Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.81421

Detection of *Listeria innocua* in the dairy processing chain: resistance to antibiotics and essential oils

Mayara Cardoso da ROSA[1], Raiza IACUZIO[2], Giovana Rueda BARBOSA[2], Rodrigo de Castro Lisboa PEREIRA[3], Melina CRUZADO-BRAVO[4], Vera Lucia Mores RALL[5], Deyse Christina VALLIM[3], Nathália Cristina Cirone SILVA[2*]

Abstract

Listeria monocytogenes is a food pathogen responsible for many foodborne disease outbreaks. *Listeria innocua* is similar to *L. monocytogenes,* could be considered biologically similar to *L. monocytogenes* and could be used to predict its behavior. The aims of this work were to isolate *Listeria* spp. in dairy processing plants, perform genotypic characterization of virulence, evaluate the antimicrobial resistance profile, and verify the sensitivity of the strains to essential oils. Sampling was held in six dairy processing plants in the region of Campinas, Brazil. Seventeen *Listeria innocua* strains were isolated, all negative for virulence genes *inl*A, *inl*B, *inl*C, *inl*J, *act*A, *plc*A, and *plc*B. The strains were resistant to kanamycin (5.9%), penicillin (11.8%), ampicillin (11.8%), cefoxitin (58.8%), clindamycin (76.50%) and oxacillin (100%). The susceptibility of the strains to commercial essential oils (*Rosemarinus officinalis, Cymbopogon citratus, Eucalyptus citirodora, Mentha piperita, Piper nigrum, Vetiveria zizanioides, Cymbopogon nardus, Cymbopogon martinii* and *Pogostemon cablin*) was evaluated by Minimum Inhibitory Concentration (MIC) test. The results showed *Cymbopogon citratus, Cymbopogon martini, Cymbopogon nardus* and *Mentha piperita* oils were most efficient. The resistance of *Listeria innocua* isolated in dairy processing plants is worrisome, and the use of essential oils could be an alternative for development of new drugs and products.

Keywords: food safety; *Listeria* infection; resistance; food processing; dairy.

Practical Application: It could be an alternative to the use of chemical products in the sanitizing processes of surfaces in contact with food.

1 Introduction

Foodborne illnesses are a public health problem and contamination with microorganisms can occur at any stage in the process of food production. It is estimated that one in ten people worldwide get sick and that 420,000 die every year after ingesting contaminated food (World Health Organization, 2016).

In the dairy industry, different pathogens are frequently isolated from milk and dairy products, mainly gram positive and negative bacteria. A gram-positive bacteria usually isolated in dairy products is *Listeria monocytogenes,* is a major food pathogen responsible for many foodborne illness outbreaks, including from dairy products (Centers for Disease Control and Prevention, 2015, 2016, 2017). The pathogen was responsible for the most cases of hospitalization and death in Europe (European Food Safety Authority, 2012).

L. innocua is similar to *L. monocytogenes*, but is non-pathogenic for humans (Picart et al., 2002) and has a similar response when compared to *L. monocytogenes* in chemical and physical stresses and in biofilm formation, and both species share the same natural environments (Friedly et al., 2008; Silva-Angulo et al., 2015; Costa et al., 2018). These similarities mean that *L. inoccua* could be considered as biologically similar for *L. monocytogenes* and can be used in experiment to assess its susceptibility to essential oil. In addition, if some strains of *L. innocua* are susceptible or resistant to antibiotics that automatically *L. monocytogenes* are similar. Since *L. inoccua* is non-pathogenic, it is preferred to study over L. *monocytogenes* to predict its behavior (Costa et al., 2018).

Phenotypic antimicrobial resistance profile is relevant for public health. Although *Listeria* sp. are generally susceptible to a wide variety of antimicrobial agents Zhang et al. (2007), strains of *Listeria* spp. resistant to antibiotics have already been reported (Zhang et al., 2007; Moreno et al., 2014; Obaidat & Stringer (2019), which can affect the treatment.

L. monocytogenes is considered a microbiological hazard in food production, which justifies the monitoring of this microorganism or its possible indicator within the food production chain.

Received 19 Nov., 2021
Accepted 06 June, 2022
[1] *Departamento de Agroindústria, Alimentos e Nutrição, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo – USP, Piracicaba, SP, Brasil*
[2] *Departamento de Ciência de Alimentos e Nutrição, Faculdade de Engenharia de Alimentos – FEA, Universidade Estadual de Campinas – UNICAMP, Campinas, SP, Brasil*
[3] *Laboratório de Zoonoses Bacterianas, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil*
[4] *Escuela Profesional de Ingeniería Agroindustrial, Facultad Ciencias Agrarias, Universidad Nacional Autónoma de Chota, Chota, Cajamarca, Perú*
[5] *Departamento de Microbiologia e Imunologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Botucatu, SP, Brasil*
*Corresponding author: ncirone@unicamp.br

Additionally, research on agents that may have an inhibitory action over this species, such as essential oils, once the demand for new natural products is growing.

