Article

Co-Occurrence of *L. monocytogenes* with Other Bacterial Genera and Bacterial Diversity on Cleaned Conveyor Surfaces in a Swine Slaughterhouse

Tamazight Cherifi 1,2,3, Julie Arsenault 2,4,*, Sylvain Quessy 1,3 and Philippe Fravalo 1,2,3,5,*

---

1. Introduction

The presence of *Listeria monocytogenes* in food processing plants is a major concern for industries and food control authorities [1–3]. The ubiquitous nature of this bacterium combined with its ability to grow in harsh conditions, including high salt concentrations, high acidity levels, and large temperature ranges, makes its control within food production environments particularly challenging [4]. Indeed, the ability of *L. monocytogenes* to persist within food-processing environments was reported in many studies [4–7], and the circumstances associated with this persistence phenomenon appear to be complex [8]. *L. monocytogenes'* biofilm formation and resistance to commonly used disinfectants in food industries may partly explain this persistence [5–7,9]. Some authors found an increased ability for biofilm formation in *L. monocytogenes* persistent strains. Moreover, the detection of genes conferring resistance to disinfectants, such as quaternary ammonium compounds (QACs), was correlated with persistence phenotypes in strains isolated from food processing plants [2,10,11]. Another factor that could influence the persistence of *L. monocytogenes* in the food industry is the structure and composition of bacterial communities present...
in biofilms, which are expected to include species other than *L. monocytogenes* [12,13]. As an example, it was reported that microbial communities dominated by Gram-negative bacterial species identified on a salmon slaughterhouse conveyor belt seemed to have an inhibitory effect on *L. monocytogenes* in a mixed biofilm [13]. This previous study was based on a culture-based method to identify communities present on conveyor surfaces. Consequently, only the most abundant and cultivable bacteria were detected, which limited the ability to explore links and/or interactions between *L. monocytogenes* and other bacteria present on biofilms from meat conveyor surfaces. While bacteria from specific species could influence the occurrence of other species for many reasons—such as competing for the same resources [14,15]—the identification of co-occurrences between *L. monocytogenes* and other species might help us find innovative solutions for controlling the proliferation and/or persistence of this pathogen.

So far, there is scant information about the co-occurrence of *L. monocytogenes* and other bacteria species over time in the food production environment. Studies that were conducted include one that showed *L. monocytogenes* was outcompeted by a mixture of bacterial species present on wooden shelves mainly due to competition for nutrients [16,17]. Inhibition of *L. monocytogenes* growth could also be the result of bacteriocin secretion by other species, such as *Enterococcus faecium* [18]. Other studies, in contrast, demonstrated positive interactions between *L. monocytogenes* and other bacterial genera [19,20]. Indeed, for example, *Flavobacterium* spp., which was part of the background microbiota of a seafood processing plant, was reported to enhance the colonization of *L. monocytogenes* on stainless steel surfaces [19]. Finally, others reported no interaction between *L. monocytogenes* and other bacteria such as *Pseudomonas fluorescens* [12,21].

The aim of this study was to describe, independent of the cultivability of the bacteria, (1) the microbiota diversity on meat conveyor surfaces from a swine slaughterhouse after cleaning and disinfection operations and (2) the co-occurrence of *Listeria* spp. with other bacterial communities present on meat conveyor surfaces.

2. Materials and Methods

2.1. Sample Collection, Processing, and Culture of *Listeria monocytogenes*

Samples were collected in the cutting facility of one swine slaughterhouse during four visits and after cleaning and disinfection operations. The disinfection procedures were applied by industry employees as part of their routine activities and consisted of an application of QACs-based disinfectants at a concentration between 150 and 200 ppm, on a daily basis, after each meat-cutting process.

On each visit, the belt of three meat conveyors was sampled at the beginning, middle, and end of the conveyor. Each sampling consisted of a swabbing of 1 m² of the belt surface after mechanical mobilization (brushing) using prewarmed wet swabs with a D/E (Dey-Engley) neutralizing broth (Innovation Diagnostic, Saint-Eustache, QC, Canada) to neutralize a broad spectrum of disinfectants and antiseptics. A total of 48 swab samples were collected. Each swab was shaken vigorously in 100 mL of sodium chloride solution (0.9% of NaCl in nuclease-free water). A volume of 10 mL was transferred into a separate tube and centrifuged, and the supernatants were discarded. The tubes containing pellets were immediately stored at −80 °C for DNA extraction. The detection of *L. monocytogenes* was performed on the 90 mL of remaining suspension according to the Compendium of Analytical Methods, MFHPB-30 [22] with few modifications. Briefly, all samples were enriched in 90 mL of 2X concentrated University of Vermont media 1 (UVM-1; Innovation Diagnostics, Saint-Eustache, QC, Canada) and incubated at 30 °C for 48 h. A second enrichment with Fraser broth media (Innovation Diagnostics, Saint-Eustache, QC, Canada) was used at 37 °C for 24 h. The cultures were plated on a selective COMPASS *Listeria* agar (Innovation Diagnostics, Saint-Eustache, QC, Canada) media, and typical colonies were streaked on sheep blood agar (Oxoid, Nepean, ON, Canada). The confirmation of *Listeria monocytogenes* was performed by a polymerase chain reaction (PCR) used for serogrouping [23]. In addition to these swab samples, one control, consisting of a clean
swab and brush transported during the sampling visit, was collected on two sampling visits (1 and 4) and submitted to the same bacteriological and molecular analyses as the swab samples.

