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Poultry Salmonella Sensitivity to Antibiotics

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ABSTRACT

The objects of study were microorganisms, isolated from pathological material of chicken breeds "RoSS 208" and "Luong Phuong"; ducklings breeds "C. V. super M" and a "Khaki Campbell"; quails breed "Coturnix". In 499 samples of the birds material we discovered 153 microorganisms cultures of the genus Salmonella, which were 30.66 % of the total number of selected microorganisms cultures, including S. typhimurium – 60 (39.22 %) strains; S. albany – 32 (of 20.92 %); S. enteritidis – 20 (13.07 %); S. hadar – 9 (5.88%); S. agona and S. thompson – 5 (3.27 %); S. indiana – 4 (2.61 %); S. heidelberg, S. mbandaka and S. shalkwijk – 3 (1.96 %); S. give – 2 (1.31 %); S. derby and S. havana – 1 (0.65 %); other serovar – 5 (3.27 %). It was found that 70.0 % of the studied strains were sensitive to drugs of the fluoroquinolone group (nalidixic acid, norfloxacin, ciprofloxacin, enrofloxacin), 66.67 % – cephalosporins (ceftazidim). 83.33 % of strains were resistant to tetracycline drugs (tetracycline); 63.33 % - β-lactams (ampicillin); 56.67 % - aminoglycosides (gentamicin, kanamycin, streptomycin); 46.67 % - sulfonamides (trimethoprim). The minimum inhibitory concentration ("MIC") were established at the intersection of the growth suppression zone and the test strip "E-test": ampicillin-512 μg/ml; tetracycline – 64 μg/ml; gentamicin – 2 μg/ml; ciprofloxacin – 1 μg/ml; norfloxacin – 0.38 μg/ml and ceftazidim – 0.19 μg/ml. The amplitude values ranged from 0.019 μg/ml to 512 μg/ml.

Key words: Bacteria Salmonella / Antibiotic susceptibility / Resistance of Salmonella / Poultry / Chicken salmonellosis / Duckling salmonellosis / Quails salmonellosis.

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INTRODUCTION

Widespread and arbitrary use of antibacterial drugs in veterinary medicine, including the production of food for animals, is one of the main factors contributing to the development and spread of microbial resistance to antibacterial drugs.[1,2,3,4] Salmonellosis in animals and humans can occur due to 2500 serovars.[5] Resistance of *Salmonella* to antibiotics has dramatically increased worldwide recent years.[6] Poultry and poultry products are known reservoirs for pathogenic microorganisms, and numerous reports describe the prevalence of *Salmonella* associated with live poultry, the production environment and processing plants.[7,8]

Antibiotics were used in poultry to treat infections, and to counteract the adverse effects of stress reactions. Growing *Salmonella* resistant strains in human are associated with widespread use of antibacterial drugs in animal feed production.[9] The presence of antimicrobial residues in meat has several consequences for human health, as a possible contribution is the development of bacteria resistance to antibacterial drugs.[10] Bacterial biofilms are closely associated with clinical infections and promote drug resistance.[11] In biofilms, bacteria have increased antibiotic resistance compared to the plankton form, which leads to low treatment efficiency.[12,13] Biofilm is usually defined as a structured community of bacterial cells enclosed in a polymer matrix of its own production and attached to an inert or living surface.[14] Bacterial biofilms promote drug resistance, in particular, multi-resistant strains of *Salmonella* formed biofilms for the period from 18 to 30 hours. The results showed a potential antibacterial effect of Berberine on *Salmonella* strains. A partial index of inhibitory concentration of 0.75 revealed a synergistic effect of Berberine and Ciprofloxacin against strains of Salmonella. Berberine increases the antimicrobial activity of Ciprofloxacin against *Salmonella*, and their combination may reduce the dose of Ciprofloxacin required for therapy, providing an additional benefit to avoid drug resistance.[15]

Changes in enzymatic traits are associated with obtaining genetic information with lactose operon, plasmid "Bluescript" in the composition of this operon contains the gene of resistance to ampicillin, so it is recommended to use a duplex polymerase chain reaction based on the identification of polymorphic sites of isolates genes.[1] The emergence and spread of antibacterial-resistant bacteria has been identified as a global public health priority and underlines the importance of an epidemiological surveillance system for monitoring antibiotic resistance.[16,17,18]

This can be a public health risk in the transmission of resistant strains of *Salmonella* to humans through the consumption of contaminated poultry products.[19] Growing resistant strains of *Salmonella* isolated from cases of salmonellosis in humans were associated with the

widespread use of antibacterial drugs in the production of food products for animals.[2,20]

Therefore, the purpose of this study was to identify the *Salmonella* stains, isolated from the pathology of chickens, ducklings, quails. In addition, we analyzed the antimicrobial sensitivity to antibacterial drugs.

