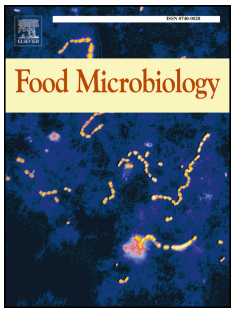

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Microbial composition and dynamics in environmental samples from a ready-to-eat food production facility with a long-term colonisation of *Listeria monocytogenes*

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ABSTRACT

Listeria monocytogenes is a foodborne pathogen of significant concern for the food industry due to its remarkable ability to persist through safety control efforts, posing a subsequent health threat to consumers. Understanding the microbial communities coexisting with *L. monocytogenes* in food processing environments provides insights into its persistence mechanisms. We investigated the microbial communities on non-food contact surfaces in a facility producing ready-to-eat foods, known to harbour a ST121 *L. monocytogenes* strain over multiple years. A 10-week sampling period was coordinated with the company and public health authorities. Metagenomic analysis revealed a stable microbial composition dominated by *Pseudomonas fluorescens*. While highly related populations were present in high-care production zones, distinctive taxa characteristic of specific areas were observed (e.g., *Sphingomonas aerolata*). Although *Listeria* spp. were not detected in metagenomes, they were detected in cultured samples, suggesting low relative abundance in factory settings. The findings suggest that a stable resident microbiota, with distinct adaptations to different areas within the factory, was selected for by their collective ability to survive control efforts in this environment. *Listeria* spp. was a member of this microbial community, albeit at low abundance, and may likewise benefit from the mutualism of the overall microbial community.

Keywords: *Listeria monocytogenes*, food processing environments, microbial ecology, metagenomics, food safety

1. INTRODUCTION

Material surfaces in food production environments (FPEs) can be colonized by communities of microbes that persist over time despite the multitude of approaches used to control microbial hazards, such as rigorous cleaning and disinfection routines and the use of controlled temperature in the facilities. These microbial communities, or resident microbiota, consist mainly of non-pathogenic microorganisms but can also contain pathogenic bacteria which are usually in very low abundance [1, 2]. Amongst foodborne pathogens, *Listeria monocytogenes* is a significant concern for food businesses and health and food safety authorities. *L. monocytogenes* causes invasive listeriosis, a relatively low-incidence foodborne infection, where for example, England and Wales respectively reported 0.39 and 0.16 cases of listeriosis per 100,000 population in 2021 [3]. However, *L. monocytogenes* ranks among the top three causes of foodborne diseases resulting in hospital admissions in Western countries [4], and has an alarmingly high case fatality rate of, for example, 17.5% in England and Wales in 2021 [3].

Controlling *L. monocytogenes* poses challenges to food businesses, as it is a highly resilient microorganism with distinctive physiological characteristics. Notably, this pathogen thrives at low temperatures, tolerates low pH levels and high salt concentrations, and can become tolerant to biocides [5]. These characteristics contribute to its survival and persistence in food environments, particularly in humid and low-temperature areas, even after regular cleaning and disinfection [5, 6]. The persistent presence of this pathogen in FPEs poses a significant risk for the contamination of food products, as *L. monocytogenes* may transmit from the environment into foods, or may already be present in food ingredients and then remain in finished food products.

This issue of controlling *L. monocytogenes* becomes particularly important in ready-to-eat (RTE) food manufacturing settings because products are consumed without further cooking and are often stored at refrigeration temperatures in which *L. monocytogenes* can reproduce. Significant foodborne outbreaks can occur when contaminated RTE foods are served widely to vulnerable

populations [7], with notable examples occurring in Canada in 2008 [8] and the UK in 2019 [9]. Similarly, pre-packed sandwiches served in hospitals have been identified as the most common food vehicle leading to listeriosis in England and Wales [10]. Importantly, different *L. monocytogenes* subtypes have been assessed at varying levels of food safety risk; for instance the most virulent strains have a more full complement of virulence genes such as LIPI-1 and *inlA* (as commonly seen with sequence types (ST) ST1, ST4 and ST87), whereas those strains with a truncated *inlA* (as commonly seen with ST9 and ST121) are also associated with food environments and can be assessed as lower risk for causing illness [11].

The resident microbiota that coexists with *L. monocytogenes* in environmental settings may have a role in the survival of this pathogen. For example, a protective effect of *Pseudomonas* biofilms on *L. monocytogenes* has been observed, which suggested that control of *Pseudomonas* in the FPEs could reduce contamination of *L. monocytogenes* [12, 13]. Studies using amplicon sequencing or metagenomics alongside culture enrichments have explored the resident microbiota coexisting with *L. monocytogenes* in various FPEs [2], including fish and meat processing settings, dairy factories [14] and fruit packing facilities [15]. Despite the well-known health risks associated with *L. monocytogenes*-contaminated RTE foods for human health [16], there has been limited research on the microbial communities coexisting with *L. monocytogenes* in RTE-food production facilities [17].

Culture-based isolation followed by subtyping using whole genome sequencing (WGS) are increasingly being used to support surveillance, source attribution and outbreak response activities [18]. For example, in the UK a case of listeriosis was reported in 2017 where a hospitalised patient consumed contaminated sandwiches and developed listeriosis [19]. Subsequent investigation by UKHSA using WGS revealed that the ST121 *L. monocytogenes* strain isolated from the patient was genetically indistinguishable to isolates found in food products and environmental samples originating from a UK-based company (hereafter, 'Company X'). Furthermore, the presence of the ST121 *L. monocytogenes* and other *Listeria* species within the FPE of Company X were revealed over

2017-2020 [19], indicating a persistent colonization of the factory premises and subsequent contamination of equipment, food contact surfaces, and food products. Following the investigation, the company implemented corrective measures, including a thorough assessment of the factory cleaning procedures and layout, which led to a substantial reduction in food product contamination [19]. Despite efforts in eliminating this pathogen, the ST121 strain was still detected on non-food contact surfaces of the factory in 2021 during follow-up surveillance activities (results not shown), presenting an opportunity to study and understand the microbial factors related to its persistence.

In this study, we used shotgun metagenomics to investigate the resident microbiota in Company X, a RTE-food factory with persistent *L. monocytogenes* contamination. Sampling was coordinated over a 10-week period with the company and with public health authorities, and changes to the microbiota resulting from cleaning and disinfection were evaluated. Additionally, we assessed the feasibility of applying a quasimetagenomics approach (involving bacterial culture enrichments prior to sequencing) for source tracking of *Listeria* spp. and *L. monocytogenes* in FPE environmental samples. An understanding of the microbial composition and dynamics in food production environments using innovative sequencing techniques can contribute to novel and improved strategies for preventing and managing *L. monocytogenes* contamination.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Environmental samples were collected by UKHSA officers from areas of a RTE-factory that had shown recurrent contamination with *L. monocytogenes*.

An initial trial sampling event was performed in November 2021 (T0) to test the performance of the swabs for metagenomic analysis. Samples were obtained by swabbing 10 x 10 cm areas according to

UKHSA environmental surface sampling microbiological guidelines [20, 21] using 3M™ Sponge-Stick embedded in 10 mL neutralizing buffer as sampling media. In addition, maximum recovery media (MRM) and neutralizing buffer collected from sterile swabs were inoculated with 10^8, 10^7 and 10^6 CFU/mL of *L. monocytogenes* serovar 1/2a strain 396044 and used as control to test DNA extraction and sequencing procedures. Based on these trials, the dry Sponge-Stick embedded in MRM demonstrated adequate performance in the DNA extraction and sequencing workflows, and this method was selected for continued sampling.

The samples were obtained from non-food contact surfaces located in 10 different sites (Supplementary_Site_description) in the high care zone of the factory (designated area of the factory where products are handled under controlled environment to minimize the risk of contamination), which is divided in two areas: preparation (where the food ingredients are stored and kept at a controlled temperature of 4 °C) and production (where the ingredients are combined and packed, kept at a controlled temperature of 10 °C) (Supplementary Fig. 1). A drain (located at the production area of the factory) and 9 floor surfaces (4 located in the preparation area, 5 located in the production area of the factory) were sampled and sent under refrigeration to the Quadram Institute (Norwich, United Kingdom) in accordance with the Food Standards Agency Food Law Code of Practice [22] for further processing.

Samples for characterization of the environmental microbial populations were collected over 10 weeks: T1 occurred in February 2022 during normal operation of the factory; T2 and T3 occurred two months later with T2 samples collected in the morning during operation of the factory and T3 samples collected in the afternoon on the same day after routine cleaning had occurred; the final samples (T4) were collected one week following T2/T3 (Supplementary_Sample_metadata). Samples were obtained as described above, using the 3M™ dry Sponge-Stick embedded in 10 mL MRM as sampling buffer.

Upon receipt at the laboratory, all swab samples were homogenised for 1 min in a stomacher (400 Circulator Lab Blender, Seward) and the buffer was aseptically collected and divided into aliquots for further processing. For each sample, a 800 µL aliquot was stored at -20 °C and used for total DNA extraction and a 2 mL aliquot was stored in 20% glycerol at -80 °C for further microbiological testing.

2.2. Listeria enrichment and isolation

Listeria enrichments were performed according to UKHSA guidelines [23] with some modifications. Briefly, a primary enrichment was prepared by inoculating 1 mL of sample stored in glycerol in half Fraser broth (Thermo Fisher Scientific) at 1 in 10 proportion. After 24 h incubation at 30 °C, a secondary enrichment was prepared by inoculating 300 µL of the primary enrichment into 30 mL of Full Fraser broth (Thermo Fisher Scientific). The theoretical limit of detection of the modified method is 10 CFU per swab instead of 1 CFU per swab. A positive and negative control, consisting of media inoculated with *L. monocytogenes* serovar 1/2a strain 396044 [19] and uninoculated media respectively, were used and treated as the rest of the samples. Both, primary and secondary enrichments were incubated for 48 h each. Using a 10 µL loop, both enrichments were sub-cultured after 6, 24 and 48 h incubation to Listeria chromogenic (ALOA) agar (Thermo Fisher Scientific). Presumptive *Listeria* spp. and presumptive *L. monocytogenes* colonies were selected from the ALOA plates, which were subsequently re-streaked to new ALOA plates to obtain pure colonies, and subsequently preserved in 20% glycerol at -80 °C. Aliquots (800 µL) were collected from primary and secondary enrichments after 6, 20, 22, 24, 26, 28, 30 and 48 h incubation and stored at -20 °C for subsequent DNA extraction.

