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Title: Evaluation of the microbiological contamination of food processing environments through implementing surface sensors in an Iberian pork processing plant: An approach towards the control of Listeria monocytogenes.

Article Type: Research Paper

Keywords: sampling; surface sensors; microbial contamination; Listeria monocytogenes; ecology

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Abstract: Food safety is one of the biggest concerns of food industrial development due to the risk of foodborne diseases, which are one of the most relevant health problems in the contemporary world and an important cause of reduced economic productivity. One of the main sources of microbial contamination in food products are industrial surfaces, which are colonized by pathogenic microorganisms capable of forming biofilms, making surfaces into reservoirs and potential sources of cross-contamination to food products. A study was conducted to determine the microbiological contamination from different microbial groups on different industrial surfaces in a meat processing plant through implementing a sensor-based sampling system, with a focus on detecting L. monocytogenes. The results obtained showed two main groups of areas with greater and lesser degrees of microbiological contamination, determined as the total aerobic counts of the microbial group with the highest contribution. The areas considered as major contributors to microbial contamination were three of the sampled floors and the storage cabinet for tools, demonstrating to be important sources of possible cross-contamination. A total of four L. monocytogenes presences were obtained during sampling. Moreover, a direct relation was observed between aerobic counts and detecting L. monocytogenes, and three possible hypotheses were formulated to explain the connection. Last, a safety zone marking the limits beyond which the surface can be considered as a safety risk was established, although more studies are needed to demonstrate if these limits can be used as an internal hygienic surface control. The use of SCH sensors as a surface sampling system for the food industry have been shown to work effectively and with relative ease.

Editorial Board
Journal of Food Control

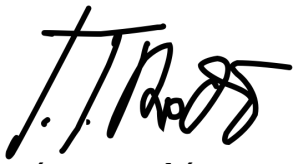
Dear Sirs,

We send a copy of our paper entitled: “Evaluation of the microbiological contamination of food processing environments through implementing surface sensors in an Iberian pork processing plant. An approach towards the control of *Listeria monocytogenes*”, to this Journal, since it could cover one of the aims and scope, the food safety. This topic is in the spotlight at these moments, it is for this reason that we consider that it can be a subject of general interest.

This paper has been developed by my direction at the “Universitat Autònoma de Barcelona”. The aim was to consider the potential of surface sensors for being used as potent technology to control microbiological contamination of food processing environment and, therefore, to apply any type of corrective action that helps to improve the hygiene of the surfaces by not obtaining the presence of certain pathogens. For the purposes of developing the study, surface sensors were installed on an Iberian pork processing plant, and thirteen different areas were monitored for nine different groups of microorganisms. Based on the results obtained, a direct relation was observed between aerobic counts and detecting *L. monocytogenes*, and three possible hypotheses were formulated to explain the connection. One of the possible explanations is the presence of certain inhibitors, which could be exploited, in the future, as a potent technology to control bacterial adherence and biofilm formation in the food industry. Furthermore, a safety zone marking the limits beyond which the surface can be considered as a safety risk was established. We consider that the paper is on the interest for academics and the food industry for further develop biofilm control strategies.

We would be very appreciated if you may consider this work for its publication in Journal of Food Control.

Sincerely yours.



Dr. José Juan Rodríguez Jerez
Bellaterra, October 30th 2018

RESPONSE TO REVIEWERS

We thank the reviewers for their interest in our work and for all the helpful comments. The reviewers have brought up some good points and we really appreciate the opportunity to improve the manuscript.

We have tried to do our best to respond to the points raised and, we hope that these revisions improve the study such that the reviewers now deem it worthy of publication.

All the changes proposed through the revision of the reviews are highlighted in red in order for you to clearly identify what has been changed.

Reviewers' comments:

Reviewer #1: Manuscript Number: FOODCONT-D-18-02665

Overall: This was an excellent study and is a very well-written paper.
Response:
Thank you for all these suggestions that will greatly improve the quality of the manuscript.

Suggestions:
1) In the title, change the period to a colon and add a period to the very end.
Response:
We have introduced this change in the manuscript.

2) Line 75: Remove 'the' before the work 'biofilms'
Response:
We have introduced this change in the manuscript.

3) Lines 81-84: Could the authors please provide more clarity on the second interaction —the current wording is not clear.
Response:
We have changed the wording. Now, between Lines 85-88, you will find: “on the other hand, cooperative interactions that include, among others, metabolic interactions that facilitate the production of biofilm matrix due to different microorganisms could provide different components with which the protective matrix will be composed”.