MATERIALS AND METHODS

The objects of study were microorganisms, isolated from pathological material of chicken breeds "RoSS 208" and "Luong Phuong"; ducklings breeds "C. V. super M" and a "Khaki Campbell"; quails breed "Coturnix" from the provinces of Hanoi, Bacninh, Quangninh Vietnam.

Samples were collected from pathological poultry material. Muller-Kauffmann Tetrathionate-Novobiocin Broth (Biokar Diagnostic) broth, which supports the growth of salmonella serovars, pathological material, and subcultures on the media: "Rappaport -Vassiliadis Medium"-"MSRV-agar" (Biokar Diagnostic), "Ram bach agar", Xylose Lysine Deoxycholate agar -"XLD – agar" (Merck) was performed in accordance with standard methods. Gram-negative bacteria that form on the "XLD – agar" reddish colonies with black center and transparent zone were further processed and identified by biochemical tests like Catalase test, Citrate test, Triple Sugar iron test, Urease test, Motility test, Methyl-Red and Voges-Proskauer test, Gas production test, Sulfide production test and confirmed.

The sensitivity of microorganisms to antibiotics was studied in accordance with the guidelines "Methodical Instructions 4.2.1890-4 Determination of the micro-organisms sensitivity to antibacterial drugs." Determination of the minimum inhibitory concentration ("MIC") of antibiotics was performed using "E-test" (AB-Biodisk, Sweden).

The study of the sensitivity of microorganisms to antibacterial drugs by disco-diffusion method was carried out on the medium "Muller–Hinton" (HiMedia) using standard paper discs with antibacterial drugs (Ampicillin, Gentamicin, Kanamycin, Nalidixic acid, Norfloxacin, Streptomycin, Tetracycline, Trimethoprim, Ceftazidim, Ciprofloxacin, Enrofloxacin).

For inoculation we used a standard bacterial suspension equivalent to 0.5 according to the Mcfarland standard, diluted 100 times on a nutrient broth, the concentration of the microorganism in it was approximately 1.5 x 10^8 CFU/ml. Suspension was applied with a pipette to the surface of the Petri dish with a Muller–Hinton medium in the volume of 1-2 ml, evenly distributed over the surface by swaying, after which the excess was removed with a pipette, the surface of the medium was dried. Application of paper disks was carried out using sterile tweezers or an automatic dispenser. Immediately after the application of paper discs, Petri dishes were placed in a thermostat upside down and cultivated at 37°C for 18-24 hours. Diameter of the growth inhibition zones was measured to an accuracy of 1.0 mm. When measuring the growth inhibition zones, they were oriented to the zone of complete suppression of visible growth.

Determination of the minimum inhibitory concentration ("MIC") of antibiotics was performed using "E-test" (AB-Biodisk, Sweden), which is a narrow strip of polymer (0,5x6,0 cm), which is applied to the gradient of antibacterial drugs concentrations (from minimum to maximum). Suppression of microbial growth around the "E-test" strip was taken into account in the area where the concentration of antibacterial drugs diffusing from the strip is higher than the minimum inhibitory concentration, thus forming a teardrop-shaped inhibition zone. The values of the antibacterial drugs concentration in each area of the strip are printed on the outer surface of the "E-test". The value of the minimum inhibitory concentration was taken into account in the area where the boundary of the growth inhibition zone came close to the strip.

The experimental data were statistically processed using the program "Statistica" for PC Microsoft Excel 2007. In the results statistical analysis of experimental studies the differences were considered significant at $p \leq 0.05$.

RESULTS

In 499 samples of the birds material, including 115 samples of chickens, 300 – ducklings, 84 – quails, we discovered 153 microorganisms cultures of the genus *Salmonella*, which were 30.66 % of the total number of selected microorganisms cultures. The etiological structure of poultry salmonellosis is shown in FIG 1.

