ORIGINAL ARTICLE

Evaluation of Contamination of Poultry Meat and Semi-finished Products with *Campylobacter* Bacteria

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Abstract

Purpose: The scope of the paper was the assessment of the contamination (or monitoring) of poultry meat and semi-finished products with *Campylobacter* ("from the retail network and farms" may be added), using the culture identification technique and the polymerase chain reaction method, as well as the evaluation of the antibiotic resistance of strains isolated from poultry meat and semi-finished products. **Materials and Methods:** The experimental part of the work was carried out on the basis of the Institute of Veterinary and Sanitary Expertise, Biological and Food Safety of the Moscow State Institute of Food Production and the Federal Publicly Funded Institution of Science "FIC of Nutrition and Biotechnology" at the Nutriicrobiome Biosafety and Analysis Laboratory. The GOST ISO 10272-1-2013 "microbiology of food and animal feeds. Methods for the detection and counting of *Campylobacter* spp., Part 1. Detection method" was used in the work performed to isolate and identify the *Campylobacter* spp., Pure growths were identified per totality of cultural, morphological, and biochemical signs confirming the belonging of the isolated cultures to the bacteria of the *Campylobacter* genus. The belonging of *Campylobacter* spp., isolated cultures was also determined by polymerase chain reaction (PCR) with electrophoretic detection of amplification products in an agarose gel. The sensitivity to antibiotics was determined according to the clinical guidelines "determination of the sensitivity of microorganisms to antimicrobial agents" (IACMAC, 2014) by disc diffusion method using standard discs. **Results:** It was established that out of the 28 samples of poultry meat and semi-finished products, *Campylobacter* bacteria were found in 4 samples of the half-finished product "drumette" in the weights of 10 and 1.0 g. **Conclusions:** The results of the PCR analysis confirmed the belonging of the isolated cultures to *Campylobacter Jejuni* species. In turn, the PCR is a faster method than the culture technique and allows giving a definitive answer within a few hours.

Key words: Antibiotic resistance, *Campylobacter*, contamination, microbiological evaluation, polymerase chain reaction, poultry meat

INTRODUCTION

In Russia, the industrial poultry farming is one of the most important and rapidly developing sectors of agriculture. Poultry meat makes up more than 43% of the total volume of raw meat, and demand for it has been constantly growing. Thanks to the State Program of Development of Agriculture and Regulation of Agricultural Products, Raw Materials and Food Markets for 2008-2012, as well as the target program of the department "Development of Poultry Farming in the Russian Federation for 2010-2012," the share of poultry meat in the total meat increased more than twice as compared with 1990. Thanks to government support and investment in the industry, in the near future the Russian poultry farming may become an export-oriented one.[1]

However, the proportion of nutritional diseases with the alimentary transmission has increased dramatically over the

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past 10 years. These diseases cause both the physical damage to the human body, often leading to serious complications and even death, and the economic losses to the state. According to the WHO, the consumption of unsafe foods causes more than 200 diseases - from diarrhea to cancer. Every year, 600 million (approximately one in ten) people worldwide fall ill due to the consumption of substandard food containing pathogens. Out of them, 420 thousand die. This has resulted in the loss of 33 million years of healthy life to date. Children under 5 years old are subject to the highest risk: They make up 40% of all cases, and every year 125 thousand children die of nutrition-related diseases**.** The diseases with lesions of the gastrointestinal tract constitute the greatest proportion: 550 million people annually, out of them 230 thousand with deaths.[2,3]

The nutritional diseases, whose etiologic factors are *Salmonella* spp., *Campylobacter* spp., and enterohemorrhagic *Escherichia coli* have acquired the highest prevalence in the recent years.[4]

Intestinal *Campylobacter* is widespread all over the world, and according to the WHO data for the period 2007-2015 it significantly exceeded the salmonellosis incidence.[3] However, in contrast to salmonellosis, most often it is recorded in high-income countries [Figure 1].

People aged 25-64 fall sick most often (57% of cases), and children under 5 years old account for 11% of cases, but in this group, the highest mortality is observed. The sex distribution is 50:50 (according to the WHO report for 2007-2015).

