Chapter 5

Biofilms of *Salmonella* and *Campylobacter* in the Poultry Industry

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65254

Abstract

Biofilm is characterized by a bacterial population firmly adhered to a surface involved by a self-produced matrix of extracellular polymeric substance. These communities provide longer survival and resistance to adverse conditions such as presence of antibiotics and disinfectants. Various foodborne microorganisms are capable of forming such structures, including *Salmonella* and *Campylobacter*, which are the major contaminants at the poultry industry. This biomass can affect the water transport system and pipes, and once the agent is established at the industry, it can form biofilms in any processing area. There are intrinsic and extrinsic mechanisms, and also molecular aspects involved in the biofilm formation. The adoption of several strategies may exhibit effectiveness to prevent the cell adhesion, such as the use of surfaces resistant to biofilm formation. In case of preexisting biofilms, there are physical, chemical, and biological methods used to control and eliminate them. Nanotechnology has emerged as another effective measure as nanometals affect the essential activities of microorganisms. These findings highlight the difficulty in controlling biofilms, due to the strategies used by these agents to adapt and survive in sessile form, causing recurring contamination throughout the poultry chain production, deterioration in the final product and infections in the human host.

Keywords: prevention, control, public health, microbial adhesion, anti-biofilm agents

1. Introduction

The industry destined to food production has a great challenge to maintain the safety of the products to be marketed. Among these challenges, there are the failures that may occur during

the cleaning process, which may favor the permanence of microorganisms that are able to form biofilms. Several microorganisms transmitted by food are able to form these structures, among them stands out gender representatives *Salmonella* and *Campylobacter*, which are major contaminants of poultry products and the agents that represent the highest risk of foodborne infections to humans [1].

Biofilms are defined as a process of bacterial cells adhesion to a living or inert surface. These cells clump together forming bacterial communities, which are surrounded by a polymer matrix composed mainly by polysaccharides, as well as proteins and nucleic acids [2, 3]. This extracellular matrix promotes the biofilm protection, inhibiting access of biocidal agents, concentrating nutrients, and preventing dehydration [4].

Typically, biofilms consist of microorganisms in mixed cultures under symbiotic conditions, which are considered more resistant to chemical agents commonly used for cleaning and sanitizing, as well as to other harsh conditions such as refrigeration, acidity, saltiness, and antibiotics [5–7].

Since *Salmonella* and *Campylobacter* are present in the industry environment, they can form biofilms on produced food, and also in processing areas, such as walls, floors, pipes, and drains, and in contact surfaces, such as stainless steel, aluminum, nylon, rubber, plastic, polystyrene, and glass [8, 9].

The broiler slaughter industry generates residue rich in protein and lipids, which are deposited on surfaces [10], favoring the formation of biofilms of these pathogens responsible for frequent public health problems. Thus, these bacteria end up becoming a potential source of contamination within the industry that can be transferred to food or to their packaging, becoming a constant threat of recontamination [11].

Biofilms cause economic losses due to food spoilage and damage to equipment by biocorrosion, and also for damages caused in humans arising from foodborne infections [12].

The infections by *Salmonella* and *Campylobacter* have similar characteristics, with presence of diarrhea, abdominal cramps, and fever between 12 and 72 hours after infection. The illness usually lasts 4–7 days, and in most people, the recovery takes place spontaneously, without treatment. In some cases, diarrhea can be more serious with the need for hospitalization. In these patients, the microorganisms can spread from the intestines to the bloodstream and then to other body sites, with the risk of death. In cases of campylobacteriosis, patients may develop Guillain-Barré syndrome (GBS), which causes flaccid paralysis and risk of death from respiratory insufficiency [13].

There are intrinsic and extrinsic mechanisms involved in the formation of these communities, such as the material, type, and shape of the surface, the electric charge, hydrophobicity, and hydrodynamics. There are also different characteristics that determine the maturity of the biofilm, including the environment, mobility, growth rate, the capacity of cell signaling, and the production of extracellular polymer matrix [14]. Besides these, the molecular aspects are also extremely important, as the presence of *luxS* gene and activation of the *quorum-sensing* system in *Campylobacter* and genes encoding extracellular matrix components, Curli fimbriae and cellulose in *Salmonella* [15, 16].

http://dx.doi.org/10.5772/65254

The biofilm maturation allows the development of a primitive homeostasis and circulatory system, with exchange of genetic material and metabolic cooperation coordinated by *quorum-sensing*. This system is the mechanism used by bacteria to withstand the changes in their environment and therefore use specific strategies that allow adaptation to environmental stress [17].