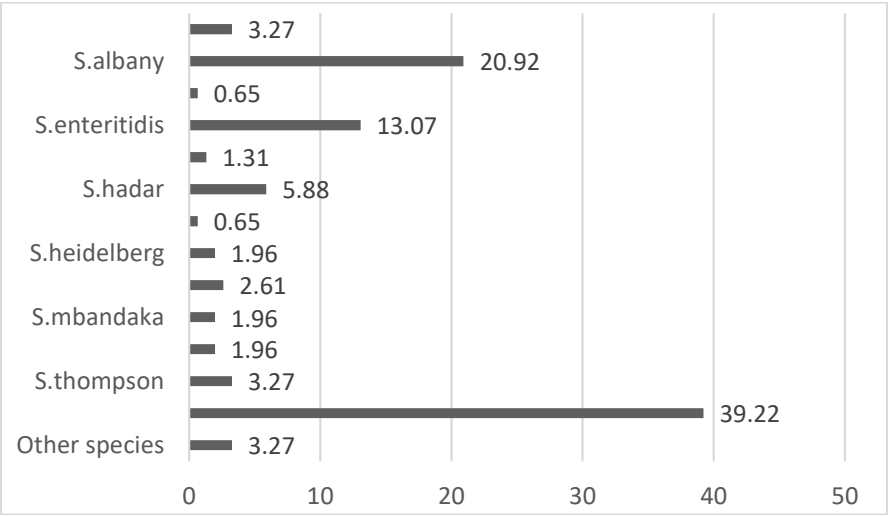

FIG. 1. Study of the etiological structure of poultry salmonellosis.

Among the isolated cultures of microorganisms after serological identification *S. typhimurium* were 60 (39.22 %) strains; *S. albany* – 32 (20.92 %); *S. enteritidis* – 20 (13.07 %); *S. hadar* – 9 (5.88 %); *S. adopa* and *S. thompson* – 5 (3.27 %); *S. indiana* – 4 (2.61 %); *S. heidelberg*, *S. mbandaka* and *S. shalkwijk* – 3 (1.96 %); S. give – 2 (1.31 %); *S. derby* and *S. havana* – 1 (0.65 %); other species – 5 (3.27 %).

52 cultures of microorganisms of genus *Salmonella* were isolated from 115 samples of chicken pathological material, which is 45.21 % of the total number of selected cultures of microorganisms. *S. albany* were 32 (61.54 %) strains; *S. typhimurium* and *S. enteritidis* – 5 (9.62 %); *S. agona* and *S. shalkwijk* – 3 (5.77 %); *S. hadar* – 2 (3.85 %); *S. derby* – 1 (1.92 %); other species – 1 (1.92 %).

74 strains of *Salmonella* were isolated from 300 samples of ducklings patmaterial, predominant serotypes were *S. typhimurium* – 51 (68.92 %) strains, *S. enteritidis* – 7 (9.46 %), *S. hadar* – 5 (6.76 %), *S. indiana* – 4 (5.41 %), *S. give* and *S. mbandaka* – 2 (2.70 %), other species – 3 (4.05 %).

27 strains of *Salmonella* (32.14%) were isolated from 84 samples of quail patmaterial, including *S. enteritidis* of 8 (29.63 %) strains; *S. thompson* – 5 (18.52 %); *S. typhimurium* – 4 (14.81 %); *S. heidelberg* – 3 (11.11 %); *S. hadar* and *S. agona* – 2 (7.41 %); *S. mbandaka* and *S. havana* – 1 (3.70 %); others species – 1 (3.70 %) (TABLE 1).

TABLE 1. Results of serological identification of Salmonella.

Material / Bacterial Strains	Chickens		Ducks		Quails		Total	
	Ab.	*%*	*Ab.*	*%*	*Ab.*	*%*	*Ab.*	*%*
S. agona	3	5.77	-	-	2	7.41	5	3.27
S. albany	32	61.54	-	-	-	-	32	20.92
S. derby	1	1.92	-	-	-	-	1	0.65
S. enteritidis	5	9.62	7	9.46	8	29.63	20	13.07
S. give	-	-	2	2.70	-	-	2	1.31
S. hadar	2	3.85	5	6.76	2	7.41	9	5.88
S. havana	-	-	-	-	1	3.70	1	0.65
S. heidelberg	-	-	-	-	3	11.11	3	1.96
S. indiana	-	-	4	5.41	-	-	4	2.61
S. mbandaka	-	-	2	2.70	1	3.70	3	1.96
S. shalkwijk	3	5.77	-	-	-	-	3	1.96
S. thompson	-	-	-	-	5	18.52	5	3.27
S. typhimurium	5	9.62	51	68.92	4	14.81	60	39.22
others species	1	1.92	3	4.05	1	3.70	5	3.27

