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STUDY OF ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA* SPP. ISOLATED FROM FOOD AND BIOLOGICAL MATERIAL

Liliia VYGOVSKA, Vitalii NEDOSEKOV, Valerii USHKALOV, Oksana BOYKO, Oleksandra KEPPLE, Yuriy VISHOVAN, Sergiy TERESHCHENKO, Liliana DAVYDOVSKA, Sergiy BOIANOVSKIY, Valentina MOMOT, Tatyana KORCHOVA

Ukrainian Laboratory of Quality and Safety of Agricultural Products,
National University of Life and Environmental Sciences of Ukraine

Corresponding author: Liliia Vygovska, e-mail: lnvygovska@gmail.com

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Key words: *Salmonella spp., biological properties, antibiotics, resistance, susceptibility.*

Introduction. *Antimicrobial resistance is a global public health problem that the world is facing today.*

Material and methods. *Isolation and identification of Salmonella spp. were performed according to DSTU EN 12824: 2004; DSTU ISO 18593: 2006 standards; Nutrient media were manufactured in accordance with DSTU EN ISO 11133: 2014 standard. The antibiotic susceptibility of cultures was determined by the disk diffusion method. The research findings were both studied and interpreted in accordance with EUCAST recommendations.*

Results. *Out of 10 samples (material was obtained from animals, humans and foods), 10 cultures of Salmonella spp. were isolated and analyzed. The cultures were mainly susceptible to semisynthetic and inhibitor-protected penicillins, cephalosporins, carbapenems and more often to tetracyclines, macrolides, lincosamides. Most Salmonella spp. strains were intermediate susceptibility to aminoglycosides, 30% of Salmonella spp. strains were susceptible to pefloxacin (II) and only 10% were susceptible to ciprofloxacin (II).*

Conclusions. *The obtained data indicate the screening feasibility of antibiotic susceptibility of Salmonella spp. including a wide range of tested drugs, which may be important in determining the antibiotic therapy.*

Cuvinte cheie: *Salmonella spp., proprietăți biologice, antibiotice, rezistență, sensibilitate.*

STUDIEREA SENSIBILITĂŢII LA ANTIBIOTICE A SPECIILOR DE *SALMONELLA*, IZOLATE DIN MATERIALE BIOLOGICE ŞI PRODUSE ALIMENTARE

Introducere. *Actualmente, rezistența antimicrobiană este o problemă globală de sănătate publică.*

Material și metode. *Izolarea și identificarea Salmonella spp a fost efectuată conform standardelor DSTU EN 12824:2004; DSTU ISO 18593:2006; mediile nutritive fiind preparate în conformitate cu standardul DSTU EN ISO 11133:2014. Sensibilitatea tulpinilor la antibiotice a fost determinată prin metoda disc-difuzimetrică, iar studiul și interpretarea rezultatelor au fost realizate în conformitate cu recomandările EUCAST.*

Rezultate. *Din 10 probe (material prelevat de la animale, oameni și din alimente) au fost izolate și studiate 10 tulpini de Salmonella spp. Preponderent, tulpinile sunt sensibile la penicilinele de semisintetice și cele protejate de inhibitori, cefalosporine, carbapeneme; mai frecvent prezentând rezistență la tetracicline, macrolide și lincosamide. Pentru aminoglicozide însă majoritatea tulpinilor Salmonella spp. au prezentat sensibilitate intermediară, astfel, din 30% tulpini Salmonella spp. sensibile la pefloxacină (II), doar 10% au fost sensibile la ciprofloxacină (II).*

Concluzii. *Datele obținute indică fezabilitatea screening-ului sensibilității Salmonella spp. la antibioticele din cea mai largă listă posibilă de preparate testate, impunându-se drept acțiune oportună în determinarea antibioticoterapiei.*

INTRODUCTION

Over the XXI century, infectious diseases are still the main causes of death worldwide (1, 2). Recently, the spread of antibiotic-resistant microorganisms has become a public concern. Antimicrobials have been used in the production of livestock products for therapeutic and prophylactic purposes, which contributed to the development of adaptive mechanisms to the applied antibiotics. This, in turn, served as an impetus for the production of new antimicrobial agents.

The Ukrainian agriculture sector shows a considerable sample size among global production volumes (3, 4). An increase in both the demand and production volume of agricultural products is accompanied by a free section of the continental boundaries of semi-finished or finished agri-food products (5). Migration of people, birds and terrestrial wild animals also has contributed to the microbial long-distance transfer (5, 6, 7, 8). All these factors have differently promoted the spread of multi-resistant microbial strains to the existing antibiotics both due to their transportation by living organisms over long distances, and due to the production of the agro-industrial complex.