2.2. DNA Extraction, 16S rDNA Construction Library, and Bioinformatics Analysis

The conserved pellets from the step above were used for phenol chloroform DNA extraction protocol. Briefly, bacteria were lysed with a lysis buffer (Tris-Hcl, EDTA, NaCl, and SDS) and content was extracted using glass beads by vortexing using the FastPrep procedure (MP Biomedical, Solon, OH, USA). The phenol chloroform isoamylic (25:24:1 v:v) solution was added to the supernatant. After mixing by inversion for 2 min, the solution was centrifuged, and the pellets were discarded. An additional 2 min of mixing was conducted, and, finally, the solution was centrifuged to collect the supernatant. Ammonium acetate (10 mM) and cold ethanol 100% were added to the supernatant, and the suspension was kept at −80 °C for 24 h for DNA precipitation. After precipitation, the tubes were centrifuged at 21,004 × g for 15 min, and the pellets were washed with ethanol before drying. The DNA was solubilized in 50 µL of nuclease-free water and stored at −20 °C before PCR amplification of the 16S rDNA V4 region. The primers 515F/806R pair [24] was used to amplify the V4 region, and the libraries were prepared using the NEXTERA kit (Illumina, Inc., San Diego, CA, USA) following recommendations from the manufacturer. The Illumina MiSeq technology was used to perform the paired-end sequencing at Genome Quebec Innovation Center (https://www.genomequebec.com/en/home/, accessed on 12 April 2016).

Data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) 2 v 2017.12 pipeline [25]. The demultiplexed reads were filtered and denoised using Dada2 v 2017.12.1 software [26]. The first 13 low-quality bases and the last 10 ones (at position 240) from both of the reverse (R1) and forward (F1) reads were trimmed during this step based on the quality plots that QIIME 2 generated. The resulting two separated tables containing amplicon single variants (ASVs) and sequences of each ASV were used for the downstream analyses.

ASVs table and their corresponding sequences were clustered into operational taxonomic units (OTU) using VSEARCH [27] v2018.8.0 and a closed-reference clustering with identity cutoff set at 99% against the SILVA database [28]. To minimize the impact of potential false positives due to cross-contamination, we used the decontam R package [29] to detect and remove potential OTUs detected as contaminants, using default settings.

Taxonomic composition of samples was determined by performing a pre-trained, naïve Bayes classifier on the reference taxonomy and sequences from SILVA database with 99% identity [28]. The feature classifier sklearn-classify [28] was used for taxonomic classification.

2.3. Diversity of OTUs by Sampling Visit and Listeria monocytogenes Culture Status

The alpha and beta diversities of OTUs were estimated for each sample rarefied at 20,000 sequences per sample. This threshold was defined based on the sample having the lowest number of sequences (n = 20,287) and was supported by the exploration of the rarefaction curves performed using phylloseq R package [30] v1.26.1, considering that this sample size was sufficient to reach the curve plateau for the majority of samples (Supplementary Figure S1 and Supplementary Table S1).

The alpha diversity, Shannon diversity index [31], and Shannon evenness index [32] were estimated in each rarefied sample. For the two indices, the median values of samples were compared between visits using a Kruskal–Wallis test followed by pairwise comparisons with Bonferroni adjustment for multiple testing. The median values were also compared according to the L. monocytogenes culture status of samples using a Wilcoxon test.

For the beta diversity, Bray–Curtis and Jaccard dissimilarity indices were estimated to explore the quantitative (community abundance) and qualitative (presence/absence) dissimilarities of OTUs in each sample, respectively, using phylloseq package in R. The Bray–Curtis
and Jaccard indices were compared between visits and according to L. monocytogenes culture status using pairwise PERMANOVA analyses with 999 permutations.

2.4. Description of the Taxonomy Composition

To explore the taxonomy composition of the bacterial communities in OTUs from sampled conveyor surfaces, the relative abundance of their genera was described using the phyloseq package. All OTUs with missing values for the genus rank were excluded. The relative abundance of the most frequent genus in OTUs according to the sampling visits and to the L. monocytogenes culture status was also evaluated.