About the problems involved in the presence of biofilms, preventing their development and their elimination represents greater security to the produced food and consumer. The adoption of several strategies may exhibit effectiveness in eliminating the use of more resistant surfaces for biofilm formation [18]. In the prevention and in cases of previous formed biofilms, physical, chemical, and biological methods can be used, being the combination of the three methods considered most effective [19]. Nanotechnology has emerged as another alternative as nanometals affect the essential activities of *Campylobacter* and *Salmonella* [20].

This data reinforces the difficulty in *Salmonella* and *Campylobacter* control in sessile forms within the chain production of food, especially in poultry products industry.

2. Characteristics of biofilms

Biofilms are formed by an aggregation of bacteria that adhere to each other and secrete extracellular polymeric substances (EPS), as a pellicle formed in the air-liquid interface. These structures are established in response to various environmental conditions and can be developed by multiple signaling strategies. They are usually composed of a mixture of different species of bacteria [21].

There are five stages that make up the cycle of formation of these structures: (a) free phase, (b) fixing the surface, (c) microcolony, (d) macrocolony, and (e) dispersion (**Figure 1**).

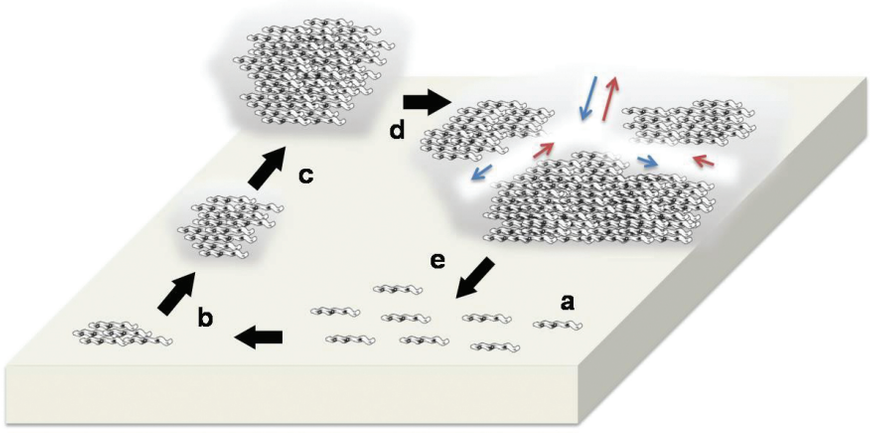

Figure 1. Stages of biofilm formation cycle in *Campylobacter*: (a) planktonic phase, (b) adhesion to the surface, (c) microcolony, (d) macrocolony, and (e) dispersion. The arrows indicate the path taken by nutrients (blue) and excreta (red) inside the tubules formed in the mature biofilm.

The first stage is characterized by reversible fixing facilitated by flagellar motility that allows the range to the surface. This connection is of low intensity and allows full movement of the bacterium, but also allows it to be easily removed by cleaning processes [22]. This weak initial interaction of the bacteria with the substrate involves hydrophobic interactions, electrostatic forces, and van der Waals force, which determine the adhesion between the bacterial cell and the surface [23].

The second stage is called irreversible fixation, moment when gradual increasing in the bond strength occurs by means of continuous production of exopolymers and adhesins. At this stage, the cell removal from the surface requires the action of mechanical force such as scraping, or by chemical treatment. The most important components for this period are exopolymers, adhesins, and DNA (extracellular DNA with structural function) [24]. This phase involves stronger dipole-dipole, hydrogen bonds, hydrophobic interactions, covalent, and ionic bonds [23].

In *Campylobacter*, there is an exponential increase in the expression of genes related to flagellar motility (*flaA* and *flaB*), and adhesion such as *cadF* and *peb4*. Bacterial motility plays a key role in the migration and starts the formation of microcolonies mediated by *quorum-sensing* mechanism [25, 26].