4) Line 91: '…different surfaces found in the food industry.'
Response:
We have introduced “found” in the manuscript.

5) Line 107: Clarify '…controlling surfaces…'

Response:
We wanted to highlight the importance for the food industry to control the surfaces, which are a relevant source of microbial contamination to food products. We tried to clarify this in the manuscript. Now, between Lines 114-115, you can find: “Due to the importance for the food industry to control surfaces in order to ensure food safety”.

6) Line 139: change 'remaining in' to 'experiencing'
Response:
We have introduced this change in the manuscript.

7) Lines 145-146: Please provide more clarification—'As one of the main steps…' 'steps' needs to refer to an action, such as …'cleaning food contact surfaces…' or …sanitizing food contact surfaces…'. Or is the sentence trying to convey 'As one of the main concerns…in the [ ] testing program…'
Response:
We have expressed ourselves in an incorrect way. In fact, what we wanted to highlight here was that, one of the concerns within the monitoring plans of *L. monocytogenes* is, precisely, the control of food contact surfaces, because of the importance of cross contamination in the transmission of this pathogen. We have changed “steps” for “concerns”. You can find this correction in Line 156.

8) Lines 147-153: 'Once the SCH sensors were installed on the selected surfaces…(since there were three positions). Could be made more concise by replacing with: 'The SCH sensors hold three stainless steel coupons AISI 316 (2 cm in diameter and 1 mm thick) grade B. Once the sensors were installed on the selected surfaces (Table 1), one sample was collected every week.'
Response:
Thank you, we think this is more concise and understandable. We have introduced it in the manuscript (Lines 158-161).

9) Line 156: Change 'Once the samples were taken, different…' to 'Once sampled, different…'
Response:
We have introduced this change in the manuscript.

10) Line 163: 'For the quantification,…' remove 'the'
Response:
We have introduced this change in the manuscript.

11) Line 164: Change 'mediums' to 'media'
Response:
We have introduced this change in the manuscript.

12) Line 165: Change 'To do this,' to 'For this method,'

Response:
We have introduced this change in the manuscript.

13) Line 169: Add a comma after 'During incubation,'
Response:
We have introduced this change in the manuscript.

14) Line 171: Add a comma after '…positive wells,'
Response:
We have introduced this change in the manuscript.

15) Line 176: Change 'Last' to 'Finally'
Response:
We have introduced this change in the manuscript.

16) Line 183: Could simplify from '…confirmed by carrying out biochemical tests (rhamnose fermentation) and by VIDAS…' to '…confirmed by rhamnose fermentation and by VIDAS…'
Response:
Thank you, it is now more concise. You can find this correction in Line 194.

17) Line 197: Could the authors describe a bit more the 13 areas sampled over 74 led to n=988?
Response:
We are very sorry about that. It was a numerical confusion. The total number of samples is n=962. We have changed the number in Line 157 and Line 207.

18) Line 218: Replace 'Through an enzymatic reaction, this product is able to release oxygen gas from the decomposition of hydrogen peroxide present in the biodetector, which is retained in a thickener forming a clearly visible foam that marks the zones…' with 'Via an enzymatic reaction, this product is able to release oxygen gas from the decomposition of hydrogen peroxide present in the biodetector. The oxygen gas is retained in a thickener, forming a clearly visible foam that marks the zones…'
Response:
Thank you. We have adapted this suggestion in the manuscript.

19) Line 231: Add a comma after 'study' in '…product used in the present study…'
Response:
We have introduced this change in the manuscript.

20) Lines 232-233: Could authors please clarify—is the sentence trying to convey that the dye does not work when fats and proteins are present?
Response:

Yes, it seems that this product is capable of staining parts of the extracellular matrix produced by microorganisms, but nevertheless, it does not stain residual organic matter present on a surface.

21) Line 234: Add commas '…which, being water-soluble,…'
Response:
We have introduced this change in the manuscript.

22) Line 241: '…using these sampling methods has important…' (because 'has' is referring back to 'using')
Response:
Sorry about that, we did not notice it. We have introduced this change in the manuscript.

23) Line 249: Change: 'In this study it appears…' to 'In their study, it appears…'
Response:
We have introduced this change in the manuscript.

24) Line 250: Change: '…and those that were dragged could have been…' to '…and those that were removed from the surface may have been…'
Response:
We have introduced this change in the manuscript.

25) Line 252: Change: 'However, if these stainless steel coupons are recovered directly by agitation with glass beads, the vibration causes the beads to hit the surface causing friction and so the microorganisms are detached, resulting in a real count of the…' to 'However, if the stainless steel coupons undergo agitation with glass beads, the vibration causes the beads to hit the surface creating friction to detach the microorganisms, resulting in a real count of the…'
Response:
Thank you for this suggestion. We have adapted it in the manuscript (Lines 251-253).