2 Materials and methods

2.1 Sampling and microbiological analysis

A total of two samplings were performed in six dairy processing plants in Campinas region, São Paulo State, Brazil from raw milk, pasteurized milk, processing environment surfaces (floor, cold chamber, drains, pasteurization and coagulation tank, table, molds, boxes, shovels) and Minas frescal cheese, totalizing 164 samples. Sampling from the environment and equipment were conducted using swabs moistened in peptone (0.1%) with neutralizing sanitizers, when needed, in areas delimited by sterile molds. Drains and tools were sampled without a delimited area. Immediately after collection, the swabs were placed in tubes containing 15 mL of buffered L*isteria* enrichment broth (BLEB). The collected samples were refrigerated and transported in isothermal boxes to *Higiene e laticinios* laboratory (*Escola Superior de Agricultura "Luiz de Queiroz"*, São Paulo University) for analysis.

The milk and cheeses were homogenized (25 g or 25 mL of sample) with 225 mL of BLEB in a *Stomacher* and then incubated (48h/30 °C). The environmental samples collected with swabs were homogenized with stomacher and incubated for 48 h/30 °C. After 4 hours of incubation, antimicrobials acriflavine (10 mg/L); nalidixic acid (40 mg/L); cycloheximide (50 mg/L) were added to the enrichment broth (BLEB) containing the samples. The method described by the FDA in the Bacteriological Analytical Manual (BAM) (Hitchins et al., 2017) was used for the analysis. Each sample incubated in BLEB, were placed on to Oxford and ALOA agars, followed by incubation at 35 °C/24-48 h and 37 °C/24 h, respectively.

Typical *Listeria* colonies were selected and hemolysis test were performed following Gram staining, catalase and motility test. The isolates were subjected to carbohydrate fermentation tests with alpha-methyl-d-mannoside, D-xylose, D-rhamnose, D-mannitol and D-glucose. In addition, three to five colonies were taken to perform the detection test.

2.2 Molecular detection of virulence factors

The bacterial DNA was extracted using InstaGene™ DNA Purification Matrix (Bio-Rad, Hercules, CA, USA). The DNA samples were stored at -20 °C until further analysis. Polymerase chain reaction (PCR) was performed with primers for the 23S rRNA and for the *hly* gene specific for the *Listeria* genus and *L. monocytogenes* species, respectively, according as previously reported to Hudson et al. (2001).

Positive isolates for 23S rDNA and/or *hly* were tested for *inl*A, *inl*B, *inl*C, *inl*J, *act*A, *plc*A, *plc*B, *prf*A genes, as described in Table 1.

2.3 Phenotypic antimicrobial resistance

The antibiotics susceptibility of strains was tested by disk diffusion technique according to Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical & Laboratory Standards Institute, 2015), Mueller–Hinton agar (Difco) was used for analysis. Tests and results were performed following recommendations for *Staphylococcus* sp (Obaidat et al., 2015). The antibiotics

Table 1. Primer and their properties used for the confirmation of the *L. monocytogenes* species and for the detection of the regulatory gene.

Gene	Sequence of primers (5´→3´)	Annealing temperature (°C)	bp	Reference
inlA	F – AATCTAGCACCACTGTCGGG R - TGTGACCTTCTTTTACGGGC	50	733	Rousseaux et al. (2004)
inlB	F - TGGGAGAGTAACCCAACCAC R - GTTGACCTTCGATGGTTGCT	40	884	Liu et al. (2007)
inlC	F - AATTCCCACAGGACACAACC R - CGGGAATGCAATTTTTCACTA	40	517	Liu et al. (2007)
inlJ	F - TGTAACCCCGCTTACACAGTT R - AGCGGCTTGGCAGTCTAATA	40	238	Liu et al. (2007)
hlyA	F – GCAGTTGCAAGCGCTTGGAGTGAA R - GCAACGTATCCTCCAGAGTGATCG	60	456	Paziak-Domańska et al. (1999)
actA	F – CGCCGCGGAAATTAAAAAAAGA R - ACGAAGGAACCGGGCTGCTAG	60	839	Schoder et al. (2003)
iap	F - ACAAGCTGCACCTGTTGCAG R - TGACAGCGTGTGTAGTAGCA	60	131	Schoder et al. (2003)
plcA	F - CTCGTGAGCTTTGTGATACC R - GATTGGCGTCTTAGGACTTGCAGG	55	1773	Roche et al. (2005)
plcB	F - ATTGGCGTGTTCTCTTTAGG R - TTAATACGGAACATAACGCG	55	1103	Roche et al. (2005)
prfA	F - GGGGTACCCCTCGTACTCAACTTAACATC R - GCTCTAGAGCAAACTCCATCGCTCTTCCAG	33	1285	Roche et al. (2005)
luxS	F – TCCGCAAGCTCTTTTGCGCCT R - TGTTTTAGCGAAGACTTTAGCCGATGT	57,5	117	Bonsaglia et al. (2014)

bp: base pair; F: Forward; R: Reverse.

tested were clindamycin (2 µg), oxacillin (1 µg), sulfazotrim (25 µg), cefoxitin (30 µg), gentamicin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), rifampicin (5 µg), vancomycin (30 µg), kanamycin (30 µg), tetracycline (30 µg), ampicillin (10 µg), cephalothin (30 µg) and erythromycin (15 µg), all antibiotics were acquired by LABORCLIN.

