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Antimicrobial resistance of *Salmonella* isolates recovered from animal products

N. B. Shadrova¹, O. V. Pruntova², E. A. Korchagina³

FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

SUMMARY

The article provides data on antimicrobial resistance (AMR) of Salmonella isolates recovered from animal products tested in the Laboratory for Microbiological Testing of the FGBI "ARRIAH" from 2019 to 2020. 106 isolates of Salmonella enterica subsp. enterica were recovered from 4,500 tested samples of raw materials and products of animal origin, 23% of them were untyped, and 77% belonged to 17 serological variants. Isolates of S. enteritidis (n = 37) and S. virchow (n = 9) serovariants dominated among the typed cultures of Salmonella, which is consistent with the data from other authors. Antimicrobial susceptibility of the microorganisms was determined in a disk diffusion test in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing. Different Salmonella serovars demonstrated different proportions of susceptible and resistant isolates, in terms of antibiotics from ten pharmacological groups. The largest number of polyresistant isolates was noted in Salmonella serovars S. virchow, S. nigeria, S. infantis, S. colindale. Both resistant and polyresistant Salmonella isolates were most often isolated from poultry products. S. typhimurium serovar, which is referred to in literature as polyresistant, was resistant to one or two antimicrobial agents as the research demonstrates. Isolates of 9 Salmonella serovars out of 17 (65%) showed resistance to nalidixic acid. 97% (n = 36) of S. enteritidis isolates were resistant to this antimicrobial agent. Isolates of S. colindale serovar (n = 2) were resistant to 8 antimicrobials. Untyped Salmonella isolates were resistant to 9 antibiotics, 2 cultures out of them showed resistance to ciprofloxacin.

Keywords: Salmonella, serovariant, antibiotic, susceptibility, resistance

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For correspondence: Natalya B. Shadrova, Candidate of Science (Biology), Head of Microbiology Laboratory, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, e-mail: shadrova@arriah.ru.

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Антибиотикорезистентность изолятов сальмонелл, выделенных из продуктов животного происхождения

Н. Б. Шадрова¹, О. В. Прунтова², Е. А. Корчагина³

ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

РЕЗЮМЕ

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¹ https://orcid.org/0000-0001-7510-1269, e-mail: shadrova@arriah.ru

² https://orcid.org/0000-0003-3143-7339, e-mail: pruntova@arriah.ru

³ e-mail: korchagina@arriah.ru

¹ https://orcid.org/0000-0001-7510-1269, e-mail: shadrova@arriah.ru

² https://orcid.org/0000-0003-3143-7339, e-mail: pruntova@arriah.ru

³ e-mail: korchaqina@arriah.ru

S. colindale. Резистентные и полирезистентные изоляты сальмонелл наиболее часто выделяли из продукции птицеводства. Серовар S. typhimurium, который в источниках литературы определяют как полирезистентный, в представленных исследованиях был устойчив к одному или двум антимикробным препаратам. У изолятов 9 сероваров сальмонелл из 17 (65%) отмечена устойчивость к налидиксовой кислоте. Доля резистентных к данному средству изолятов S. enteritidis составила 97% (n = 36). Изоляты серовара S. colindale (n = 2) были устойчивы к 8 антимикробным препаратам, S. papuana (n = 5) — к 6 антибиотикам, а S. agona (n = 3) — к 5 антибактериальным средствам. Нетипируемые изоляты сальмонелл были резистентны к 9 антибиотикам, из которых 2 культуры проявили устойчивость к ципрофлоксацину.

Ключевые слова: Salmonella, серовариант, антибиотик, чувствительность, резистентность

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Для корреспонденции: Шадрова Наталья Борисовна, кандидат биологических наук, заведующий лабораторией микробиологических исследований ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, e-mail: shadrova@arriah.ru.

INTRODUCTION

Salmonellosis is a widespread infection of humans and animals caused by various representatives of *Salmonella enterica* [1–3]. According to the government report "On sanitary and epidemiological well-being of the population in the Russian Federation in 2020", the overall incidence of salmonellosis showed a decrease. Compared with 2019, the 2020 incidence decreased by 1.6 times and accounted for 14.71 cases per 100 thousand [4, 5]. However, salmonellosis is still a matter of concern. Based on the data provided by the reference center for monitoring of salmonellosis, 27 *Salmonella* serotypes were isolated from infected humans in 2020, 17 serotypes were isolated from food staples, 16 from environmental objects. As in previous years, *Salmonella* was most often isolated from poultry products [4].