In the study of *Salmonella* sensitivity isolated from birds to antibacterial drugs found that 3 (10.0 %) were sensitive to drugs group β-lactams (Ampicillin); 7 (23.33 %) - aminoglycosides (Gentamicin, Kanamycin, Streptomycin); 9 (30.0 %) - sulfonamides (Trimethoprim); 3 (10.0 %) - tetracycline (Tetracycline); 21 (70.0 %) - fluoroquinolones (Nalidixic acid, Norfloxacin, Ciprofloxacin, Enrofloxacin); 20 (66.67 %) - cephalosporins (Ceftazidim).

Moderate resistance was in 8 (26.67 %) strains to β-lactam drugs; 6 (20.0 %) - aminoglycosides; 7 (23.23 %) - sulfonamides; 2 (6.67 %) - tetracyclines; 4 (13.33 %) - fluoroquinolones; 6 (20.0 %) - cephalosporins. Resistance to antibiotics was in 19 (63.33 %) strains to the group β-lactams; 17 (56.67 %) aminoglycoside; 14 (46.67 %) - sulfonamides; 25 (83.33 %) - tetracycline; 5 (16.67 %) - fluoroquinolone; 4 (13.33 %), cephalosporins (TABLE 2).

TABLE 2. Results of the Salmonella sensitivity to antibacterial drugs

Antibiotics	Total number test strains	Research results					
		Sensitive		*Moderate resistant*		*Resistant*	
		Ab.	(%)	Ab.	(%)	Ab.	(%)
β-lactams							
Ampicillin	30	3	10.0	8	26.67	19	63.33
Aminoglycosides							
Gentamicin	30	18	60.0	2	6.67	10	33.33
Kanamycin	30	3	10.0	15	50.0	12	40.0
Streptomycin	30	1	3.33	0	0	29	96.67
Sulfonamides							
Trimethoprim	30	9	30.0	7	23.33	14	46.67
Tetracyclines							
Tetracycline	30	3	10.0	2	6.67	25	83.33

Fluoroquinolones							
Nalidixic acid	30	18	60.0	1	3.33	11	36.67
Norfloxacin	30	26	86.67	3	10.0	1	3.33
Ciprofloxacin	30	21	70.0	4	13.33	5	16.67
Enrofloxacin	30	18	60.0	10	33.33	2	6.67
Cephalosporins							
Ceftazidime	30	20	66.67	6	20.0	4	13.33

The minimum inhibitory concentration ("MIC") were established at the intersection of the growth suppression zone and the test strip "E-test": Ampicillin-512 µg/ml; Tetracycline – 64 µg/ml; Gentamicin – 2 µg/ml; Ciprofloxacin – 1 µg/ml; Norfloxacin – 0.38 µg/ml and Ceftazidim – 0.19 µg/ml. *Salmonella* sensitivity to Ceftazidim started from 0.19 µg/ml, Norfloxacin - 0.38 µg/ml, Ciprofloxacin -1 µg/ml, Gentamicin - 2 µg/ml. Resistance was to ampicillin (256 µg/ml) and tetracycline (64 µg/ml). Amplitude values ranged from 0.019 µg/ml to 512 µg/ml (TABLE 3).