2.4 Strains susceptibility to essential oils

The susceptibility of the isolates to commercial essential oils (*Rosemarinus officinalis*, *Cymbopogon citratus*, *Eucalyptus citirodora*, *Mentha piperita*, *Piper nigrum*, *Vetiveria zizanioides*, *Cymbopogon nardus*, *Cymbopogon martinii* and *Pogostemon cablin*) was evaluated by Minimum Inhibitory Concentration (MIC) test.

For the MIC evaluated, the assays were conducted according to the methodology described by Iacuzio (2019), the isolates were grown in Tripticase Soy Agar with 0.6% Yeast Extract (TSA-YE; Difco) at 35 °C/24 h. Bacterial colonies from fresh pure culture were mixed with sterile saline solution (0.85%) to prepare the turbidity of each inoculum, which was adjusted to McFarland (0.5; ~108 CFU/mL) standards, using a densitometer (Densichek, BioMérieux, Durham, NC). The bacterial suspension was further diluted to ~105CFU/mL concentration. The MIC was determined by serial dilution using 96-well plates and the bacterial suspension incubated with essential oils at concentrations of 10 to 0.0049%, in final volumes of 100 µL of Trip Soy Broth (TSB- Difco) containing up to 2.5% of Tween 80. Sterility controls, positive growth and oils controls were included.

After the determination of the MIC, the minimum bactericidal concentration (MBC) was determined (Hafidh et al., 2011). The conversion of the values obtained in the Minimum Inhibitory Concentration test from percentage to $mg.ml^{-1}$ was performed according to Silva et al. (2011).

2.5 Statistical analyses

For the MIC was used a factorial ANOVA (8x4). Where 8= essential oils and 4= strains used. Significant factors ($p \leq 0.05$) were then subjected to multiple comparisons using the Duncan test. Statistical analyses were performed using XLSTAT 2018 software for Microsoft Excel® (Microsoft®, WA, USA).

3 Results and discussion

3.1 Isolation of Listeria sp. and virulence factors

Seventeen isolates were obtained from 5 samples (3.1% of the samples) with biochemical profile characteristic of *L. innocua*: Gram-positive rods, catalase positive, characteristic motility, positive for alpha-methyl-d-mannoside and D-glucose fermentation, negative for D-xylose, D-rhamnose, D-mannitol fermentation and without hemolysis in blood agar. These isolates derived from floor (from different processing plants), from drain (of the same plant, but different samplings); and one from a storage box. In these isolates, a PCR was performed for the 23S rDNA region and *hly* gene, with all isolates being positive for the 23s rDNA and negative for the *hly* gene. Also, the isolates were submitted to PCR for virulence genes *inlA*, *inlB*, *inlC*, *inlJ*, *actA*, *plcA*, *prfA*, found mostly in *L. monocytogenes*, but that may be present in other species of the genus (Gouin et al., 1994; Johnson et al., 2004). These genes are associated with the mechanism of pathogenicity and multiplication of this microorganism in the host (Silva et al., 2015; Iglesias, 2014). All isolates were negative for the virulence genes analyzed.

3.2 Phenotypic antibiotic resistance

Table 2 show the results obtained in this study regarding the phenotypic profiles found for antibiotic resistance. It was possible to observe that isolates from the same sample, showed different resistance profile.

Table 2. Resistance profile of the isolates of *L. innocua*.

Strain	Dairy	Sampling	Origin	Species	VAN	TET	ERI	PEN	AMP	CLO	CIP	SUT	CFO	CLI	RIF	OXA	GEN	KAN	CFL
1	E	2	drain	*L. innocua*	S	S	S	R	R	S	S	S	S	S	S	R	S	R	S
2	F	1	box	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
3	C	2	floor	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
4	C	2	floor	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
5	C	2	floor	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
6	C	2	floor	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
7	C	2	floor	*L. innocua*	S	S	S	I	S	S	S	S	R	R	S	R	S	S	S
8	C	2	floor	*L. innocua*	S	S	S	R	R	S	S	S	I	R	S	R	S	I	S
11	E	1	drain	*L. innocua*	S	S	S	S	S	S	S	S	S	I	S	R	S	S	S
12	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	R	R	S	R	S	S	S
13	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
15	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	R	R	S	R	S	S	S
16	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	I	R	S	R	S	S	S
17	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
18	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	R	R	S	R	S	S	S
19	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	I	I	S	R	S	S	S
20	F	2	floor	*L. innocua*	S	S	S	S	S	S	S	S	R	R	S	R	S	S	S

S = Sensitive; R = Resistant; I = Intermediate; VAN: vancomycin; TET: tetracycline; ERI: erythromycin; PEN: penicillin; AMP: ampicillin; CLO: chloramphenicol; CIP: ciprofloxacin; SUT: sulfazotrim; CFO: cefoxitin; CLI: clindamycin; RIF: rifampicin; OXA: oxacillin; GEN: gentamicin; KAN: kanamycin; CFL: cephalothin.

Considering the antibiotics tested, resistance was observed for kanamycin (5.9%), penicillin (11.8%), ampicillin (11.8%), cefoxitin (58.8%), clindamycin (76.50%) and oxacillin (100%).