Salmonella is susceptible to a wide range of antimicrobials, due to the structural features of the cell wall. As Gram-negative microorganisms, the bacteria are susceptible to: beta-lactams (aminopenicillins, carboxypenicillins, inhibitor-protected penicillins, cephalosporins, monobactams, carbapenems), aminoglycosides (streptomycin, kanamycin, gentamicin, tobramycin, amikacin), quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin, etc.), tetracyclines (tetracycline, doxycycline), polymyxins, sulfonamides and co-trimoxazole, nitrofurans (nifuroxazide, nifuratel, furazolidone) and products of other groups (fosfomycin, chloramphenicol) [6–8].

According to the report "On sanitary and epidemiological well-being of the population in the Russian Federation in 2020", from 2015 to 2020, the following serotypes were predominantly recovered: *S. enteritidis* (64.7%), *S. ty-phimurium* (4.8%) and *S. infantis* (3.2%). A study of antimicrobial susceptibility of *S. enteritidis* isolates recovered in 2020 revealed that 58.7% of cultures are resistant to colistin, and 75% — to ciprofloxacin. Polyresistance was noted in 4.1% of isolates, and two cultures of *S. enteritidis* demonstrated resistance to polymyxins, monobactams, penicillins, second-, third-, forth-generation cephalosporins. 85% of the isolated *S. infantis* cultures showed resis-

tance to more than three classes of antibiotics, while all the isolates were susceptible to glycocyclines, polymyxins, carbopenems, first-generation cephalosporins and third-generation aminoglycosides. About half of *S. typhimurium* isolates were susceptible to all antimicrobials, and 30% of cultures were resistant to two groups of antibiotics (penicillins, tetracyclines) [4].

The World Health Organization estimates that half of all the antibiotics produced in the world are used to not only treat people, but also those animals and birds who are used to produce products for human consumption. Consequently, the number of strains resistant to antimicrobials is steadily increasing [9].

The issue of antimicrobial resistance has now become extremely alarming in Russia, as well as in the EU, the USA, Canada, etc., and in order to solve it, the Government of the Russian Federation developed the "Strategy for preventing spread of antimicrobial resistance in the Russian Federation until 2030", approved by Order No. 2045-r of the Government of the Russian Federation, September 25, 2017 [10].

According to the WHO, *S. enterica* bacteria belongs to the microorganisms that have resistant serotypes found in the food chain [11].

Such antimicrobials as chloramphenicol, ampicillin, and co-trimoxazole were used at different historical periods of human and veterinary medicine to treat salmonellosis. In the years that followed, the pathogen developed resistance to traditional medicinal products, therefore, fluoroquinolones and extended-spectrum cephalosporins (ESC) were adopted for treatment of salmonellosis [12]. Currently, the *Enterobacteriaceae* family demonstrates a steadily increasing resistance to most antimicrobials used for treatment of infectious diseases in animals and humans. In this regard, it is mandatory to determine antimicrobial susceptibility of bacteria isolated not only from affected animals, but also from animal products. European countries and the USA ensure continuous control over antimicrobial resistance in microorganisms, including *Salmonella*. First,

they monitor resistance to quinolones and ESP, and polyresistance to antimicrobials [13, 14]. Therefore, detection of *Salmonella* isolates resistant to antimicrobials in animal products is an urgent problem.

The purpose of this work was to determine antimicrobial resistance of *Salmonella* isolates recovered from raw materials of animal origin from 2019 to 2020.

MATERIALS AND METHODS

Strains of microorganisms. Strains Salmonella typhimurium ATCC 14028 and Escherichia coli ATCC 25922 deposited in the collection were used to control nutrient media and Salmonella isolation methods.

Isolates of bacteria. 106 *Salmonella* isolates recovered from animal products and food products for microbiological tests in 2019–2020 were used in the work.

