Antibiotic resistance in the pathogenic bacteria isolated from environmental samples of the food production facilities

Yuliya Yushina, *Elena* Zaiko*, *Anzhelika* Makhova, *Dagmara* Bataeva, and *Nasarbay* Nasyrov

V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

Abstract. Foodborne pathogens, such as *Salmonella spp. and Listeria monocytogenes*, may be present at various stages of production. The overuse of antibiotics over the past few decades has led to the emergence of many antibiotic-resistant bacteria, including foodborne pathogens. We investigated the sensitivity of *Salmonella spp and Listeria monocytogenes* to various antibiotics. Studied strains of *L. monocytogenes and Salmonella* spp. they showed resistance to tetracycline, which totaled 66.7%. High resistance of strains to ampicillin was also noted (57.14%). All strains were sensitive to amikacin, azithromycin, Meropenem, ciprofloxacin, moxifloxacin, norfloxacin. All strains of Salmonella spp. were resistant to ampicillin and penicillin G. Resistance to streptomycin and trimethoprim/sulfamethoxazole was 91.7%. All strains of L. monocytogenes were resistant to nalidixic acid. 22.2% of the strains showed resistance to tetracycline and cefepime. 11.1% of the strains were resistant to chloramphenicol and trimethoprim/sulfamethoxazole.

1 Introduction

Despite the pathogenic bacteria-control measures, they are still dangerous to human health. Pathogens such as *Salmonella spp* and *Listeria monocytogenes* are the main causative agents of foodborne illness associated with food [1.2]. Antibacterial treatment is a prevalent approach in contemporary medicine for combating bacterial infections. The problem with antibiotic treatment is the emergence of antibiotic resistance in bacterial populations, which can render previously effective drugs ineffective and limit treatment options. Antibiotics are fed to the animals via feed in low or sub-therapeutic concentrations, and 75%–90% of the antibiotics administered this way are lost to the environment via urine and feces of the farm animals [3]. The data shows that between 2014 and 2016 nearly a million people died due to antimicrobial-resistant infections and nearly 300 million premature deaths by 2050 [4]. In Europe, an estimated 25,000 deaths are due to antibiotic-resistant bacteria [5].

Bacteria may develop resistance due to the exposure to sub-lethal levels of antibiotics in their surroundings, or alternatively, bacteria may directly acquire resistance mechanisms from other bacteria via DNA transfer mechanisms. The use of antibiotics in food and

* Corresponding author: e.zaiko@fncps.ru

agriculture has direct as well as indirect effects on the development of antibiotic resistance by bacteria associated with animals which can enter into the food chain through meat and fish [6]. The EFSA report [7] emphasizes that *Salmonella spp.* is the second most commonly reported gastrointestinal infection in humans after campylobacteriosis. *L. monocytogenes* is the most serious zoonotic disease with a high rate of hospitalization (92%) and mortality (17.6%) [8]

Foodborne pathogenic bacteria are currently being monitored worldwide for resistance to important classes of antibiotics [9]. The aim of this study was to evaluate the occurrence of antibiotic-resistant *L. monocytogenes* and *Salmonella spp.* in different environmental samples within food entreprises.

2 Experimental materials and methods

2.1 Objects

The objects of the study were 12 strains of *Salmonella spp.* and 9 strains of *L. monocytogenes* isolated from various environmental samples at food production facilities in 2021.

