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Research article

Antimicrobial resistance of *Salmonella* strains isolated from food products of animal origin in Ukraine between 2018-2021

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Abstract

Salmonellosis is a zoonotic infection of humans and animals. In recent years, an increase in the number of resistant strains of *Salmonella* from animals and animal products have been recorded. We conducted microbiological testing, and antibiotic resistance profile of 34 *Salmonella* strains isolated from foods of animal origin between 2018 to 2021 in Ukraine. Twenty isolates were successfully assigned to six different serovars [*S.* Enteritidis (n=13, *S.* Infantis (n=2), *S.* Schwarzengrund (n=1), *S.* Livingstone (n=2), *S.* Vuadens (n=1) and *S.* Hadar (n=1)], and 14 isolates remained untyped. Most of the isolated strains were recovered from poultry products. The strains were tested for six antibacterial groups: quinolones, aminoglycosides, β-lactams, cephalosporins, phenols, and dihydrofolate reductase inhibitors by disc diffusion method. The highest level of resistance was recorded to 4^{th} generation cephalosporins, e.g., cefepime (88.2%). The lowest level of resistance was to cefotaxime and ceftriaxone (5.9%). No resistance was found to imipenem and aminoglycoside antibiotics, particularly gentamicin and amikacin. The study results are of concern because antibiotic-resistant bacteria and their genes can cause human infections by penetration and transmission at any stage of the food production cycle. Further research is required to monitor the contamination of food products of animal origin with salmonellosis pathogens, including antibiotic-resistant strains.

Keywords: *Salmonella* spp., Antibiotic resistance, Disc-diffusion method

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Introduction

Salmonellosis is a severe foodborne disease that poses a significant threat to global food safety leading to social and public health problems (Nair and Kollanoor Johny, 2019; Park et al., 2020; Castellanos et al., 2020). *Salmonella* (*S.*) currently includes more than 2600 characterized serovars, and *S. enterica* is the most pathogenic (Milczarek et al., 2019; Chang et al., 2020). According to the European Food Safety Authority (EFSA), salmonellosis was the second most prevalent zoonotic infection in humans after campylobacteriosis within the European Union (EU) in 2021 (https://www.efsa.europa.eu/en/topics/topic/salmonella).

In Europe, the increase in human cases of salmonellosis is associated with the spread of *Salmonella* through poultry and eggs (Abdulhaleem et al., 2019; Nair and Kollanoor Johny, 2019; Li et al., 2021; WHO, 2022). The spread of salmonellosis among people is also facilitated by expanding the public catering network, violation of food preparation and storage regulations, and low levels of personal hygiene and sanitary practices in certain segments of the human population (Garkavenko et al., 2021). It is worth mentioning that antimicrobials are widely used in animal husbandry as growth promotors and in food production to reduce contamination (Kotsyuba et al., 2014; Korotkevych, 2016; Jajere, 2019). A complete ban on antimicrobial growth promotors was issued in the EU in 2003 and enacted in 2006 (EU, 2003). However, according to the World Organization for Animal Health (WOAH), several countries in Europe and Central Asia continued to use antibiotics as growth promoters in animals, with bacitracin and tylosin being the most commonly used drugs (Florez-Cuadrado et al., 2018). Excessive and improper use of antibiotics over a long period has led to antimicrobial resistance (AMR) development. AMR has become a global public health problem, and resistant bacteria and their genes can be transmitted to humans via foods (Lalruatdiki et al., 2018; Jajere, 2019; Kakatkar et al., 2021; Quansah and Chen, 2021).

The epidemiological situation of salmonellosis in Ukraine is currently assessed as unfavorable, with a tendency to further deterioration (Donchenko and Nadykta, 2007; Kostenko et al., 2012; Jakubchak, 2014). The incidence of salmonellosis in the neighboring countries of Ukraine, particularly Russia, is characterized by a general downward trend in the incidence rate, in 2021, compared to 2020. The rate did not change significantly and amounted to 13.61 per 100 thousand people. According to the federal service for supervision of consumer rights protection and human well-being, in January-August 2022, 16360 cases of salmonellosis were detected in Russia (11.17 per 100 thousand) https://www.rospotrebnadzor.ru/activities/recommendations/details.php?ELEMENT_ID=23393). The registered incidence of salmonellosis in Kazakhstan and Belarus ranges from 13.7 to 55 cases per 100 thousand population, respectively (Kozyreva et al., 2014). In Poland, 8269 cases of salmonellosis were registered in sanitary and epidemiological surveillance in 2021 (Adriana, 2022). However, the epidemiological situation and the drug efficacy of *Salmonella* strains are still not well investigated in Ukraine.