Antibiotics. To determine the antimicrobial susceptibility of bacteria, standard paper disks with the following antibiotics were used: azithromycin (15 μ g), nalidixic acid (30 μ g), streptomycin (10 μ g), tetracycline (30 μ g), amikacin (30 μ g), amoxicillin (20 μ g), trimethoprim/sulfamethoxazole (23.75/1.25 μ g), ampicillin (10 μ g), gentamicin (10 μ g), cefotaxime (30 μ g), levomycetin (30 μ g), imipenem (10 μ g), meropenem (10 μ g), ciprofloxacin (5 μ g), kanamycin (30 μ g) (Saint Petersburg Pasteur Institute, Russia).

Salmonella isolation and identification. The bacteria were isolated from samples of animal products and food products according to GOST 31659-2012 "Food products. Method for the detection of Salmonella spp.", using nutrient media produced by the FBIS SRCAMB (Russia).

For non-selective pre-enrichment, a sample of the product weighing (25 \pm 0.1) g prepared for the study was put into a sterile bag containing 225 cm³ of buffered peptone water and was homogenized for 1 min. The inocula were incubated at a temperature of (37 ± 1) °C for (18 ± 1) hours. After the pre-enrichment, 1 cm³ samples were inoculated into test tubes with 10 cm³ of RVS (Rappaport - Vassiliadis Medium) and 10 cm³ of selenite medium. Inocula on RVS were incubated at a temperature of (41.5 ± 1) °C, and on a selenite medium – at (37 ± 1) °C for (24 ± 1) hours. After incubation, the content of each tube was streaked with a loop on two media: bismuth-sulfite-agar (BSA) and XLD (xylose-lysine-deoxycholate agar). The inocula were incubated at a temperature of (37 ± 1) °C for (24 ± 1) hours. The fact that the grown colonies belonged to Salmonella was confirmed by enzyme immunoassay using Singlepath® rapid tests (Merck KGaA, Germany) and mini VIDAS analyzer (bioMerieux SA, France).

Serological identification of Salmonella. The serological formula of the isolated Salmonella enterica cultures was determined in agglutination test on glass with diagnostic mono- and polyvalent O- and H-sera adsorbed for the PETSAL® test (FSUE SPbSRIVS FMBA, Russia). The serological variant of the strain was determined based on the serological formula in accordance with the Kaufman – White scheme.

Determination of antimicrobial susceptibility. The identified cultures were tested for antimicrobial susceptibility using disk diffusion test according to MUC 4.2.1890-04

¹ GOST 31659-2012 Food products. Method for the detection of *Salmonella* spp. Available at: https://docs.cntd.ru/document/12000982

"Determination of the antimicrobial susceptibility of microorganisms" and clinical recommendations "Determination of antimicrobial susceptibility of microorganisms". The bacterial suspension (0.5 according to the McFarland turbidity standard) was evenly distributed on the surface of the Mueller – Kauffman agar. Disks with antibiotics were put on the surface of agar inoculated with the tested culture (maximum 6 disks per 1 dish). After the disk application, Petri dishes were placed upside down in a thermostat and incubated at a temperature of (35 ± 1) °C for 18-24 hours.

The results were evaluated by microbial growth "inhibition zone" around the disks. The diameter of the microbial growth "inhibition zone", taking into account the diameter of the disk itself, was determined with an accuracy of 1 mm.

Interpretation and analysis of the results. Based on the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) the bacterial isolates were divided into the following groups: susceptible, resistant to one and two antibiotics, polyresistant – resistant to three or more antibiotics [15]. The group of resistant isolates included extremely resistant cultures, which were resistant to 6 and 7 antimicrobials [16].

Statistical processing of the test results. The experiments were performed in triplicate. The obtained data were statistically processed in standard Microsoft Exel 2010 analysis package.

RESULTS AND DISCUSSION

From 2019 to 2020, 4,500 samples of animal products were tested in the FGBI "ARRIAH" Laboratory for Microbiological Research in order to detect contamination with *Salmonella*. As a result, 106 isolates were recovered and identified by growth and biochemical properties as *Salmonella* bacteria, which belonged to 17 serological variants from groups B, C, D, E, and 24 isolates of *Salmonella enterica* (which belonged to serogroups B, C and E) were untyped.

