



Influence of the food matrix on dual-species biofilm of *Listeria monocytogenes* and *Pseudomonas fluorescens*

Daniel Leirias Caurio, Danieli Quadros da Silva, Luana Dornelles, Nathasha Noronha Arechavaleta and Amanda de Souza da Motta*

Instituto de Ciências Básicas da Saúde, Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Rio Grande do Sul, Rua Sarmiento Leite, 500, 90050-170, Porto Alegre, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: amanda.motta@ufrgs.br

ABSTRACT. In the food industry, the formation of biofilms results in serious microbial recontamination problem. This work aimed to study *Listeria* sp. obtained from sliced products and food handling surfaces, for the preparation of food of animal origin to be consumed cold. *Listeria monocytogenes*, *Listeria innocua* and *Listeria seeligeri* were detected. In the evaluation of antibiotic susceptibility, the isolates were sensitive to ampicillin and penicillin, which were the main antibiotics used in treatment. The isolates showed the ability to form biofilm in microplate, in stainless steel and polypropylene surfaces, with a variation of sessile cells between 4.29 ± 0.23 and $7.03 \pm 0.01 \log_{10}$ CFU cm^{-2} in TSBYE. This ability was also observed in food matrix composed of UHT whole milk in mono-species cultivation and in associated cultivation of *L. monocytogenes* with *Pseudomonas fluorescens*. The application of sanitizers peracetic acid and sodium hypochlorite revealed efficiency in the eradication of adhered cells and biofilms formed in stainless steel surfaces. Therefore, *Listeria* sp. showed to be persistent and able to form biofilm in mono-species cultivation or associated with *P. fluorescens*, under different conditions. Taking these aspects into account, the need for proper hygiene in the food production process is highlighted in order to avoid risks to the consumers health.

Keywords: adhesion; *L. innocua*; *L. seeligeri*; stainless steel; polypropylene; sanitizing.

Received on December 20, 2021.

Accepted on June 20, 2022.

Introduction

The growth in the frequency of foodborne diseases has been reported in recent years, with *Listeria* sp. one of the microorganisms implicated in outbreaks. This bacterium is found in different environments such as the Laboratories for the preparation of food of animal origin. These settings handle animal products ready for consumption and are important environments to be studied considering the risk of bacteria permanence on processing surfaces (Skowron et al., 2019).

Listeria sp. is a Gram-positive, non-sporulated, facultatively anaerobic bacillus, which has the ability to grow at temperatures from 2.5 to 44°C, as well as at pH between 6.0 and 8.0, and tolerate different salt concentrations (Skowron et al., 2019; European Food Safety Authority, & European Center for Disease Prevention and Control [EFSA/ECDC], 2019). Twenty-eight species are currently described for the genus *Listeria*, named as follow: *L. aquatica*, *L. booriae*, *L. cornellensis*, *L. cossartiae*, *L. costaricensis*, *L. denitrificans*, *L. farberii*, *L. fleischmannii*, *L. floridensis*, *L. goaensis*, *L. grandensis*, *L. grayi*, *L. immobilis*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. monocytogenes*, *L. murrayi*, *L. newyorkensis*, *L. portnoyi*, *L. riparia*, *L. rocourtiae*, *L. rustica*, *L. seeligeri*, *L. thailandensis*, *L. valentina*, *L. weihenstephanensis* and *L. welshimeri* (Parte, Sardà Carbasse, Meier-Kolthoff, Reimer, & Göker, 2020).

The persistence capacity of this microorganism is due to its ability to adhere to surfaces and form biofilm (Ramires et al., 2021). The term biofilm is defined as a structured community of bacterial cells, within a microbial polymeric matrix, associated with a surface, providing protection to the bacteria. *Listeria* has been described as biofilm forming bacteria in different environments, and they can survive the sanitization processes, negatively impacting food safety (Skowron et al., 2019; Alonso, Harada & Kabuki, 2020). In view of this, the present work aimed to identify species of *Listeria* isolated from in Laboratories preparation of food of animal origin and evaluate the potential of biofilm formation under different conditions.

Material and methods

Listeria sp. isolates and growing conditions

Fourteen species of *Listeria* sp. were studied. From them, 12 species were isolated from samples of animal products, sliced foods and food handling surfaces in Laboratories for the preparation of food of animal origin in Porto Alegre – RS/Brazil. These isolates were kept in 20% glycerol at a temperature of -20°C. For reactivation, the bacteria were transferred to tubes with 'Trypticase Soy Broth', plus 0.6% 'Yeast Extract' (TSBYE - Oxoid) incubated at the temperature of 37°C for 24 hours. After their growth, the bacteria were seeded on 'Trypticase Soy' Agar plates with 0.6% 'Yeast Extract' (TSAYE - Oxoid) and incubated at 37°C for 24 hours. Isolates were identified using the 'Matrix Assisted Laser Desorption/Ionization' method – 'Time of Flight Mass Spectroscopy' (MALDI-TOF/MS). This technique was performed using the chemical extraction method, according to the ethanol/formic acid protocol (Pyz-Lukasik et al., 2021). This procedure was carried out at the Institute of Basic Health Sciences of the Federal University of Rio Grande do Sul, using the equipment MALDI Biotyper 4.0 software MBT OC (MicroFlex LT - Bruker Daltonics). Spectra were analyzed by using MALDI Biotyper automation control, the Bruker Biotyper version 4.0 softwares (flexControl and MBT RTC), and the library.

Antimicrobial susceptibility test

For the evaluation, the Kirby-Bauer method was used. Bacterial suspensions were standardized by the McFarland 0.5 scale and seeded on a Mueller-Hinton (MH) Agar surface with 5% defibrinated horse blood. Antibiotic discs containing penicillin (1 IU) were used; ampicillin (2 µg); meropenem (10 µg); erythromycin (15 µg) and sulfazotrin (25 µg), as recommended by the European Committee on Antimicrobial Susceptibility Testing [EuCAST] (2019). Were also used ciprofloxacin discs (5 µg) clindamycin (2 µg); tetracycline (30 µg) as per Clinical and Laboratory Standards Institute 28th Edition [CLSI] (2018). The plates were incubated at 35 ± 1°C for 18 to 20 hours and interpreted according to the recommendations.

Biofilm formation of *Listeria* on microtiter plate assay

The ability of biofilm formation *in vitro* was determined as described by (Stepanovic, Cirkovic, Ranin, & Svabic-Vlahovic, 2004). The polystyrene microplate assay is an indirect measurement of the total biomass of cell adhesion or biofilms formed, and consequently, a measurement of biofilm *in vitro*. The sterile polystyrene microtiter plates (96 wells) were filled with 180 µL of TSBYE broth. The species of *Listeria* sp. were resuspended in 0.85% saline solution and adjusted to 0.5 McFarland scale and 20 µL of this solution was inoculated per well, in octuplicate. For the negative control, only TSBYE broth was used, followed by the positive control *Staphylococcus epidermidis* ATCC 35984 (Schmidt, Estes, McLaren, & Spangehl, 2018). The microtiter plates were incubated at 37°C for 48 hours. Subsequently, they were washed with 200 µL of 0.85% saline solution and fixation of the bacteria was performed with 200 µL of methanol for 20 minutes. Afterwards, the microplate was stained with 200 µL of crystal violet (0.1%) for 15 minutes, followed by washing with sterile distilled water. After drying the microplates, the colored button fixed to the bottom of the wells was resuspended in 200 µL of 95% ethanol and kept at rest for 30 minutes. The optical density (OD) of the suspension was quantified with the aid of a microplate spectrophotometer reader at a wavelength of 595 nm (Model: Anthos 2010 Type 17 550 S. N° 17 550 4894). The classification for biofilm formation followed the criteria described by Chusri, Phatthalung and Voravuthikunchai (2012). The mean OD of the negative control represented by 0.131 ± 0.004 was the cutoff point. The isolates were classified as follows: non-biofilm formers (NF): samples whose OD was less than the cutoff point. Weak biofilm formers (WF): samples with average OD above the cutoff, but less than or equal to twice the cutoff. Moderate biofilm formers (MF): samples with mean OD above twice the cutoff point, but less than or equal to 4 x the cutoff point. Strong biofilm formers (SF): samples with a mean OD greater than 4 x the cutoff point value represented by 0.524 ± 0.016.

