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RESEARCH ARTICLE OPEN ACCESS

Screening of Epidemiologically Significant Mechanisms of Antibiotics to β-Lactams in *Enterobacteriaceae* - Pathogens of Zoonoses

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Abstract

Among the acquired mechanisms of resistance to antibiotics of microorganisms, the production of beta-lactamases, enzymes that inactivate penicillins, cephalosporins, carbapenems, and monobactams, is widespread. Most often, such beta-lactamases, in particular ESBL (extended-spectrum beta-lactamases), are capable of destroying III and IV generations of cephalosporins. One of the important ESBL producers is *Escherichia coli* and, to a lesser extent, Salmonella enteritidis, which are clinically significant in animals and humans. The purpose of the study was to screen ESBL DDM using cephalosporin markers and screening of mobile extrachromosomal factors of bacterial heredity – plasmids (potentially dangerous factors of genetic transport) in isolates of *E. coli* and *S. enteritidis*, polyresistant to aminoderms, from environmental objects, patho- and biological material, raw materials and products of animal origin. Results of our studies have shown the level of their distribution among animals, poultry, since from 13 field isolates of *E. coli* isolated from the milk of cows with mastitis and pathological material from pigs, ESBL production was found in 3 strains (23.1%) and from 18 field isolates of S. enteritidis isolated from pathological material from poultry, ESBL production was found in 2 strains (11.1%). Based on the results of molecular genetics studies, the presence of resistance plasmids (R-plasmids) in 9 field *E. coli* isolates was confirmed, 4 of which produced acquired beta-lactamases, incl. ESBL and 8 field isolates of S. enteritidis, 7 of which confirmed the presence of acquired carbapenemases.

Keywords: Antibiotic resistance, antibiotics, beta-lactamases of extended-spectrum (ESBL), carbapenemases, *E. coli*, plasmids, *S. enteritidis*

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INTRODUCTION

Currently, there is a worldwide trend of increasing resistance to antibiotics in pathogens of zoonotic diseases to threatening levels[1]. New mechanisms of resistance in pathogenic bacteria are emerging and spreading everywhere, with great risks associated with a reduction in the effectiveness of human and animal treatment and an increase in its cost. Antibiotic resistance is gaining momentum due to their misuse, low level of prevention and control of bacterial infections, due to the ingress of antibiotic-resistant bacteria into the food chain due to contamination of raw materials and food[2].

Measures to reduce the resistance of bacteria and limit their spread have to be taken at all levels of society. At the session of the United Nations General Assembly in New York in September 2016, the Heads of State pledged to deploy broad and coordinated action to address the root causes of antibiotic resistance, especially in the human and animal health sectors and agriculture with further development of national action plans to address antibiotic resistance. Ukraine is also involved in these.

One of the initiatives proposed by the WHO to improve the situation is particularly relevant and concerns the Global Action Plan on antimicrobial resistance (GLASS). This system is based on standardized approaches to the collection, analysis and exchange of data on antibiotic resistance on a global scale using the data obtained for decision-making at the local, national and regional levels[3-6].

Thus, a detailed study and analysis of the resistance of zoonotic pathogens obtained by passive monitoring after microbiological studies of patho- and biomaterial, samples of raw materials and products of animal origin, poultry and environmental facilities for Ukraine is an urgent problem today.

Leading experts of the World Health Organization (WHO) in the field of antibiotic resistance recommend the development and implementation of effective approaches to overcome this dangerous global phenomenon by conducting comprehensive monitoring studies of the resistance of microorganisms to antibiotics. Antibiotic-resistant microflora enters the body in different ways. One of these is through raw materials and food products of animal origin contaminated with antibiotic-resistant microorganisms[7-10]. Clinically significant beta-lactam antibiotics (beta-lactams) are now widely used in foods of animal origin. This is a large group of antibiotics containing a beta-lactam ring. The group of beta-lactams includes large subgroups of cephalosporins, carbapenems, monobactams and penicillins. The mechanism of action of beta-lactam antibiotics is based on the violation of the microorganisms' cell wall synthesis. The target of their action is trans- and carboxypeptidases, which promote the synthesis of the main component of the outer membrane of gram-positive and gram-negative bacteria – peptidoglycan[11,12].