2.2 Screening of the Isolates for Resistance to Antimicrobial Preparations

The sensitivity of *Salmonella* isolates to antimicrobial preparations was determined using the disk diffusion test on Muller-Hinton agar plates in accordance with recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2023) [10], harmonized with recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST) [11]. The following 12 antimicrobial preparations were tested: Amikacin (30 mcg), Azithromycin (15 mcg), Cefepime (30 mcg), Ceftazidime (30 mcg), Ceftriaxone (30 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Erythromycin (15 mcg), Imipenem (10 mcg), Meropenem (10 mcg), Moxifloxacin (5 mcg), Nalidixic acid (30 mcg) Norfloxacin (10 mcg), Penicillin G (10 iu), Streptomycin (10 mcg), Sulphamethoxazole/trimethoprim (25 mcg), Tetracycline (30 mcg), Tobramycin (10 mcg), Lincomycin (15 мкг). All antibiotic-containing paper disks were manufactured by the St.-Petersburg Pasteur Institute, Russia. *Escherichia Coli* ATCC 25922 was used to control the quality of research. The isolates were classified as sensitive, intermediate, or resistant according to CLSI (2023). The following breakpoints were used for *L. monocytogenes*: ampicillin < 16 мм; erythromycin < 25 mm; sulphamethoxazole/trimethoprim < 29 mm. There are no criteria for *L. monocytogenes* for other antimicrobial preparations, the criteria for *Staphylococcus aureus* were used [12 ,13] including Imipenem < 22 mm; ciprofloxacin < 21 mm. The isolates resistant to three or more different classes of antimicrobial preparations were considered multidrug-resistant (MDR).

3 Results

The studied strains of *L. monocytogenes* and Salmonella spp. showed resistance to tetracycline, which in total amounted to 66.7% (Figure 1). High resistance of strains was also noted to ampicillin. (57,14%). All strains were sensitive to Amikacin, Azithromycin, Meropenem, Ciprofloxacin, Moxifloxacin, Norfloxacin.

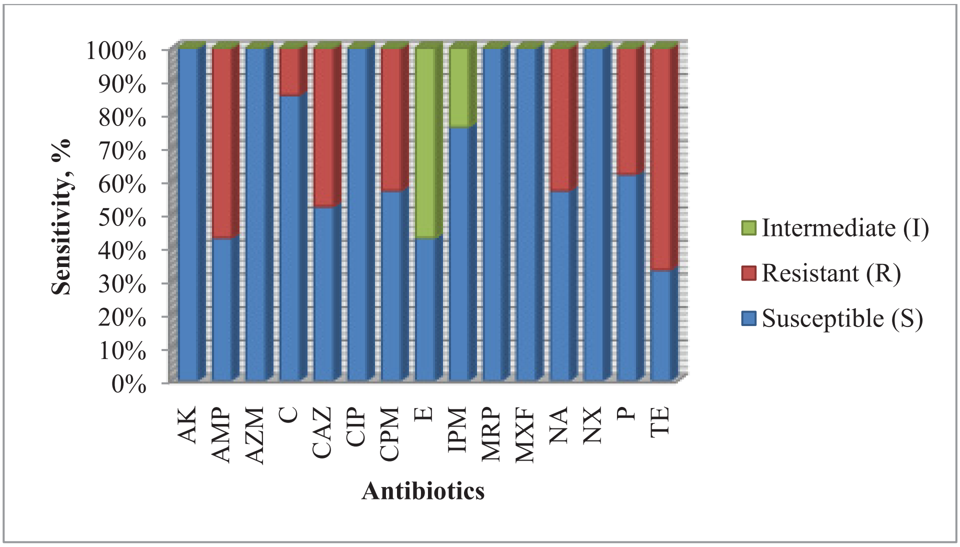

Fig. 1. Antibiotic sensitivity of pathogenic microorganisms. Note: Amikacin (AK), Azithromycin (AZM), Cefepime (CPM), Ceftazidime (CAZ), Ceftriaxone (CTR), Chloramphenicol (C), Ciprofloxacin (CIP), Erythromycin (E), Imipenem (IPM), Meropenem (MRP), Moxifloxacin (MX), Nalidixic acid (NA), Norfloxacin (NX), Penicillin G (P), Streptomycin (S), Sulphamethoxazole/trimethoprim (SX), Tetracycline (TE), Tobramycin (TOB), Lincomycin (LINC)

The strains showed intermediate sensitivity to Erythromycin and Imipenem, 57.14% and 23.8%, respectively. Cephalosporin resistance has been identified: 42,85% to Cefepime, 46,7% to Ceftazidime. A slightly lower percentage of strain resistance was to Penicillin G and Chloramphenicol, 38.0% and 14.29%, respectively.