Biofilm formation of *Listeria* on SS and PP in TSBYE culture medium

The study used AISI 304 stainless steel (SS) surfaces and polypropylene (PP) surfaces, both containing 1 cm². Surfaces were sanitized as described by Alonso et al. (2020) and Bogo, Costa, Frazzon and Motta (2020) with modifications. For the experiment, selected bacterial colonies were transferred to TSBYE culture medium and incubated at 37°C for 24 hours. Subsequently, an aliquot of 25 µL was transferred to 100 mL of TSBYE, obtaining $\approx 3 \log_{10}$ CFU mL⁻¹ (T0). Three surfaces of each material were immersed in separate tubes containing

10 mL with inoculum in TSBYE and incubated in an oven, without shaking, at 37°C for up to 72 hours. Every 24 hours, one surface from each tube was aseptically removed, washed in sterile dH₂O to remove planktonic cells and then immersed in 10 mL of 0.85% saline solution and vortexed for 3 min. to remove the sessile cells (Alonso & Kabuki, 2019). The evaluation of sessile cells (\log_{10} CFU cm⁻²) and planktonic cells (\log_{10} CFU mL⁻¹) were performed at T0, 24, 48, and 72 hours. The Petri dishes containing TSAYE were incubated at 37°C for 24 hours (Miles, Misra, & Irwin, 1938).

Evaluation of biofilm formation capacity on SS and PP in UHT whole milk substrate

The surfaces were prepared according to the previous experiment, using the substrate UHT whole milk (commercial) at different temperatures. In incubations at 37°C and room temperature (RT), with an average of 28.6°C, sessile and planktonic cells were evaluated at times: T0, 24, 48 and 72 hours. As for the inoculum kept at refrigeration temperature, with an average of 7.9°C, the evaluations were at: T0, 72, 120 and 168 hours.

L. monocytogenes with *P. fluorescens* in dual-species biofilm formation on SS and PP surfaces in UHT milk

The bacterial species used were *L. monocytogenes* QF Oxford and *P. fluorescens* PL7.1 (Bogo et al., 2020). These microorganisms were cultivated at optimal growth temperatures for 24 hours, standardizing the initial inoculum in $\approx 3 \log_{10}$ CFU mL⁻¹ for both species (T0) and associated (1:1). The surfaces were incubated at RT (27.4°C) with evaluations at the T0, 24, 48 and 72 hours and in refrigeration (8.2°C) with evaluations at: T0, 72, 120 and 168 hours. Counts were performed in selective culture media: Agar *Listeria* Oxford and Agar Cetrimide. Plates were incubated at RT for 24 hours and cell numbers expressed as described above.

Evaluation of sanitizers for removal of the dual-species biofilm on SS"

The evaluation of sanitizers on the dual-species biofilm (*L. monocytogenes* and *P. fluorescens*) on SS surface in UHT milk after 72 hours was according to the methodology described by Young-Min, Seung-Youb and Sun-Young (2012) and Ga-Hee and Dong-Hyun (2016). The sanitizers used were: peracetic acid at a concentration of 0.2% (Peroxoni Top -Launer Chemical Industry and Commerce LLC-Brazil - Active content % Peracetic Acid ≥ 15.00) and Sodium Hypochlorite (Water Ypê Chlorine Active - Amparo LLC-Brazil - chlorine content % 2.0 - 2.5 p/p) applied as indicated by the manufacturers. For that, the surfaces were washed three times in dH₂O sterile to remove planktonic cells. The SS surfaces containing sessile cells were immersed in tubes with 10 mL of the sanitizing product for 10 minutes at RT, followed by the same procedure in dH₂O sterile in negative control condition. After this time, the surfaces were washed three times in sterile dH₂O to remove the sanitizers and were immersed in 10 mL of saline solution 0.85% NaCl. Following, they were vortexed for 3 minutes to remove the sessile cells after the action of the sanitizing agent. At this stage, the sessile cell count was performed using the method described by Miles et al. (1938). The counts were carried out onto medium Plate Count Agar (PCA), Agar *Listeria* Oxford and Agar Cetrimide, after incubated at RT for 24 hours.

Statistical analysis

Data were analysis using SPSS, version 18.0. Comparisons of qualitative data were performed using the chi-square test with adjusted residual analysis. For descriptive analysis, quantitative data with symmetrical distribution were expressed as means and standard deviations of means (\pm SD). All data showed a symmetrical distribution by the ShapiroWilk test. Categorical variables were described as absolute (n) and relative (n %) frequencies. Bivariate analyzes for comparisons of quantitative variables between the different treatments were conducted using Student's t tests for independent samples, one-way or two-way analysis of variance (ANOVA) with post hoc Bonferroni, when applicable. Bivariate analysis for comparisons of quantitative variables between different analysis times were conducted using Student's t tests for paired samples. The significance level was set at 5% for all analysis.

Results

Identification of *Listeria* sp. species

A total of 14 bacteria were used in this study: four species belonging to *L. monocytogenes*, four of *L. innocua* and four belonging to species *L. seeligeri*. In addition to these isolates, the standard strains *L. monocytogenes* ATCC 35152 and *L. monocytogenes* ATCC 7644 were also used in the study (Kumar et al., 2009; Wiktorczyk, Grudlewska, Skowron, Gryń, & Gospodarek-Komkowska, 2018). The bacterias *L. monocytogenes* 17D78/03, *L.*

monocytogenes 4C, *L. innocua* 6B, *L. innocua* L07, *L. innocua* L10, *L. innocua* L13, *L. monocytogenes* 4B, *L. seeligeri* BQ Oxford, *L. seeligeri* BP Oxford, *L. seeligeri* BP Palcam and *L. seeligeri* MP Oxford and *L. monocytogenes* QF Oxford were studied and their respective origins were listed in Table 1. The collections were carried out together with the 'Food Surveillance Service of the Health Secretariat' of Porto Alegre, according to State Law 6503/72 and State Decree 23430/74-RS.

Table 1. Species of *Listeria* sp. identified in MALDI-TOF/MS with their code and origins, with the respective classifications regarding the potential of biofilm formation in microplate.