According Troxler et al. (2000), the species of *Listeria* were naturally sensitive or intermediate to tetracyclines, aminoglycosides, penicillins (except oxacillin), carbapenems, macrolides, lincosamides, glycopeptides, chloramphenicol and rifampicin. This work presents the resistance of 100% of strains to oxacillin. The resistance observed to other antibiotics could be a result of horizontal gene transfer, once is growing the bacterial resistance. Other studies also observed that all *L. innocua* isolates were resistant to oxacillin and a high percent (75%) of them were resistant to clindamycin (Davis & Jackson, 2009; Osman et al., 2016).

Prates (2010) evaluated the antibiotic-resistance profile of *Listeria* spp. in dairy processing plants, and 20 isolates from equipment, processing environment and cheese have been tested for 15 antimicrobials. In that study, it was observed that all isolates were resistant to at least one antimicrobial and it also showed resistance to oxacillin, corroborating the present study. According to this same author, antibiotic resistance may occur via plasmids and transposons, which confer resistance to antimicrobials and can also be transferred from one bacterium to the other.

Other studies observed resistance or intermediate resistance of *L. monocytogenes* isolated from food (Iglesias, 2014; Moreno et al., 2014) which is a concern for consumers and public health.

3.3 Susceptibility of the isolates to essential oils

Figure 1 show the average means of the MIC obtained for each oil (*Rosemarinus officinalis*, *Cymbopogon citratus*, *Eucalyptus citirodora*, *Mentha piperita*, *Piper nigrum*, *Vetiveria zizanioides*, *Cymbopogon nardus*, *Cymbopogon martinii* and *Pogostemon cablin*) used in relation to susceptibility of strains evaluated.

According to ANOVA showed in Figure 1: *Cymbopogon citratus, Cymbopogon martini, Cymbopogon nardus and Mentha piperita* oils had the lowest MIC and they did not present a significant difference, i.e., it requires the least amount of active compound, which means it was more efficient in inhibiting the microorganisms tested (MIC ranged 0.6 to 1.8 mg.ml^{-1}). *Piper nigrum* oil was the one that needed the highest quantity of the active compound (above 25 mg.mL^{-1}) and statistically different with *Rosemarinus officinalis* and *Vetiveria zizanioides*. For the remaining oils, the concentrations of the active compounds necessary for inactivation of the bacteria tested ranged from 3 to 6 mg.mL^{-1}.

The action of the essential oils on bacteria is related to the disruption of the cytoplasmic membrane, damage to the protein in the membrane, coagulation of the cytoplasm, change in the flow of electrons, interruption of the proton-motive force and change in the active transport and reduction of the intracellular ATP pool (Nazzaro et al., 2013). The biological activity of essential oils depends on their composition, as well as on the microorganism's characteristics (Caixeta, 2010; Caputo et al., 2020).

Citral is the major component of *Cymbopogon* sp. essential oils. The mechanism of action of citral are not totally clear, but there is evidence that this component can disrupt the cell membrane integrity causing effects on the homeostasis of the microbial cell (Somolinos et al., 2010; Caixeta, 2010; Sharma et al., 2019).

Oliveira et al. (2010) analyzed the composition of *C. citratus* and *C. nardus* essential oils, finding that monoterpenes as major chemical constituents. For *C. citratus* essential oil, the major constituents observed were: geranial and neral, which isomerically form citral. Minor components were 2-undecanone, linalol,

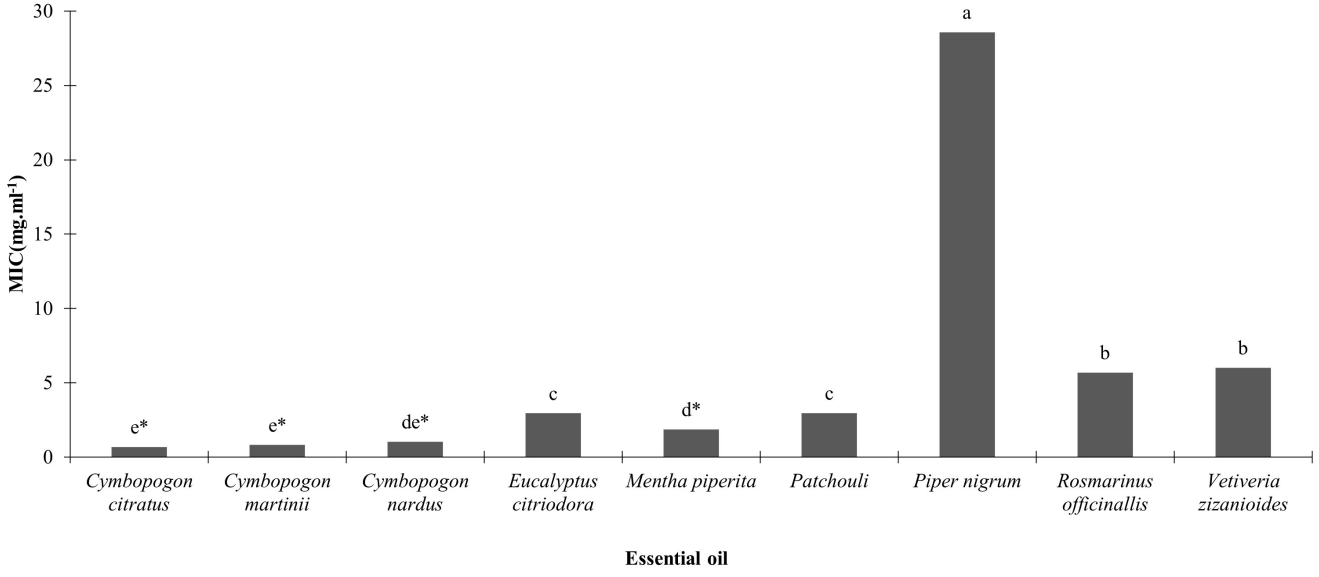

Figure 1. MIC average of essential oils used on L. *inocua* strains. Difference statistically significant ($P < 0.05$) is indicated by different letter according to Duncan test. *Statistically equal.

