JOURNAL
OF HALAL QUALITY AND CERTIFICATION

Rigorous Sanitary Measures to Reduce *Campylobacter* in Chicken Production as an Alternative to the Prophylaxis Use of Antibiotics

Elma Hrustemović[1*], Faruk Čaklovica[1], Vesna Jaki-Tkalec[2], Sanja Furmeg[2], Zrinka Štritof[3], Nikola Paripović[3], Usman Mir Khan[4]

[1]*Faculty of Veterinary Medicine, University of Sarajevo, Zmaja od Bosne 90, Sarajevo 71000, Bosnia and Herzegovina*
[2]*Croatian Veterinary Institute, Križevci Veterinary Institute, Ivana Zakmardija Dijankovečkog 10, Križevci 48260, Croatia*
[3]*Faculty of Veterinary Medicine, University of Zagreb, Vjekoslava Heinzela 55, Zagreb 10000, Croatia*
[4]*National Institute of Food Science and Technology, University of Agriculture Faisalabad (UAF) 38000, Pakistan*

Corresponding author: Elma Hrustemović
E-mail address: elma_hrustemovic@hotmail.com

Review paper

ABSTRACT

Campylobacteriosis is a leading cause of food poisoning in Europe, the USA, Australia and New Zealand, and identifying reservoirs of infection is important in disease prevention. Although campylobacteriosis is considered rare in Africa, research indicates that chicken meat is contaminated in African countries as well. Monitoring the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in broiler meat production indicates variability depending on the country, season, source of the sample and hygiene in primary production (farms) and broiler meat processing (slaughterhouses/production plants). Therefore, the purpose of this review was to improve the understanding of the impact of implementing appropriate sanitation measures on reducing contamination with *Campylobacter* species in primary production and processing of chicken meat, in facilities with implemented HACCP and Halal control systems. The alignment with EU legislation regulates hygiene criteria in primary production and processing of chicken meat. The application of rigorous hygiene measures at all stages of chicken meat production can reduce the number of thermophilic *Campylobacter* species on broiler chicken carcasses. By setting the recommended EU critical limit of 1000 CFU/g for contamination of broiler neck skin in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

Keywords: *Antibiotics, Campylobacter, primary production and processing of chicken meat, sanitary measures*

Introduction

The cessation of the use of antibiotics in broiler farming would contribute to reducing the spread of antibiotic resistance, which together with campylobacteriosis represents a serious threat to public health. The application of rigorous sanitary measures, although a long-known procedure, can now be said to be a novelty in the production process because it contributes to reducing the spread of campylobacteriosis and antibiotic resistance. Today, rigorous hygiene measures in broiler farming and slaughterhouses are used as an alternative to the prophylactic use of antibiotics in broiler farming, but also as a means of biostimulation of chickens and preventing the spread of campylobacteriosis and antibiotic resistance (WHO 2023).

The Nordic countries have perfected a control system that includes prevention at the level of primary production (farm), which is in line with the European Food Criteria (EU) 2017/1495. Preventive biosecurity measures (vaccination, carvacrol in feed and drinking water, water disinfection) and hygiene can be implemented on broiler farms. Broilers are tested for the presence of *Campylobacter* near slaughter age in order to further implement preventive measures depending on the status of the flock. In the case of a positive flock, it is recommended that the meat of the positive flock be frozen (Olsen et al. 2024).

Meunier et al. (2016) state in their study that primary broiler production is crucial in ensuring the sanitary quality of meat and that it is necessary to ensure good sanitation of the facilities where chickens are kept. Chicken farms are a major source of *Campylobacter* spp. because they colonize the cecum of chickens. Control at the farm level is very important, but also very difficult to implement (Baali et al. 2020). On the other hand, negative broiler flocks, which do not have *Campylobacter* species in the intestines, after slaughtering without strict hygiene at different stages of primary processing, can produce carcasses contaminated with *Campylobacter* species above the limit values, indicating subsequent contamination via workers' hands or from surfaces in slaughterhouses (Wieczorek & Osek 2015).

In slaughterhouses and production plants, the most important measure for controlling campylobacteriosis remains the application of rigorous sanitary measures, which some authors' state can produce broiler carcasses free of *Campylobacter* spp. even in positive flocks (Szott et al. 2020). Hue et al. (2010) investigated the risk factors that lead to increased contamination of broiler carcasses with *Campylobacter* species during slaughter and found that the most critical stage in the slaughterhouse is evisceration. As a solution to the problem, it is proposed to rinse broiler carcasses after evisceration and perform slaughter according to the contamination status of the broiler flock with *Campylobacter* spp.

Positive flocks result in carcass contamination. It is important that farms and slaughterhouses implement strict biosecurity and hygiene measures in accordance with European food criteria. The limit value for contamination of the neck skin of broiler chickens with *Campylobacter* spp. in slaughterhouses should not exceed 1000 CFU/g (EU 2017/1495 & EFSA 2011). Therefore, the purpose of this review was to improve the understanding of the impact of implementing appropriate sanitation measures on reducing contamination of *Campylobacter* species in primary production and processing of chicken meat, in facilities with implemented HACCP and Halal control systems.