Microorganism (species)	Code	Origin	Classification	OD±SD
<i>Staphylococcus epidermidis</i> * #	ATCC 35984	American Type Culture Collection	SF	2.797±0.009
<i>L. monocytogenes</i> #	ATCC 35152	American Type Culture Collection	SF	0.683±0.010
	ATCC 7644	American Type Culture Collection	MF	0.355±0.006
<i>L. monocytogenes</i>	17D78/03	food isolate	MF	0.361±0.006
	QF Oxford	sliced cheese isolate	SF	0.598±0.010
	4B	Chicken carcass isolate	SF	0.643±0.020
	4C	food isolate	MF	0.448±0.009
<i>L. innocua</i>	L10	Isolated from raw buffalo milk	MF	0.401±0.008
	L13	Isolated from raw buffalo milk	MF	0.401±0.004
	6B	food isolate	MF	0.445±0.010
	L07	Isolated from raw buffalo milk	MF	0.312±0.006
<i>L. seeligeri</i>	BQ Oxford	Cheese Countertop isolate	MF	0.325±0.010
	BP Palcam	Ham Countertop isolate	MF	0.321±0.008
	BP oxford	Cheese Countertop isolate	MF	0.423±0.005
	MP Oxford	Food handler hands isolated	WF	0.179±0.006

*Positive Control SF; #ATCC; NF-Non-biofilm former; WF- Weak biofilm former; MF- Moderate biofilm former; SF- Strong biofilm former; Negative Control 0.131±0.004; OD - optical density.

Evaluation of antimicrobial susceptibility profile

In the assessment of susceptibility to antibiotics, the bacteria studied showed sensitivity to Ampicillin (2 µg); Penicillin (1 IU); Tetracycline (30 µg); Meropenem (10 µg) and Erythromycin (15 µg). However, it was observed that from the 14 (100%) bacteria studied, nine (64.3%) were resistant to at least one antibiotic tested. From this resistant group, the isolates *L. monocytogenes* 4C and *L. seeligeri* BP Oxford, presented a profile for clindamycin (2 µg) and sulfazotrim (25 µg). The *L. monocytogenes* ATCC 35152, *L. monocytogenes* 4B, *L. innocua* 6B, *L. innocua* L10, *L. innocua* L07, were resistant to clindamycin (2 µg). The bacteria *L. monocytogenes* ATCC 7644 and 17D78/03, *L. innocua* L13 and *L. seeligeri* BQ Oxford showed intermediate sensitivity to clindamycin (2 µg), and the isolate *L. seeligeri* BQ Oxford also presented a profile to ciprofloxacin (5 µg).

Evaluation of the biofilm-forming ability of *Listeria* by microplate method

In the evaluation of biofilm formation by the microplate method, three *L. monocytogenes* isolates presented a strong biofilm former profile. (SF): *L. monocytogenes* 4B (chicken carcass), *L. monocytogenes* ATCC 35152, *L. monocytogenes* QF Oxford (sliced cheese). The *L. seeligeri* MP Oxford, isolated from food handler hands, presented a weak biofilm former (WF). The other bacteria showed results as moderate biofilm former (MF). Data is described in Table 1.

Evaluation of the ability to form biofilms on SS and PP in TSBYE culture medium

Biofilm formation of *L. monocytogenes* ATCC 35152, *L. monocytogenes* 4B and *L. monocytogenes* QF Oxford on SS and PP surfaces occurred in all times observed. The mean of planktonic cell values for the two surfaces observed during the study time of up to 72 hours was $8.76 \pm 0.40 \log_{10}$ CFU mL⁻¹. The sessile cell count on SS surface was between 5.20 ± 0.31 and $5.52 \pm 0.08 \log_{10}$ CFU cm⁻² for *L. monocytogenes* 4B, between 5.41 ± 0.44 and $4.29 \pm 0.23 \log_{10}$ CFU cm⁻² for the *L. monocytogenes* ATCC 35152 strain, and between 5.36 ± 0.59 and $5.68 \pm 0.24 \log_{10}$ CFU cm⁻² in the *L. monocytogenes* QF Oxford in the 72 hours studied time. On PP surface the sessile cell values were between 6.22 ± 0.51 and $6.72 \pm 0.07 \log_{10}$ CFU cm⁻² for *L. monocytogenes* 4B, between 6.44 ± 0.37 and $6.62 \pm 0.41 \log_{10}$ CFU cm⁻² for the strain *L. monocytogenes* ATCC 35152, and between 6.86 ± 0.14 and $7.03 \pm 0.01 \log_{10}$ CFU cm⁻² in the isolate *L. monocytogenes* QF Oxford at the times studied.

When comparing the three bacteria on the SS surface, there was no difference between groups for \log_{10} CFU cm⁻² in 24 hours (one-way ANOVA, $F(2.6) = 0.362$, $p = 0.710$) or in 48 hours (one-way ANOVA, $F(2.6) = 3.782$, $p = 0.087$). On the other hand, it was evidenced that in 72 hours there was a difference for the \log_{10} CFU

cm⁻² (One-way ANOVA, $F(2.6) = 40.981$, $p \leq 0.0001$), where the *L. monocytogenes* ATCC 35152 group had a lower mean than the *L. monocytogenes* 4B and *L. monocytogenes* QF Oxford groups (Bonferroni post hoc, $p \leq 0.005$). For the PP surface, there was no difference between groups for log₁₀ CFU cm⁻² in 24 hours (one-way ANOVA, $F(2.6) = 2.577$, $p = 0.156$), 48 hours (one-way ANOVA, $F(2.6) = 3.739$, $p = 0.088$) or 72 hours (one-way ANOVA, $F(2.6) = 0.620$, $p = 0.569$).

Biofilm formation by *L. monocytogenes* QF Oxford on SS and PP surfaces in UHT milk under different conditions

At a temperature of 37°C, the mean planktonic cell population in tubes containing SS and PP surfaces was 8.24 ± 0.40 log₁₀ CFU mL⁻¹ for times up to 72 hours. The sessile cell population on SS surfaces ranged from 3.10 ± 0.07 to 3.48 ± 0.23 log₁₀ CFU cm⁻² (Figure 1A). On PP surfaces, the values of the sessile cells were between 4.75 ± 0.59 to 5.07 ± 0.44 log₁₀ CFU cm⁻² (Figure 1B), in the times studied.

