meat technology
Founder and publisher:
Institute of Meat Hygiene and Technology, Belgrade

UDK: 579.67(65)
637.52/.58.065(65)
ID: 158133001
https://doi.org/10.18485/meattech.2024.65.2.5

Original Scientific Paper

Biofilm production and acetic acid sensitivity of *Staphylococcus aureus* and *Escherichia coli* isolated from poultry slaughterhouse environment, broiler carcasses and offal in Algeria

Saïd Derbal[1,2] and Mourad Hanfer[3,4]*

[1] *Laboratory of Environment, Health and Animal Production (LEHAP), Institute of Veterinary and Agronomic Sciences, University of Batna 1, 05000 Batna, Algeria*
[2] *Pharmaceutical Sciences Research Center, 25016 Ali Mendjeli, Constantine, Algeria*
[3] *Laboratory of Biology and Environment, Faculty of Nature and Life Sciences, University of Constantine 1, 25000 Constantine, Algeria*
[4] *Faculty of Nature and Life Sciences, University of Batna 2, 05078 Fesdis, Batna, Algeria*

ARTICLE INFO

Keywords:
Acetic acid
Bacteria
Biofilm
Broiler
Environment
Slaughterhouses

ABSTRACT

The environment of poultry slaughterhouses, broiler carcasses and offal can act as reservoirs and spread various zoonotic bacterial pathogens such as *Staphylococcus aureus* and *Escherichia coli*. The objectives of this study were to determine the prevalence of *S. aureus* and *E. coli* in broiler carcasses and offal, and the environment of poultry slaughterhouses, and to evaluate the capacity for biofilm formation and sensitivity to acetic acid of certain bacterial isolates. A total of 210 samples were taken from different parts of the carcasses (wings, thighs and breasts) and offal (livers and hearts) of broiler chickens, and 19 environmental samples were collected from various compartments of poultry slaughterhouses (walls, floors and equipment) to determine the prevalence of *S. aureus* and *E. coli*. Fourteen *S. aureus* strains and 14 *E. coli* strains isolated from broiler products, as well as 14 *S. aureus* strains and 14 *E. coli* strains isolated from the environment of poultry slaughterhouses, were specifically selected to evaluate their ability to form biofilms. The tube and the tissue culture plate methods were used to evaluate biofilm forming capacity, while the minimum inhibitory concentration (MIC) of acetic acid on these bacterial isolates was determined by the agar dilution method. The total quantities of biofilm produced by the different categories of bacterial strains were compared by statistical analysis. The prevalences of *S. aureus* and *E. coli* were 100% in broiler carcass and offal samples, while in environmental samples, the prevalence of *E. coli* was 94.73% and that of *S. aureus* was 78.94%. Using the tube method, 35.71% of *S. aureus* strains demonstrated strong biofilm production, 50% demonstrated moderate production and 14.28% demonstrated weak production. No strain was categorized as non-biofilm producing. Similarly, for *E. coli* strains, 32.14% had strong biofilm production, 21.42% moderate production, and 46.42% weak production, with no strain being non-biofilm producing. Using the tissue culture plate method, 39.28% of *S. aureus* strains had moderate biofilm production, while 60.71% showed weak production. No isolates were identified as having strong production or being non-biofilm producers. For *E. coli* strains, 14.28% showed strong biofilm production, 39.28% moderate production, and 46.42% weak production, with no isolate being categorized as a non-biofilm producer. The two methods made it possible to detect biofilm production by all studied bacterial isolates. The tube method revealed a higher rate of isolates with strong biofilm production (33.92%) compared to the tissue culture plate method (7.14%). In contrast, the tube method recorded a lower rate of isolates exhibiting moderate biofilm production (35.71%) compared to the tissue culture plate method (39.28%).

Corresponding author: Saïd Derbal, *said.derbal@univ-batna.dz*

Paper received: July 19th 2023. Accepted: January 25th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

Similarly, the tube method showed a lower rate of isolates with weak biofilm production (30.35%) compared to the tissue culture plate method (53.57%). Regarding measures of total biofilm produced, environmental bacteria presented a not significantly higher value (total optical density (OD)=12.45) than did bacteria isolated from broilers (total OD=11.83). Likewise, the total quantity of biofilm produced by all 14 *E. coli* (total OD=12.78) was numerically but not significantly higher than that produced by all *S. aureus* isolates (total OD=11.5). Among the isolates from broilers, the 14 *E. coli* strains produced a numerically not significantly higher amount of biofilm (total OD=6.76) than the 14 *S. aureus* strains (total OD=5.07). The minimum inhibitory concentration (MIC) of acetic acid was ≤0.08% for all bacterial isolates, except for two *S. aureus* isolates, for which the minimum inhibitory concentration was 0.16%. In conclusion, *S. aureus* and *E. coli* are frequently present in the environment of poultry slaughterhouses and in broiler products. All bacterial isolates demonstrated an ability to form biofilms. These bacteria were very sensitive to acetic acid, which is therefore considered an ideal agent for disinfection of the poultry slaughterhouses environment and decontamination of broiler carcasses.

1. Introduction

Broiler flocks are an essential food source in Algeria, but they can also serve as disease reservoirs and spread various zoonotic bacteria. Among these zoonotic bacteria, *Staphylococcus aureus* and *Escherichia coli* is particularly known as major pathogens capable of causing infections in animals and humans. In addition, these bacteria are responsible for recurrent foodborne infections in meat and poultry products in Algeria, resulting in significant economic losses. Food poisoning caused by *S. aureus*, *E. coli*, *Salmonella enterica subsp.* Enteritidis and *S.* Typhimurium, mainly linked to the consumption of contaminated chicken meat, is one of the main public health problems in developing countries (*Mead*, 2004; *Antunes et al.*, 2016; *Bortolaia et al.*, 2016). Despite efforts by the poultry industry to reduce foodborne illnesses associated with chicken products, they remain one of the main culprits associated with foodborne illnesses in Algeria.

S. aureus and *E. coli* have the ability to form biofilms that protect them against hostile conditions such as temperature variations, limitations or deprivation of nutrients, as well as dehydration *(Idrees et al.*, 2021). The formation of biofilm reduces bacterial susceptibility to antimicrobial agents and the host's immune defences, thus making infections difficult to eliminate. Additionally, upon infection, dispersal of biofilm cells can lead to spread to secondary sites and worsen infection (*Lister and Horswill*, 2014). Consequently, biofilm formation by *S. aureus* on medical devices and host tissues (*Lister and Horswill*, 2014), as well as by *E. coli* in the urinary tract (*Ballén et al.*, 2022), can lead to chronic infections. These chronic biofilm-related infections often lead to a significant increase in morbidity and mortality (*Moormeier and Bayles*, 2017).

Additionally, *S. aureus* and *E. coli* are capable of forming biofilms on poultry processing surfaces, which can lead to cross-contamination of slaughtered broiler carcasses and offal. It should be noted that the knife blade used for neck cutting could be a potential source of cross-contamination during slaughter (*Mead et al.*, 1994). Moreover, pathogenic bacteria demonstrate the ability to survive in hot water, thus increasing the risk of transmission of viable microorganisms between carcasses during scalding (*Henry et al.*, 2012). The plucking phase is emerging as a significant source of cross-contamination (*Morar et al.*, 2014), favoured by the surface of the rubber fingers that facilitates the transfer of bacteria to the carcasses (*Fries*, 2002). Evisceration requires special attention due to its high potential for bacterial cross-contamination, with faecal contamination from this step being one of the main concerns in poultry processing (*Brizio and Prentice*, 2015). When inspectors manually handle carcasses, there is an accidental risk of increasing cross-contamination between carcasses (*Oosterom et al.*, 1983). Furthermore, *Lillard* (1990) reported that the cooling tank is a major site of cross-contamination between carcasses.

The presence of biofilms in the poultry slaughterhouse environment is a major problem due to the link between biofilms and the survival and pathogenicity of bacteria (*Ducková et al.*, 2023), as is the ability of biofilm bacteria to persistently contaminate carcasses, offal of slaughtered broilers and even poultry slaughterhouse workers. Biofilm formation appears to play a key role in many foods poisoning cases, particularly those involving contaminated broilers from the poultry slaughterhouse environment, where *S. aureus* and *E. coli* are frequently implicated (*Mead*, 2004; *Bortolaia et al.*, 2016). Biofilm formation in poultry slaughterhouses

compromises the effectiveness of cleaning, disinfection and decontamination of slaughtered broiler carcasses (*Ducková et al.*, 2023), posing a serious threat to the white meat industry. This threat can be avoided by the application of a good manufacturing practice (GMP) program that is mainly based on the exclusion and elimination of unwanted and foreign materials, as well as the inhibition and destruction of undesirable microorganisms (*de Oliveira et al.*, 2016).

