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PREVALENCE AND ANTIBIOTIC RESISTANCE OF MICROORGANISMS OF THE ENTEROBACTERIACEAE FAMILY ISOLATED FROM DOGS WITH PARVOVIRUS ENTERITIS AND CLINICALLY HEALTHY CONTROLS IN THE NORTHERN REGION OF THE REPUBLIC OF KAZAKHSTAN

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Abstract

One of the main problems in the treatment of infectious diseases in pets is the spread of strains of microorganisms resistant to antimicrobial drugs. The aim of the study was to isolate conditionally pathogenic microorganisms of the *Enterobacteriaceae* family from dogs with parvavirus enteritis and clinically healthy animals, with the determination of phenotypic and genotypic resistance to antimicrobial drugs. In the period from March 2021 to March 2023, biological material from dogs with parvavirus enteritis (n = 152) and healthy dogs (n = 196), taken in veterinary clinics of Kostanay, was analyzed. Eighty-two isolates of conditionally pathogenic microorganisms of the *Enterobacteriaceae* family were isolated and studied from 348 biomaterial samples taken from dogs with enteritis. Conditionally pathogenic *Enterobacteriaceae* were isolated in 26.3% of cases (40 strains), among them: 65% of *E.coli* strains, 12% - *Klebsiella*, 10% - *Proteus*, 8% - *Citrobacter*, 5%- *Enterobacter*. Forty two microorganisms were isolated from healthy dogs: 83% are *E.coli* microorganisms, 5% are *Klebsiella* and *Proteus* isolates, and

2% are *Citrobacter* and *Enterobacter* microorganisms. In all isolated isolates, the resistance/sensitivity to the action of antibacterial drugs was determined by the disco-diffuse method. It was revealed that all isolated strains of microorganisms showed sensitivity to the action of streptomycin, belonging to the group of aminoglycosides, showed resistance to tetracycline, doxycycline, ofloxacin, ampicillin, amoxicillin. Resistance genes have been identified to beta-lactams, aminoglycosides, tetracyclines, and sulfonamides. The resistance gene to fluoroquinolones was isolated from 1 *E. coli* isolate, from a healthy animal. Resistance genes were not detected in the DNA of *Citrobacter*, *Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs. The most common genes were genes encoding resistance to aminoglycosides - 28% of strains, to beta-lactams - 21.9%, to tetracyclines - 13.4% of animals. It was concluded that the uncontrolled and frequent use of antibacterial drugs of the beta-lactam group and tetracyclines in dogs leads to the spread of genotypic resistance among microorganisms of the *Enterobacteriaceae* family.

Key words: antibiotic resistance; *E. coli*; *Enterobacteriaceae*; *Klebsiella*; parvovirus enteritis; *Proteus*; the resistance gene.

Basic position and Introduction

Parvovirus infection characterized by severe enteritis and vomiting, as well as dehydration, fever, leukopenia and diarrhea. Treatment of this infection is mainly symptomatic, antimicrobial and antiemetic drugs are also used. One of the main problems in the treatment of infectious diseases in pets is the spread of strains of microorganisms resistant to antimicrobial drugs [1, 2]. Infectious diseases caused by such strains of microorganisms are characterized by a long course and worsen the further prognosis of the disease. If the drug used in the treatment of the disease is not effective, then it is necessary to use other stronger antimicrobial agents that are unsafe [3]. All this increases the risk of the spread of resistant strains of microorganisms in the environment.

Every year the list of drugs increases, in particular, antibiotics used in the treatment of diseases of small domestic animals (cats and dogs), the exception is not drugs used in human medicine (cephalosporins and fluoroquinolones) [4]. The transmission of microorganisms that have developed resistance to these drugs at the genetic level occurs between pets, owners and veterinary staff, where pets can act as reservoirs of bacteria, which creates the possibility for interspecific transmission of resistant forms of microorganisms. All of this may affect the use of antimicrobials in human medicine [5]. The increase and spread of antimicrobial resistance in domestic animals leads to an increased risk of therapeutic failures, i.e. inefficiency of treatment, increased costs of animal treatment and health complications [6].

In the infectious pathology of dogs, a significant part falls on viral infections. According to many researchers, parvovirus enteritis of dogs, despite the widespread use of effective vaccines, remains the most dangerous and most common viral infection, with 100% morbidity at all ages, 10% and 91% mortality in adult dogs and puppies, respectively [7, 8]. It leads to significant economic losses,

which consist of direct losses due to high mortality of especially valuable animals, a decrease in service and breeding qualities in sick and ill individuals, as well as the costs of diagnostic, therapeutic and preventive measures.

