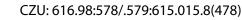
- Сон И.М., Литвинов В.И., Стародубов В.П., Сельцовский П.П. Эпидемиология туберкулеза. Москва, 2003. 286 с.
- Яворский К., Чобану А., Болотникова В. и др. Аналитическая оценка заболеваемости туберкулезом и другими болезнями органов дыхания в период 2012-2014 гг. и реализация стратегии РАL в Республике Молдова. В: Вестник Смоленской Государственной медицинской академии, 2015, том 14, № 2. ISSN 2225-6016.
- Яворский К.М., Болотникова В.А., Московчук А.Ф. и др. Туберкулез и другие бронхолегочные заболевания: реалии сегодняшнего дня и перспективы развития фтизиопульмонологической помощи населению Республики Молдова. В: Туберкулез и болезни легких. Съезд фтизиатров России с международным участием 27-29 мая 2015 года. 2015, № 5, с. 200-201. ISSN 2075-1230.
- 11. *Global tuberculosis control*: WHO report 2001. Geneva, 2001.
- Global tuberculosis control: surveillance, planning, financing. WHO report 2005. Geneva, World Health Organization (WHO/HTM/TB/2005.349).
- 13. Global tuberculosis report 2018 WHO, ww.who.int/ tb/publications/global_report/en/
- 14. Rapoartele statistice F.2 Privind bolile infecțioase și parazitare, anii 1998-2018.
- 15. Registre de evidență a bolilor infecțioase, f.060, anii 1998–2018.
- 16. Fișa de anchetare epidemiologică a focarului de boală infecțioasă, f.357/e, anii 1998–2018.

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PREVALENCE AND ANTIMICROBIAL RESISTANCE PROFILES OF SALMONELLA SPP. AND CAMPYLOBACTER SPP. IN HUMAN AND BROILER CHICKEN IN REPUBLIC OF MOLDOVA

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Summary

Foodborne diseases represent a significant public health burden and important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide. In same time, antimicrobial resistance (AMR) of the Salmonella spp. and Campylobacter spp. is an increasingly threat to global public health that requires action across all countries, government sectors and society, which is linked to irrational antimicrobial use in human sector and food animals. We aimed to get insight on the occurrence and AMR of Salmonella spp. and Campylobacter spp. isolated from humans with diarrhea and broiler chicken in Moldova, as such obtained results can evidence base and help national policy making to make decisions and take necessary measures for reducing the spread of strains resistant to antimicrobial.

Keywords: Salmonella spp., Campylobacter spp., antimicrobial resistance.

Rezumat

Prevalența și profilurile de rezistență antimicrobiană ale Salmonella spp. și Campylobacter spp. izolate de la oameni și din carnea de pui în Republica Moldova

Bolile cu factor de transmitere alimentar reprezintă o povară importantă pentru sănătatea publică, constituie o cauză majoră a morbidității și mortalității și un impediment semnificativ pentru dezvoltarea socioeconomică la nivel mondial. În același timp, rezistența antimicrobiană (AMR) a speciilor Salmonella spp. și Campylobacter spp. este o amenințare din ce în ce mai mare pentru sănătatea publică la nivel global, care necesită acțiuni în toate țările, în sectoarele guvernamentale și în societate, amenințare rezultată din utilizarea irațională a antimicrobienelor în sectorul uman și în hrana animalelor. Ne-am propus să înțelegem apariția AMR a Salmonella spp. și Campylobacter spp. izolat de la oameni cu diaree și din carne de pui broiler din Moldova, astfel că rezultatele obținute pot deveni o bază pentru elaborarea politicilor naționale, prin care s-ar lua decizii și măsurile necesare pentru reducerea răspândirii tulpinilor rezistente la antimicrobiene.

Cuvinte-cheie: Salmonella spp., Campylobacter spp., rezistență la preparatele antimicrobiene

Резюме

Распространенность и профили антимикробной резистентности Salmonella spp. и Campylobacter spp., изолированных от людей с диареей и цыплят-бройлеров в Республике Молдова

Болезни пищевого происхождения представляют собой серьезное бремя для общественного здравоохранения и являются важной причиной заболеваемости и смертности, а также значительным препятствием для социально-экономического развития во всем мире. В то же время устойчивость Salmonella spp. и Campylobacter spp. к противомикробным препаратам во всем мире становится все более серьезной угрозой для общественного здравоохранения, которая требует действий во всех странах, государственных секторах и обществе, что связано с нерациональным использованием противомикробных препаратов в человеческом секторе и в кормах для животных. Мы стремились получить представление о происхождении устойчивости к противомикробным препаратам Salmonella spp. и Campylobacter spp., изолированных от людей с диареей и цыплят-бройлеров, так как полученные результаты могут послужить доказательной базой и помочь в разработке национальной политики для принятия решений и необходимых мер для сокращения распространения штаммов, устойчивых к противомикробным препаратам.

Ключевые слова: Salmonella spp., Campylobacter spp., антимикробная резистентность

Introduction

Foodborne diseases are globally important, as they result in considerable morbidity, mortality, and economic costs [1,2].