With regard to the decontamination of broiler carcasses, several methods have been developed to reduce the levels of bacterial contamination. Currently, most methods focus on washing and sanitizing procedures with agents like hot water, chlorine, short-chain organic acids, quaternary ammonium and sodium hypochlorite (*Dickson and Anderson*, 1992). Alternative processes, such as gamma irradiation and the use of cold water, are also effective (*Dickson and Anderson*, 1992).

The hazard analysis and critical control point (HACCP) system guarantees regular monitoring of the entire chicken processing procedure, optimizes hygiene control, checks control parameters and records the results, ensures compliance with hygiene legislation, raises awareness of personnel to food safety requirements, and establishes uniform operational standards throughout the industry. However, it does not completely resolve the drawback of microbiological risks associated with processing operations, which are often difficult to control effectively. To overcome this gap, the HACCP system must be put in place after the implementation of good hygienic practice (GHP), GMP and sanitation standard operating procedure (SSOP) programs. GHP/GMP/SSOP are operational prerequisite programs (oPRPs) used for the analysis and control of the facility and its environment, personnel, the cleaning and disinfection process, equipment and utensils, as well as storage and distribution (*de Oliveira et al.*, 2016). GHP/GMP/SSOP programs are based on the exclusion and elimination of unwanted and foreign materials, with the inhibition and destruction of pathogenic microorganisms. The integration of GHP/GMP/SSOP programs followed by the HACCP system enables process hygiene requirements and impacts on meat safety, thus ensuring control of foodborne diseases (*de Oliveira et al.*, 2016). The decontamination of carcasses could also be added as a food safety management choice, usually when batches of high-risk broilers from farms with a low level of biosecurity are destined for slaughter; decontamination of such animals should contribute to the reduction of foodborne infections in humans (*Dinçer and Baysal*, 2004).

Different interventions have been put in place to effectively reduce the bacterial load on broiler carcasses. The interventions are classified as either physical or chemical interventions, the latter including the use of organic acids (*Loretz et al.*, 2010). Organic acids are weak acids, most of which have no defined limits in terms of acceptable daily intake for humans. The antimicrobial activity of organic acids relies on two main mechanisms: cytoplasmic acidification with subsequent uncoupling of energy production and regulation, and accumulation of the dissociated acid anion to toxic levels. It is likely that the interaction of these mechanisms leads to the inhibition of microbes (*Mani-López et al.*, 2012). For many years, organic acids have been successfully used for the decontamination of beef, pork and poultry products against various bacteria (*Mani-López et al.*, 2012). Table 1 shows some studies on organic acids used for decontamination of broiler carcasses. They have proven to be safe, simple, effective and economical meat decontamination agents, highly recommended on a large scale (*Raftari et al.*, 2009). The use of acetic acid is a well-known method for the decontamination of poultry carcasses and offal, as well as for the disinfection of poultry slaughterhouses. This is an efficient and commonly used approach in the industry (*Idrees et al.*, 2021).

The emergence of multi-drug resistant bacteria contaminating the environment of poultry slaughterhouses and broiler carcasses in Algeria, such as *methicillin-resistant S. aureus* (MRSA) (*Bounar-Kechih et al.*, 2018) and extended-spectrum β-lactamase-producing *E. coli* (*Aberkane et al.*, 2023), has led to the search for solutions to eliminate these bacteria, in particular by using other molecules with antibacterial activity. The use of organic acids, such as acetic acid, could solve this problem (*Nkosi et al.*, 2021). Therefore, it is important to assess the ability of these bacteria to form biofilm and their sensitivity to acetic acid, in order to develop effective disinfection and decontamination strategies.

Numerous studies have demonstrated the antimicrobial effectiveness of acetic acid against *S. aureus* and *E. coli* present in broiler meat (*Abdul Wahid*, 2008; *Bin Jasass*, 2008; *Sakhare et al.*, 1999). However, research on acetic acid's effectiveness against strains isolated from the poultry slaughterhouse environment is limited. In addition, no study has yet been carried out in Algeria to assess acetic acid's effectiveness against bacteria isolated from carcasses, offal of broiler chickens or the environment of local poultry slaughterhouses.

Table 1. Organic acids used for decontamination of broiler carcasses

Organic acids	Application of organic acids	Antibacterial effectiveness of organic acids	References
Citric acid ($C_6H_8O_7$)	Cloacal washing of broiler carcasses with citric acid (5% and 10%, w/v).	Reduction in the number of psychrophilic or mesophilic bacteria on carcasses, of 0.88 $\log_{10}$ CFU cm^2 and 0.56 $\log_{10}$ CFU cm^2 for both concentrations respectively.	*Meredith et al*., 2013
	Treatment of previously inoculated chicken breast pieces by vacuum-infusion with 150.0 mM citric acid.	Reduction of *S. Typhimurium* counts to almost undetectable levels on day 6 of storage (100 CFU/g) and to undetectable levels after day 9 of storage at 4 °C.	*Over et al*., 2009
Lactic acid ($C_3H_6O_3$)	Cloacal washing of broiler carcasses with lactic acid (5%, v/v).	Reduction in the number of *Campylobacter* on carcasses by 0.66 $\log_{10}$ CFU cm^2.	*Meredith et al*., 2013
	Washing broiler carcasses with lactic acid (1% and 3%, v/v).	Reduction in the number of aerobic mesophilic bacteria, coliforms and *E. coli* on carcasses, of 1.259 log CFU, 1.685 log CFU, 2.023 log CFU and 2.502 log CFU, 3.876 log CFU, 3.820 log CFU compared to the control samples, for both concentrations respectively. Total elimination of *Salmonella* with both concentrations.	*Halil & Abdurrahman Üsame*, 2000
Propionic acid ($C_3H_6O_2$)	Immersing freshly inoculated chicken thighs in a propionic acid solution (1% and 2%, v/v).	Reduction in the number of *L. monocytogenes* of 2.72 log CFU on the thighs compared to the controls, with the 2% concentration, after 3 days of storage.	*González-Fandos & Herrera*, 2013a
Succinic acid ($C_4H_6O_4$)	Immersion of broiler breasts in 80 mL of a *Salmonella* cocktail at 10^7 CFU/mL for 2 min, then transferred into sterile beakers containing 250 mL of succinic acid (2% and 5%, v/v) for 5 min.	Reduction in *Salmonella* counts from 1.27 to 1.47 log CFU/g and from 2.00 to 3.20 log CFU/g on breasts compared to controls, with both concentrations respectively.	*Radkowski et al*., 2018
Malic acid ($C_4H_6O_5$)	Soaking freshly inoculated chicken thighs in a malic acid solution (1% and 2%, v/v) for 5 min.	Reduction at 4 °C in the number of *L. monocytogenes* of approximately 1.66 log CFU on the thighs compared to controls, with the 2% concentration	*González-Fandos & Herrera*, 2013b
	Soaking broiler chicken thighs previously inoculated in a malic acid solution (1% and 2%, v/v).	Reduction in the number of *C. jejuni* by 1.18 log CFU on the thighs compared to controls, with the 2% concentration.	*González-Fandos & Maya*, 2015
Tartaric acid ($C_4H_6O_6$)	Application of tartaric acid (0.5% and 1%, v/v) to broiler breast skin previously inoculated, under simulated scald (50°C for 2 min).	Reduction in the number of *S.* Typhimurium on the skin by 2.64 and 1.23 log CFU log CFU for both concentrations respectively.	*Tamblyn & Conner*, 1997b
	Vacuum-infusion of chicken breast pieces previously inoculated, in tartaric acid (150 mM).	Reduction of *S.* Typhimurium counts on meat to almost undetectable levels by the 6^{th} day of storage (100 CFU/g) and to undetectable levels after the 9^{th} day of storage at 4°C.	*Over et al*., 2009

Legend: CFU – colony-forming unit; mM – millimolar

Consequently, our study aimed to determine the prevalence of *S. aureus* and *E. coli* in the poultry slaughterhouse environment and in the carcasses and offal of broiler chickens in Algeria, to evaluate, using two distinct methods, the capacity of selected bacterial isolates to form biofilms, and to determine the isolates' sensitivity to acetic acid by determining the concentration minimal inhibitory (MIC).

2. Materials and Methods

2.1 Sampling and detection of S. aureus and E. coli

Sampling was carried out in poultry slaughterhouses located in Algeria. In total, 210 samples were taken from different parts of broiler carcasses and offal, from 14 farms. For each farm, 15 samples were collected, including 3 wings, 3 thighs, 3 breasts, 3 livers and 3 hearts. This sampling procedure was carried out in the drying room. In addition, 19 environmental samples were taken from various compartments of poultry slaughterhouses, including 3 walls (scalding and plucking room, evisceration and washing room, and conditioning room), 6 floors (reception room, stunning and bleeding room, scalding and plucking room, evisceration and washing room, drying room and conditioning room), as well as 10 pieces of equipment (bleeding knife, scalding tank, plucking machine, finisher fingers, head remover, evisceration knife, leg cutter, recovery cart, worker hand and recovery table).