The recovered isolates belong to the following serovariants. According to the literature, *S. enteritidis* is the main causative agent of salmonellosis in both animals and humans (35%) [17–19]. Most of the cultures in our work (34.9%) also belonged to this serovariant. Isolates of *S. virchow* serovar were detected in 9 samples (8.5%). Isolates of such serovars as *S. nigeria*, *S. papuana* and *S. infantis* were identified in 7 (6.6%), 5 (4.7%) and 5 (4.7%) samples, respectively (Table). Serotype *S. typhimurium*, clinically important for both animals and humans, was detected in only two samples, which accounted for 1.9%.

Untyped isolates of *Salmonella enterica* subsp. *enterica* were identified in 24 samples of animal products, which accounted for 22.6%. Serovar *S. agona*, which caused two outbreaks of salmonellosis in 2017–2018 in the EU member countries, was isolated in our studies from 3 samples (2.8%) [20].

Determination of Salmonella isolates susceptibility to antibiotics from various pharmacological groups. When choosing antimicrobials to check antimicrobial resistance

² MUC 4.2.1890-04 Determination of antimicrobial susceptibility of microorganisms. Moscow: Federal Center of State Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia. 2004. 91 p. Available at: https://fcgie.ru/download/elektronnaya_baza_metod_dokum/muk_1890-04.pdf.

³ Determination of antimicrobial susceptibility of microorganisms. 2018. 206 p. Available at: https://flm.kz/files/14062184925c1281c1dfd6b.pdf.

Table Serological variants of Salmonella most often recovered from animal products (n = 74)

Serological variant	Number of isolates	% of the total number of isolates recovered from food products
S. enteritidis	37	34.9
S. virchow	9	8.5
S. nigeria	7	6.6
S. papuana	5	4.7
S. infantis	5	4.7
S. derby	4	3.8
S. agona	3	2.8
S. colindale	2	1.9
S. typhimurium	2	1.9

of 106 Salmonella isolates, we considered the use of these products for human and veterinary medicine and took into account EUCAST recommendations for determination of antimicrobial susceptibility of the Gram-negative microorganisms.

As a result, it was found that *Salmonella* isolates are susceptible to 3 antibiotics from the aminoglycosides group (amikacin – 100%, gentamicin – 100% and kanamycin – 98%), to carbapenems (imipenem – 100% and meropenem – 100%), as well as to cefotaxime – 97%. The susceptibility of *Salmonella* cultures to levomycetin was 92%, azithromycin – 91%, ciprofloxacin – 90%. 87% of isolates were susceptible to ampicillin and 84% to streptomycin (Fig. 1).

Seventy-four percent of isolates demonstrated resistance to nalidixic acid, and 45% of cultures demonstrated resistance to trimethoprim/sulfamethoxazole.

As Figure 2 shows, only ten out of the total number of the tested isolates (n = 106) were susceptible to all groups of antimicrobials, which was 9%, whereas 96 isolates (91%) were resistant. At the same time, the proportion of isolates

resistant to one group of antimicrobials was 38% (40 isolates).

Twenty cultures (19%) showed resistance to 2 groups of antibiotics. Thirty- six isolates (34%) were polyresistant, i.e. resistant to three or more antimicrobials. This group included extremely resistant isolates, i.e. those that are resistant to 6 antibiotics: ciprofloxacin, streptomycin, nalidixic acid, cefotaxime, levomycetin and kanamycin. Extremely resistant isolates (resistant to 7 antimicrobials) accounted for 4% of the total number of polyresistant.

Determination of antimicrobial resistance in Salmonella isolated from samples of animal products. Most Salmonella isolates were recovered from poultry meat (Fig. 3), whereas the proportion of resistant cultures was 90%, including 3 extremely resistant ones. Out of 35 isolates recovered from meat semi-finished products, 32 (91%) were resistant. In the group of isolates recovered from pork and pork semi-finished products, only one out of 13 was susceptible to antimicrobials and one was defined as extremely resistant.

Thus, most polyresistant *Salmonella* cultures were found in poultry meat samples (50%), including extremely resistant ones, alongside with that, most recovered isolates were resistant to many groups of antimicrobials. Isolates resistant to amoxicillin were recovered only from pork samples – 69% (n = 9).