myrcene, geraniol, (E)-β-ocimene and (Z)-β-ocimene. For *C. nardus* essential oil, citronellal, geraniol and citronellol were the dominant components followed by β-elemene, γ-cadinene, δ-cadinene, citronellyl acetate, germacrene D, α-muurolene, limonene, geranial, linalool and neo-isopulegol. The author's results suggest that *C. citratus* and *C. nardus* essential oils could be alternatives to sanitize industrial stainless steel surfaces contaminated by *L. monocytogenes* (Oliveira et al., 2010).

Many studies reported the antimicrobial activity of *Cymbopogon* spp. against bacteria (Bassolé et al., 2011; Boeira et al., 2020), which reveal for these genera an important alternative for antibiotics and food preservatives.

While, Igarashi (2010) evaluated the antimicrobial activity of 12 essential oils in relation to 12 strains of *Listeria monocytogenes*, among other microorganisms isolated from chicken carcasses, a MIC of 0.8% could be observed for the essential oil from *Mentha piperita* in relation to the microorganism of interest. From the results obtained in the present study, it can be affirmed that the essential oil extracted from *Mentha piperita* has greater effectiveness as antimicrobial agent against strains of *Listeria* spp. isolated from the processing environment of Minas frescal cheese, since the MIC ranged from 0.078% and 0.312% (0.7 to 2.8 $mg.ml^{-1}$), depending on the strain. This fact can be observed due to variations in the composition of the essential oils used in the studies, which can be caused by numerous factors, such as growing conditions, external factors and methodology used in the harvesting and extraction of the essential oils, as well as due to factors inherent to the strains identified and analyzed themselves, such as species variability, expression or suppression of resistance genes, among other factors.

From the results obtained in the present study, it can be affirmed that the essential oil extracted from *Mentha piperita* has greater effectiveness as antimicrobial agent against the isolates tested, while the oils from *Piper nigrum* and *Eucaluptus citriodora* were less efficient.

According to Singh et al. (2015) and Valeriano et al. (2012), antibacterial activity maybe associated with the compounds present in higher amounts, such as menthol, menthone, methyl acetate, isomenthone. The high antimicrobial activity of the essential oil of the *Mentha* spp. genus is also associated with the mechanisms of action in bacterial cells and their approach to multiple targets, acting on and causing damages to the cytoplasmic membrane's functions, to respiratory activity and to the efflux pump (Guedes & Souza, 2017).

In general, essential oils exhibit antimicrobial properties which makes them appropriate alternatives to conventional medicines (Chaves et al., 2008). Besides that, there is a demand for natural food additive options which highlight the use of EOs as potential alternative antimicrobials (Smaoui et al., 2016).

The use of *Mentha piperita* as biopreservation has been studied, its essential oil used alone limited the microbial deterioration of minced meat (Smaoui et al., 2016). This shows the antimicrobial action of the oil and its prospective use in food, which values the current work, since *Listeria* sp. is an important foodborne pathogen (Centers for Disease Control and Prevention, 2017).

Based on the results obtained, and with the prospect of an expansion in the market of minimally processed products with less additives such as preservatives, the use of essential oils stands out as an alternative to conventional antimicrobials or as an additional safety barrier in ready-to-eat foods. Depending on the product, these compounds can be inserted into the formulation itself (Pesavento et al., 2015; Guedes & Souza, 2017) or in the packaging, with the goal of reducing contamination levels mainly in the product's most shallow layer, as well as the gradual release of these compounds during its shelf life (Atarés & Chiralt, 2016).

4 Conclusion

The isolation of *Listeria* spp. from the environment and utensils of dairy processing plants implies that these are suitable niches for the development of other dangerous species, such as *L. monocytogenes*. As we observed resistance of isolates, the monitoring of the antimicrobial resistance of these microorganisms is of paramount importance. The tests performed and the results obtained from essential oils, especially *Cymbopogon citratus*, *Cymbopogon martini*, *Cymbopogon nardus* and *Mentha piperita*, indicate their great potential for being employed as an alternative to chemical additives already used by the food industry, improving the safety value of the products when added in low quantities or with different approaches.

Conflict of Interest

No conflict of interest declared.

Acknowledgements

We thank FAPESP for the fellowship granted to student Mayara Cardoso de Rosa (process no.: 2016/12322-0) and PRP/UNICAMP (process no.: 2069/17) for financing the study.

References

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology*, 48, 51-62. http://dx.doi.org/10.1016/j.tifs.2015.12.001.