2.3. DNA extraction

Total microbial DNA was obtained from 800 µL of sampling buffer homogenates for the direct metagenomic sequencing, from 800 µL of *Listeria* enrichment cultures for the quasimetagenomics

approach, and from 800 µL of overnight pure cultures of *Listeria* for WGS of the isolates. In all instances, DNA was extracted using the Maxwell® RSC PureFood Pathogen Kit on a Maxwell® RSC 48 automated extraction system (Promega) according to manufacturer's instructions. A negative control consisting of buffer obtained from sterile swabs or from uninoculated Half-Fraser and Fraser broth was included on each DNA extraction batch. DNA concentration was measured using the QuantiFluor® dsDNA kit on a GloMax® Explorer plate reader (Promega).

2.4. Sequencing

The environmental samples were analysed by direct metagenomic analysis to characterise the total environmental microbial population and/or by quasimetagenomics to identify and subtype *Listeria* genomes. The total DNA extracted either directly from environmental sampling buffer homogenates, from *Listeria* enrichments, or from *Listeria* isolates was sequenced at the Quadram Institute (Norwich, United Kingdom). DNA was normalised and used to prepare 150 bp paired-end sequencing libraries with the Illumina DNA Prep kit (Illumina Inc, CA, USA). Sequencing was performed on an Illumina Nextseq500 instrument using a Mid Output Flowcell and the NSQ® 500 Mid Output KT v2 (300 cycle) following the Illumina recommendations.

DNA from selected *Listeria* enrichment timepoints was also sequenced using Oxford Nanopore long-read sequencing technology. Native barcoded libraries were generated using the Nanopore Sequencing Ligation kit (SQK-LSK109, Oxford Nanopore Technologies) with the Native Barcoding Expansion 96 kit (EXP-NBD196, Oxford Nanopore Technologies). Libraries were sequenced on a PromethION instrument using R9.4.1 flow cells and raw sequencing data was collected with ONT MinKNOW software (v4.0.5). Subsequently, base calling and de-multiplexing was carried out using Guppy v6.06 (Oxford Nanopore Technologies)

2.5. Bioinformatic analysis

195 2.5.1. Read quality filtering and taxonomic classification.

196 Illumina raw reads derived from direct sequencing of the environmental samples were quality
197 filtered and contaminating chicken and human reads were removed, using the genomes *Gallus*
198 *gallus* GRCg6a and *Homo sapiens* hg38 as reference and the pipeline KneadData v 0.10.0 [24].
199 Illumina raw reads derived from *Listeria* enrichments and pure cultures were quality filtered with
200 fastp [25]. Filtered reads were taxonomically classified with MetaPhlAn4 (v 4.0.3) [26] using default
201 parameters.

202 Nanopore reads were processed with Porechop [27] to remove adapters; the trimmed reads were
203 then quality filtered with Filtlong [28] to keep the 95% of reads with best quality. Read quality was
204 assessed with NanoStat [29]. Taxonomic classification of the filtered nanopore reads was performed
205 with the online BugSeq pipeline [30]. To ensure comparability with MetaPhlAn4 analysis, unclassified
206 reads were not taken into consideration when calculating relative abundances.

207

208 2.5.2. Direct mapping of metagenomic reads against reference genomes.

209 To assess the potential detection of *Listeria* spp. genomes within our study, unfiltered metagenomic
210 reads were mapped against the draft genomes of the identified *Listeria* species and subsequently
211 genome coverage was calculated using MATAFILER [31], mapping was performed using bowtie2 and
212 alignments post-processed as described in [32]. The genome was considered to be present in the
213 metagenomes if the median coverage of the contigs within the N90 was >2.

214

215 2.5.3. Genome, Metagenome-assembled genomes (MAGs) and binning.

216 Genome assembly of *Listeria* isolates was performed on filtered reads with Shovill [33] using Spades
217 [34] as assembler. Hybrid metagenome-assembled genomes were obtained from the *Listeria*
218 enrichments using OPERA-MS v.0.9.0 [35] on the filtered reads using default parameters. The

219 resulting assemblies were binned with metaWRAP v1.3.0 [36], which includes MaxBin2 and
220 CONCOCT as binning tools and a binning refinement module. The quality of the WGS and bins was
221 assessed with CheckM [37] and QUAST [38] and taxonomic classification was performed with GTDB-
222 tk [39] with the GTDB database (Release 95).

223

224 2.5.4. MLST classification

225 MLST classification of Illumina raw reads derived from the *Listeria* enrichments was performed with
226 MetaMLST v1.2.2 [40] while nanopore reads were classified with krocus v1.0.1. [41]. Metagenome-
227 assembled genomes derived from enrichments and genomes derived from *Listeria* isolates were
228 typed with MLST v2.16.1 [42] against the PubMLST database [43].

229

230 2.5.5. SNP distance calculation

231 The genomes of the *L. monocytogenes* isolates obtained in this study were compared with those of
232 the 4 *L. monocytogenes* isolates obtained from the environment of the same factory in 2017 [19].
233 SNPs differentiating reference isolates and study isolates were detected using Snippy [44] and the
234 outputs were combined into a core SNP alignment using the snippy-core function and counted with
235 snp-dist [45].

236

237 *2.6. Statistical analysis*

238 The following analysis was performed for data derived from direct sequencing: species richness and
239 the alpha diversity indexes Shannon and Simpson were estimated using the *diversity* function within
240 the R package *vegan* (version 2.6-4) [46] and compared with the *wilcox.test* and *kruskal.test*
241 functions on the R package *stats* (version 4.2.2) [47]. Differences in microbiome composition were

evaluated by Principal Coordinate Analysis (PCoA) of the beta diversity Bray-Curtis distance, calculated with the *pco* function and the *vegdist* function within the R packages *ecodist* [48] and *vegan* respectively. The PCoA was plotted with *ggplot* and data ellipses were drawn based on the factory area with the *stat_ellipse* function, both contained in the R package *ggplot2* [49]. Metagenome dissimilarities between area of the factory or time points of collection were tested by Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) with the *adonis* function in the R package *vegan*.

To analyse associations between taxa, relative abundances were transformed to centered log ratios using the *clr* function [50] and correlation test was performed with the *corr.test* and the Spearman method within the *psych* package [51].

Relative abundance at genus or species level was plotted using *ggplot*. Differences in relative abundance were calculated using the ANCOMBC R package and considered significant when the adjusted p-value (q-value) was less than 0.01 [52]. The 15 most abundant taxa were extracted using the *top_taxa* function within the R package *microbiome* [53]. All R analyses were performed with R version 4.2.2.

3. RESULTS

3.1. *Selection of swabs with maximum recovery media for direct metagenomic analysis*

To assess the viability of a direct sequencing workflow for profiling of the resident microbiota in a RTE-food factory, trial samples were collected from 10 non-food contact surfaces located within the high-care zone of the factory that had previously exhibited recurring culture positive findings for *L. monocytogenes*.

For the initial sampling event (T0), 10 environmental samples were collected using swabs immersed in neutralizing buffer, matching routine microbiological sampling approaches [20, 21]. The efficiency

of DNA extraction and library preparation methods were tested on these samples. Additionally, mock samples were created by spiking 10^8, 10^7 and 10^6 CFU of *L. monocytogenes* serovar 1/2a strain 396044 into two types of sterile sampling medium (neutralizing buffer and MRM). Notably, after extraction, all environmental swabs and mock samples yielded quantifiable DNA (0.66-5.98 ng/µL and 0.12-3.06 ng/µL respectively; Supplementary_Swab_testing). No statistical differences were observed in the DNA yielded from neutralizing and MRM swabs (p-value = 0.437). However, differences were observed in the number of sequencing reads: T0 swab samples did not yield any reads, while mock samples made with neutralising buffer yielded 94-136 reads and mock samples made with MRM yielded 17,752-149,752 reads (p-value = 0.0216). None of the reads from the neutralising buffer samples were classified as *Listeria,* whereas 2,800-68,709 (47.06-99.96%) reads were assigned to *Listeria* genus from the MRM samples (p-value = 0.0336) (Supplementary_Swab_testing). Based on these trial results where neutralisation buffer inhibited direct sequencing efforts, in all subsequent sampling events, dry swabs immersed in MRM were utilised.

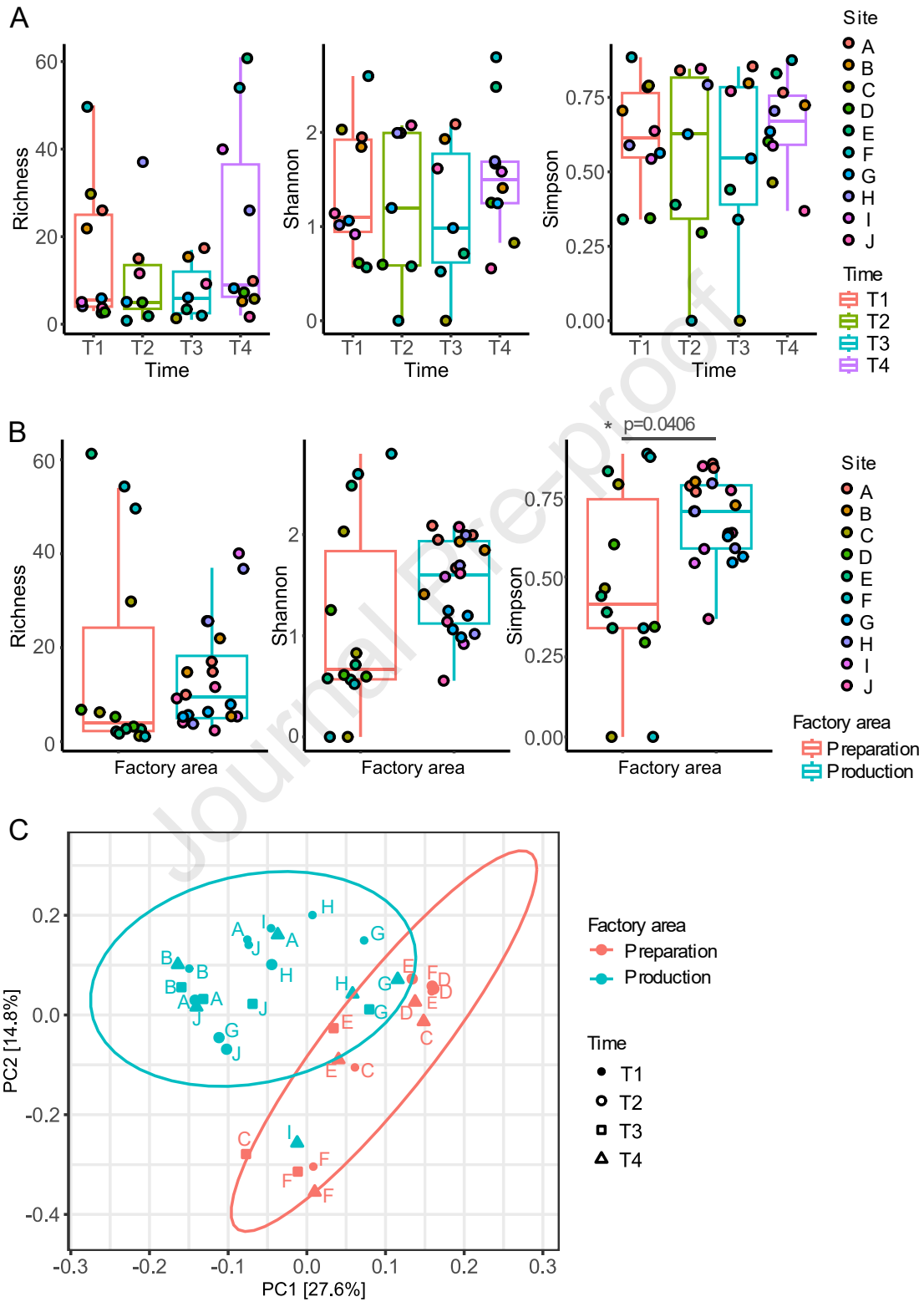
3.2. *Microbial composition of the factory environment remains stable at standard operation and post-cleaning phases, but differs between areas of the factory*