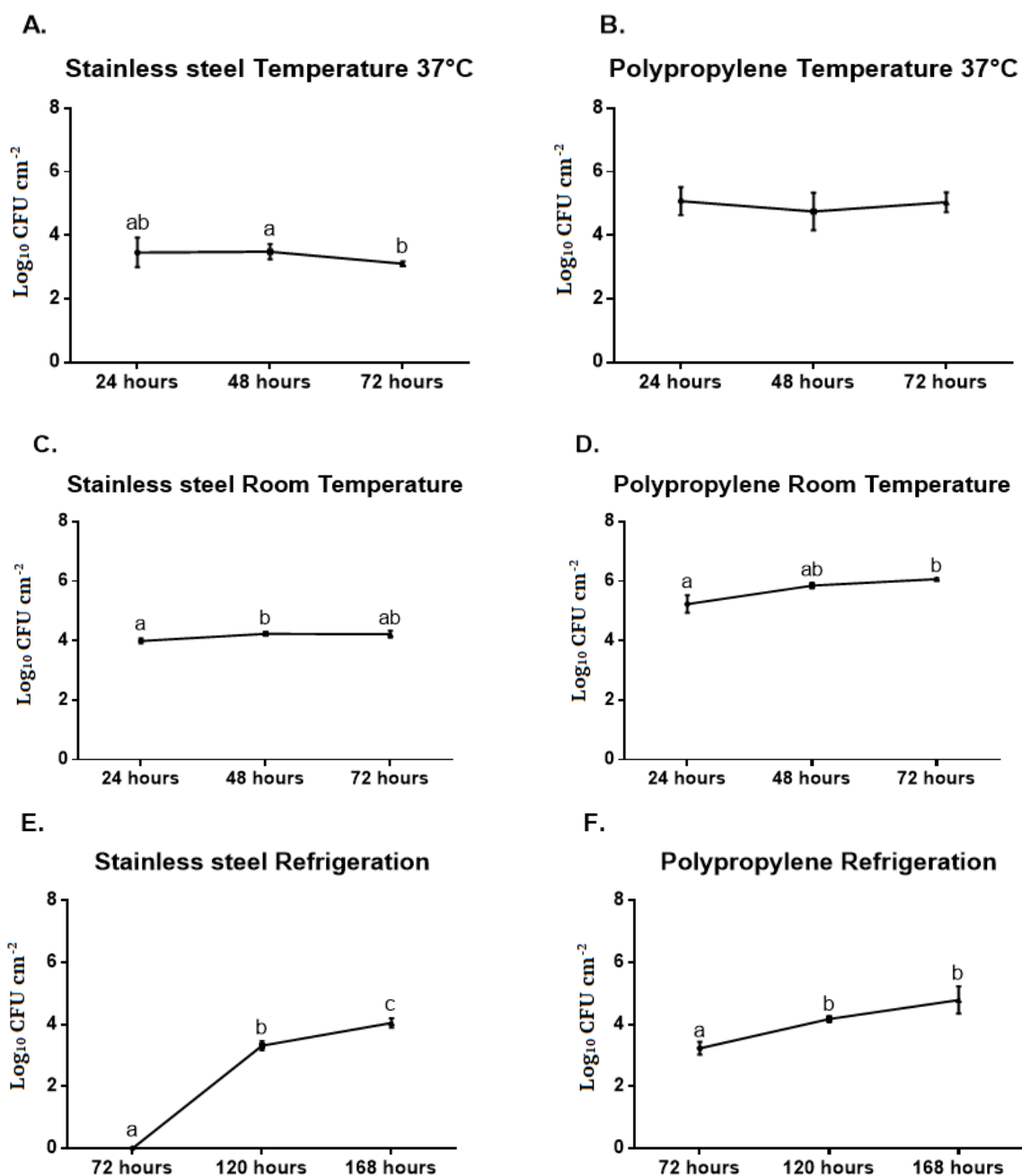


Figure 1. Biofilm formation by *Listeria monocytogenes* QF Oxford on SS surfaces (A, C, E) and PP surfaces (B, D, F) in UHT milk at 37°C (A, B), room temperature (C, D), and refrigeration temperature (E, F). CFU – colony forming unit. ^{Ab} Different letters indicate statistical difference between the analyzed groups.

When paired analyzes compared the different times analyzed, the \log_{10} CFU cm^{-2} of the *L. monocytogenes* QF Oxford group on the SS surface with the UHT whole milk medium showed a significant reduction in the time of 72 hours when compared to the time of 48 hours (t test of Student for paired samples, $p = 0.025$), showing no difference for the other analyses. In the evaluation of the biofilm formation capacity at RT, the mean of the planktonic cells values in SS and PP was $8.54 \pm 0.10 \log_{10}$ CFU mL^{-1} in times of up to 72 hours. The sessile cell counts on SS surface ranged from 3.99 ± 0.08 to $4.23 \pm 0.02 \log_{10}$ CFU cm^{-2} (Figure 1C), and 5.23 ± 0.029 to $6.07 \pm 0.03 \log_{10}$ CFU cm^{-2} on PP surface at the times studied (Figure 1D).

In the present study, the capacity of biofilm formation at refrigeration temperature with times of 72, 120 and 168 hours was also evaluated. At these times, the mean of the planktonic cells values in the tubes containing the SS and PP surfaces was $7.16 \pm 1.30 \log_{10}$ CFU mL^{-1} . In SS surfaces, sessile cell values were not observed in 72 hours of incubation. The results, from 120 and 168 hours, were 3.31 ± 0.14 to $4.04 \pm 0.14 \log_{10}$ CFU cm^{-2} of sessile cells respectively (Figure 1E). The values for the sessile cells in the PP surfaces ranged from 3.23 ± 0.20 to $4.78 \pm 0.43 \log_{10}$ CFU cm^{-2} at the times studied (Figure 1F).

At 37°C , the \log_{10} CFU cm^{-2} of the *L. monocytogenes* QF Oxford group decreased in 72 hours compared to 48 hours on the SS surface (Student's t-test for paired samples, $p = 0.025$, Figure 1A), with no changes observed on the PP surface (Figure 1B). At RT, the \log_{10} CFU cm^{-2} of the Oxford QF group increased in 48 hours compared to 24 hours on the SS surface (Student's t-test for paired samples, $p = 0.043$, Figure 1C) and in 72 hours compared to 24 hours on the PP surface (Student's t-test for paired samples, $p = 0.035$, Figure 1D).

At refrigeration temperature, the \log_{10} CFU cm^{-2} of the *L. monocytogenes* QF Oxford group, on the SS surface, increased in 120 hours compared to 72 hours (Student's t-test for paired samples, $p = 0.001$, Figure 1E) and in 168 hours versus 120 hours (Student's t-test for paired samples, $p = 0.049$, Figure 1E). An increase in \log_{10} CFU cm^{-2} of the *L. monocytogenes* QF Oxford group, in PP, was evidenced at 120 and 168 hours compared to 72 hours, when kept at refrigeration temperature (Figure 1F).

Evaluation of biofilm formation capacity by *L. monocytogenes* QF Oxford associated with *P. fluorescens* PL7.1 on SS and PP with UHT milk substrate at different temperatures

In the evaluations at RT, the mean of planktonic cells of *L. monocytogenes* QF Oxford and *P. fluorescens* PL7.1 was $8.17 \pm 0.40 \log_{10}$ CFU mL^{-1} at the times studied. At RT, SS evaluations showed sessile cell counts between 3.99 ± 0.21 and $4.84 \pm 0.58 \log_{10}$ CFU cm^{-2} for the isolate *L. monocytogenes* QF Oxford and between 3.54 ± 0.07 and $6.07 \pm 0.23 \log_{10}$ CFU cm^{-2} for the bacteria *P. fluorescens* PL7.1 (Figure 2A). In the PP surfaces, the sessile cell values were between 5.46 ± 0.37 and $5.66 \pm 0.11 \log_{10}$ CFU cm^{-2} for the isolate *L. monocytogenes* QF Oxford and between 5.40 ± 0.29 and $6.71 \pm 0.43 \log_{10}$ CFU cm^{-2} for *P. fluorescens* PL7.1 (Figure 2B).

In the evaluations at refrigeration temperature, the mean values of planktonic cells of *L. monocytogenes* QF Oxford and *P. fluorescens* PL7.1 were $6.71 \pm 1.10 \log_{10}$ CFU mL^{-1} at the times studied. In SS surfaces, the values of sessile cells of the isolate *L. monocytogenes* QF Oxford were not recovered in 72 hours of incubation. The results from 120 and 168 hours were 3.04 ± 0.60 and $3.85 \pm 0.40 \log_{10}$ CFU cm^{-2} of sessile cells respectively. For *P. fluorescens* PL7.1 the sessile cell values were between 4.40 ± 0.07 to $4.73 \pm 0.55 \log_{10}$ CFU cm^{-2} at the times studied (Figure 2C).