The swabs were subjected to bacteriological analysis using Chapman agar (BIOKAR®, France) and Hektoen agar (BIOKAR®, France) for the isolation of *S. aureus* and *E. coli* strains, respectively. The bacterial strains were identified using standard microbiological tests and biochemical tests using API Staph strips (BioMérieux®, France) for *S. aureus* and API 20E strips (BioMérieux®, France) for *E. coli*.

2.2 Bacterial strain selection

Fourteen strains each of *S. aureus* and *E. coli*, isolated from broiler products, were carefully chosen to explore their ability to form biofilm. Each broiler farm was represented by one strain each of *S. aureus* and *E. coli*. Another 14 strains each of *S. aureus* and *E. coli*, isolated from different sources in the poultry slaughterhouses environment, were also specifically selected to evaluate their ability to form biofilm. The selected bacterial isolates were stored at −80 °C in tryptic soy broth (TSB) (BIOKAR®, France) containing 20% (V/V) glycerol for subsequent analyses. Before each experiment, the bacterial isolates were

Table 2. Origin of selected bacterial strains used in the study

Bacterial strain number	Environment		Broiler	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	Wall (scalding and plucking room)	Wall (scalding and plucking room)	Wing	Wing
2	Wall (evisceration and washing room)	Wall (evisceration and washing room)	Wing	Wing
3	Wall (conditioning room)	Wall (conditioning room)	Thigh	Thigh
4	Floor (reception room)	Floor (reception room)	Thigh	Thigh
5	Floor (scalding and plucking room)	Floor (scalding and plucking room)	Breast	Breast
6	Floor (evisceration and washing room)	Floor (evisceration and washing room)	Breast	Breast
7	Floor (drying room)	Floor (drying room)	Breast	Breast
8	Bleeding knife	Head remover	Liver	Liver
9	Scalding tank	Scalding tank	Liver	Liver
10	Plucking machine	Plucking machine	Liver	Liver
11	Evisceration knife	Evisceration knife	Liver	Liver
12	Leg cutter	Leg cutter	Heart	Heart
13	Recovery cart	Recovery cart	Heart	Heart
14	Recovery table	Recovery table	Heart	Heart

thawed and subcultured on nutrient agar (BIOKAR®, France). Table 2 shows the origin of the selected bacterial strains for the biofilm study.

2.3 Qualitative detection of biofilm by the tube method

All selected isolates of *S. aureus* and *E. coli* were evaluated for their ability to form a biofilm using the tube method, as described by *Christensen et al.* (1982), which allows qualitative detection of the biofilm. A single colony of isolate was inoculated into test tubes containing 3 ml of tryptic soy broth supplemented with 1% glucose (TSBG) (BIOKAR®, France). The tubes were then incubated at 37 °C for 48 h. A negative control containing only TSBG (BIOKAR®, France) without bacterial inoculum was also included. After incubation, the tubes were decanted, washed with phosphate-buffered saline (pH 7.2) and dried. Then, the tubes were stained with a solution of gentian violet (0.1%) for 15 min, followed by rinsing with distilled water. The tubes were dried in an inverted position. Assessment of biofilm formation was performed visually and results were scored according to control strains. The formation of a biofilm was considered positive when a visible film was observed covering the wall and the bottom of the tube. The biofilm-producing capacity of the different isolates was classified, according to the intensity of the biofilm's violet color, as none, weak, moderate or strong (*Hassan et al.*, 2011). Each experiment was performed in triplicate and repeated three times.

2.4 Quantitative assay of biofilm formation by the tissue culture plate method

This test was performed using the method of *Christensen et al.* (1985), which allows the quantitative detection of biofilm formation. Isolated bacteria from fresh agar plates were inoculated into 10 mL of tryptic soy broth supplemented with 1% glucose (TSBG) (BIOKAR®, France). The broths were incubated overnight at 37 °C. Using a flat-bottomed polystyrene 96-well tissue culture plate (3 wells for each strain), each well was filled with 20 µL of the previous night's culture (equivalent to 0.5 McFarland standard) and then topped up with 180 µl of sterile TSBG medium (BIOKAR®, France). Wells inoculated with sterile TSBG medium (BIOKAR®, France) were used as a negative control. After aerobic incubation for 24 h at 37 °C, the contents of each well were removed by gentle tapping, then the wells were carefully washed three times with 0.2 mL of phosphate-buffered saline (pH 7.2) to eliminate detached bacteria. Then, each well was filled with 200 µL of methanol 99% to fix the adherent bacteria for 15 min. The plates were decanted, left to dry, then stained for 7 min with 0.2 mL of crystal violet (0.1%). Excess dye was rinsed off with tap water. After the plates were air-dried, the dye bound to adherent cells was resolubilized with 160 µL of ethanol per well. The optical density (OD) of each well was measured at 630 nm using a microplate reader (Mindray MR-96A®). Absorbance values were measured twice: before the ethanol addition, then after the ethanol addition. According to the absorbance values, the adhesion ability of each bacterial isolate was classified into four categories: none, weak, moderate and strong. The cut-off absorbance value (optical density (ODc)) was taken as three standard deviations (SD) above the mean OD of the negative control. Each experiment was performed in triplicate and repeated three times. The interpretation of biofilm production (formation) was carried out according to the criteria of *Stepanovic et al.* (2007). Table 3 shows the classification of bacterial cell adhesion and biofilm formation in the tissue culture plates.

2.5 Determination of the minimum inhibitory concentration (MIC) of acetic acid

The minimum inhibitory concentration (MIC) of acetic acid was determined against all selected bacterial isolates of *S. aureus* and *E. coli* using the agar dilution method based on the guidelines of the Clinical and Laboratory Standards Institute

Table 3. Classification of bacterial cell adhesion and biofilm formation in the tissue culture plate method

Average value of OD	Adhesion	Biofilm formation
OD ≤ Odc	None	None
ODc < OD ≤ 2ODc	Weak	Weak
2 ODc < OD ≤ 4ODc	Moderate	Moderate
4 ODc < OD	Strong	Strong

Legend: OD – optical density; ODc – cut-off absorbance value of optical density

(*CLSI*, 2018) with the use of Muller-Hinton (MH) agar (BIOKAR®, France). Acetic acid was incorporated into MH agar plates at the following concentrations: 2.5%, 1.25%, 0.63%, 0.31%, 0.16% and 0.08% (v/v). Then, a standardized bacteria suspension (adjusted to 0.5 McFarland standard) containing a concentration of 5×10^8 CFU mL^{-1} was prepared. This standardized bacteria suspension was diluted to approximately 10^7 CFU mL^{-1}, and 2 µL of this dilution were spotted at several points onto MH agar plates with acetic acid, so each spot contained approximately 10^4 CFU. An agar plate without antibacterial agent was used as a control. After aerobic incubation at 37 °C for 24 h, the agar plates were visually examined to assess growth. The growth of the isolate indicates that it is resistant to the acetic acid concentration incorporated into the MH agar.

2.6 Statistical analysis

To quantify biofilm formation using the tissue culture plate method, experiments were independently repeated three times, with three replicate of plate wells for each bacterial strain. In order to compare the total quantities of biofilm produced by the different categories of bacterial strains, a statistical analysis was carried out using the IBM SPSS Statistics V28 software. A one-way analysis of variance (ANOVA) test, followed by a t-test paired two sample for means, was used to assess differences in biofilm mass. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1 Bacterial isolates

All samples of broiler carcasses and offal tested positive for the presence of *S. aureus* and *E. coli*, thus establishing a prevalence of 100% for these two bacteria. Regarding environmental samples, *E. coli* was detected in 18 out of 19 samples, with a prevalence of 94.73%, while *S. aureus* was identified in 15 out of 19 samples, showing a prevalence of 78.94 %. In detail, the three samples from the walls revealed the presence of three strains each of *S. aureus* and *E. coli*. For the six soil samples, five strains of *S. aureus* and six strains of *E. coli* were detected, while in the ten equipment samples, seven strains of *S. aureus* and nine strains of *E. coli* were identified.