26) Line 261: Add '…placed on different surfaces identified as problematic where they…'
Response:
We have introduced “identified as problematic” in the manuscript (Line 260).

27) Line 277: Change and add: '…storage cabinet, and so they are areas where…' to '…storage cabinet, and are therefore areas where…'
Response:
We have introduced this change in the manuscript.

28) Lines 303-305: Change: 'The data were grouped monthly based on the weekly counts obtained from the predetermined sampling points to obtain representative graphs

to observe the evolution of the contamination of the different microbial groups over time.' to 'The data were grouped monthly based on the weekly counts obtained to observe graphically the evolution of contamination of the different microbial groups over time.'

Response:

Thank you for this contribution. We have introduced it in the manuscript.

29) Line 310: Add an 's' to 'carcases'

Response:

We have introduced this change in the manuscript.

30) Line 336: It would be interesting to do a community analysis to identify whether there is another bug (or other bugs) whose presence enhances Lm regardless of LD—lots to study!

Response:

Thank you very much for this comment. In fact, it is something that we are just beginning to apply in another study that is the follow-up of this one. We will observe what microbiota can be especially inhibitory or potentiating of this pathogen and we will try to design products that can eliminate microbiota that does not interest us, and promote growth of the microbiota that exercise a role that interests us. But, as you say, lots to study yet!!

31) Lines 359-360: What are the materials of the floors and carts? (Just curious)

Response:

The material of the carts is 316 stainless steel. However, the floor is made of polished cement and is covered with an epoxy paint.

32) Line 365: Please clarify which plant is being referred to in 'In the studied plant'

Response:

We were referring to the industry under study. We have clarified this in the manuscript. Now, you can find in Line 377: “In the studied Iberian pork processing plant,”.

33) Line 376-377: In 'A relationship between the counts of this microbial group…' is 'this microbial group' referring to aerobes?

Response:

Yes. We did not write aerobes to avoid repeating this name too much. However, if you consider that it is necessary to include it so that it is more understandable, we will change it.

34) Lines 419-424: Please clarify

Response:

What we are trying to refer here is that, based on the results obtained, if a safety zone is established between aerobic counts ranging 1.5 logs to 4 logs (that is, that the counting on surface of mesophilic aerobes is in this range), *L. monocytogenes* is not able to grow

optimally and therefore is not detected. This may be due to the fact that when there are enough counts of mesophilic aerobic microorganisms, the growth of pathogens that have been shown to be uncompetitive as for example *L. monocytogenes*, is displaced. That is, if there is resident aerobic microbiota, due to competition in terms of space, nutrient, etc., it can displace the growth of uncompetitive microorganisms. In addition, throughout the study, we also observed that other pathogens such as *Salmonella* spp., *S. aureus* or *E. coli* did not increase with these values of optimal aerobic counts (so, at the same time, it is not causing other problems such as the growth of other pathogens).

We tried to clarify in the text by adding one sentence to reinforce the argument. You can find between Lines 431-439: "For that reason, the establishment of an aerobic surface counts as a residential microbiota of food processing environments that does not exceed 4 logs of CFU could imply a constant displacement of poorly competitive pathogens such as *L. monocytogenes*. On this regard, obtaining aerobic counts within this range would be interesting to generate processing environments free of non-competitive pathogens, especially if potential inhibitors of these pathogenic bacteria are found. Furthermore, the range established between these aerobic counting values did not conditioned the increase of other pathogens studied, such as *Salmonella* spp., *S. aureus* or *E. coli*".

35) Line 452: Add '…was observed for the food industry surfaces studied.'

Response:

We have introduced "studied" in the manuscript (Line 467).

36) Line 480: Please add a space between 'survival' and 'mechanisms'

Response:

We have introduced this change in the manuscript.

37) Line 514: Make 'Pathogenesis' lowercase

Response:

We have introduced this change in the manuscript.

38) Line 594: Italicize E coli

Response:

We have introduced this change in the manuscript.

39) Line 595: Italicize L mono

Response:

We have introduced this change in the manuscript.

40) There appears to be a 3-D shading effect on the data points in the graph making it appear that there are more data than there are.

Response:

We have checked the graphs but we do not find any shading effect, so we do not know how to improve them. As the other reviewers did not observed that, we will not change it. Nevertheless, if you consider that it is a problem, we will try to use another software to generate again all the graphs.