2.5. Description of Bacterial Communities

To explore the relationships between microbial communities, particularly between Listeria and other genera, a network was created from positive and/or negative correlations between bacterial genera. Rare genera were first filtered to remove all OTUs corresponding to the genus with fewer than 20 occurrences across all samples. The filtered table was then used to construct the network based on three indicators: Spearman correlation, Bray–Curtis, and Kullback–Leibler dissimilarities. This network was built using the CoNet program [33] and implemented with Cytoscape software v3.3.0 [34]. A threshold of 1500 top- and bottom-scoring (for anti-correlations) links was included for each of the three indicators. For each link between two genera, 100 renormalized permutations and bootstrap score distributions were computed. Brown’s method was used to merge all measure-specific p-values of the indicators [35], with false discovery rate controlled at 5% using the Benjamini–Hochberg correction. Only links with confirmed associations for all 3 indicators were kept in the final network. The resulting network was visualized in Gephi v 0.9.2 [36] using the Yifan Hu algorithm [37]. Nodes (i.e., genera with significant interactions) were clustered using the constant Potts model with the resolution set at 0.1 [38] to identify the community structure of the network, with the size of each node representing the relative abundance of the genus. A second network was created from the previous one limited to the nodes directly or indirectly connected to Listeria spp. For better clarity of the visualization, the network was filtered based on the Spearman correlation coefficient used in the analysis as the weight of the link. Thus, only nodes with a correlation coefficient higher than 0.45 were kept.

3. Results

L. monocytogenes was detected by a culture-based method in 13 (27.1%) out of the 48 samples. The first and second visits showed the highest proportion of positive samples (Table 1).

Table 1. Bacteriological detection of L. monocytogenes in samples from swine slaughterhouse conveyor surfaces collected after cleaning and sanitation, by sampling visit.

| Sampling Visit | Date of Sampling | Number of Samples | Number (%) of Positive Samples to L. monocytogenes (%) |
|----------------|------------------|------------------|-----------------------------------------------------|
| S1             | 23 May 2014      | 12 a             | 6 (50)                                               |
| S2             | 18 January 2015  | 12 b             | 5 (41.7)                                             |
| S3             | 7 February 2015  | 12               | 1 (8.33)                                             |
| S4             | 28 February 2015 | 12               | 1 (8.33)                                             |
| Total          |                  | 48               | 13 (27.1)                                            |

*a 2 samples were not sequenced, b one sample was not sequenced.

From the 48 samples, three samples were not sequenced due to the small amount of extracted DNA. The number of sequences in the remaining 45 samples after quality control procedures using Dada2 ranged from 20,287 to 229,410 sequences per sample (Supplementary Table S1). All downstream analyses were performed based on the metadata table shown in Supplementary Table S1.
3.1. Diversity of the Microbiota on Conveyor Surfaces Was Different between Visits

Shannon diversity and evenness indices showed a significant difference of both richness and evenness in OTUs (Kruskal–Wallis, \( p = 0.00005 \) and \( p = 0.00009 \), respectively) between sampling visits. Samples from visit S1 were less diverse compared to all other sampling visits (Kruskal–Wallis, \( p < 0.05 \), Figure 1a). Moreover, a significantly higher diversity and higher evenness were observed in S2 compared to S4 (Figure 1a). Alpha diversity comparisons between culture-positive and culture-negative samples for \textit{L. monocytogenes} showed no significant differences (Figure 1b).

When richness estimations were compared between visits by pairwise PERMANOVA analysis based on Bray–Curtis and Jaccard indices, the diversities were significantly different between all pairs of visits (pseudo-\( F \) test, \( p = 0.001 \) for both indices), except between S2 and S3 (Table 2; Figure 2a,b). Diversity comparisons between the group of samples positive and negative for \textit{L. monocytogenes} showed no difference (Figure 2c,d).
Table 2. Pairwise PERMANOVA analysis based on Bray–Curtis and analysis of variance using Jaccard distance matrices with 999 permutations.

| Sampling Visits Compared | Bray–Curtis | $p$-Value | Jaccard |
|--------------------------|-------------|-----------|---------|
| S1 vs. S2                | 0.001       | 0.001     |         |
| S1 vs. S3                | 0.001       | 0.003     |         |
| S1 vs. S4                | 0.001       | 0.001     |         |
| S2 vs. S3                | 0.401       | 0.152     |         |
| S2 vs. S4                | 0.001       | 0.001     |         |
| S3 vs. S4                | 0.009       | 0.002     |         |

Figure 2. Cont.
was composed of 13 other genera, covering 21.1% of the nodes (Figure 4b). According to our results, the Listeria genus had no direct positive correlation with any other bacterial genera (Figure 4b), but direct negative correlations were present between Listeria and the six genera, Herminiimonas, Bryobacter, Caulobacter, None (genus not identified), Sphingomonas, and Mycobacterium, belonging to the six phyla, Proteobacteria, Acidobacteria, Proteobacteria, Elusimicrobia, Proteobacteria, and Actinobacteria, respectively (Figure 4b). Interestingly, Proteobacteria and Actinobacteria were the phyla which had the highest numbers of direct associations with Listeria, while Acidobacteria, Bacteroidetes, and Elusimicrobia had only two, one, and one genus, respectively, interacting with Listeria. From the Proteobacteria
phylum, the *Sphingomonas* genus accounted for the most abundant genera interacting negatively with *Listeria* (Figure 4b).