Epidemiology

Campylobacteriosis, epidemiologically speaking, occurs during periods of high air temperatures and high humidity. It can be said that it is a seasonal disease (Cortés et al. 2022). The reproduction of *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli*) in the intestines of chickens is most intense in late summer and early autumn in temperate climates. While in subtropical and tropical areas, high temperatures and high humidity are almost always present, so campylobacteriosis occurs in these areas throughout the year (Urdaneta et al. 2023).

Chicken meat is one of the important sources of protein for people around the world, therefore the health safety of chicken meat is of great importance. Biosecurity and hygiene measures in the production and processing of chicken meat must be at an enviable level in accordance with the recommended hygiene criteria for the chicken meat production process in accordance with European regulations, especially during the campylobacteriosis epidemic season (Olsen et al. 2024).

The Nordic and Baltic countries have developed the best control system for *Campylobacter* spp. in the chicken meat production chain, where in Norway, but also in Iceland, microbiological control of meat is carried out weekly during the campylobacteriosis season. In Iceland, the limit for *Campylobacter* spp. in neck skin is set at 500 CFU/g instead of 1000 CFU/g (EU) 1495/2017, and for this purpose, rigorous hygiene measures are implemented in slaughterhouses and production plants (Olsen et al. 2024). Campylobacteriosis outbreaks have long been associated with developed European countries, Sweden, Denmark, then the United States and Australia (Hanafy et al. 2022), and outbreaks have been rarely reported in Africa, Asia and the Middle East, with the exception of Thailand (Mason et al. 2017). In the region, in Croatia, an increase in campylobacteriosis epidemics has been recorded since 2007, when the reporting of campylobacteriosis became a legal obligation (Furmeg et al. 2021).

According to reports from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the number of cases of campylobacteriosis in Europe has been increasing annually since 2005 (Table 1) (EFSA & ECDC 2009–2021). Today, the Nordic countries have developed the best control system for *Campylobacter* spp. at the farm, slaughterhouse and production plant levels, where rigorous sanitary measures are applied in the chicken meat production process, and without the use of antibiotics in chickens in breeding (Lopes et al. 2018; Olsen et al. 2024). The limit for contamination of chicken neck skin for *Campylobacter* spp. in slaughterhouses is set at 1000 CFU/g in accordance with EU 2017/1495.

Table 1. Number of reported cases of campylobacteriosis in Europe per year

Annual reports	**Growth in campylobacteriosis cases from 2005 to 2021**		
	Annual growth (%)	Number of cases	Sources
2005	6-7	195 426	EFSA & ECDC (2009)
2007	2-3	200 507	EFSA & ECDC (2009)
2010	5-6	212 064	EFSA & ECDC (2012)
2011	3-4	220 209	EFSA & ECDC (2013)
2014	7-8	236 851	EFSA & ECDC (2015)
2016	3-4	246 307	EFSA & ECDC (2017)
2018	0-1	246 571	EFSA & ECDC (2019)
2019 (COVID-19)	Recorded decline since 2019	120 946	EFSA & ECDC (2021)
2021	/	/	/

Rigorous sanitary procedures applied in primary production and processing of chicken meat, based on control systems best developed in Scandinavian countries, and based on compliance with EU legislation, have been proven to contribute to protection against campylobacteriosis and prevent the spread of antibiotic resistance. Based on research to date, these methods have proven effective in most EU countries. By setting the recommended EU critical limit of 1000 CFU/g for contamination of the neck skin of broilers in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

Biological characteristics

Campylobacter

The pathogenicity of *Campylobacter* species depends on the degree of virulence of the bacterial species/strain. Thermophilic *Campylobacter* spp. (mainly *C. jejuni* and *C. coli*) are extremely virulent and only a small number of bacteria are required to cause campylobacteriosis in consumers of chicken meat (Finsterer 2022). These bacterial species possess virulence factors, i.e. a capsule, polysaccharide and oligosaccharide membranes, toxin production, various biochemical properties, protease enzymes, etc. All of the above virulence factors can encode genes for toxins, as well as genes for acquiring antibiotic resistance. On the other hand, there are mechanisms that pathogenic *Campylobacter* species use to exert their pathogenicity. *Campylobacter* species have the ability to invade, produce toxins, adhere to the intestinal mucosa and initial reproduction (the mechanism by which they colonize the intestines of chickens) (Habib et al. 2023).

In order for bacteria to possess these pathogenic mechanisms at all, they must possess genes: genes for invasion and colonization such as *cadF*, *ciaB*, *pldA* (Buiatte et al. 2023). The presence of flagella is also one of the mechanisms of bacterial pathogenicity by which bacteria move to their final destination, in this case the intestinal epithelium. Chemotaxis represents directed movement, translocation is movement towards organs, and invasion is passage through the epithelium (Habib et al. 2023). Pathogenesis is the way in which infection occurs and depends on virulence factors, the infectious dose of bacteria and the immunity of the attacked macroorganism (Jama & Ketley 2023).