Campylobacteriosis and salmonellosis are the two most frequently reported food borne diseases worldwide and chicken meat is considered to be one of the most important food vehicles. The burden of the diseases and the cost of control measures are highly significant in many countries and contamination with zoonotic Campylobacter and Salmonella has the potential to severely disrupt trade between countries [3].

World Health Organization (WHO) estimates, that each year, almost 1 in 10 people fall ill and 33 million of healthy life years are lost. According to WHO, diarrheal diseases are resulting from eating unsafe food, with 550 million people falling ill yearly (including 220 million children under the age of 5 years) and Campylobacter is 1 of the 4 key global causes of them. The true incidence of gastroenteritis due to *Campylobacter* spp. is poorly known, particularly in Low- and Middle-income countries. Studies in high-income countries have estimated the annual incidence at between 464 and 963 per 1000 population [4].

Nontyphoidal salmonellae are a leading cause of bacterial diarrhea worldwide; they are estimated to cause approximately 153 million cases of gastroenteritis and 57,000 deaths globally each year. Nontyphoidal salmonellosis refers to illnesses caused by all serotypes of Salmonella except for Typhi, Paratyphi A, Paratyphi B, and Paratyphi C. Usually, people fall ill from through the consumption of food or water contaminated with animal feces. Transmission can also occur through direct contact with infected animals or their environment and directly between humans [5].

Antimicrobial resistance (AMR) is a major threat to global health and the world economy, and poses a unique challenge to humanity. Resistant microorganisms exist in humans, animals, food, and the environment. This makes AMR a complex epidemiological issue. The main cause of AMR is antimicrobial use. A comprehensive, collaborative and coordinated collection and analysis of data from multiple domains. WHO, FAO, OIE and the OECD need to develop targets and goals to promote appropriate use of antimicrobials in human, animal and plant health to prevent infection. Application of improved hygiene standards along the food production chain, «from farm to fork», is of paramount importance to control the dissemination of all zoonotic foodborne pathogens, and thus the drug resistant ones [6].

The AMR Review calculated that resistant bacteria already kill more than 700.000 people worldwide. This study has estimated the global economic cost of antimicrobial drug resistance by 2050. The results show a considerable human and economic cost. Initial research, looking only at part of the impact of AMR, shows that a continued rise in resistance by 2050 would lead to 10 million people dying every year and a reduction of 2% to 3,5% [8].

A public health related monitoring system for antibiotic resistance is set up in Moldova, including the antimicrobial susceptibility testing for Salmonella. The Technical Assessment conducted by the European Centre for Communicable Disease Control (ECDC) noted that level of disease recognition and reporting was critically low for some diseases, including Campylobacter. No information is available on the occurrence of Campylobacter infection in Moldova, from humans.

This study conducted a survey to obtain a preliminary understanding of the occurrence and AMR of Salmonella and Campylobacter among human patients with diarrhea in Moldova, with the overall objective of informing policy-making on food safety and AMR in Moldova.

Materials and methods

Studies to evaluate the presence of Salmonella and Campylobacter microorganisms among humans and in poultry in Moldova and antimicrobial resistance assessment was conducted during October-February 2018.

87 fecal samples were collected from the Municipal Clinical Hospital for Contagious Disease for Children, from the patients with acute diarrhea (acute enterocolitis, gastroenteritis, gastro-enterocolitis, hemocolitis, and food poisoning). Samples were collected before initiating antimicrobial treatment and analyzed in the laboratory of extremely contagious acute diarrheal diseases and zooanthroponosis in compliance with the existing and accepted methodology in the country [9].

In collaboration with the National Food Safety Agency, 75 samples of poultry meat were collected from 11 domestic producers (60 samples) and 4 import manufacturers (15 samples). Samples of poultry meat have been taken from slaughterhouses and slaughter centers after the technological process, as well as from the warehouses of the finished product in compliance with the sampling requirements stipulated in normative documents. A study for the detection of Salmonella and Campylobacter in poultry meat was conducted in the Sanitary Microbiology Laboratory using standardized methods. All samples were coded by the seal and sent to the laboratory on the day of collection with the accompanying documentation. From total 75 investigated samples – 49 samples (65,3%) were collected and sent for research on the day of production, 26 samples (34,7%) were sampled and shipped in the frozen state (from 3 to 12 months from date of manufacture). Until the investigation, all samples were stored in the refrigerator at 2-8 °C, previously frozen samples were defrosting [13-16].

The preliminary biochemical identification of Salmonella spp. was performed using the Kligler medium (*HiMedia*, India) based on the possibility of isolated strains to ferment glucose and lactose and produce hydrogen sulfide and using O.B.I.S. Salmonella test (*Oxoid*, UK) for the differentiation of microorganisms with a similar appearance of colonies (*Citrobacter* spp., *Proteus* spp.). For the full biochemical identification, were used commercial biochemical tests API 20E (*bioMerieux*, France).

Salmonella isolates from poultry meat and humans were sera-identified according to the general recommendations for salmonella serotyping and the Kauffmann-White scheme using both poly- and monovalent salmonella serum (O and H sera).