Reviewer #2:

This is an interesting manuscript that provides some insight into methods to detect microbial surfaces on surfaces of meat processing plant. Sensor based sampling systems are relatively new and this manuscript highlights their use in a pork processing environment. My recommendation is to include some information about the SCH sensor. There is insufficient information in the manuscript to judge how this system works. The only information I can find on line is in Spanish which is not helpful for readers who cannot read Spanish.

Response:

With the objective of making more understandable the explanation of the surface sensors, we have thought to include an image. In this sense, we have introduced the Figure 1 with the design of the sensor and, as a consequence of the introduction of this new figure, we have changed the order of the rest of figures. Furthermore, we have added more information regarding these sensors. You can find between Lines 100-102: "These sensors are stainless steel coupons that are attached to a stainless steel base, thanks to the action of a neodymium magnet which is coated with epoxy paint (Figure 1)".

In the highlights, the last highlight does not read well. I suggest "Competitive inhibition between surface resident microbiota and pathogenic bacteria requires study"

Response:

Thank you so much. We have changed the last highlight for the one you suggested.

Reviewer #3:

This is an original article, which reports the microbiological contamination of food processing environments using a sensor-based sampling system. Authors report a direct relation between aerobic counts and the presence of *Listeria monocytogenes* on food industry surfaces. As referred in the manuscript more studies are necessary to demonstrate this relation and which of the possible hypotheses formulated is confirmed.

We know abstract has a limited number of words, but we considered that the results referred in the abstract could be improved, including more information (ex: which areas had a greater and a lesser degree of microbial contamination). A conclusion about the use of SCH sensors should also be referred. The last sentence of the abstract is true but the limits were stablished based on four situations were *L. monocytogenes* was detected

and this is clearly insufficient. The conclusions mentioned in the abstract should be in line with the conclusions presented in the paper.

Response:

We completely agree with your observations. We have tried to improve the abstract with these suggestions. Now, between Lines 24-34, you can find: "The areas considered as major contributors to microbial contamination were three of the sampled floors and the storage cabinet for tools, demonstrating to be important sources of possible cross-contamination. A total of four *L. monocytogenes* presences were obtained during sampling. Moreover, a direct relation was observed between aerobic counts and detecting *L. monocytogenes*, and three possible hypotheses were formulated to explain the connection. Last, a safety zone marking the limits beyond which the surface can be considered as a safety risk was established, although more studies are needed to demonstrate if these limits can be used as an internal hygienic surface control. The use of SCH sensors as a surface sampling system for the food industry have been shown to work effectively and with relative ease".

In introduction authors give the necessary information about the theme and the aim of the study is clearly defined. Despite this, in our opinion, authors should consider to delete sentences in lines 110-112. The first sentence is material and methods and the last we think was not an aim of the work.

Response:

Thank you. We have eliminated these sentences in the manuscript. Now, you can find as the aim of the study (Lines 114-117): "Due to the importance for the food industry to control surfaces in order to ensure food safety, the main aim of the present study was to implement and assess a novel technology to evaluate the microbiological contamination of surfaces in an Iberian pig processing plant".

The authors described material and methods with the necessary elements for their understanding and indicating the bibliography followed. Authors refer that were collected and analyzed 988 samples from 13 different areas and over 74 weeks. We think that it would be useful to explain how authors reached the number of 988 samples.

Response:

We are very sorry about that. It was a numerical confusion. The total number of samples is n=962. We have changed the number in Line 157 and Line 207.

Authors analyzed the results in different perspectives and tried to obtain as much information as possible. In our opinion the first paragraph of point 3.1 should be included in point 2.2 of Material and Methods and deleted from results and discussion section. In point 3.2 are referred the results of the microbial contamination but the authors do not discuss them.

Response:

We have changed the first paragraph of point 3.1 to point 2.2 of the M&M section. Furthermore, we have introduced discussion into the point 3.2. Now, you can find between Lines 301-313: "Eisel, Linton, & Muriana (1997) evaluated the microbial load

of aerobes, total coliforms and *E. coli* from various food contact surfaces, equipment, walls and floors, determining that the walls and floors represented the most contaminated surfaces. The floors are an important source of microbial contamination, since the resident microorganisms can be transferred to the different areas of an industry, through the footwear of the workers, who circulate inside the establishment spreading them. Drains and soils can provide a favorable environment for microbial growth and, consequently, be an important reservoir, as has been demonstrated for *Pseudomonas* spp. and *Aeromonas* spp. (Hoodt & Zottola, 1997), *Salmonella* spp. (Rivera-Betancourt et al., 2004) or for *L. monocytogenes* (Ciccio et al., 2012). Floors can be a direct source of propagation, especially if cleaning is done with high pressure water. This practice can spread microorganisms by suspending them in the air as small drops of water (Barros, Nero, Monteiro, & Beloti, 2007)".