Prophylaxis use of antibiotics in broiler farming

The use of antibiotics (AB) in primary broiler production is present worldwide. Antibiotics are used as a supplement to the diet of chickens in order to biostimulate, increase growth and shorten fattening time (Santos-Ferreira et al. 2022). The use of non-resorbable antibiotics bacitracin and spiramycin in broiler farms reduced the risk of infection and cross-contamination within the flock (Milanov et al. 2016). By slaughtering a healthy flock of broilers in slaughterhouses, the risk of cross-contamination of chicken meat is reduced (Wieczorek & Osek 2015). Non-absorbable antibiotics perform enterodisinfection in broilers, improve their performance, their health, the microbiological correctness of chicken meat and protect the health of consumers. Many authors warn that the benefits of using antibiotics in broiler prophylaxis are much smaller than the negative effects (Santos-Ferreira et al. 2022). Denmark and Sweden, countries where campylobacteriosis was a pressing public health problem, saw the disadvantages of this practice and were the first to ban the use of AB in broilers for prophylactic purposes (Olsen et al. 2024).

Antibiotic residues in chicken meat can lead to the spread of antibiotic resistance (ABR). Cooking chicken meat containing antibiotic residues in the meat can create more dangerous chemical structures, and the consumption of such meat can lead to autoimmune disorders in consumers (Senčić et al. 2021). Antibiotic residues in chicken meat are not the only danger for consumers. Antibiotic resistance genes can spread through microbial contaminants of chicken meat to bacteria of human origin. Also, intestinal bacteria can develop various mechanisms of antibiotic resistance. The use of antibiotics can cause mutations. Antibiotic resistance genes can be transmitted to offspring vertically and to almost all bacteria by horizontal transfer (Gharbi et al. 2021). The European Union has banned the use of antibiotics in broiler prophylaxis since 2006 (FDA 2017), so finding an alternative to the prophylactic use of antibiotics is of crucial importance for broiler chicken producers. A natural agent that should have a biostimulating effect should not be harmful to the health of chickens, consumers, and should also not pollute the environment (Santos-Ferreira et al. 2022).

Antibiotic resistance

Thermophilic Campylobacter spp. can acquire antibiotic resistance genes from other bacteria in the immediate vicinity. They have the ability to accumulate genes in the genome, but they are also prone to mutations caused by the use of antibiotics because they do not possess genes that protect bacterial DNA (umuCD, vsr) or genes that serve for DNA repair (mutH, mutL) (Al-Khresieh et al. 2022). Vertical gene transfer from parent to offspring and horizontal gene transfer to all bacteria in the immediate vicinity are known. Horizontal gene transfer is enabled by conjugation, transformation and transduction (Habib et al. 2023). Bacterial conjugation occurs with the help of sexual cells, and is enabled by plasmids, transposons and integrons. For horizontal gene transfer to occur at all, bacteria must possess conjugated *tet*(O) genes within the *pTet* plasmid. Transformation is possible after the death of the bacterial cell, where the bacterial DNA is released through the lysis process and gene transfer to other competent bacteria can occur, only 1% of bacteria have this ability. Transduction is the transfer of bacterial DNA with the help of phage bacterial viruses to other competent bacteria (Habib et al. 2023).

In *Campylobacter* species, DNA mutations are not uncommon and can be caused by various mutagens, most often antibiotics. Therefore, uncontrolled use of various types of antibiotics, e.g. from the fluoroquinolone class, can cause mutations of local *gyrA* genes associated with fluoroquinolone resistance.

Table 2. Mechanisms of acquisition of antibiotic resistance in *Campylobacter* species (Markey et al. 2013)

Antibiotics: class/type	**Antibiotic resistance of *Campylobacter* species**
	Biochemical mechanisms of antibiotic resistance
I β lactams: penicillins, amoxicillin, amoxicillin with clavulanic acid, ampicillin, ampicillin sulbactam, cephalosporins I, II, III and IV generation, reserve carbapenems: carbapenem, imipenem, meropenem, ertapenem. **Glycopeptides: vancomycin, teicoplanin, telavancin, antitumor antibiotic belomycin.** **Polypeptides: polymyxin B, polymyxin E or colistin, bacitracin.**	I Enzyme alteration and modification
II Quinolones and fluoroquinolones: ciprofloxacin, norfloxacin, marbofloxacin. **Aminocoumarins: novobiocin.** **Rifamycins: rifampicin or rifampin.**	II Change in DNA gyrase and topoisomerase IV
III and IV Nitrofurans: nitrofurantoin. **Tetracyclines: tetracycline, doxycycline.** **Aminoglycosides: gentamicin, reserve amikacin, kanamycin, neomycin, etc.** **Lincosamides: clindamycin, lincomycin.** **Macrolides: azithromycin, erythromycin, clarithromycin.**	III Target modification or change of the target enzyme and IV Modification of ribosome structure
V To all antibiotic classes and disinfectants	V Active efflux (pump or carrier protein)
VI Sulfonamides and trimethoprim	VI Alteration of metabolic pathway
VII To all antibiotic classes	VII Alteration of cell membrane permeability