The results of sensitivity to antibiotics among the studied Salmonella are presented in table 1. All strains of Salmonella spp. were resistant to Ampicillin and Penicillin G. A resistance to Streptomycin and Trimethoprim/sulfamethoxazole was amounted to 91.7%. 83.3% of the tested *Salmonella spp.* were resistant to tetracycline. 75% of strains showed resistance to Lincomycin, and 18.75% to Chloramphenicol. The full sensitivity of *Salmonella spp*. showed to all antibiotics of the Fluoroquinolone and Cephalosporin groups, such as Ciprofloxacin, Moxifloxacin, Norfloxacin, Cefepime, Ceftazidime. All strains of Salmonella spp. were sensitive to Aminoglycosides, such as Amikacin and Tobramycin.

Table 1. Antimicrobial resistance of *Salmonella* isolated from environmental samples.

Antimicrobial Class	**Antimicrobial agent**	**No. of strains (%)**		
		Resistance (R)	**Intermediate (I)**	**Susceptible (S)**
Penicillins	Ampicillin	12 (100)	0 (0)	0 (0)
Monobactams Carbapenems	Imipenem	0 (0)	0 (0)	12 (100)
Aminoglycosides	Amikacin	0 (0)	0 (0)	12 (100)
	Streptomycin	11 (91,7)	0 (0)	1 (8,3)
	Tobramycin	0 (0)	0 (0)	12 (100)
Folate pathway antagonists	Trimethoprim/ sulfamethoxazole	11 (91,7)	0 (0)	1 (8,3)
Inhibits protein synthesis	Chloramphenicol	2 (18,75)	0 (0)	10 (81,25)
Macrolides and azalides	Azithromycin	0 (0)	0 (0)	12 (100)
	Erythromycin	0 (0)	12 (100)	0 (0)

Continuation of Table 1.

Antimicrobial Class	**Antimicrobial agent**	**No. of strains (%)**		
		Resistance (R)	**Intermediate (I)**	**Susceptible (S)**
β-Lactam/β-lactamase inhibitor combinations	Meropenem	0 (0)	0 (0)	12 (100)
Tetracyclines	Tetracycline	10 (83,3)	0 (0)	2 (16,7)
Fluoroquinolone	Ciprofloxacin	0 (0)	0 (0)	12 (100)
	Moxifloxacin	0 (0)	0 (0)	12 (100)
	Norfloxacin	0 (0)	0 (0)	12 (100)
Cephalosporins	Cefepime	0 (0)	0 (0)	12 (100)
	Ceftazidime	0 (0)	0 (0)	12 (100)
Penicillins	Penicillin G	12 (100)	0 (0)	0 (0)
Lincosamide	Lincomycin	9 (75)	2 (16,7)	1 (8,3)
	Nalidixic acid	0 (0)	0 (0)	12 (100)

All strains of L. monocytogenes were resistant to nalidixic acid. 22.2% of the strains showed resistance to tetracycline and cefepime. 11,1% of strains were resistant to chloramphenicol и trimethoprim/sulfamethoxazole.

Table 2. Antimicrobial resistance of *L. monocytogenes* isolated from environmental samples.