Such antibiotics are inactivated by the action of special enzymes - beta-lactamases, and the detection of mechanisms of resistance to beta-lactams is of great epidemiological importance. Beta-lactamases include enzymes: carbapenemases (beta-lactamases, hydrolyzing penicillins, some cephalosporins, carbapenems and monobactams), extended-spectrum beta-lactamases (ESBL, hydrolyzing cephalosporins of the third and fourth generation, and aztreonam, in addition to cephamycin and carbapenems) and acquired AmpC beta-lactamases (hydrolyze penicillins, third-generation cephalosporins and monobactams and do not hydrolyze fourth-generation cephalosporins)[13,14].

In most countries of the world and in Europe, in particular, experts of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) for national reference centers of health and veterinary authorities recommend the detection of ESBL-producing bacteria and their characteristics[5,15].

ESBL beta-lactamases are resistant to cephalosporins and, as studies show, have a high level of polyresistance to antibiotics[16].

Numerous studies have shown that the genes that encode the synthesis of the vast majority of already identified beta-lactamases are contained on extrachromosomal elements of bacterial heredity – plasmids[17].

The problem today is the anthropogenic pressure of beta-lactam antibiotics and the increased risk of the spread of plasmid associated antibiotic resistance genes of zoonotic *Enterobacteriaceae*. Therefore, the monitoring of

plasmid-associated genes in R-plasmims will be able to provide information on the possibility of developing alternative ways to combat antibiotic resistance of zoonotic pathogens[18-20].

Thus, the study of the prevalence of *enterobacteriaceae* circulating in Ukraine with acquired mechanisms of antibiotic resistance will promote a standardized approach to data collection, analysis and exchange on a global scale and ensure the implementation of the tasks of the National Action Plan for Combating Antibiotic Resistance to Antimicrobials.

The purpose of the study was to screen ESBL DDM using cephalosporin markers and screening of mobile extrachromosomal factors of bacterial heredity – plasmids (potentially dangerous factors of genetic transport) in isolates of *E. coli* and *S. enteritidis*, polyresistant to aminoderms, from environmental objects, patho- and biological material, raw materials and products of animal origin.

MATERIALS AND METHODS

Test materials used in the study. Isolates of *Enterobacteriaceae*: 13 strains of *E. coli* and 18 strains of *S. enteritidis*. In particular: isolates of *E. coli* - 7 strains «1/44», «9/97», «10/98», «11/99», «12/100», «13/101», «14/102» isolated from mastitis cow's milk; 2 strains «5/73», «6/77» isolated from dog's and elephant's faeces; 3 strains «7/80», «15/124» і «S14/58» isolated from pathological material from poultry (chickens, quails) and pig; 1 strain «16/175» isolated from animal feed samples (granulated soybean meal); isolates of *S. enteritidis* - 8 strains «1/3», «26/145», «29/171.a», «37/245», «48/284», «45/265», «48/284», «49/286», «58/347», «59/348» isolated from food (chicken filet, milk-containing cheese product, ready to eat fish; shawarma from chicken fillet; read to eat rise; Eclair cakes, ready to eat chicken); 3 strains «57/346», «71/422» and «51/300» isolated from poultry samples (pathological material, meconium and faeces) and 6 strains «9/20», «14/71», «16/78», «22/114», «46/275», «47/276» isolated from environmental objects (chick box papers, dust from the poultry building). Specialists of the State Research Institute selected isolates of *Enterobacteriaceae* for passive monitoring during 2019 for Laboratory Diagnostics and Veterinary Sanitary Examination (Kyiv).

Mechanisms of setting the disc-diffusion method to determine the general profiles of antibiotic resistance, acquired mechanisms of resistance with confirmation of ESBL products, quality control of studies and screening of experimental isolates for the presence of plasmid DNA. Determination of general profiles of antibiotic resistance was performed by disco-diffusion method (DDM) using disks with antibiotics: tetracycline (30 µg / disk), nalidixic acid (30), ampicillin (10), cefoxitin (30), gentamicin (10), ciprofloxacin (5), chloramphenicol (30), imipenem (10), sulfamethoxazole (25), ceftazidime (10), cefotaxime + clavulanic acid (30/10), cefotaxime (5 and 30), cefepime (30), ceftazidime + clavulanic acid (30/10), aztreonam (30), trimethoprim (5), ceftriaxone (30), amikacin (30), amoxiclav (10). Diameters of growth retardation zones (mm) of experimental cultures of Enterobacteriaceae were measured and interpreted taking into account the recommendations of the EUCAST[5,19].