On the other hand, uncontrolled use of macrolide antibiotics can cause mutations in 23S rRNA, which is associated with macrolide resistance. Since Campylobacter spp. can accumulate genes in the genome, there is a possibility that they can acquire antibiotic resistance genes from other competent bacteria, mainly from gram-positive bacteria, by horizontal transfer. They can possess *ermB*, *optrA*, *fexA* and *cfrC* genes in their genome. The *erm*(B) genes are associated with acquired resistance to erythromycin (macrolides), *tet*(O) to tetracycline (tetracyclines) (Qin et al. 2023). Sometimes it is only the presence of the gene that is at issue, which is why it is important to examine the presence of phenotypic, not just genotypic, resistance. The presence of the gene indicates the possibility of *Campylobacter* spp. resistance to antibiotics, and indicates the possibility of preventing antibiotic resistance, which is achieved by reducing or completely stopping the use of a particular antibiotic in chickens, even for therapeutic purposes. It is important to use antibiotics responsibly and rationally in agriculture, veterinary medicine and medicine. To date, *Campylobacter jejuni* has ˜1650 conjugated genes in its genome, and this number is increasing (Hitchcock et al. 2022). Bacteria can also possess antibiotic resistance mechanisms without the presence of resistance genes in the genome of the bacterial cell. Resistance mechanisms are mainly related to the mechanism of action of the antibiotic whose use has caused resistance (Table 2) (Markey et al. 2013).

Control of Campylobacteriosis along the Food Chain

Biological contaminants and preventive measures

The improved European system for the control of *Campylobacter* spp. in raw chicken meat sets a limit for contamination of chicken neck skin with *Campylobacter* spp. in slaughterhouses of 1000 CFU/g based on Regulation (EU) 2017/1495. Preventive measures to prevent contamination of chicken meat with *Campylobacter* spp. can be divided into three levels of protection. The first and most important level of safety that ensures the health safety of chicken meat is control at the farm level. Sanitary control in chicken farming is the most important preventive measure with a strong effect, because flocks positive for *Campylobacter* spp. produce positive broiler carcasses in slaughterhouses and production plants (Sibanda et al. 2018).

The second measure is the serological categorization of *Campylobacter* species from chicken meat, since campylobacteriosis is mainly caused by the consumption of infected chicken meat (Taha-Abdelaziz et al. 2023). The third measure is the implementation of hygiene and sanitation measures in slaughterhouses, but with a somewhat smaller impact on the safety of chicken meat if it is a positive flock slaughtered. It includes the application of hygiene and sanitation standards during slaughter, carcass processing and packaging (Sibanda et al. 2018).

The fourth measure is of medium effect, which includes washing broiler carcasses under a strong stream of drinking water, which is important during evisceration because it leads to decontamination of the carcass. The fifth preventive measure is cooling the meat to +4°C of medium effect, because *Campylobacter* spp. are mesophilic bacteria (Olsen et al. 2024). On farms, aldehydes and cresols are most commonly used in the sanitation process (Table 3) (Butucel et al. 2022). The second line of defense in the fight against human campylobacteriosis is represented by hygienic and sanitary measures applied during transport, processing of broiler chicken carcasses and during packaging (Table 4) (Perez-Arnedo & Gonzalez-Fandos 2019; Olsen et al. 2024).

Table 3. Disinfectants used on chicken farms (Butucel et al. 2022)

Disinfectant	**Characteristics of disinfectants** Positive and negative properties
Aldehydes, a well-known representative of formaldehyde (HCHO)	Bactericide, fungicide, virucidal. Disinfection of floors, walls and equipment is achieved at a concentration of 1-2%. It leaves no residue, but is toxic, corrosive, has a pungent odor and is inhibited by organic detritus. Neutralization of too pungent odor is carried out with 25% ammonia. Formaldehyde is the only one in a gaseous state, and in combination with potassium permanganate it is used for fumigation of eggs.
Monohydroxyl phenols – Cresols, representative of carbolic acid (C6H5OH)	Bactericides, fungicides, virucides and sporocides. Used for disinfection of floors, walls and equipment. Leaves a residue. Mildly toxic and inexpensive, but corrosive and moderately inactivated by organic detritus. A solution of cresol in potassium soap is often used for disinfection.
Carboxylic acids, the main representative is acetic acid (CH3COOH)	Bactericides, fungicides, virucides, but not sporicides. Insignificant residual activities. They are not inhibited by organic detritus. They have a good synergism of action in combination with detergents. Disadvantages: toxicity, corrosion, high cost and strong smell.
Halogen elements, the main representative is chlorine (Cl), chlorine lime (CaCl(OCl))	It has an antimicrobial effect on almost all microorganisms, but it is the best bactericide. Chlorine is a cheap disinfectant, leaves no residue, but is toxic, corrosive and inhibited by organic detritus. In its gaseous state, it is used for water disinfection, and in the form of chlorine lime/lime, calcium hypochlorite is used for sanitation.
Halogen elements, represented by iodine (I)	Bactericide, fungicide and viruicide. It leaves no residue. Disadvantages are toxicity, medium corrosivity and inhibition in organic detritus.