### Figure 3.

Relative frequency of different taxonomic profiles identified from OTUs in cutting facility conveyor surfaces. (a) Relative abundance of the top sixteen genera according to the sampling periods. (b) Relative abundance of the top sixteen genera according to *L. monocytogenes* culture results.
only two, one, and one genus, respectively, interacting with *Listeria*. From the *Proteobacteria* phylum, the *Sphingomonas* genus accounted for the most abundant genera interacting negatively with *Listeria* (Figure 4b).

**Figure 4.** Network construction based on Spearman correlation, Bray–Curtis dissimilarities, and Kullback–Leibler dissimilarities. (a) General network of all associations between genera clustered according to constant Potts model (communities sharing most links between each other). The size of each node refers to the abundance of the genus, the thickness of the link refers to its weight (presented here by the Spearman correlation coefficient), and the color of each node refers to positive (green) and negative interactions (red), (b) Network construction based on the direct associations between *Listeria* genus and the other genera and indirect associations between those genera and others. Links colored in red represent negative associations between the genera while green links represent positive associations. The node labelled “none” refers to the unidentified genus from the *Elusimicrobia* phylum.
4. Discussion

In this study, we described the diversity of the microbiota present on swine meat conveyor surfaces from four sampling visits. We also identified, in silico, the co-occurrence of this microbiota with the *Listeria* genus, including *Listeria monocytogenes*, as confirmed by a culture-based method.

The proportion of culture-positive samples for *L. monocytogenes* observed in this study was in the same range as reported in other studies [4]. The relative abundance of *Listeria* spp. revealed by 16S metagenomic in culture-positive samples for *L. monocytogenes* was higher than in culture-negative samples. This concordance between the two methods may suggest that *L. monocytogenes* was the most prevalent species among *Listeria* spp. present on conveyor surfaces. Moreover, classification at the species level only identified *L. monocytogenes* among the *Listeria* genus (Supplementary Figure S2).

Diversity analyses showed significant differences in both composition and organization of microbial communities depending on the sampling visits. Continuous new sources of bacteria from incoming materials or persons entering the processing environment should be considered in interpreting such diversity. It was reported, for example, that carcasses contaminated by *L. monocytogenes* from internal organs, such as tonsils and tongue, during the slaughtering process may contaminate plant environments [39,40]. Additionally, the plant workers can be an important source of contamination of food processing plant environments and products since humans are known to be an important reservoir, and the risk of contamination is associated with the level of sanitary practices [41].

From one batch to another, the microbiota composition changed, and these modifications were particularly noticeable due to the long intervals of time in our study (i.e., from three weeks to eight months between two consecutive visits). In addition, fluctuations in humidity, temperature, nutrient access, shear forces, and other physicochemical stresses could affect microbiota composition on conveyor surfaces over time, and one may think that only bacteria that can withstand these fluctuations could establish a resident niche, as supported by Fagerlund, Møretrø, Heir, Briandet, and Langsrud [12].

Overall, there was no significant difference in diversity indices of background microbiota between the culture-positive and culture-negative samples and *L. monocytogenes*. In our conditions, the presence of *L. monocytogenes* was not affected by the variability (relative abundance of species) and variety (number of different species) of species composing the microbiota of the meat conveyor surfaces.

According to taxonomic classification, *Proteobacteria* was the most abundant phylum, followed by *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria*. Few data are available to confirm these distributions. Despite most phyla being the same as observed in another study, *Actinobacteria* was reported as one of the most abundant phyla detected in meat samples [42]. This discrepancy could be due to their sampling period, which was before cleaning and sanitation on meat conveyor surfaces, whereas our sampling was done after cleaning and sanitation. It was previously reported that such factors may affect the composition of the bacterial community [12].

Among the 114 identified genera, the Gram-negative *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Chryseobacterium*, and *Caulobacter* were the most abundant ones across all sampling visits. These genera have already been pointed out as the most abundant background microbiota of meat conveyor surfaces [12], as well as of processing plant equipment in fish production [2,12,13]. Furthermore, *Pseudomonas* and *Acinetobacter* were reported previously for their ability to survive cleaning and their high biofilm-forming ability [12], which may help to explain their high abundance. The *Sphingomonas* genus was not detected when culture-based approaches were used, while sequence-based, cultivation-independent approaches found this genus as the most abundant, with *Pseudomonas* spp. [43]. The failure to detect *Sphingomonas* with a culture-based method may be the result of higher requirements in growth factors, such as Mg$^{2+}$ for some *Sphingomonas*, as suggested by some researchers [44], and/or to interspecies competition [45]. Our findings confirmed this genus
forms a significant part of the microbial communities and underlined the complementarity of both methods for description of bacterial composition in a given environment.