Studies of recent decades have significantly expanded the understanding of epizootology and specific prevention of parvovirus enteritis as a monoinfection [9]. However, such important issues as the peculiarities of the course of the pathological process and the treatment of dogs with parvovirus enteritis complicated by associations of conditionally pathogenic bacteria remain poorly understood.

The aim of the study was to isolate conditionally pathogenic microorganisms of the *Enterobacteriaceae* family from dogs with parvavirus enteritis and clinically healthy animals, with the determination of phenotypic and genotypic resistance to antimicrobial drugs.

Materials and Methods

The research work was carried out in the period from March 2021 to March 2023. The selection of biomaterial from dogs was carried out in veterinary clinics of the city of Kostanay. Laboratory studies were carried out in the Department of Microbiological Analysis of the Research Institute of Applied Biotechnology of the A. Baitursynov KRU.

The object of research is biological material from dogs clinically healthy and diagnosed with parvavirus enteritis. A total of 348 samples were examined - flushes from the oral cavity and anal opening.

All animals underwent a clinical examination according to generally accepted methods, with the necessary diagnostic tests, a blood test, rapid tests to confirm infectious pathology (tests for parvovirus enteritis) and sampling from animal biological material to isolate conditionally pathogenic microorganisms with determination of resistance to antimicrobial drugs. After the final diagnosis was made, the animals were treated with data on sensitivity to antibacterial drugs.

Isolation and accumulation of pure cultures of microorganisms was carried out using universal chromogenic, differential diagnostic media. The identification of *E.coli*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Proteus* cultures was performed according to the approved guidelines for the microbiological diagnosis of diseases caused by microorganisms of the *Enterobacteriaceae* family [10], as well as in accordance with the Bergi bacterial determinant.

To isolate microorganisms from the studied material, crops were sown on BCH, incubated for 18-20 hours at a temperature of 36-37 ° C, after which they were transplanted onto cups with chromogenic CHROMagarOrientation, which were re-cultivated. When distinct colonies characteristic of the growth of *E.coli*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Proteus* appeared on this medium, smears were prepared and colored by Gram. When gram-negative straight rods with rounded ends were found in smears typical in morphology, their biochemical properties were studied (Table 1).

Table 1 – Differentiation of *Enterobacteriaceae* by enzymatic properties

	The microorganism / Test or substrate	*Escherichia coli*	*Klebsiella*	*Citrobacter*	*Enterobacter*	*Proteus*
According to the results of seeding on a combined medium (Kligler)	Lactose (bevel)	+/-	+	+/x	+	-
	Glucose (column)	+/-	+	+	+	x
	Hydrogen sulfide	-	-	+/-	-	+/-
Additional tests to determine ancestral affiliation	Simmons Citrate	-	+/-	+	+	+/-
	Lactose	+	+/-	+/-	+	-
	Mannitol	+	+	+	+	-
	Mobility	+/-	-	+	+	+/-
	Indole	+/-	-/+	-/+	-	+/-
	Urea by Christensen	-	+	-/+	+/-	+/-
	Test with methyl red	+	-/+	+	-	+
	Voges-Proskauer Test	-	-/+	-	+	-

+ 90% or more positive reactions
- 90% or more negative reactions
+/- more often positive, less often negative
-/+ more often negative, less often positive
X Various biochemical reactions

The ability of bacteria to ferment lactose, glucose, as well as to form gas and hydrogen sulfide was determined by the change in the color of the medium, the appearance of gas bubbles in the Kliegler medium. The change in the medium was taken into account after 24-hour incubation at t 37 ° C.

The enzymatic properties of bacteria were studied on Gis media with lactose, mannitol. 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 studied isolates was studied by their growth when seeded with an injection into semi-liquid agar. The change in media during growth was taken into account after 2 days of incubation.

The Voges-Proscauer test was performed based on the detection of acetoin by adding α-naphthol and potassium hydroxide to a 2-day culture of microorganisms on Clark's medium. In the presence of oxygen, acetoin is oxidized into a diacetyl-forming compound of red color.

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 BCH, with a positive reaction, the formation of a red ring was observed.