HCHO = molecular formula for formaldehyde
C6H5OH = molecular formula for carbolic acid
CH3COOH = structural formula for acetic acid
Cl = symbol for the chemical element chlorine
CaCl(OCl) = structural formula for chlorine lime, calcium chloride hypochlorite/caporite
I = symbol for the chemical element iodine

Table 4. Hygienic and sanitary measures applied during slaughter and carcass processing (Perez-Arnedo & Gonzalez-Fandos 2019; Olsen et al. 2024)

Slaughter and primary processing stages			**Hygienic and sanitary measures during slaughter and carcass processing**
	Dirty phases	Pure phases	Hygiene and sanitary measures
I phase	The slaughter "Eat the meat of animals whose blood has been shed and over which the name of Allah has been mentioned" (Bukhari and Muslim) (Halal slaughter).		Sanitary processing of the cages, healthy flocks are slaughtered, the reception/slaughter house is separated, the slaughtering is done immediately. Bleeding 1-3 min without treatment.
I phase	Skinning Plucking Hygiene washing of carcasses with removal of head and legs		The pools must be hygienically clean, dried and the water used must be potable. The pools for skinning and plucking represent an important risk factor for cross-contamination of carcass skin with *Campylobacter* spp. and it is very important that the pools and the water are prepared with strict biosecurity measures recommended by the veterinary service. Before processing the carcasses, they are washed under a stream of clean potable water.
II phase	Evisceration		Evisceration is a dirty stage with a high risk of cross-contamination. It is important to prevent gastric and intestinal effusion. When removing organs, sterile knives are used, and all working surfaces must be treated sanitaryly, as well as the workers' hands.
III phase		Draining Cooling Confectionery	Correct carcasses go for cooling, and defective ones are discarded. Minor damage can be cut/trimmed. Final wash. Strain and cool for 1 hour at 4◦C, without tying. Packaging (name, address of the manufacturer and veterinary control number).

Sanitation and Risk Analysis

Sanitation in broiler chicken production should be performed only by trained workers. Sanitation, which is commonly used in European countries, reduces the bacterial load by 95% and involves mechanical washing with detergent, followed by the use of disinfectants (Table 5) (Quinn et al. 2011). Hot air drying reduces the number of bacteria (Butucel et al. 2022). Another method of sanitation involves mechanical cleaning, without the use of disinfectants (Burbarelli et al. 2015). Sanitation removes microorganisms, which contributes to the control of the environment in which broiler chicken meat is produced. Sanitation is a legally prescribed hygiene and health measure in chicken meat production that improves the safety of the final product (Olsen et al. 2024).

Mechanical cleaning is an important phase of sanitation, especially in broiler farming (farms). The purpose of mechanical cleaning is to remove organic matter that inactivates disinfectants. In the dry phase, the equipment is first dismantled and moved. Then, the equipment and parts of the facility are blown out with air using a compressor. Coarse, polypropylene brushes and brooms are used for mechanical brushing of floors, walls and equipment (Luyckx et al. 2015). In the wet phase, detergents that break down organic and mineral deposits are used. Basic and acidic detergents are used. By breaking down organic matter, the full antimicrobial potential of the disinfectant is used. After washing and drying, disinfectants are used. An atomizer (sprayer) is used to disinfect the facility. In this way, the facility is prepared for the reception of one-day-old chicks (Gichure et al. 2022).

The implementation of hygiene and sanitation measures must be constantly monitored and controlled (Olsen et al. 2024). After proper sanitation, swabs are taken to show the effectiveness. In case of positive swabs, disinfection should be repeated until the standard is achieved (Luyckx et al. 2015). In EU countries, producers are required to submit results on the number of *Campylobacter* spp. in chicken meat to the government, which improves meat safety control. Chicken meat contaminated with *Campylobacter* spp. above the limit value is more often subject to controls on the implementation of the HACCP system, until the analysis results are satisfactory (Olsen et al. 2024).

Table 5. Disinfectants and mechanisms of action (Quinn et al. 2011)

Disinfectant	**Different mechanisms of action of disinfectants** Mechanisms of action
Alcohols and aldehydes	They coagulate bacterial proteins
Monohydroxyl phenols – Cresols, carbolic acid (C6H5OH), Halogen elements: fluorine (F), chlorine (Cl), bromine (Br), iodine (I), astat (At) **Peroxide halogen elements: hydrogen peroxide (H2O2) and potassium permanganate (KMnO4), Carboxylic acids: acetic (CH3COOH), lactic (CH3CH2COOH), etc.**	They damage the bacterial cell through the oxidation process
Cationic (quaternary ammonium compounds), anionic, nonionic and amphoteric surfactants **Detergents (acids and bases)**	They damage the bacterial cell by surface activity. They hydrolyze internal cell structures.