To evaluate the microbial populations in the factory environment, the metagenomes of 40 non-food contact surfaces in the high-care zone of the factory were compared between sampling timepoints (T1, T2, T3 and T4; with 10 samples per timepoint) and between areas of the factory (16 samples correlated to the preparation area and 24 samples to the production area). DNA concentrations in the samples extracted from environmental swabs varied from 0.02 to 9.24 ng/µL and no DNA was detected in blank samples. Shotgun sequencing of the DNA from the 40 environmental swab samples resulted in 75 million reads across all samples (average of 1,741,932 per sample, Supplementary_Direct_Sequencing). After reads were quality filtered, 9 samples resulted in less than

3K reads (Supplementary_Direct_Sequencing) suggesting failure during sequencing. The 9 failed samples included the 3 negative controls and 3 samples from each of timepoint T2 and T3; and within each timepoint of T2 and T3, 2 samples were from the production area and 1 from the preparation area. Samples with less thank 3K sequences failed taxonomic classification with MetaPhlAn4 and were not included for further metagenomic analysis. In the remaining 34 experimental samples, variation in microbial composition at different timepoints and between the two main areas of the high-care zone of the factory were analysed for alpha diversity indexes (richness, Shannon and Simpson). There were no significant differences for alpha diversity over the sample collection time course (Fig. 1A; Supplementary_stats_a-diversity). Between different areas of the factory, similar medians were also observed for α-indexes although statistically significant differences were observed for the Simpson index (p-value = 0.0406) (Fig. 1B).

To visualise differences in the microbial composition between groups (e.g. preparation vs. production areas or samples taken at the same site at different time points), Bray-Curtis similarity distances were compared using PCoA (Fig. 1C). The first and second principal components explained 42.4% of the differences in microbial composition. Samples collected from the same sites tended to cluster together independently of the time in which they were collected (adonis p-value = 0.175) whereas the microbial taxa in samples collected from the two areas of the factory were related but clustered into separate groups with a significant difference (adonis p-value = 0.001).

309



310

311 **Figure 1**. **Changes in bacterial diversity in the different areas of the factory over ten weeks.** A and
312 B) Alpha diversity evaluated with richness, Shannon and Simpson indexes depending on A) time and
313 B) area of the factory. Each boxplot represents the interquartile range (IQR) of the alpha diversity
314 indexes values and the whiskers represent the minimum and maximum values. C) Principal
315 Coordinate Analysis of the Bray-Curtis distance level. The ellipses represent normal data ellipses for
316 the samples in the preparation and production areas of the factory. Statistical comparisons with the
317 A) Kruskal-Wallis, B) Wilcoxon and C) Adonis tests are presented in supplementary materials.
318 Significant differences for alpha diversity (p-value < 0.05) are indicated in the graphs with an asterisk
319 (*).

320

321 *3.3. Pseudomonas was the most abundant and stable genus on floor surfaces while other*
322 *taxonomic groups were differentially abundant between areas of the factory.*

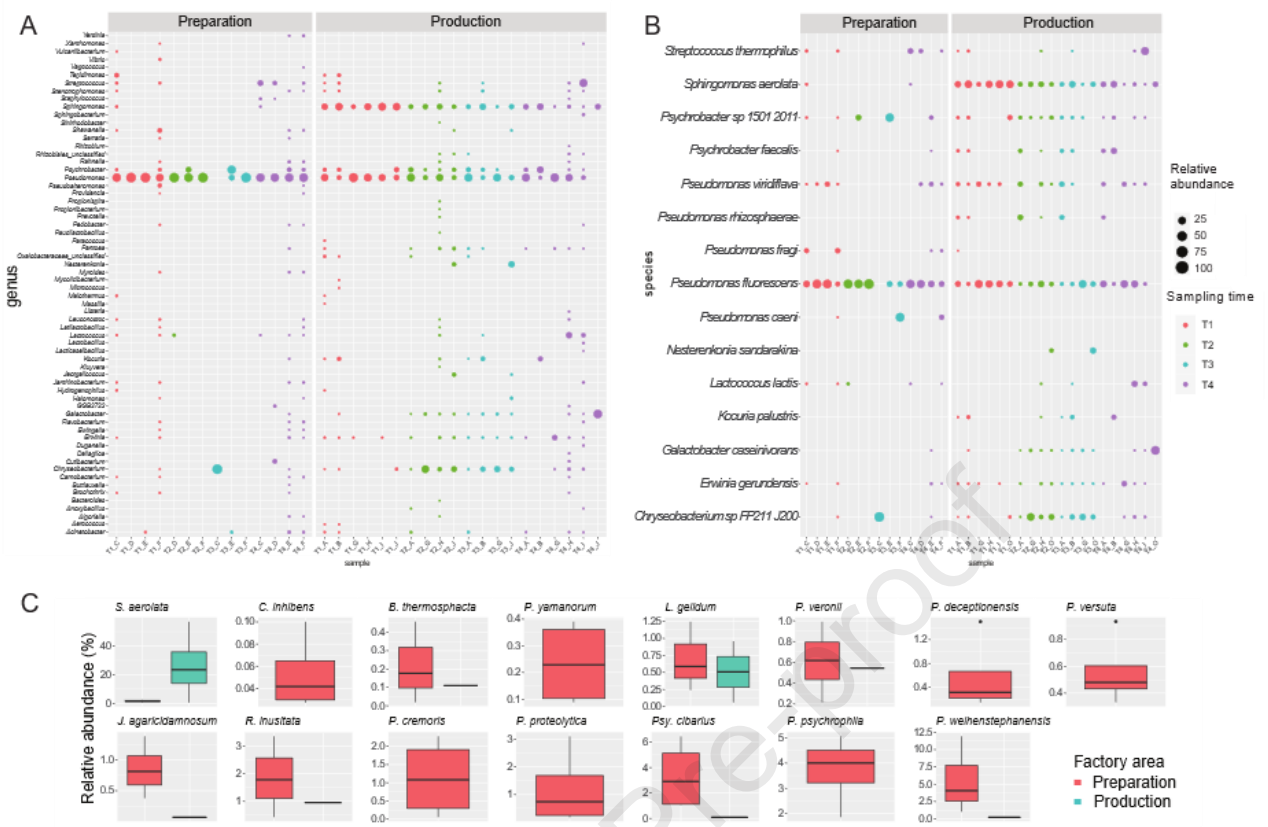
323 The most prevalent and abundant genus observed in the sampling period was *Pseudomonas*, with
324 *Psychrobacter, Sphingomonas,* and *Chryseobacterium* also being highly represented, particularly in
325 the production area (Fig. 2A). When sequences were classified to the species level, *Pseudomonas*
326 *fluorescens* was most abundant, with *Pseudomonas caeni*, *Pseudomonas viridiflava*, and
327 *Pseudomonas rhizosphaerae* also being observed in fewer samples and at less abundance (Fig. 2B).
328 For other genera, *Sphingomonas aerolata*, *Chryseobacterium* spp, *Psychrobacter faecalis*,
329 *Psychrobacter* sp. 1501 2011, *Streptococcus thermophilus* and *Galactobacter caseinivorans* were
330 observed in multiple samples (Fig. 2B). Amongst these taxa, only *P. fluorescens* was consistently
331 present in at least 90% of the samples, accounting for a relative abundance of ≥1%. Further, the
332 relative abundance of *P. fluorescens* remained remarkably consistent between both the production
333 and preparation areas of the factory, with no significant differences observed
334 (Supplementary_ANCOMBC). In addition, the relative abundance of this genus was stable over time

as indicated by the absence of significant differences between timepoints (Supplementary_ANCOMBC).

Conversely, significant differences in the abundance of other taxonomic groups were found between the two areas of the factory (Fig. 2C and Supplementary Fig. 2). For instance, *S. aerolata* was one of the most prevalent microorganisms in the production area, exhibiting a higher abundance there compared to the preparation area. Within the preparation area, multiple species of *Pseudomonas* were more abundant (Fig. 2C). No differences in the relative abundance of any of the taxonomic groups were identified from the metagenomes among any of the timepoints, even when comparing T2 and T3, which corresponded to periods before and after cleaning (Supplementary_ANCOMBC).

Considering the metagenome from all sampling points, there was a strong positive correlation between certain individual species of *Pseudomonas* with other species within the same genus, and also with species within the genus *Psychrobacter, Flavobacter* and *Serratia* (Supplementary Fig. 3 and Supplementary_Significant_correlations). Additionally, a strong positive correlation between species of *Pseudomonas* and *Brochothrix thermosphacta* and species within the genus *Rahnella* and *Providencia* were observed (Supplementary Fig. 3 and Supplementary_Significant_correlations).

Notably, *Listeria* spp. was not detected in any of the metagenomes using MetaPhlAn4 or by direct mapping against genomes of *L. monocytogenes* and *L. seeligeri* isolated in this study. If present in the samples, *Listeria* was at very low abundance and below the threshold of detection with the direct metagenomic sequencing approach.



354 **Figure 2. Relative abundance of the environmental samples.** Bubble plots representing the relative
355 abundance of A) genus and B) the 15 most abundant species identified in the factory environment.
356 Each column represents a sample and samples have been grouped by area of the factory and
357 coloured by time of collection of the sample. C) Comparison of the relative abundance at production
358 and preparation areas for species exhibiting significant differences in abundance, as determined by
359 ANCOM-BC analysis. Each boxplot represents the interquartile range (IQR) of the relative abundance
360 and the whiskers represent the minimum and maximum values.