The antibiotic sensitivity of isolated isolates of *E.coli*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Proteus* was studied by applying standard antibiotic discs to a freshly sown lawn of the culture using Muller–Hinton agar. The results were taken into account after 18-24-hour incubation at a temperature of 37 ° C by the presence of microbe growth retardation zones around the discs, which, according to the instructions, indicates either the sensitivity of the pathogen to the drug or its resistance to this antibiotic (Table 2). Interpretation of the results was carried out:

- according to methodical instruction 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs" [11];
- in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 11.0 [12];
- in accordance with the recommendations of the Institute of Clinical and Laboratory Standards (CLSI) [13]

Table 2 - Interpretation of the results of determining the sensitivity of *Enterobacteriaceae*

№	Name of disks with drugs	≤R	I	S≥	Interpretation of the base
1	2	3	4	5	6
1	Ampicillin (AMP) 10 mcg	14		14	Eucast 11.0
2	Amoxicillin (ACC) 25 mcg	15	15-20	21	methodical instruction
3	Cefoperazone (CPR) 75 mcg	15	16-20	21	CLSI, МУК
4	Cefoxitin (CFN) 30 mcg	9		19	Eucast 11.0
5	Cefpodoxime (CFM) 10 mcg	17		17	CLSI
6	Meropenem (MPN) 10 mcg	16		22	Eucast 11.0
7	Streptomycin (STR) 10 mcg	11	12-14	15	CLSI
8	Kanamycin (CAN) 30 mcg	13	14-17	18	CLSI, methodical instruction
9	Gentamicin (GEN) 10 mcg	17		17	Eucast 11.0
10	Levomycetin (LEV), 30 mcg	17		17	Eucast 11.0
11	Tetracycline (TET) 30 mcg	19		19	Eucast 11.0
12	Doxycycline (DOC C) 30 mcg	0	11-13	14	CLSI
13	Enrofloxacin (ENR) 5 mcg	17	18-21	22	methodical instruction
14	Ciprofloxacin (CIP) 5 mcg	22		25	Eucast 11.0
15	Norfloxacin (NOR) 10 mcg	22		22	Eucast 11.0
16	Ofloxacin (OF) 5 mcg	22		24	Eucast 11.0
17	Hemifloxacin (HEME) 5 mcg	15	16-19	20	CLSI
18	Nalidixic acid (NC) 30 mcg	13	14-18	19	CLSI
19	Trimethoprim/sulfamethoxazole (KTZ) 1.25/23.75	11		14	Eucast 11.0
20	Furazolidone (FRN) 300 mcg	14	15-16	17	CLSI

21	Furadonin (FD) 300 mcg	14	15-16	17	CLSI

Determination of resistance genes

DNA material for molecular research was obtained by bacterial lysis according to the recommendations of the Reference Laboratory for Resistance to Antibacterial Drugs of the European Union (Community Reference Laboratory for Antimicrobial Resistance) with minor changes. Identification of genes encoding antimicrobial resistance was carried out by PCR.

Results

In a clinical study, parvovirus enteritis was detected in 152 animals. The group of clinically healthy animals (n=196) included dogs whose owners applied to a veterinary clinic for preventive examination, as well as vaccination and deworming. Biological material was taken from all animals (flushes from the mouth and anus). Eighty two isolates of opportunistic *Enterobacteriaceae* were isolated and studied from 348 samples of biomaterial. Among them: 61 (57.9%) strains of *E.coli*, 7 (10.5%) strains of *Klebsiella*, 4 (8.8%) strains of *Citrobacter*, 4 (12.3%) strains of *Enterobacter* and 6 (10.5%) strains of *Proteus* (Figure 1).