In the PP surfaces, the sessile cell values of the isolate *L. monocytogenes* QF Oxford were not observed in 72 hours of incubation. At 120 and 168 hours, the values were 2.89 ± 0.75 and $3.82 \pm 0.33 \log_{10}$ CFU cm^{-2} sessile cells respectively. For *P. fluorescens* PL7.1 the values of sessile cells in PP were between 5.60 ± 0.17 to $5.77 \pm 0.01 \log_{10}$ CFU cm^{-2} at the times studied (Figure 2D).

There was a higher \log_{10} CFU cm^{-2} of sessile cells from the *L. monocytogenes* QF Oxford group in 24 hours in SS and at RT compared to the sessile cells from the *P. fluorescens* PL7.1 group (Student's t-test for independent samples, $p = 0.028$). An increase in sessile cells within 72 hours was also observed in SS surfaces at RT in the *P. fluorescens* PL7.1 group compared to the *L. monocytogenes* QF Oxford group (Student's t-test for independent samples, $p = 0.028$) (Figure 2A). Similarly, in the PP surfaces and at RT, the *P. fluorescens* PL7.1 group presented higher \log_{10} CFU cm^{-2} of sessile cells in 72 hours (Student's t-test for independent samples, $p = 0.038$) (Figure 2B). In SS and PP at refrigeration temperature, the *P. fluorescens* PL7.1 group presented higher \log_{10} CFU cm^{-2} of sessile cells at 72, 120 and 168 hours compared to the *L. monocytogenes* QF Oxford group (Student's t test for independent samples, $p \leq 0.0001$) (Figure 2C and 2D).

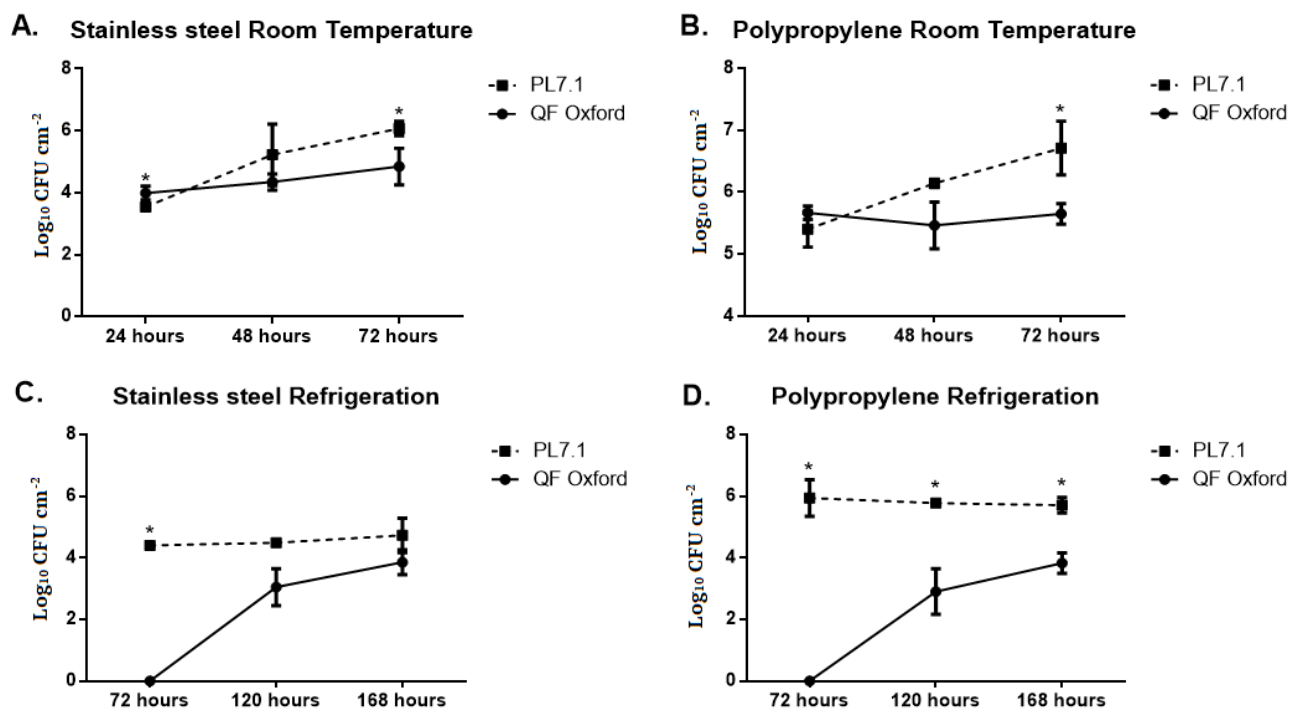


Figure 2. Evaluation of capacity of biofilm formation with association of *Listeria monocytogenes* QF Oxford, and *Pseudomonas fluorescens* PL7.1 in SS (A) and PP (B) in substrate in UHT milk at RT (A, B) at 24, 48, 72 hours and refrigeration temperature at 72, 120, 168 hours (C, D). CFU – colony forming unit. *Statistical difference between analyzed groups.

Evaluation of sanitizers in the eradication of biofilm in SS

On SS surfaces containing $4.23 \pm 0.14 \log_{10}$ CFU cm⁻² of sessile cells of the bacteria *L. monocytogenes* QF Oxford and $5.46 \pm 0.05 \log_{10}$ CFU cm⁻² of sessile cells of the bacteria *P. fluorescens* PL7.1 were immersed in peracetic acid and sodium hypochlorite. After the application time, the sessile cells were counted, for which no growth was observed in the treated group. At 72 hours, in the bacterium *L. monocytogenes* QF Oxford, a group effect (one-way ANOVA, $F(4.9) = 2686.580$, $p \leq 0.0001$) was observed, with a mean of \log_{10} CFU cm⁻² of the *L. monocytogenes* QF Oxford group higher than the control group and all higher than the sanitizers (peracetic acid and sodium hypochlorite) (post hoc Bonferroni, $p \leq 0.05$). The same effect was evidenced at 72 hours in the *P. fluorescens* PL7.1 bacteria being the mean \log_{10} CFU cm⁻² of the PL7.1 group (one-way ANOVA, $F(4.9) = 8811.621$, $p \leq 0.0001$, Bonferroni post hoc, $p \leq 0.05$).

Discussion

The present study analyzed species of *Listeria* sp. from food samples, slices and food handling surfaces in laboratories for the preparation of foods of animal origin for cold consumption in the city of Porto Alegre – RS/Brazil. The occurrence of these bacteria in ready-to-eat foods or in places such as manipulation benches indicates that safety measures to prevent contamination must be improved immediately. The species of *L. monocytogenes*, *L. innocua*, *L. seeligeri* are reported by other works identifying these microorganisms in samples similar to the present study (Skowron et al., 2019; Osman, Kappel, Fox, Orabi, & Samir, 2020; Gwida et al., 2020). In Brazil, Pessoa et al. (2020), described the presence of *Listeria* sp. in Italian type salamis, ready for consumption. Souza et al. (2020) reported the presence of *L. monocytogenes* and *L. innocua* in chicken carcass isolates.