The formation of microcolonies is detected in the third stage, which occurs approximately after 2 hours, to *Salmonella*, and 4 hours to *Campylobacter*, and after the fixing step, with a diameter ranging from 0.5 to 2 mm. The cells become sessile and the *quorum-sensing* mechanism is activated upregulating the expression of the *luxS* gene, engaged in self-induction of biofilm formation. At that time, extrinsic factors act directly on the adhesion capacity, such as environmental conditions (aerobic, anaerobic, and microaerophilic), the type of available nutrients and the attachment surface (stainless steel, glass, and polypropylene) [21].

The maturation step is accompanied by the formation of macrocolonies resulting from microbial growth and recruitment of other environmental microorganisms. At this time, diffusion through the matrix of exopolymers is slower than the cellular metabolism and the resulting chemical gradients create microniches. Inside them, the death of bacterial cells is evident in the central region, permitting the formation of cavities where motility is possible for planktonic forms. *In vitro* that stage lasts for up to 3 days, if exchanged for fresh media and is accompanied by the formation of channels that allow the exchange of nutrients and excretions [24, 27]. **Figure 2** shows a schematic structure of bacterial biofilm *in vitro*.

The last phase is the dispersion of planktonic forms for formation of new biofilms. This process is done passively, independently of oxygen concentration [24, 28].

In human and animal hosts, the *C. jejuni* and *Salmonella* are capable of forming microcolonies on the intestinal epithelium within a few hours after infection. Biofilm formation in the intestinal absorption surfaces prevents normal functions in the ileal mucosa and can contribute to the symptoms of the disease. The ability of adhesion and chemotaxis to mucin, which comprises intestinal mucus, allows the formation of "bacterial blankets" inside and under these layers of mucus after 3–4 hours of exposure [22].

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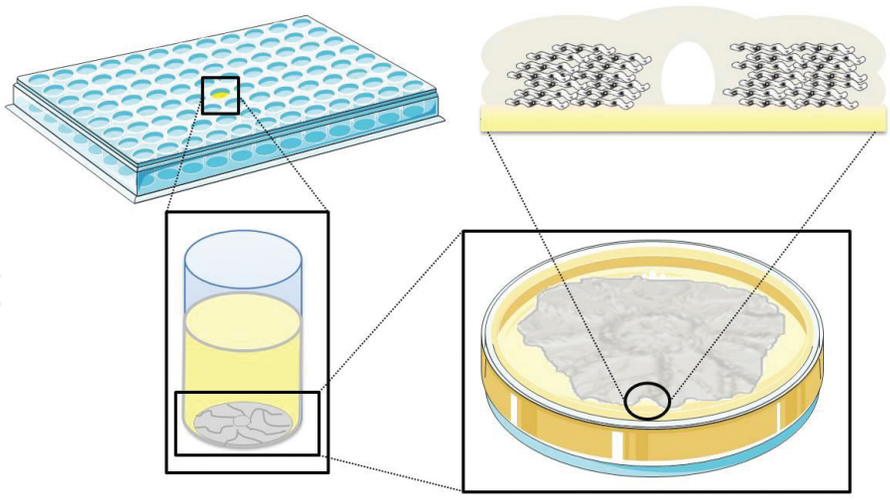

Figure 2. *In vitro* analysis of bacterial biofilms formation. The testing protocol is based on 10^7 UFC.mL^{-1} in specific culture media (TSB broth, Mueller Hinton broth, Bolton broth, Brucella broth, and Chicken juice broth) incubated for 24 hours (*Salmonella*) or 48 hours (*Campylobacter*) in plates of 96 wells at a temperature of 37℃ under aerobic (*Salmonella*) or microaerophilic (*Campylobacter*) conditions. Subsequently, there is the formation of a bacterial mass on the bottom of the wells, which characterize the structure of a biofilm. After about 96 hours of incubation, the formation of substances transport channels is already established and becomes visible in electronic confocal and scan microscopies.

Outside the host, sessile form of *Campylobacter* and *Salmonella* allows better survival under stressful conditions of temperature, oxygen, and nutrients in abiotic environments, in different food matrices, and especially, in chicken meat and in the presence of antibacterial agents. Thus, its spread through the food chain becomes easier. The reasons that allow this survival success are multifactorial, but include especially the reduction of metabolic activity and decreasing of the adsorbent action of extracellular polymer matrix, which reduces the amount of antimicrobial and sanitizing agent required to interact with the biofilm cells, and specific factors expressed by the cells in the biofilm, such as efflux pumps [8, 29].