The purpose of this work is to study the biological properties and assess the antibiotic resistance among various groups *Salmonella* spp. cultures, isolated from biological samples taken from Kiev, Volyn region.

MATERIAL AND METHODS

Salmonella spp. strains were isolated and identified from the research materials according to: DSTU EN 12824:2004 Microbiology of food products and animal nutrition. Horizontal method for detecting *Salmonella* spp.; DSTU 4769:2007 Bacteriological study of pathological material from animals. Methods of detecting salmonella; MU 4.2.2723-10 "Laboratory diagnostics of salmonella, detection of *Salmonella* in food products and environmental objects", 2010; DSTU ISO 18593:2006 "Microbiology of food products and animal nutrition. The study of microbial fingerprinting and washings from the surface". Antibiotic susceptibility testing by disc diffusion method, using HiMedia's discs. The research findings were studied and interpreted in accordance with EUCAST guidelines (9).

RESULTS

The microbial cultures were isolated during the microbiological assessment of materials taken from Kiev, Volyn region.

The study confirmed 6 samples of biomaterial, obtained from birds and 6 *Salmonella* spp. strains were identified: *S.* Typhimurium (from geese), *S.* Virchow (from ducks), *S.*Virchow (from chickens), *S.* Gallinarum, S. Pullorum (from chickens), *S.* Enteritidis (from chickens) *S. Dublin* (from calf); two cultures – from food samples (*S. Enteritidis*, which are rare *Salmonella* species (F-67+) and two cultures (*S.* Enteritidis, *S.* Typhimurium) found in people with symptoms of food poisoning).

The bacterial cultures formed a uniform turbidity and a small amount of white amorphous sediment in the Pepted Meat Broth, which was easily broken-down while being shaken. The cultures of *S.* Dublin, *S.* Gallinarum, *S.* Pullorum formed growth rings. The bacterial cultures formed transparent, tender, greyish and 3-4 mm S-colonies in Meat Infusion Agar.

The laboratory animals (white mice weighing 16-18 g) which were subcutaneously administered doses of 0.5×10^9 CFU/cm^3 died in 100% of experimental animals within 8 hours.

The research findings showed that the studied cultures in 100% of cases were susceptible to ampicillin and ampicillin/sulbactam, whereas were resistant to benzylpenicillin and methicillin; 90% of strains were resistant to oxacillin; 50% of cultures were susceptible to ticarcillin and 10% – resistant to ticarcillin/clavulanic acid; 30% of strains were susceptible and 60% were resistant to amoxicillin; 30% of cultures were susceptible 30% – resistant to piperacillin; 20% of strains were susceptible and 80% – resistant to carbenicillin.

The structural similarity between cephalosporins and penicillins causes the same mechanism of antimicrobial action and cross-allergies in some patients (10, 11). The studied *Salmonella* spp. strains showed susceptibility to most of cephalosporins; no direct dependence of levels of antimicrobial susceptibility to various generation of drugs was found: 80% of the studied strains showed susceptibility and 10% showed resistance to cefazolin (I); 60% of cultures showed susceptibility and 30% showed resistance to cephalexin (I); 90% of the studied bacterial strains were susceptible and 10% of cultures were resistant to cefaclor (II).

20% of the studied cultures were susceptible and 60% are resistant to cefuroxime (II); 90% of the studied cultures were susceptible to cefamandole (II), no resistant cultures were detected; 80% of the studied cultures were susceptible and 20% are resistant to cefixim (III); 30% of the studied cultures were susceptible and 30% were resistant to cefoperazone (III); 80% of the cultures of the studied cultures showed susceptibility to cefotaxim (III), no resistant cultures were detected; 50% of the studied bacterial strains showed susceptibility and 20% were resistant to ceftazidime (III); 20% of the studied strains were susceptible and 20% were resistant to ceftriaxone (III); 100% of the studied strains showed resistance to cefepim (IV).

Due to the natural activity of carbapenems (imipenem and meropenem) against enterobacteria, the studied cultures were found susceptible to imipenem (70%) and meropenem (80%).

No *Salmonella* spp. resistant to carbapenems was detected.

