

Article

Microbial Ecology Evaluation of an Iberian Pig Processing Plant through Implementing SCH Sensors and the Influence of the Resident Microbiota on *Listeria monocytogenes*

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Featured Application: An interesting control strategy for *L. monocytogenes* biofilm presence on surfaces of the food industry may be the growth of certain microbial communities that displace the pathogen.

Abstract: There is a whole community of microorganisms capable of surviving the cleaning and disinfection processes in the food industry. These persistent microorganisms can enhance or inhibit biofilm formation and the proliferation of foodborne pathogens. Cleaning and disinfection protocols will never reduce the contamination load to 0; however, it is crucial to know which resident species are present and the risk they represent to pathogens, such as *Listeria monocytogenes*, as they can be further used as a complementary control strategy. The aim of this study was to evaluate the resident surface microbiota in an Iberian pig processing plant after carrying out the cleaning and disinfection processes. To do so, surface sensors were implemented, sampled, and evaluated by culture plate count. Further, isolated microorganisms were identified through biochemical tests. The results show that the surfaces are dominated by *Bacillus* spp., *Pseudomonas* spp., different enterobacteria, *Mannheimia haemolytica*, *Rhizobium radiobacter*, *Staphylococcus* spp., *Aeromonas* spp., lactic acid bacteria, and yeasts and molds. Moreover, their probable relationship with the presence of *L. monocytogenes* in three areas of the plant is also explained. Further studies of the resident microbiota and their interaction with pathogens such as *L. monocytogenes* are required. New control strategies that promote the most advantageous profile of microorganisms in the resident microbiota could be a possible alternative for pathogen control in the food industry. To this end, the understanding of the resident microbiota on the surfaces of the food industry and its relation with pathogen presence is crucial.

Keywords: food industry; surface sensors; resident microbiota; microbial ecology; *Listeria monocytogenes*; ecological interrelations

1. Introduction

Despite all the efforts to eliminate the microorganisms present on the surfaces in the food industry, certain microbial communities can persist forming the resident microbiota. Important preventative measures against bacterial persistence are a judicious use of water, keeping the premises at a cold temperature, and frequent cleaning and disinfection [1]. In fact, multiple studies show the transfer capacity of microorganisms between food, surfaces, hands, and utensils, among others, highlighting the

relevant role of cross-contamination in foodborne diseases [2–7]. Disinfection does not aim to sterilize surfaces but to reduce their microbial contamination to a safe, suitable level for their use [1]. There is a wide variety of bacterial families and variability in terms of the obtained results since many factors are at play such as the nature of the worked product. Nevertheless, *Pseudomonas* spp., *Enterobacteriaceae*, *Acinetobacter* spp., *Bacillus* spp., *Staphylococcus* spp., and lactic acid bacteria generally dominate on the surfaces of food facilities [8]. Persistent microorganisms can reach the final products through cross-contamination and consequently spoil them.

These resident microorganisms can either inhibit the proliferation of pathogens or, on the contrary, enhance their establishment in mixed biofilms [9]. Any microorganism, pathogen, or spoilage such as *Pseudomonas* spp. and *Listeria monocytogenes* can form biofilms [10]. The resident microbiota can have a significant effect on the probability of finding *L. monocytogenes* on food premises [11]. For example, in the presence of a natural microbiota on wooden shelves, inoculated *L. monocytogenes* remained stable or even decreased to 2 log (CFU/cm²) after twelve days of incubation at 15 °C under all conditions tested. However, *L. monocytogenes* increased to 4 log (CFU/cm²) when the resident biofilm was thermally inactivated [12], suggesting that the ecosystem residing in wooden shelves is able to control certain pathogens. *L. monocytogenes* can also frequently be isolated after sanitation and still remain the most challenging microbial threat to the food industry, including the meat processing industry [13]. *L. monocytogenes* persistence appears to be strongly linked to the manufacture of products and not to the sustained arrival of raw material. Ortiz et al. showed that some clones survived in a studied manufacturing area for three years [14]. On the other hand, resident bacteria may play a role in the persistence and spread of antimicrobial resistance genes [15]. For these reasons, progress in the identification of established bacteria in food processing environments is essential. Few studies have characterized the resident species and their interactions with foodborne pathogens such as *L. monocytogenes*. On a practical level, conventional methods for surface sampling are used, such as swabs or sponges, which in certain cases do not guarantee the complete recovery of cells within biofilms [16]. Additionally, the most common procedure is to culture samples in a non-specific media at a temperature of 30 °C, thus missing the opportunity to identify psychrotrophic microorganisms. Another relevant aspect is the time lapse and temperature between collecting and analyzing the samples because these can alter the results [8]. Last, the proposed approach to the microbiological control of food contact surfaces has previously been the maximum reduction of the microbial load. Products and strategies have been designed to maximize cleaning and disinfection operations. However, a potentially interesting approach that has not yet been considered is the use of microorganisms with the ability to compete with pathogens, thereby preventing their growth. A recent study proposed that the hygienic theory of the surfaces traditionally used in the food industry could be reconsidered using this type of microorganisms, provided they have no type of spoilage effect on the food products [16].