The behavior of these pathogens in mixed and single cultures differs significantly. Biofilms formed by different species of bacteria, such as those found in food industries, represent a substantial risk, since they can protect each other during the application of chemical agents [30]. It is true that in mixed cultures with *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli*, *Bacillus* sp., and *Enterococcus faecalis* survival and persistence of *Campylobacter* is quite evident. In addition, structural changes and in metabolic activity of microcolonies can be observed. Additional examples are the commensalism of *C. jejuni* with *Pseudomonas aeruginosa*, which promotes the increase of tolerance to environmental oxygen concentrations by *C. jejuni* and the increase of *Campylobacter* survival in water biofilms composed of different species of protozoa [31–33].

3. Biofilms in poultry production

Salmonella and *Campylobacter* are microorganisms that are usually contaminants of chicken flocks. Some risk factors are the key to raising levels of infection, including inadequate hygiene

measures, negligence of biosecurity standards, the presence of other animals on the premises, and others. However, in addition to environmental issues, there is also the persistence of the microorganism in the form of biofilm on the premises [34].

The absence of water is lethal to the growth of microorganisms, but the existence of a minimum supply of water within the farm may be sufficient for the establishment of biofilm. Drinking fountains are more conducive to bacterial attachment, being the portions covered with rubber with greater biomass, because of the facility to adhere [1].

The control of biofilm formation in the water distribution systems contribute significantly to improve the health of birds, minimizing the need of antibiotic treatments. All chemicals that are added to the drinking water of the poultry, such as dietary supplements and medicines, settle in biofilms and then may spread and generate residues even after its use has been completed. In that way, cleaning of the drinking fountains of poultry is a practice of extreme importance to be adopted in the farms, to ensure that the chickens receive good quality water [35]. The treatment of water supplied in poultry has gained increasing popularity in Europe [36], and today it is an essential measure to control pathogens in the poultry production chain.

In order to reduce microbial contamination the producers treat the water that will be destined to the birds with various chemicals such as: chlorine, chlorine dioxide, organic acids, peracetic acid, and hydrogen peroxide. However, these substances have action only under appropriate conditions of temperature and pH [37] and for a limited period of time, so it should be repeated periodically.

Some producers use chemical control methods in water intended for poultry only once or twice a week, and others limit this kind of processing to the end of the production cycle, i.e., there is a lack of standardization and proper methodology that undertake control efficiency and reduction of pathogens in the water, and potentiates the transmission of pathogens by water [37].

Studies have shown that one of the most common sources of contamination are from biofilms developed inside the water supply pipe, where a variety of microorganisms proliferate surrounded by mud, and are adhered to surface and continuously release planktonic cells in water [38].

Ventilation systems (coolers) are also a favorable area for microbial aggregation, especially in situations where there is a preexisting biofilm [1].

Higher biofilm formation rates are observed at the chicken processing steps because of large amount of moisture in the environment. Several critical points are identified during production, such as plastic curtains, mats, scalding tanks, chiller, and stainless steel tools [18].

A research with *Salmonella* spp. strain isolated from a poultry slaughterhouse in Brazil, determined that the canvas was the most suitable material for the induction of growth of the matrix by this microorganism, followed by polystyrene, and lastly, stainless steel [9].

The fact that the chicken reaches the abattoir harboring pathogens such as *Salmonella* and *Campylobacter*, increase the chances of contamination of equipment during processing.

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Campylobacter genotyping studies clearly show the similarity of strains present in the intestine of poultry, and in environmental samples of the final product [34, 39].

The survival of *Campylobacter* in the chicken's skin is another form of adaptation of the microorganism. This agent is able to fix into the deep crevices of the skin and follicles feather of the bird. These recesses provide ideal conditions for bacteria to adhere, colonize, form biofilms, and remain protected in the carcass, even at low temperatures [40].

Thus, it must consider that proper control of cleaning methods used in the poultry production system is of paramount importance and should include strict compliance with the established biosecurity protocols. This applies mainly to difficult decontamination environments such as feed mills, agricultural environments, and farms [41, 42].