Therefore, this study aimed to determine the antibiotic susceptibility profile of different serological variants of *Salmonella* and the prevalence of their resistant strains in food products of animal origin produced in Ukraine between 2018 and 2021.

Materials and methods

Sampling and Salmonella spp. identification

During 2018-2021, microbiologists of state laboratories of the State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP) collected samples from 24 regions during the official veterinary and sanitary control and supervision of food safety of animal origin. From 2018 to 2021, 336 food samples were contaminated with *Salmonella* spp. According to the regulation of SSUFSCP, only 10% of isolated strains were subjected to serotyping and antimicrobial susceptibility testing (AST). Therefore, 34 (10%) *Salmonella* strains isolated from food products of animal origin (Table 1) in the study period were selected for further serotyping and antibacterial susceptibility testing. Identification and serotyping of *Salmonella* strains were based on EN ISO 6579-1:2017/A1:2020 method "Microbiology of the food chain- Horizontal method for detecting, enumeration and serotyping of *Salmonella*- Part 1: Detection of *Salmonella* spp.- Amendment 1 Broader range of incubation temperatures, an amendment to the status of Annex D, and correction of the composition of MSRV and SC (ISO 6579-1:2017/Amd 1:2020)".

Antimicrobial susceptibility profiles

The antimicrobial susceptibility was determined by the disc diffusion method (DDM) on Mueller-Hinton agar (MHA) manufactured by HiMedia, Mumbai, Maharashtra, India. The preparation of Muller-Hinton agar plates was carried out according to the manufacturer's instructions. Preparation of bacterial suspension was carried out by suspension method using Densi-La-Meter. For this purpose, daily colonies of *Salmonella* were suspended in an 11 mL sterile physiological solution and adopted to an optical density of 0.5 McFarland units (MFU). The suspension was thoroughly homogenized, and 0.5 mL of the bacterial culture suspension was applied to the surface of MX agar, after which discs with antibiotics were placed on the agar surface.

The antibiotics disks used were belonging to quinolones [norfloxacin (NX) 10 μg, ciprofloxacin (CIP) 5 μg]; aminoglycosides [gentamicin (GEN) 10 μg, amikacin (AMK) 30 μg]; β-lactams [amoxicillin (AML) 10 μg, ampicillin (AMP) 10 μg, amoxiclav (AMC) 10 μg, ticarcillin (TI) 75 μg, imipenem (I) 10 μg]; cephalosporins [cefoxitin (CX) 30 μg, ceftriaxone (CRT) 30 μg, cefotaxime (CTX) 5 μg, ceftazidime (CAZ) 10 μg, cefepime (CPM) 30 μg]; phenolic [chloramphenicol (C) 30 μg]; and dihydrofolate reductase inhibitor [trimethoprim (TR) 5 μg]. All isolates and antibiotic discs were incubated at 36 $\pm$1 °C for 18-22 hours. Using a special ruler by HiMedia, Mumbai, Maharashtra, India, the diameters of the growth inhibition zones of the studied *Salmonella* isolates were measured to the nearest millimeter, and the results were interpreted. The sensitivity of *Salmonella* spp. strains to antibacterial drugs were assessed following EUCAST recommendations https://www.eucast.org/ast_of_bacteria. Quality control of the studies was carried out with a control strain of *Escherichia coli* ATCC 25922.

Results

Identification and serotyping of Salmonella isolates

In total, 34 *Salmonella* strains were subjected to AST. Thirteen isolates were identified as *S.* Enteritidis and mostly recovered from chicken. Moreover, two isolates of *S.* Livingstone from semi-finished meat products, two *S.* Infantis isolates from chicken, one *S.* Vuadens from turkey, one *S.* Hadar from duck meat, one *S.* Schwarzengrund from ready-to-cook meat, and 14 were untypable. Fourteen *Salmonella* spp. were untyped and mostly recovered from poultry meat, semi-finished meat products, poultry, beef meat, and chickens (Table 1).

Antimicrobials sensitivity testing based on antimicrobial groups

Salmonella was significantly resistant to β-lactams, e.g., nine isolates were resistant to ticarcillin, five to

Table 1: Spectrum of *Salmonella* spp. isolated from food of animal origin during 2018-2021 in Ukraine.