For the identification and differentiation of Campylobacter strains from humans, the following tests were performed: microscopy (Gram), mobility (Brucella broth, *HiMedia*), catalase, oxidase, susceptibility testing to nalidixic acid and cephalothin (cephazolin), sodium Hippurate hydrolysis test. The inoculated media were incubated for 24-48 hours to 41,5 °C under microaerophilic and hypercapnia conditions.

The tests specified in ISO [16] have been used for the confirmation of suspected colonies of *Campylobacter* spp. obtained from the poultry meat research: morphology (Gram microscopy), mobility (Brucella broth, *HiMedia*), possibility of microaerobic growth at 25 °C and aerobic conditions at 41,5 °C (Collagen Blood Agar, *HiMedia*) and presence of oxidase. In addition, O.B.I.S. Campy test (*Oxoid*, UK) for gram-state screening and L-alanyl aminopeptidase (L-ALA) activity in order to differentiate microorganisms from *Campylobacteraceae* from other microorganisms with similar morphology.

The antimicrobial susceptibility of Campylobacter and Salmonella isolates were tested in accordance with the EUCAST methodology [19] using the disc-diffusion method commercial discs (*HiMedia*, India). Salmonella isolates have been tested in 19 antimicrobial agents, which are part of 7 classes of antimicrobial group: Penicillin's (Ampicillin 10 µg, Piperacillin 30 µg, Amoxiclav 30 µg); Cephalosporins (Cefotaxime 5 µg, Cefoxitin 30 µg, Ceftazidime 10 µg, Cefepime 30 µg); Carbapenems (Ertapenem 10 µg, Meropenem 10 µg); Fluoroquinolones (Ciprofloxacin 5 µg, Norfloxacin 10 µg, Ofloxacin 5 µg, Levofloxacin 5 µg); Aminoglycosides (Gentamicin 10 µg, Amikacin 30 µg, Tobramycin 10 µg, Netilmicin 10 µg); Amphenicols (Chloramphenicol 30 µg) and Sulfanilamide (Co-Trimoxazole 25 µg). The quality control of the sensitivity assessment procedure was assured by the Escherichia coli ATCC 25922 microorganism test.

The antimicrobial susceptibility of Campylobacter isolates was tested using the disc-diffusion method [19] with commercial discs (*HiMedia*, India) containing erythromycin (15 μ g disc load), tetracycline (30 μ g disc load), and ciprofloxacin (5 μ g disc load), as per the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19].

We used *Campylobacter coli* ATCC 33559, *Campylobacter jejuni* ATCC 33291 for quality control.

Results and discussion

Isolation of *Salmonella* and *Campylobacter* from human patients

Of the 87 human samples, 9 (10,4%) were positive for *Salmonella* spp. The following Salmonella serovars have been identified: 5 serovars from group O:9 (D1) (*Salmonella Enteritidis*) and 4 serovars from group O:4 (B) (*Salmonella Typhimurium*) and 7 (8,0%) for *Campylobacter* spp. (3 strains of *Campylobacter coli* and 4 strains of *Campylobacter jejuni*). The results are presented in *Table 1*. Positive for both Salmonella spp. and Campylobacter spp. not were detected none of the samples.

Following the study of 75 samples of poultry analyzed in 6 samples (8%), Salmonella was detected, including: 3 salmonella (17,6%) was found from chicken carcasses, 1 salmonella from chicken meat (8,3%), chicken neck (10%) and chicken caecum with content (2%). In 5 cases, Salmonella was detected from fresh samples and in one case from the frozen sample with a longer shelf-life. Campylobacter suspect colonies were not confirmed by the tests used and therefore Campylobacter was not detected from the poultry samples tested in this study. The results are presented in Table 2. The following Salmonella serovars have been identified: 2 serovars from gr. 0:7 (S. Othmarschen, S. Isangi), 2 serovars from gr. O:4 (S. Lagos, S. Pasing) and 2 strains of S. Serftenberg (gr. 0:3,19) and correlate with the multiannual monitoring data on the circulation of Salmonella serotypes

in the environment and food on the territory of Moldova (S. Othmarschen, S. Lagos and S. Senftenberg are sporadically recorded). For the first time, S. Isangi serotypes (frozen chicken carcass) and S. Pasing (chicken caecum with content) were identified.

Antimicrobial susceptibility testing of Salmonella isolates

Table 3 presents the summary results of antimicrobial susceptibility testing of the 9 Salmonella isolates from human patients and 6 Salmonella isolates from poultry. In total, 88,8% of isolates from humans were resistant to amoxiclav, 77,7% to ampicillin, 66,6% to cefoxitin, 55,5% to piperacillin and 55,5% to ceftazidime. Of these isolates, 100% were susceptible to netilmicin and none was resistant and intermediate susceptible; 88,8% were susceptible to cefotaxime, 88,8% to gentamicin and 88,8% to chloramphenicol.