Monitoring Genotypic Antibiotic Resistance

The presence of antibiotic resistance determinants in *Campylobacter* spp. isolates from chicken meat, such as antibiotic resistance genes, various mutations, pose a challenge for food safety. The importance of monitoring *Campylobacter* spp. isolates from chicken meat, changes within the genome, the presence of new antibiotic resistance genes is important to prevent the spread of antibiotic resistance. The presence of antibiotic resistance genes in *Campylobacter* spp. indicates uncontrolled use of antibiotics, which, in the case of genotypic resistance, should be withdrawn from use (Habib et al. 2023).

In a study on the association between genotypic and phenotypic antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from meat and cecum of various animals in the United States, Zhao et al. (2015) reported whole genome sequencing data from 2000 to 2013, where 114 isolates (82 *C. coli* and 32 *C. jejuni*) were characterized for the detection of antibiotic resistance genes and mutations. In resistant isolates of *C. jejuni* and *C. coli*, 18 acquired antibiotic resistance genes were detected, *tet*(O), *bla*OXA-61, *cat*A, *lnu*(C), *aph*(2″)-Ib, *aph*(2″)-Ic, *aph*(2')-If, *aph*(2″)-Ig, *aph*(2″)-Ih, *aac*(6')-Ie-aph(2″)-Ia, *aac*(6')-Ie-aph(2″)-If, *aac*(6')-Im, *aad*E, *sat*4, *ant*(6'), *aad*9, *aph*(3')-Ic i *aph*(3')-IIIa. Mutations in the regions of the native gyrA gene and in the 23S rRNA were recorded. Genotypes were tested by whole genome sequencing, and phenotypes were determined by microdilution. A high degree of agreement was found between the results of genotypic and phenotypic methods used to determine antibiotic resistance (99.2%), for fluoroquinolones and tetracyclines (100%), macrolides: erythromycin (95.4%), azithromycin (98.7%), aminoglycosides: aminoglycosides: amicosides.78%. clindamycin: (98.7%), ketolides: telithromycin (98.7%). The results of the study suggest that whole genome sequencing is a reliable method for monitoring antibiotic resistance.

In a 16-year retrospective study examining the genotypes and phenotypes of antibiotic resistance in *Campylobacter coli* isolates from different sources in Brazil, Gomes et al. (2023) reported that the antibiotic resistance genes *bla*OXA-605 / *bla*OXA-61/ (54%), *tet*(O) (22.2%), *cme*B (9.5%), *aad*E-Cc (6.3%), *aph* (3') – IIIa (1.6%), *sat*4 (1.6%) i *aad*9 (1.6%) were found to be co-resistant. Mutations in the gyrA gene regions (T86I in QRDR) were detected in 8 (12.7%) C. coli strains. Genotypes were tested using whole genome sequencing (WGS), and phenotypes were examined using disk diffusion. Comparison of genotypic and phenotypic methods revealed concordance for fluoroquinolones (100%), tetracyclines (92.9%), β-lactams (82.4%), and aminoglycosides (80%).

Monitoring the Impact of Sanitation on the Reduction of *Campylobacter*

The purpose of this review is to improve the understanding of the impact of implementing appropriate sanitation measures on reducing *Campylobacter* species contamination in primary chicken production and processing, in facilities with implemented HACCP and Halal control systems. Also, this research can improve the understanding of the global public health problem, which is antibiotic resistance of *Campylobacter* spp. isolates from chicken meat. By implementing rigorous sanitation in primary chicken production of broilers, it is possible to stop the use of antibiotics in primary chicken production (farms) in countries where this is still the practice.

In accordance with the EU Regulation, which prescribes hygiene criteria in the primary production and processing of chicken meat, antibiotics are not used on chicken farms, but other biosecurity measures (vaccines, carvacrol in chicken feed, monoclonal antibodies) and rigorous hygiene and sanitary measures. In order to check the hygiene of chicken meat production and processing, a critical limit of 1000 CFU/g (EU 2017/1495) has been set for contamination of chicken neck skin in slaughterhouses. Laboratory tests for the presence of *Campylobacter* spp. in chicken meat, in slaughterhouses with HACCP and Halal standards must be performed once a week. In case the 52 analyses are satisfactory, laboratory tests are performed every other week. Only drinking water may be used for the purpose of decontamination of chicken carcasses (EU 2017/1495). Based on a report by the European Food Safety Authority, by setting a critical limit of 1000 CFU/g for contamination of broiler neck skin in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90% (EFSA 2011).