Type of food	*Salmonella* serotypes isolated from foods of animal origin (No.34)													
	S. Enteritidis		*S.* Infantis		*S.* Schwarzengrund		*S.* Livingstone		*S.* Vuadens		*S.* Hadar		Untyped	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Poultry meat	8	23.5	2	5.9	-	-	-	-	1	2.9	1	2.9	11	32.4
Semi-finished meat products	-	-	-	-	1	2.9	2	5.9	-	-	-	-	2	5.9
Beef meat	-	-	-	-	-	-	-	-	-	-	-	-	1	2.9
Prepared fish meat	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Prepared poultry meat	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Prepared rice meal	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Dairy products	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Confectionery	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-
Total	13	38.2	2	5.9	1	2.9	2	5.9	1	2.9	1	2.9	14	41.2

amoxiclav, three to amoxicillin, and two to ampicillin. Six isolates out of 34 tested were resistant to quinolones, e.g. three isolates were resistant to norfloxacin and three to ciprofloxacin. Two isolates (5.9%) showed resistance to trimethoprim, and eight isolates (23.5%) showed resistance to chloramphenicol (Figure 1 & Figure 2). No isolates were resistant to imipenem and aminoglycoside antibiotics, particularly gentamicin and amikacin. According to the results of our study, it was established that the studied strains showed the greatest resistance to cephalosporins, e.g., 28 (82.4%) isolates were resistant to cefepime, nine (26.5%) to ceftazidime, eight (23.5 %) to cefoxitin and two (5.9%) isolates each to ceftriaxone and cefotaxime.

Antibiotic sensitivity testing based on tested samples

It should be noted that 73.5% of *Salmonella* spp. isolates were isolated from poultry products, particularly broiler fillets, chicken wings, chicken quarters, duck bones, and turkey thighs. The most significant resistance to cefepime was demonstrated by *Salmonella* isolates obtained from poultry meat (88.2%). Eight isolates recovered from sausages, milk-containing cheese products, ready-made fish dishes, minced pork, and chicken showed resistance to cefoxitin, and nine isolates isolated from sausages, a milk-containing cheese product, chicken showed resistance to ceftazidime. High resistance to ticarcillin was found (29.4%) in isolates obtained from the meat of broilers, ducks, and sausages.

Resistance to β-lactam antibiotics, in particular, amoxiclav, was found in 23.5% of the studied isolates, which were isolated from chicken, duck meat, sausages, shawarma, ready-made fish dishes, and milk-containing cheese products. *Salmonella* showed resistance to chloramphenicol in 23.5% of isolates obtained from beef, chicken, and duck bone. It should be noted that the use of chloramphenicol is prohibited for use in animal husbandry. Two *Salmonella* isolates from poultry products showed resistance to trimethoprim, which was 5.9% of the total number of tested strains. No strains of *Salmonella* resistant to gentamicin, amikacin, and imipenem were detected.

Antibiotic sensitivity testing based on Salmonella serotypes

A total of 62.5% of *S.* Enteritidis isolates showed resistance to cefepime, 12.5% showed resistance to ceftazidime and ticarcillin, and 6.25% showed resistance to ciprofloxacin and ticarcillin. Among the studied *S.* Infantis isolates, 12.5% were resistant to cefepime, and 6.25% were resistant to ciprofloxacin and cefoxitin. *S.* Hadar (5.9%) was isolated from duck meat. Resistance was found to five antibacterial drugs, which amounted to 6.25% to amoxicillin, ampicillin, amoxiclav, ticarcillin, and cefepime. Only one isolate of *S.* Enteritidis, which was isolated from the confectionery, was sensitive to all the antibiotics used in the study.

Five isolates, of which three *S.* Enteritidis, one untyped *Salmonella* spp., and one *S.* Vuadens, showed resistance only to cefepime. Resistance to cefepime and cefoxitin was recorded in isolates of *Salmonella* spp. untyped and *S.* Infantis. A similar profile was observed in *S.* Infantis; however, this isolate was also resistant to ciprofloxacin. Two isolates of *S.* Livingstone and *S.* Schwarzengrund showed resistance to cefepime and ticarcillin. It is important to note that seven strains (20.6%) showed resistance to three antibiotics in different combinations. One (2.9%) untyped *Salmonella* spp. strain isolated from poultry products showed resistance to six antibiotics (amoxiclav, ceftazidime, cefepime, cefotaxime, chloramphenicol, and trimethoprim), and one (2.9%) *Salmonella* spp. untyped isolate obtained from poultry products showed resistance to nine antibacterial drugs (norfloxacin, amoxicillin, ampicillin, amoxiclav, ticarcillin, cefoxitin, ceftazidime, cefepime, and chloramphenicol).