4. Intrinsic mechanisms of *Campylobacter*

For the biofilm establishment, both environmental variations and the microorganism itself correlate with genes that are expressed by bacteria in sessile form.

Table 1 illustrates some of the molecular mechanisms involved directly in biofilm formation and the link between this mechanism and the flagellar apparatus.

The several genes that encode different flagellar proteins clearly show the necessity of flagella on biofilm formation in *C. jejuni*, since they are overexpressed in this form of growth. The absence of *flaA*, *flaB*, *flaC*, *flhA*, or *fliS* results in the formation of weak biofilms. There are many flagella functions for biofilm formation and are linked to the motility, adhesion, and secretion of substances, suggesting its importance in the early stages of formation of sessile structure [43].

The lack of genes involved in flagella expression activation, as well as those that are involved in chemotaxis (*cheA*, *cheY*, and *cetB*) also reduces the self-bonding [44].

The biofilm matrix is composed basically by exopolysaccharides (EPS), proteins and DNA and thus the regulatory genes of these molecules production and the availability of nutrients define quantitative variations in this composition [21].

In addition to the flagella, the genes that decisively affect biofilm formation in *Campylobacter* are associated with the envelope/cell surface. Mutations that alter the expression and the protein secretion of surface also impair biofilm formation [45, 46].

The stress response generates metabolic changes in the expression of nearly the totality of the bacterial genome. Both, increasing the response to stress protein expression and decreasing of metabolic activity express the conditions of sessile microorganism and contributes to tolerance to the harsh conditions that bacteria are submit in biofilms. Iron uptake and stress response proteins are highly expressed in biofilms. Metabolic protein expression will vary, depending on the need, suggesting that distinct changes in metabolism mark the transition between modes of planktonic and sessile growth [47, 48].

	Gene	Proteic product	Biofilm	Motility
Flagella adhesion	*flaA*	Larger flagellin	+	+
	flaB	Flagellin	+	=
	flaC	Flagellin homologous, adhesion	+	=
	fiA	Flagellar sigma factor	+	=
	fibA	Flagellar proteic secretor apparatus	+	+
	flaG	Flagellin homologus	+	=
	fliS	Flagellar chaperone	+	+
	cadF	Adhesin	+	+
Metabolism	*pta*	Acetyltransferase phosphate	+	+
	ackA	Acetate kinase	+	+
	phoX	Alkaline phosphatase	−	=
Regulation/stress response	*csr A*	*RNA-binding* regulatory protein	+	+
	spoT	Binfunctional synthetase II	−	=
	ppk1	Poly-P kinase 1	−	=
	ppk2	Poly-P kinase 2	−	=
	Cj1556	Transcriptional regulator	+	+
	luxS	Autoinducer-2 synthase	+	+
	sodB	Oxidative stress regulator	+	=
	dnaJ	Thermic stress regulator	+	+
	cbrA	Metabolic growth regulator	+	=
	htrA	Osmotic shock regulator	+	=
	p19	Iron uptake	+	=
Proteic envelop/secretion	*pgp1*	Carboxypeptidase	+	+
	peb4	Peptidyl-prolyl isomerase	+	+
	tatC	Arginine transporter	+	+

+, Increase in phenotype;
−, Decrease in phenotype;
=, Remains the same phenotype.
Source: [50] with modifications.

Table 1. Phenotypic classification of biofilm formation capacity and flagellar motility according to the presence of specific genes.

5. Intrinsic mechanisms of *Salmonella*

Salmonella is equipped with external cellular components, such as flagella, fimbriae, and pili, which play an important role in the accession process of the cells to surfaces, environmental persistence, in biofilm formation, and in colonization and cell invasion [49].

Even in the case of intrinsic mechanisms of *Salmonella*, Hamilton et al. [50] observed the existence of several genes and proteins involved in bacterial attachment, motility, detection,

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and response to oxygen availability, transport and response to stress, being differently expressed in biofilms than the planktonic cells.