DDM (disco-diffusion method) with cephalosporin diagnostic markers in the combination recommended by EUCAST, ceftriaxone (30) and ceftazidime (10) was used to determine ESBL products in experimental *Enterobacteriaceae*[5].

Quality control of the studies was carried out with a test culture of *E. coli* ATCC 25922, recommended by EUCAST as a control strain for studies of isolates of the *Enterobacterales* genus. In order to screen bacteria for the presence of plasmid DNA, we used the method of DNA imaging according to Eckhardt (1978) and preparative isolation of plasmid DNA. For preparative obtaining plasmid DNA preparations, we used the method of alkaline lysis by Birnboim and Dolly (1979). The obtained plasmid DNA was visualized by agarose gel electrophoresis and stored at -20°C. Visualization of plasmid DNA preparations and DNA fragments using the method of electrophoresis in 1% agarose gel and Tris-borate buffer solution. After electrophoresis, the gel was stained in buffer with 0.5 µg/ml ethidium bromide, washed with water and the DNA was observed under ultraviolet light.

RESULTS

In order to identify epidemiologically significant mechanisms of antibiotic resistance to beta-lactams in isolates of *E. coli* and *S. enteritidis*

Table 1. Antibiotic profile of field isolates of *E. coli* strains

The name of *E. coli* strain	Tetracycline	Ceftazidime	Nalidixic acid	Ampicillin	Cefoxitin	Gentamicin	Ciprofloxacin	Chloramphenicol	Imipenem	Sulfamethoxazole
«1/44»	R	S	R	R	S	S	S	S	S	X
«5/73»	S	S	S	S	S	S	S	S	S	R
«6/77»	S	S	R	S	S	S	S	S	S	R
«7/80»	R	S	R	R	S	S	R	S	S	R
«9/97»	R	R	S	S		S	S	S	S	S
«10/98»	R	MR	R	S	R	S	S	S	S	S
«11/99»	S	S	R	S	X	S	S	S	S	S
«12/100»	R	R	S	S	S	S	S	S	S	S
«13/101»	R	S	R	S	S	S	S	S	S	S
«14/102»	R	S	R	S	R	S	S	S	S	S
«15/124»	R	S	R	R	X	S	S	S	S	R
«16/175»	S	MR	S	S	X	S	S	S	S	S
S «14/58»	X	R	X	R	S	R	R	R	S	X

Note. R- resistant; S-sensitive; MR – moderately resistant; X – not tested.

Table 2. Screening for ceftazidime-resistant *E. coli* isolates for confirmation of ESBL-producers

No	Type of experimental isolate and ID number (species/ general)	ESBL Screening		Screening results
		cephalosporin test marker: (combination cefotaxim and ceftazidime)		ESBL production was confirmed by the values of the diameters of the zones of growth inhibition ceftriaxone (<23) and ceftazidime (<22), yes / no
		Cefotaxim (30 µg/disk)	Ceftazidime (10 µg/disk)	
According to the EUCAST documents range of resistance for the control strain of *E. coli* ATCC 25922		< 21	< 22	no
E. coli ATCC 25922 (actual test results)		21	20	non-ESBL-product (negative quality control) no
1.	*E. coli* strain «9/97»	19	10	ESBL-product
2.	*E. coli* strain «12/100»	14	13	ESBL-product
3.	*E. coli* strain «S14/58»»	0 (self-growth)	20	ESBL-product

- pathogens of zoonoses isolated from the environment, pathological material, raw materials, products of animal origin and poultry industry under passive monitoring, the identified isolates were studied comprehensively for sensitivity to antibiotics. Table 1 shows the antibiotic resistance profiles of *E. coli* field isolates.

Analysis of the study results showed that among the field isolates of *E. coli* 50% were strains polyresistant to antibiotics (resistance up to 3 or more varieties of antibiotics). Among the studied strains of *Escherichia* in 3 of them - "9/97", "12/100" and S «14/58» , resistance (R) to ceftazidime was detected and in 2 more strains "10/98", "16/175" the value of the diameters of the zones of growth inhibition was within the range of moderate resistance (MR).