In total, 50% of Salmonella isolated from poultry meat were resistant to ampicillin, 50% to cefepime, 33,3% to amoxiclav, 16,6% to ceftazidime and 16,6% to ciprofloxacin. All isolates were susceptible to cefotaxime, cefoxitin, levofloxacin, norfloxacin, to all antimicrobial preparations tested in the aminoglycoside class, ampicillin and sulfanilamide. Intermediate sensitivity to piperacillin (33,3%), cefepime, ceftazidime and ofloxacin (16,6%) was observed.

Table 3 provides an overview of the AMR profiles and the frequencies observed for the Salmonella isolates from humans and from poultry.

None of the Salmonella isolated from humans were sensitive to all antimicrobials included in the susceptibility testing. All Salmonella isolated were resistant to at least one antimicrobial agent. Of the Salmonella isolates from human, 22.2 % were resistant to one class of antimicrobial agents. One isolated of Salmonella was resistant to 2 classes, 4 classes and 5 classes of antimicrobial agents.

Of the 6 strains of Salmonella isolated from poultry meat, only 1 strain (16,6%) demonstrated sensitivity to all antimicrobial preparations used in the study. Strains resistant to all antimicrobial agents have not been detected. Of the Salmonella isolated from poultry meat 33,3% were resistant to only one antimicrobial preparation, 33,3% were resistant to 1 class of antimicrobial preparations and 50% were resistant to 2 classes of antimicrobial preparations. In this study, strains of Salmonella isolated from poultry have not demonstrated resistance to three and more classes of antimicrobial preparations concomitantly.

Summary results of the antimicrobial susceptibility testing of the Campylobacter isolates are presented in *Table 4*.

Table 5 provides an overview of the AMR profiles and the frequencies observed for the Campylobac-

ter isolates from humans. All isolates from humans were resistant to at least one antimicrobial class. In result none Campylobacter isolated from humans were resistant to all three antimicrobial classes, i.e. multi-resistant (*Table 6*). The results of this study confirm that Salmonella and Campylobacter are etiologically significant factors of clinical diarrheal diseases among the population of Moldova, which is in line with data from several studies conducted in different countries Analysis of the results shows that meat products and their semi-finished products can be a source of human infection, 8% of the tested samples have been infected with Salmonella.

The Food-borne Diseases Active Surveillance Network (FoodNet) of the United States Centers for Disease Control and Prevention reported that incidence of Salmonella infection was highest among the total food poisoning cases (CDC, 2010). Especially, Salmonella enterica serovars Enteritidis and Typhimurium are the most frequently identified bacteria in human salmonellosis (Chung et al., 2003; Cheong et al., 2007). The results of the multi-annual monitoring carried out in Moldova correlate with the published data of the prevalent Salmonella serovars. Annually, the share of S. Enteritidis in food and other environmental objects is from 28% to 82%, S. Typhimurium from 2% to 45% (annual national report, National Center of Public Health) and in most cases detected in research into food poisoning. The results of our survey confirm that Salmonella spp. and Campylobacter spp. are important etiological agents of clinical diarrheal disease among humans in Moldova.

In this study from humans were isolated only two serotypes, Salmonella Typhimurium and Salmonella Enteritidis. The Salmonella Typhimurium and Salmonella Enteritidis serovars represent 98,6% of the serological structure of Salmonella spp. in humans (2014 – 95,6%, 2015 – 93,2%, 2016 – 95,7%). But, according to the annual epidemiological surveillance data of the acute diarrheal diseases in 2017 were registered significant changes in the etiological structure of salmonellosis in the human population. The intense migration of the population, the import of food condition the circulation of unusual serotypes such as: S. Agona, S. Derby, S. Kingston, S. Montevideo, S. Kentucky, S. Nchanga. The results indicate that in the onset of salmonellosis in Moldova an important role may represent broiler chicken, as in many other countries [20, 21].

Salmonella serovars found in this study (*S. Othmarschen, S. Lagos* and *S. Senftenberg* are sporadically recorded) correlate with the multiannual monitoring data on the circulation of Salmonella serotypes in the environment and food on the territory of R. Moldova. The prevalent serotypes of Salmonella (*S. Enteritidis*, *S. Typhimurium*) were not detected, conditioned by several factors, which require a more detailed study. From year to year the range of isolated salmonella in the republic are filled with new serovars. In this study, new serotypes for Moldova were also identified: *S. Isangi* and *S. Pasing*.

The variety of isolated strains of salmonella complicates the task of the veterinary service to detect and eliminate sources of infected products in the country's trade network, and poses the task of public health monitoring for the regular monitoring of the circulation of pathogenic strains of ADD in humans and their sensitivity to antibiotics.

According to published data [22], the prevalence of Campylobacter in chicken in European countries is from 11-12% (Finland, Denmark) to 70-76% (Spain, Austria, France).

Conducted study, contributed to implementation for the first time in the country the microbiological method of isolation and identification of Campylobacter spp. Thus, at patients with acute diarrheal diseases included in the study were detected 7 strains of *Campylobacter* spp. with prevalence of *Campylobacter jejuni*.

Campylobacter was not detected from the poultry samples tested in this study. Actually, national legislation does not provide for the determination of Campylobacter in poultry [23], but at the same time ISO standards are approved as national standards for Campylobacter detection by both the detection method and the counting method [16].