For most infections caused by *L. monocytogenes* the treatment of choice is ampicillin, penicillin, erythromycin, gentamicin or sulfamethoxazole-trimethoprim (sulfazotrim) (Durán-Garrido, Gómez-Palomo, Estades-Rubio, & Jiménez-Garrido, 2020; Rzepniewski, Goluchowska, Idziakowska, & Najman, 2020). In the assessment of susceptibility to antimicrobials, among the eight antimicrobials tested, ampicillin and penicillin proved to be efficient in eliminating this bacterium. However, we observed that 64.3% of the bacteria used in the study were resistant to at least one antibiotic tested. Clindamycin resistance with *L. monocytogenes* isolates is widely described in other works (Oliveira et al., 2018; Arslan & Selin, 2019). Resistance to sulfazotrim is important as it is the second-choice drug in patients allergic to penicillin

(Demoliner et al., 2015; Olaimat et al., 2018). These antimicrobial resistance factors mentioned are especially related to the acquisition of genes such as *mefA*, *erm*, *lnuA* and *lnuB*, *dfrD*, *dfrG*, and *dhfr*. Another important aspect of sulfazotrim resistance is the conjugation of plasmid IP501 and transfer of the *Tn6198* transposon among *Enterococcus faecalis* to *L. monocytogenes*, making it resistant to the sulfazotrim compound (Oliveira et al., 2018; Maung et al., 2019; Matereke & Okoh, 2020).

An important form of *Listeria sp.* to resist to the environment is the potential for biofilm formation (Chusri et al., 2012). From the species of *L. monocytogenes*, three of them presented a profile of strong biofilm formers. The *L. innocua* specie presented themselves as biofilm moderate formers, whereas on the *L. seeligeri*, three of them presented a moderate profile of biofilm former and one was classified as weak microplate biofilm former. The microplate assay is an indirect reading technique of cell adhesion and biofilm formation *in vitro*. The studies carried out with this technique presented varying levels regarding the classification based on OD, with spectra between weak, moderate and strong biofilm formers. Nevertheless, ratified by the mentioned studies, it is an important technique especially for screening with bacteria that may have the ability to adhere to surfaces or the ability to form biofilms (Nilsson, Ross, & Bowman, 2011; Dygico, Gahan, Grogan, & Burgess, 2020; Osman et al., 2020).

An important parameter in determining whether there is bacterial adhesion or mature biofilm formation is the number of microorganisms present on the surface. Counts upper $5 \log_{10}$ CFU cm^{-2} are considered to have mature biofilm (Wiktorczyk et al., 2018; Alonso & Kabuki, 2019; Ramires et al., 2021). In the present study we observed that three *L. monocytogenes* (4B, ATCC 35152 e QF Oxford) which revealed the ability to form biofilm in the microplate test, were also able to form biofilm on surfaces after 24 hours of incubation, in TSBYE culture medium at 37°C, both in SS material and in PP. Studies with these surfaces describe the formation of biofilm, with sessile cell values similar to the present work (Alonso & Kabuki 2019; Ripolles-Avila, Cervantes-Huaman, Hascoët, Yuste, & Rodríguez-Jerez, 2019; Skowron et al., 2019; Ramires et al., 2021).

Substrates such as culture media can inhibit or favor the growth of microorganisms and change the composition of the biofilm, depending on the chemical structures involved (Bassi, Cappa, Gazzola, Orrù, & Cocconcelli, 2017). In this study, we evaluated the capacity of *L. monocytogenes* to form biofilm in the UHT milk, where we observed a reduction in the value of CFU cm^{-2} of sessile cells compared to the culture medium. Alonso and Kabuki (2019) observed a reduction of approximately $1 \log_{10}$ CFU cm^{-2} in the number of sessile cells of *L. monocytogenes* in 24 hours of incubation, in whey protein, when compared to the substrate culture medium. In this study we observed a reduction of 1.93 ± 0.05 and $1.96 \pm 0.04 \log_{10}$ CFU cm^{-2} in the values of sessile cells in SS and PP surfaces, respectively, when cultivated in UHT milk compared to cultures in TSBYE medium in 24 hours of evaluation. This reduction in \log_{10} CFU cm^{-2} can be attributed to milk protein, due to its composition, in disputing the same location of the surfaces, influencing the bacterial adhesion capacity (Bassi et al., 2017; Alonso et al., 2020). Therefore, the difference in values related to the surfaces can be influenced by the porosity and surface texture between the two materials tested (Marik, Zuchel, Schaffner & Strawn, 2020; Dygico et al., 2020).

In the adhesion process, the temperature factor is also an important influencer. In this study, we observed that *L. monocytogenes* QF Oxford developed better at RT, on both surfaces with a higher rate of \log_{10} CFU cm^{-2} at RT with averages of 28.6°C when compared to the other temperatures tested. The temperature around 25 and 30°C is reported as the highest sessile cell count of this microorganism when similar materials are used (Dygico et al., 2020; Mendez, Walker, Vipham, & Trinetta, 2020). This factor is influenced by the motility expressed in *L. monocytogenes* at temperatures close to the environment, and reduced when exposed to temperatures of 37°C (Mendez et al., 2020; Muchaamba, Stephan, & Taurai, 2021).

This cooperative interaction between bacterial species can change the composition of the biofilm matrix increasing resistance, the biomass of the biofilm formed, the adherence, or present an environment of microbial competition (Govaert, Smet, Walsh, & Impe, 2019). The evaluations described between *L. monocytogenes* and *P. fluorescens* in biofilms showed that *L. monocytogenes* was able to adhere to the SS surface under multi-species conditions, however along the period the number of sessile cells of *L. monocytogenes* obtained a reduction, but did not influenced the population level of *P. fluorescens* (Maggio et al., 2021). *L. monocytogenes* with *B. cereus* in dual-species biofilm, can reach counts above $5 \log_{10}$ CFU cm^{-2} by cooperation. On the other hand, in the presence of *B. cereus*, an antagonistic substance producer, *L. monocytogenes* can adhere, without promoting biofilm formation (Alonso et al., 2020). When analyzing a study of cooperating biofilm cells by *L. monocytogenes* and *P. fluorescens*, the population of *L. monocytogenes* showed an average of 1-2 \log_{10} CFU cm^{-2} higher when compared to monospecies culture (Puga, Dahdouh, SanJose, & Orgaz, 2018).

Studies have evaluated biofilm formation in SS and PP at temperatures of 7, 23 and 30°C for 24, 48 and 72 hours in two species of psychrotrophic bacteria, reporting that both cultures of *P. fluorescens* were capable of adhering to both types of surfaces. The count of bacterial cells adhering to the PP surface was higher than those adhering to SS for both isolates, due to the type of surface of the materials tested (Bogo et al., 2020). These results corroborate the values found in this study.

Sanitary practices in food production environments aim to eliminate microorganisms that may be pathogenic or harmful to food. Chlorine-based solutions such as hypochlorite and peracetic acid are disinfectants widely used as sanitizers in the food industry (Demoliner et al., 2015). Thus, the present work evaluated the effectiveness of peracetic acid and sodium hypochlorite sanitizers in the eradication of adhered cells and biofilm in associated culture. These two products were effective when applied for 10 minutes on surfaces containing $4.23 \pm 0.14 \log_{10}$ CFU cm^{-2} of *L. monocytogenes* QF Oxford sessile cells and biofilms with $5.46 \pm 0.05 \log_{10}$ CFU cm^{-2} of *P. fluorescens* PL7.1 sessile cells. Studies that evaluated peracetic acid in biofilms formed by *L. monocytogenes* isolated from a cheese processing factory, indicated the efficiency of this sanitizer in biofilms formed in SS (Lee et al., 2019). Similar results were observed in this study, indicating that application for 10 minutes is effective in eliminating adhered *L. monocytogenes* cells and *P. fluorescens* biofilms in associated culture.