It should be noted that polyresistant *Salmonella* isolates were found in all groups of animal products, but in beef samples, their number was the smallest.

When studying antibiotic resistance of 53 Salmonella isolates recovered from poultry products, it was found that all the isolates were susceptible to three antibiotics of the aminoglycoside group (gentamicin – 100%, amikacin – 100%, kanamycin – 100%), and the carbapenem antibiotics (meropenem – 100% and imipenem – 100%). In addition, 52 isolates (98%) were found to be susceptible to cefotaxime, 48 isolates (91%) to azithromycin and 49 isolates (92%) to levomycetin (Fig. 4).

Resistance to a quinolone drug (nalidixic acid) was reported in 46 *Salmonella* isolates (87%) recovered from poultry meat samples, and resistance to fluoroquinolones (ciprofloxacin) was reported in 2 isolates.

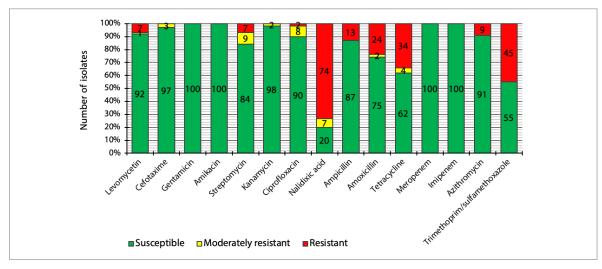


Fig. 1. Antimicrobial susceptibility of Salmonella isolates recovered from raw materials of animal origin

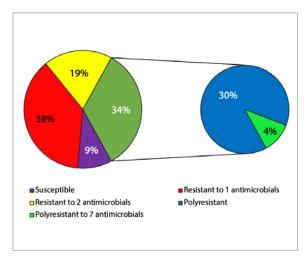


Fig. 2. Proportion of Salmonella isolates susceptible and resistant to antimicrobials (%)

The analysis of antimicrobial resistance in *Salmonella* isolates of various serovariants (Fig. 5) showed that most *S. enteritidis* serovar isolates were resistant to 1 and 2 antibiotics (35 isolates – 95%), only one isolate was polyresistant.

The largest number of polyresistant isolates was observed in the following serovars *S. virchow* (56%), *S. nigeria* (71%), *S. infantis* (40%), and *S. papuana* serovar was represented only by polyresistant isolates (100%), out of which 1 culture was resistant to 5 antimicrobials.

One of *S. colindale* serovar isolates was resistant to 7 antimicrobials.

In the course of the research, it was found that 36 isolates (97%) of *S. enteritidis* serovar are resistant to nalidixic acid, which belongs to the quinolone group and is the highest priority drug included in the WHO list of critically important antimicrobials in human medicine [21]. It is worth noting that resistance to nalidixic acid was also observed in isolates of *S. virchow, S. infantis, S. nigeria* and *S. papuana*.

Resistance to amoxicillin was reported in 5 isolates of *S. enteritidis* (14%), to trimethoprim/sulfamethoxazole – in 3 isolates (8%) and to tetracycline – in 1 culture (Fig. 5).

All 9 *S. virchow* isolates were resistant to nalidixic acid (100%), and 7 isolates (78%) showed no susceptibility to trimethoprim/sulfamethoxazole. *S. nigeria* isolates (n = 7) were 100% resistant to nalidixic acid, and 86% resistant to trimethoprim/sulfamethoxazole. *S. papuana* isolates (n = 5) showed resistance to 6 antimicrobials, while all cultures were resistant to trimethoprim/sulfamethoxazole. *S. infantis* isolates (n = 5) were resistant to nalidixic acid (4 isolates), to tetracycline (2 isolates) and to trimethoprim/sulfamethoxazole (3 isolates).

Isolates of *S. agona* (n=3) were resistant to amoxicillin, ampicillin, nalidixic acid, streptomycin and tetracycline (Fig. 6). All isolates of *S. colindale* (n=2) were resistant to nalidixic acid (100%) and to 7 antimicrobials. Two isolates of *S. typhimurium* serovar were reported to be resistant to nalidixic acid and azithromycin. Untyped *Salmonella* isolates showed no susceptibility to 9 antibiotics. At the same time, 2 isolates were resistant to ciprofloxacin.