Antimicrobial Class	**Antimicrobial agent**	**No. of strains (%)**		
		Susceptible (S)	**Intermediate (I)**	**Resistance (R)**
Penicillins	Ampicillin	9(100)	0 (0)	0 (0)
Monobactams Carbapenems	Imipenem	4(44,5)	5(55,5)	0 (0)
Aminoglycosides	Amikacin	9(100)	0 (0)	0 (0)
	Streptomycin	9(100)	0 (0)	0 (0)
Folate pathway antagonists	Trimethoprim/ sulfamethoxazole	8(88,9)	0 (0)	1(11,1)
Inhibits protein synthesis	Chloramphenicol	8(88,9)	0 (0)	1(11,1)
Macrolides and azalides	Azithromycin	9(100)	0 (0)	0 (0)
	Erythromycin	9(100)	0 (0)	0 (0)
β-Lactam/β-lactamase inhibitor combinations	Meropenem	9(100)	0 (0)	0 (0)
Tetracyclines	Tetracycline	7(77,8)	0 (0)	2(22,2)
Fluoroquinolone	Ciprofloxacin	9(100)	0 (0)	0 (0)
	Moxifloxacin	9(100)	0 (0)	0 (0)
	Norfloxacin	9(100)	0 (0)	0 (0)
Cephalosporins	Cefepime	0 (0)	7(77,8)	2(22,2)
	Ceftriaxone	0 (0)	9(100)	0 (0)
Penicillins	Penicillin G	8(88,9)	0 (0)	1(11,1)
	Nalidixic acid	0 (0)	0 (0)	9(100)

Strains of *L. monocytogenes* were sensitive to aminoglycosides (amikacin, streptomycin), macrolides and azalides (azithromycin, erythromycin), fluoroquinolone (ciprofloxacin, moxifloxacin, norfloxacin). No resistance was noted to such antibiotics as ampicillin and meropenem. Intermediate sensitivity was found to imipenem in 55.5% of strains, to cefepime in 77.8%, and to ceftriaxone in 100% of strains.

2.3 Multidrug-resistance

Among the studied microorganisms, multidrug resistance was established. The results are presented in Figure 2.

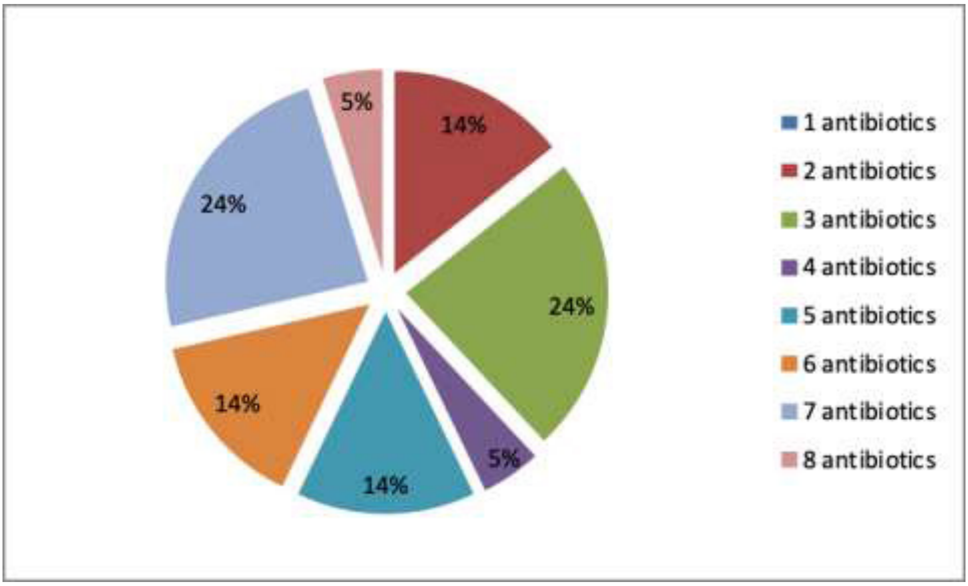

Fig. 2. Results of the analysis of multidrug resistance.

None of the tested strains demonstrated full sensitivity to all tested antibiotics. At least 1 strain was resistant to two antibiotics; there were 14%. Also, 14% of the strains showed resistance to 5 and 6 different antibiotics. The maximum amount of resistance to antibiotics was found in 5% of microorganisms to 8 antibiotics.