The obtained isolated of *Listeria* sp., showed to be persistent, with adhesion capacity and biofilm formation on surfaces used in food industries. Furthermore, they proved to be resistant to second option antimicrobials in the treatment of infections caused by *L. monocytogenes*, which could represent a risk to consumers.

Conclusion

Biofilm mono-species of *L. monocytogenes* can also colonize on SS and PP surfaces in TSBYE broth. *L. monocytogenes* also form biofilm in dual-species with *P. fluorescens* PL7.1 in several temperatures.

The sanitizers peracetic acid and sodium hypochlorite, showed efficiency in the eradication of biofilm on SS, when applied according to the manufacturers' recommendations. Therefore, surfaces and foods when handled inappropriately can harbor these microorganisms, and as potential biofilm formers they increase the persistence of *Listeria* sp. in these locations. We emphasize the importance of proper hygiene in the food production process in order to minimize the risks to consumer health.

References

- Alonso, V. P. P., & Kabuki, D. Y. (2019). Formation and dispersal of biofilms in dairy substrates. *International Journal of Dairy Technology*, 72(3), 472-478. DOI: <https://doi.org/10.1111/1471-0307.12587>
- Alonso, V. P. P., Harada A. M. M., & Kabuki, D. Y. (2020). Competitive and/or cooperative interactions of *Listeria monocytogenes* with *Bacillus cereus* in dual-species biofilm formation. *Frontiers in Microbiology*, 11(1), 177. DOI: <https://doi.org/10.3389/fmicb.2020.00177>
- Arslan, S., & Selin, B. (2019). Prevalence and antimicrobial resistance of *Listeria* species and subtyping and virulence factors of *Listeria monocytogenes* from retail meat. *Journal of Food Safety*, 39(1), e12578. DOI: <https://doi.org/10.1111/jfs.12578>
- Bassi, D., Cappa, F., Gazzola, S., Orrù, P. S., & Cocconcetti, P. S. (2017). Biofilm formation on stainless steel by *Streptococcus thermophilus* UC8547 in milk environments is mediated by the proteinase PrtS. *Applied and Environmental Microbiology*, 83(8), 1-12. DOI: <https://doi.org/10.1128/AEM.02840-16>
- Bogo, M., Costa, G. A., Frazzon, A. P. G., & Motta, A. S. (2020). Evaluation of the adhesion potential of psychrotrophic bacteria isolated from refrigerated raw buffalo milk: simulating storage conditions. *Buffalo Bulletin*, 39(2), 215-228.
- Chusri, S., Phatthalung, P. N., & Voravuthikunchai, S. P. (2012). Anti-biofilm activity of *Quercus infectoria* G. Olivier against methicillin-resistant *Staphylococcus aureus*. *Letters in Applied Microbiology*, 54(6), 511-517. DOI: <https://doi.org/10.1111/j.1472-765X.2012.03236.x>
- Clinical and Laboratory Standards Institute [CLSI]. (2018). *Performance standards for antimicrobial susceptibility testing* (28th ed., CLSI supplement M100S). Wayne, PA: CLSI.
- Demoliner, F., Damasceno, K. S., Oliveira, D. P., Hallal, E. D., Mello, J. F., Lameiro, K. R., & Gandra, E. A. (2015). Resistance to disinfectants and antibiotics of *Pseudomonas* spp. and *Listeria* spp. biofilms on polystyrene and stainless steel. *African Journal of Microbiology Research*, 9(27), 1706-1715. DOI: <https://doi.org/10.5897/AJMR2015.7498>

- Durán-Garrido, F. J., Gómez-Palomo, J. M., Estades-Rubio, F.J., & Jiménez-Garrido, C. (2020). Infección por *Listeria monocytogenes* en paciente con sarcoma pélvico. *Revista Española de Casos Clínicos en Medicina Interna*, 5(2), 81-83. DOI: <https://doi.org/10.32818/reccmi.a5n2a9>
- Dygico, L. K., Gahan, C. G., Grogan, H., & Burgess, C. M. (2020). The ability of *Listeria monocytogenes* to form biofilm on surfaces relevant to the mushroom production environment. *International Journal of Food Microbiology*, 317(1), 108385. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2019.108385>
- European Committee on Antimicrobial Susceptibility Testing [Eucast]. (2019). *Clinical Breakpoint Tables v. 9.0*. Retrieved from https://www.eucast.org/clinical_breakpoints
- European Food Safety Authority, & European Center for Disease Prevention and Control [EFSA / ECDC]. (2019). The European Union one health 2018 zoonoses report. *EFSA Journal*, 17(12), e05926. DOI: <https://doi.org/10.2903/j.efsa.2019.5926>
- Ga-Hee, B., & Dong-Hyun, K. (2016). Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. *Food Microbiology*, 55(1), 47-54.
- Govaert, M., Smet, C., Walsh, J. L., & Impe, J. F. M. V. (2019). Dual-species model biofilm consisting of *Listeria monocytogenes* and *Salmonella Typhimurium*: development and inactivation with cold atmospheric plasma (CAP). *Frontiers in Microbiology*, 10(1), 2524. DOI: <https://doi.org/10.3389/fmicb.2019.02524>
- Gwida, M., Lüth, S., El-Ashker, M., Zakaria, A., El-Gohary, F., Elsayed, M., ... Al Dahouk, S. (2020). Contamination Pathways can be traced along the poultry processing chain by whole genome sequencing of *Listeria innocua*. *Microorganisms*, 8(3), 414. DOI: <https://doi.org/10.3390/microorganisms8030414>
- Kumar, S., Parvathi, A., George, J., Krohne, G., Karunasagar, I., & Karunasagar, I. (2009). A study on the effects of some laboratory-derived genetic mutations on biofilm formation by *Listeria monocytogenes*. *World Journal of Microbiology and Biotechnology*, 25(3), 527-531. DOI: <https://doi.org/10.1007/s11274-008-9919-8>
- Lee, B-H., Cole, S., Badel-Berchoux, S., Guillier, L., Feliz, B., Krezdorn, N., ... Piveteau, P. (2019). Biofilm formation of *Listeria monocytogenes* strains under food processing environments and pan-genome-wide association study. *Frontiers in Microbiology*, 10(1) 2698. DOI: <https://doi.org/10.3389/fmicb.2019.02698>
- Maggio, F., Rossi, C., Chaves-López, C., Serio, A., Valbonetti, L., Pomilio, F. & Paparella, A. (2021). Interactions between *L. monocytogenes* and *P. fluorescens* in dual-species biofilms under simulated dairy processing conditions. *Foods*, 10(1), 176. DOI: <https://doi.org/10.3390/foods10010176>
- Marik, C. M., Zuchel, J., Schaffner, D. W., & Strawn, L. K. (2020). Growth and survival of *Listeria monocytogenes* on intact fruit and vegetable surfaces during postharvest handling: a systematic literature review. *Journal of Food Protection*, 83(1), 108-128. DOI: <https://doi.org/10.4315/0362-028X.JFP-19-283>
- Matereke, L. T., & Okoh, A. I. (2020). *Listeria monocytogenes* virulence, antimicrobial resistance and environmental persistence: a review. *Pathogens*, 9(7), 528. DOI: <https://doi.org/10.3390/pathogens9070528>
- Maung, A. T., Mohammadi, T. N., Nakashima, S., Liu, P., Masuda, Y., Honjoh, K. I., & Miyamoto T. (2019). Antimicrobial resistance profiles of *Listeria monocytogenes* isolated from chicken meat in Fukuoka, Japan. *International Journal of Food Microbiology*, 304(1), 49-57. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2019.05.016>
- Mendez, E., Walker, D. K., Vipham, J., & Trinetta, V. (2020). The use of a CDC biofilm reactor to grow multi-strain *Listeria monocytogenes* biofilm. *Food Microbiology*, 92(1), 103592. DOI: <https://doi.org/10.1016/j.fm.2020.103592>
- Miles, A. A., Misra, S. S., & Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *The Journal of Hygiene*, 38(6), 732-749. DOI: <https://doi.org/10.1017/s002217240001158x>
- Muchaamba, F., Stephan, R., & Taurai, T. (2021). *Listeria monocytogenes* Cold shock proteins: small proteins with a huge impact. *Microorganisms*, 9(5), 1061. DOI: <https://doi.org/10.3390/microorganisms9051061>
- Nilsson, R. E., Ross, T., & Bowman, J. P. (2011). Variability in biofilm production by *Listeria monocytogenes* correlated to strain origin and growth conditions. *International Journal of Food Microbiology*, 150(1), 14-24. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2011.07.012>
- Olaimat, A. N., Al-Holy, M. A., Shahbaz, H. M., Al-Nabulsi, A. A., Abu- Ghoush, M. H., Osaili, T. M., ... & Holley, R. A. (2018). Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 17(5), 1277-1292. DOI: <https://doi.org/10.1111/1541-4337.12387>
- Oliveira, T. S., Varjão, L. M., Silva, L. N. N., Pereira, R. C. L., Hofer, E., Vallim, D. C., & Castro Almeida, R. C. (2018). *Listeria monocytogenes* at chicken slaughterhouse: Occurrence, genetic relationship among