In order to produce healthy chicken meat in slaughterhouses, it is very important that strict hygiene and sanitation measures are implemented on chicken farms. These are prophylactic measures to prevent the spread of diseases in chickens, while at the same time omitting the prophylactic use of antibiotics in chickens, which contributes to the spread of antibiotic resistance of bacteria (Umaraw et al. 2017). Appropriate sanitary conditions on chicken farms improve the intestinal microbiota of chickens. When a large number of pathogens are present in the intestines of chickens, 20% of energy is consumed. If there is a healthy microbiota of chickens, feed conversion is improved and chickens gain weight (biostimulation) even when antibiotics are not used (de Castro Burbarelli et al. 2017). Cessation of the use of antibiotics in chickens for the purpose of protection against campylobacteriosis, other bacterial diseases, and for the purpose of biostimulation (antibiotics as growth promoters) prevents the spread of antibiotic resistance, a global public health problem (Nastasijević et al. 2020). Based on monitoring the spread of antimicrobial resistance in *Campylobacter* spp., there are still no such reports of antimicrobial resistance to currently available disinfectants, but the possibility of the emergence of disinfectant resistance can never be ruled out. For this purpose, it is recommended not to use the same disinfectant for a long period of time (Davies & Wales 2019).

In Bosnia and Herzegovina and the region, such research has not been conducted, and we do not have data on the impact of sanitary measures on chicken growth, health and ensuring the health safety of chicken meat in slaughterhouses. It has not yet been investigated whether chicken producers have stopped using antibiotics in primary production (farms) in Bosnia and Herzegovina and turned to other alternative solutions to antibiotics in order to ensure the biostimulation of chickens and the health safety of meat. Although it seems that sanitation is something that is already known and has been used for a long time in the chicken meat production process, reporting on the prevalence of *Campylobacter* spp. in chicken meat tells us that some European countries have seriously approached solving this public health problem, while some have not yet achieved satisfactory results. Therefore, it is important to apply rigorous hygiene and sanitary measures in the process of primary production and processing of chicken meat, in order to achieve satisfactory results.

The best system for controlling *Campylobacter* spp. in chicken meat, according to EU criteria, developed by the Nordic countries, where campylobacteriosis has been one of the biggest public health problems for many years. Today, the Nordic and Baltic countries can boast a significantly lower prevalence of *Campylobacter* spp. in chicken meat, because they have developed the best control system (EU 2017/1495). According to the latest reports, the prevalence of *Campylobacter* spp. in chicken meat in Estonia were very low, the results are excellent (1.8%), followed by Iceland, which also recorded a low prevalence (2.1%) (Lopes et al. 2018; Mäesaar et al. 2014). On the other hand, some European countries that also act according to the latest Regulation (EU) 2017/1495, do not have satisfactory results in the prevalence of *Campylobacter* spp. in chicken meat. High prevalences were recorded in France (76%), Spain (70%), Turkey (56.1%), Poland (50%), Italy (34.1%) and Belgium (29%) (Ozbey & Tasdemi 2014; Tedersoo et al. 2022; Wieczorek et al. 2012). Such results indicate inadequate biosecurity measures, poor hygiene in the chicken meat production process, poor control of insects, rodents that serve as vectors on farms and slaughterhouses (Horvat et al. 2022; Olsen et al. 2024).

It is most important that good hygiene and sanitation standards are applied on farms, but that these measures continue to be applied during transport, slaughter, primary processing of broiler carcasses and packaging. Proper sanitation of broiler chicken farms will ensure a healthy microbiota of chickens, prevent colonization of *Campylobacter* spp. 42 days of fattening. If a healthy chicken flock is produced, healthy chicken meat will also be ensured, if hygiene and sanitation measures are continued along the entire chicken meat production chain. However, if a sick chicken flock is produced, highly contaminated with *Campylobacter* spp. and the most rigorous chicken meat control measures in slaughterhouses will rarely have satisfactory results in terms of the prevalence of *Campylobacter* spp. in chicken meat (Olsen et al. 2024).

If proper sanitation is not provided in the facilities where chickens are kept (sanitation of floors, walls, equipment, drinkers and feeders), the proper application of Good Manufacturing Practices and Good Hygiene Practices, the HACCP concept in slaughterhouses, will not have much significance in ensuring the sanitary quality of chicken meat. The application of the HACCP concept in slaughterhouses prevents cross-contamination and is very important for ensuring the microbiological safety of chicken meat if healthy flocks are slaughtered (Castañeda-Gulla et al. 2020). Protection against cross-contamination of chicken meat is an important segment in ensuring the sanitary quality of meat (Borges et al. 2020).

Meunier et al. (2016) state in their research that primary broiler production is key to ensuring the sanitary quality of meat and that it is necessary to ensure good sanitation of facilities where chickens are kept, because positive flocks affect increased contamination during slaughter and carcass processing. They also state that the application of nutrients, organic, inorganic acids, probiotics, bacteriocins, bacteriophages, essential oils and immunization of chickens contributes to the biosecurity of meat.

Burbarelli et al. (2015) analyzed two models of farm cleaning that would affect the microbiological status of the environment and the increase in growth in broilers. In the first model, they used mechanical cleaning, dry and wet, and in the second model, they used high-pressure water washing with detergents and, after rinsing, they used two disinfectants from the aldehyde and cresol group: glutaraldehyde 250g/L, formaldehyde 185g/L and p-chloro-m-cresol 210 g/L, which represents the European cleaning model that has proven to be more effective in reducing pathogens.