Owing to the fact that according to EUCAST, resistance of *Enterobacteriaceae*, including *E. coli*, to most cephalosporins is an indicator of the presence of acquired mechanisms of resistance, we were interested in deeper studies to confirm the production of extended-spectrum beta-lactamases in ceftazidime-resistant strains of *E. coli* "9/97" and "12/100" and check the moderate sensitivity to ceftazidime strains of *E. coli*

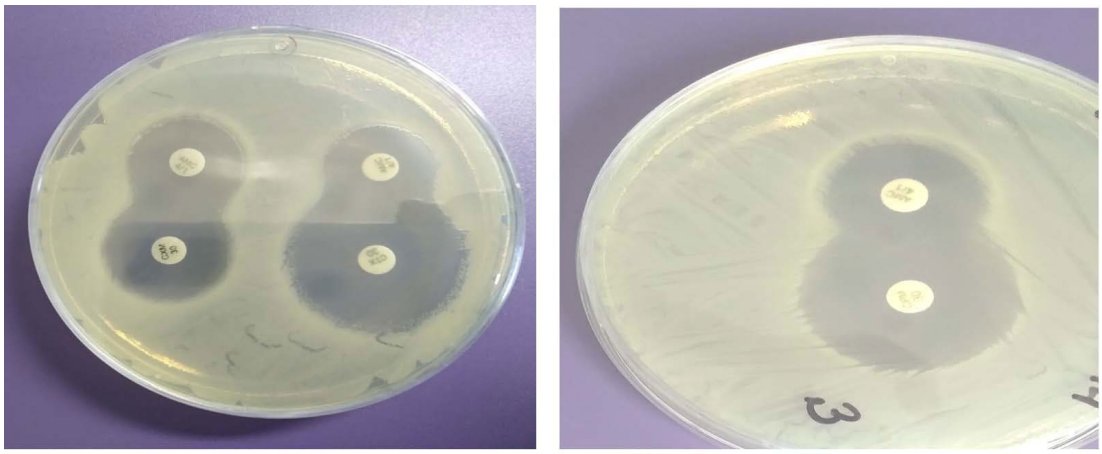

Fig. 1. Double-disc synergy test (DDST) with cephalosporins - ceftazidime, cefotaxime, cefepime and clavulanic acid, the "keyhole" effect , *E. coli* strain «9/97».

Table 3. Antibiotic profile of ceftazidime- resistant field isolates of *S. enteritidis*

-	Strain's name																	
	«1/3»	«9/20»	«14/71»	«16/78»	«22/114»	«26/145»	«29/171a»	«37/245»	«45/265»	«46/275»	«47/276»	«48/284	«49/286»	«51/300»	«57/346»	«58/347»	«59/348»	«71/422»
Ciprofloxacin	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	MR	MR	R
Ampicillin	S	S	S	R	S	S	R	S	S	S	R	S	S	S	P	S	S	R
Trimethoprim	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S
Ceftazidime	S	S	S	S	S	S	MR	S	S	S	MR	R	S	R	R	MR	MR	R
Amikacin	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	R
Cefepime	R	S	S	S	R	S	R	S	S	S	R	S	S	R	R	R	R	R
Gentamicin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
Chloramphenicol	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R
Imipenem	S	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S
Aztreonam	S	S	S	S	R	R	S	S	R	R	R	S	S	S	R	R	R	R
Nalidixic acid	R	R	R	R	R	R	R	R	R	R	R	R	R	R	X	X	X	X
Amoxiclav	R	R	R	R	R	R	R	R	R	R	R	R	R	R	X	X	X	X
Cefoxitin	S	S	S	S	S	R	R	S	S	S	R	S	S	S	R	R	R	R

Note. R – resistant; S-sensitive; MR – moderately resistant, X – not tested.

Table 4. Screening for ESBL-products in *S. enteritidis* isolates with cephalosporin markers

No	Type of experimental isolate and ID number (species/ general)	ESBL Screening		Screening results
		cephalosporin test marker: (combination cefotaxim and ceftazidime)		ESBL-producers confirmed by the diameter size of the zones of growth inhibition ceftriaxone (<23) and ceftazidime (<22), yes / no
		Cefotaxim (30 µg/disk)	Ceftazidime (10 µg/disk)	
According to the EUCAST documents range of resistance for the control strain of *E. coli* ATCC 25922		<21	<22	no
E. coli ATCC 25922 (actual test results)		30	25	non-ESBL-product (negative quality control) no
1.	*S. enteritidis* strain «51/300»	30	colonies in the zone of growth inhibition	no
2.	*Salmonella enteritidis* strain «57/346»	14	13	ESBL-product
3.	*Salmonella enteritidis* strain «71/422»	0 (self-growth)	20	ESBL-product

for possible ESBL products. Therefore, following the EUCAST methodology for the detection of resistance mechanisms in Enterobacteria of the I-st group (*E. coli*, *Salmonella* spp., *Klebsiella* spp., *Shygella* spp., *P. mirabilis*), in particular the production of carbapenemases of extended-spectrum (ESBL), we conducted an ESBL screening using a cephalosporin test with a combination of ceftriaxone and ceftazidime, as shown in Table 2.