This study was a first step in the approval and implementation of the method of detection of Campylobacter in poultry meat. The study revealed some gaps that need to be evaluated and considered in organizing the laboratory test in the presence of Campylobacter namely: the impact of the season study/investigation, the place of sampling, the state of the collected samples, the sampling process/scheme, etc. The sampling period which held during October-February 2018 (which is not the main season of acute bacterial intestinal infections among humans in Moldova), and not the summer and early autumn when usually most cases of salmonellosis and campylobacteriosis are reported so we can conclude that the real burden of salmonellosis and campylobacteriosis in the human population is higher.

Data from the literature highlights that one of the best predictors of Campylobacter prevalence is the season, the highest prevalence observed in June [22, 25], other factors related to broiler growth, such as growth practices, biosecurity or bird health, may be important [26].

There are different data on Campylobacter survival in frozen conditions. According to the literature [28, 29] some studies show that differences in the prevalence of C. jejuni in fresh and frozen samples is a strong indicator that freezing or frozen storage is detrimental to C. jejuni survival and subsequent isolation in the laboratory. Freezing may reduce the number of live campylobacter and chicken mushrooms (Garenaux et al., 2009; Sampers et al., 2010) and that usually freezing chicken or skin for 24 hours causes a reduction in the number of campylobacter (Oyarzabal et al., 2010; Sampers et al., 2010). However, other studies [30] show that no difference in the recovery of C. jejuni from fresh, chilled or frozen samples after selective enrichment has been detected, showing that this microorganism can survive under the storage conditions tested.

Among the polyetiological group of intestinal diseases, diseases caused by resistant bacteria, whose occurrence is provoked by the use of antibiotics in agriculture and the use of livestock products, become more and more dangerous these days. Prevention, detection and medical care for the emergence of diseases caused by resistant strains of microorganisms require a comprehensive approach, including cooperation and information support between public health and the veterinary service. To control the occurrence, spread and circulation of antibiotic-resistant strains, monitoring of the use of antimicrobial agents in livestock production is necessary. The World Health Assembly of 2015 called upon all states to develop by 2017 individual action plans to combat the emergence of resistant strains of microorganisms.

Based on the veterinary service within National Agency for Food Safety date antimicrobials are routinely used in poultry production and are also available without a veterinary prescription. The antimicrobial classes most commonly used are tetracyclines, aminoglycosides, fluoroquinolones and Penicillin's. These classes of antimicrobials are considered critically important because are also used in the treatment of human pathologies. FBD caused by resistant bacteria are well documented in humans, and can be linked with antimicrobial use in food animals.

According to the EFSA data antimicrobials such as ampicillin, sulfonamides and tetracyclines have been widely used for many years in veterinary medicine to treat infections in production animals. Generally, moderate to high levels of resistance to these antimicrobials are reported by European countries from producing animals and meat products thereof. Despite the limited number of patients (i.e. only 87) and poultry meat (i.e. only 75), tested strains on antimicrobial susceptibility does not permit to draw some accurate conclusions about the resistance to these preparations tested.

Thereby, in the study Salmonella isolates from humans presented resistance to more classes of antimicrobials (to two classes and four classes), this can lead to deficiencies in the treatment of these infections especially invasive cases of salmonellosis. Most *Campylobacter* spp. isolates from humans were resistant to two classes antimicrobial which indicates the importance of the antimicrobial susceptibility testing for prescribing appropriate therapy and avoiding therapeutic failure.

Data obtained from the study of the resistance of Salmonella strains detected in poultry to antimicrobial preparations shows the high-moderate resistance to ampicillin (50%) and the low resistance (16,6%) to ciprofloxacin and correlates with the data published by EFSA. Of all 6 strains of Salmonella isolated from poultry meat, only 1 strain (16,6%) demonstrated sensitivity to all antimicrobial preparations used in the study.

Strains resistant to all antimicrobial agents have not been determined. Of Salmonella isolated from poultry meat, 2 strains (33,3%) were resistant to one class of antimicrobial preparations and 3 strains (50%) were resistant to 2 classes of antimicrobial preparations. In this study strains of Salmonella isolated from poultry meat have not demonstrated resistance to three and more classes of antimicrobial preparations concurrently. The data obtained demonstrates the actuality of the study and the need for further study of antimicrobial resistance in zoonotic and indicator bacteria from humans and food.

Conclusion

1. The study is a first step in studying the prevalence of *Salmonella* spp. and *Campylobacter* spp. in poultry in Republic of Moldova, including antimicrobial susceptibility testing, and is the reason to discuss the need to organize deeper studies with the application of the provisions of the relevant documents.

2. The study has shown the existence of the problem in the field of food chain safety from a microbiological point of view and the need to intensify the mutual cooperation of stakeholders (such as ANSA and ANSP) on the monitoring of *Salmonella* and *Campylobacter* circulation in the food chain and the environment and impact assessment on public health.