Discussion

Salmonella is often resistant to antibacterial drugs; therefore, it is essential to determine the sensitivity of these pathogens to antibiotics (Van Boeckel et al.,

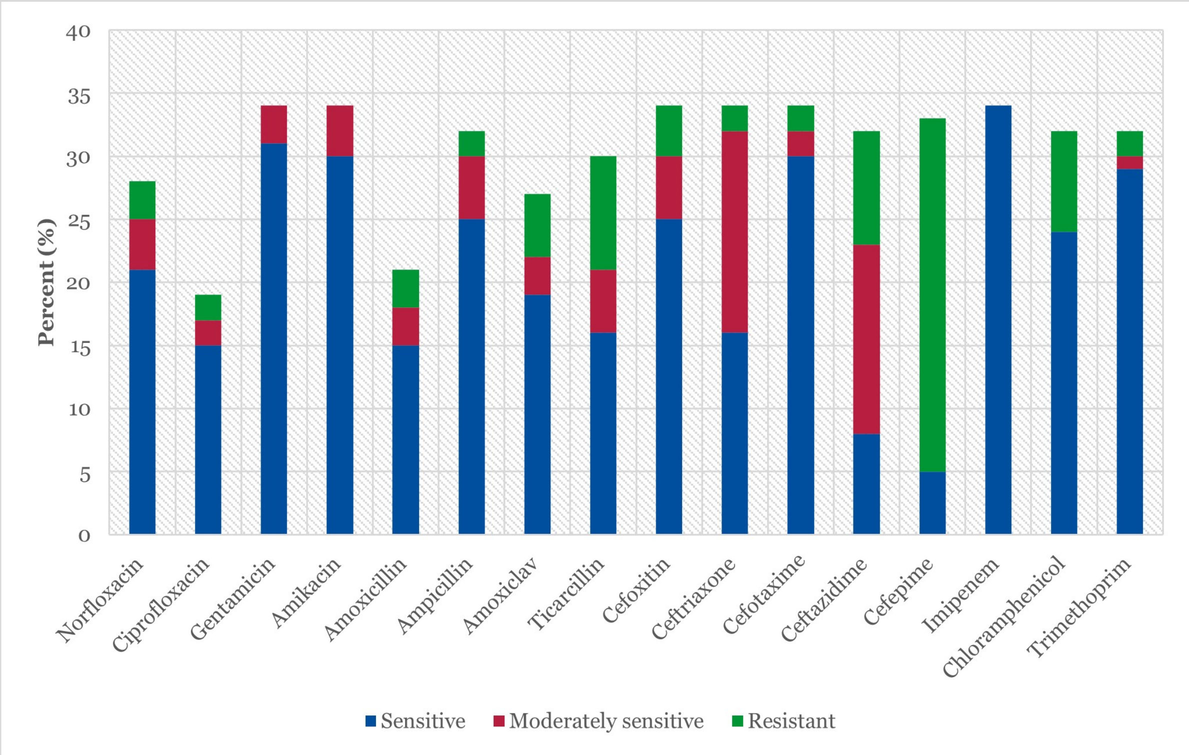

Figure 1: The number of sensitive, moderate, and resistant *Salmonella* isolates (n= 34) to different antibacterial drugs.

2015). High antibiotic resistance of *Salmonella* is registered worldwide (Rublenko, 2020). This is evidenced by the reports of the European Food Safety Agency and the Global Surveillance System for Antimicrobial Resistance (GLASS) (EFSA and ECDC, 2018; EFSA and ECDC, 2019, 2020). In addition, the resistance of *Salmonella* isolates to fluoroquinolones was documented in several countries of Africa and the eastern region of the Mediterranean coast, as well as in the countries of Central America (Bilge et al., 2018; Jaja et al., 2019). In 2018, salmonellosis was the second most common zoonotic disease in the European Union, with 91,857 confirmed human cases, and the most common cause of foodborne outbreaks, accounting for 30.7% of all cases (Majowicz et al., 2010; EFSA and ECDC, 2020).