Overlapping with a macro quantitative study of the contamination load on the surfaces of an Iberian pig processing plant carried out by Ripolles-Avila et al., the objective of this study was to analyze the resident microbiota in the same thirteen areas of two meat processing plants [16]. The specific purpose was to identify the resident microorganisms (aerobic mesophilic bacteria, lactic acid bacteria, and yeasts and molds) after the cleaning and disinfection processes by means of implementing SCH surface sensors (SCH; Hygiene Control Sensor). Another objective was to compare the existing species in the different areas that could have a positive or negative effect on the presence of *L. monocytogenes*. As a long-term aim, this study was conducted to investigate the presence of possible inhibitors or enhancers of this persistent foodborne pathogen in the industry's microbiota to reinforce the control strategies of *L. monocytogenes* and optimize cleaning and disinfection protocols.

2. Materials and Methods

2.1. Study Approach

The study was carried out in two Iberian pig processing plants (A and B) belonging to the same company. The activity in Plant A is mainly the slicing and packaging of raw meat provided by Plant B to produce cured meat products ready for consumption. The latter has a slaughterhouse which can slaughter 300 animals per day. The production process is generally based on the slaughter of the animals producing carcasses, which is followed by the meat cutting phase. After refrigeration, these products go for salting, chopping, or pickling to make sausages. The process finishes with curing or ripening and further dispatch [14]. This industry was one of the first Spanish slaughterhouses to export pork meat products to the United States. The company has difficulty controlling *L. monocytogenes*, which is repeatedly found in final products such as “chorizo”, a Spanish traditional sausage.

This ecological analysis overlapped temporally (16 non-consecutive weeks) with another long-term quantitative study of the same surfaces. Ripolles-Avila et al. monitored the microbial contamination of both plants for twenty-one months (May 2016–January 2018), taking a total of approximately 980 samples collected weekly from the thirteen locations on the surfaces (Table 1) where the SCH sensors were installed (Premiumlab, Barcelona, Spain) [16].

Table 1. Work surfaces from Plants A and B where the SCH sensors were installed (coded from 1 to 13) [16].

Processing Plant	ID	Surface
A	1	Sump in the deboning room
	2	Slicer A
	3	Sump in the slicing room
B	4	Floor of the carcasses airing room
	5	Storage cabinet for tools
	6	Floor of the work room
	7	Floor of the fresh meat carts cleaning room
	8	Floor of the cured meat carts cleaning room
	9	Slicer B
	10	Iberian sausage transportation carts
	11	Slide of vacuum machine
	12	Floor of the heat treatment room
	13	Sink

2.2. Surface Sensors

The SCH sensors were AISI 316 grade 2B stainless steel coupons (2 cm in diameter and 1 mm thick) coupled to a base through the action of neodymium magnets and coated with epoxy paint. These bases, which were also made of stainless steel, could hold three coupons simultaneously, thus facilitating their weekly analysis for three consecutive weeks. The supports were welded to the areas to be evaluated (Figure 1). This tool enabled the coupons to be in the same conditions as the rest of the surfaces in that area. The sensors were then subjected to the same contamination and cleaning/disinfection protocol as the surfaces to be sampled [17], allowing the natural biofilms that may have formed on the surfaces to be analyzed. To this effect, Moen et al. indicated that stainless steel coupons are suitable for analyzing the natural microbiota of the surrounding environment and they have been used in different subsequent studies [18]. More concretely, a variation of just 5.1% on species richness between the sensors and the sinks (the real study material of their research) was demonstrated.



Figure 1. Examples of their placement in the collaborating industry: (a) Fresh meat carts cleaning room floor; (b) slicer B; and (c) vacuum machine slide. (d) Design of a SCH surface sensor [19].

2.3. Sampling Procedure and Recovery of Adhered Microorganisms

Stainless-steel coupons that had been present in the facilities for three weeks were collected every week for sampling. The sensor was extracted from its support using a sterile magnetized bar and deposited aseptically in a sterile flask. The flasks carrying the sensors were sent to the laboratory in an expanded polystyrene box to isolate the samples thermally.

The technique of recovery by agitation with pearls (UNE-EN 13697:2015) was chosen to detach the microorganisms from the sensors. The samples were transferred to sterile flasks containing 3.5 g of glass beads (2 mm diameter) and 9 mL of peptone water (BioMérieux, Marcy l’Etoile, France). The flasks were vortexed for 1.5 min at a frequency of 40 Hz. This stirring technique enables a high recovery of microorganisms, resulting in a real microbial load count and high reproducibility [17]. Decimal dilutions of the resulting suspension were done in peptone water and transferred to different culture media. The samples were sowed on Plate Count Agar plates (PCA; Oxoid, Madrid, Spain) and left for 48 h at 30 °C before proceeding with the identification of the resulting colonies. This process was also performed on Man, Rogosa and Sharpe agar (MRS, Oxoid, Madrid, Spain) for the isolation of lactic acid bacteria.