361

362 3.4. *Culture enrichments reveal the persisting ST121 strain of Listeria monocytogenes and*
363 *coexisting Listeria seeligerii*

364 In the absence of detectable *Listeria* in the metagenomes of environmental samples taken from
365 areas recently confirmed to harbour *Listeria* spp., culture enrichments were performed for all 50
366 environmental samples to identify and type *Listeria* spp. To note, although samples from T0

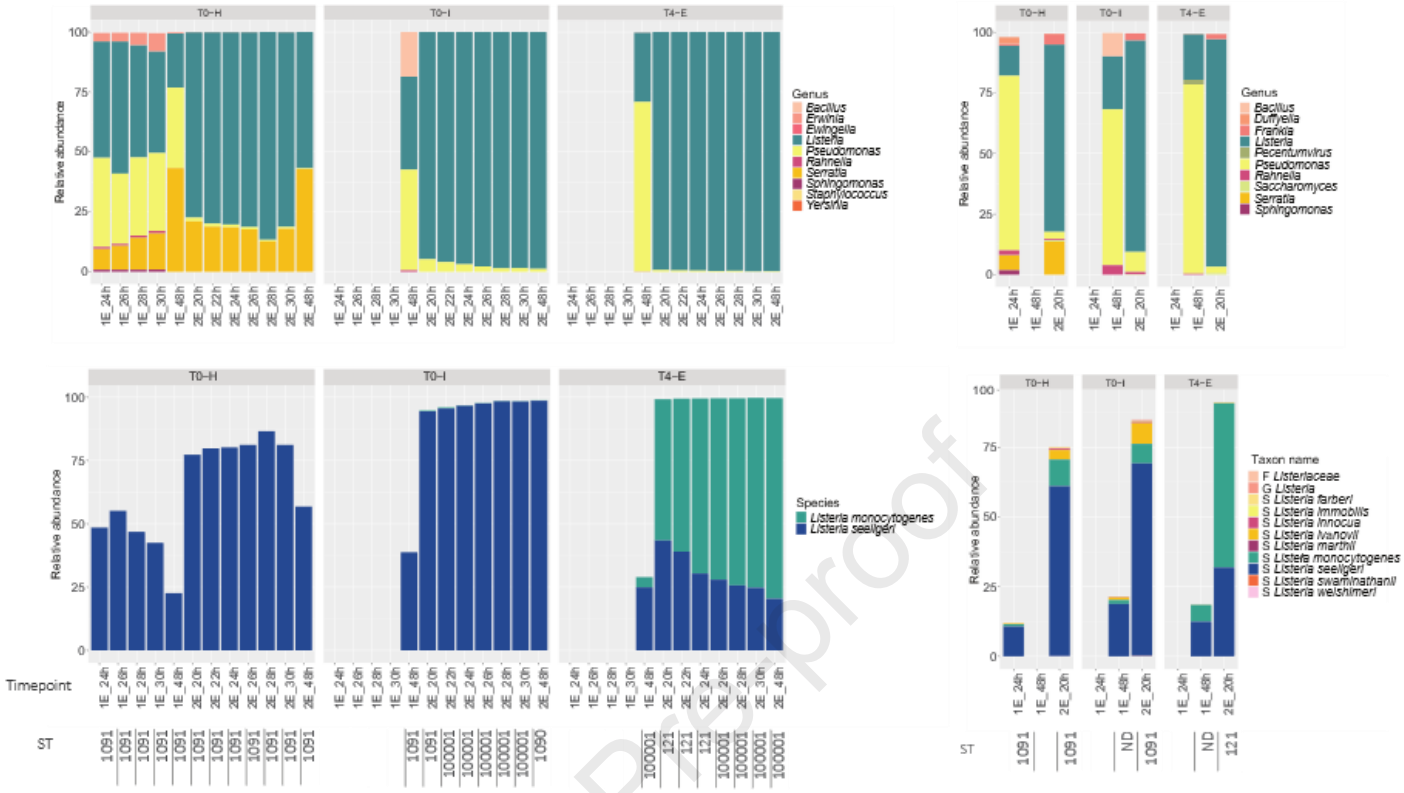
containing neutralizing buffer were not suitable for direct metagenomic analysis, they remained appropriate for culture enrichments. *L. seeligeri* was isolated from 17 of 50 samples, and notably, *L. monocytogenes* was also isolated from 3 of these samples (Sites H and I at T0 and Site E at T4) (Supplementary – Listeria_detection_standard), one of them (Site E at T4) being part of the samples included in the microbial composition analysis. All *L. monocytogenes* isolates were classified by MLST as ST121 (Supplementary_WGS_isolates) with 9 or less SNPs observed between genomes of environmental isolates recovered from the same factory in 2017 (Supplementary_SNP_distance). The lineage of *L. seeligeri* isolates could not be traced back to strains identified in earlier years at the facility since genomes of this species from this factory had not been previously subjected to sequencing.

To investigate if the presence of *Listeria* spp. correlated with differences in the microbial composition of the direct metagenomes of environmental samples, alpha diversity indexes (richness, Shannon and Simpson) and Bray-Curtis similarity distances were compared using PCoA (Supplementary Fig. 4). The alpha diversity indexes were similar in all samples, whether *Listeria* was detected or not, although slightly higher richness was observed in *Listeria* positive samples (p-value = 0.0447) (Supplementary_stats_a-diversity and Supplementary Fig. 4A). The overall microbial composition of the samples was also independent of the presence of *Listeria* spp. (adonis p-value = 0.062) (Supplementary_stats_b-diversity and Supplementary Fig. 4B).

3.5. *Co-occurring genera and Listeria species limited the quasimetagenomic classification of Listeria monocytogenes sequence types.*

As an alternative method for the detection of *Listeria* in the environmental samples, we assessed the performance of a quasimetagenomics approach (sequencing of *Listeria* culture enrichments) in terms of ST identification. Aliquots of the *Listeria* enrichments were collected at timepoints during culture and sequenced with Illumina and nanopore platforms. For the 3 *L. monocytogenes* positive

samples, all 16 enrichment timepoints collected during the primary and secondary enrichments were sequenced using Illumina technology to observe the effects of culture enrichment on the quasimetagenome. Furthermore, 2 selected enrichment timepoints per sample were sequenced with nanopore technology to observe any methodological attributes of long versus short read sequencing during enrichment. Notably, for the *L. monocytogenes* positive samples, library preparation was successfully achieved only after a minimum of 24 h of enrichment (Fig. 3 and Supplementary_Quasimetagenomics). Sequencing of the enrichments resulted in 1.9 – 10.4 million Illumina reads and 19.32 – 57.94 thousand nanopore reads per sample (Fig. 3 and Supplementary_Quasimetagenomics). For the 14 *L. seeligeri* positive samples, selected enrichment timepoints (primary enrichment after 20, 24, 28 and 48h and secondary enrichment after 20 and 48h of incubation) were sequenced with Illumina technology (Supplementary Fig. 5 and Supplementary_Quasimetagenomics). None of the primary enrichments after 20 and 24h produced successful libraries, while the successful ones resulted in a range of 1.85 – 12.15 million Illumina reads for this set of samples.



406 **Figure 3. Microbial dynamics of *Listeria* enrichments in samples positive for *L. monocytogenes* and**
407 ***L. seeligeri.*** Barplots show the relative abundance of A) and B) the 10 most abundant genera and C)
408 and D) the 10 most abundant *Listeria* species identified in the enrichment using A) C) Illumina or B)
409 D) nanopore sequencing. Timepoints 1E and 2E indicate primary and secondary enrichments
410 respectively. The tables in panels C) and D) show the ST identified from raw reads (100001 refers to
411 new loci-sequences or new STs [40]).

412

413 As anticipated, the proportion of *Listeria* reads amongst all microbial reads varied as the
414 enrichments progressed, increasing in the secondary enrichments (0.75 – 61.57 % *Listeria* short
415 reads in primary enrichments; 15.12 – 99.87 % *Listeria* short reads in secondary enrichments) (Fig. 3
416 and Supplementary Fig. 5). Additionally, the proportion of *Listeria* reads and the composition of the
417 enrichments varied between samples (Fig. 3A and Supplementary Fig. 5). Similar to the findings in
418 the direct metagenomes obtained without culture, *Pseudomonas* reads were the most abundant in

the primary enrichments, having higher relative abundance than *Listeria* (12.84 - 99.2 % *Pseudomonas* reads in primary enrichments) and were then greatly reduced in the secondary enrichments (0 - 9.08 % *Pseudomonas reads* in the secondary enrichments). Other taxa such as *Serratia, Erwinia, Rahnella, Erwingella, Yersinia, Bacillus* and *Staphylococcus* were also detected in the primary and secondary enrichments in at least one sample at minimum 1% relative abundance (Fig. 3A and Supplementary Fig. 5).

When sub-classifying *Listeria* sequence reads to the species level, *L. seeligeri* was the most abundant in all samples except T4-E (0.3 – 33.2 % *L. seeligeri* reads in primary enrichments; 11.1 – 90.4 % *L. seeligeri* reads in in secondary enrichments). In the secondary enrichment of sample T4-E, one of the three samples from which *L. monocytogenes* ST121 was isolated, sequence reads for *L. monocytogenes* were most abundant (1.9 – 68.8 %) (Fig. 3C). In this sample, *L. monocytogenes* was first detected in the first enrichment after 48 h of incubation. Surprisingly, in the other two *L. monocytogenes* positive samples (T0-H and T0-I) the percentage of *L. monocytogenes* reads observed was below 0.00437 % (Fig 3C).

For the 3 samples from which *L. monocytogenes* ST121 was isolated using culture enrichment, classification of *Listeria* sequence reads from quasimetagenomes to the ST level was attempted by analysing raw Illumina reads and raw nanopore reads from sequences obtained at different timepoints during the primary and secondary enrichments. ST classification was also attempted using hybrid metagenome-assembled genomes (MAGs) that were prepared by combining reads from the short and long-read technologies. For two samples (T0-H and T0-I) the *Listeria* reads were dominated by *L. seeligeri*, and accordingly, *L. seeligeri* STs were classified as either ST1090 or ST1091 (Fig. 3C). Similarly, *L. monocytogenes* was not detected in these two samples by direct mapping against the genome of *L. monocytogenes* isolated in this study. Accordingly, with less than 9000 *L. monocytogenes* reads observed at any timepoint with any sequencing technology, in these same two samples ST classification for *L. monocytogenes* was not achieved. Alternatively, short and long read

444 datasets from sample T4-E were identified as ST121, and a hybrid assembly was possible that
445 covered 84.76% of the *L. monocytogenes* genome (Supplementary_Quasimetagenomics and
446 Supplementary_Quasimetagenomics_MAGs_QC).