4 Discussions and conclusion

The study aimed to assess the prevalence of antibiotic-resistant *L. monocytogenes* and *Salmonella spp.* in various environmental samples in food industries. This is important as strains present in the environment may contaminate food, posing a potential risk of infection to humans. This research has indicated that foodborne pathogens found in food establishments often exhibit significant levels of resistance to antibiotics.

The reduced antibiotic susceptibility demonstrated in the *Salmonella* population (Table 1) may be the cause of treatment failure in some clinical situations. More than 90% of the strains were resistant to streptomycin. Streptomycin is not routinely used to treat salmonellosis; but it is commonly used as a growth promoter in animals. For this reason, streptomycin could be useful as a marker for resistant isolates moving up the food chain [14]. Our results show that resistance was very low to such an important group of antibiotics as fluoroquinols. Salmonella infections are commonly treated using ciprofloxacin, a fluoroquinolone compound known for its wide-ranging effectiveness [15]. Two more antibiotics, such as tetracycline and streptomycin, showed a high level of resistance in this study. This suggests healthcare providers and veterinarians should gradually stop using these two antibiotics in both clinical and animal settings. Antibiotics used to treat patients with salmonella infections include ciprofloxacin, azithromycin, and ceftriaxone [16]. All studied *Salmonella* were sensitive to azithromycin and ceftriaxrn.

Listeria monocytogenes can be present in a variety of meat processing facilities such as slaughterhouses and meat processing plants [17]. The prevalence of L. monocytogenes in meat processing environments may also increase or decrease due to sanitary measures [17]. Proper cleaning and sanitation procedures reduce the prevalence of *L. monocytogenes* in meat processing plants. However, this pathogen is often found even after sanitation procedures. This shows the persistence of some strains and insufficient cleaning process [18]. The cleaning process uses detergents to remove the microbial agent from environmental objects in food production facilities. However, there are problems with the presence of hard-to-reach places [19]. Hard-to-reach places are places (surfaces) where disinfectants do not reach properly, and as a result, proper cleaning and disinfection does not occur. Several studies have shown that L. monocytogenes has the ability to withstand commonly used sanitation in food processing plants [20,21]. According to these studies, the resistance of L. monocytogenes to disinfectants in food processing plants is attributed to the development of biofilms that can endure in the facility for extended periods.

Listeria strain resistant to ampicillin has been identified in the United States [22]. However, in our study, all strains were sensitive to ampicillin. *Listeria monocytogenes* resistant to streptomycin, erythromycin, kanamycin, sulfonamide and rifampicin have also been reported from various countries [23]. Multidrug resistance has also been observed in strains isolated from food and environmental samples around the world [24]. In addition, *L. monocytogenes* isolated from food and animals (n = 167) in the USA were resistant to ciprofloxacin, tetracycline, sulfonamide, and nalidixic acid at 1.8%, 9%, 73%, and 100%, respectively [25]. In our work, L. monocytogenes were sensitive to the antibiotics Amikacin, Streptomycin, Azithromycin, Erythromycin, Ciprofloxacin, Moxifloxacin, Norfloxacin, Ampicillin and Meropenem. In the work of Pavel Andriyanov et al., strains of Listeria monocytogenes were sensitive to gentamicin, kanamycin, neomycin, streptomycin, vancomycin and teicoplanin. Efficacy was also established against all tested strains of clarithromycin, levofloxacin and a combination of amoxicillin and clavulanic acid, as well as trimethroprim and sulfamethoxazole [26]. However, in our study, some strains showed resistance to trimethroprim and sulfamethoxazole, clavulanic acid.

The paper shows a significant spread of acquired resistance, including multiple resistance, among pathogenic strains circulating at production facilities. Increased antibiotic resistance among strains of L. monocytogenes and Salmonella spp. correspond to the global trend of increasing the prevalence of antibiotic resistance among foodborne pathogens. It is becoming increasingly clear that antibiotic resistance will remain a major obstacle to be addressed in the near future.

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