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

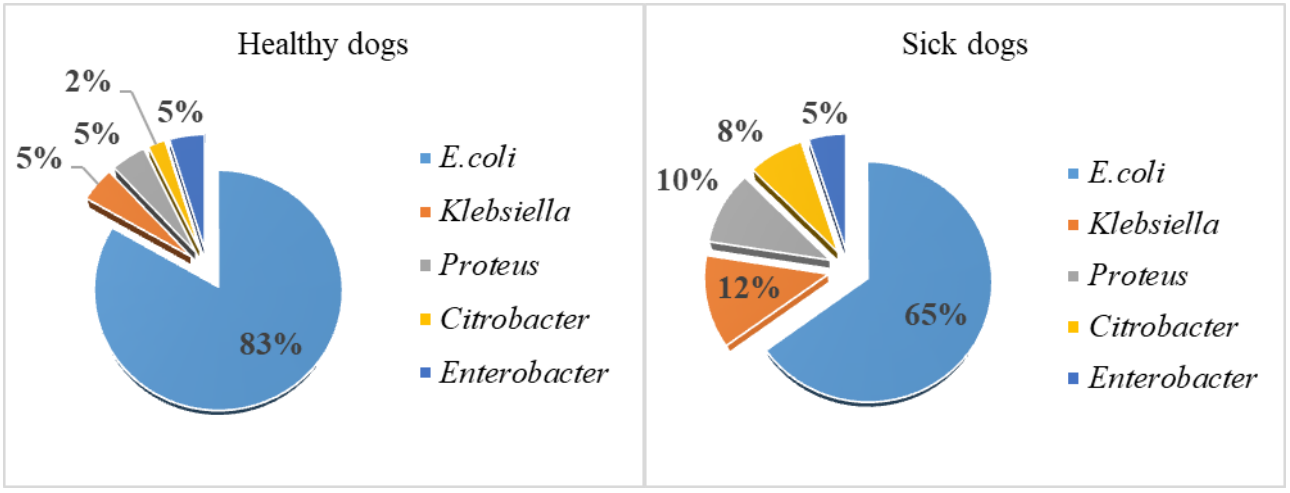

Figure 1- Percentage of isolates isolated from dogs

From 152 samples of biomaterial from dogs with enteritis, conditionally pathogenic enterobacteria were isolated in 26.3% of cases (40 strains). Among them: 26 (65%) *E.coli* strains, 5 (12%) *Klebsiella* strains, 4 (10%) *Proteus* strains, 3 (8%) *Citrobacter* strains, 2 (5%) and *Enterobacter* strains.

Forty two microorganisms were isolated from 196 samples of biomaterial from healthy dogs, which is 21.4% of the total number of samples. Among 83% (35 strains) are *E.coli* microorganisms, 5% of *Klebsiella* and *Proteus* isolates (2 strains each), and 2% of *Citrobacter* and *Enterobacter* microorganisms.

Antibiotic resistance. The next stage of the research was to determine the sensitivity to antibacterial drugs of

the isolated strains and the determination of resistance genes. The results of antibiotic resistance of microorganisms of the *Enterobacteriaceae* family isolated from dogs with parvovirus enteritis and clinically healthy controls are shown in Figures 2 and 3.

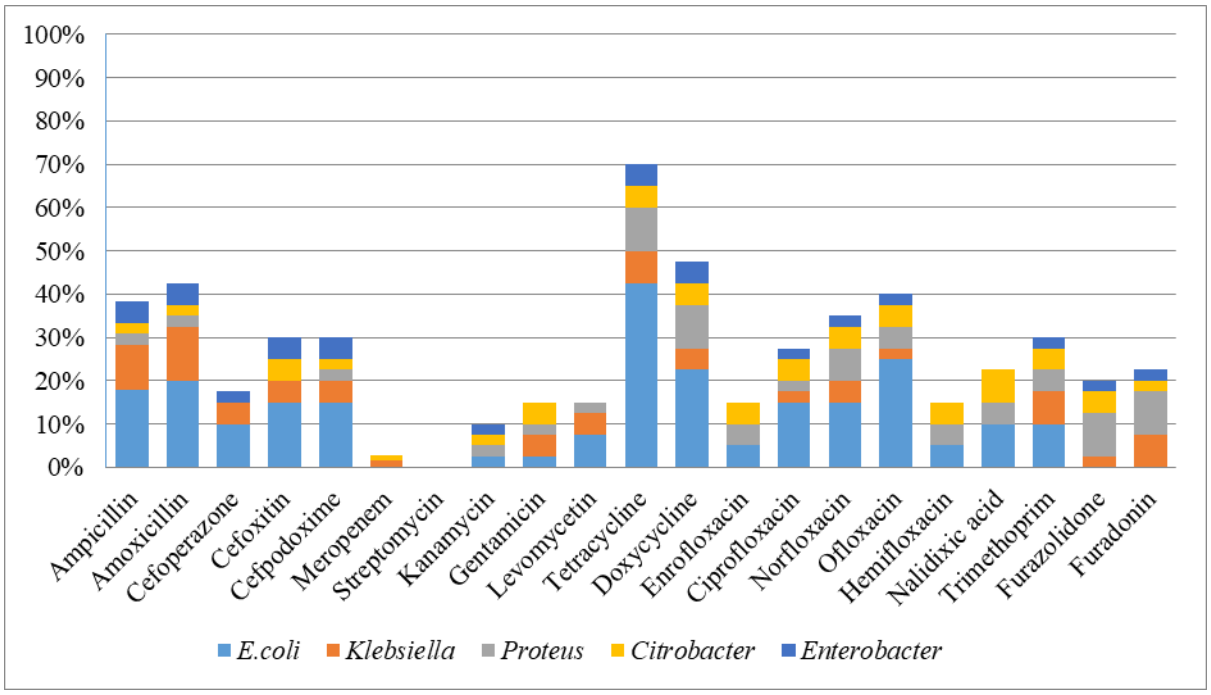

Figure 2 – Results of antibiotic resistance of strains isolated from dogs with paravavirus enteritis