3.2 Qualitative test for the detection of biofilm with the tube method

Based on the results of the qualitative biofilm tube test, *S. aureus* and *E. coli* isolates were classified according to their ability to produce biofilm in TSBG medium. For *S. aureus*, of the tested 28 isolates, 10 (35.71%) showed strong biofilm production, 14 (50%) showed moderate production, 4 (14.28%) showed weak production, and no isolate was a non-biofilm producer. As for *E. coli*, of the tested 28 isolates, 9 (32.14%) showed strong biofilm production, 6 (21.42%) showed moderate production,

Table 4. Biofilm production capacity of bacteria assessed by the tube method

Bacteria	Origin	Biofilm production capacity			
		None	Weak	Moderate	Strong
S. aureus	Environment 14 isolates	0/14 (0%)	4/14 (28.57%)	8/14 (57.14%)	2/14 (14.28%)
	Broiler 14 isolates	0/14 (0%)	0/14 (0%)	6/14 (42.85%)	8/14 (57.14%)
	Total 28 isolates	0/28 (0%)	4/28 (14.28%)	14/28 (50%)	10/28 (35.71%)
E. coli	Environment 14 isolates	0/14 (0%)	13/14 (92.85%)	1/14 (7.14%)	0/14 (0%)
	Broiler 14 isolates	0/14 (0%)	0/14 (0%)	5/14 (35.71%)	9/14 (64.28%)
	Total 28 isolates	0/28 (0%)	13/28 (46.42%)	6/28 (21.42%)	9/28 (32.14%)

Table 5. Biofilm production capacity of bacteria assessed by the tissue culture plate method

Bacteria	Origin	Biofilm production capacity			
		None	Weak	Moderate	Strong
S. aureus	Environment 14 isolates	0/14 (0%)	6/14 (42.85%)	8/14 (57.14%)	0/14 (0%)
	Broiler 14 isolates	0/14 (0%)	11/14 (78.57%)	3/14 (21.42%)	0/14 (0%)
	Total 28 isolates	0/28 (0%)	17/28 (60.71%)	11/28 (39.28%)	0/28 (0%)
E. coli	Environment 14 isolates	0/14 (0%)	7/14 (50%)	6/14 (42.85%)	1/14 (7.14%)
	Broiler 14 isolates	0/14 (0%)	6/14 (42.85%)	5/14 (35.71%)	3/14 (21.42%)
	Total 28 isolates	0/28 (0%)	13/28 (46.42%)	11/28 (39.28%)	4/28 (14.28%)

13 (46.42%) showed weak production, and no isolate was a non-biofilm producer. The complete results of the biofilm-producing capacity of all bacterial isolates by the tube method are shown in Table 4.

3.3 Quantitative assay of biofilm formation with the method of tissue culture plate method

The bacterial isolates were classified according to the results obtained with the tissue culture plate method. For *S. aureus*, of the 28 tested isolates, 11 (39.28%) showed moderate biofilm production, 17 (60.71%) showed weak production, and no isolates were classified as having strong production or not producing biofilm. Regarding *E. coli*, of the 28 tested isolates, 4 (14.28%) showed strong biofilm production, 11 (39.28%) showed moderate production, 13 (46.42%) showed weak production, and no isolates were classified as non-biofilm producing. The complete results of the biofilm-producing capacity of all bacterial isolates by the tissue culture plate method are presented in Table 5.

3.4 Comparison of detection methods

Both methods detected biofilm production by all bacterial isolates, but with differences in the amounts of biofilm produced. The number of isolates with strong biofilm production was higher with the tube method 19/56 (33.92%) compared to the tissue culture plate method, which detected only 4/56 (7.14%). In contrast, the number of isolates with moderate biofilm production was lower with the tube method 20/56 (35.71%) compared to the tissue culture plate method, which identified 22/56 (39.28%). Similarly, the number of isolates with weak biofilm production was lower with the tube method 17/56 (30.35%) compared to the tissue culture plate method which

Table 6. Comparative screening of *S. aureus* and *E. coli* isolates producing biofilm by the tube and tissue culture plate methods

Classification of biofilm production	Number of isolates (%) according to biofilm formation	
	Tube (qualitative method)	Tissue culture plate (quantitative method)
Strong	19/56 (33.92%)	4/56 (7.14%)
Moderate	20/56 (35.71%)	22/56 (39.28%)
Weak	17/56 (30.35%)	30/56 (53.57%)
None	0/56 (0%)	0/56 (0%)

revealed 30/56 (53.57%). Table 6 presents a comparison of types of biofilm produced by *S. aureus* and *E. coli* isolates as assessed by the tube and tissue culture plate methods.

3.5 Comparison of biofilm production by different categories of bacteria

Overall, the total amount of biofilm produced by environmental bacteria (total OD=12.45) was higher than that produced by bacteria isolated from broilers (total OD=11.83), but this difference was not statistically significant ($p>0.05$). Similarly, the total amount of biofilm produced by all *E. coli* isolates (total OD=12.78) was higher than that produced by all *S. aureus* isolates (total OD=11.5), but this difference was also not statistically significant ($p>0.05$).

Regarding the comparison of the total amounts of biofilm produced by the different categories of bacteria, the 14 strains of *E. coli* isolated from broilers produced the greatest amount of biofilm (total OD=6.76), followed by the 14 strains of *S. aureus* isolated from the environment (total OD=6.43), the 14 strains of *E. coli* isolated from the environment (total OD=6.02) and finally the 14 strains of *S. aureus* isolated from broilers (total OD=5.07). However, none of these differences were statistically significant ($p>0.05$). Table 7 shows the OD of biofilm produced by each bacterial strain and by the different categories of bacteria.

3.6 Minimum inhibitory concentration (MIC) of acetic acid

The study of the minimum inhibitory concentration (MIC) of acetic acid on all *S. aureus* and *E. coli* isolates revealed that all isolates were susceptible to all tested concentrations, with the exception of two isolates of *S. aureus* isolated from broiler livers which were resistant at the concentration of 0.08%.

Table 7. Optical density of biofilm produced by each bacterial strain and by different categories of bacteria

Bacterial strain number	Environment		Broiler	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
1	0.57466667	0.51666667	0.356	0.86433333
2	0.53933333	0.89833333	0.252	0.83566667
3	0.21466667	0.45733333	0.80366667	0.77166667
4	0.27166667	0.33766667	0.35233333	0.93633333
5	0.43666667	0.306	0.592	0.47533333
6	0.66433333	0.347	0.29833333	0.22433333
7	0.30833333	0.39566667	0.232	0.24133333
8	0.30366667	0.335	0.37866667	0.46966667
9	0.60033333	0.43466667	0.26233333	0.47466667
10	0.31833333	0.431	0.428	0.49433333
11	0.60266667	0.44566667	0.27133333	0.16966667
12	0.73933333	0.35233333	0.28366667	0.26433333
13	0.56433333	0.34366667	0.354	0.24033333
14	0.29233333	0.42433333	0.211	0.29833333
Total	6.43066666	6.02533334	5.07533333	6.76033332
	12.456		11.83566665	

Table 8. Minimum inhibitory concentration of acetic acid on each bacterial strain

Bacterial strain number	Environment		Broiler	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	≤0.08%	≤0.08%	≤0.08%	≤0.08%
2	≤0.08%	≤0.08%	≤0.08%	≤0.08%
3	≤0.08%	≤0.08%	≤0.08%	≤0.08%
4	≤0.08%	≤0.08%	≤0.08%	≤0.08%
5	≤0.08%	≤0.08%	≤0.08%	≤0.08%
6	≤0.08%	≤0.08%	≤0.08%	≤0.08%
7	≤0.08%	≤0.08%	≤0.08%	≤0.08%
8	≤0.08%	≤0.08%	≤0.08%	≤0.08%
9	≤0.08%	≤0.08%	≤0.08%	≤0.08%
10	≤0.08%	≤0.08%	0.16%	≤0.08%
11	≤0.08%	≤0.08%	0.16%	≤0.08%
12	≤0.08%	≤0.08%	≤0.08%	≤0.08%
13	≤0.08%	≤0.08%	≤0.08%	≤0.08%
14	≤0.08%	≤0.08%	≤0.08%	≤0.08%

Thus, for all *E. coli* isolates, the MIC of acetic acid was ≤0.08%. For 26 isolates of *S. aureus*, the MIC of acetic acid was also ≤0.08%, while for the other two strains of *S. aureus* (isolated from broiler livers), the MIC of acetic acid was 0.16%. Table 8 shows the MIC of acetic acid for each bacterial strain.

4. Discussion

S. aureus is the most pathogenic species of the genus *Staphylococcus*. It is very common in the commensal state; it colonizes the skin, the digestive tract and the nasal cavities of humans and warm-blooded animals. However, it can become pathogenic and be responsible for localized suppurative infections, life-threatening infections and food poisoning in humans. It can survive in the external environment and it can be found in poultry slaughterhouses, which amplifies transmission phenomena. Also, it is considered a notorious pathogen due to its antibiotic resistance and phenotypic adaptability, as a result of its ability to develop biofilms.