2.4. Identification Methodology

To identify a representative sample of the resident microorganisms on these surfaces, random isolations of 10% were made of each of the morphologically different colonies on the PCA plates. In the first phase, all the colonies to be isolated were picked with a flamed inoculation needle and transferred to a Tryptic Soy Agar medium (TSA; Oxoid, Madrid, Spain). This process was carried out for eight weeks. Three consecutive sows were then achieved in TSA agar for between 18–24 h at 37 °C to purify each isolated microorganism. Afterwards, different tests such as catalase, oxidase, KOH test, and optical microscopy observation with Gram stain were performed with the aim of using the information as the basis for the choice of subsequent biochemical tests [20]. The microorganisms were conserved in inclined TSA agar tubes under refrigeration conditions (± 4 °C) until they were identified. In a third phase, a representative amount of the samples was identified using the BD BBL™ Crystal™ identification system for Gram positive and API® 20E and 20NE kits for Gram-negative Enterobacteriaceae and non-Enterobacteriaceae, respectively. The possible yeasts and molds found in the previous stage were cultured in Sabouraud Glucose Agar with chloramphenicol medium (SAB; Sigma-Aldrich, Madrid, Spain) (five to seven days at room temperature) and identified by API® 20C AUX. Later, the identification of lactic acid bacteria was undertaken. The same procedure was carried out with MRS agar (48 h at 30 °C). An API® 50 CHL test was carried out on all the catalase negative bacteria isolated from the MRS to identify the species present. The instructions of use provided by the

manufacturing companies were followed for all the mentioned kits, inoculating multiple test strips that harbored a battery of specific biochemical tests, as performed by other researchers such as [21].

2.5. Statistical Analysis of the Data

The relationship between the studied areas was determined by means of the similarity of the mesophilic aerobes profile. Following other authors such as Feligini et al., these locations were classified into clusters through a hierarchical clusters analysis [22]. In this case, the analysis of hierarchical conglomerates was the most appropriate approach since the number of clusters was not known a priori and the number of areas to be classified was small (eleven areas). Thus, the statistical program measured the proximity between two conglomerates by calculating the average of the distances between objects in the two groups. A matrix of proximity between the objects was generated from the Euclidean distances between all the sites. Last, the distances between clusters of sampled areas were represented by a dendrogram.

3. Results and Discussion

3.1. Identified Species

A total of 523 microorganisms were isolated from PCA and MRS agar culture media. Of these, 240 catalase positive isolates were discarded from the MRS medium since only the presence of lactic acid bacteria was investigated in this medium. Two-hundred microorganisms with different profiles were identified from these isolates, including mesophilic aerobic bacteria, lactic acid bacteria, and yeasts and molds. The results of the identifications are shown below in Tables 2–4, ordered from the greatest to the least presence on the surfaces.

Overall, in terms of mesophilic aerobes, there was a higher proportion of Gram-negative bacteria (57.27%) (Table 2). According to the study presented by Møretro et al., Gram-negative bacteria such as *Pseudomonas* spp. have a greater capacity to form biofilms than Gram-positive bacteria [13].

The presence of the genera *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Aeromonas* spp., *Serratia* spp., *Enterobacter* spp., *Ralstonia* spp., *Proteus vulgaris*, or *Stenotrophomonas maltophilia* has also been described in raw meat cold stores [23]. In the present study, the major bacterial genera found were *Bacillus* spp. (28.18% of the isolated bacteria) and *Pseudomonas* spp. (21.82%). The species identified within the genera *Bacillus* spp. were *Bacillus subtilis* (86.96%), *Bacillus megaterium* (6.52%), and *Bacillus licheniformis* (6.52%). The identified species of the genus *Pseudomonas* spp. were *Pseudomonas fluorescens* (40.00%), *Pseudomonas luteola* (40.00%), and *Pseudomonas stutzeri* (20.00%). These results are in concordance with other surfaces studies on which *Pseudomonas* spp. was predominant [24–27]. Stellato et al. also evaluated a beef and pork processing plant, identifying *Pseudomonas* spp. and several species of enterobacteria as major components of the surface microbiota [28]. In addition, according to Marouani-Gadri et al., the dominant genera in another meat industry (beef slaughterhouse and cutting room) were *Staphylococcus* spp. and *Bacillus* spp. [29]. Like in the present study, *Lactobacillus* spp., *Staphylococcus* spp., and *Enterobacter* spp. were also present.

Mannheimia haemolytica (9.17%), *Rhizobium radiobacter* (7.34%), *Staphylococcus* spp. (6.42%), and *Aeromonas* spp. (5.50%) (Table 2) were also found in the Iberian pig processing plant, in line with Hoodt and Zottola and Mertz et al. for *Aeromonas* spp. [27,30]. In another slaughterhouse, also analyzed after the cleaning and disinfection processes, the identified genera from a non-selective medium were *Aerococcus* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Serratia* spp. [13]. Furthermore, in another study conducted by Maes et al., the most abundant genera of the microbiota present on the food contact surfaces were *Pseudomonas* spp., *Microbacterium* spp., *Stenotrophomonas* spp., *Staphylococcus* spp., and *Streptococcus* spp. [26]. All these findings suggest that the type of microbiota found can be different depending on the surface area and the food industry, which may have a direct influence on the final hygiene of the food product.