*Listeria* and *Staphylococcus* were among the most abundant genera in the *Firmicutes* phylum detected on these cleaned meat conveyor surfaces. Considering previous studies describing *Listeria* (and particularly *L. innocua* and *L. monocytogenes*) as a weak competitor in presence of *Pseudomonas* and *Acinetobacter* [12,13], it was expected that this genus would be less abundant in our communities, particularly when both *Pseudomonas* and *Acinetobacter* were well represented. The co-occurrence of *Listeria* and *Pseudomonas* genera reported in this study has to then be considered in accordance with other studies where an increase in the number of cells of *L. monocytogenes* was reported with the growth of *Pseudomonas putida* or *P. fragi* biofilms [12,45]. Moreover, when *Pseudomonas* and *Listeria* were grown in a mixed biofilm with other genera, it was shown that *Pseudomonas* dominated but did not eliminate *Listeria* from the biofilm [13]. Our results suggest that *Listeria* formed niches within *Pseudomonas* biofilm and could withstand cleaning and disinfection procedures and other harsh conditions. All the aforementioned statements were, however, based on culture-based methods, which are limited for identifying several bacteria from a given sample. Indeed, the strong competition that occurs during enrichment steps in cultures, as well as the viable but non-cultivable properties of some bacterial species on surfaces, underlines the need for non-culture-based analysis. For this reason, we aimed to study these interactions considering all bacterial populations using the 16S rDNA sequence-based methods. It is worth noting that these methods cannot distinguish between dead and viable bacteria. Thus, once possible associations are identified, the interactions might need to be confirmed, e.g., via cultivation-based methods.

At a community network level, considering all bacterial populations and based on correlation and dissimilar methods, only negative correlations between *Listeria* and other genera were detected. Such results were previously reported where, among twenty-nine bacterial species recovered from a dairy environment, sixteen induced reduction of biofilm formation by *L. monocytogenes* [46]. Such findings suggest that these populations are not mutualistic; either they do not share similar niches or a competition exists, as reported previously [47]. The competition between species could be related to nutrient limitation and augmented by antimicrobial compound production, as reported by Giaouris et al. [9]. Negative associations were often reported with *Listeria* spp., particularly for *L. monocytogenes*, since its presence, particularly in biofilm co-culture, was repeatedly hampered [13,48,49]. *Listeria monocytogenes* is recognized as a weak competitor when placed in a co-culture [2,12,13], which is consistent with our results. It is noteworthy that, except for *Sphingomonas*, these bacteria have never been cultivated along with *Listeria* spp. The low abundance of these genera compared to *Pseudomonas* and *Sphingomonas* and the difficulty of cultivation or non-cultivable status of some of these genera, such as certain species of *Herminiimonas* [50] and *Pseudoclavibacter* [51], could be the reasons why they were not detected in classical bacteriology studies. The negative association between *Listeria* and *Sphingomonas* is supported by a previous study where *Sphingomonas* inhibited the growth of *L. monocytogenes* by production of astaxanthin [52]. Interestingly, species from the *Paracoccus* genus, such as *P. marcusii*, which have a negative interaction with *Listeria* genus, are reported to produce astaxanthin as well [53,54]. Similar, negative associations with the *Listeria* genus or *L. monocytogenes* were observed with other species such as *Lactococcus lactis* [48].

5. Conclusions

In summary, this study showed significant differences in composition and abundances of residual microbiota on pork meat conveyor surfaces over time, which highlights the non-stable bacterial community composition in the meat industry environment. However, the core bacteria population, represented by the most abundant genera, was often the same over time in this environment. From this background microbiota, a total of six genera, such as *Sphingomonas*, were found to interact negatively with *Listeria* spp., which could be explained by various factors, such as growth inhibition, absence of shared habitats,
or competition, but further studies based on cultured methods are needed to interpret these negative interactions. These results provide interesting information regarding the relationship between background microbiota and *Listeria* spp. in order to identify potential bacterial species inhibiting its persistence within the food production environment.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/microorganisms10030613/s1, Supplementary Table S1: Sample identification with number of sequences, OTUs, *Listeria monocytogenes* culture-base status, and Shannon measure and its evenness. Supplementary Table S2: Total number of nodes and links resulting from the network construction based on the Spearman correlation, Bray–Curtis, and Kullback–Leibler dissimilarities. Supplementary Figure S1: Rarefaction curve of OTUs for each sample collected from meat conveyor surfaces. Supplementary Figure S2: Relative frequency of bacterial species identified from OTUs detected in cutting facility conveyor surfaces according to positive samples in *Listeria monocytogenes* culture-based method.