In our study, all *S. aureus* isolates produced biofilm, which is consistent with results from other studies. For example, *Bernier-Lachance et al.* (2020) reported that all 15 MRSA from chicken meat were able to form biofilms. Moreover, in the study conducted by *Igbinosa et al.* (2023), the biofilm-producing capacity of 110 MRSA strains isolated from poultry meat was assessed. The results revealed that 27 (24.55%) were weak biofilm producers, 18 (16.36%) were moderate biofilm producers and 39 (35.45%) were strong biofilm producers. Previous studies, such as those by *Knobloch et al.* (2002) and *Rewatkar and Wadher* (2013), also reported that the biofilm-forming capacity in *S. aureus* varies depending on the detection method used.

E. coli naturally occurs in the digestive tract of humans and warm-blooded animals, most often without causing any disease. It is a so-called commensal bacterium normally present in the intestinal microbiota. While the majority of *E. coli* strains are harmless, some have acquired virulence factors that make them pathogenic and capable of causing severe food poisoning in humans, especially in young children and the elderly. They can be found in the form of biofilm in the poultry slaughterhouse environment, once it has been soiled by poultry droppings. Contaminated feathers constitute an important means of introducing *E. coli* into the poultry slaughterhouse environment (*Rigby et al.*, 1980). *E. coli* contamination of the poultry slaughterhouse environment can also result from splashes and aerosols generated when washing carcasses (*Sofos et al.*, 2013).

According to our results, all *E. coli* isolates produced biofilm, which is in agreement with the results of the study conducted by *Crecencio et al.* (2020). That study evaluated the biofilm-forming capacity of 88 *E. coli* strains isolated from chilled raw chicken meat cuts. Their results revealed that 70.44% of the strains were able to form biofilms (moderate to strong), of which 31 strains were strong biofilm producers. Several other studies have also reported the ability of avian pathogenic *E. coli* (APEC) and avian faecal *E. coli* (AFEC) strains to form biofilm. For example, studies by *Al-Marri et al.* (2021), *Crecencio et al*. (2020) and *Skyberg et al.* (2007) confirmed this biofilm-producing capacity in these bacteria.

The ability of *E. coli* to produce biofilm varies depending on the experimental conditions. *Cremet et al.* (2013) pointed out that the detection rate of biofilm-producing strains differs depending on the method used, and that biofilm production is influenced by atmospheric and nutrient factors. Additionally, *Reisner et al.* (2006) reported the impact of environmental and genetic factors on biofilm formation. *Skyberg et al.* (2007) also noted that the ability to form biofilm differed depending on the pathotype of *E. coli* and nutrient conditions. According to *Oosterik et al.* (2014), biofilm formation by APEC strains is affected by serogroup and surface material. It is also important to emphasize that measured biofilm formation by *E. coli* depends on the method used, the specific strain and is strongly modulated by the culture conditions, as indicated by *Naves et al.* (2008).

These studies revealed significant differences in the biofilm-forming ability between various strains of *S. aureus* and *E. coli*, whether from chicken meat or other sources, which confirms our results. Our study employed two distinct methods to evaluate biofilm production capacity, namely the tube and the tissue culture plate methods. Both methods revealed the propensity of all selected bacterial strains to produce biofilms. In accordance with *Hassan et al.* (2011), the tissue culture plate method was more effective than the tube method for analysing biofilm production capacity, as demonstrated in the study of 110 clinical isolates. Furthermore, the study of *Knobloch et al.* (2002) established a significant correlation between the tube method and the tissue culture plate method for strong biofilm-producing strains, based on the analysis of 128 *S. aureus* isolates.

In our study, the minimum inhibitory concentration (MIC) of acetic acid was less than or equal to 0.08% for all bacterial isolates, except for two isolates of *S. aureus* which had an MIC of 0.16%. These results are encouraging and satisfactory, in comparison with other studies. For example, *Fraise et al.* (2013) reported that acetic acid was effective at dilutions as low as 0.166% against various bacterial pathogens. Similarly, in the study by *Amrutha et al.* (2017), the MIC of acetic acid was 1.5% for *E. coli* and 1% for *Salmonella* spp. Another study by *Ouattara et al.* (1997) showed that concentrations of acetic acid ranging from 0.1% to 1% (w/v) completely inhibited the growth of several common bacteria implicated in meat spoilage. These results reinforce the effectiveness of acetic acid as an inhibitory agent against pathogenic bacteria.

Several studies have demonstrated the antibacterial effectiveness of spraying broiler carcasses with acetic acid (Table 9). In addition, *Bin Jasass* (2008) reported that portions of chicken previously immersed in a suspension of *E. coli* were soaked in different concentrations of acetic acid (0.5%, 1% and 1.5%). Those authors revealed a reduction in the total number of *E. coli* of 0.7, 1.1 and 1.4 log CFU cm^{-2}, respectively, on the surface of soaked chicken meat. These studies thus confirm the effectiveness of this antimicrobial agent, acetic acid, in reducing bacterial contamination on chicken carcasses without altering their appearance (*Abdul Wahid*, 2008).

The effectiveness of acetic acid against other bacterial species known to be causative agents of food poisoning has been confirmed by several studies. For example, *Zhao and Doyle* (2006) demonstrated that acetic acid concentrations of 0.5%, 1%, 1.5% and 2% reduced *C. jejuni* counts by 0.5 log CFU/ml in 2 min in a suspension at 48 °C, and that a concentration of 2% reduced *C. jejuni* counts by 1.4 log CFU/g for up to 45 s on chicken wings at 48 °C. In addition, the study by *Tamblyn and Conner* (1997a) revealed that acetic acid concentrations of 0.5%, 1%, 2%, 4% and 6% exhibited bactericidal activity against *S.* Typhimurium on the skin of poultry. Acetic acid treatment was applied during simulated cooling (0 °C for 60 min), post-treatment immersion (23 °C for 15 s) or scalding (50 °C for 2 min). This bactericidal activity was dependent on the concentration and the method of application. *Salmonella*, whether firmly or loosely attached to the skin of poultry, demonstrated superior resistance to acetic acid compared to freely-suspended *Salmonella*. Notably, a concentration of 4% acetic acid was needed to eliminate approximately 2 log levels of *S.* Typhimurium attached to the skin of broilers.

The effectiveness of acetic acid against bacteria responsible for food poisoning can be influenced by several factors. *Oh et al*. (2009) observed a significant increase in resistance to acetic acid (400 mM)

in *E. coli* O157:H7 isolates from various sources, as the temperature decreased to 15 °C, for a given pH. No significant differences ($p \geq 0.05$) were observed between the various strains. All strains of *E. coli* O157:H7 showed reductions of between 1.8 to 4.5 log levels at pH 3.3 and 30 °C after 25 minutes. Anaerobic incubation was the most protective condition for all strains of *E. coli* O157:H7, compared to other atmosphere conditions. Furthermore, *McKellar and Knight* (1999) reported the effectiveness of acetic acid on 19 strains of enterohemorrhagic *E. coli*, isolated from humans and food, after 24 h. Outbreak strains showed significantly greater survival ($p \leq 0.05$) upon acid treatment than did strains isolated from fermented foods, high pH, or animal or human isolates. Significant differences ($p \leq 0.05$) were observed between serotypes as well as between O157:H7 and other serotypes after 3 or 6 h of exposure to acetic acid. In another study conducted by *Lee and Kang* (2009), various combinations between three factors, namely heat (55 °C), acetic acid (0.25%, v/v) and salt (3%, w/v), were tested and compared to individual treatments to eliminate *E. coli* O157:H7 in laboratory media. On combining salt with heat, no significant further reduction of *E. coli* O157:H7 was measured (there was no additive effect over the effect of heat alone). However, the combination of acid and heat resulted in a more significant reduction in *E. coli* O157:H7 (synergistic effect). When salt was combined with acid treatment, the salt provided protection against the acid treatment (antagonistic effect), thus resulting in less reduction of *E. coli* O157:H7 in the combined treatment compared to the individual acid treatment.