The conclusions taken by authors are clear and realistic, taking into account the limitations inherent to the study.
In our opinion the documents is easy to read and we did not find errors. I suggest authors the following change in lines 41-42 - "…food contact surfaces, equipment and utensils…" instead of "…food contact surfaces, equipment and facilities…"
Response:
Thank you for these comments. We have introduced, as well, this change in the manuscript.

Thank you for all contributions that have made us to highly improve the manuscript,

Dr. José Juan Rodríguez Jerez

Evaluation of the microbiological contamination of food processing environments through implementing surface sensors in an Iberian pork processing plant: An approach towards the control of *Listeria monocytogenes*.

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Abstract

Food safety is one of the biggest concerns of food industrial development due to the risk of foodborne diseases, which are one of the most relevant health problems in the contemporary world and an important cause of reduced economic productivity. One of the main sources of microbial contamination in food products are industrial surfaces, which are colonized by pathogenic microorganisms capable of forming biofilms, making surfaces into reservoirs and potential sources of cross-contamination to food products. A study was conducted to determine the microbiological contamination from different microbial groups on different industrial surfaces in a meat processing plant through implementing a sensor-based sampling system, with a focus on detecting *L. monocytogenes*. The results obtained showed two main groups of areas with greater and lesser degrees of microbiological contamination, determined as the total aerobic counts of the microbial group with the highest contribution. The areas considered as major contributors to microbial contamination were three of the sampled floors and the storage cabinet for tools, demonstrating to be important sources of possible cross-contamination. A total of four *L. monocytogenes* presences were obtained during sampling. Moreover, a direct relation was observed between aerobic counts and detecting *L. monocytogenes*, and three possible hypotheses were formulated to explain the connection. Last, a safety zone marking the limits beyond which the surface can be considered as a safety risk was established, although more studies are needed to demonstrate if these limits can be used as an internal hygienic surface control. The use of SCH sensors as a surface sampling system for the food industry have been shown to work effectively and with relative ease.

Keywords: sampling; surface sensors; microbial contamination; *Listeria monocytogenes*; ecology.

1. Introduction

The fact that meat and meat products are able to transmit pathogenic microorganisms is currently a frequent topic of debate, despite the numerous attempts already made to improve meat hygiene and safety (Bae et al., 2011). Considering the global trend of meat consumption, effectively managing food hygiene to reinforce consumer confidence and protect public health is extremely relevant (Kim & Yim, 2017; Sofos & Geornaras, 2010). An important prerequisite for preventing the microbiological contamination of food products is to hold to high hygienic standards in the food-processing environment, especially in the areas where there are food contact surfaces, equipment and utensils (Osimani, Garofalo, Clementi, Tavoletti, & Aquilanti, 2014). Cleaning and disinfection procedures are critical and must be performed to established guidelines otherwise they potentially allow cross-contamination to occur, which not only reduces a product's shelf-life but also increases the risk of foodborne diseases related to the presence of pathogens (Moore & Griffith, 2002; Reij & Den Aantrekker, 2004). A surveillance programme to control foodborne infections and intoxications in Europe reported that the main contributing factor to outbreaks was cross-contamination (Tirado & Schmidt, 2001), indicating that enormous efforts must be made to control this otherwise it could become an increasing tendency over time. The most relevant cross-contamination vehicle between food contact surfaces and food products are biofilms (Giaouris et al., 2014). Biofilms are considered as a community of cells adhered to a surface, substrate or each other, which in turn are encompassed by a self-produced matrix formed by different components including exopolysaccharides, proteins and eDNA, and where the same community presents a differential phenotype with respect to planktonic modes (Donlan & Costerton, 2002; Lazazzera, 2005). Cell detachment from the biofilm structure when food comes into contact with a contaminated surface, and

food product contamination by aerosols originating from contaminated equipment, are the two main factors contributing to cross-contamination (Chmielewski & Frank, 2003).