Of the 40 studied strains of opportunistic microorganisms, 70% were resistant to tetracycline - 28 strains (17 - *E.coli*, 4- *Proteus*, 3- *Klebsiella*, 2 *Citrobacter* and *Enterobacter*), 47.5% were resistant to doxycycline (9 strains - *E.coli*, 4- *Klebsiella*, 2 *Proteus*, *Citrobacter* and *Enterobacter*), to amoxicillin – 42.5% (7 strains - *E.coli*, 4- *Klebsiella*, 2 *Enterobacter* and 1 *Proteus*, *Citrobacter*), ofloxacin - 40% (10 strains - *E.coli*, 2- *Citrobacter* and *Proteus*, and 1 strain of *Klebsiella* and *Enterobacter*), 37.5% were resistant to ampicillin (7 *E.coli*, 4- *Klebsiella*, 2- *Enterobacter*, 1 strain - *Proteus*, *Citrobacter*), 35% to norfloxacin (6 strains of *E.coli*, 3- *Proteus*, 2- *Klebsiella* and *Citrobacter*, and 1- *Enterobacter*), 30% of microorganisms were resistant to cefpodoxime (6- *E.coli*, 2 strains of *Enterobacter* and *Klebsiella*, 1 *Citrobacter* and *Proteus* strain,), cefoxitin (6 - *E.coli*, 2 strains of *Klebsiella*, *Citrobacter* and *Enterobacter*) and trimethoprim/sulfamethoxazole (4- *E.coli*, 3- *Klebsiella*, 2 – *Citrobacter* and *Proteus* and 1- *Enterobacter*), 27.5% to ciprofloxacin (6 strains of *E.coli*, 2 – *Citrobacter* and 1 *Klebsiella*, *Enterobacter*, *Proteus*) and nalidixic acid (4- *E.coli*, 3- *Citrobacter* and 2- *Proteus*), 22.5% were resistant to furadonin (4- *Proteus*, 3- *Klebsiella* and 1 *Citrobacter* and *Enterobacter*), 20% were resistant to furazolidone (4- *Proteus*, 2- *Citrobacter* and 1 *Klebsiella* and *Enterobacter*), 17.5% to

cefoperazone (4 *E.coli*, 2- *Klebsiella* and 1 *Enterobacter*), 15% of microorganisms were resistant to gentamicin (2 strains of *Citrobacter* and *Klebsiella*, 1 each – *Proteus* and *E.coli*), levomycetin (3 *E.coli*, 2 *Klebsiella* and 1 *Proteus*), enrofloxacin and hemifloxacin (2 strains of *E.coli*, *Citrobacter* and *Proteus*), to kanmycin – 10% (1 strain of *Proteus*, *Citrobacter*, *E.coli* and *Enterobacter*), 5% - 1 strain of *Citrobacter* and 1 strain of *Klebsiella* was resistant to meropenem. No streptomycin-resistant strains were detected.