3. The burden of foodborne diseases in our country, re-emergence of antibiotic-resistant foodborne bacteria requires concerted efforts in terms of collaboration, funding, awareness and commitment from government, and policy makers,

4. Harmonization of national legislation on the monitoring of zoonoses and zoonotic agents and microbiological criteria for foodstuffs to the requirements of European directives.

5. The method of detection and counting of *Campylobacter* spp. and the *Salmonella* counting method have to be implemented in Laboratories at national (ANSP, ANSA) and territorial (CPH) level and clinical laboratories.

Table 1

Type and number of samples and the frequency of isolation of Salmonella and Campylobacter

Sample (n=87)	Salmonella positive (n=9)			Campylobacter positive (n=7)				
	S. Enteritidis S. Tiphymur		murium	С. је	ijuni	C. coli		
Human, faeces from patients with diarrhea	no	%	no	%	no	%	no	%
	5	5,7	4	4,5	4	4,5	3	3,7

Table 2

Type and number of samples and the frequency of isolation of Salmonella and Campylobacter from poultry

Cample ture	Samples tested	Salmonel	la positive	Campylobacter positive		
Sample type	n	n	%	n	%	
Poultry, carcasses, chicken and turkey meat, neck and offal of chickens, intestinal content (caecum)	75	6	8,0	-	-	

Table 3

	Isolates from huma			humans (; (n=9)		Isolates from poultry (n=6)					
Antimicrobial	1	R		Ι	_	S	R		1		S	
	n	%	n	%	n	%	n	%	n	%	n	%
Penicillin's												
ampicillin	7	77.7	0	0	2	22.2	3	50	0	0	3	50
piperacillin	5	55.5	2	22.2	2	22.2	0	0	2	33.3	4	66.6
amoxiclav	8	88.8	0	0	1	11.1	2	33.3	0	0	4	66.6
Cephalosporins												
cefotaxime	1	11.1	0	0	8	88.8	0	0	0	0	6	100
cefepime	3	33.3	4	44.4	2	22.2	3	50	1	16.6	2	33.3
ceftazidime	5	55.5	2	22.2	2	22.2	1	16.6	1	16.6	4	66.6
cefoxitin	6	66.6	1	11.1	2	22.2	0	0	0	0	6	100
Quinolones												
ciprofloxacin	2	22.2	1	11.1	6	66.6	1	16.6	0	0	5	83.3
levofloxacin	1	11.1	3	33.3	3	33.3	0	0	0	0	6	100
norfloxacin	3	33.3	2	22.2	4	44.4	0	0	0	0	6	100
ofloxacin	1	11.1	3	33.3	3	33.3	0	0	1	16.6	5	83.3
Aminoglycosides												
gentamicin	0	0	1	11.1	8	88.8	0	0	0	0	6	100
amikacin	0	0	4	44.4	5	55.5	0	0	0	0	6	100
tobramycin	1	11.1	1	11.1	7	777	0	0	0	0	6	100
netilmicin	0	0	0	0	9	100	0	0	0	0	6	100
Amphenicols												
chloramphenicol	1	11.1	0	0	8	88.8	0	0	0	0	6	100
Carbapenems												
meropenem	1	11.1	1	11.1	7	77.7	0	0	0	0	6	100
ertapenem	2	22,2	3	33,3	4	44,4	0	0	0	0	6	100

Antimicrobial susceptibility of Salmonella spp. isolates from humans and from poultry

Note. I – intermediate susceptible; R – resistant; S – susceptible.

Table 4

Resistance profiles of Salmonella isolates from humans and from poultry

AMD profile	Isolates from I	humans (N=9)	Isolates from poultry (N=6)		
AMR profile	n	%	n	%	
Susceptible to all antimicrobial agents	0	0	1	1,66	
Resistant to all antimicrobial agents	0	0	0	0	
Resistant to at least one antimicrobial agent	9	100	2	33,3	
Resistant to one class of antimicrobial agents <i>Penicillin's</i> (AP/AUG/	2	22,2	2	33,3	
PTZ)					
Ampicilin (AP)	7	77,7	1	16,6	
Amoxiclav (AUG)	8	88,8	1	16,6	
Piperacilin (PTZ)	5	55,5			
Resistant to two classes of antimicrobial agents	1	11,1	3	5,0	
Penicillin's (AP/AUG/PTZ) /Quinolones (CIP/LEV/NOR)	1	11,1			
Ampicillin (AP)/cefepime (CPM)	3	33,3	1	16,6	
Ampicillin (AP)/amoxiclav (AUG)/cefepime (CPM)/ceftazidime (CAZ)	3	33,3	1	16,6	
Cefepime (CPM)/ciprofloxacin (CIP)	1	11,1	1	16,6	
Resistant to three classes of antimicrobial agents	0	0	0	0	
Resistant to four classes of antimicrobial agents	1	11,1	0	0	
Penicillin's (AP/AUG/PTZ) / Cephalosporins (CTX/CPM/CAZ/FOX) / Car-					
bapenems (MEM/IMI), Amphenicols (C)	1	11,1			