**Author Contributions:** Conceptualization, T.C., J.A., S.Q. and P.F.; Data curation, T.C. and J.A.; Formal analysis, T.C.; Funding acquisition, P.F.; Methodology, T.C. and J.A.; Project administration, S.Q. and P.F.; Resources, S.Q. and P.F.; Software, T.C. and J.A.; Supervision, S.Q.; Validation, T.C., J.A., S.Q. and P.F.; Visualization, T.C. and J.A.; Writing—original draft, T.C.; Writing—review and editing, T.C., J.A., S.Q. and P.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC RDCP) 520873-17. The funding organizations did not have any influence either in the design of the study, in the analysis and interpretation of data, or in the writing of the manuscript.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Publicly available datasets were analyzed in this study. These data can be found here: PRJNA728720.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Sofos, J.N. Challenges to meat safety in the 21st century. *Meat Sci.* 2008, 78, 3–13. [CrossRef]
2. Cherifi, T.; Carrillo, C.; Lambert, D.; Miniaï, I.; Quessy, S.; Larivièrre-Gauthier, G.; Blais, B.; Fravalo, P. Genomic characterization of *Listeria monocytogenes* isolates reveals that their persistence in a pig slaughterhouse is linked to the presence of benzalkonium chloride resistance genes. *BMC Microbiol.* 2018, 18, 220. [CrossRef] [PubMed]
3. Hadjicharalambous, C.; Grispoldi, L.; Goga, B.C. Quantitative risk assessment of *Listeria monocytogenes* in a traditional RTE product. *EFSA J.* 2019, 17, e170906. [PubMed]
4. Ortiz, S.; Lopez, V.; Villatoro, D.; Lopez, P.; Davila, J.C.; Martinez-Suarez, J.N. A 3-year surveillance of the genetic diversity and persistence of *Listeria monocytogenes* in an Iberian pig slaughterhouse and processing plant. *Foodborne Pathog. Dis.* 2010, 7, 1177–1184. [CrossRef]
5. Moretro, T.; Schirmer, B.C.T.; Heir, E.; Fagerlund, A.; Hjemli, P.; Langsrud, S. Tolerance to quaternary ammonium compound disinfectants may enhance growth of *Listeria monocytogenes* in the food industry. *Int. J. Food Microbiol.* 2017, 241, 215–224. [CrossRef] [PubMed]
6. Martínez-Suárez, J.V.; Ortiz, S.; López-Alonso, V. Potential Impact of the Resistance to Quaternary Ammonium Disinfectants on the Persistence of *Listeria monocytogenes* in Food Processing Environments. *Front. Microbiol.* 2016, 7, 638. [CrossRef] [PubMed]
7. Fox, E.M.; Leonard, N.; Jordan, K. Physiological and transcriptional characterization of persistent and nonpersistent *Listeria monocytogenes* isolates. *Appl. Environ. Microbiol.* 2011, 77, 6559–6569. [CrossRef]
8. Carpentier, B.; Cerf, O. Review—Persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int. J. Food Microbiol.* 2011, 145, 1–8. [CrossRef]
9. Giaouris, E.; Heir, E.; Hebraud, M.; Chorianopoulos, N.; Langsrud, S.; Moretro, T.; Habimana, O.; Desvaux, M.; Renier, S.; Nychas, G.J. Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Sci.* 2014, 97, 298–309. [CrossRef]
10. Dutta, V.; Elhanafi, D.; Kathariou, S. Conservation and distribution of the benzalkonium chloride resistance cassette bcrABC in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 2013, 79, 6067–6074. [CrossRef] [PubMed]
11. Elhanafi, D.; Dutta, V.; Kathariou, S. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998–1999 outbreak. *Appl. Environ. Microbiol.* 2010, 76, 8231–8238. [CrossRef] [PubMed]
12. Fagerlund, A.; Møretø, T.; Heir, E.; Briandet, R.; Langsrud, S. Cleaning and disinfection of biofilms composed of \textit{Listeria monocytogenes} and background microbiota from meat processing surfaces. \textit{J. Appl. Environ. Microbiol.} \textbf{2017}, \textit{83}, e01046-17. [CrossRef] [PubMed]

13. Fagerlund, A.; Langsrud, S.; Schirmer, B.C.; Møretø, T.; Heir, E. Genome analysis of \textit{Listeria monocytogenes} sequence type 8 strains persisting in salmon and poultry processing environments and comparison with related strains. \textit{PLoS ONE} \textbf{2016}, \textit{11}, e0151117. [CrossRef] [PubMed]

14. Zupaničić, J.; Raghupathi, P.K.; Houf, K.; Burmole, M.; Sørensen, S.J.; Gunde-Cimerman, N. Synergistic Interactions in Microbial Biofilms Facilitate the Establishment of Opportunistic Pathogenic Fungi in Household Dishwashers. \textit{Front. Microbiol.} \textbf{2018}, \textit{9}, 21. [CrossRef] [PubMed]

15. Elias, S.; Banin, E. Multi-species biofilms: Living with friendly neighbors. \textit{FEMS Microbiol. Rev.} \textbf{2012}, \textit{36}, 990–1004. [CrossRef]

16. Guillier, L.; Stahl, V.; Hezard, B.; Notz, E.; Briandet, R. Modelling the competitive growth between \textit{Listeria monocytogenes} and biofilm microflora of smear cheese wooden shelves. \textit{Int. J. Food Microbiol.} \textbf{2008}, \textit{128}, 51–57. [CrossRef] [PubMed]

