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**DETERMINATION OF SENSITIVITY AND RESISTANCE TO ANTI-MICROBIAL ENTEROBACTERIA FOR DISEASES OF THE GASTROINTESTINAL TRACT IN DOGS AND CATS IN THE CITY OF KOSTANAY**

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Our article presents the data of scientific research conducted within the framework of the project "Prevalence of determinants of resistance to antibacterial drugs" of the grant funding of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan for 2021-2023. Samples were taken from the pharynx and anus of small domestic animals (cat, dog) with inflammatory diseases of the gastrointestinal tract, who sought therapeutic help in veterinary clinics of the city of Kostanay, to determine the sensitivity of the microflora to antimicrobial drugs, to identify resistant bacteria and for the effectiveness of the treatment. Since antimicrobial therapy is widely used in the treatment of small pets. Knowledge of the secreted enterobacteria in diseases of the gastrointestinal tract of cats and dogs and preferences for antimicrobial resistance is an important factor for successful therapy. Enterobacteria were detected in the studied animals (n - 52). In clinical samples from the pharynx and anal opening in both animal species, the main bacterium secreted was *E. coli*. Rare bacterial agents were *Proteus* and *Klebsiella* bacteria, which showed high resistance to most antimicrobial drugs.

Our research can help clinical practitioners in making rational decisions when treating antimicrobial drugs.

Key words: enterobacteriaceae, cats, dogs, antibiotic resistance.

**ОПРЕДЕЛЕНИЕ ЧУВСТВИТЕЛЬНОСТИ И РЕЗИСТЕНТНОСТИ К ПРОТИВОМИКРОБНЫМ ПРЕПАРАТАМ ЭНТЕРОБАКТЕРИЙ ПРИ ЗАБОЛЕВАНИЯХ ЖКТ У СОБАК И КОШЕК В ГОРОДЕ КОСТАНАЙ**

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В данной статье приводятся данные научных исследований, которые проводятся в рамках проекта «Распространенность детерминант устойчивости к антибактериальным препаратам» грантового финансирования Комитета науки Министерства образования и науки Республики Казахстан на 2021-2023 годы. Пробы с зева и анального отверстия, мелких домашних животных (кошка, собака) с заболеваниями желудочно-кишечного тракта воспалительного характера, хозяева которых обратились за терапевтической помощью в ветеринарные клиники города Костанай, были взяты для определения чувствительности микрофлоры к антимикробным препаратам, выявления резистентных бактерий и для эффективности проводимого лечения. Так как антимикробная терапия широко применяется в лечении мелких домашних животных. Знание выделяемых энтеробактерий при заболеваниях желудочно-кишечного тракта кошек и собак и тенденций к устойчивости к противомикробным препаратам является важным фактором для проведения успешной терапии. У исследуемых животных (n - 52) были определены энтеробактерии. В клинических образцах из зева и анального отверстия у обоих видов животных основной выделяемой бактерией была *E.coli*. Редко встречающимися бактериальными агентами были бактерии *Протея* и *Клебсиелла*, которые показали высокую резистентность к большинству противомикробных препаратов.

*Наше исследование, может помочь врачам клинической практики в принятии рациональных решений при терапии антимикробных препаратов.*

*Ключевые слова: энтеробактерии, кошки, собаки, антибиотикорезистентность.*

### **ҚОСТАНАЙ ҚАЛАСЫНДА ИТТЕРМЕН МЫСЫҚТАРДА АҚЖ-НЫҢ АУРУЛАРЫ КЕЗІНДЕ МИКРОБТАРҒА ҚАРСЫ ПРЕПАРАТТАРҒА ЭНТЕРОБАКТЕРИЯЛАРДЫҢ СЕЗІМТАЛДЫҒЫМЕН РЕЗИСТЕНТТІЛІГІН АНЫҚТАУ**

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*Біздің мақалада ғылыми зерттеулер Қазақстан Республикасы Білім және ғылым министрлігі Ғылыми комитетінің 2021-2023 жылдарға арналған гранттық қаржыландыруының "Бактерияға қарсы препараттарға төзімділік детерминанттарының таралуы" жобасы шеңберінде жүргізілді.*