Bassolé, I. H. N., Lamien-Meda, A., Bayala, B., Obame, L., Ilboudo, A. J., Franz, C., Novak, J., Nebié, R. C., & Dicko, M. H. (2011). Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine*, 18(12), 1070-1074. http://dx.doi.org/10.1016/j.phymed.2011.05.009. PMid:21665450.

Boeira, C. P., Piovesan, N., Flores, D. C. B., Soquetta, M. B., Lucas, B. N., Heck, R. T., Alves, J S., Campagnol, P. C. B., Santos, D., Flores, E. M. M., Rosa, C. S., & Terra, N. N. (2020). Phytochemical characterization and antimicrobial activity of *Cymbopogon citratus* extract for application as natural antioxidant in fresh sausage. *Food Chemistry*, 319, 126553. http://dx.doi.org/10.1016/j.foodchem.2020.126553. PMid:32197214.

Bonsaglia, E. C. R., Silva, N. C. C., Fernades, A. Jr., Araújo, J. P. Jr., Tsunemi, M. H., & Rall, V. L. M. (2014). Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. *Food Control*, 35(1), 386-391. http://dx.doi.org/10.1016/j.foodcont.2013.07.023.

Caixeta, S. D. (2010). *Ação de óleos essenciais de Curcuma longa L. e Bixa orellana L. sobre Pseudomonas aeruginosa e Listeria monocytogenes, planctônicas e sesseis em polipropileno* (Tese de doutorado). Universidade Federal de Lavras, Lavras.

Caputo, L., Smeriglio, A., Trombetta, D., Cornara, L., Trevena, G., Valussi, M., Fratianni, F., De Feo, V., & Nazzaro, F. (2020). Chemical composition and biological activities of the essential Oils of *Leptospermum petersonii* and *Eucalyptus gunnii. Frontiers in Microbiology*, 11, 409. http://dx.doi.org/10.3389/fmicb.2020.00409. PMid:32351456.

Centers for Disease Control and Prevention – CDC. (2015). *Multistate outbreak of listeriosis linked to soft cheeses distributed by Karoun Dairies, Inc. (final update).* Atlanta: CDC.

Centers for Disease Control and Prevention – CDC. (2016). *Multistate outbreak of listeriosis linked to packaged salads produced at Springfield, Ohio dole processing facility.* Atlanta: CDC.

Centers for Disease Control and Prevention – CDC. (2017). *Multistate outbreak of listeriosis linked to soft raw milk cheese made by vulto creamery (final update).* Atlanta: CDC.

Chaves, A. V., He, M. L., Yang, W. Z., Hristov, A. N., McAllister, T. A., & Benchaar, C. (2008). Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. *Canadian Journal of Animal Science*, 88(1), 117-122. http://dx.doi.org/10.4141/CJAS07061.

Clinical & Laboratory Standards Institute – CLSI. (2015). *Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement.* Wayne: CLSI.

Costa, A., Lourenco, A., Civera, T., & Brito, L. (2018). *Listeria innocua* and *Listeria monocytogenes* strains from dairy plants behave similarly in biofilm sanitizer testing. *Lebensmittel-Wissenschaft + Technologie*, 92, 477-483. http://dx.doi.org/10.1016/j.lwt.2018.02.073.

Davis, J. A., & Jackson, C. R. (2009). Comparative antimicrobial susceptibility of *Listeria monocytogenes, L. innocua*, and *L. welshimeri*. microbial drug resistance. *Microbial Drug Resistance*, 15(1), 27-32. http://dx.doi.org/10.1089/mdr.2009.0863. PMid:19216646.

European Food Safety Authority – EFSA. (2012). *EFSA: the European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012.* Parma: EFSA.

Friedly, E. C., Crandall, P. G., Ricke, S., O'Bryan, C. A., Martin, E. M., & Boyd, L. M. (2008). Identification of *Listeria innocua* surrogates for *Listeria monocytogenes* in hamburger patties. *Journal of Food Science*, 73(4), M174-M178. http://dx.doi.org/10.1111/j.1750-3841.2008.00719.x. PMid:18460133.

Gouin, E., Mengaud, J., & Cossart, P. O. (1994). The virulence gene cluster of Listeria *monocytogenes* is also present in *Listeria ivanovii*, an animal pathogen, and *Listeria seeligeri*, a nonpathogenic species. *Infection and Immunity*, 62(8), 3550-3553. http://dx.doi.org/10.1128/iai.62.8.3550-3553.1994. PMid:8039927.

Guedes, J. P. S., & Souza, E. L. (2017). Investigation of damage to *Escherichia coli, Listeria monocytogenes* and *Salmonella* Enteritidis exposed to *Mentha arvensis* L. and *M. piperita* L. essential oils in pineapple and mango juice by flow cytometry. *Food Microbiology*, 76, 564-571. http://dx.doi.org/10.1016/j.fm.2017.09.020. PMid:30166188.

Hafidh, R. R., Abdulamir, A. S., Vern, L. S., Bakar, F. A., Abaas, F., Jahanshiri, F., & Sekawi, Z. (2011). Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *Open Microbiol Journal*, 5, 96-106.