Reservoir is represented by wild and domestic birds, in which *Campylobacter* spp., are the commensals of the gastrointestinal tract. There is the evidence of commensalism of some subspecies of *Campylobacter* in the gut of pigs.[5]

The main route of transmission is the nutritional one. The main transmission factors are products made from animal-based raw materials, as well as contaminated water.[6] Poultry meat plays a leading role in the transmission of *Campylobacter* spp., - up to 78% of human cases of nutritional *Campylobacter*iosis originate from an infected chicken. According to Dawkins MS: People consume nearly 60 billion chickens yearly and, despite the intensive efforts to improve the biosecurity practice on farms over the past decade, 71.2% of chicken flocks in the EU sent for slaughter during 2008 were contaminated with *Campylobacter* spp., and the incidence among people continues to grow. Moreover, the highest concentration of pathogen is noted in the skin (over 1,000 of colony forming units/g) in the neck and the cloaca. Furthermore, the source of the pathogen may be the beef, pork, milk, and other foods [Figure 2] that have not been sufficiently treated by heat or are contaminated because of non-compliance with technological processes and conditions of storage and sale of finished products.[7,8]

According to the results of the audit conducted by the CDC in 2011, *Campylobacter* bacteria were detected in 47% of

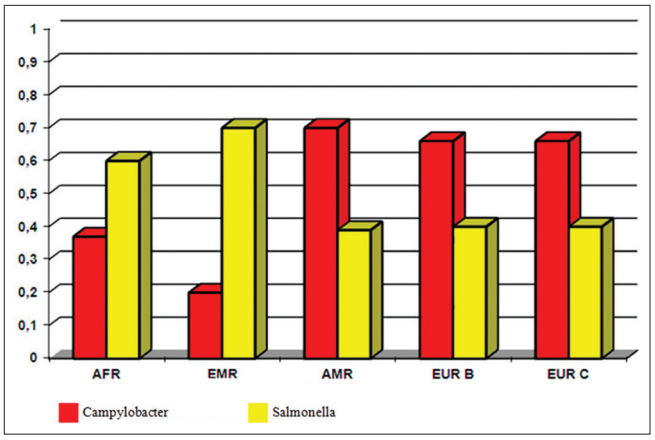

Figure 1: Distribution of sickness cases by regions. AFR: African region, EMR: Central Asian region, AMR: North American region, EUR B: Western Europe, EUR C: Eastern Europe, including Russia

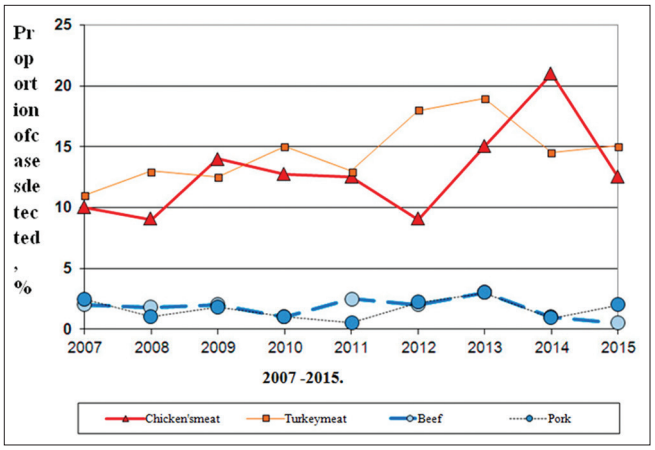

Figure 2: The product distribution according to the degree of contamination

raw chicken samples sold in the retail network. According to the reports of EU member states for 2013, *Campylobacter* were isolated from 31.4% of raw chicken meat samples. In the period of 2014-2015, according to surveys conducted by the British Agency for Food Standards, 73.3% of the tested products contained *Campylobacter*, and the frequency of pathogen detection in the packaging swabs was 7%.[3,9]

MATERIALS AND METHODS

The experimental part of the work was carried out at the Institute of Veterinary and Sanitary Examination, Biological and Food Safety FSBEU "Moscow State Institute of Food Production" and FSBUN "FIC of Food and Biotechnology" in the biosafety and nutrimicrobiom analysis laboratory.

A total of 28 samples were examined, including 12 - from the chicken carcass, 8 - from soup sets, consisting of the dorsal part and the skin, 4 - skin trimmings, and 4 - drumette. All

samples were obtained from various farms of the Moscow and Tambov regions.

Defining by the culture method was performed according to GOST ISO 10272-1-2013 «Microbiology of food items and animal feed. Methods of detection and counting *Campylobacter* spp., bacteria. Part 1: Method of detection. Furthermore, the bioMerieux (France) API CAMPY diagnostic systems (strips) were used for the specific differentiation of *Campylobacter* isolated cultures. The species confirmation of the *Campylobacter* spp., isolated cultures was also performed using polymerase chain reaction (PCR) with the electrophoretic detection of amplification products in the agarose gel. Determination of sensitivity to antibiotics was performed according to clinical guidelines, "Determination of the sensitivity of microorganisms to antimicrobial agents" (IACMAC 2014) using the disk diffusion method with the standard disks.