The most studied cultures exhibited a moderate resistance to aminoglycosides: 20% of cultures were susceptible and 30% were resistant to streptomycin (I); 60% – susceptible and 10% – resistant to gentamicin (I); 20% – susceptible and 20% – resistant to kanamycin (I); 20% of cultures are susceptible and 80% – intermediate susceptible to neomycin (I); 40% –and 10% – resistant to tobramycin (II); except for netilin (II), to which 100% of the cultures were susceptible, and amikacin (III), which showed an inhibitory effect on 20% of the studied *Salmonella* spp. strains

Salmonella spp. have natural resistance to macrolides (12, 13). Our studies also proved that *Salmonella* spp. is resistant to macrolides. However, cultures of *S.* Gallinarum, *S.* Pullorum, *S.* Dublin, *S.* Virchow (Q) were susceptible to azithromycin (15); *S.* Virchow (Q) strain was susceptible to clarithromycin.

Most of the *Salmonella* spp. showed natural resistance to tetracyclines. The culture of *S.* Gallinarum Pullorum was susceptible to tetracycline, doxycycline; *S.* Typhimurium *(F)* showed sensitivity to Tetracycline.

High concentrations of linquamides may also exhibit bactericidal effects to relatively high susceptible microorganisms (13, 14). The studies conducted on microbial resistance to lincosamides showed susceptibility to lincomycin and clindamycin, except for *S.* Virchow (Q) strain.

Depending on the mechanism of action, quinolones differ completely from other AMP drugs. 50% of *Salmonella* spp. were susceptible to nalidixic acid (I), norfloxacin (II), levofloxacin (III), and gatifloxacin (IV). The remaining drugs showed a susceptibility of 30% (pefloxacin (II) to 10% (ciprofloxacin (II), ofloxacin (II), lomefloxacin (II)) of the studied cultures of *Salmonella* spp.

Pathogenic microorganisms rarely develop antibiotic resistance to nitrofurans. (19, 20, 21). The study results showed that 40% of the studied cultures were susceptibile to furazolidone.

All the studied *Salmonella* spp. strains were susceptibile to chloramphenicol

DISCUSSIONS

Ten *Salmonella* spp. strains were isolated from 10 samples of food products and biological material of various origin. Among the isolated cultures, 2 isolates belong to *S.* Enteritidis, 2- to *S.* Typhimurium, 2- to *S.* Virchow, 1- to *S.* Dublin, *S.* Gallinarum, *S.* Pullorum, *S.* Muenchen, *Salmonella* F-67+. Cultural-morphological, enzymatic and antigenic properties of the selected cultures correspond to species characteristics; all bacterial cultures proved to be pathogenic in white mice.

The cultures were predominantly susceptible to semisynthetic and inhibitor-protected penicillins, cephalosporins and carbapenems; cultures showed resistance to tetracyclines, macrolides, and lincosamides in most cases. As regarding the aminoglycosides, most of *Salmonella* spp. strains showed intermediate resistance; up to 50% of studied *Salmonella* spp. strains were susceptible to some quinolones of different generations, however, cultures showed resistance to an overwhelming number of cases; 30% of the selected *Salmonella* spp. cultures were susceptible to amoxicillin, whereas the cultures were non-susceptible to ampicillin, a screening recommended by EUCAST (version 8) on the enterobacteria sensitivity to amoxicillin. From 30% of *Salmonella* spp. strains susceptible to pefloxacin (II) (EUCAST screening recommendations on susceptibility of *Salmonella* spp. to ciprofloxacin), 10% cultures showed susceptibility to ciprofloxacin (II).

The study analysis found that the studied cultures isolated from food products, sick and dead animals, and biomaterial obtained from people were characterized by natural susceptibility to

antibiotics; no cases of acquired resistance was found within this study. This may indicate that, the current rational use of antibiotic therapy may be effective.

CONCLUSIONS

1. The data obtained may indicate the need for screening studies on the susceptibility of *Salmonella* spp. strains to antibacterial drugs, while the list of studied drugs should be expanded as much as possible. It might be important when choosing an appropriate antibiotic therapy.

CONFLICT OF INTERESTS

All authors declare no competing interests.

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Liliia VYGOVSKA, ORCID 0000-0001-6745-5753
Vitliy NEDOSEKOV, ORCID 0000-0001-7581-7478, SCOPUS Author ID 5718580555
Valerii USHKALOV, ORCID0000-0001-5694-632X, SCOPUS Author ID 36130483300
Oksana BOYKO, ORCID 0000-0001-8113-9423
Oleksandra KEPPLE, ORCID 0000-0002-8123-3310, Web of Science Researcher ID B-2919-2018
Yuriy VISHOVAN, ORCID 0000-0003-1128-593X
Sergiy TERESHCHENKO, ORCID 0000-0002-5786-8711
Sergiy BOIANOVSKIY, ORCID 0000-0002-4621-5192