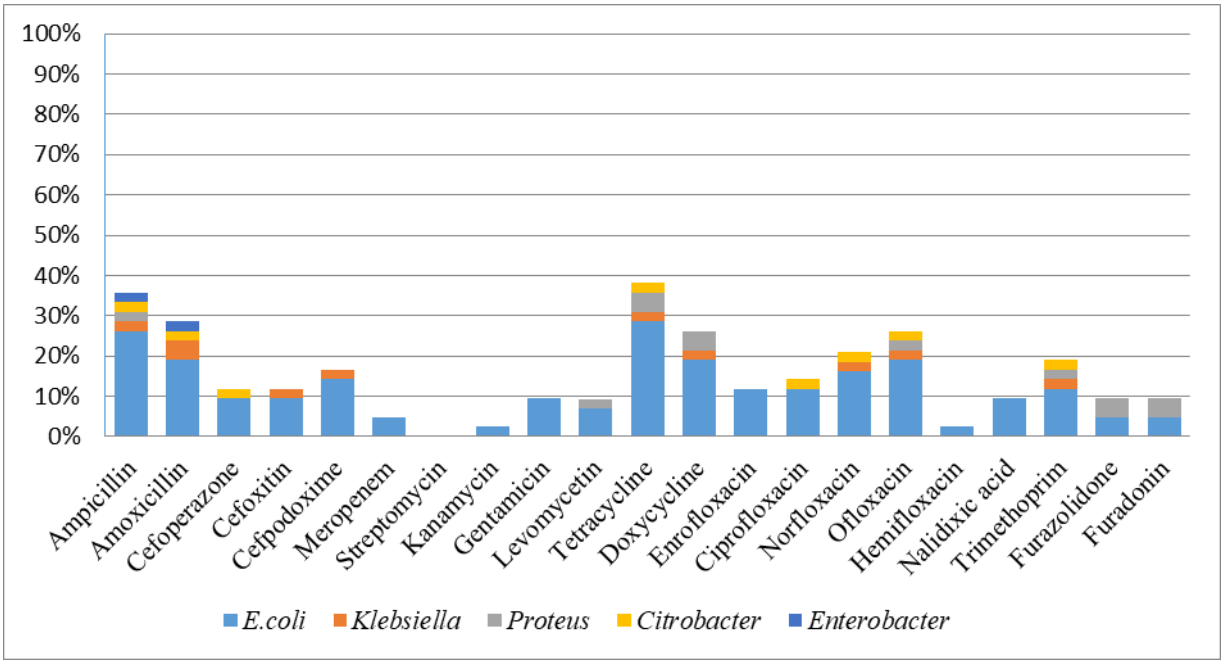

Figure 3 – Results of antibiotic resistance of strains isolated from healthy dogs

Of the 42 studied strains of *Enterobacteriaceae* isolated from healthy dogs, 38% were resistant to tetracycline - 16 strains (12 - *E.coli*, 2- *Proteus* and 1 strain of *Klebsiella* and *Citrobacter*), 35.7% - to ampicillin (11 *E.coli* and 1 strain of *Proteus*, *Klebsiella*, *Citrobacter*, *Enterobacter*), 28.6% - to amoxicillin (8- *E.coli*, 2- *Klebsiella* and 1 – *Citrobacter* and *Enterobacter*), 26.2% to doxycillin (8- *E.coli*, 2- *Proteus*, 1- *Klebsiella*) and ofloxacin (8- *E.coli*, 1 – *Proteus*, *Klebsiella* and *Citrobacter*), 21.4% to norfloxacin (7 *E.coli*, 1 strain of *Klebsiella* and *Citrobacter*), to trimethoprim/sulfamethoxazole – 19% (5 strains of *E.coli* and 1 *Proteus*, *Klebsiella*, *Citrobacter*), to cefpodoxime – 16.7% (6 *E.coli* and 1 *Klebsiella*), 14.3% to ciprofloxacin (5 *E.coli* and 1 strain of *Citrobacter*), 12% of microorganisms were resistant to cefoperazone (4 strains of *E.coli* and 1 *Citrobacter*), cefoxitin (4 strains of *E.coli* and 1 *Klebsiella*) and enrofloxacin (5 strains of *E.coli*), 9.5% of microorganisms are resistant to gentamicin (*E.coli*), levomycetin (3 *E.coli* and 1 *Proteus*), nalidixic acid (*E.coli*), furazolidone and furadonin (2 strains of *E.coli* and *Proteus* for each drug), 4.8% - to meropenem (*E.coli*), 1 isolate *E.coli* (2.4%) was resistant to

the action of kanamycin and hemifloxacin. No streptomycin-resistant strains were detected.

To determine the genetic profiles of resistance of microorganisms, primers were used, which were selected by us taking into account the use of classes of antibiotics and antimicrobials in veterinary practice.

As a result of the conducted studies, 82 samples that showed phenotypic resistance to antibacterial drugs were tested by PCR for the presence of genes encoding resistance. The results are presented in table 3.

Table 3 - Microbial resistance genes

Group of antibiotics	Gene	*E.coli*	*Klebsie-lla*	*Citro-bacter*	*Entero-bacter*	*Proteus*	*E.coli*	*Klebsie-lla*	*Citro-bacter*	*Entero-bacter*	*Proteus*	Total
		animals with enteritis					healthy animals					
Beta-lactams	BlaTEM	5	2	1		1	3	1				13
	OXA	1					3	1				5
Aminoglycosides	StrA	4			1		2	1				8
	StrB	4			1		1	1				7
	aadB					1	1					2
	aphA1	2	1		1		2					6
Tetracyclines	tetA	2	1	1			4					8
	tetB	3										3
Sulfonamides	SUL1		1			1						2
	SUL3	1				1	3					5
Fluoroquinolones	qepA						1					1
	qnrA											0

Based on the data presented in Table 3, it can be seen that resistance genes were identified for all the studied groups of antibiotics in the DNA of microorganisms of the *Enterobacteriaceae* family isolated from dogs. Most often, in 28% of strains, genes encoding resistance to aminoglycosides were isolated (in 37.5% of animals with enteritis and in 19% of healthy animals). Beta-lactam resistance genes were found in 21.9% of isolates (in 25% of sick dogs and in 19% of healthy ones). Genes encoding resistance to tetracyclines were detected in 13.4% of cases (in 17.5% of dogs with enteritis and in 9.5% of healthy animals). Genes encoding resistance to sulfonamides were also identified in 10% of cases from sick animals and in 7.1% in healthy ones. The smallest number of genes were identified that cause resistance to fluoroquinolones (qepA, n=1). It should be noted that in the DNA of *Citrobacter*, *Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs, no genes encoding resistance to antibacterial drugs have been identified

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Discussion

The acquisition and spread of resistance to antibacterial drugs by microorganisms has been noted for many years and has become one of the most important therapeutic problems in human medicine and veterinary medicine. Our study analyzed the spread of antimicrobial resistance in strains of microorganisms of the *Enterobacteriaceae* family isolated from healthy domestic dogs and dogs with diagnosed parvavirus enteritis.