Table 2. Percentage of identified genera and species from Plate Count Agar (PCA) and its corresponding Gram stain (Gram positive or negative bacteria).

Identified Species	% vs Isolated Bacteria	% vs Isolated Microorganisms
Molds and yeasts	-	29.03
<i>Bacillus</i> spp. ¹	28.18	20.00
<i>Pseudomonas</i> spp. ²	21.82	15.48
<i>Mannheimia haemolytica</i>	9.09	6.45
<i>Rhizobium radiobacter</i>	7.27	5.16
<i>Staphylococcus</i> spp.	6.36	4.52
<i>Aeromonas</i> spp.	5.45	3.87
<i>Leifsonia aquatica</i>	3.64	2.58
<i>Serratia</i> spp.	3.64	2.58
<i>Enterobacter asburiae</i>	3.64	2.58
<i>Proteus vulgaris</i>	1.82	1.29
<i>Corynebacterium</i> spp.	1.82	1.29
<i>Helcococcus kunzii</i>	0.91	0.65
<i>Aerococcus urinae</i>	0.91	0.65
<i>Gardnerella vaginalis</i>	0.91	0.65
<i>Stenotrophomonas maltophilia</i>	0.91	0.65
<i>Ewingella americana</i>	0.91	0.65
<i>Ralstonia pickettii</i>	0.91	0.65
<i>Vibrio</i> spp.	0.91	0.65
<i>Ochrobactrum anthropi</i>	0.91	0.65
Total	100.00	100.00
Gram stain	%	
Gram positive	42.73	-
Gram negative	57.27	-
Total	100.00	-

¹ Blue indicates that the bacteria are Gram-positive. ² Orange indicates that the bacteria are Gram-negative.

Table 3. Identified lactic acid bacteria from Man, Rogosa and Sharpe agar (MRS), in number of isolates and respective percentage from the total of lactic acid bacteria.

Identified Species	Total	%
<i>Leuconostoc mesenteroides</i> ssp. <i>cremoris</i>	23.0	51.1
<i>Lactobacillus delbrueckii</i> ssp. <i>delbrueckii</i>	8.0	17.8
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	6.0	13.3
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>	4.0	8.9
<i>Lactobacillus acidophilus</i>	1.0	2.2
<i>Pediococcus damniosus</i>	1.0	2.2
Non-identified	2.0	4.4
Total	45.0	100.0

It has been demonstrated that food industry surfaces, including those of the equipment, present an entire microbial ecosystem both during production and after cleaning and disinfection. The microbiota present is partly a reflection of the raw material used [31]. In this case, starter cultures are used to produce raw-cured meat. A typical starter for the preparation of raw-cured sausages is composed of *Lactobacillus sakei*, *Staphylococcus xylosum*, and *Staphylococcus carnosus* [32]. In this regard, different species of *Lactobacillus* spp. which can be used as starters were found in both Plant A and Plant B of the present study (Table 3), thus indicating that microbiota from food is transferred to the surfaces. Regarding lactic acid bacteria, the predominant species were *Leuconostoc mesenteroides* ssp. *cremoris*, which commonly cause the spoilage of food packaged in a modified atmosphere and stored in cold conditions and were detected in abundance in a study carried out on sausages [25,33,34].

Table 4. Identified molds and yeasts from cultures in PCA, in number of isolates and respective percentage from the total of lactic acid bacteria.

Identified Species	Total	%
<i>Candida zeylanoides</i>	27.0	61.4
<i>Cryptococcus uniguttulatus</i>	3.0	6.8
<i>Candida krusei</i>	2.0	4.5
<i>Candida cifferri</i>	2.0	4.5
<i>Candida</i> spp.	2.0	4.5
<i>Candida pelliculosa</i>	1.0	2.3
<i>Candida magnoliae</i>	1.0	2.3
<i>Cryptococcus terreus</i>	1.0	2.3
Non-identified	5.0	11.4
Total	44.0	100.0

In this study, of the close to 30% of yeasts and molds, *Candida* spp. was the most abundant yeast followed by *Cryptococcus* spp. (Table 4). These results also coincide with other studies [24,35]. However, few studies that characterize the surface microbiota mention the isolated species of yeasts and molds, like Chevallier et al. and Talon et al. [36,37].