*Мысықтар мен иттердің асқазан-ішек жолдарының аурулары кезінде бөлінетін энтеробактерияларды анықтау және микробтарға қарсы препараттарға төзімділік деңгейін білу, бұл терапияны сәтті жүргізудің маңызды факторы болып табылады. Ветеринарлық емханасына асқазан мен ішектің қабыну аурулары бар барлық жануарларға, емдік көмек көрсету үшін және емдеудің тиімділігін бағалау үшін микрофлораның антибиотиктерге сезімталдығына арналған дақылдар жүргізілді. Зерттелетін жануарларда (n - 52) энтеробактериялар анықталды. Жануарлардың екі түрінің де жұтқыншақ пен анустан алынған клиникалық үлгілерде негізгі бөлінетін бактерия *E. coli* болып анықталды.*

*Протеус және Клебсиелла бактериялары сирек кездесті, бірақ олар көптеген микробқа қарсы препараттарға жоғары төзімділігін көрсетті.*

*Біздің зерттеуіміз клиникалық тәжірибе дәрігерлеріне микробқа қарсы терапия кезінде ұтымды шешім қабылдауға көмектесе алады.*

*Түйінді сөздер: энтеробактериялар, мысықтар, иттер, антибиотиктерге төзімділік.*

Relevance. The microflora of the gastrointestinal tract of mammals includes a huge number of commensal bacteria that are normally present in the intestine as well as many protozoa and fungi. This microbiota has a profound effect on the immune system and can vary over time depending on the course of the disease or the diet of the animals.

Microorganisms of the family Enterobacteriaceae, although natural inhabitants of the intestinal tract of mammals, can cause diseases of the gastrointestinal tract itself as well as of the urinary tract, soft tissues, ears, skin and respiratory tract in cats and dogs [1, p. 349]. For uncomplicated infections, antibacterial drugs such as ampicillin, amoxicillin-clavulanate, or first- and second-generation cephalosporins are the treatment option, while third-generation cephalosporins and fluoroquinolones (enrofloxacin or ciprofloxacin) remain suitable for therapy of severe infections [2, p. 263]. ESBL production in enterobacteriaceae is the main mechanism, in the formation of resistance of gram-negative bacteria to beta-lactam drugs - to penicillins, cephalosporins of generations I-V [3, p. 57]. The appearance of extended spectrum  $\beta$ -lactamases (ESBL) producing enterobacteriaceae in sick and healthy small domestic animals is a serious problem for infection control in veterinary medicine [4, p.196]. Meanwhile, studies have shown that highly virulent multidrug-resistant enterobacteriaceae in humans, such as *Escherichia coli* and *Klebsiella pneumoniae* can also be isolated in domestic animals [5, p. Recently dogs and cats have been increasingly considered a reservoir and source of transmission of antibiotic-resistant bacteria [7, p. 279,8, p. 1679, 9, p. 189, 10, p. 182]. However, the degree and importance of antimicrobial resistance in cats and dogs are still poorly understood [11, p. 170]. Antimicrobial resistance in pets will lead to an increased risk of therapeutic failures, i.e. ineffective treatment, increased animal treatment costs and health complications [12, p. 395].

The aim of the study was to determine the sensitivity to antimicrobial drugs and the trend of development of resistance to antibacterial drugs enterobacterium in dogs and cats in diseases of the gastrointestinal tract.

Materials and methods of research.

The research work was carried out in the period from March to August 2021. Sampling of biomaterial from sick cats and dogs with dyspeptic phenomena was carried out in veterinary clinics of Kostanay city.

Extraction and identification of bacteria was carried out on the basis of the Research Institute of Applied Biotechnology of Kostanay Regional University named after A. Baitursynov.