Among the most important genes associated with biofilm formation, there is the *csg*, responsible for coding the Curli fimbriae, described to be highly aggregative and play an important role in biofilm formation by *Salmonella*, to promote the initial interaction between the cell and the surface and the subsequent cell-cell interaction [51]. CsgD previously referred to AgfD, is the main unit of control and integration for biofilm formation in *Salmonella*, regulating the expression of specific matrix components associated to biofilm [52]. The *csg*D gene acts in addition to the regulation of fimbriae, activation of cellulose, determining the formation of a dense extracellular matrix. This gene activates the transcription of *adrA* gene, which stimulates the production of a protein that acts on the enzymatic activity of the cellulose biosynthesis. Thus, it forms a highly hydrophobic compressed network and composing rigid lining cells in the extracellular matrix of *Salmonella* biofilms [53].

6. Extrinsic factors linked to biofilms

About the environmental conditions, there are many factors that determine the production, development, and maintenance of biofilms, including the pH, temperature, type of material and the surface roughness, the presence of organic and inorganic compounds, the condition of dynamic flow, osmotic pressure, oxygen concentration, concentration and bioavailability of nutrients, and the presence of antimicrobial agents in the medium. This is due to the fact that different environmental conditions will generate different responses in gene regulations of the bacteria and thus the behavior of biofilms [54].

The sessile mode of growth is enhanced in low quantity of nutrient conditions. This fact is noted by elevated LPS production in the matrix. An example is related to the use of excessive nutritive media, such as Bolton, Brucella, and Brain Heart Infusion broths, *in vitro*, that are less prone to biofilm formation in *C. jejuni* and *Salmonella*, than Mueller Hinton, Chicken juice, and Tryptone Soya broth, less rich in nutrients. The carbon deficiency, nitrogen and phosphorus, is related to increasing formation of biofilms [27, 55].

The role of temperature in the formation of biofilms is more complex, varies among species and the changes are related to other environmental conditions [56].

For *Campylobacter*, unlike what happens in other enterobacteria including *Salmonella*, a dynamic flow condition does not lead to a better fixation. Thus, *in vitro* assays are carried out in static conditions, since the agitation does not allow connection of the microorganisms to form biofilms. In contradictory ways, in mixed cultures of biofilms, agitation rates can be high and equal to 2.5 mL/min [57].

The physicochemical properties of the surface exert a strong influence on the adherence of microorganisms. In general, the bacteria adhere more easily to the hydrophobic surfaces like plastics, than hydrophilic surfaces such as glass or metal [58].

The osmotic stress inhibits biofilm formation and leads to dispersion of the existing structure. The addition of NaCl (sodium chloride), glucose, or sucrose significantly decreases the formation of biofilm on *C. jejuni*, being these induced to transition to coccoid morphology [55].

The effect of oxygen tension in biofilm formation of *C. jejuni* appears to vary widely among strains. The oxygen seems to promote the early stages of biofilm formation, which occurs more rapidly in the first 24 hours. However, after 48 hours there are no significant differences in aerobic and microaerophilic conditions [25, 56].

7. The importance of the *quorum-sensing*

The *quorum-sensing* mechanisms directly influence the formation of biofilms. This communication of numerous bacteria via small signal molecules directs bacterial population and regulates the expression of virulence genes, toxin production, motility, chemotaxis, and biofilm formation, which can contribute to the adaptation and bacterial colonization [59].

Molecules of *quorum-sensing* AI-1 (autoinducer 1) are signaling to mediate specific intraspecies communication. AI-2 mediates communication intra and inter-particular species of Gram-positive and Gram-negative bacteria [59].

For *C. jejuni*, it has shown that AI-2 in the supernatant level increases during the exponential growth phase and decreases during entry into stationary phase. This molecule is produced by *Campylobacter* in different food matrices at various temperatures and corresponds to the expression of *luxS* gene, which is overexpressed in cultures of Chicken juice at low temperatures [60].

Both *quorum-sensing* mechanisms (AI-1 and AI-2) contribute to the regulation of biofilm formation against the growth of planktonic cells, which in turn promotes bacterial colonization, persistence under adverse conditions and expression of virulence factors. Thus, the detailed investigation of AI-mediated mechanisms could serve as a new tool to be used in therapeutic applications or reducing the amount of human pathogens in the transmission path for the termination of bacterial communication [61].

8. Biofilm control

Given the biofilms resistance of *Salmonella* and *Campylobacter* to disinfectants and antibiotics, it is important to evaluate and develop alternative strategies to prevent their formation.