Hitchins, A. D., Jinneman, K. & Chen, Y. (2017). Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods. In A. D. Hitchins, K. Jinneman, & Y. Chen. *Bacteriological Analytical Manual (BAM)*. Washington D.C.: FDA U.S Food & Drug. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration

Hudson, J., Lake, R., Savill, M., Scholes, P., & McCormick, R. (2001). Rapid detection of *Listeria monocytogenes* in ham samples using immunomagnetic separation followed by polymerase chain reaction. *Journal of Applied Microbiology*, 90(4), 614-621. http://dx.doi.org/10.1046/j.1365-2672.2001.01287.x. PMid:11309074.

Iacuzio, R. (2019). *Desenvolvimento e análise da estabilidade de biopolímero ativo adicionado de óleos essenciais como agentes antimicrobianos para acondicionamento de queijo minas frescal* (Dissertação de mestrado). Departamento de Ciência de Alimentos, Universidade Estadual de Campinas, Campinas.

Igarashi, M. C. (2010). *Desenvolvimento de filme comestível a base de alginato incorporado ao agente antimicrobiano óleo essencial de cravo: aplicação em alimentos* (Dissertação de mestrado). Universidade de São Paulo, São Paulo.

Iglesias, M. A. (2014). *Caracterização molecular e sensibilidade a antimicrobianos de Listeria monocytogenes isoladas em carcaças bovinas no sul do Brasil* (Dissertação de mestrado). Universidade Federal de Pelotas, Pelotas.

Johnson, J., Jinneman, K., Stelma, G., Smith, B. G., Lye, D., Messer, J., Ulaszek, J., Evsen, L., Gendel, S., Bennett, R. W., Swaminathan, B., Pruckler, J., Steigerwalt, A., Kathariou, S., Yildirim, S., Volokhov, D., Rasooly, A., Chizhikov, V., Wiedmann, M., Fortes, E., Duvall, R. E., & Hitchins, A. D. (2004). Natural atypical *Listeria innocua* strains with *Listeria monocytogenes* pathogenicity island 1 genes. *Applied and Environmental Microbiology*, 70(7), 4256-4266. http://dx.doi.org/10.1128/AEM.70.7.4256-4266.2004. PMid:15240309.

Liu, D., Lawrence, M. L., Austin, F. W., & Ainsworth, A. J. (2007). A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. *Journal of Microbiological Methods*, 71(2), 133-140. http://dx.doi.org/10.1016/j.mimet.2007.08.007. PMid:17884210.

Moreno, L. Z., Paixão, R., Gobbi, D. D. S., Raimundo, D. C., Ferreira, T. P., Moreno, A. M., Hofer, E., Reis, C. M. F., Matté, G. R., & Matté, M. H. (2014). Characterization of antibiotic resistance in *Listeria* spp. Isolated from slaughterhouse environments, pork and human infections. *Journal of Infection in Developing Countries*, 8(4), 416-423. http://dx.doi.org/10.3855/jidc.4188. PMid:24727506.

Nazzaro, F., Fratianni, F., Martino, L., Coppola, R., & de Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451-1474. http://dx.doi.org/10.3390/ph6121451. PMid:24287491.

Obaidat, M. M., & Stringer, A. P. (2019). Prevalence, molecular characterization, and antimicrobial resistance profiles *of Listeria monocytogenes, Salmonella* enterica, and *Escherichia coli* O157:H7 on dairy cattle farms in Jordan. *Journal of Dairy Science*, 102(10), 8710-8720. http://dx.doi.org/10.3168/jds.2019-16461. PMid:31351714.

Obaidat, M. M., Salman, A. E. B., Lafi, S. Q., & Al-Abboodi, A. R. (2015). Characterization *of Listeria monocytogenes* from three countries and antibiotic resistance differences among countries and *Listeria monocytogenes* serogroups. *Letters in Applied Microbiology*, 60(6), 609-614. http://dx.doi.org/10.1111/lam.12420. PMid:25808878.

Oliveira, M. M. M., Brugnera, D. F., Cardoso, M. G., Alves, E., & Piccoli, R. H. (2010). Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. *Food Control*, 21(4), 549-553. http://dx.doi.org/10.1016/j.foodcont.2009.08.003.

Osman, K. M., Samir, A., Abo-Shama, U. H., Mohamed, E. H., Orabi, A., & Zolnikov, T. (2016). Determination of virulence and antibiotic

resistance pattern of biofilm producing *Listeria* species isolated from retail raw milk. *BMC Microbiology*, 16(1), 263. http://dx.doi.org/10.1186/s12866-016-0880-7. PMid:27821054.

Paziak-Domańska, B., Bogusławska, E., Wieckowska-Szakiel, M., Kotłowski, R., Różalska, B., Chmiela, M., Kur, J., Dąbrowski, W., & Rudnicka, W. (1999). Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. *FEMS Microbiology Letters*, 171(2), 209-214. http://dx.doi.org/10.1111/j.1574-6968.1999.tb13434.x. PMid:10077846.

Pesavento, G., Calonico, C., Bilia, A. R., Barnabei, M., Calesini, F., Addona, R., Mencarelli, L., Carmagnini, L., Di Martino, M. C., & Lo Nostro, A. (2015). Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control*, 54, 188-199. http://dx.doi.org/10.1016/j.foodcont.2015.01.045.

Picart, L., Dumay, E., & Cheftel, J. C. (2002). Inactivation of *Listeria innocua* in dairy fluids by pulsed electric fields: influence of electric parameters and food composition. *Innovative Food Science & Emerging Technologies*, 3(4), 357-369. http://dx.doi.org/10.1016/S1466-8564(02)00055-3.