3.2. Ecological Profiles of the Different Areas: Cluster Analysis

According to the analysis by hierarchical clusters, the ecological profiles of the sampled areas can be grouped into four clusters, although only two of them are relevant. The first cluster was composed of areas 13 (sink), 5 (storage cabinet for tools), 9 (slicer B), 3 (sump in the slicing room), and 4 (carcasses airing room floor). The distance between these areas is short, so their ecological profiles can be considered as similar (Figure 2). The second main cluster is made up of areas 6 (work room floor), 7 (fresh meat carts cleaning room floor), and 10 (Iberian sausage transportation carts). The areas 8 (cured meat carts cleaning room floor) and 2 (slicer) form the other two clusters, each one on its own. These two profiles were remarkably different from the rest and could therefore be considered as outliers. As the technique of classification by hierarchical conglomerates is sensitive to the presence of outliers they can appear as two differentiated clusters.

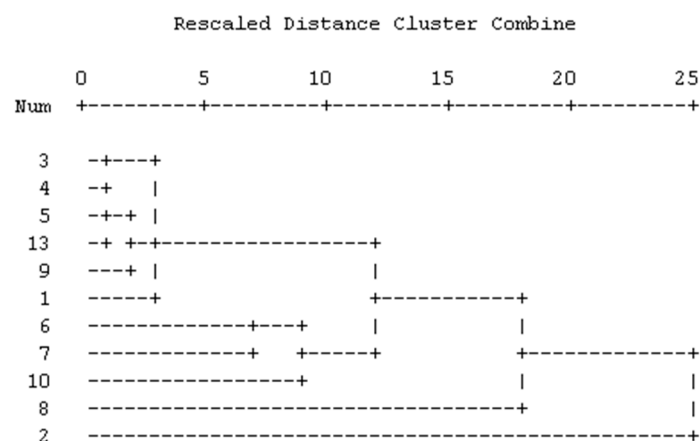


Figure 2. Analysis by hierarchical clusters showing the degree of similarity of the ecological profile of the sampled areas. There are two main clusters: One grouping with areas 3, 4, 5, 13, 9, and 1 and another with areas 6, 7, 7, and 10.

Ecological profiles could not be established in areas 11 (vacuum machine slide) and 12 (heat treatment room floor) (Table 1) due to the lack of growth on the PCA plates. In fact, in the quantitative study carried out by Ripolles-Avila et al. these two areas were described as the least microbiologically contaminated [16].

Cluster 1 grouped the areas dominated by *Pseudomonas* spp. (Figure 3). These areas may have similar ecological profiles because they intervene in successive stages of the productive process. The carcasses are taken from the carcass airing room to the cutting room where the manipulated tools are stored in the storage cabinet. Therefore, how the different areas may be related to each other and how the movement of microbiota between them can occur are very relevant, especially in the transfer of pathogens such as *L. monocytogenes*. Area 13 (sink) also had the same proportion of *Bacillus* spp. and *Pseudomonas* spp., so it could be considered as belonging to both clusters. The work room receives products from the cutting room and processes such as chopping are carried out there. In this conglomerate, four of the six areas presented 13 to 50% of *Aeromonas* spp. and less abundantly *Bacillus* spp., *Rhizobium radiobacter*, *Corynebacterium* spp., and *Leifsonia aquatica*.