Listeria monocytogenes is a foodborne pathogen that is considerably significant for the food industry (Jemmi & Stephan, 2006; Larsen et al., 2014; Ripolles-Avila, Hascoët, Guerrero-Navarro, & Rodríguez-Jerez, 2018; Todd & Notermans, 2011). For instance, listeriosis is the only foodborne disease that has shown a notable increasing tendency in the EU / EEA over the last 5 years (2012-2016), with a 9.3 % increment in confirmed cases between 2015 and 2016 (EFSA-ECDC, 2017). In their last scientific report, the European Food Safety Authority (EFSA) and the European Centre for Disease Control and Prevention (ECDC) stated that out of a total of 360,524 confirmed cases of human zoonoses occurring in Europe in 2016, 2,536 (0.70 %) corresponded to *L. monocytogenes*. Although this percentage of cases may seem low compared to other pathogens, its high mortality rate amongst human populations with vulnerable immune systems, the elderly and its connection with stillbirth and miscarriage makes it a considerable public health issue (Lourenço, Rego, Brito, & Frank, 2012).

It has been reported that the communities of microorganisms that form biofilms in food-processing environments are constituted by multiple species ranging from pathogenic to food spoilage microorganisms (Røder, Sørensen, & Burmølle, 2016; Srey, Jahid, & Ha, 2013). Two main interactions are observed within these structures (Giaouris et al., 2015): on the one hand, competitive interactions such as limiting nutrient sources or producing compounds that supress the growth of other species (Rendueles & Ghigo, 2012); and on the other hand, cooperative interactions that include, among others, metabolic interactions that facilitate the production of biofilm matrix due to different

87 microorganisms could provide different components with which the protective matrix
88 will be composed.(Mitri, Xavier, & Foster, 2011; Nadell, Foster, & Xavier, 2010).
89
90 For the food industry, surface sampling is absolutely essential for evaluating and
91 controlling the microbial contamination of food contact surfaces. Surface sampling
92 techniques have until now been based on swabs or sponges and consist of extracting the
93 microorganisms from the surface and contact plates (Kasuga et al., 2011; Valentine et
94 al., 2008). However, these traditional methods can present some standardization
95 problems when collecting samples from the different surfaces found in the food
96 industry. With the goal of overcoming these standardization problems, a surface sensor
97 named SCH (Hygiene Control Sensor, Premiumlab) was developed to monitor the
98 biological contamination of surfaces and offer an alternative to traditional methods
99 when analysing product safety and quality (Ripolles-Avila, Ríos-Castillo, & Rodríguez-
100 Jerez, 2018). These sensors are stainless steel coupons that are attached to a stainless
101 steel base, thanks to the action of a neodymium magnet which is coated with epoxy
102 paint (Figure 1). The sensors are installed by fixing them onto the food contact surface
103 to be evaluated, where they remain exposed within the facility allowing the surfaces to
104 be evaluated in real industry conditions, from contamination of the environmental
105 process to on-site cleaning. As these sensors are tools for surface sampling they must be
106 further analysed to quantify the cells within the biofilms or to detect determinate
107 microorganisms present on the structures. When using these sensors, the
108 microorganisms present on the surfaces must be recovered. To do so, the surface
109 fragments must be placed in saline solution and shaken (with glass beads or sonication)
110 with the aim of recovering the microorganisms that have adhered to the surface

(González-Rivas, Ripolles-Avila, Fontecha-Umaña, Ríos-Castillo, & Rodríguez-Jerez, 2018; Ripolles-Avila, Cervantes-Huaman, Hascoët, Yuste, & Rodríguez-Jerez, 2019).

Due to the importance for the food industry to control surfaces in order to ensure food safety, the main aim of the present study was to implement and assess a novel technology to evaluate the microbiological contamination of surfaces in an Iberian pig processing plant.

2. Materials and Methods

2.1. Processing plant

The study was carried out at an industrial processing plant that has a medium-sized industrial slaughterhouse annex for pork meat products. This industry is one of the only industries in Spain allowed to export products made from pork to the United States. The main products made at this plant are cured, either as whole pieces (hams, shoulders and loins) or as the Spanish traditional sausages "salchichón" and "chorizo". Among these 5 product categories, there are more than thirty distinct products with different formulations, sizes, pH (ranging from 4.3 to 6.3) and even ripening conditions. As all the products have a water activity value of below 0.92 they are not considered proficient at supporting *L. monocytogenes* growth (Ortiz et al., 2010), so a limit of 100 CFU per gram is admitted for these products following EU regulations.

2.2. Selecting the surfaces to be sampled and SCH sensor installation

The main idea was to detect the surfaces in the selected industrial processing plant that could be a source of contamination for the food products and for installing the surface

sensors (SCH) (Laboratorios Ordesa, Barcelona, Spain) to monitor the level of microbiological contamination. A microbial biodetector (i.e. BioFinder, iTram Higiene www.itramhigiene.com/en/) was employed to determine what surfaces to analyse. This product detected the presence of biofilms on the different industrial surfaces, thus indicating the areas where it was best to install the SCH sensors. These sensors are metal pieces on a stainless steel base that are fixed to the selected surfaces as points to be evaluated, experiencing in the same conditions as the rest of the installation being soiled and cleaned, and therefore also contaminated, in the same way. This procedure allowed us to reproduce microbial biofilms formed by the wild strains typical of the collaborating processing plant.