TABLE 3. Minimum inhibitory concentration to antibiotics (n=30)

Antibiotics	MIC_{50}	MIC_{90}	Amplitude values
Ampicillin	1.5	512	0.75-512
Gentamicin	1.5	2	0.75-256
Norfloxacin	0.023	0.38	0.016-64
Tetracycline	3	64	1-512
Ceftazidime	0.125	0.19	0.064-512
Ciprofloxacin	0.125	1	0.064-64

Minimum inhibitory concentration (MIC)

CONCLUSION

Antibiotics are widely used to treat infections in humans, animals, food, and plants. The uncontrolled and broad use of antibiotics against pathogens has resulted in the occurrence of antibiotic-resistant bacteria. According to the literature, *Salmonella*, circulating among poultry and isolated from poultry products and raw materials, have resistance to many antibacterial drugs, especially Ampicillin, Tetracycline and Streptomycin. Thus, 114 serotypes of *Salmonella (Corvallis, Brancaster and Albany)* isolated from poultry were considered multiresistant. Resistance was established to Sulfonamides (96.5%), Ampicillin (89.5%), Tetracycline (85.1%), Chloramphenicol (75.4%), Trimethoprim (68.4%), Trimethoprim-Sulfametoxazole (67.5%), Streptomycin (58.8%) and Nalidixic acid (44.4%). All strains could produce biofilms. Strong producers of biofilm were 69.3% of the studied strains.[21]

Salmonella resistance was reported for Doxycycline (100%), Oxytetracycline (97.62%), Neomycin (8.10%), Erythromycin (83.33%), Tetracycline (78.57%) and Ceftizoxime (35.71%). Also resistance was established to Norfloxacin (26.19%), Ampicillin (21.43%), Azithromycin (21.43%), Ciprofloxacin (19.05%), Colistin (4.76%), Streptomycin (16.67%), Cefotaxime (14.19%), Enrofloxacin (14.29%), Amoxiclavu (14.29%), Gentamicin (7.14%), Chloramphenicol (4.76%), Amikacin (4.76%) and Ceftazidim.[8,10,17,22,23,24]

Studying the circulation of antibiotic-resistant strains in the environment, *Salmonella spp.* bacteria were 27.0 %, including *S. enteritidis* – 5.9 %; *S. typhimurium* – 19.1 %, the most common resistance to Tetracycline – 100.0 %; Doxycycline – 100.0 %; Ampicillin – 100.0 %; Sulfametoxazole – 92.9%; Nalidixic acid – 85.8 %; Ceftazidim – 78.6 %; Neomycin – 64.3%; Streptomycin – 50.0 %; Gentamicin – 28.6 %, all isolates were sensitive to Ciprofloxacin.[25] It was found that 45.0 % of isolates showed multiple resistance to 1-5 drugs; 29.0 % – to 6-10 drugs; 22.0 % – to 11-15 drugs.[26]

189 *Salmonella* strains showed high resistance to the following drugs: Nalidixic acid (99.5%), Ampicillin (87.8%), Tetracycline (51.9%), Ciprofloxacin (48.7%), Trimethoprim / Sulfametoxazole (48.1%) and Spectinomycin (34.4%). Moreover, 60.8% of isolates were resistant to multiple drugs, and multidrug-resistant strains increased from 44.7% to 78.6%.[20]

Products from broiler chickens at the slaughterhouse were contaminated with multiple-resistant *Salmonella* strains, which can come from animals producing food, and cross-contamination during slaughter, which contributes to the spread of resistance genes throughout the production chain.[1,27,28]

21 strains of *Salmonella* isolated from broiler carcasses, 20 strains (95.2%) were resistant to Ampicillin, 18 strains (85.7%) to Nalidixic acid, 17 (81%) to Cefotaxime and 13 strains (61.9%) to Tetracycline. 19 strains of Salmonella isolated from egg-laying chickens, 19 strains (100%) were resistant to Nalidixic acid and Tetracycline, 18 strains (94.7%) to Ampicillin and 13 strains (68.4%) to Ciprofloxacin. Resistant Salmonella strains can pose a risk to public health because they are widely found in raw chicken carcasses of commercial broilers and egg-laying chickens.[3]

Multiple resistance to antibacterial drugs is one of the factors of distribution and circulation of microorganisms in the environment and the food chain, largely due to the ability to form biofilms.[29,30] Bacteria in biofilms increase antibiotic resistance compared to the plankton form, which leads to low treatment efficiency. [4,12,31] The emergence and spread of bacterial resistance has been identified as a global public health priority and underlines the importance of the surveillance system for monitoring antibiotic resistance.[6,17,32]

The adding of antibiotics to animal and poultry feed causes

the accumulation of drugs in the body of animals and animal products.[16,18,33,34]

The results of the research will create a database of antibiotic resistance drift in *Salmonella* sp. and determine recommendations for the use of antibacterial agents to reduce resistance to antibacterial drugs.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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