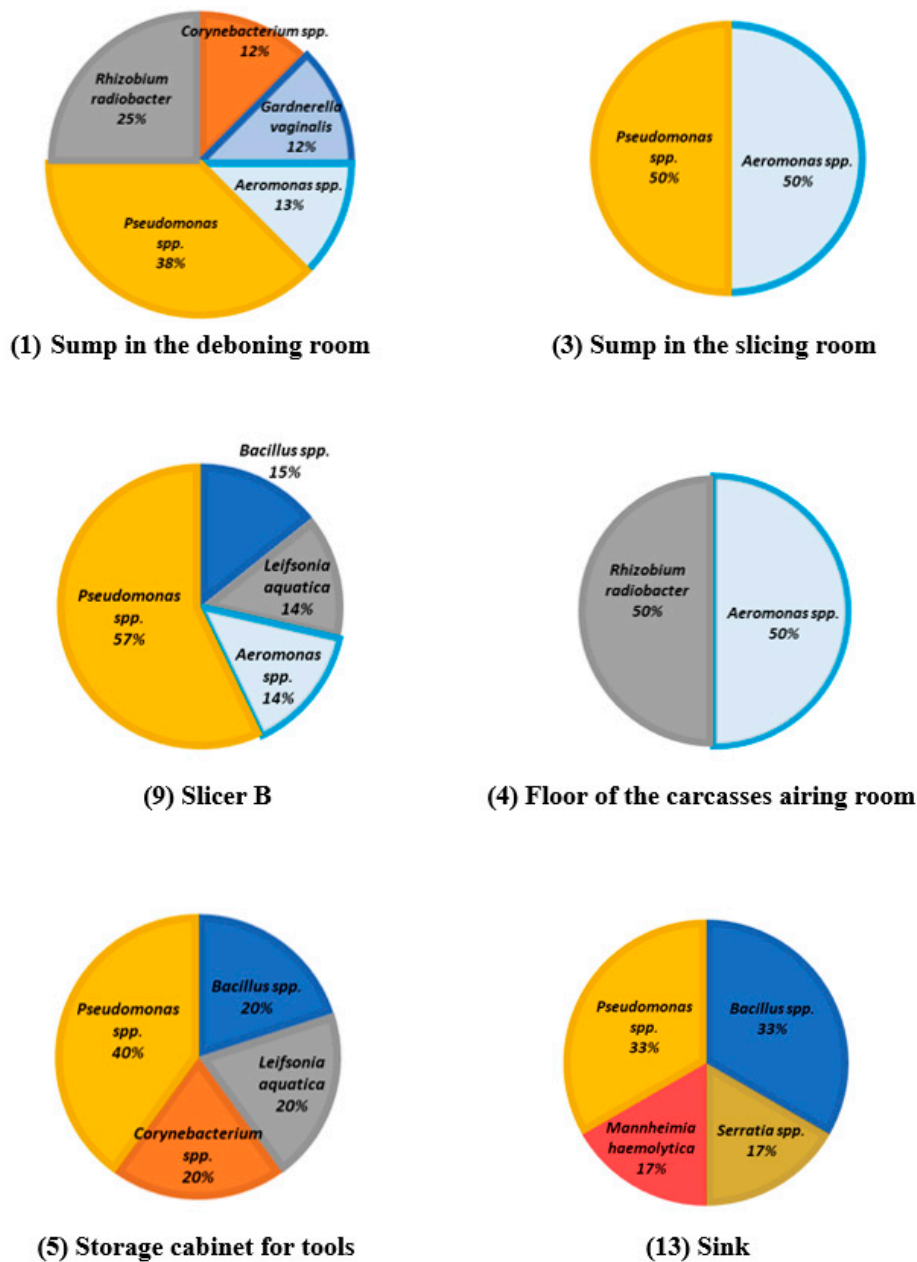


Figure 3. Individual profiles of the areas categorized as cluster 1. *Pseudomonas* spp. was predominant in most areas and there was also a notable presence of *Aeromonas* spp.

Like in the present study, Hoodt and Zottola identified the presence of *Pseudomonas* spp. and *Aeromonas* spp. in the sinks [30]. In other studies, *Pseudomonas* spp. [27,37], *Enterobacteriaceae*, and yeasts and molds have been found on knives, tables, and chopping machines [35]. *Pseudomonas* spp. is commonly found in soil and water where it plays an important role in the degradation of organic material. They are part of the normal microbiota of human skin and are found in the respiratory tract and intestines [38]. *Aeromonas* spp. is also ubiquitous, usually found in water sources, soil, arthropods, mollusks, mammals, birds, fish, and insects [39]. *Corynebacterium* spp., present in the sump in the deboning room and tools storage cabinet, also has a global distribution and is found in soils, water, animals, and plants, especially in temperate areas [40]. The less common *Leifsonia aquatica*, isolated from slicer B and the tool storage cabinet (Figure 3), could come from the water since it inhabits the aquatic environment. It is characterized by having a low growth rate and the capacity to form biofilms [41].

In this second cluster, areas 6 (work room floor), 7 (cured meat carts cleaning room floor), and 10 (Iberian sausage transportation carts) were mostly colonized by *Bacillus* spp. (Figure 4). This genus includes many of the most ubiquitous bacteria [42]. These areas are physically close and the same personnel and equipment, such as the transportation carts, raw material, and fresh products, circulate there. The fresh meat is processed in the work room and it is transported by the carts, so the same residues can be found in the work room as in the fresh meat carts cleaning room floor. Microbial communities are representative of each processing area and are influenced to a large extent by food debris, liquid effluents, and wash water [25]. *R. radiobacter*, isolated in the three areas that comprise this cluster, is also a ubiquitous bacterium and is usually found in soil, plants, etc. [43]. *Serratia* spp., another common microorganism in the area between the work room floor and the sink, has also been isolated from industries that manufacture sausages [44]. *S. liquefaciens* is one of the dominant enterobacteria in raw meat working plants. Another species of the genus *Serratia* spp. that is sometimes identified is *S. marcescens* [44]. The results obtained in the present study are in concordance with these findings, as both species were found on the surfaces in Plant B. *Enterobacter* spp., belonging to the family *Enterobacteriaceae* and widely distributed in nature, were found between the floor of the fresh meat cart cleaning room and the Iberian sausage transportation carts. They can be found in soil, water, and as part of the microbiota of animals, insects, and the human gastrointestinal tract [45].

Clusters 3 and 4 correspond to areas 8 (cured meat carts cleaning room floor) and 2 (slicer A), respectively. The analysis by hierarchical clusters determined that these two profiles were distant from the other two main clusters (1 and 2). This could be explained by the presence of non-isolated species such as *Staphylococcus* spp., *Stenotrophomonas maltophilia* in the other areas, and the strong presence of *M. haemolytica* in area 8 (cured meat carts cleaning room floor) (Figure 5). The atypical profile of location (8) could be explained by the fact that the carts transport a different raw material. They do not displace fresh products, but rather genera that have undergone a process of healing or maturation in which the microbiota is modified [35]. In addition, this room is on the upper floor of Plant B, unlike almost all the other areas where sensors were located (except for slicer B).