According to Podavalenko et al. (2021), the incidence of salmonellosis in Ukraine is highly prevalent. Outbreaks of salmonellosis are often registered. *S.* Enteritidis is the most isolated serovar from clinical material of patients, carriers, and environmental objects ($p<0.05$). Risk groups for salmonellosis are children ($p<0.05$) and the rural population ($p<0.05$) (Podavalenko et al., 2021). *Salmonella* was also isolated from meat in China and showed resistance to ampicillin in 39% of cases, chloramphenicol in 20%, and trimethoprim in 9%. Although none of the isolates in this study showed resistance to β-lactams except ampicillin, about a third of the isolates were quinolone-resistant (Chen et al., 2004).

EFSA reports showing a high level of resistance of *Salmonella* isolates recovered from the meat of broilers, laying hens, and turkey to ciprofloxacin in 2016 (43.7%) and 2017 (64.7%) (EFSA and ECDC, 2018). In 2018, the indicators of resistance to ciprofloxacin in isolates obtained from broilers were 51.8%, in turkeys was 42.7%, and in laying hens was 16.2%. In *Salmonella* obtained from pigs and cattle, resistance to ciprofloxacin varied from 10.3 to 12.7%, respectively (EFSA and ECDC, 2019). Third-generation cephalosporins have higher therapeutic effects, but the misuse of these antibiotics has increased the number of resistant strains of *S.* Enteritidis is mainly caused by the production of extended-spectrum β-lactamases (Tenover, 2006). This phenomenon represents an ever-increasing threat to public health and a challenge that must be addressed by developing alternative therapeutic strategies (Yahav et al., 2007).

According to Ying Fu, 38 isolates with resistance to cefepime were simultaneously resistant to ceftazidime (100%), cefotaxime (100%), and ampicillin (100%). Also, they showed high resistance to nalidixic acid (97.37%), sulfafurazole (76.32%), streptomycin (63.16%), and trimethoprim-sulfamethoxazole (52.63%), followed by tetracycline (44.74%) and chloramphenicol (28.95%). The identical cefepime-resistant isolates showed sensitivity to amoxicillin-clavulanic acid (15.79%), gentamicin (13.16%), and amikacin (13.16%). They all showed intermediate sensitivity to ciprofloxacin, ofloxacin, polymyxin B, and imipenem (Fu et al., 2020). A small number of isolates were resistant to trimethoprim (5.9%). Resistance to gentamicin was found at a low level in isolates from pigs, broilers, laying hens, and turkeys, i.e., 5.9%,

