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Campylobacter Spp. In Poultry Production in Russia: The Study of Contamination by Traditional Microbiological Methods and Quantitative PCR Assay

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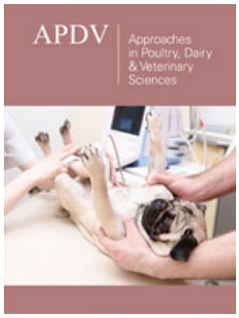

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Mini Review

During the past decades, in most countries the incidence of *campylobacteriosis* had increased, the number of its cases began to prevail over the other common diarrheal bacterial infections - salmonellosis and shigellosis [1]. The worldwide spread of *campylobacteriosis* and the great socio economic damage from this disease explain its inclusion in the list of emergent foodborne infections by the World Health Organization [2]. *Campylobacter* are ubiquitous bacteria, they are present in poultry or animals. Six taxa of the genus *Campylobacter* form a genetically related group of thermophilic *campylobacteria* with optimal growth temperature +42 °C and with the ability to infect humans and warm-blooded animals [3]. The most epidemiological significance represents *Campylobacter* jejuni, which cause up to 90% of laboratory confirmed cases of food borne *campylobacteriosis* [4]. The study of the mechanisms of survival and resistance of *C. jejuni* allows to predict the intensity of reproduction of bacterial populations in food raw materials and finished products, to evaluate the ability of pathogen to overcome the protective barriers of the organism and, consequently, the degree of risk associated with the use of new types of technological impacts in food production. These data are necessary for the development of an effective microbiological monitoring system at all stages of agricultural production, processing and storage of food products. The study of the antibiotic resistance mechanisms and stress tolerance of *Campylobacter* spp. will allow to intensify the use of risk assessment methodology and to implement effective monitoring of contamination of food with *campylobacteria*.

Considering the significance of the problem, studies included a screening assessment of the prevalence and nature of contamination of food products and production environment by *Campylobacter* spp., the study of phenotypic and genetic profiles of *C. jejuni* and expression of antibiotic resistance and pathogenicity under the influence of stress factors, including the use of antimicrobial agents. Research included the revealing of the patterns of the tolerance formation and adaptation to unfavorable environmental factors, the development of new methodological approaches allowing fast detection of *C.jejuni* in various food products. In total, more than 300 samples were analyzed, including broiler chicken meat and chicken raw by-products (chilled and frozen), turkey, quail, as well as more than 50 swabs from the surfaces of equipment at poultry processing plants.

The highest level of *campylobacter* detection (over 45%) was observed in raw poultry, including dressed chicken, turkeys, quails and semi-finished products. The study revealed a common pattern of Campylobacter penetration in raw materials and food products with inadequate sanitary treatment of certain sites of production: in most cases *Campylobacter* spp. was isolated from samples contaminated with coliforms (over 60% of samples), in addition, *Salmonella* spp. was detected in 17% of samples.

The frequency of contamination of poultry with pathogens is largely depends on the cooling technique of the carcasses [5]. The use of the immersion method of cooling creates

conditions for the cross contamination with pathogens through a water bath (45% of positive samples with *Campylobacter* spp). *Campylobacter* was also detected quite often (in 27% of samples) with the use of combined supercooled water and hydroaerosols. Contamination by pathogens was the lowest in evaporative cooling method with the use of antimicrobial hydroagricoles (less than 5% positive samples), allowing to recognize this method as the most promising for the production of microbiologically safe products. The study of equipment surfaces showed that the *Campylobacter* spp. detection frequency was 38,7%, *Salmonella* spp. - 12,9%.