As a result of the conducted studies, 348 samples of biological material (flushes from the oral cavity and anus) were selected from dogs in veterinary clinics of Kostanay, among them clinically healthy dogs (n=196) and dogs with parvavirus enteritis (n=152). 23.6% of animals (n=82) were found to carry conditionally pathogenic microorganisms of the *Enterobacteriaceae* family, including 48.7% (40 isolates) isolated from sick animals and 51.3% (42 isolates) from healthy ones. Among them: 61 (57.9%) strains belonged to *E.coli*, 7 (10.5%) strains of *Klebsiella*, 4 (8.8%) strains of *Citrobacter*, 4 (12.3%) strains of *Enterobacter* and 6 (10.5%) strains of *Proteus*. The results obtained by us correlate with the data obtained in a number of studies in Western Europe, where the prevalence of colonization by *Enterobacteriaceae* in dogs (including healthy and sick animals) ranged from 3.1 to 55% [14, 15]. From 152 samples of biomaterial from dogs with enteritis, conditionally pathogenic *Enterobacteriaceae* were isolated in 26.3% of cases (40 strains). Among the microorganisms isolated from the biomaterial of sick animals, 65% of the strains belonged to *E.coli*, 12% of the strains to *Klebsiella*, 10% of the strains to *Proteus*, 8% - *Citrobacter*, 5% - *Enterobacter*. Forty two microorganisms were isolated from 196 samples of biomaterial from healthy dogs, which is 21.4% of the total number of samples. Among them, 83% are *E.coli* microorganisms, 5% are *Klebsiella* and *Proteus* isolates, and 2% are *Citrobacter* and *Enterobacter* microorganisms.

In all isolated isolates, antimicrobial resistance/sensitivity was determined by the disco-diffuse method. Analyzing the data obtained during this study, it should be noted that the maximum number of microorganisms (53.6%) isolated from both sick animals and healthy ones showed resistance to the action of tetracycline. These results are consistent with a number of studies conducted over the past decade in the UK and Portugal, where *Enterobacteriaceae* also had the maximum percentage of resistant strains to this antibacterial drug [16, 17]. *E.coli* isolates showed increased resistance to ampicillin, amoxicillin, cefpodoxime, as well as low resistance to carbapenems (meropenem), these results correlate with the results of studies obtained in Australia [18]. The maximum resistance to the action of amoxicillin (100%) and ampicillin (71%) and the minimum to the action of meropenem were also found in microorganisms of the genus *Klebsiella*. Studies conducted in 2017-2019 in Thailand and Portugal confirm that this microorganism has a high resistance to this drug [19; 20]. There are not many studies on the prevalence of resistance to antibacterial drugs in

Citrobacter isolates from dogs all over the world. The results of our studies show that the maximum number of microorganisms were resistant to nalidixic acid, tetracycline, ciprofloxacin, norfloxacin, ofloxacin, trimetaprim/sulfamethoxazole. In studies conducted in Nigeria, the data were partially similar, *Citrobacter* showed resistance to amoxicillin, ciprofloxacin [21]. All microorganisms of the genus *Proteus* have resistance to tetracycline, doxycycline and nitrofurans, as well as a decrease in resistance to norfloxacin and hemifloxacin. Similar results were obtained in Japan, where similar resistance to the action of antibacterial drugs was obtained in *Proteus* and *Enterobacter* [22, 23]. It was revealed that *Enterobacter* strains exhibit the greatest resistance against ampicillin, amoxicillin, tetracycline and doxycycline.

It should be noted that in general, the largest number of all isolated microorganisms showed resistance to the group of tetracyclines and beta-lactams, which, according to the State register of Veterinary drugs and feed additives of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, are the drugs of choice in the treatment of infectious diseases of animals. In a study conducted in Spain, beta-lactams were the antimicrobial drugs most commonly prescribed to dogs [24].