Prates, D. F. (2010). *Ocorrência, caracterização sorológica e avaliação do perfil de resistência a antibióticos em Listeria spp. isoladas em laticínios processadores de queijos no sul do Rio Grande do Sul* (Dissertação de mestrado). Universidade Federal de Pelotas, Pelotas.

Roche, P. S. M., Gracieux, E., Milohanic, I., Albert, I., Virlogeux-Payant, I., Témoin, S., Grépinet, A. O., Kerouanton, C., Jacquet, P., Cossart, P., & Velge, P. (2005). Investigation of specific substitutions in virulence genes characterizing phenotypic groups of low-virulence field strains of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 71(10), 6039-6048. http://dx.doi.org/10.1128/AEM.71.10.6039-6048.2005. PMid:16204519.

Rousseaux, S., Olier, M., Lemaitre, J. P., Piveteau, P., & Guzzo, J. (2004). Use of PCR-restriction fragment length polymorphism of inlA for rapid screening of *Listeria monocytogenes* strains deficient in the ability to invade Caco-2 cells. *Applied and Environmental Microbiology*, 70(4), 2180-2185. http://dx.doi.org/10.1128/AEM.70.4.2180-2185.2004. PMid:15066811.

Schoder, D., Winter, P., Kareem, A., Baumgartner, W., & Wagner, L. (2003). A case of sporadic ovine mastitis caused by *Listeria monocytogenes* and its effect on contamination of raw milk and raw milk cheeses produced in the on- farm dairy. *The Journal of Dairy Research*, 70(4), 395-401. http://dx.doi.org/10.1017/S0022029903006277. PMid:14649410.

Sharma, R., Rao, R., Kumar, S., Mahant, S., & Khatkar, S. (2019). Therapeutic potential of citronella essential oil: a review. *Current Drug Discovery Technologies*, 16(4), 330-339. http://dx.doi.org/10.2174/1570163815666180718095041. PMid:30019646.

Silva, A., Genovés, S., Martorell, P., Zanini, S. F., Rodrigo, D., & Martinez, A. (2015). Sublethal injury and virulence changes in *Listeria monocytogenes* and *Listeria innocua* treated with antimicrobials carvacrol and citral. *Food Microbiology*, 50, 5-11. http://dx.doi.org/10.1016/j.fm.2015.02.016. PMid:25998809.

Silva, N. C. C., Barbosa, L., & Seito, L. N. (2011). A FJ: Antimicrobial activity and phytochemical analysis of crude extracts and essential oils from medicinal plants. *Natural Product Research*, 26, 1-5.

Silva-Angulo, A. B., Zanini, S. F., Rosenthal, A., Rodrigo, D., Klein, G., & Martínez, A. (2015). Comparative study of the effects of citral on the growth and injury of *Listeria innocua* and *Listeria monocytogenes* cells. *PLoS One*, 10(2), e0114026. http://dx.doi.org/10.1371/journal.pone.0114026. PMid:25643164.

Singh, R., Shushni, M. A. M., & Belkheir, A. (2015). Antibacterial and antioxidant activity of *Mentha piperita* L. *Arabian Journal of Chemistry*, 8(3), 322-328. http://dx.doi.org/10.1016/j.arabjc.2011.01.019.

Smaoui, S., Hsouna, A. B., Lahmar, A., Ennouri, K., Mtibaa-Chakchouk, A., Sellem, I., Najah, S., Bouaziz, M., & Mellouli, L. (2016). Bio-preservative effect of the essential oil of the endemic *Mentha piperita* used alone and in combination with BacTN635 in stored minced beef meat. *Meat Science*, 117, 196-204. http://dx.doi.org/10.1016/j.meatsci.2016.03.006. PMid:26995774.

Somolinos, M., Garcia, D., Condon, S., Mackey, B., & Pagan, R. (2010). Inactivation of *Escherichia coli* by citral. *Journal of Applied Microbiology*, 108(6), 1928-1939. http://dx.doi.org/10.1111/j.1365-2672.2009.04597.x. PMid:19891710.

Troxler, R., von Graevenitz, A., Funke, G., Wiedemann, B., & Stock, I. (2000). Natural antibiotic susceptibility of Listeria species: L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri and L. welshimeri strains. *Clinical Microbiology and Infection*, 6(10), 525-535. https://doi.org/10.1046/j.1469-0691.2000.00168.x.

Valeriano, C., Oliveira, T. L. C., Carvalho, S. M., Cardoso, M. G., Alves, E., & Piccoli, R. H. (2012). The sanitizing action of essential oil-based solutions against *Salmonella* enterica serotype Enteritidis S64 biofilm formation on AISI 304 stainless steel. *Food Control*, 25(2), 673-677. http://dx.doi.org/10.1016/j.foodcont.2011.12.015.

World Health Organization – WHO. (2016). *FoodBorne diseases*. Retrieved from http://www.who.int/topics/foodborne_diseases/en/

Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A. A., & Meng, J. (2007). Characterization *of Listeria monocytogenes* isolated from retail foods. *International Journal of Food Microbiology*, 113(1), 47-53. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.07.010. PMid:16996156.