Analysis of the ESBL screening results showed that *E. coli* strains "9/97", "12/100" and S «14/58» are resistant to ceftazidime, which was determined by the general profile of antibiotic resistance and after screening testes

Table 5. Results of plasmid DNA screening in field isolate strains of *S. enteritidis* and *E. coli*

Enterobacteriaceae type	Strain's name	The presence of plasmid DNA described by :	
		Eckhard's method	alkaline lysis method (Birnboim and Destiny's)
S. enteritidis	«1/3»	-	-
	«9/26»	+	+
	«14/71»	-	-
	«16/78»	+	+
	«22/114»	+	+
	«26/145»	+	+
	«29/171a»	+	+
	«37/245»	-	-
	«45/265»	+	+
	«46/275»	+	+
	«47/276»	+	+
E. coli	«1/44»	+	+
	«5/73»	-	-
	«6/77»	-	-
	«7/80»	+	+
	«9/97»	+	+
	«10/98»	+	+
	«11/99»	+	+
	«12/100»	+	+
	«13/101»	+	+
	«14/102»	+	+
	«15/124»	+	+
	«16/175»	-	-

with cefatoxime, to confirm the ESBLs it was found that the designated strains have acquired resistance mechanisms and perhaps produce beta-lactamases of extended-spectrum. After conducting targeted studies to confirm ESBL production by test bacteria using the double-disc synergy test (DDST) with cephalosporins - ceftazidime, cefotaxime, cefepime and clavulanic acid, in all cases the "keyhole" effect was revealed: inhibition zones around any of cephalosporin disks are augment in the direction of the disk containing clavulanic acid, which confirmed the production of such an enzyme (Fig. 1).

Table 3 shows data on the general profiles of antibiotic resistance of ceftazidime- resistant field isolates of *S. enteritidis*.

Analysis of the research findings of the general antibiotic resistance profiles of field isolates of *S. enteritidis* showed that 72.2% of strains were polyresistant (3 or more) to the applied antibiotics. Attention has to be paid to the absence of any effect of amoxiclav and nalidixic acid on *S. enteritidis* isolates, i.e. they have 100.0% resistance to these antibiotics.

Resistance of *Enterobacteriaceae* of the first group (*E. coli*, *Salmonella* spp., *Klebsiella* spp., *Shygella* spp., *P. mirabilis*) to most cephalosporins is an indicator of the presence of acquired resistance mechanisms, and according to the analyzed results of the antibioticogram of *S. enteritidis* strains "51/300", «57/346» and «71/422» proved to be resistant to ceftazidime. To confirm ESBLs in ceftazidim-resistant *S. enteritidis*, we performed an ESBL screening, the results of which are shown in Table 4.

Analysis of the experimental results confirmed the acquired resistance in *S. enteritidis* strains "57/346" and "71/422", because after the application of a specific cephalosporin test in combination with ceftazidime and ceftriaxone, these strains had showed resistance to these antibiotics. Ceftazidime-resistant strain

S. enteritidis "51/300" had showed sensitivity to ceftriaxone, therefore ESBL production was not confirmed.

While analyzing results of DDM quality control with cephalosporin markers in combination of ceftriaxone (30 µg / disk) and ceftazidime (10 µg / disk), we confirmed the absence of ESBLs in the test culture of *E. coli* ATCC 25922, which meets the EUCAST recommendations and allows to recognize reliable the test results on detection of ESBLs in field isolates of *S. enteritidis* and *E. coli.*

It is known that the vast majority of beta-lactamases, in particular ESBL, are products of expression of plasmid-associated genes for