The object of research was the biomaterial from cats and dogs which came to the veterinary clinic with clinical manifestations of gastro-intestinal diseases (dyspeptic phenomena). The subject of the study was Enterobacteriaceae: Escherichiacoli, Klebsiella, Enterobacter, Citobacter, Proteus. A total of 160 animals were examined, animals with detected Enterobacteriaceae were used for research in an amount of 52.

All the animals were examined clinically according to standard methods, with the necessary diagnostic tests: ultrasound investigation, biochemical oxidation control, fast tests to rule out infectious pathologies (ICA tests for parvovirus enteritis, coronavirus infection, feline panleukopenia) and samples taken to determine sensitivity to antibacterial agents for bacteria isolated from clinical specimens. After making a final diagnosis with data on sensitivity to antibacterial agents, the animals were treated.

Samples were collected from the pharynx and anus using disposable tampon probes embedded in a dry sterile tube (tubser). Rectal sampling was performed by inserting the tip of the sterile tampon probe 1.0-1.5 cm deep into the anal sphincter. Then we made careful rotary movements around the axis for 5-6 seconds to collect material from the crypts of the anus (anal folds), then carefully extracted the tampon and placed it in a tube. Oral swabs were taken with a tampon probe along the posterior pharyngeal wall, along the soft palate and cheek fold with rotary movements, and immediately closed in a tube.

Microbiological examination for isolation and identification of bacteria was performed according to the approved methodological guidelines for the diagnosis of diseases caused by bacteria of the Enterobacteriaceae family using Bergey's bacteria identifier.

For this purpose, universal chromogenic and differential-diagnostic media were used. Inoculations were performed on MPB to isolate microorganisms from the test samples, incubated at 37°C for 18-20 hours, then transferred to chromogenic CHROMagar™ Orientation medium, which were cultured again. After the detection of well-defined colonies, Gram stained smears were prepared. If bacilli were found in the smears, their biochemical properties were studied.

Antimicrobial sensitivity was determined using the disk diffusion method using Mueller-Hinton agar. We interpreted (Table 1): according to methodical instructions 4.2.1890-04 "Determination of susceptibility of microorganisms to antimicrobial drugs"; according to recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST) [15] and recommendations of Clinical and Laboratory Standards Institute (CLSI) [16].

Figure 1 – List of antibacterial drugs in determining the sensitivity of Enterobacteriaceae

№	Name of drug discs	≤R	I	S≥	Interpretation base
1	Ampicillin (10 micrograms)	14		14	Eucast 11.0
2	Amoxicillin (25 micrograms)	15	15-20	21	НД-ПМП-1
3	Cefoperazone (CPR) (75 micrograms)	15	16-20	21	CLSI, МУК
4	Cefoxitin (CPN) (30 micrograms)	9		19	Eucast 11.0
5	Cefpodoxime (CFM), 10 micrograms	17		17	CLSI
6	Streptomycin (10 micrograms)	11	12-14	15	CLSI
7	Kanamycin (30 micrograms)	13	14-17	18	CLSI, МУК
8	Gentamicin (10 micrograms)	17		17	Eucast 11.0
9	Levomecetin (LEV), 30 micrograms	17		17	Eucast 11.0
10	Tetracycline (30 micrograms)	19		19	Eucast 11.0
11	Doxycycline (30 micrograms)	0	11-13	14	CLSI
12	Enrofloxacin (ENR), 5 micrograms	17	18-21	22	МУК
13	Ciprofloxacin (CIP), 5 micrograms	22		25	Eucast 11.0
14	Norfloxacin (NOR), 10 micrograms	22		22	Eucast 11.0
15	Ofloxacin (OF), 5 micrograms	22		24	Eucast 11.0
16	Hemifloxacin (GEM), 5 micrograms	15	16-19	20	CLSI
17	Nalidixic acid (NK), 30 micrograms	13	14-18	19	CLSI
18	Trimethoprim/sulfamethoxazole 1.25/23.75	11		14	Eucast 11.0
19	Furazolidone (FRN), 300 micrograms	14	15-16	17	CLSI
20	Furadonin (FD), 300 micrograms	14	15-16	17	CLSI

The β-lactamase-producing bacteria were determined by chromogenic culture (CHROMagarESBL, CHROMagarC3GR) and interpreted according to the instructions for the use of chromogenic media.