Luyckx et al. (2015) in their study of the effectiveness of four cleaning models implemented in broiler houses with the aim of preventing zoonoses, discovered critical points of disinfection: drinkers, cracks in the floors and holes for water drainage. Such studies indicate that before sanitation, all holes in chicken houses that are difficult to access for sanitation and allow the development of microorganisms should be sanitized. Drinkers, due to the constant presence of water, often due to the presence of biofilm, can be a source of pathogenic microorganisms and it is important to remove organic matter, bifilms, by pressure washing, which will increase the effectiveness of disinfectants.

Hue et al. (2010) investigated the risk factors that lead to increased contamination of broiler carcasses with *Campylobacter* species during slaughter and found that the most critical stage in the slaughterhouse is evisceration. As a solution to the problem, it is proposed to rinse broiler carcasses after evisceration and perform slaughter according to the *Campylobacter* contamination status of the broiler chicken flock. Positive flocks determine carcass contamination. Cross-contamination occurs during transport, slaughter and primary processing of broiler carcasses (Perez-Arnedo & Gonzalez-Fandos 2019).

Conclusions

Rigorous sanitary procedures applied in primary production and processing of chicken meat, based on control systems best developed in the Nordic and Baltic countries, and based on compliance with EU legislation, have been proven to contribute to protection against campylobacteriosis and prevent the spread of antibiotic resistance. Testing the correlation of genotypic and phenotypic acquired antibiotic resistance in *Camplobacter* spp. isolates from raw chicken meat may be an appropriate control measure, in case of suspicion of misuse of antibiotics in chicken farming (farms) as growth promoters. Based on previous research, these methods have proven effective in most EU countries. By setting the recommended EU critical limit of 1000 CFU/g for contamination of the neck skin of broilers in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

Acknowledgment

This study was made possible thanks to a grant from the Ministry of Science, Higher Education and Youth of the Sarajevo Canton in 2022 and a grant for doctoral students from the Federal Ministry of Education and Science in 2021.

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Stroge sanitarne mjere za smanjenje kampilobaktera u proizvodnji pilića kao alternativa profilaktičkoj upotrebi antibiotika

Elma Hrustemović[1*], Faruk Čaklovica[1], Vesna Jaki-Tkalec[2], Sanja Furmeg[2], Zrinka Štritof[3], Nikola Paripović[3], Usman Mir Khan[4]

[1]Veterinarski fakultet, Univerzitet u Sarajevu, Zmaja od Bosne 90, Sarajevo 71000, Bosna i Hercegovina
[2]Hrvatski veterinarski institut, Veterinarski institut Križevci, Ivana Zakmardija Dijankovečkog 10, Križevci 48260, Hrvatska
[3]Veterinarski fakultet, Univerzitet u Zagrebu, Vjekoslava Heinzela 55, Zagreb 10000, Hrvatska
[4]Nacionalni institut za nauku o hrani i tehnologiju, Poljoprivredni univerzitet u Faisalabadu (UAF) 38000, Pakistan

Autor za korespondenciju: Elma Hurstemović
E-mail adresa: elma_hrustemovic@hotmail.com

Pregledni rad

Sažetak

Kampilobakterioza je vodeći uzrok trovanja hranom u Europi, SAD-u, Australiji i Novom Zelandu, a otkrivanje rezervoara infekcije važno je u prevenciji bolesti. Iako se kampilobakterioza smatra rijetkom u Africi, istraživanja ukazuju na kontaminaciju pilećeg mesa i u afričkim zemljama. Praćenje prevalencije *Campylobacter jejuni* i *Campylobacter coli* u proizvodnji mesa brojlera ukazuje na varijabilnost u zavisnosti od zemlje, sezone, porijekla uzorka i higijene u primarnoj proizvodnji (farme) i preradi mesa brojlera (klaonice/proizvodni pogoni). Stoga je svrha ovog preglednog rada bila poboljšati razumijevanje utjecaja koji primjena odgovarajućih sanitarnih mjera ima na smanjenje kontaminacije *Campylobacter* vrsta u primarnoj proizvodnji i preradi pilećeg mesa, u objektima sa implementiranim HACCP i Halal kontrolnim sistemima. Usklađivanjem sa zakonodavstvom EU uređuju se higijenski kriterijumi u primarnoj proizvodnji i preradi pilećeg mesa. Primjenom rigoroznih higijenskih mjera u svim fazama proizvodnje pilećeg mesa može se smanjiti broj termofilnih vrsta *Campylobacter* na trupovima brojlerskih pilića. Postavljanjem preporučene kritične granice EU od 1000 CFU/g za kontaminaciju kože vrata brojlera u klaonicama, incidencija kampilobakterioze može se smanjiti za 40% do 90%.

Ključne riječi: Antibiotici, *Campylobacter, primarna proizvodnja i prerada pilećeg mesa, sanitarne mjere*