447

448 DISCUSSION

449 Previous studies have described the microbiome of FPEs such as abattoirs and meat
450 processing plants [54-59], fermented-products production facilities [14, 54, 59-63] and fruit and
451 vegetables processing plants [15, 64, 65], and identified distinctive microbiomes depending on the
452 type of commodities [59, 66]. However, very few studies describe the FPE of RTE meals [67], despite
453 the importance of this type of food in the contamination with foodborne pathogens during
454 processing [16]. The primary objective of this study was to gain insight into the resident microbial
455 populations coexisting with *L. monocytogenes* in a FPE where this pathogen has been persistently
456 contaminating non-food contact surfaces. The study aimed to assess the composition of these
457 populations in various areas within a factory setting and to evaluate the impact of cleaning and
458 disinfection procedures on the overall microbial population. It is noteworthy that multiple areas of
459 this factory have been monitored by UKHSA for several years, including food contact and non-food
460 contact surfaces, and for the purpose of this study, only areas that have shown persistent
461 contamination with *L. monocytogenes* and other *Listeria* spp. have been included. While *L.*
462 *monocytogenes* is known to endure in FPEs by concealing in difficult-to-clean areas such as slicers,
463 wheels or cracks on the materials or areas with standing water [68], the floor surfaces included in
464 this study do not exhibit apparent challenging to clean areas that would account for *L.*
465 *monocytogenes* evading the cleaning process.

466

A 10-week sampling period was coordinated with the company and with public health authorities to advance the understanding of a persisting food safety risk. Using metagenomics, we identified a resident microbiota in the high-care zone of the facility that was dominated by *Pseudomonas, Sphingomonas* and *Psychrobacter.* In particular, the species *P. fluorescens* was the most abundant in all but two of the factory sites. *Pseudomonas* spp. and *Psychrobacter* spp. are frequently the dominant taxa in various food processing environments, such as abattoirs, meat-processing, dairy, and fruit-processing factories [55, 58, 59, 64], due to their tolerance to low temperatures [69] and their ability to form biofilms [70, 71], which often results in contamination of food products [72, 73]. To our knowledge, the presence of *Sphingomonas* spp. has not been described in FPEs, although some members of this genus have been isolated from other anthropized environments [74-76]. Additionally, certain *Sphingomonas* species have been described as psychrotolerant [74], which may explain their ability to survive in RTE food processing environments.

Notably, the application of the cleaning and disinfection procedures during the study period, which included among other procedures, scrubbing with a degreaser followed by the application of a troclosene sodium-based disinfectant, did not result in major shifts in the composition of the microbial populations observed by metagenomic analysis. Although this study was not designed to assess the efficacy of the cleaning and disinfection procedures, as the methodology does not differentiate between viable and non-viable microorganisms nor was sampling statistically powered, a strikingly stable population was observed. *Pseudomonas* has been described as one of the most abundant taxa detected on surfaces after cleaning and disinfection procedures in facilities that process meat [77] and minimally processed vegetables [65], as detected by culture-dependant and culture-independent approaches respectively. Similarly, studies using RNA-based amplicon sequencing coupled with plate counts have shown that cleaning and sanitizing procedures are less efficient at removing psychrotrophic bacteria, including *Pseudomonas*, while subsequent ozonation reduced the viable bacteria within this group [56]. In studies that monitored FPEs over longer periods of time, shifts in microbial populations were observed. For example, the microbiome of a

493 newly opened meat processing plant was disrupted once the meat-cutting activities started [55] and
494 changes in the microbiome of a fruit processing facility over seasons were hypothesised to be caused
495 by changes in: the natural environment where the fruit is produced, the water used within the
496 facilities, or the personnel working in the facilities [15]. The absence of substantial changes in the
497 microbial community over time in the facility manufacturing RTE-meals could be explained by a low
498 inflow of foreign microbiota coupled with the high adaptation of the microbiota to the environment.
499 Unlike other FPEs where a large influx of raw materials carrying a high microbial load occurs,
500 facilities manufacturing RTE-meals introduce pre-cooked or pre-processed ingredients, potentially
501 limiting the entry of external microbiota into the facility. It is also likely that as microbes are
502 naturally introduced into FPEs, a rapid selection occurs for those capable of surviving and persisting
503 through the control measures such as cleaning and disinfection. Simultaneously, these microbes
504 must compete with the resident microbiota that have already established successful niches within
505 the environment. For example, in conjunction with the persistent ST121 clone observed in Company
506 X, multiple non-ST121 *L. monocytogenes* strains were transiently observed in the facility between
507 2017 and 2020 but were not detected during our sampling time-points in 2021 and 2022. This
508 suggests that these strains were susceptible to the cleaning and disinfection methods and did not
509 become persistent [19].

510 Although the presence of some taxa was consistent across the factory, there was a clear
511 distinction between the two areas within the high-care zone, reflecting the adaptation of the
512 populations to different environments. Although there was movement of personnel between them,
513 potentially allowing for the transmission of taxa, both areas were kept at different temperatures and
514 this factor has likely contributed to the differential selection between microbial populations.
515 *Sphingomonas aerolata,* one of the most abundant members of the resident microbiota of this
516 factory, was significantly more prevalent in the production area of the facility (detected in 20
517 samples in the production area, with relative abundances ranging from 0.67 to 56.94 % compared to
518 only two samples from the preparation area, where its relative abundance ranged from 0.89% to

519 2.51%). *S. aerolata* is a psychrotolerant bacteria firstly isolated from the air of a building [74] and to
520 our knowledge, this is the first time that has been detected in food processing environments,
521 although it has been detected by 16S rRNA amplicon sequencing in fruits [78]. Other taxa, mainly
522 within the *Pseudomonas* genus, showed higher abundance in the preparation area, although these
523 species represented a minor proportion of the whole community (<0.125%). Adaptation of the
524 microbial communities to different types of food processing environments [59] and different
525 environments within food production facilities [14, 55] have been described before, highlighting the
526 importance of monitoring individual ecological niches within the same facilities to identify the key
527 microbial taxa characteristic of FPEs.

528 There is evidence that biofilms present in FPEs could harbour foodborne pathogens
529 (potentially at low abundance) and support their survival against cleaning and disinfection
530 treatments [77, 79]. In our study, the sampled sites were selected based on the recurrent detection
531 of *Listeria* spp. and *L. monocytogenes* through culture enrichment during an extended period of
532 enhanced surveillance [19]. Although these taxa could not be detected from the metagenomes,
533 culture-dependent methods revealed the presence of *Listeria* spp. in all sites, except for two, and of
534 *L. monocytogenes* in three of the sites, in at least one occasion. *L. monocytogenes* and most other
535 pathogens are present in very low abundances in food and food processing environments compared
536 to resident microflora and the only way to detect pathogens in these sample types is by culture
537 enrichment [14, 54]. Notably, only the partial volume of the swabs was used to obtain metagenomic
538 DNA, further increasing the limit of detection. A wide range of taxa have been described to coexist
539 with *L. monocytogenes* and *Listeria* spp. In meat processing plants, known biofilm-producers such as
540 *Pseudomonas, Acinetobacter* and *Janthinobacterium* were associated with the presence of *Listeria*
541 spp. [58] while *Psyschromonas, Shewanella, Lactococcus, Lactobacillus* or *Yersinia* were associated
542 with *Listeria* in fish, meat and dairy plants [54]. *Pseudomonas*, *Stenotrophomonas* and
543 *Microbacterium* were identified as potential indicators of *L. monocytogenes* presence in fruit packing
544 facilities (Rolon 2023). In this study we have identified *P. fluorescens* as the most abundant and

prevalent taxa in the sites monitored. The interactions between *L. monocytogenes* and *Pseudomonas* spp. within biofilms have been studied in laboratory models [12, 13, 80, 81], highlighting the protective effect of *Pseudomonas* spp., particularly *P. fluorescens,* towards *L. monocytogenes*. The effect of other members of the resident microbiota in FPEs, such as *Xantomonadaceae* or *Flavobacteriaceae,* can either enhance or inhibit the survival of *L. monocytogenes,* respectively [82, 83]. All these studies indicate the importance of the background microbiota in FPEs in the survival of *L. monocytogenes* and our results contribute to building the knowledge into understanding these communities.

There is a growing usage case for monitoring and characterising *L. monocytogenes* in FPEs using whole-genome sequences from bacterial isolates, which offers advantages such as the high level of discrimination between strains and the possibility to detect antimicrobial and biocide resistance and virulence genes. However, this is a time-consuming process, as recovering the pathogen from a food or environmental sample can take between 2 and 4 days [23]. To accelerate the process of obtaining *Listeria* genomes, the use of quasimetagenomic sequencing has been proposed [84]. The use of a quasimetagenomic approach is not only an opportunity to speed the source tracking of *L. monocytogenes*, but also to capture the diversity of *Listeria* strains in a sample [85], although there is debate wether the enrichment could select for specific *Listeria* strains [85-87].Previous studies have successfully used quasimetagenomic sequencing to obtain and subtype *L. monocytogenes* MAGs from environmental swabs, with the proportion of *L. monocytogenes* reads ranging from 0.02% to 91.82%. In some cases, *Pseudomonas* or *Enterococcus* dominated the quasimetagenomes and the *L. monocytogenes* genome coverage was insufficient to perform SNP analysis [66]. In our study, quasimetagenomics from liquid culture enrichments was trialled as an additional means to identify and then sequence type *Listeria* spp. that were known to be present in samples (via routine culture) but where *Listeria* spp. were not detectable using direct metagenomics. In the majority of samples containing *L. seeligeri*, the corresponding STs were observed using quasimetagenomics. However, *L. monocytogenes* STs were classified in only a single sample, either by taxonomic

classification or by direct mapping against reference genomes, despite sufficient sequencing depth (equivocal to 90x coverage, but with a limited number of reads classified as *L. monocytogenes,* Fig. 3). Even with enrichment, with a quasimetagenomic dataset there may be diminished detectable sequence yields for a given pathogen when the originating amounts of pathogen were low [85]. During enrichment *L. monocytogenes* can be overgrown by other *Listeria* species present in the original culture [88]. It is likely that *L. seeligeri* was the predominant species in the original sample and this species has retained the higher proportion during the enrichment culture. The limited amount of sample did not allow enumeration of the *Listeria* colony forming units present in the original samples. Moreover, certain genera, notably *Pseudomonas*, are only partially inhibited by Half Fraser broth [89], which further challenges the isolation and subtyping of *L. monocytogenes* from primary enrichments.

Metagenomics supported exploration of the microbiological dynamics within the facility, identifying dominant organisms that have been selected for and have endured in that environment, while low abundance organisms like *Listeria* required culture efforts to detect, but likewise have been successful in enduring through the food safety control efforts imposed over many years. In this stable microbial population, there may be mutualism between the resident members that has likely supported adaptation to the specific conditions of this industrial niche.

The mechanisms of this mutualism and the impact of environmental conditions on creating resilient populations can be investigated using models that mimic these complex populations, with the goal of informing food safety interventions on persisting microbial populations.