**Study Results:**

Of all the animals during the study period (n=160), 83 animals were admitted at clinical examination and anamnestic questioning with symptoms of digestive tract lesions (vomiting, diarrhea, congestion of gut contents). Vomiting was recorded in 52 animals, of which viral enteritis was established in 12 cases. Non-inflammatory gastrointestinal diseases such as gastric and intestinal atony, hypotonia, intestinal obstruction, intoxication were recorded in 23 cases. Inflammatory diseases of the stomach and intestines accounted for 52 animals (62.7% of all patients with GIT symptoms) of which cats 16% and the remaining 84% were dogs.

In addition to clinical examination, OAC and ultrasound examination, antibiotic susceptibility samples were taken from all animals. Clinical samples submitted during the study period (Table 2) were taken from pharynx 54% and anal 46%, the reason for this unevenness of sampling was animal aggression. In cats, 8 samples were taken from the mouth and 8 from the anus and in dogs, 43 samples were taken from the mouth and 41 rectal samples.

Figure 2 – Bacterial isolates from samples of cats and dogs with gastrointestinal diseases

Clinical specimens (swabs)	n	Escherichiacoli, кол-во /%	Klebsiella, кол-во /%	Enterobacteraerogene s, кол-во /%	Citobacter, кол-во /%	Proteus, кол-во /%
<b>cats</b>						
from the mouth	8	6/ 87,5	-	2/12,5	-	-
from the anus	8	6/ 87,5	2/12,5	-	-	-
<b>dogs</b>						
from the mouth	43	17/ 39,5	6/ 13,9	13/30,2	11/25,6	
from the anus	41	25/ 60,9	6/14,6	6/14,6	6/14,6	6/14,6

Fifty-eight clinical enterobacteria were isolated in animals: from sick cats (n=8) and dogs (n=50) treated for various gastrointestinal pathologies. The most common bacterial agent in cats and dogs was Escherichiacoli. In 6 cats out of 8(87.5%), Escherichiacoli were isolated in oral and anal samples. In dogs, E.coli was isolated in 39.5%(17) of oral samples and 60.9%(25) from the anus. There is a frequent isolation of Enterobacteraerogenes from the oral cavity in dogs in 30.2% (13) and in cats in 12.5% (2). The next most frequent in dogs is Citobacter, which in 25.6% of cases was isolated from the oral cavity and 14.6% from rectal swabs. In cats, however, the bacterium was not isolated. Further, in dogs 13.9% Klebsiella was isolated from the pharynx and 14.6% from the anus, in cats only from the anus 12.5%. Proteus in dogs was isolated only from rectal smears - 14.6%, in cats it was not isolated.

The sensitivity of bacteria to antimicrobial agents is presented in Table 3.

Figure 3 – Sensitivity of microorganisms to antimicrobials isolated from clinical specimens of dogs

Antibiotics	Microorganisms (%)														
	<i>E.coli</i>			Klebsiella spp.			Proteus spp.			Enterobacteraerogenes			Citobacter spp.		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
<b>Beta-lactams</b>															
Ampicillin	57	-	43	50	-	50	100	-	-	86	-	14	15	-	85
Amoxicillin	50	14	29	100	-	-	100	-	-	86	-	14	15	-	85
Cefoperazone	14	7	79	86	-	14	18	-	82	14	-	86	10	25	65
Cefoxitin	36	-	64	50	-	50	-	-	100	14	-	86	15	-	85
Cefpodoxime	29	-	71	43	-	57	18	-	82	14	-	86	15	-	85
<b>Aminoglycosides</b>															
Streptomycin	14	-	86	-	-	100	36	-	64	14	-	86	15	-	85
Kanamycin	-	-	100	-	-	100	82	-	18	50	-	50	15	-	85
Gentamicin	-	-	100	50	-	50	82	-	18	14	-	86	15	-	85
<b>Amphenicols</b>															
Levomycetin	29	-	71	50	-	50	82	-	18	86	-	14	15	-	85
<b>Tetracyclines</b>															