The surfaces to be evaluated were selected in two different ways. First, based on previous microbiological results obtained by the industry according to where high levels of microbial contamination had previously been found; and second through using BioFinder. Via an enzymatic reaction, this product is able to release oxygen gas from the decomposition of hydrogen peroxide present in the biodetector. The oxygen gas is retained in a thickener, forming a clearly visible foam that marks the zones of a surface where there is microbial activity, thus detecting the points of an installation where biofilms are present (Ripolles-Avila et al., 2018).

2.3. Sampling procedure

As one of the main concerns in the *L. monocytogenes* testing program in the food industry is the food contact surfaces, these were regularly analysed with a total of 962 samples collected over an almost 2-year period (May 2016 – February 2018). The SCH sensors hold three stainless steel coupons AISI 316 (2 cm in diameter and 1 mm thick)

grade B. Once the sensors were installed on the selected surfaces (Table 1), one sample was collected every week. The sample was one of these coupons, and when the coupons to be sampled were removed two others remained in the base. The coupons were always in the processing plant for three weeks before being collected (since there were three positions).

2.4. Microbiological analysis

Once sampled, different microbiological analyses were carried out, not only to quantify the total aerobic count, *Enterobacteria*, coliforms/*Escherichia coli*, coagulase positive *Staphylococcus* sp., lactic acid bacteria, and yeast and moulds, but also to detect *Salmonella* spp. and *L. monocytogenes*. The samples were transferred to sterile flasks containing glass beads and peptone water (PW, bioMérieux, Marcy l'Etoile, France) after which they were vortexed for 90 seconds at a frequency of 40 Hz to dislodge all the attached cells from the surface to be able to quantify or detect the distinct microbial groups. For quantification, the resulting suspension was decimally diluted in PW and transferred to different culture media. In the case of the aerobic plate count, the rapid method TEMPO system was employed. For this method, 1 mL of the corresponding dilution factor was transferred to a TEMPO vial previously hydrated with 3 ml of sterile distilled water, which was then vortexed to homogenize its content and transferred by the TEMPO Filler Unit into an enumeration card with 48 wells of 3 different volumes. During incubation, microorganism growth modifies a fluorescent signal in the medium, which is detected by the TEMPO Reader Unit. Depending on the number and size of the positive wells, the system calculates the number of microorganisms present in the sample. Quantification of the rest of the microbial groups was carried out according to the following standards: ISO 21528 part 2, ISO 4832, ISO 6888 part 3, ISO 15214 and

185 ISO 21527 respectively for *Enterobacteria*, coliforms/*E. coli*, coagulase positive
186 *Staphylococcus* spp., lactic acid bacteria, and yeast and moulds. Finally, the presence of
187 *Salmonella* spp. and *L. monocytogenes* was detected following ISO method 6579 and
188 ISO method 11290 part 2, respectively.
189
190 Where the presence of *L. monocytogenes* was obtained, several colonies were randomly
191 selected and isolated so that the subtypes could be subsequently identified to detect the
192 different strains of *L. monocytogenes* present in the sample. In all cases, the
193 presumptive identification of *L. monocytogenes* was carried out by ALOA culture. The
194 isolates were confirmed by rhamnose fermentation and by VIDAS immunoassay
195 (bioMérieux, Marcy l'Etoile, France) specifically for *L. monocytogenes*. Once
196 confirmed, the strains were cultivated three times in Soya Tripticase Agar (TSA, Oxoid,
197 Madrid, Spain) overnight at 37 ºC.
198
199 **2.5. Polymerase chain reaction serotyping**
200 The isolates were classified in polymerase chain reaction (PCR)–based serotypes using
201 a multiplex PCR assay, as described by Doumith et al., (2004). This PCR uses four
202 primer pairs specific to the four major serotypes of *L. monocytogenes*, 1/2a (or 3a), 1/2b
203 (or 3b), 1/2c (or 3c), and 4b (or 4d and 4e), in addition to one primer pair specific to
204 *Listeria* spp. (Doumith et al., 2004).
205
206 **2.6. Statistical analysis**
207 The samples were taken over 74 weeks from the 13 different areas (n = 962) in the two
208 processing plants evaluated in the study. The results obtained from the microbiological
209 counting analyses were expressed in logarithmic units per square centimetre, while the

results for detecting the two pathogens were expressed as 0 or 1 depending on whether they were absent or present, respectively.