- isolates, and evaluation of antimicrobial susceptibility. *Food Control*, 88(1), 131-138. DOI: <https://doi.org/10.1016/j.foodcont.2018.01.015>
- Osman, K. M., Kappell, A. D., Fox, E. M., Orabi, A., & Samir, A. (2020). Prevalence, pathogenicity, virulence, antibiotic resistance, and phylogenetic analysis of biofilm-producing *Listeria monocytogenes* isolated from different ecological niches in Egypt: food, humans, animals, and environment. *Pathogens*, 9(1), 5. DOI: <https://doi.org/10.3390/pathogens9010005>
- Parte, A. C., Sardà Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C., & Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, 70(11), 5607-5612. DOI: <https://doi.org/10.1099/ijsem.0.004332>
- Pessoa, C. A. S. S., Aragão, B. B., Costa, C. A., Santos, H. C. S., Silva, M. G. V., & Moura, A. P. B. L. (2020). Ocorrência de *Listeria* spp. em salame tipo italiano comercializados em supermercados do bairro de Casa Amarela, Recife-PE. *Medicina Veterinária*, 14(4), 341-345. DOI: <https://doi.org/10.26605/medvet-v14n4-2724>
- Puga, C., Dahdouh, E., SanJose, C., & Orgaz, B. (2018). *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. *Frontiers in Microbiology*, 9(1), 1706. DOI: <https://doi.org/10.3389/fmicb.2018.01706>
- Pyz-Lukasik, R., Gondek, M., Winiarczyk, D., Michalak, K., Paszkiewicz, W., Piróg-Komorowska, A., ... Ziomek, M. (2021). Occurrence of *Listeria monocytogenes* in Artisanal Cheeses from Poland and Its Identification by MALDI-TOF MS. *Pathogens*, 10(6), 632. DOI: <https://doi.org/10.3390/pathogens10060632>
- Ramires, T., Kleinub ing, N. R., Iglesias, M. A., Soares, H. R., Nuncio, A. S. P., Kroning, I. S., ... Silva, W. P. (2021). Genetic diversity, biofilm and virulence characteristics of *Listeria monocytogenes* in salmon sushi. *Food Research International*, 140(1), 109871. DOI: <https://doi.org/10.1016/j.foodres.2020.109871>
- Ripolles-Avila, C., Cervantes-Huaman, B.H., Hascoët, A.S., Yuste, J., & Rodríguez-Jerez, J.J. (2019). Quantification of mature *Listeria monocytogenes* biofilm cells formed by an in vitro model: A comparison of different methods. *International Journal of Food Microbiology*, 289(1), 209-214. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2018.10.020>
- Rzepniewski, P., Goluchowska, N., Idziakowska, E. G., & Najman, B. K. (2020). Neonatal listeriosis-infection leading to death of the newborn. a case report. *Biomedical Journal of Scientific & Technical Research*, 29(2), 22311-22313. DOI: <https://doi.org/10.26717/BJSTR.2020.29.004778>
- Schmidt, K., Estes, C., McLaren, A., & Spangehl, M. J. (2018). Chlorhexidine antiseptic irrigation eradicates *Staphylococcus epidermidis* from biofilm: an in vitro study. *Clinical Orthopaedics and Related Research*, 476(3), 648-653. DOI: <https://doi.org/10.1007/s11999-0000000000000052>
- Skowron, K., Wiktorczyk, N., Grudlewska, K., Kwiecińska-Piróg, J., Wałęcka-Zacharska, E., Paluszak, Z., & Gospodarek-Komkowska, E. (2019). Drug-susceptibility, biofilm-forming ability and biofilm survival on stainless steel of *Listeria* spp. strains isolated from cheese. *International Journal of Food Microbiology*, 296(1), 75-82. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2019.02.021>
- Souza, C. O. S. S., Roça, R. O., Pinto, J. P. A. N., Sakate, R. I., Matos, A. V. R., & Moura, G. F. (2020). Ocorrência de *Listeria monocytogenes* em frangos alternativos. *Brazilian Journal of Development*, 6(6), 34791-34804. DOI: <https://doi.org/10.34117/bjdv6n6-137>
- State Decree No. 23,430, of October 24. (1974). Approves Regulation that provides for the promotion, protection and recovery of Public Health.
- State Law No. 6,503, of December 22. (1972). Provides for the promotion, protection and recovery of Public Health. Euclides Triches, Governor of the State of Rio Grande do Sul.
- Stepanovic, S., Cirkovic, I., Ranin, L., & Svabic-Vlahovic, M. (2004). Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. *Letters in Applied Microbiology*, 38(5), 428-432. DOI: <https://doi.org/10.1111/j.1472-765X.2004.01513.x>
- Wiktorczyk, N., Grudlewska, K., Skowron, K., Gryń, G., & Gospodarek-Komkowska, E. (2018). The effect of blood on the ability of biofilm formation by *Listeria monocytogenes* strains. *Medical Research Journal*, 3(1), 28-31. DOI: <https://doi.org/10.5603/MRJ.2018.0005>
- Young-Min, B., Seung-Youb, B., & Sun-Young, L. (2012). Resistance of pathogenic bacteria on the surface of stainless steel depending on attachment form and efficacy of chemical sanitizers. *International Journal of Food Microbiology*, 153(3), 465-473. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2011.12.017>