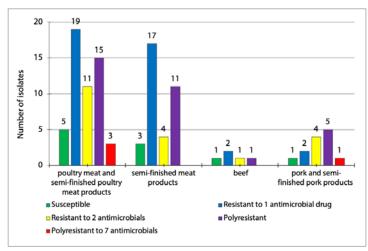


Fig. 3. Antimicrobial susceptibility of Salmonella isolates recovered from products of animal origin

According to the government report "On sanitary and epidemiological well-being of the population in the Russian Federation in 2020", more than 60% of *Salmonella* isolates were found to be resistant to antimicrobials, of which 75% of *S. enteritidis* isolates were resistant to ciprofloxacin [4]. Our study showed that the proportion of isolates resistant to antibiotics was 91%, while 97% of *S. enteritidis* isolates were resistant to nalidixic acid.

It should be noted that a significant part of *Salmo-nella* isolates are polyresistant. These data are consistent with those ones from the relevant EU reports, which note polyresistance in monophasic variants of *S. typhimurium* (56.7% of isolates), *S. kentucky*, *S. infantis*, *S. typhimurium* and *S. enteritidis*.

Strains isolated from food products are more likely to be polyresistant, unlike strains isolated from humans (41.6% vs. 15.8%). Most of these strains are represented by serovars *S. typhimurium* (over 60%) and *S. infantis*

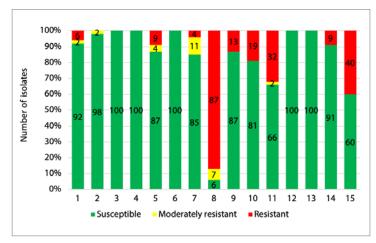


Fig. 4. Antimicrobial resistance of Salmonella isolates recovered from poultry meat and semi-finished poultry meat products:

1 – levomycetin, 2 – cefotaxime, 3 – gentamicin, 4 – amikacin, 5 – streptomycin,
6 – kanamycin, 7 – ciprofloxacin, 8 – nalidixic acid, 9 – ampicillin, 10 – amoxicillin,
11 – tetracycline, 12 – meropenem, 13 – imipenem, 14 – azithromycin,
15 – trimethoprim/sulfamethoxazole

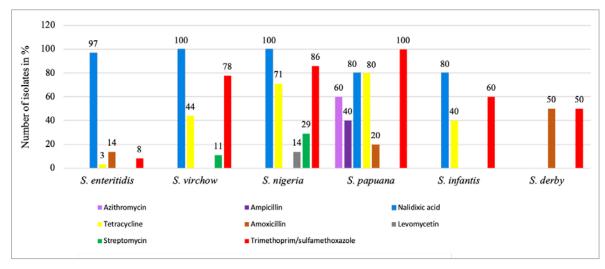


Fig. 5. Resistance of isolates of the following serovars S. enteritidis, S. virchow, S. nigeria, S. papuana, S. infantis and S. derby to antimicrobials

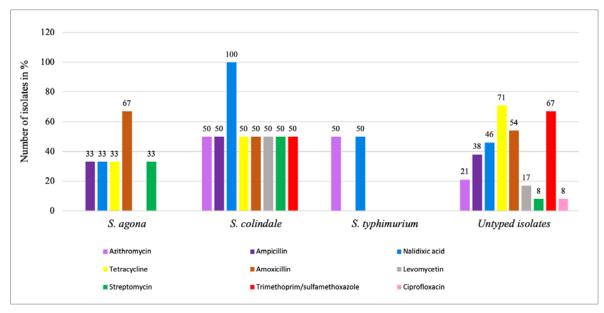


Fig. 6. Resistance of isolates of serovars S. agona, S. colindale, S. typhimurium and untyped cultures to antimicrobials

(over 80%). In general, *Salmonella* resistance to high concentrations of ciprofloxacin has increased [22].

In 2020, isolates of *S. enteritidis* serovariant were most often recovered from animal products in the Russian Federation, which is consistent with the data we obtained. This serovariant was most often recovered (52%) from poultry meat samples, followed by *S. typhimurium* and *S. infantis*, respectively.