The equipment design and the choice of the materials and coatings used in the food industry are extremely important in preventing biofilm formation. This is because even adopting the most effective cleaning and sanitizing programs, it is not possible to compensate for problems caused by faulty equipment, which have inaccessible corners, cracks, crevices, valves, and joints, which are vulnerable points for biofilm accumulation [62].

The use of well-designed equipment associated with the adoption of effective hygiene measures allow the removal of unwanted material from surfaces, including microorganisms, foreign materials, and residues from cleaning products [63, 64].

New technologies for detecting the presence of biofilms have been developed in order to control the colonization of surfaces by bacteria and identify the early stages of biofilm formation and development [65]. A research performed by Ref. [65] developed a mechatronic sensor to surface capable of providing various information such as the presence of biofilms in the early stages, presence of cleaning products in the surface, and differentiation of the type of cleaning employed (biological or chemical).

Once the biofilm is already established, it should be emphasized cleaning processes using mechanical action, which is one of the main measures for their elimination or control [66], because the friction acts on the matrix disruption, exposing deeper layers and making the microorganisms more accessible.

Generally, disinfectants do not penetrate the biofilm matrix after an inefficient cleaning procedure and, therefore, does not destroy all the biofilm cells [64], reaching only the outer layers. Cleaning is the first step and very important to improve the sanitation of equipment and facilities [67]. It is important to remove effectively the food wastes that may contain microorganisms or promote microbial growth.

The use of high temperature may reduce the need for application of mechanical forces, such as turbulence in the wash water. The chemicals commonly used for cleaning are surfactants or alkalis, used to suspend and dissolve the food residues by reducing the surface tension, emulsify fats, and denature proteins [66].

In addition to the mechanical action, other measures must be taken to prevent and control microbial adhesion. In this sense, the facilities, equipment, and utensils should be washed daily and disinfected with the use of microbicides substances previously approved by legislation.

However, there are studies showing that even using the recommended concentration of sanitizing, resistance of bacteria in biofilms still exists. A study performed by Ref. [9] evaluated the bactericidal capacity of peracetic acid on *Salmonella* biofilm and noted that 44.11% (15/34) of the strains were susceptible to the disinfectant in the concentration of 0.2%. In other strains, that were resistant to disinfectants (55.89%, 19/34), more concentrated solutions of the disinfectant were tested, 0.3–0.5%, and for the concentration of 0.5% was observed that was the maximum sanitizing capacity, with no resistant isolate to the agent. This results, generates concern, because when it is used at the indicated concentration by the manufacturer and by the regulatory agency (0.2%), there is inefficiency of the product, and may result in contamination of the surfaces, maintaining the bacteria in the cutting processing environment of chicken.

The disinfection is the use of products for elimination of microorganisms, especially pathogenic. The purpose of disinfection is to reduce the microbial load remaining on the surface after cleaning and prevents their proliferation before restarting the production process. Disinfectants must be effective, safe, and easy to handle, they should be removed from surfaces easily, using water, leaving no residue in the final product that may affect the consumer [68].

Mechanism of action	Examples	Compounds
Blocking in the bacterial adhesion	Policides	2-pyridone bicyclic
	Iron chelators	Lactoferrin; plant extracts, tannins
QS inactivation	Competition for receptor sites/AIs degration	Furanones halogenated and peptide inhibitor of RNA III (RIP)
Mature Biofilms—Matriz	Enzymes	Proteinase, typsin, DNAase, sodium metaperiodate
	Alteração no pH	Detergentes ácidos/alcalinos
Mature Biofilms- Biomass	Nanoparticles	Zinc, silver, titanium, gold.
	Antiseptics	Chlorhexidine, triclosan.
	Bioactive	AMP, terpinen-4-o1.

Source: [81] with modification.

Table 2. Key targets to combat microbial biofilms and examples of agents.

The chemicals currently used in disinfection processes belong to the following types: acidic compounds, biocides, aldehyde-based, caustics, chlorine, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenols, biguanides, and surfactants [64, 69]. Some examples of agents that may be used to control and/or eliminate biofilms of *Salmonella* and *Campylobacter* are shown in **Table 2**.