Tetracycline	7	-	93	100	-	-	82	-	18	14	-	86	50	-	50
Doxycycline	58	$\frac{2}{1}$	21	100	-	-	82	-	18	-	$\frac{1}{4}$	86	15	-	85
Fluoroquinolones															
Enrofloxacin	29	7	64	-	86	-	82	-	18	14	-	86	15	-	85
Ciprofloxacin	36	-	64	100	-	-	18	-	82	14	-	86	15	-	85
Norfloxacin	36	-	64	100	-	-	100	-	-	14	-	86	15	-	85
Ofloxacin	71	7	22	100	-	-	82	-	18	86	-	14	15	-	85
Hemifloxacin	21	-	79	-	100	-	82	-	18	14	-	86	85	-	15
Quinolones															
Nalidixic acid	29	-	71	50	-	50	82	-	18	14	-	86	85	-	15
Sulfonamides															
Trimethoprim/ sulfamethoxazole	36	-	64	36	-	64	82	-	18	14	-	86	-	25	75
Nitrofurans															
Furazolidon	-	-	100	-	-	100	82	-	18	14	-	86	60	-	40
Furadonin	-	-	100	100	-	-	64	-	36	86	-	14	60	-	40

R - resistant, I - intermediate, S – sensitive

Table 3 shows that rather high resistance to beta-lactams of the first generations was shown by all groups of microorganisms except Citobacter. Multiple drug resistance was observed in Klebsiella and Proteus, and Klebsiella showed 100% resistance to amoxicillin, tetracycline, doxycycline, ciprofloxacin, norfloxacin and fudonin. Proteins are resistant to amoxicillin, ampicillin, and norfloxacin. The bacteria Enterobacter aerogenes and Cito Baster showed low antimicrobial resistance. E.coli as a common microorganism showed high resistance to ampicillin, amoxicillin, doxycycline and ofloxacin. Good and effective drugs in suppression of E.coli activity are antibiotics of aminoglycoside and nitrofurans groups.

Microflora sensitivity test results in cats were as follows: E.coli, also a common microorganism, was 100% sensitive to nitrofurans (furazalidone, furadonin), and showed high resistance (75%) to tetracycline-type antibiotics (tetracycline, doxycycline), was low resistant to all other groups of antibiotics.

Klebsiellasp. was highly resistant to tetracycline-type antibiotics, fluoroquinolones, and aminoglycosides, low resistant to nitrofurans and sulfonamides. Enterobacter aerogenes is highly resistant to  $\beta$ -lactam antibiotics of the first groups Ampicillin and Amoxicillin, tetracyclines, Ofloxacin and Fudonin.

#### Conclusion

Bacterial culture and antimicrobial susceptibility testing of clinical specimens in veterinary practice is important both for diagnosing bacterial growth and for guiding treatment toward rational and judicious use of antimicrobial agents. Antibiotic therapy is widely used in the treatment of small pets.

In our studies in cats and dogs, E. coli was the most common isolate in most cases. In cats, E. coli was isolated in 87.5% of pharyngeal and anus smears. In dogs E.coli was isolated in 39.5% of oral specimens and 60.9% of anus specimens. Enterobacter aerogenes were most often isolated from the oral cavity in dogs in 30.2% and in cats in 12.5%, and from the anus in dogs in 14.6. Citobacillus in the oral cavity of dogs was isolated in 25.6% of cases and from rectal swabs in 14.6%. It did not occur in cats. Klebsiellasp. from the pharynx in 13.9% and 14.6% from the anus, in cats only from the anus in 12.5%. In dogs Proteusspp. was isolated only from anal swabs in 12.5%, in cats it was not isolated.

The isolation of microflora in diseases of the gastrointestinal tract of cats and dogs is one of the successful factors of therapeutic intervention. Resistance to pet antimicrobials will lead to an increased risk of therapeutic failure, i.e. ineffective treatment, increased animal treatment costs and health complications. Our study, can help clinical practitioners make rational decisions when using antimicrobials.