Half of *S. typhimurium* isolates was susceptible to all antibiotics, while 30% had resistance to two classes of antimicrobials (penicillins, tetracyclines). Antimicrobial resistance was noted in 90% of *S. infantis* isolates, while resistance to 3 or more medicinal preparations was reported in 59% of them. At the same time, all the studied isolates were susceptible to glycocycline, polymyxins, carbopenems, first-generation cephalosporins and third-generation aminoglycosides [4].

In recent years, there have been reports on Salmonella isolates resistant to antimicrobials widely used in human

and veterinary medicine, such as sulfonamides (30.5%), tetracyclines (28.8%) and ampicillin (25.9%). The resistance of individual *Salmonella* serovars to these compounds varied from low in *S. enteritidis* (4.5–7.8%) to high in the monophasic variant *S. typhimurium* (86–88%) and *S. kentucky* (71–76%) [23–27].

CONCLUSION

One hundred and six isolates of *S. enterica* subsp. *enterica* were recovered from 4,500 samples of animal products tested in the FGBI "ARRIAH" Laboratory for Microbiological Research from 2019 to 2020. 77% out of the isolates belonged to 17 serological variants, and the rest were untyped (23%). Isolates of *S. enteritidis* serovariants dominated (n = 37) and *S. virchow* (n = 9), which is consistent with the data provided by other authors.

While determining antimicrobial susceptibility of Salmonella isolates, we detected differences in the ratio

between susceptible and resistant *Salmonella* of different serological variants in relation to antibiotics of ten pharmacological groups.

Salmonella isolates of all the studied serovariants were found to be susceptible to the following antibiotics: amikacin (100%), gentamicin (100%), kanamycin (98%), imipenem and meropenem (100%).

Salmonella isolates were resistant to quinolones (nalidixic acid) – 74% and to sulfonamides (trimethoprim/sulfamethoxazole) – 45%. The proportion of isolates resistant to one group of antimicrobials was 38%, and 34% were polyresistant, including cultures resistant to 7 antibiotics (4%).

The largest number of polyresistant isolates was noted in *Salmonella* serovars *S. virchow, S. nigeria, S. infantis, S. colindale,* and *S. papuana* serovar was represented only by polyresistant isolates (100%).

Resistant and polyresistant *Salmonella* isolates was most often recovered from poultry products. Cultures resistant to ciprofloxacin were found only in poultry meat. *Salmonella* isolates resistant to nalidixic acid and trimethoprim/sulfamethoxazole were detected in all groups of animal products, and the maximum number of such isolates was recovered from poultry meat – 87% (n = 46) and 40% (n = 21), respectively. Isolates resistant to amoxicillin were recovered only from pork samples – 69% (n = 9).

Serovar *S. typhimurium*, which is known as polyresistant according to the literature, was resistant to one or two antimicrobials in our studies.

Isolates of 9 serovars showed resistance to nalidixic acid, while the proportion of *S. enteritidis* isolates resistant to this product was 97% (n=36). Isolates of *S. colindale* serovar (n=2) were resistant to 8 antimicrobials, isolates of *S. papuana* (n=5) were resistant to 6 antibiotics, isolates of *S. agona* (n=3) – to 5 medicinal products. The untyped *Salmonella* isolates were resistant to 9 antibiotics, including ciprofloxacin (2 isolates).

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Natalya B. Shadrova, Candidate of Science (Biology), Head of Laboratory for Microbiological Testing, FGBI "ARRIAH", Vladimir, Russia

Olga V. Pruntova, Doctor of Science (Biology), Professor, Chief Expert of the Information and Analysis Centre, FGBI "ARRIAH", Vladimir. Russia.

Evgenia A. Korchagina, Leading Biologist, Laboratory for Microbiological Testing, FGBI "ARRIAH", Vladimir, Russia.

Шадрова Наталья Борисовна, кандидат биологических наук, заведующий лабораторией микробиологических исследований ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Прунтова Ольга Владиславовна, доктор биологических наук, профессор, главный эксперт информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Корчагина Евгения Александровна, ведущий биолог лаборатории микробиологических исследований ФГБУ «ВНИИЗЖ», г. Владимир, Россия.