After the cultivation time, the cups with inoculation were examined for signs of growth. Several types of colonies were detected. On coal agar: (1) Small white waxy opaque convex, the medium surface covered with white translucent bloom, the medium has a sharp, unpleasant smell, (2) small-dew translucent whitish flat, resembling drops of condensation. On Preston agar: (1) Small-dew flat with irregular shape, somewhat like spreading in the course of a stroke, (2) convex round waxy, yellow in the middle, and pale at the edges - translucent, the medium has a sharp odor and a hemolysis area, and (3) white waxy, with signs of creeping growth. Suspicious colonies were tested for oxidase and catalase.

The smears were prepared from the colonies similar with *Campylobacter* by cultural properties and giving a positive response to the oxidase and catalase and stained by Gram. A total of 64 smears were microscope, and in two of them, the multiple Gram-negative coccoid forms of thin and small single polymorphonuclear curved rods were found. Based on these data, a preliminary conclusion about the presence of *Campylobacter* spp., in two samples was made. To obtain pure cultures, the Columbia blood agar was reseeded to the surface.

On blood agar: Single, small-dew, translucent, spreading colonies, and a weak zone of hemolysis. In smears: Gram-negative small thin polymorphonuclear curved rods.

When cultured on TSA and in broth, there were no visible signs of growth for Brucella. That confirms the appurtenance of the isolated microorganisms to thermotolerant *Campylobacter* species.

The study of biochemical properties of the isolated strains was conducted using the bioMerieux api Campy diagnostic kit.

To confirm the appurtenance of the isolated species to *Campylobacter jejuni*, the PCR analysis was performed, as one of the highly specific molecular biological techniques available today.

RESULTS

PCR results have confirmed that the isolated microorganisms are the *C. jejuni* [Table 1].

In the study of antibiotic susceptibility, the multiple antibiotic resistance was evident in the isolated strains.

The cultures were resistant to penicillins, cephalosporins, and fluoroquinolones, which is consistent with the available literature data. Erythromycin - the drug of choice in the treatment of nutritional *Campylobacter*iosis - was ineffective against isolated strains. However, the cultures were sensitive to tetracyclines and aminoglycosides, although the available literature data indicate the stability of most strains to these drugs.

DISCUSSION OF THE STUDY RESULTS

It was established that out of 28 samples of poultry and semi-finished products the *Campylobacter* bacteria had been detected in 4 semi-finished product samples "drumette" in 10 and 1.0 g weights. Based on the study of biochemical properties with the use of the bioMerieux api Campy diagnostic kit, it has been found that the isolated cultures belong to thermotolerant *C. jejuni* subsp. *Jejuni*. The results of the PCR analysis confirmed the appurtenance of the isolated cultures to *C. jejuni* form.

In its turn, the PCR technique is faster as compared to the culture method and allows giving a definitive answer within a few hours. Isolates of *Campylobacter* spp., had a multiple antibiotic resistance and were sensitive to aminoglycosides,

Table 1: PCR results

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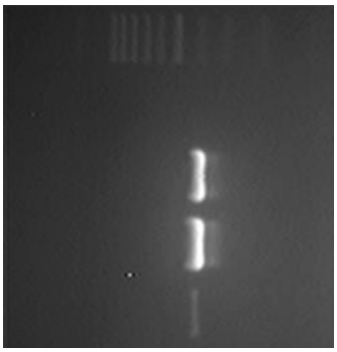
Sample	Result	Phoresis
Molecular weight marker		
Negative control of allocation		
Sample 1	+	
Sample 2	+	
Positive control	+	
Molecular weight marker		
Negative control	-	

PCR: Polymerase chain reaction

tetracyclines, and fenicoles. Among the macrolides, the azithromycin proved to be effective.

CONCLUSION

Dynamic development of the poultry farming in Russia and the increased consumption of poultry by the population, the growing incidence of food *Campylobacteriosis*, often severe complications of the disease, high contamination of raw materials and the lack of sufficiently accurate and simple methodology for the detection of pathogens in food cause the relevance of obtaining microbiologically safe poultry products. For this purpose, it is proposed to carry out a constant microbiological control both in the procurement of raw materials and on the stages of production, storage and sale of food products.

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