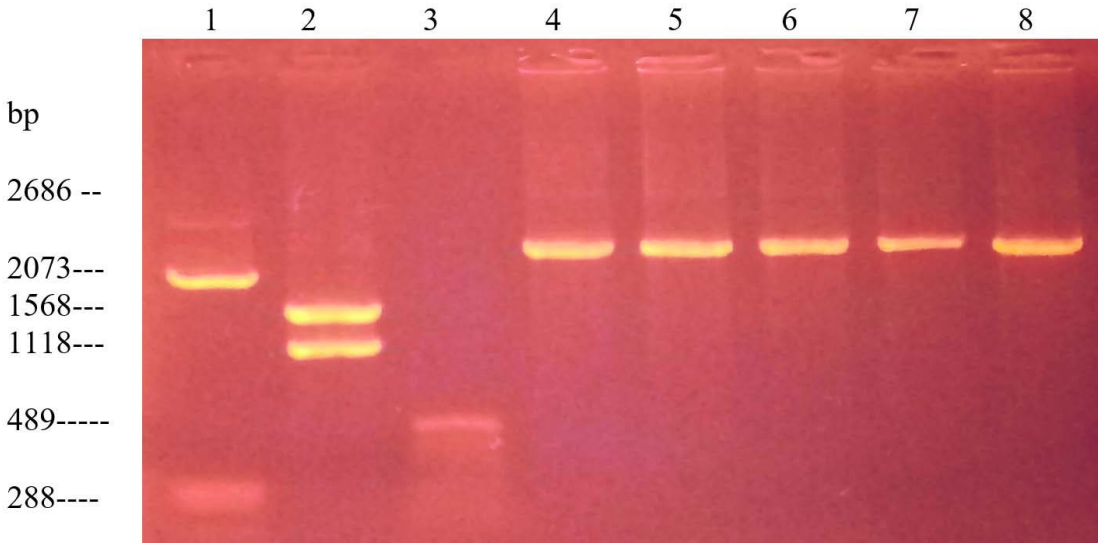

Fig. 2. Electrophoregram of DNA markers and isolated DNA plasmid of field isolates of *S. enteritidis*: 1– plasmid marker pUC19, digested with the enzyme Mva I (2686, 2073,288 bp); 2 - plasmid marker pUC19, digested with the enzyme Bgl I (1568, 1118 bp); 3 - plasmid marker pUC19, digested with with the enzyme Msp I (489 BP); 4 - *S. enteritidis* 16/78; 5 - *S. enteritidis* 22/114; 6 - *S. enteritidis* 26/145; 7 - *S. enteritidis* 29 / 171a; 8 - *S. enteritidis* 45/265