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## ИЗУЧЕНИЕ БИОЛОГИЧЕСКОЙ БЕЗОПАСНОСТИ МЯСА КРУПНОГО РОГАТОГО СКОТА МИКРОБИОЛОГИЧЕСКИМИ, ТОКСИКОЛОГИЧЕСКИМИ И РАДИОЛОГИЧЕСКИМИ ПОКАЗАТЕЛЯМИ

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В данной статье представлены показатели традиционной оценки биологической безопасности мясмолодых телок, мяса молодняка быков и мяса крупного рогатого скота, положительно реагирующего на бруцеллез в сравнительном аспекте. Уровень и характер изменений в мясе имеют решающее влияние на качество мяса в результате развития автолитических, микробиологических и окислительных процессов. Микробиологическое исследование показало, что количество мезофильных аэробных и факультативно-анаэробных микроорганизмов незначительно превышает норму в образцах мяса КРС, положительно реагирующие на бруцеллез. Токсикологическое и радиологическое исследование образцов говядины проводилось с привлечением лабораторного специалиста в соответствии с требованиями ГОСТ. По результатам токсикологического и радиологического исследования образцов мяса говядины в наших образцах содержание Pb, Cd и Cs не превышало заданных значений, т.е. соответствовало требованиям ГОСТ. Вывод: было исследовано на показатели КМАФАМ в, не более г, Salmonella, в 25 г продуктах БГКП, (кишечные палочки), 0,01 г продуктахh S.Aurei, 25 г продуктах Clostridia, в 1 г продуктах. Изучены качественные показатели: Свинец, Рb, Кадмий, Cd, Цезий-137, Cs. Используются классические и новейшие технологии исследования.

Ключевые слова: мясо, радиология, токсикология, микробиология, кишечные палочки.

## ІРІ ҚАРА МАЛ ЕТІНІҢ БИОЛОГИЯЛЫҚ ҚАУІПСІЗДІГІН МИКРОБИОЛОГИЯЛЫҚ, ТОКСИКОЛОГИЯЛЫҚ ЖӘНЕ РАДИОЛОГИЯЛЫҚ КӨРСЕТКІШТЕРМЕН ЗЕРТТЕУ

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Бұл мақалада салыстырмалы аспектіде бруцеллезге оң әсер ететін жас қашарлардың етінің, бұқалардың жас төлінің және ірі қара малдың етінің биологиялық қауіпсіздігін дәстүрлі бағалау көрсеткіштері ұсынылған. Еттің өзгеру деңгейі мен сипаты автолитикалық, микробиологиялық және тотығу процестерінің дамуы нәтижесінде ет сапасына шешуші әсер етеді. Микробиологиялық зерттеу мезофильді аэробты және факультативті-анаэробты микроорганизмдердің саны бруцеллезге оң әсер ететін ірі қара мал етінің үлгілеріндегі нормадан сәл асып түсетінін көрсетті. Сиыр етінің үлгілерін токсикологиялық және радиологиялық зерттеу ГОСТ талаптарына сәйкес зертханалық маманның қатысуымен жүргізілді. Сиыр етінің үлгілерін токсикологиялық және радиологиялық зерттеу нәтижелері бойынша біздің үлгілерімізде Рb, Cd және Cs мазмұны Берілген мәндерден аспады, яғни ГОСТ талаптарына сәйкес келді. Қорытынды: Кмафам в, г артық емес, Salmonella, 25 г БГКП өнімдерінде, (E. coli), 0,01 г өнімдеріh S. Aurei, 25 г Clostridia өнімдерінде, 1 г өнімдерде зерттелген. Сапалық көрсеткіштер зерттелді: қорғасын, Рb, Кадмий, Cd, Цезий-137, Cs. Классикалық және жаңа зерттеу технологиялары қолданылды.

Түйінді сөздер: ет, радиология, токсикология, микробиология.