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AUTHORS CONTRIBUTION

MD: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualization, original draft, review & editing; HA: data curation, resources; TLV: formal analysis; AVG: conceptualization, investigation, data curation, review & editing; NLM: formal analysis, investigation, review & editing; OO: investigation; LMS: investigation; JF: formal analysis; NS: investigation; JM: conceptualization; FH: conceptualization, formal analysis, review & editing; FJ: conceptualization, review & editing; MG: conceptualization, funding acquisition, review & editing. All authors contributed to the revision of the manuscript and approved the final version.

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DATA AVAILABILITY

The datasets supporting the conclusions of this article are available in the NCBI repository, under the BioProjects PRJNA1060911 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1060911) and PRJNA1061071 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1061071).

643 REFERENCES

644 1. Møretrø, T. and Langsrud, S.. Residential bacteria on surfaces in the food industry and their
645 implications for food safety and quality. *Compr Rev Food Sci Food Saf*. 2017, **16**(5): p. 1022-
646 1041. https://doi.org/10.1111/1541-4337.12283
647 2. Fagerlund, A., Langsrud, S. and Møretrø T. Microbial diversity and ecology of biofilms in food
648 industry environments associated with *Listeria monocytogenes* persistence. *Curr Opin Food*
649 *Sci*, 2021. **37**: p. 171-178. https://doi.org/10.1016/j.cofs.2020.10.015
650 3. UK Health Security Agency. Research and analysis. Listeriosis in England and Wales:
651 summary for 2021, 2021. https://www.gov.uk/government/publications/listeria-
652 monocytogenes-surveillance-reports/listeriosis-in-england-and-wales-summary-for-2021.
653 Accessed 12 Jan 2024.
654 4. Koopmans, M.M., Brouwer, M.C., Vazquez-Boland, J.A., and van de Beek, D. Human
655 Listeriosis. *Clin Microbiol Rev*. 2023, **36**(1). https://doi.org/10.1128/cmr.00060-19
656 5. Ferreira, V., Wiedmann, M., Teixeira, P. and Stasiewicz, M.J. *Listeria monocytogenes*
657 persistence in food-associated environments: epidemiology, strain characteristics, and
658 implications for public health. *J Food Prot*, 2014. **77**(1): p. 150-70.
659 https://doi.org/10.4315/0362-028X.JFP-13-150
660 6. Mazaheri, T., Cervantes-Huamán, BRH., Bermúdez-Capdevila, M., Ripolles-Avila, C.,
661 Rodríguez-Jerez, J.J. *Listeria monocytogenes* biofilms in the food industry: Is the current
662 hygiene program sufficient to combat the persistence of the pathogen? *Microorganisms*,
663 2021. **9**(1). https://doi.org/10.3390/microorganisms9010181
664 7. Silk, B.J., McCoy, M.H., Iwamoto, M. and Griffin, P.M. Foodborne listeriosis acquired in
665 hospitals. *Clin Infect Dis*, 2014. **59**(4): p. 532-40. https://doi.org/10.1093/cid/ciu365
666 8. Government of Canada. Report of the independent investigator into the 2008 listeriosis
667 outbreak, 2009. https://www.canada.ca/en/news/archive/2009/07/report-independent-
668 investigator-into-2008-listeriosis-outbreak.html Accessed 12 Jan 2024.

669 9. Public Health England. Investigation into an outbreak of *Listeria monocytogenes* infections
670 associated with hospital-provided preprepared sandwiches, UK May to July 2019 2020.
671 October 2020.
672 10. McLauchlin, J., Grant, K.A. and Amar, C.F.L. Human foodborne listeriosis in England and
673 Wales, 1981 to 2015. *Epidemiol Infect* 2020, **148**: p. e54.
674 https://doi.org/10.1017/S0950268820000473
675 11. FAO and WHO. 2022. *Listeria monocytogenes* in ready-to-eat (RTE) foods: attribution,
676 characterization and monitoring – Meeting report. Microbiological Risk Assessment Series
677 No. 38. https://doi.org/10.4060/cc2400en
678 12. Sterniša, M., Gradišar Centa, U., Drnovšek, A., Remškar, M., Smole Možina, S. *Pseudomonas*
679 *fragi* biofilm on stainless steel (at low temperatures) affects the survival of *Campylobacter*
680 *jejuni* and *Listeria monocytogenes* and their control by a polymer molybdenum oxide
681 nanocomposite coating. *Int J Food Microbiol*, 2023. **394**: p. 110159.
682 https://doi.org/10.1016/j.ijfoodmicro.2023.110159
683 13. Thomassen, G.M.B., Reiche, T., Hjorungnes, M. and Mehli, L. High disinfectant tolerance in
684 *Pseudomonas* spp. biofilm aids the survival of *Listeria monocytogenes. Microorganisms*,
685 2023. **11**(6). https://doi.org/10.3390/microorganisms11061414
686 14. Dzieciol, M., Schornsteiner, E., Muhterem-Uyar, M., Stessl, B., Wagner, M. and Schmitz-Esser
687 S. Bacterial diversity of floor drain biofilms and drain waters in a *Listeria monocytogenes*
688 contaminated food processing environment. *Int J Food Microbiol*, 2016. **223**: p. 33-40.
689 https://doi.org/10.1016/j.ijfoodmicro.2016.02.004
690 15. Rolon, M.L., Tan, X., Chung, T., Gonzalez-Escalona, N., Chen, Y., Macarisin, D., LaBorde, L.F.
691 and Kovac J. The composition of environmental microbiota in three tree fruit packing
692 facilities changed over seasons and contained taxa indicative of *L. monocytogenes*
693 contamination. *Microbiome*, 2023. **11**(1): p. 128. https://doi.org/10.1186/s40168-023-
694 01544-8

16. Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P. S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Lindqvist, R, Nørrung, B., Ricci, A., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., and Wahlström, H. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA J*, 2018. **16**(1): p. e05134. https://doi.org/10.2903/j.efsa.2018.5134

17. Beccalli, M.P., Picozzi, C., Mangieri, N., Vigentini, I., Foschino, R. Assessment of microbial populations in the manufacture of vacuum-packaged ready-to-eat roast beef and in a related production plant. *J Food Prot*, 2019. **82**(1): p. 58-64. https://doi.org/10.4315/0362-028X.JFP-18-147

18. Kubota, K.A., Wolfgang, W.J., Baker, D.J., Boxrud, D., Turner, L., Trees, E., Carleton, H.A. and Gerner-Smidt, P. PulseNet and the changing paradigm of laboratory-based surveillance for foodborne diseases. *Public Health Rep*, 2019. **134**(2_Suppl): p. 22s-28s. https://doi.org/10.1177/0033354919881650

19. McLauchlin, J., Aird, H., Amar, C., Boyd, G., Brindle, A., Dallman, T., Jalava, K., Painset, A., Simbo, A. and Swindlehurst, M. Listeriosis associated with pre-prepared sandwich consumption in hospital in England, 2017. *Epidemiol Infect*, 2021. **149**: p. e220. https://doi.org/10.1017/S0950268821001916

20. Public Health England. Detection and enumeration of bacteria in swabs and other environmental samples. National Infection Service Food Water and Environmental Microbiology Standard Method. 2017.

21. Public Health England. Examining food, water and environmental samples from healthcare environments. Microbiological guidelines. 2020. https://www.gov.uk/government/publications/examining-food-water-and-environmental-samples-healthcare-settings Accessed 12 Jan 2024.

22. Food Standards Agency. Food Law Code of Practice. https://www.food.gov.uk/about-us/food-and-feed-codes-ofpractice. 2017.

23. Public Health England. Detection and enumeration of *Listeria monocytogenes* and other *Listeria* species. National Infection Service Food Water and Environmental Microbiology Standard Method. 2018.

24. Huttenhower, C. KneadData. https://github.com/biobakery/kneaddata. 2021.

25. Chen, S., Zhou, Y., Chen, Y. and Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 2018. **34**(17): p. i884-i890. https://doi.org/10.1093/bioinformatics/bty560

26. Blanco-Míguez, A., Beghini, F., Cumbo, F., McIver, L.J., Thompson, K.N., Zolfo, M., Manghi, P., Dubois, L., Huang, K.D., Thomas, A.M., Nickols, W.A., Piccinno, G., Piperni, E., Punčochář, M., Valles-Colomer, M., Tett, A., Giordano, F., Davies, R., Wolf, J., Berry, S.E., Spector, T.D., Franzosa, E.A., Pasolli, E., Asnicar, F., Huttenhower, C. and Segata, N.Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nat Biotechnol*, 2023 Nov;41(11):1633-1644. https://doi.org/10.1038/s41587-023-01688-w

27. Wick, R. Porechop: adapter trimmer for Oxford Nanopore reads. https://github.com/rrwick/Porechop. 2017.

28. Wick, R. Filtlong: quality filtering tool for long reads. https://github.com/rrwick/Filtlong. 2017.

29. De Coster, W., D'Hert, S., Schultz, D.T., Cruts, M. and Van Broeckhoven, C. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics*, 2018. **34**(15): p. 2666-2669. https://doi.org/10.1093/bioinformatics/bty149

30. Fan, J., Huang, S. and Chorlton, S.D. BugSeq: a highly accurate cloud platform for long-read metagenomic analyses. *BMC Bioinformatics*, 2021. **22**(1): p. 160. https://doi.org/10.1186/s12859-021-04089-5

31. Hildebrand, F. MATAFILER. https://github.com/hildebra/MATAFILER.2017.

32. Hildebrand, F., Moitinho-Silva, L., Blasche, S., Jahn, M.T., Gossmann, T.I., Huerta-Cepas, J., Hercog, R., Luetge, M., Bahram, M., Pryszlak, A., Alves, R.J., Waszak, S.M., Zhu, A., Ye, L., Costea, P.I., Aalvink, S., Belzer, C., Forslund, S.K., Sunagawa, S., Hentschel, U., Merten, C., Patil, K.R., Benes, V. and Bork, P. Antibiotics-induced monodominance of a novel gut bacterial order. *Gut,* 2019. **68**(10):1781-1790. https://doi.org/10.1136/gutjnl-2018-317715

33. Seeman, T., Kwong, J., Gladman., S and Goncalves da Silva, A. Shovill: Assemble bacterial isolate genomes from Illumina paired-end reads. https://github.com/tseemann/shovill. 2017.

34. Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A. and Korobeynikov, A. Using SPAdes De Novo Assembler*. Curr Protoc Bioinformatics*, 2020. **70**(1): p. e102. https://doi.org/10.1002/cpbi.102

35. Bertrand, D., Shaw, J., Kalathiyappan, M., Ng A.H.Q., Kumar, M.S., Li, C., Dvornicic, M., Soldo, J.P., Koh, J.Y., Tong, C., Ng, O.T, Barkham, T., Young, B., Marimuthu, K., Chng, K.R., Sikic, M. and Nagarajan, N. Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. *Nat Biotechnol*, 2019. **37**(8): p. 937-944. https://doi.org/10.1038/s41587-019-0191-2

36. Uritskiy, G.V., DiRuggiero, J. and Taylor J. MetaWRAP-a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome*, 2018. **6**(1): p. 158. https://doi.org/10.1186/s40168-018-0541-1

37. Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. and Tyson, G.W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res,* 2015. **25**(7): p. 1043-55. https://doi.org/10.1101/gr.186072.114

38. Gurevich, A., Saveliev, V., Vyahhi, N. and Tesler, G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*, 2013. **29**(8): p. 1072-5. https://doi.org/10.1093/bioinformatics/btt086

39. Chaumeil, P.A., Mussig, A.J., Hugenholtz, P. and Parks, D.H. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics*, 2019. **36**(6): p. 1925-7. https://doi.org/10.1093/bioinformatics/btz848

40. Zolfo, M., Tett, A., Jousson, O., Donati, C. and Segata, N. MetaMLST: multi-locus strain-level bacterial typing from metagenomic samples. *Nucleic Acids Res*, 2017. **45**(2): p. e7. https://doi.org/10.1093/nar/gkw837

41. Page, A.J. and Keane, J.A. Rapid multi-locus sequence typing direct from uncorrected long reads using Krocus. *PeerJ*, 2018. **6**: p. e5233. https://doi.org/10.7717/peerj.5233

42. Seemann, T. mlst. https://github.com/tseemann/mlst.

43. Jolley, K.A. and Maiden, M.C. BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics*, 2010. **11**: p. 595. https://doi.org/10.1186/1471-2105-11-595

44. Seeman, T., Snippy. https://github.com/tseemann/snippy. 2014.

45. Seeman, T., Klötzl, F., Page, A. snp-dist. https://github.com/tseemann/snp-dists.

46. Oksanen, J., Simpson, G.L., Blanchet, F.G.0, Kindt, R.K., Legendre, L.P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., de Caceres, M., Durand, S., Evangelista H.B.A., FitzJohn, R., Friendly M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.H., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J. vegan: Community echology package. https://cran.r-project.org/web/packages/vegan/index.html. 2022.

47. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.

48. Goslee, S.C. and Urban, D.L. The ecodist package for dissimilarity-based analysis of ecological data. *J Stat Softw*, 2007. **22**(7): p. 1-19. https://doi.org/10.18637/jss.v022.i07

49. Wickham, H., Sievert, C. and Pedersen, T.L. ggplot2 : Elegant Graphics for Data Analysis, in Use R! *Springer Cham*, 2016.

50. Boogaart, K.G.V.D. Centered log ratio transform. https://search.r-project.org/CRAN/refmans/compositions/html/clr.html.

51. Revelle, W. psych: Procedures for psychological, psychometric, and personality research. https://CRAN.R-project.org/package=psych. 2023.

52. Lin, H. and Peddada, S.D. Analysis of compositions of microbiomes with bias correction. *Nat Commun*, 2020. **11**(1): p. 3514. https://doi.org/10.1038/s41467-020-17041-7

53. Leo Lahti, S.S.E.A. Tools for microbiome analysis in R. http://microbiome.github.com/microbiome. 2017.

54. Rodríguez-López, P., Bernárdez, M., Rodríguez-Herrera, J.J., Comesaña, A.S. and Cabo M.L. Identification and metagenetic characterisation of *Listeria monocytogenes*-harbouring communities present in food-related industrial environments*. Food Control*, 2019. **95**: p. 6-17. https://doi.org/10.1016/j.foodcont.2018.07.023

55. Cobo-Díaz, J.F., Alvarez-Molina, A., Alexa, E.A., Walsh, C.J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter, P. and Alvarez-Ordóñez, A. Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. Microbiome, 2021. **9**(1). https://doi.org/10.1186/s40168-021-01131-9

56. Botta, C., Ferrocino, I., Pessione, A., Cocolin, L. and Rantsiou, K. Spatiotemporal distribution of the environmental microbiota in food processing plants as impacted by cleaning and sanitizing procedures: the case of slaughterhouses and gaseous ozone. *Appl Environ Microbiol*, 2020. **86**(23). https://doi.org/10.1128/AEM.01861-20

57. Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F.F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M. and Selberherr, E. The sources and transmission routes of microbial populations throughout a meat processing facility. *NPJ Biofilms Microbiomes*, 2020. **6**(1): p. 26. https://doi.org/10.1038/s41522-020-0136-z

58. Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Fleischmann, S., Selberherr, E., Thalguter, S., Quijada, N.M., Dzieciol, M., Wagner, M. and Stessl, B. Co-occurrence of *Listeria* spp. and spoilage associated microbiota during meat processing due to cross-contamination events. *Front Microbiol*, 2021. **12**: p. 632935. https://doi.org/10.3389/fmicb.2021.632935

59. Alvarez-Molina, A., Cobo-Díaz, J.F., Alexa, E.A., Crispie, F., Prieto, M., López, M., Cotter, P.D. and Alvarez-Ordóñez, A. Sequencing-based analysis of the microbiomes of Spanish food processing facilities reveals environment-specific variation in the dominant taxa and antibiotic resistance genes. *Food Res Int*, 2023. **173**(Pt 2): p. 113442. https://doi.org/10.1016/j.foodres.2023.113442

60. Bokulich, N.A., Bamforth, C.W. and Mills, D.A. Brewhouse-resident microbiota are responsible for multi-stage fermentation of American coolship ale. *PLoS One*, 2012. **7**(4): p. e35507. https://doi.org/10.1371/journal.pone.0035507

61. Bokulich, N.A. and Mills, D.A. Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Appl Environ Microbiol*, 2013. **79**(17): p. 5214-23. https://doi.org/10.1128/AEM.00934-13

62. Bokulich, N.A., Ohta, M., Richardson, P.M. and Mills, D.A. Monitoring seasonal changes in winery-resident microbiota. *PLoS One*, 2013. **8**(6): p. e66437. https://doi.org/10.1371/journal.pone.0066437

63. Schön, K., Schornsteiner, E., Dzieciol, M., Wagner, M., Müller, M. and Schmitz-Esser, S. Microbial communities in dairy processing environment floor-drains are dominated by

product-associated bacteria and yeasts. *Food Control*, 2016. **70**: p. 210-215. https://doi.org/10.1016/j.foodcont.2016.05.057

64. Tan, X., Chung, T., Chen, Y., Macarisin, D., LaBorde, L. and Kovac, J. The occurrence of *Listeria monocytogenes* is associated with built environment microbiota in three tree fruit processing facilities. *Microbiome*, 2019. **7**(1): p. 115. https://doi.org/10.1186/s40168-019-0726-2

65. Valentino, V., Sequino, G., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., De Filippis, F., Ercolini, D. Evidence of virulence and antibiotic resistance genes from the microbiome mapping in minimally processed vegetables producing facilities. *Food Res Int*, 2022. **162**(Pt B): p. 112202. https://doi.org/10.1016/j.foodres.2022.112202

66. Kocurek, B., Ramachandran, P., Grim, C.J., Morin, P., Howard, L., Ottesen, A., Timme, R., Leonard, S.R., Rand, H., Strain, E., Tadesse, D., Pettengill, J.B., Lacher, D.W., Mammel, M. and Jarvis, K.G. Application of quasimetagenomics methods to define microbial diversity and subtype *Listeria monocytogenes* in dairy and seafood production facilities. *Microbiol Spectr*, 2023: p. e0148223. https://doi.org/10.1101/2022.11.07.515551

67. Pothakos, V., Stellato, G., Ercolini, D. and Devlieghere F. Processing environment and ingredients are both sources of *Leuconostoc gelidum*, which emerges as a major spoiler in ready-to-eat meals. *Appl Environ Microbiol*, 2015. **81**(10): p. 3529-41. https://doi.org/10.1128/AEM.03941-14

68. Simmons, C.K. and Wiedmann, M. Identification and classification of sampling sites for pathogen environmental monitoring programs for *Listeria monocytogenes:* Results from an expert elicitation. *Food Microbiol*, 2018. **75**: p. 2-17. https://doi.org/10.1016/j.fm.2017.07.005

69. Zhang, Y., Wei, J., Yuan, Y. and Yue, T. Diversity and characterization of spoilage-associated psychrotrophs in food in cold chain. *Int J Food Microbiol*, 2019. **290**: p. 86-95. https://doi.org/10.1016/j.ijfoodmicro.2018.09.026

70. Rossi, C., Serio, A., Chaves-López, C., Anniballi, F., Auricchio, B., Goffredo, E., Cenci-Goga, B.T., Lista, F., Fillo and S., Paparella,A. Biofilm formation, pigment production and motility in *Pseudomonas spp.* isolated from the dairy industry. *Food Control*, 2018. **86**: p. 241-248. https://doi.org/10.1016/j.foodcont.2017.11.018

71. Wagner, E.M., Fischel, K., Rammer, N., Beer, C., Palmetzhofer, A.L., Conrady, B., Roch, F.F., Hanson, B.T., Wagner, M., Rychli, K.. Bacteria of eleven different species isolated from biofilms in a meat processing environment have diverse biofilm forming abilities. *Int J Food Microbiol*, 2021. **349**. https://doi.org/10.1016/j.ijfoodmicro.2021.109232

72. Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., Ercolini, D. Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. *Appl Environ Microbiol*, 2016. **82**(13): p. 4045-4054. https://doi.org/10.1128/AEM.00793-16

73. Bloomfield, S.J., Palau, R., Holden, E.R., Webber, M.A. and Mather, A.E. Genomic characterization of *Pseudomonas* spp. on food: implications for spoilage, antimicrobial resistance and human infection. *BMC Microbiol*, 2024. **24**(1): p. 20. https://doi.org/10.1186/s12866-023-03153-9

74. Busse, H.J., Denner, E.B.M., Buczolits, S., Salkinoja-Salonen, M., Bennasar, A. and Kämpfer P. *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas. Int J Syst Evol Microbiol*,, 2003. **53**: p. 1253-1260. https://doi.org/10.1099/ijs.0.02461-0

75. Kim SJ, Moon JY, Lim JM, Ahn JH, Weon HY, Ahn TY, Kwon SW. *Sphingomonas aerophila* sp. nov. and *Sphingomonas naasensis* sp. nov., isolated from air and soil, respectively *Int J Syst Evol Microbiol*, 2014. **64**: p. 926-932. https://doi.org/10.1099/ijs.0.055269-0

76. Lee, H., Kim, D.U., Lee, S., Yun, J., Park, S., Yoon, J.H., Park, S.Y. and Ka, J.O. *Sphingomonas carri* sp. nov., isolated from a car air-conditioning system.*Int J Syst Evol Microbiol*, 2017. **67**(10): p. 4069-4074. https://doi.org/10.1099/ijsem.0.002250

77. Fagerlund, A., Møretrø, T., Heir, E., Briandet, R. and Langsrud, S. Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. *Appl Environ Microbiol*, 2017. **83**(17). https://doi.org/10.1128/AEM.01046-17