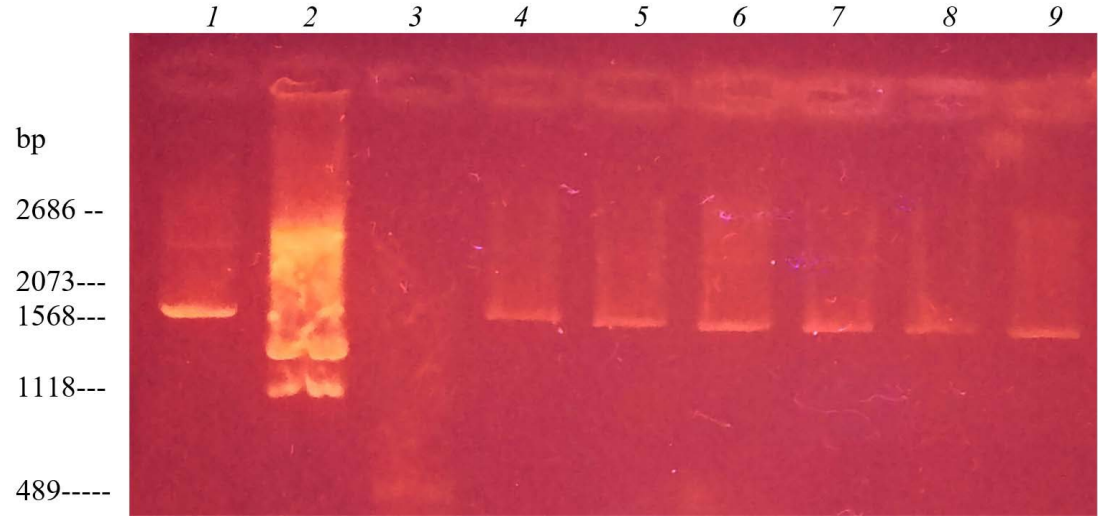

Fig. 3. Electrophoregram of DNA markers and isolated DNA plasmid by Birnboim-Dolis' alkaline lysis method from *E. coli* field isolates: 1 - plasmid marker pUC19, digested with the enzyme Mva I (2686, 2073 BP); 2 - plasmid marker pUC19, digested with the enzyme Bgl I (1568, 1118 BP); 3 - plasmid marker pUC19, digested with the enzyme Msp I (489 BP); 4 - *E. coli* 1/44; 5 - *E. coli* 7/80; 6 - *E. coli* 9/97; 7 - *E. coli* 10/98; 8 - *E. coli* 12/100; 9 - *E. coli* 13/101

antibiotic resistance of R-plasmids. Therefore, it was important to study the presence of plasmids in examined field isolates of bacteria.

Studies have shown that the method of alkaline lysis is an effective method for the preparative isolation of plasmids from isolates of *S. enteritidis* and *E. coli.* Eckhard's method is suitable for screening on the presence of plasmids directly only during the agarose gel electrophoresis.

As shown in Table 5, plasmid DNA was detected in the field isolate strains of *S. enteritidis* and *E. coli*.

The results showed that among the isolates of *S. enteritidis* eight strains were plasmid containing (Fig. 1). Since the studied plasmids were low molecular weight, it was assumed that they belong to the class of R-plasmids.

Analysis of plasmid-containing field isolates of *S. enteritidis* showed the presence of seven strains of this species of Salmonella, carbapenemase and the absence of all plasmid-containing strains of ESBLs. Thus, we can assume that the identified plasmids carry genes that encode the synthesis of carbapenemases in *Salmonella* and therefore there is a need to develop methods for molecular genetic typing of plasmid-associated genes encoding the synthesis of such enzymes.

Regarding the screening of antibiotic resistance plasmids in 12 isolates of *E. coli*, Fig. 2 shows that nine strains had low molecular weight plasmids.

Such isolates of *Enterobacterial* pathogens, due to their polyresistance, pose a potential danger to humans and animals in the spread and acquisition among identical populations of microorganisms sensitive to antibiotics, plasmid-associated genes of antibiotic resistance. It was found that among the epidemiologically significant beta-lactamases studied in *E. coli* field isolates there were four plasmid-containing strains, of which - two ESBL-producing and two - produce other acquired beta-lactamases. The other five plasmid-containing strains of *E. coli* are potential carriers of non-β-lactamase-encoding antibiotic resistance genes.

DISCUSSION

Resistance of *Enterobacteriaceae*, including *E. coli* and *Salmonella* spp., to a number of beta-lactam antibiotics is rapidly spreading and is becoming a serious problem in veterinary and human medicine[22,23]. It is becoming obvious that the effectiveness of detecting resistance to these antibiotics using traditional methods remains quite low. This has been confirmed by numerous studies, as well as in our case[20], because the detection of resistance in microorganisms by determining only the general profile of antibiotics does not allow to determine whether such microorganisms have already acquired plasmid-mediated genes of antibiotic resistance.

ESBL-producing *E. coli* isolates are increasingly identified in farm animals, in food products of animal origin, which confirms the hypothesis that animals are natural resistant sources of infection with ESBL-producing *Enterobacteriaceae*[22,23]. In this case, this hypothesis is confirmed by the results of our studies, because ESBL-producing strains of *E. coli* "9/97" and "12/100" were isolated from mastitis cow's milk and strain S « 14/58» from pathological material from pig. Thus, 13 strains of *E. coli*, mono- and polyresistant to ADR, were isolated from livestock products, 23,1% of which had acquired resistance mechanisms, which was confirmed by ESBL products during the screening with cephalosporin markers and the presence of plasmid-mediated resistance genes in these strains, according to the results of antibiotic resistance plasmids screening.

Screening results for ESBL production in ceftazidime-resistant field isolates of *S. enteritidis* showed the presence of this enzyme in strains "57/346", "71/422", which was 11.1% of the tested isolates.

Infections caused by zoonotic pathogens resistant to antibiotics are a serious problem for veterinary and humane medicine; because the effectiveness of treatment is reduced or absent, there is a risk of life-threatening conditions in animals and humans, increasing the duration of treatment with more expensive antibiotics or finding alternative treatment methods. This leads to significant budget expenditures in the economics and social sphere[24].