17. Mariani, C.; Oulahal, N.; Chamba, J.-F.; Dubois-Brissonnet, F.; Notz, E.; Briandet, R. Inhibition of \textit{Listeria monocytogenes} by resident biofilms present on wooden shelves used for cheese ripening. \textit{Food Control} \textbf{2011}, \textit{22}, 1357–1362. [CrossRef]

18. Arthara, K.; Cassens, R.G.; Luchansky, J.B. Characterization of bacteriocins from Enterococcus faecium with activity against \textit{Listeria monocytogenes}. \textit{Int. J. Food Microbiol.} \textbf{1993}, \textit{19}, 123–134. [CrossRef]

19. Bremer, P.J.; Monk, I.; Osborne, C.M. Survival of \textit{Listeria monocytogenes} attached to stainless steel surfaces in the presence or absence of \textit{Flavobacterium}. \textit{J. Food Prot.} \textbf{2001}, \textit{64}, 1369–1376. [CrossRef]

20. Shedleur-Bourguignon, F.; Thériault, W.P.; Longpré, J.; Thyboeau, A.; Fravalpo, P. Use of an ecosystem-based approach to shed light on the heterogeneity of the contamination pattern of \textit{Listeria monocytogenes} on conveyor belt surfaces in a swine slaughterhouse in the province of Quebec, Canada. \textit{Pathogens} \textbf{2021}, \textit{10}, 1368. [CrossRef]

21. Overney, A.; Jacques-André-Coquin, J.; Ng, P.; Carpentier, B.; Guillier, L.; Firmesse, O. Impact of environmental factors on the culturability and viability of \textit{Listeria monocytogenes} under conditions encountered in food processing plants. \textit{Int. J. Food Microbiol.} \textbf{2017}, \textit{244}, 74–81. [CrossRef]

22. Pagotto, F.; Hebert, K.; Farber, J. Isolation of \textit{Listeria monocytogenes} and Other \textit{Listeria} spp. from Foods and Environmental Samples; HPB Methods for the Microbiological Analysis of Foods, Method MFHPB-30; Health Canada, Health Products and Food Branch: Ottawa, ON, Canada, 2011; Volume 2.

23. Kérioulant, A.; Marault, M.; Petit, L.; Grout, J.; Dao, T.T.; Brisabois, A. Evaluation of a multiplex PCR assay as an alternative method for \textit{Listeria monocytogenes} serotyping. \textit{J. Microbiol. Methods.} \textbf{2010}, \textit{80}, 134–137. [CrossRef]

24. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. \textit{ISME J.} \textbf{2012}, \textit{6}, 1621–1624. [CrossRef] [PubMed]

25. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. \textit{Nat. Methods} \textbf{2010}, \textit{7}, 335–336. [CrossRef] [PubMed]

26. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. \textit{Nat. Methods} \textbf{2016}, \textit{13}, 581–583. [CrossRef] [PubMed]

27. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A versatile open source tool for metagenomics. \textit{PeerJ} \textbf{2016}, \textit{4}, e2584. [CrossRef]

28. Quast, C.; Puechse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. \textit{Nucleic Acids Res.} \textbf{2013}, \textit{41}, D590–D596. [CrossRef] [PubMed]

29. Davis, N.M.; Proctor, D.; Holmes, S.P.; Reiman, D.A.; Callahan, B.J. Simple statistical identification and removal of contaminant sequences in marker-genome and metagenomics data. \textit{Microbiome} \textbf{2018}, \textit{6}, 226. [CrossRef] [PubMed]

30. McMurdie, P.J.; Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. \textit{PLoS ONE} \textbf{2013}, \textit{8}, e61217. [CrossRef] [PubMed]

31. Shannon, C.E. A mathematical theory of communication. \textit{Bell Syst. Tech. J.} \textbf{1948}, \textit{27}, 379–423. [CrossRef]

32. Pielou, E.C. \textit{An Introduction to Mathematical Ecology}; Wiley-Interscience: New York, NY, USA; London, UK, 1969; p. 286.

33. Faust, K.; Raes, J. CoNet app: Inference of biological association networks using Cytoscape. \textit{F1000Research} \textbf{2016}, \textit{5}, 1519. [CrossRef] [PubMed]

34. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. \textit{Genome Res.} \textbf{2003}, \textit{13}, 2498–2504. [CrossRef] [PubMed]

35. Brown, M.B. 400: A Method for combining non-independent, one-sided tests of significance. \textit{Biometrics} \textbf{1975}, \textit{31}, 987–992. [CrossRef]

36. Bastian, M.; Heymann, S.; Jacomy, M. Gephi: An open source software for exploring and manipulating networks. In Proceedings of the International AAAI Conference on Web and Social Media, San Jose, CA, USA, 17–20 May 2009.