78. Wicaksono, W.A., Buko, A., Kusstatscher, P., Cernava, C., Sinkkonen, A., Laitinen, O.H., Virtanen, S.M., Hyöty, H. and Berg, G. Impact of cultivation and origin on the fruit microbiome of apples and blueberries and implications for the exposome. *Microb Ecol*, 2023. **86**(2): p. 973-984. https://doi.org/10.1007/s00248-022-02157-8

79. Fernández-Gómez, P., Oliveira, M., Cobo-Díaz, J.F., González-Raurich, M., Múgica-Vidal, R., Alba-Elías, F., Prieto, M., Alvarez-Ordóñez, A. and López, M. The background microbiota and sanitization agent drive the fate of *Listeria monocytogenes* in multispecies biofilms formed on a plasma-polymerized coating applied on stainless steel. *Int J Food Microbiol*, 2023. **386**: p. 110017. https://doi.org/10.1016/j.ijfoodmicro.2022.110017

80. Overney, A., Jacques-André-Coquin, J., Ng, P., Carpentier, B., Guillier, L., Firmesse, O. Impact of environmental factors on the culturability and viability of *Listeria monocytogenes* under conditions encountered in food processing plants. *Int J Food Microbiol*, 2017. **244**: p. 74-81. https://doi.org/10.1016/j.ijfoodmicro.2016.12.012

81. Puga, C.H., Dahdouh, E., SanJose, C. and Orgaz, B. *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. *Front Microbiol*, 2018. **9**: p. 1706. https://doi.org/10.3389/fmicb.2018.01706

82. Rolon, M. L., Voloshchuk, O., Bartlett, K. V., LaBorde, L. F., and Kovac, J. Multi-species biofilms of environmental microbiota isolated from fruit packing facilities promoted

922 tolerance of *Listeria monocytogenes* to benzalkonium chloride. *Biofilm*, 2024. **7**, 100177.
923 https://doi.org/10.1016/j.bioflm.2024.100177
924 83. Carpentier, B. and Chassaing, D. Interactions in biofilms between *Listeria monocytogenes*
925 and resident microorganisms from food industry premises. *Int J Food Microbiol*, 2004. **97**(2):
926 p. 111-22. https://doi.org/10.1016/j.ijfoodmicro.2004.03.031
927 84. Ottesen, A., Ramachandran, P., Chen, Y., Brown, E., Reed, E. and Strain E. Quasimetagenomic
928 source tracking of *Listeria monocytogenes* from naturally contaminated ice cream. *BMC*
929 *Infect Dis*, 2020. **20**(1): p. 83. https://doi.org/10.1186/s12879-019-4747-z
930 85. Wagner, E., Fagerlund, A., Langsrud, S., Møretrø, T., Jensen, M.R. and Moen, B. Surveillance
931 of *Listeria monocytogenes*: early detection, population dynamics, and quasimetagenomic
932 sequencing during selective enrichment. *Appl Environ Microbiol*, 2021. **87**(24): p. e0177421.
933 https://doi.org/10.1128/AEM.01774-21
934 86. Bruhn, J.B., Vogel, B.F. and Gram, L. Bias in the *Listeria monocytogenes* enrichment
935 procedure: lineage 2 strains outcompete lineage 1 strains in University of Vermont selective
936 enrichments. *Appl Environ Microbiol*, 2005. **71**(2): p. 961-7.
937 https://doi.org/10.1128/AEM.71.2.961-967.2005
938 87. Locatelli, A., Lewis, M.A. and Rothrock, M.J. The distribution of *Listeria* in pasture-raised
939 broiler farm soils is potentially related to University of Vermont medium enrichment bias
940 toward *Listeria innocua* over *Listeria monocytogenes. Front Vet Sci*, 2017. **4**.
941 https://doi.org/10.3389/fvets.2017.00227
942 88. Besse, N.G., Barre, L., Buhariwalla, C., Vignaud, M.L., Khamissi, E., Decourseulles, E.,
943 Nirsimloo, M., Chelly, M. and Kalmokoff, M. The overgrowth of *Listeria monocytogenes* by
944 other *Listeria* spp. in food samples undergoing enrichment cultivation has a nutritional basis.
945 *Int J Food Microbiol*, 2010. **136**(3): p. 345-51.
946 https://doi.org/10.1016/j.ijfoodmicro.2009.10.025

947 89. Al-Zeyara, S.A., Jarvis, B. and Mackey, B.M. The inhibitory effect of natural microflora of food
948 on growth of *Listeria monocytogenes* in enrichment broths. *Int J Food Microbiol*, 2011.
949 **145**(1): p. 98-105. https://doi.org/10.1016/j.ijfoodmicro.2010.11.036

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951

952 FIGURE LEGENDS

953 **Figure 1**. **Changes in bacterial diversity in the different areas of the factory over ten weeks.** A and
954 B) Alpha diversity evaluated with richness, Shannon and Simpson indexes depending on A) time and
955 B) area of the factory. Each boxplot represents the interquartile range (IQR) of the alpha diversity
956 indexes values and the whiskers represent the minimum and maximum values. C) Principal
957 Coordinate Analysis of the Bray-Curtis distance level. The ellipses represent normal data ellipses for
958 the samples in the preparation and production areas of the factory. Statistical comparisons with the
959 A) Kruskal-Wallis, B) Wilcoxon and C) Adonis tests are presented in supplementary materials.
960 Significant differences for alpha diversity (p-value < 0.05) are indicated in the graphs with an asterisk
961 (*).

962 **Figure 2. Relative abundance of the environmental samples.** Bubble plots representing the relative
963 abundance of A) genus and B) the 15 most abundant species identified in the factory environment.
964 Each column represents a sample and samples have been grouped by area of the factory and
965 coloured by time of collection of the sample. C) Comparison of the relative abundance at production
966 and preparation areas for species exhibiting significant differences in abundance, as determined by
967 ANCOM-BC analysis. Each boxplot represents the interquartile range (IQR) of the relative abundance
968 and the whiskers represent the minimum and maximum values.

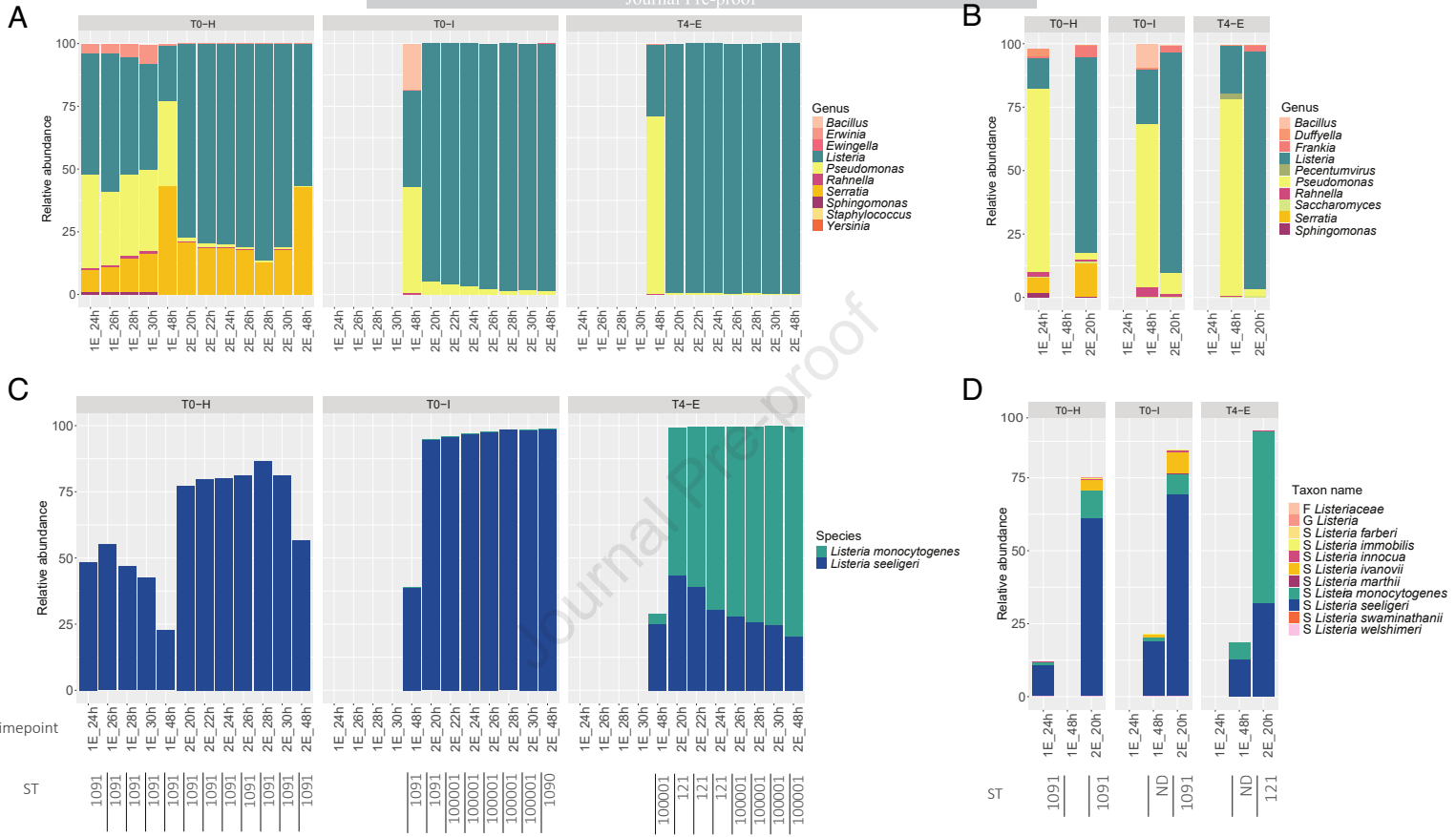
969 **Figure 3. Microbial dynamics of *Listeria* enrichments in samples positive for *L. monocytogenes* and**
970 ***L. seeligeri.*** Barplots show the relative abundance of A) and B) the 10 most abundant genera and C)

and D) the 10 most abundant *Listeria* species identified in the enrichment using A) C) Illumina or B) D) nanopore sequencing. Timepoints 1E and 2E indicate primary and secondary enrichments respectively. The tables in panels C) and D) show the ST identified from raw reads (100001 refers to new loci-sequences or new STs [40]).

Supplementary materials

Supplementary.xlsx

Supplementary_figures.PDF



Highlights

- Microbiota on non-food contact surfaces remains stable in factory environment.
- Distinctive microbiota observed in different factory areas.
- Microbiota adapted to environmental conditions within factory.
- *Listeria monocytogenes* and *Listeria* spp. are present on surfaces at low abundance.

Declarations of interest: none