Antimicrobial susceptibility of Campylobacter spp. isolates from humans

Antimicrobial agent		Isolates from humans (N=7)								
		R		1	S					
ugent	n	%	n	%	n	%				
Macrolides: erythromycin	5	71.4	0	0	2	28.5				
Tetracyclines: tetracycline	3	42.8	0	0	4	57.1				
Quinolones: ciprofloxacin	4	57.1	1	14.2	2	28.5				

Resistance profiles for Campylobacter spp. isolates from humans

AMD profile	Campylobacter isolates from humans (N=7)				
AMR profile	n	%			
Susceptible to all antimicrobial agents	0	0			
Resistant to at least one antimicrobial agent	7	100			
Resistant to one class of antimicrobial agents:	2	28.5			
Tetracyclines (TE)	1	14.2			
Macrolides (E)	1	14.2			
Resistant to two classes of antimicrobial agents:	5	71.4			
Tetracyclines (TE)/Quinolones (CIP)	1	14.2			
Macrolides (E)/Quinolones (CIP)	3	42.8			
Macrolides (E)/Tetracyclines (TE)	1	14.2			
Resistant to three or more classes of antimicrobial agents	0	0			

Bibliography

- Kirk M.D., Pires S.M., Black R.E., et al. World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases. In: *PLoS Med.*, 2015; vol. 12(12): e1001921. https://www.ncbi.nlm.nih.gov/ pubmed/26633831
- Stein C., Kuchenmüller T., Hendrickx S., et al. The Global Burden of Disease assessments-WHO is responsible? In: *PLoS Negl. Trop. Dis.*, 2007; vol. 26; nr. 1(3): e161. https://www.ncbi.nlm.nih.gov/pubmed/18160984
- 3. Guidelines for the control of campylobacter and salmonella in chicken meat. CAC/GL 78-2011.
- World Health Organization. The global view of campylobacteriosis: report of an expert consultation, Utrecht, Netherlands, 9-11 July 2012. http://www.who.int/iris/handle/10665/80751
- Jennifer C., Hunter K., Watkins F. Salmonellosis (Nontyphoidal). Chapter 3. Infectious Diseases. https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-ravel/salmonellosis-nontyphoidal
- 6. A European One Health Action Plan against Antimicrobial Resistance (AMR). https://ec.europa.eu/health/ amr/sites/amr/files/amr_action_plan_ 2017_en.pdf
- 7. Jim O'Neill. Antimicrobial Resistance: Tackling a crisis for the future health and wealth of nations. In: *The Review on Antimicrobial Resistance*. December 2014. https://amr-review.org
- 8. Melnic V., Cojocaru R., Evtodienco V. *Diagnosticul de laborator al infecției cu Campylobacter (indicații meto-dice)*. Chișinău, 2005. 21 p.
- Evtodienco V. ş.a. Diagnosticul microbiologic al infecțiilor intestinale (indicații metodice). Chişinău, 2012. 83 p.
- 10. *Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella.* Part 1: Detection of *Salmonella* spp. SM EN ISO 6579-1:2017

- Microbiology of the food chain. Horizontal method for detection and enumeration of Campylobacter spp. Part 1: Detection method. SM SR EN ISO 10272-1:2014
- 12. European Committee on Antimicrobial Susceptibility Testing. *Breakpoint tables for interpretation of MICs and zone diameters*. Version 8.0.
- Abdukhalilova G., Kaftyreva L., Wagenaar A., et al. Occurrence and antimicrobial resistance of Salmonella and Campylobacter in humans and broiler chicken in Uzbekistan. In: *Public Health Panorama*, 2016, vol. 2, pp. 340-347.
- 14. WHO estimates of the global burden of foodborne diseases. Foodborne diseases burden epidemiology reference group 2007–2015. Geneva: World Health Organization, 2015.
- 15. Skarp C.P., Hänninen M.L., Rautelin H.I. Campylobacteriosis: the role of poultry meat. In: *Clin. Microbiol. Infect.*, 2016, vol. 22(2), pp. 103-109.
- 16. HG nr. 221 din 16.03.2009 cu privire la aprobarea Regulilor privind criteriile microbiologice pentru produsele alimentare. In: *Monitorul Oficial*, nr. 59-61 din 24.03.2009, art. 272.
- 17. Jorgensen F., Ellis-Iversen J., Rushton S., et al. Influence of Season and Geography on *C. jejuni* and *C. coli* Subtypes in Housed Broiler Flocks Reared in Great Britain. In: *Appl. Environ. Microbiol.*, 2011, vol. 77, no. 11, pp. 3741-3748.
- Patrick M.E., et al. Effect of climate on incidence of Campylobacter spp. in humans and prevalence in broiler flocks in Denmark. In: Appl. Environ. Microbiol., 2004, vol. 70, pp. 7474–7480.
- 19. Swart A.N., Mangen M.-J.J., Havelaar A.H. *Microbiological criteria as a decision tool for controlling Campylobacter in the broiler meat chain*. RIVM Letter report 330331008/2013
- 20. Franklín G., et al. The influence of freezing and duration of storage on Campylobacter and indicator bacteria in broiler carcasses. In: *Food Microbiology*, 2006, vol. 23(7), pp. 677-683.