The strategies most used in industry involve the removal of biofilms already installed, by removing the matrix and/or bacterial biomass. As a first step is quoted to use hygienic processes with enzymatic detergents and compounds that promote the sudden change in pH and subsequent matrix liquefaction [70].

The use of enzyme-based detergents may be useful to improve the cleaning process. However, due to the heterogeneity in biofilm matrices, it is necessary to know the exact composition for which suitable enzymatic treatments can be applied [71], so that a mixture of different enzymes can increase the spectrum action on biofilm degradation. These enzymatic processes have the advantage of disaggregate biofilm agglomerates, rather than just remove them from the surface, as is the case of mechanical action.

Another important point to be analyzed for the elimination of bacteria in mature biofilm is the involvement of strain-dependent characteristics, since there are molecular intrinsic factors that may act by preventing the effectiveness of the agents, hindering its penetration depending on the composition of the matrix, and also the mechanism of action of the applied agent.

In general, the policides act by inhibiting adhesin and essential fimbriae synthesis in the process of fixing the bacteria to surfaces. The iron chelating agents prevent the availability of this element in the initial process of accession, essential for the biofilm formation. Inactiva-

tion of the *quorum-sensing* system involves the use of compounds that compete for binding sites of self-inducing molecules or direct degradation of these molecules.

The surfactants and biosurfactants are also alternatives that can be used in combating biofilm formation. A study of [72] reported that pretreated surfaces with surfactants may have potential higher than 90% in the prevention of bacterial adhesion, and biosurfactants such as rhamnolipids and short chain fatty acids can promote rupture on biofilms [73, 74]. Since surfactin from *Bacillus subtilis* disperses and prevents the formation of biofilms of *Salmonella enterica, E. coli*, and *Proteus mirabilis* [75].

The nanoparticles, as well as the antimicrobial peptides (AMPs), appears as a current strategy for the removal of biomass of biofilms, since they are stable at high temperature and pressures, have inactivates potential, can easily penetrate the matrix, are less likely to develop resistance, have minimal effect on the human cells and can be used to extend the shelf life of fresh and meat products [76, 77].

Combinations of different treatments, with different types of actions are also useful. For example, ultrasound waves [78] were associated with the improvement performance of proteolytic enzymes. These processes target the biofilm matrix, causing the disaggregation and dispersion of the biomass. However, they are not efficient in eliminating these microorganisms, which can adhere to the surface again and restart a new cycle of the biofilm formation.

Alternatively, under increasing interest for biofilm control is to use bacteriophages, which are viruses with high specificity that infect and lyse bacteria and diffuse easily into the matrix layers, including in mature biofilms [79–81]. This technology is still in development, so information about the bacteriophage action in biofilms is still scarce [82]. However, it is known that the infection of biofilm by a phage depends on their chemical composition and also environmental factors such as temperature, growth phase, media, and phage concentration [83].

Studies on the use of natural antimicrobials as antibiofilm substances, for example, compounds extracted from aromatic plants [84], which are recognized as safe for not leave toxic residues for the consumer and does not change the quality of final product. These compounds have demonstrated their antimicrobial activity in planktonic bacteria and some is being evaluated for its potential in eradicating biofilms.

A research performed by Ref. [85] tested the influence of carvacrol, a broad spectrum antimicrobial found in essential oils of herbs such as oregano and thyme, on biofilm of *S. aureus* and *S*. Typhimurium in stainless steel, and found that carvacrol has inhibitory effect on both species, with the effectiveness of the product associated with the species and stage of biofilm formation, the concentration of application, and the form of treatment.

The use of combined actions involving two or more types of chemical, physical, and natural treatments have been reported as the measure of control with more effectiveness against biofilm formation [86]. These treatments can synergistically enhance and broaden the spectrum of actions to eradicate biofilms.

9. Conclusion

Despite several options for new treatments to prevent and remove biofilms, further studies need to be carried out continuously to understand the dynamics of these structures.

Whereas biofilms are constant sources of contamination of production systems for spoilage and pathogens, having economic and public health impacts, prevention should be included in the objectives of the quality of industrial controls. Among the actions required in all strategies, should be included the frequent monitoring, and internal policies to ensure compliance with the preestablished hygiene plans, particularly, respecting the intervals between cleaning processes.

Acknowledgements

To Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support.

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