Acetic acid has long been known to be used as an antiseptic, disinfectant and food preservative due to its antimicrobial potential. The proper use of acetic acid in broiler processing can help minimize the risk of food poisoning. However, exposure of *S. aureus* and *E. coli* to acetic acid could result in resistance gene acquisition and the development of resistance, which is problematic due to limited broiler disinfection options. Biofilm formation complicates disinfection of acetic acid-resistant bacteria, as biofilms are a favourable environment for the exchange of these resistance determinants. The use of acetic acid in poultry slaughterhouses can lead to a reduction in the microbial load in the environment, on broiler carcasses and in offal, but this approach must not be seen as a replacement for the proper hygiene management when slaughtering broilers. Therefore, prerequisite

Table 9. Antibacterial and antimicrobial effectiveness of spraying broiler carcasses with acetic acid ($C_2H_4O_2$)

Treatment method	Evaluation parameters	Treatment without acetic acid	Treatment with acetic acid	References
Spray wash broiler carcasses with acetic acid (0.5%), after scalding.	Total Plate Count (log CFU cm^{-2})	4.02 ± 0.26	3.71 ± 0.19	*Sakhare et al*. (1999)
	Yeast and Mold (log CFU cm^{-2})	1.98 ± 0.08	1.02 ± 0.10	
	S. aureus (log CFU cm^{-2})	1.51 ± 0.07	1.14 ± 0.09	
	Coliforms (MPN cm^{-2})	0.17 ± 0.05	0.10 ± 0.03	
Spray wash broiler carcasses with acetic acid (0.5%), after defeathering.	Total Plate Count (log CFU cm^{-2})	4.07 ± 0.28	3.79 ± 0.26	*Sakhare et al*. (1999)
	Yeast and Mold (log CFU cm^{-2})	2.19 ± 0.13	1.47 ± 0.11	
	S. aureus (log CFU cm^{-2})	1.71 ± 0.05	1.10 ± 0.07	
	Coliforms (MPN cm^{-2})	2.53 ± 0.11	1.71 ± 0.08	
Spray wash broiler carcasses with acetic acid (0.5%), after evisceration.	Total Plate Count (log CFU cm^{-2})	3.36 ± 0.07	3.10 ± 0.21	*Sakhare et al*. (1999)
	Yeast and Mold (log CFU cm^{-2})	1.86 ± 0.09	1.00 ± 0.10	
	S. aureus (log CFU cm^{-2})	1.96 ± 0.07	0.91 ± 0.10	
	Coliforms (MPN cm^{-2})	2.03 ± 0.08	1.51 ± 0.09	
Spraying broiler carcasses with acetic acid (1%).	Mesophilic Bacteria (CFU/cm^2)	27.47×10^3	10.50×10^3	*Abdul Wahid* (2008)
	Coliforms (CFU/cm^2)	2.71×10^3	1.03×10^3	
	E. coli (CFU/cm^2)	4.41×10^2	7.5×10^1	
	S. aureus (CFU/cm^2)	2.74×10^2	1.05×10^2	

Legend: CFU – colony-forming unit; MPN – most probable number

programs, such as GHP/GMP/SSOP, must be established before the implementation of the HACCP system that more closely controls the risks to human health, as well as the prevention of modifications of foodstuffs by means of control practices in all stages of white meat production (*de Oliveira et al.*, 2016).

5. Conclusion

The importance and impact of this study lie in its innovative character in Algeria, being the first to explore the capacity of biofilm formation by *S. aureus* and *E. coli* contaminating the poultry slaughterhouse environment, broiler carcasses and offal, while evaluating the microorganisms' sensitivity to acetic acid.

Contamination by *S. aureus* and *E. coli* of poultry slaughterhouses and broilers at slaughter raises serious concerns for public health. Of particular concern is that these bacteria species have the ability to form biofilms that protect/harbour pathogenic strains. The persistence of biofilm-forming bacteria throughout the chicken processing chain greatly increases the risk of contamination of broiler meat and offal. It is imperative to establish GMP and SSOP programs followed by the HACCP system, which should help reduce the presence of *S. aureus* and *E. coli* such that less biofilm is formed in the poultry slaughterhouse environment. In turn, the chicken meat produced will then carry lower levels of contamination with these two pathogenic microorganisms and so should be safer from the public health point of view. This will minimize the risk of dissemination of these bacteria and their associated genes. At the same time, it is essential to understand the mechanisms involved in the formation of biofilm by these bacteria in order to develop new strategies to effectively eliminate the biofilm. Concerted efforts in these areas will help ensure food safety and protect consumer health.

The high level of susceptibility of the bacteria isolated in our study to acetic acid suggests that it is suitable for use in poultry slaughterhouses to disinfect the environment and decontaminate broiler carcasses and offal effectively, as a method of choice for food safety management.

Proizvodnja biofilma i osetljivost na sirćetnu kiselinu *Staphilococcus aureus* i *Escherichia coli* izolovanih u klanicama za živinu, na trupovima brojlera i iznutricama u Alžiru

Saïd Derbal i Mourad Hanfer

INFORMACIJE O RADU

Keywords:
Sirćetna kiselina
Bakterija
Biofilm
Brojer
Sredina
Klanice

APSTRAKT

Klanice za živinu, trupovi brojlera i iznutrice mogu delovati kao rezervoari i širiti različite zoonotične bakterijske patogene kao što su *Staphilococcus aureus* i *Escherichia coli*. Cilj ovog istraživanja je da se utvrdi prevalencija *Staphilococcus aureus* i *Escherichia coli* na trupovima brojlera i iznutricama, kao i u klanicama za živinu, uz procenu kapaciteta za formiranje biofilma i osetljivosti na sirćetnu kiselinu pojedinih bakterijskih izolata. Uzeto je ukupno 210 uzoraka sa različitih delova trupova (krila, karabatak i grudi) i iznutrica (jetra i srca) pilića brojlera, a 19 uzoraka životne sredine je prikupljeno iz različitih odeljenja klanica živine (zidovi, podovi i oprema) da se utvrdi prevalencija *Staphilococcus aureus* i *Escherichia coli*. Za procenu sposobnosti da formiraju biofilmove, posebno je odabrano 14 sojeva *Staphilococcus aureus* i 14 sojeva *Escherichia coli*, izolovanih iz proizvoda brojlera, kao i 14 sojeva *Staphilococcus aureus* i 14 sojeva *Escherichia coli* izolovanih iz okruženja klanica. Za procenu kapaciteta formiranja biofilma korišćena je metoda epruvete i metoda ploče za kulturu tkiva, dok je minimalna inhibitorna koncentracija (MIC- minimum inhibitory concentration) sirćetne kiseline na ovim bakterijskim izolatima određena metodom razblaživanja agarom. Ukupne količine biofilma proizvedenih od strane različitih kategorija bakterijskih sojeva upoređene su statističkom analizom. Prevalencija *Staphilococcus aureus* i *Escherichia coli* bila je 100% u uzorcima trupova brojlera i iznutrica, dok je u uzorcima iz klanice prevalencija *Escherichia coli* bila 94,73%, a *Staphilococcus aureus* 78,94%.

Metodom epruvete, procena sojeva *Staphilococcus aureus* je pokazala da je 35,71% pokazalo visoku proizvodnju biofilma, dok je 50% pokazalo umerenu produkciju, a 14,28% nisku proizvodnju. Nijedan soj nije kategorisan kao ne-biofilm. Slično, za sojeve *Escherichia coli*, rezultati su pokazali da je 32,14% imalo visoku proizvodnju biofilma, 21,42% umerenu proizvodnju i 46,42% nisku proizvodnju, pri čemu nijedan soj ne proizvodi biofilm. Metodom ploče za kulturu tkiva, procena sojeva *Staphilococcus aureus* je pokazala da je 39,28% imalo umerenu produkciju biofilma, dok je 60,71% pokazalo nisku produkciju. Nisu identifikovani izolati koji imaju visoku proizvodnju ili nisu proizvođači biofilma. Za sojeve *Escherichia coli*, 14,28% je pokazalo visoku proizvodnju biofilma, 39,28% umerenu proizvodnju i 46,42% nisku proizvodnju, pri čemu nijedan izolat nije kategorisan kao proizvođač koji nije biofilm. Dve korišćene metode omogućile su otkrivanje proizvodnje biofilma kod svih proučavanih bakterijskih izolata. Metoda epruvete je pokazala veću stopu izolata sa visokom produkcijom biofilma (33,92%) u poređenju sa metodom ploče za kulturu tkiva (7,14%). Nasuprot tome, metoda epruvete je zabeležila nižu stopu izolata koji pokazuju umerenu proizvodnju biofilma (35,71%) u poređenju sa metodom ploče za kulturu tkiva (39,28%). Slično, metoda epruvete je pokazala nižu stopu izolata sa niskom produkcijom biofilma (30,35%) u poređenju sa metodom ploče za kulturu tkiva (53,57%). U pogledu ukupne količine proizvedenog biofilma, bakterije životne sredine su imale veću vrednost (ukupni OD=12,45) u poređenju sa bakterijama izolovanim iz brojlera (ukupni OD=11,83), pri čemu razlika nije značajna (p>0,05). Isto tako, ukupna količina biofilma proizvedenog kod svih izolata *Escherichia coli* (ukupni OD=12,78) bila je veća od one koju proizvode svi izolati *Staphilococcus aureus* (ukupni OD=11,5), bez značajne razlike (p>0,05). Među izolatima brojlera, 14 sojeva *Escherichia coli* imalo je najveću količinu biofilma (ukupni OD=6,76), dok je 14 sojeva *Staphilococcus aureus* imalo najmanju količinu (ukupno OD=5,07), sa neznatnom razlikom (p> 0,05). Minimalna inhibitorna koncentracija (MIC) sirćetne kiseline bila je ≤0,08% za sve bakterijske izolate, osim za dva izolata *Staphilococcus aureus*, za koja je minimalna inhibitorna koncentracija bila 0,16%. Zaključno, *Staphilococcus aureus* i *Escherichia coli* su često prisutni u okruženju klanica živine i u proizvodima od brojlera. Svi bakterijski izolati su pokazali sposobnost formiranja biofilma. Utvrđeno je da su ove bakterije veoma osetljive na sirćetnu kiselinu, koja se stoga smatra idealnim sredstvom za dezinfekciju okruženja u klanicama živine i dekontaminaciju trupova brojlera.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Abdul Wahid, A. T. (2008). Effects of acetic acid and hydrogen peroxide on the microbiological quality and skin appearance of poultry carcasses. *Basrah Journal of Veterinary Research*, 7(2), 97–100, https://doi.org/10.33762/bvetr.2008.55507