Among the isolated microorganisms, the majority of isolated strains of microorganisms (56%) had polyresistance, with the exception of 21 strains isolated from healthy animals (20 isolates of *E.coli* and 1 *Enterobacter*) and 14 isolates of *E.coli* obtained from sick dogs.

In general, as a result of studies of isolates, conditionally pathogenic *Enterobacteriaceae*, it was noticed that strains isolated from dogs with diagnosed parvavirus enteritis have a greater degree of resistance to antibacterial drugs, unlike strains isolated from healthy animals. Isolates from sick animals are on average 8.7% more resistant to the beta-lactam group than strains from healthy dogs, 4.4% more resistant to the aminoglycosides group, 5.5% more resistant to amphenicols, 25.6% more resistant to tetracyclines, 11.24% more resistant to fluoroquinolones, 18% more resistant to quinolones, 11% more resistant to sulfonamides, to nitrofurans by 11.7%.

To determine the bacterial resistance profiles, primers were used, which were selected taking into account the use of classes of antibiotics and antimicrobials in veterinary practice. As a result of DNA studies, 82 strains of opportunistic microorganisms of the *Enterobacteriaceae* family were tested by PCR for the presence of genes encoding resistance. Resistance genes were identified for all the studied groups of antibiotics in the DNA of microorganisms of the *Enterobacteriaceae* family isolated from dogs. Most often, in 28% of strains, genes encoding resistance to aminoglycosides were isolated (in 37.5% of animals with enteritis and in 19% of healthy animals). Beta-lactam resistance genes were found in 21.9% of isolates (in 25% of sick dogs and in 19% of healthy ones). Genes encoding resistance to tetracyclines were detected in 13.4% of cases (in 17.5% of

dogs with enteritis and in 9.5% of healthy animals). Genes encoding resistance to sulfonamides were also identified in 10% of cases from sick animals and in 7.1% in healthy ones. The smallest number of genes were identified that cause resistance to fluoroquinolones. It should be noted that in the DNA of *Citrobacter*, *Enterobacter* and *Proteus* isolates isolated from clinically healthy dogs, no genes encoding resistance to antibacterial drugs have been identified.

In the course of the study, it was found that in 18 DNA samples with genotypic resistance to antibiotics, their connection with phenotypic profiles is traced. However, in 6 *E.coli* samples (3 from sick animals and 3 from healthy ones), resistance genes to antibacterial drugs of the aminoglycoside group (StrA, strB, aadB, aphA1) were detected, while phenotypic resistance in these microorganisms to this drug was not detected. Probably, these may be the so-called "silent" genes, which are found in a number of studies, but have not been studied enough yet [25, 26].

Thirty seven strains of microorganisms had phenotypic resistance to the group of fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, hemifloxacin), while genotypic resistance, i.e. the presence of qepA, qnrA genes was absent. This is probably due to differences in the mechanisms of resistance [27].

Conclusion

In the Northern region of the Republic of Kazakhstan, dogs have a high prevalence of opportunistic microorganisms of the *Enterobacteriaceae* family, as in dogs with parvavirus enteritis (*E. coli* - 26 (65%), *Klebsiella* - 5 (12%), *Enterobacter* – 2 (5%), *Proteus* – 3 (8%), and 3 isolates *Citrobacter*), and in healthy animals (*E. coli* - 83%), *Klebsiella* 5%, *Enterobacter* – 2%, *Proteus* – 5% and *Citrobacter* - 2%). The study of antibiotic resistance of the isolated strains showed high resistance to beta-lactams and tetracyclines. Resistance to these groups of antibacterial drugs is due to the presence of resistance genes blaTEM, OXA, tetA and tetB in microorganisms.

In general, as a result of studies of isolates, conditionally pathogenic *Enterobacteriaceae*, it was noticed that strains isolated from dogs with diagnosed parvavirus enteritis have a greater degree of resistance to antibacterial drugs, unlike strains isolated from healthy animals. Isolates from sick animals are on average 8.7% more resistant to the beta-lactam group than strains from healthy dogs, 4.4% more resistant to the aminoglycosides group, 5.5% more resistant to amphenicols, 25.6% more resistant to tetracyclines, 11.24% more resistant to fluoroquinolones, 18% more resistant to quinolones, 11% more resistant to sulfonamides, to nitrofurans by 11.7%.

Uncontrolled and frequent use of antibacterial drugs of the beta-lactam group and tetracyclines in dogs leads to the spread of genotypic resistance among microorganisms of the *Enterobacteriaceae* family.

Thus, the results obtained made it possible to assess the existing level of prevalence of antibiotic-resistant forms of conditionally pathogenic

microorganisms detected from dogs in the Northern region of Kazakhstan and to determine their phenotypic and genotypic profile.

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