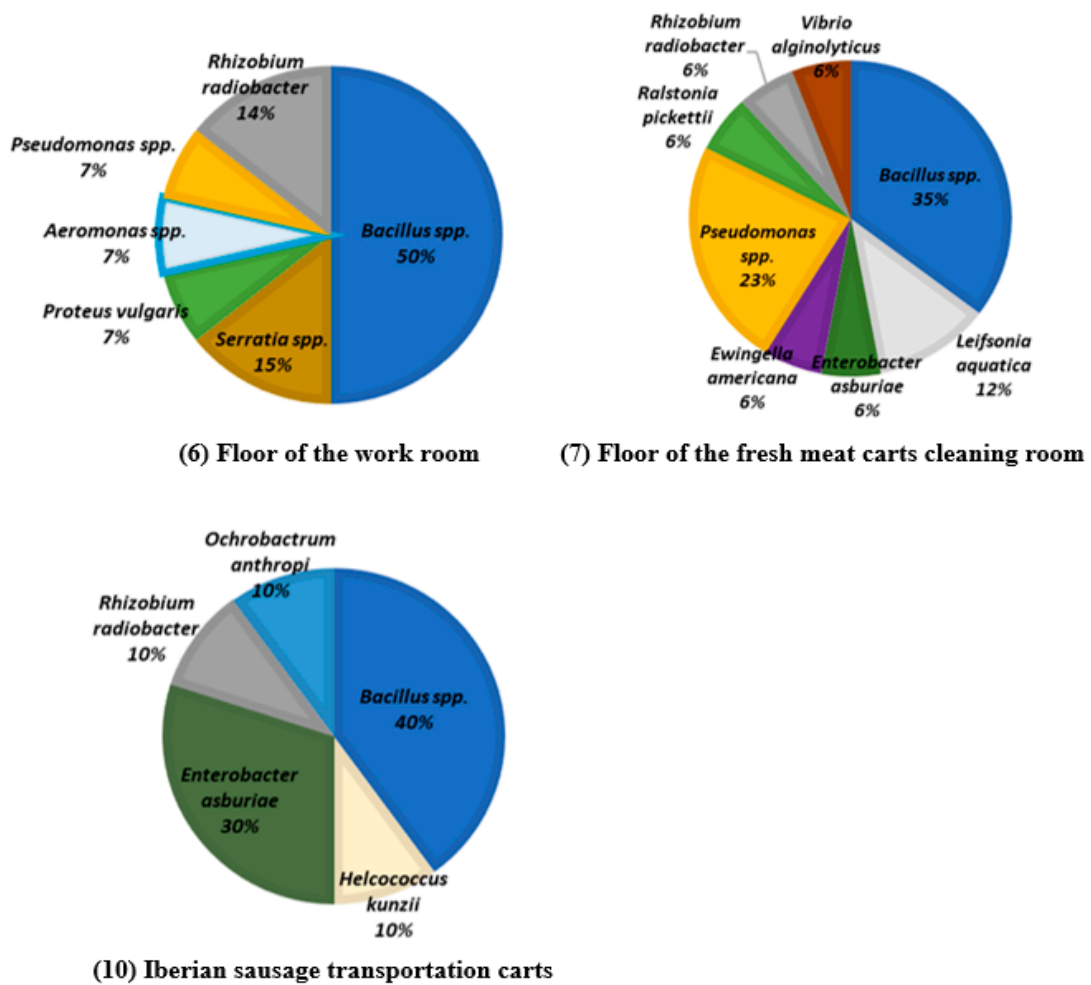


Figure 4. Individual profiles of the areas categorized as cluster 2. *Bacillus spp.* was predominant in all the areas and there was a marked presence of *Rhizobium radiobacter* and *Enterobacter asburiae*.

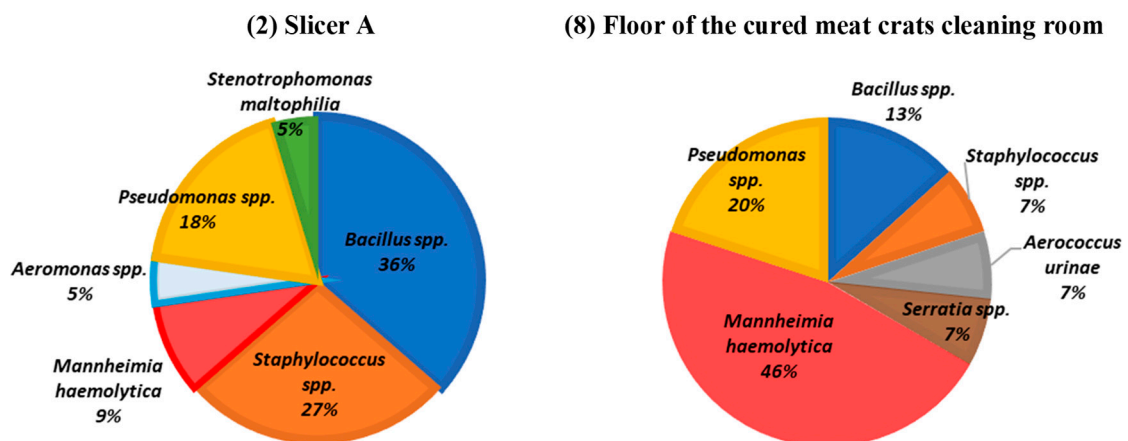


Figure 5. Individual profiles of the categorized areas, such as cluster 3, (8) cured meat carts cleaning room floor, and cluster 4, (2) slicer A.

