β-lactamase-producing *Escherichia coli* in cats and dogs in New Zealand. *Veterinary Microbiology*, *233*, 61-67. https://www.sciencedirect.com/science/article/abs/pii/S0378113518311507

Kroemer, S., El Garch, F., Galland, D., Petit, J. L., Woehrle, F., & Boulouis, H. J. (2014). Antibiotic susceptibility of bacteria isolated from infections in cats and dogs throughout Europe (2002–2009). *Comparative Immunology, Microbiology and Infectious Diseases*, *37*(2), 97-108. https://doi.org/10.1016/j.cimid.2013.10.001

Lloyd, D. H. (2007). Reservoirs of antimicrobial resistance in pet animals. *Clinical Infectious Diseases*, *45*(Supplement_2), S148-S152. https://doi.org/10.1086/519254

McEwen, S. A., & Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. *Clinical Infectious Diseases*, *34*(Supplement_3), S93-S106. https://doi.org/10.1086/340246

Murphy, C. P., Reid-Smith, R. J., Boerlin, P., Weese, J. S., Prescott, J. F., Janecko, N., & McEwen, S. A. (2012). Out-patient antimicrobial drug use in dogs and cats for new disease events from community companion animal practices in Ontario. *The Canadian Veterinary Journal*, *53*(3), 291-298. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3280785/

Pedersen, K., Pedersen, K., Jensen, H., Finster, K., Jensen, V. F., & Heuer, O. E. (2007). Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *Journal of Antimicrobial Chemotherapy*, *60*(4), 775-781. https://doi.org/10.1093/jac/dkm269

Rzewuska, M., Czopowicz, M., Kizerwetter-Świda, M., Chrobak, D., Błaszczak, B., & Binek, M. (2015). Multidrug resistance in *Escherichia coli* strains isolated from infections in dogs and cats in Poland (2007–2013). *The Scientific World Journal*, *2015*. https://doi.org/10.1155/2015/408205

SVA. (2021, April). Protocol for identification of *C. jejuni, C. coli* and C. lari by gel-based PCR. Version 1. National Veterinary Institute. https://www.sva.se/media/ju0l5ios/eurl_protocol-identification-jejuni-coli-lari_gelpcr_v1.pdf (assessed on October 22, 2021).

Saputra, S., Jordan, D., Mitchell, T., San Wong, H., Abraham, R. J., Kidsley, A., ... & Abraham, S. (2017). Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia. *Veterinary Microbiology*, *211*, 43-50. https://doi.org/10.1016/j.vetmic.2017.09.014

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., & Morris Jr, J. G. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proceedings of the National Academy of Sciences*, *99*(9), 6434-6439. http://dx.doi.org/10.1073/pnas.082188899

So, J. H., Kim, J., Bae, I. K., Jeong, S. H., Kim, S. H., Lim, S. K., ... & Lee, K. (2012). Dissemination of multidrug-resistant *Escherichia coli* in Korean veterinary hospitals. *Diagnostic Microbiology and Infectious Disease*, *73*(2), 195-199. https://doi.org/10.1016/j.diagmicrobio.2012.03.010

Soonthornsit, J., Apiratwarrasakul, S., & Phumthanakorn, N. (2022). Clinical characteristics, antimicrobial resistance and treatment outcomes of multidrug-resistant *Escherichia coli* infection in dogs and cats at a veterinary teaching hospital in Thailand. *The Thai Journal of Veterinary Medicine*, *52*(1), 207-212. https://digital.car.chula.ac.th/tjvm/vol52/iss1/22/

Thungrat, K., Price, S. B., Carpenter, D. M., & Boothe, D. M. (2015). Antimicrobial susceptibility patterns of clinical *Escherichia coli* isolates from dogs and cats in the United States: January 2008 through January 2013. *Veterinary Microbiology*, *179*(3-4), 287-295. https://doi.org/10.1016/j.vetmic.2015.06.012

Umeda, K., Hase, A., Matsuo, M., Horimoto, T., & Ogasawara, J. (2019). Prevalence and genetic characterization of cephalosporin-resistant Enterobacteriaceae among dogs and cats in an animal shelter. *Journal of Medical Microbiology*, *68*(3), 339-345. http://dx.doi.org/10.1099/jmm.0.000933

Van den Bunt, G., Fluit, A. C., Spaninks, M. P., Timmerman, A. J., Geurts, Y., Kant, A., ... & Hordijk, J. (2020). Faecal carriage, risk factors, acquisition and persistence of ESBL-producing Enterobacteriaceae in dogs and cats and co-carriage with humans belonging to the same household. *Journal of Antimicrobial Chemotherapy*, *75*(2), 342-350. https://doi.org/10.1093/jac/dkz462

Vasaikar, S., Obi, L., Morobe, I., & Bisi-Johnson, M. (2017). Molecular characteristics and antibiotic resistance profiles of Klebsiella isolates in Mthatha, Eastern Cape province, South Africa. *International Journal of Microbiology*, *2017*. https://doi.org/10.1155/2017/8486742

Wang, Y., Zhang, R., Li, J., Wu, Z., Yin, W., Schwarz, S., ... & Shen, J. (2017). Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nature Microbiology*, *2*(4), 1-7. http://dx.doi.org/10.1038/nmicrobiol.2016.260

Wedley, A. L., Dawson, S., Maddox, T. W., Coyne, K. P., Pinchbeck, G. L., Clegg, P.,... & Williams, N. J. (2017). Carriage of antimicrobial resistant *Escherichia coli* in dogs: Prevalence, associated risk factors and molecular characteristics. *Veterinary Microbiology*, *199*, 23-30. https://doi.org/10.1016/j.vetmic.2016.11.017

Xu, L., Zhai, Y., Lyu, Y., Wang, Q., An, S., Chen, J., & Chen, Y. (2014). Identification of Klebsiella pneumoniae strains harboring inactive extended-spectrum beta-lactamase antibiotic-resistance genes. *Chinese Medical Journal*, *127*(17), 3051-3057. https://mednexus.org/doi/abs/10.3760/cma.j.issn.0366-6999.20140628

Zhang, Z., Zhai, Y., Guo, Y., Li, D., Wang, Z., Wang, J., ... & Gao, Z. (2018). Characterization of unexpressed extended-spectrum beta-lactamase genes in antibiotic–sensitive Klebsiella pneumoniae isolates. *Microbial Drug Resistance*, *24*(6), 799-806. https://doi.org/10.1089/mdr.2017.0018

Zhao, S. Y., Zhang, J., Zhang, Y. L., Wang, Y. C., Xiao, S. Z., Gu, F. F., ... & Han, L. Z. (2016). Epidemiology and risk factors for faecal extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) carriage derived from residents of seven nursing homes in western Shanghai, China. *Epidemiology and Infection*, *144*(4), 695-702. https://doi.org/10.1017/s0950268815001879