Aberkane, C., Messaï, A., Messaï, C. R., & Boussaada, T. (2023). Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended spectrum β-lactamase-producing isolates in broilers in east Algeria. *Veterinary World*, 16(3), 449–454, https://doi.org/10.14202/vetworld.2023.449-454

Al-Marri, T., Al-Marri, A., Al-Zanbaqi, R., Al Ajmi, A., & Fayez, M. (2021). Multidrug resistance, biofilm formation, and virulence genes of *Escherichia coli* from backyard poultry farms. *Veterinary World*, 14(11), 2869–2877, https://doi.org/10.14202/vetworld.2021.2869-2877

Amrutha, B., Sundar, K., & Shetty, P. H. (2017). Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. *Microbial Pathogenesis*, 111, 156–162, https://doi.org/10.1016/j.micpath.2017.08.042

Antunes, P., Mourão, J., Campos, J., & Peixe, L. (2016). Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection*, 22(2), 110–121, http://dx.doi.org/10.1016/j.cmi.2015.12.004

Ballén, V., Cepas, V., Ratia, C., Gabasa, Y., & Soto, S. M. (2022). Clinical *Escherichia coli*: from biofilm formation to new antibiofilm strategies. *Microorganisms*, 10(6), 1103, https://doi.org/10.3390/microorganisms10061103

Bernier-Lachance, J., Arsenault, J., Usongo, V., Parent, É., Labrie, J., Jacques, M., Malouin, F., & Archambault, M. (2020). Prevalence and characteristics of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) isolated from chicken meat in the province of Quebec, Canada. *PLOS ONE*, 15(1), e0227183, https://doi.org/10.1371/journal.pone.0227183

Bin Jasass, F. M. (2008). Effectiveness of trisodium phosphate, lactic acid, and acetic acid in reduction of *E. coli* and microbial load on chicken surfaces. *African Journal of Microbiology Research*, (2), 50–55.

Bortolaia, V., Espinosa-Gongora, C., & Guardabassi, L. (2016). Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. *Clinical Microbiology and Infection,* 22(2), 130–140, http://dx.doi.org/10.1016/j.cmi.2015.12.003

Bounar-Kechih, S., Taha Hamdi, M., Aggad, H., Meguenni, N., & Cantekin, Z. (2018). Carriage methicillin-resistant *Staphylococcus aureus* in poultry and cattle in Northern Algeria. *Veterinary Medicine International*, 20,4636121, https://doi.org/10.1155/2018/4636121

Brizio, A. P. D. R., & Prentice, C. (2015). Chilled broiler carcasses: a study on the prevalence of Salmonella, Listeria and Campylobacter. *International Food Research Journal*, 22(1), 55–58.

Christensen, G.D., Simpson, W. A., Bisno, A. L., & Beachey, E. H. (1982). Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infection and Immunity*, 37(1), 318–326, https://doi.org/10.1128/iai.37.1.318-326.1982

Christensen, G. ., Simpson, W. A., Younger, J. A., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase negative staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. *Journal of Clinical Microbiology*, 22(6), 996—1006, https://doi.org/10.1128/jcm.22.6.996-1006.1985

CLSI, (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institute M7–A9. 9thed Wayne, PA

Crecencio, R. B., Brisola, M. C., Bitner, D., Frigo, A., Rampazzo, L., Borges, K. A., Furian, T. Q., Salle, C. T. P., Moraes, H. L. S., Faria, G. A., Da Silva, A. S., & Stefani, L. M. (2020). Antimicrobial susceptibility, biofilm formation and genetic profiles of *Escherichia coli* isolated from retail chicken meat. *Infection, Genetics and Evolution*, 84, 104355, https://doi.org/10.1016/j.meegid.2020.104355

Crémet, L., Corvec, S., Batard, E., Auger, M., Lopez, I., Pagniez, F., Dauvergne, S., & Caroff, N. (2013). Comparison of three methods to study biofilm formation by clinical strains of *Escherichia coli*. *Diagnostic Microbiology and Infectious Disease*, 75(3), 252–255, https://doi.org/10.1016/j.diagmicrobio.2012.11.019

de Oliveira, C. A. F., da Cruz, A. G., Tavolaro, P. & Corassin, C. H. (2016). Food Safety: Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis and Critical Control Point (HACCP). In Antimicrobial Food Packaging. Academic Press. pp. 129–139, https://doi.org/10.1016/B978-0-12-800723-5.00010-3

Dickson, J. S., & Anderson, M. E. (1992). Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. *Journal of Food Protection*, 55(2), 133–140, https://doi.org/10.4315/0362-028X-55.2.133

Dinçer, A. H., & Baysal, T. (2004). Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology*, 30(3), 197–204, https://doi.org/10.1080/10408410490468803

Ducková, V., Kročko, M., & Tkáčová, J. (2023). Microbial biofilms in a meat processing environment. *Meat Technology*, 64(2), 508–512, https://doi.org/10.18485/meattech.2023.64.2.98

Fraise, A. P., Wilkinson, M. A. C., Bradley, C. R., Oppenheim, B., & Moiemen, N. (2013). The antibacterial activity and stability of acetic acid. *Journal of Hospital Infection*, 84(4), 329–331, https://doi.org/10.1016/j.jhin.2013.05.001

Fries, R. (2002). Reducing *Salmonella* transfer during industrial poultry meat production. *Worlds Poultry Science Journal*, 58(4), 527–540, https://doi.org/10.1079/WPS20020038

González-Fandos, E., & Herrera, B. (2013a). Efficacy of propionic acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. *Food Control*, 34(2), 601–606, https://doi.org/10.1016/j.foodcont.2013.05.034

González-Fandos, E., & Herrera, B. (2013b). Efficacy of malic acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. *Poultry Science*, 92,1936–1941, http://dx.doi.org/10.3382/ps.2012-02968

González-Fandos, E., & Maya, N. (2015). Efficacy of malic acid against *Campylobacter jejuni* attached to chicken skin during refrigerated storage. *Journal of Food Processing and Preservation*, 40(4), 1–8, http://dx.doi.org/10.1111/jfpp.12637

Halil, T., & Abdurrahman Üsame, T. (2000). A study on the effects of chilling on the microbiological quality of poultry carcasses and surface decontamination with lactic acid. *Turkish Journal of Veterinary & Animal Sciences*, 24(6), 517–521.