3.3. Unusual Identification of *Mannheimia haemolytica*

The identification of *M. haemolytica* in the sensors located in areas 2 (slicer A), 8 (cured meat carts cleaning room floor), and 13 (sink) was unexpected. This bacterium occasionally intervenes in the porcine respiratory complex, although it is much more frequent in bovines where it also causes respiratory disease. Ahmed and Sabiel also identified *M. haemolytica* in minced beef [21].

One hypothesis is that because this opportunistic pathogen can be found in the respiratory tract of animals, it could contaminate the carcasses by means of incorrect handling in the respiratory tract removal stage. Another hypothesis could be a failure in identification when using the API[®] 20E test. In fact, Ahmed and Sabiel also used this commercial kit to identify their isolates [21]. According to the results provided by the API[®] 20E kit, in six of the ten isolates of this microorganism in this study, the probability percentage (ID %) was equal to or greater than 84.6%, reaching up to 98.5%. When API[®] kits had already been in use for twenty years, Hara et al. re-evaluated the method, concluding that it was reliable compared with traditional biochemical tests [46]. As a final aspect to be considered, *M. haemolytica* has undergone an extensive reclassification, previously known as *Bacterium bipolare multocidum*, *Bacillus bovisseptica*, and *Pasteurella haemolytica* [47]. Despite the new classification, the correct identification of the isolates continues to be difficult and laborious [48].

3.4. Influence of the Microbiota on the Presence of *L. monocytogenes*

In the quantitative study carried out by Ripolles-Avila et al., *L. monocytogenes* was isolated on five occasions from areas 5 (i.e., tools storage cabinet; serotype 4b), 8 (i.e., cured meat carts cleaning room floor; serotype 4b and 1/2a), and 10 (i.e., Iberian sausages transportation carts; serotype 1/2b) [16].

Analyzing the ecological profile of the areas where *L. monocytogenes* was found, the tools storage cabinet presented 40% of *Pseudomonas* spp., the majority genera of the community present (Figure 2). The effect of *Pseudomonas* spp. on the growth of *L. monocytogenes* has not been precisely described, since various authors have shown a positive [27], a negative, and no effect [8]. The cured meat carts cleaning room floor showed an atypical profile with the strong presence of *M. haemolytica*. It must be noted, however, that the genera with the most isolates was *Pseudomonas* spp., which has been shown to enhance the presence of *L. monocytogenes* [27,49] in some studies. It could also influence, in this sense, 7% of *Serratia* spp. since enterobacteria appear to favor *L. monocytogenes* growth [35,50]. Macroscopically, species such as *Pseudomonas* spp. can produce a viscous substance when colonizing a surface. Hoodt and Zottola suggested that these microorganisms produce a dense extracellular material that could allow the entrapment of other microorganisms [30]. These primary colonizers of meat processing plants could harbor pathogens such as *L. monocytogenes*. As for the Iberian sausage transportation carts, 30% of *Enterobacter asburiae* could be relevant since it has been observed that other enterobacteria have a positive effect on the growth of *L. monocytogenes* [35,50]. In this regard, the results obtained show that in the areas where *L. monocytogenes* was found, the surfaces contain a microbiota composed of microorganisms that could enhance *L. monocytogenes* growth. More in vitro studies are needed to demonstrate how ecological interrelations affect the formation of *L. monocytogenes* biofilms as a previous step to the application of this alternative at an industrial level. Some methodologies have been proposed for their formation and quantification that resemble real conditions [51,52]. The control of these microorganisms could also minimize the presence of this pathogen [16].

4. Conclusions

The ecological profiles of meat processing plants can vary depending on the raw material, temperature, humidity, and industrial processes carried out. The way to monitor these communities of microorganisms should be standardized both in the collection of the samples and the analysis. The microbiota of the studied Iberian pig processing plant was dominated by *Bacillus* spp. and *Pseudomonas* spp. The areas could be grouped into two main clusters and the profile of the areas and the ecological diversity was varied, enabling the correlation of the presence or absence of *L. monocytogenes*. This persistent pathogen seems to be associated with one or more specific bacterial groups such as *Pseudomonas* spp. and enterobacteria, which belong to the resident microbiota of the facilities. A high level of contamination does not always suggest the presence of these pathogens, but rather depends on the established species. Knowing the species that persist in the production plants and their interactions with *L. monocytogenes* can help to profile cleaning and disinfection programs. The control of resident microbiota is, therefore, a key element to guarantee food safety and the quality of the final products.

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