Almost all pathogens of the most common infections circulating in Ukraine produce beta-lactamases and are a factor of resistance to many, and in some cases - to all, antibiotics, especially to widely used in the treatment of these

infections, which limits or eliminates the possibility of their further application[25]. In addition, ESBL-producing strains of *E. coli* can become one of the links in the food chain in animal and human nutrition, which already carries biological risks for society as a whole.

Beta-lactamases are most often known to produce gram-negative microorganisms, although gram-positive bacteria *Staphylococcus* spp. also produce them[26].

The mechanism of beta-lactamase impact on ADR lies the binding of beta-lactamase with beta-lactam antibiotic, which triggers the hydrolysis of the amine bond of the lactam ring, which leads to inactivation of antibiotics.

The production of extended-spectrum beta-lactamases (ESBL) is one of the most common clinically significant mechanisms of resistance. ESBL includes a significant number of enzymes that are capable of cleaving oxyimino-beta-lactams, are third and fourth generation cephalosporins, aztreonam and penicillins, and are sensitive to inhibitors - clavulanic acid, sulfabactam and tazobactam[23,27].

Some scientists, to overcome the resistance of microorganisms to antibiotics, suggest developing and implementing new antibiotics in combination with beta-lactamase inhibitors[28,29].

The catastrophic situation with the spread of antibiotic resistance requires new approaches to monitoring bacterial resistance[30,31]. A comprehensive study of the resistome will help to be one step ahead of bacteria in the "race of antibiotics"[32]. Modern approaches to fattening and treatment of animals have created conditions of high selective pressure of various antibiotics, which leads to the formation of dangerous, in terms of antibiotic resistance, pool of plasmids of antibiotic resistance (R-plasmids) in probiotic and transient microflora of animals.

It is known that plasmids of the IncI2 group carrying Bla CTX-M genes can undergo interspecific migration among potential bacterial pathogens from *E. coli*[33,34]. Molecular characterization of plasmids in field isolates of the most common pathogens of the genus *Enterobacteriaceae – E. coli* and *S. enteritidis* is important for understanding the local and global distribution of their resistance to the most common beta-lactam antibiotics[33].

Genes encoding ESBL are located on plasmids. Thus, the prevalence of extended-spectrum β-lactamases (ESBL) producing *E. coli* and *Salmonella* spp. in animal populations[35] and diversity in ESBL genotypes, including *CTX-M blah, SHV blah, TEM blah, OXA* in sewage *Enterobacteriaceae*[36].

Our study has shown the presence of plasmids in the examined field isolates of *S. enteritidis* (among 13 isolates, eight had plasmins, which accounted for 72.7% of the studied material) and *E. coli* (of the 13 studied - 9 plasmid-containing, which accounted for 75.0% of the studied material). In our studies, such plasmids were classified as R-plasmids, according to their size. The scientists also attributed the plasmid DNA of *Enterobacteriaceae* to R-plasmids, according to these characteristics. In addition, the increase in bacterial mass of *S. enteritidis* and *E. coli* for plasmid screening was carried out in antibiotic-free environment, as the latter are known inducers of increased copies of R-plasmids in bacterial cells. Therefore, even in the absence of antibiotics in the culture environment, plasmid preparations with a high concentration of plasmid DNA were obtained, which testified in favor of multicopy of the studied plasmids and allows attributing them on this basis to the class of R-plasmids[34].

Among *S. enteritidis* field isolates carrying plasmids, seven strains showed the presence of carbapenemases and the absence of ESBLs in all plasmid-containing strains. Among the examined *E. coli* isolates there were four plasmid-containing strains, two of which were ESBL-producing and two ones were producing other acquired beta-lactamases. The other five plasmid-containing strains of *E. coli* are potential carriers of antibiotic resistance genes that are not associated with β-lactamase encoding. They are likely to encode tetracyclines, as these strains have this type of antibiotic resistance, which may be related to their spread in beef and chicken production[37].

Therefore, the obtained data indicate the need to continue the research aimed at identifying the mechanisms of antibiotic resistance to beta-lactams of other types in isolates of *Enterobacteriaceae* (*E. coli*, *Salmonella* spp., Ps. *aeruginosa*, and others) and the development of methods for molecular genetics of genetic type synthesis of carbapenemases in zoonotic

pathogens isolated from pathogenic and biomaterials, raw materials and products of animal origin and environmental objects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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