Hardly cultivated foodborne pathogens *Campylobacter* spp. are actively persist in the environment and can be found in foods, water or other objects [3]. The mechanism of such survival and subsequent cross-contamination is determined by the ability of pathogenic *Campylobacter* strains to change into an unculturable state and to form a biofilms, creating an inadequate view during sanitary-hygienic assessing of microbial contamination of food objects. The escaping from the inactive phase, which is characterized by loss of the ability to reproduce and reduced metabolism can be followed by the restoration of the virulent properties of the infectious agent and to initiate the infectious process as a result of consumption of contaminated product. Detection of uncultivated forms of *C. jejuni* requires of special methods of analysis confirming the viability of the bacteria regardless of their ability to form colonies on selective and nonselective media. With the constant level of the total number of microbial cells, the number of colony-forming bacteria decreases, whereas the major part of microbial population shifts in metabolic inactive condition

A common trend of reduced number of viable cells in the process of contamination and subsequent cold storage of contaminated product, until the complete disappearance of cultivated forms was established in conditions simulating the raw material contact cooling with immersion method. According to results of PCR, the number of detectable cells exceeded the number of CFU in 5-10 times, the most clearly this difference is manifested in the study of the surface contaminated food. The cultural method did not allow to detect the presence of the pathogen in 40% of tested samples, while the number of uncultivated forms reached higher values. The feasibility of application of PCR as the most appropriate method for the detection of unculturable forms in different microbial associations has been shown on the basis of analysis and generalization of the methods. It allows to turn aside the main difficulty associated with the testing of bacteria in the uncultivated state, thus providing the possibility to replace the bacterial cultivation by amplification of species-specific DNA fragment.

As the *Campylobacter* bacteria are microbial contaminants of food production, there is a potential risk of appearance of additional sources of infection due to the persistence of these pathogens. Simultaneously with the phenomenon of the existence of viable uncultivated forms these bacteria have the ability to intensify biofilm formation under the influence of various adverse conditions [6]. The assessment of the impact of some production factors (atmosphere, temperature, etc.) on the viability and intensity of biofilm formation by *C. jejuni* was studied. Most intensive processes of the biofilm formation of *C. jejuni* occurred under aerobic conditions at a temperature of 25 °C, while under the favorable for these bacteria microaerophilic conditions the biofilm formation was poorly expressed. The higher temperatures of cultivation from 37 to 42 °C to a lesser degree affected the biofilm formation process in *C. jejuni*.

The common mechanisms of the protective properties of *Campylobacter* spp. can be realized in various variants of bacterial interaction with the environment, contributing to the formation of stable variants of microorganisms in response to stress technogenic or biological factors. The most clearly defined tendency in the changing of the *C.jejuni* properties is their increased resistance to bactericidal effects, which caused by the widespread use of antimicrobial agents, including antibiotics and biocides.

Study of phenotypic profiles of *C.jejuni* antibiotic resistance, isolated from poultry, was conducted [7]. More than 90% of cultures were insensitive to nalidixic acid and ciprofloxacin, 88.6% of strains had resistance to tetracycline, 34% to erythromycin. More than 65% of strains had multiresistance to 3 - 7 antibiotics of various groups, including 40% of cultures - to 4 or more antibiotics. Five multidrug-resistant strains, simultaneously resistant to 6-8 types of antimicrobials, were detected. The high level of multiresistance of the isolated *C.jejuni* strains indicates intensive processes of formation of genetically fixed signs of variability, which is accompanied by the emergence of tolerant *campylobacter* populations and the enhancement of their pathogenic potential in conditions of widespread use of antimicrobial agents in medicine, veterinary medicine and livestock.

Under regular sanitary treatment of water and equipment using biocides, the probability of biofilm formation on the surfaces of equipment and implement is high, which reduces the effectiveness of sanitary treatment regimes designed to eliminate free-swimming microbial populations of contaminants, including agents of foodborne infections. Pathogens that survived during such processing can infect raw materials or equipment with water at various stages of the technological process. Considering the more intense adaptability of *Campylobacter* to stress, it is advisable to consider them as pathogenic indicator bacterial contaminants of poultry products and conditions of their industrial production, with its inclusion in monitoring programs and laboratory control at poultry processing plants.

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