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *The Brazilian Journal of Infectious Disease*, 15(4), 305–311, https://doi.org/10.1590/S1413-86702011000400002

Henry, I., Granier, S., Courtillon, C., Lalande, F., Chemaly, M., Salvat, G., & Cardinale, E. (2012). *Salmonella enterica* subsp. *enterica* isolated from chicken carcasses and environment at slaughter in Reunion Island: prevalence, genetic characterization and antibiotic susceptibility. *Tropical Animal Health and Production*, 45(1), 317–326, https://doi.org/10.1007/s11250-012-0221-2

Idrees, M., Sawant, S., Karodia, N., & Rahman, A. (2021). *Staphylococcus aureus* biofilm: morphology, genetics, pathogenesis and treatment strategies. *International Journal of Environmental Research and Public Health*, 18(14), 7602, https://doi.org/10.3390/ijerph18147602

Igbinosa, E. O., Beshiru, A., Igbinosa, I. H., Ogofure, A. G., Ekundayo, T. C., & Okoh, A. I. (2023). Prevalence, multiple antibiotic resistance and virulence profile of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail poultry meat from Edo, Nigeria. *Frontiers in Cellular and Infection Microbiology*, 13, 1122059, https://doi.org/10.3389/fcimb.2023.1122059

Knobloch, J., Horstkotte, M., Rohde, H., & Mack, D. (2002). Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*. *Medical Microbiology and Immunology*, 191(2), 101–106, https://doi.org/10.1007/s00430-002-0124-3

Lee, S. Y., & Kang, D. H. (2009). Combined effects of heat, acetic acid, and salt for inactivating *Escherichia coli* O157:H7 in laboratory media. *Food Control*, 20(11), 1006–1012, https://doi.org/10.1016/j.foodcont.2008.12.002

Lillard, H. S. (1990). The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. *Journal of Food Protection*, 53(3), 202–204, https://doi.org/10.4315/0362-028X-53.3.202

Lister, J. L., & Horswill, A. R. (2014). *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. *Frontiers in Cellular and Infection Microbiology*, 4(178), 1–9, https://doi.org/10.3389/fcimb.2014.00178

Loretz, M., Stephan, R., & Zweifel, C. (2010). Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey. *Food Control*, 21(6), 791–804, https://doi.org/10.1016/j.foodcont.2009.11.007

Mani-López, E., García, H. S., & López-Malo, A. (2012). Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Research International*, 45(2), 713–721, https://doi.org/10.1016/j.foodres.2011.04.043

McKellar, R. C., & Knight, K. P. (1999). Growth and survival of various strains of enterohemorrhagic *Escherichia coli* in hydrochloric and acetic acid. *Journal of Food Protection*, 62(12), 1466–1469, https://doi.org/10.4315/0362-028X-62.12.1466

Mead, G. C. (2004). Microbiological quality of poultry meat: a review. *Brazilian Journal of Poultry Science*, 6(3), 135–142, https://doi.org/10.1590/S1516-635X2004000300001

Mead, G. C., Hudson, W. R., & Hinton, M. H. (1994). Use of a marker organism in poultry-processing to identify sites of cross-contamination and evaluate possible control measures. *British Poultry Science*, 35(3), 345–354, https://doi.org/10.1080/00071669408417699

Meredith, H., McDowell, D., & Bolton, D. J. (2013). An evaluation of trisodium phosphate, citric acid and lactic acid cloacal wash treatments to reduce *Campylobacter*, total viable counts (TVC) and total enterobacteriaceae counts (TEC) on broiler carcasses during processing. *Food Control*, 32(2013), 149–152, https://doi.org/10.1016/j.foodcont.2012.11.026

Moormeier, D. E., & Bayles, K. W. (2017). *Staphylococcus aureus* biofilm: a complex developmental organism: molecular mechanisms of *S. aureus* biofilm development. *Molecular Microbiology*, 104(3), 365–376, https://doi.org/10.1111/mmi.13634

Morar, A., Imre, K., Nichita, I., Colibar, O., & Sala, C. (2014). Overview of foodborne pathogens in meat and meat products: trend in EU. Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara. *Medicina Veterinara*, 47(2), 80–89.

Naves, P., del Prado, G., Huelves, L., Gracia, M., Ruiz, V., Blanco, J., Rodríguez-Cerrato, V., Ponte, M. C., & Soriano F. (2008). Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent. *Journal of Applied Microbiology*, 105(2), 585–590, https://doi.org/10.1111/j.1365-2672.2008.03791.x

Nkosi, D. V., Bekker, J. L., & Hoffman, L. C. (2021). The use of organic acids (lactic and acetic) as a microbial decontaminant during the slaughter of meat animal species: a review. *Foods*, 10(10), 2293, https://doi.org/10.3390/foods10102293

Oh, D. H., Pan, Y., Berry, E., Cooley, M., Mandrell, R., & Breidt Jr, F. (2009). *Escherichia coli* O157:H7 strains isolated from environmental sources differ significantly in acetic acid resistance compared with human outbreak strains. *Journal of Food Protection*, 72(3), 503–509, https://doi.org/10.4315/0362-028X-72.3.503

Oosterik, L. H., Tuntufye, H. N., Butaye, P., & Goddeeris, B. M. (2014). Effect of serogroup, surface material and disinfectant on biofilm formation by avian pathogenic *Escherichia coli*. *The Veterinary Journal*, 202(3), 561–565, https://doi.org/10.1016/j.tvjl.2014.10.001

Oosterom, J., Notermans, S., Karman, H., & Engels, G. B. (1983). Origin and Prevalence of *Campylobacter jejuni* in Poultry Processing. *Journal of Food Protection*, 46(4), 339–344, https://doi.org/10.4315/0362-028X-46.4.339

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., & Bégin, A. (1997). Inhibitory effect of organic acids upon meat spoilage bacteria. *Journal of Food Protection*, 60(3), 246–253, https://doi.org/10.4315/0362-028X-60.3.246

Over, K. F., Hettiarachchy, N., Johnson, M. G., & Davis, B. (2009). Effect of organic acids and plant extracts on *Escherichia coli* **O157:H7,** *Listeria monocytogenes*, **and** *Salmonella* Typhimurium **in broth culture model and chicken meat systems.** *Journal of Food Science*, 74(9), **515–521,** https://doi.org/10.1111/j.1750-3841.2009.01375.x

Radkowski, M., Zdrodowska, B., & Gomołka-Pawlicka, M. (2018). Effect of succinic acid on elimination of Salmonella in chicken meat. *Journal of Food Protection*, 81(9), 1491–1495, https://doi.org/10.4315/0362-028X.JFP-17-446

Raftari, M., Jalilian, F. A., Abdulamir, A. S., Son, R., Sekawi, Z., &Fatimah, A. B. (2009). Effect of organic acids on *Escherichia coli* O157:H7 and *Staphylococcus aureus* contaminated meat. *The Open Microbiology Journal*, 3(1), 121–127, https://doi.org/10.2174/1874285800903010121

Reisner, A., Krogfelt, K. A., Klein, B. M., Zechner, E. L., & Molin, S. (2006). In vitro biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. *Journal of Bacteriology*, 188(10), 3572–3581, https://doi.org/10.1128/JB.188.10.3572-3581.2006

Rewatkar, A. R., & Wadher, B. J. (2013). *Staphylococcus aureus* and *Pseudomonas aeruginosa*-biofilm formation methods. *IOSR Journal of Pharmacy and Biological Sciences*, 8(5), 36–40, https://doi.org/10.9790/3008-0853640

Rigby, C. E., Pettit, J. R., Baker, M. F., Bentley, A. H., Salomons, M. O., & Lior, H. (1980). Sources of salmonellae in an uninfected commercially-processed broiler flock. *Canadian Journal of Comparative Medicine*, 44(3), 267–274.

Sakhare, P. Z., Sachindra, N. M., Yashoda, K. P., & Rao, D. N. (1999). Efficacy of intermittent decontamination treatments during processing in reducing the microbial load on broiler chicken carcass. *Food Control*, 10(3), 189–194. https://doi.org/10.1016/S0956-7135(99)00017-1

Skyberg, J. A., Siek, K. E., Doetkott, C., & Nolan, L. K. (2007). Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny. *Journal of Applied Microbiology*, 102(2), 548–554, https://doi.org/10.1111/j.1365-2672.2006.03076.x

Sofos, J. N., Flick, G., Nychas, G. J., O'Bryan, C. A., Ricke, S. C., & Crandall, P. G. (2013). Meat, Poultry, and Seafood. In *Food Microbiology: Fundamentals and Frontiers* (4 ed.). ASM Press. pp. 111–167.

Stepanovic, S., Vukovic, D., Hola, V., Di Bonaventura, G., Djukić, S., Ćirković, I., & Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by Staphylococci. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, 115(8), 891–899, https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

Tamblyn, K. C., & Conner, D. E. (1997a). Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *Journal of Food Protection*, 60(6), 629–633, https://doi.org/10.4315/0362-028X-60.6.629

Tamblyn, K. C., & Conner, D. E. (1997b). Bactericidal activity of organic acids in combination with transdermal compounds against *Salmonella typhimirium* attached to broiler skin. *Food Microbiology*, 14, 477–484, https://doi.org/10.1006/fmic.1997.0112

Zhao, T., & Doyle, M. P. (2006). Reduction of *Campylobacter jejuni* on chicken wings by chemical treatments. *Journal of Food Protection*, 69(4), 762–767, https://doi.org/10.4315/0362-028x-69.4.762

Authors info

Saïd Derbal https://orcid.org/0000-0002-1805-8775
Mourad Hanfer https://orcid.org/0000-0003-4757-0150