Table 5

Table 6

- 21. Maike T., Tereza C., et al. Effect of refrigeration and frozen storage on the Campylobacter jejuni recovery from naturally contaminated broiler carcasses. In: *Braz. J. Microbiol.*, 2010, vol. 41, no. 2. http://dx.doi. org/10.1590/S1517-83822010000200034
- Regulamentul (UE) 2017/1495 al Comisiei din 23 august 2017 de modificare a Regulamentului (CE) nr. 2073/2005 în ceea ce privește Campylobacter în carcasele de pui pentru îngrășare. https://publications.europa.eu/en/publication-detail/-/publication/218f322f-8890-11e7-b5c6-01aa75ed71a1/language-ro
- 23. Norma sanitar-veterinară privind reducerea prevalenței anumitor serotipuri de Salmonella în efectivele de carne de pui: anexa nr. 6 la HG nr. 398 din 11.06.2012.
- 24. Commission Regulation (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005 as regards Campylobacter in broiler carcases. https:// eur-lex.europa.eu/eli/reg/2017/1495/oj
- 25. HG nr. 264/2011: Regulamentul privind monitorizarea zoonozelor si a agentilor zoonotici. In: *Monitorul Oficial*, nr. 59-62 din 15.04.2011, art. 301.
- 26. Decizia de punere în aplicare a Comisiei din 12 noiembrie 2013 privind monitorizarea și raportarea rezistenței la antimicrobiene a bacteriilor zoonotice și comensale (2013/652/EU). https://eur-lex.europa.eu/legal-content/ RO/TXT/PDF/?uri=OJ:L:2013:303:FULL&from=HU
- 27. Decizia Comisiei din 19 iulie 2007 privind o contribuție financiară din partea Comunității pentru efectuarea, în statele membre, a unui studiu privind răspândirea şi rezistența antimicrobiană a *Campylobacter* spp. în rândul efectivelor de pui broiler, precum şi răspândirea *Campylobacter* spp. şi a *Salmonella* spp. în carcasele de pui broiler (2007/516/CE). https://eur-lex.europa. eu/legal-content/RO/ALL/?uri=CELEX:32007D0516

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PROBLEMELE-CHEIE ALE SITUAȚIEI EPIDEMIOLOGICE NEFAVORABILE ÎN LOCALITĂȚILE RURALE CU PRIORITATE PENTRU TUBERCULOZĂ DIN REPUBLICA MOLDOVA

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Rezumat

În pofida realizărilor medicinei contemporane, tuberculoza (TB) continuă să prezinte un mare pericol. Scopul studiului efectuat a fost depistarea particularităților situației epidemiologice și cercetarea componenței contingentului bolnavilor din satele cu situația nefavorabilă privind TB. Obiectul de studiu l-au constituit 26 de localități din raioanele Ialoveni, Nisporeni, Strășeni, Comrat și cohorta bolnavilor cu TB pulmonară primar depistați. Au fost demonstrate importanța studierii particularităților situației epidemiologice și rolul determinant al parametrilor de bază sociodemografici, care caracterizează componența cohortei. A fost accentuată necesitatea întăririi relațiilor dintre serviciul general și cel specializat, atragerii organizațiilor neguvernamentale. Rezultatele obținute și concluziile formulate pot fi folosite înpentru optimizarea programelor în lupta cu TB la nivelul raionului și al comunității.

Cuvinte-cheie: localități rurale, tuberculoză pulmonară, situație epidemiologică, acordarea ajutorului medical

Summary

The key problems of clinical and epidemiological issues in rural areas of high priority for tuberculosis in the Republic of Moldova

Despite the advances of the modern medicine, tuberculosis (TBC), as a dangerous infectious disease, still remains a serious threat. The purpose of the study was to identify the peculiarities of the epidemiological situation and the contingent of patients from the villages with a TB high-risk population. The study was conducted in 26 settlements from Ialoveni, Nisporeni, Straseni, Comrat districts and the cohort (173) of newly diagnosed patients with pulmonary TB. It is shown the importance of studying the characteristics of the epidemiological situation and the determining role of the main clinical, socio-demographic parameters that denote the cohort composition. It is underlined the necessity to strengthen the interaction of specialized and general medical services, the involvement of non-governmental organizations. The obtained results of TB control at the district and local levels.

Keywords: rural communities, pulmonary tuberculosis, epidemiological situation, medical assistance

Резюме

Ключевые проблемы клинико-эпидемиологического неблагополучия в сельских населенных пунктах высокого приоритета по туберкулезу в Республике Молдова

Несмотря на достижения современной медицины, туберкулез (ТБ), являясь опасным инфекционным заболеванием, по-прежнему представляет серьёзную угрозу. Целью исследования было выявить особенности эпидемиологической ситуации и состава контингента больных в неблагополучных по ТБ селах. Объектом исследования были 26 населенных пункта из Яловенского, Ниспоренского, Страшенского, Комратского районов и когорта (173) впервые выявленных больных ТБ легких. Показана значимость изучения особенностей эпидемиологической ситуации и детерминирующей роли основных клинических, социально-демографических параметров, характеризующих состав когорты. Сделан