37. Hu, Y. Algorithms for visualizing large networks. \textit{Comb. Probab. Comput.} \textbf{2011}, \textit{5}, 180–186.

38. Traag, V.A.; Waltman, L.; Van Eck, N.J. From Louvain to Leiden: Guaranteeing well-connected communities. \textit{Sci. Rep.} \textbf{2019}, \textit{9}, 5233. [CrossRef]
39. Autio, T.; Säteri, T.; Fredriksson-Ahomaa, M.; Rahkio, M.; Lundén, J.; Korkeala, H. *Listeria monocytogenes* contamination pattern in pig slaughterhouses. *J. Food Prot.* 2000, 63, 1438–1442. [CrossRef]

40. Nesbakken, T.; Kapperud, G.; Caugant, D.A. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. *Int. J. Food Microbiol.* 1996, 31, 161–171. [CrossRef]

41. Marriott, N.G.; Schilling, M.W.; Gravani, R.B. Food Contamination Sources. In *Principles of Food Sanitation*; Springer International Publishing: Cham, Switzerland, 2018; pp. 83–91.

42. Rodríguez-López, P.; Bernárdez, M.; Rodríguez-Herrera, J.J.; Comesana, A.S.; Cabo, M.L. Identification and metagenetic characterisation of *Listeria monocytogenes*-harbouring communities present in food-related industrial environments. *Food Control* 2019, 95, 6–17. [CrossRef]

43. Brightwell, G.; Boerema, J.; Mills, J.; Mowat, E.; Pulford, D. Identifying the bacterial community on the surface of Intralox™ belting in a meat boning room by culture-dependent and culture-independent 16S rDNA sequence analysis. *Int. J. Food Microbiol.* 2006, 109, 47–53. [CrossRef]

44. Hoo, H.; Hashidoko, Y.; Islam, M.T.; Tahara, S. Requirement of a relatively high threshold level of Mg²⁺ for cell growth of a rhizoplane bacterium, *Sphingomonas yanoikuyae* EC-5001. *Appl. Environ. Microbiol.* 2004, 70, 5214–5221. [CrossRef]

45. Giaouris, E.; Chorianopoulos, N.; Doulgeraki, A.; Nychas, G.-J. Co-culture with *Listeria monocytogenes* within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. *PLoS ONE* 2013, 8, e77276. [CrossRef] [PubMed]

46. Carpentier, B.; Chassaing, D. Interactions in biofilms between *Listeria monocytogenes* and resident microorganisms from food industry premises. *Int. J. Food Microbiol.* 2004, 97, 111–122. [CrossRef] [PubMed]

47. Yang, L.; Liu, Y.; Wu, H.; Høiby, N.; Molin, S.; Song, Z.J. Current understanding of multi-species biofilms. *Int. J. Oral Sci.* 2011, 3, 74–81. [CrossRef] [PubMed]

48. Zhao, T.; Podtburg, T.C.; Zhao, P.; Chen, D.; Baker, D.A.; Cords, B.; Doyle, M.P. Reduction by Competitive Bacteria of *Listeria monocytogenes* in Biofilms and *Listeria* Bacteria in Floor Drains in a Ready-to-eat Poultry Processing Plant. *J. Food Prot.* 2013, 76, 601–607. [CrossRef]

49. Fox, E.M.; Solomon, K.; Moore, J.E.; Wall, P.G.; Fanning, S. Phylogenetic profiles of in-house microflora in drains at a food production facility: Comparison and biocontrol implications of *Listeria*-positive and -negative bacterial populations. *J. Appl. Environ. Microbiol.* 2014, 80, 3369–3374. [CrossRef]

50. Kim, S.-J.; Park, S.-J.; Jung, M.-Y.; Kim, J.-G.; Madsen, E.L.; Rhee, S.-K. An uncultivated nitrate-reducing member of the genus *Herminimonas* degrades toluene. *J. Appl. Microbiol.* 2014, 80, 3233–3243. [CrossRef]

51. Oyaert, M.; De Baere, T.; Breyne, J.; De Laere, E.; Mariën, S.; Waets, P.; Laffut, W. First Case of *Pseudoclavibacter bifida* Bacteremia in an Immunocompromised Host with Chronic Obstructive Pulmonary Disease (COPD). *J. Clin. Microbiol.* 2013, 51, 1973–1976. [CrossRef]

52. Mageswari, A.; Subramanian, P.; Srinivasan, R.; Karthikeyan, S.; Gothandam, K.M. Astaxanthin from psychrotrophic *Sphingomonas faeni* exhibits antagonism against food-spoilage bacteria at low temperatures. *Microbiol. Res.* 2015, 179, 38–44. [CrossRef]

53. Lee, J.H.; Kim, Y.S.; Choi, T.-J.; Lee, W.J.; Kim, Y.T. *Paracoccus haeundaensis* sp. nov., a Gram-negative, halophilic, astaxanthin-producing bacterium. *Int. J. Syst. Evol. Microbiol.* 2004, 54, 1699–1702. [CrossRef]

54. Oren, A. Characterization of pigments of prokaryotes and their use in taxonomy and classification. In *Methods in Microbiology*; Elsevier: Amsterdam, The Netherlands, 2011; Volume 38, pp. 261–282.