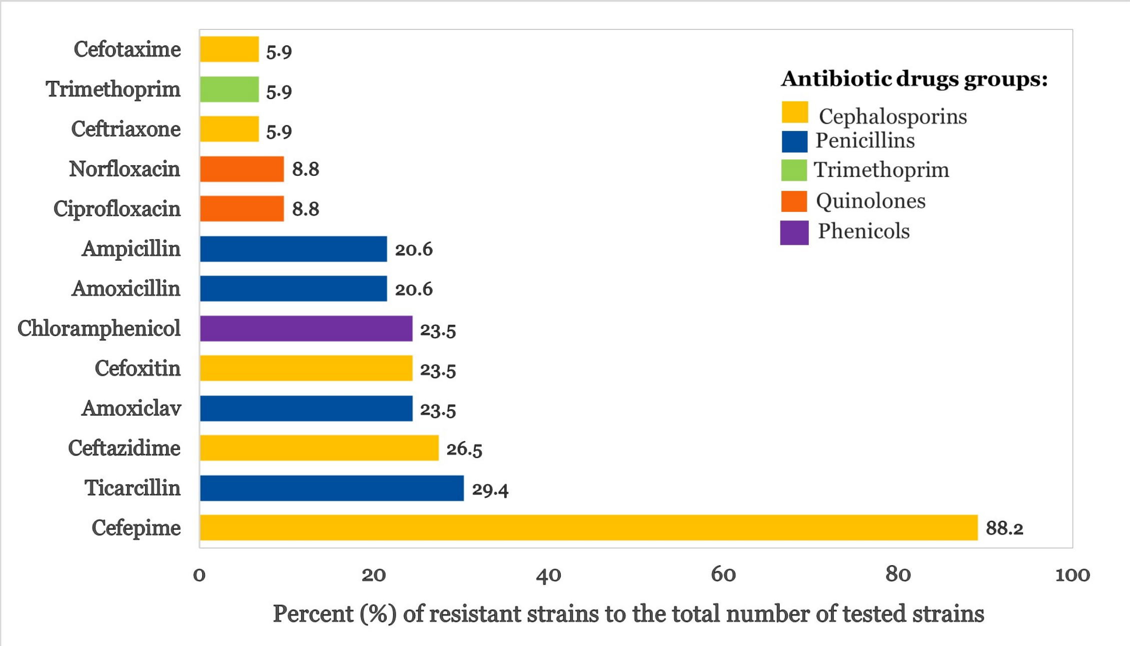

Figure 2: Percentages of antibiotic resistance of *Salmonella* spp. strains to different groups of antibacterial drugs, tested for the period 2018-2021.

2.4%, 1.1%, and 7.2%, respectively (EFSA and ECDC, 2020).

The Terrestrial Animal Health Code of the International Office of Epizootics, in section 6.7, "Harmonization of national antimicrobial resistance monitoring and surveillance programs," emphasizes the need to monitor and control antimicrobial resistance to establish and evaluate trends and sources of bacterial resistance to detect the emergence of new resistance mechanisms, and to provide data necessary for risk analysis relevant to animal and human health (Collignon et al., 2016; WHO, 2015). Although other researchers conducted similar studies in different regions of the world, these studies, which concern the antimicrobial resistance of *Salmonella* isolated from food products of animal origin, were conducted in Ukraine for the first time. Our results confirm the circulation of antibiotic-resistant *Salmonella* in Ukraine. In general, the results of our research are similar to those presented by EFSA, GLASS data; in particular, *Salmonella* isolates in food products of animal origin are usually resistant to numerous antibacterial drugs, reflecting a general trend of increasing antibiotic resistance, and also threaten to lose their effectiveness in the treatment of humans and animals from bacterial infections and pose a risk of transmission to humans through food.

In general, circulating *Salmonella* strains in Ukraine show high resistance to antibacterial drugs that belong to cephalosporins, penicillins, phenicoles, and quinolones. This causes concern since most of these antimicrobial preparations are critical for humans, such as 3rd and 4th generation cephalosporins and penicillins, and prohibited for use in productive animals, such as chloramphenicol (Collignon et al., 2016). This poses a threat to humans, as it is known that the spread of resistant strains to antimicrobial preparations can be transmitted not only within bacterial populations of *Salmonella* but also to other types of microorganisms, as well as with food to humans. Therefore, controlling the antimicrobial resistance of zoonotic agents, specifically *Salmonella*, for preserving human health is a severe problem.

Determining the susceptibility of microorganisms to antibiotics is essential to predict their effectiveness in treating bacterial infections in animals, monitor the rate of resistance spread among sensitive populations of microorganisms, and develop alternative antibiotics (Shane et al., 2017). In the field of food safety, responsible state structures should protect consumers from risks associated with the food chain and establish optimal management options to reduce them. Monitoring the sensitivity of microorganisms to antibacterial drugs will provide the necessary information on the current state of resistance of zoonotic pathogens, especially *Salmonella*, in a particular farm, region, and country as a whole. Furthermore, the monitoring data will help veterinarians choose an effective antimicrobial agent for treating bacterial infections in animals and thus contribute to more judicious use of antibacterial drugs in veterinary medicine (Stetsko, 2018).

Conclusion

In the present study, new data were obtained on the spread of antibiotic-resistant *Salmonella* isolates isolated from food products of animal origin in Ukraine. Salmonellosis is one of the most common foodborne diseases in humans, and outbreaks of the disease have been linked to milk and meat intake. Handling and raising farm animals expose individuals to the risk of serious zoonotic diseases. Additionally, animals may be habitats to *Salmonella* spp. that are multi-drug resistant, which poses a greater threat to the public's health. The risks of consuming raw or undercooked meat, and other animal products, should be clearly communicated to the general public.

Salmonellosis in animals and humans can be reduced by using strict hygiene measures at the farm level and maintaining higher standards of hygiene throughout the entire process of animal breeding and food production. Furthermore, tracking the prevalence of resistant microorganisms to antimicrobial preparations will provide valuable information that will be useful for the risk analysis process. This offers the opportunity to develop measures to reduce the spread of resistant zoonotic agents among the animal population and humans and will be necessary for implementing of a national policy on the prudent use of antimicrobial drugs in veterinary medicine.

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