The level of global contamination was evaluated to differentiate zones with greater or lesser degrees of contamination by carrying out a classification study using hierarchical clusters, in addition to an analysis of variance and later separation of means using the Tukey B test of the different variables conditioning the global contamination. A $P < 0.05$ was established as the level of significance. The data from the different sampling points were analysed monthly, including their contribution to presenting *L. monocytogenes*.

3. Results and discussion

3.1. Surface points selected and sampling method

Useful tools are required for rapidly and effectively identifying surfaces where cleaning and disinfection do not eliminate biofilms, which can facilitate hygiene control in facilities and prevent persistent contamination that may affect food (Lelieveld, Mostert, & Holah, 2005). The methodology for selecting the surfaces to be evaluated due to the presence of microorganisms and, therefore, biofilms, was based on simple visual inspection so that both costs and analysis times were reduced. TBF 300 (Betelgeux, Valencia, Spain) is a similar product based on the same methodology, but unlike the product used in the present study, it uses agents capable of dyeing the extracellular matrix, which remains colourless in the presence of habitual residues in food industries, especially fat and proteins (Orihuel et al., 2014). TBF 300, however, is not easy to clean after use, leaving the surface stained, unlike Biofinder, which, being water-soluble, is easily cleaned with water without leaving any stains or residues.

The choice of sampling method should be suitable for evaluating the work surface (González-Rivas et al., 2018). On non-porous materials, such as some plastics or stainless steel, the use of swabs or sponges to sample is appropriate, as described for *L. monocytogenes* (Krysinski, Brown, & Marchisello, 1992). However, two decades later, Ismaïl et al., (2013) highlighted that using these sampling methods has important associated disadvantages such as a slow recovery rate and low reproducibility. To this effect, Moore and Griffith, (2002) indicated that the recovery of *Salmonella* spp., inoculated on a dry surface with 3 log (CFU cm^{-2}), was between 80-90% in the case of sampling with a cotton swab, 15% in the case of sampling with a dacron swab, and 85% when sampled using sponges. Fontecha-Umaña, (2014) showed that on stainless steel coupons, swab sampling and the subsequent recovery of adhered cells had the lowest mean count and the greatest variability in the results in comparison to other methodologies. In their study, it appears that the microorganisms that tended to be attached to the surface were not dislodged effectively, and those that were removed from the surface may have been trapped in the fibres of the swab, thus retaining a large number of microorganisms. However, if the stainless steel coupons undergo agitation with glass beads, the vibration causes the beads to hit the surface creating friction to detach the microorganisms, resulting in a real count of the microbial load and greater reproducibility (Montañez-Izquierdo, 2013).

3.2. Global study of the microbiological contamination of the different sampling points

In order to assess the level of microbial contamination of the industrial surfaces and possible biofilm formation by the wild strains found in the processing plants, SCH

sensors were placed on different surfaces identified as problematic where they remained for the same exposure period in the facilities. In this way, as discussed above, the coupons that were subsequently evaluated were a true reflection of the normal work surfaces.

The average levels of microbial contamination for the different areas analysed over the 74 weeks of study, including aerobic count, Enterobacteriaceae, coliforms, *E. coli*, *Staphylococcus* coagulase positive, lactic acid bacteria, and yeast and moulds, in addition to *Salmonella* spp. and *L. monocytogenes*, are shown in Table 2. Significant differences ($P < 0.05$) were observed between the industrial areas sampled and the counts obtained for the different microbial groups, except for the counts of Enterobacteriaceae, *E. coli* and *Staphylococcus* coagulase positive ($P = 0.063$; $P = 0.080$; $P = 0.989$, respectively). The fact that the surfaces under study did not present significant differences in enterobacterial counts, but did present significant differences ($P < 0.05$) in coliform counts, could indicate that there are points where faecal contamination is more easily produced. These areas are interrelated because in all of them the sensors were installed either on the ground, in one of the sumps or in the tool storage cabinet, and are therefore areas where there is a high degree of movement from the operators of the plant.

In order to classify all the areas studied into homogeneous groups, allowing the surfaces with greater and lesser degrees of microbial contamination to be determined, a classification study by hierarchical clusters was carried out using the total microbial counts obtained by surface (i.e., including at each point the count of the different microbiological analyses) (Figure 2). The results showed two differentiated

conglomerates: first, the one that grouped the four areas corresponding to the floor of the fresh meat carts cleaning room and the floor of the cured meat carts cleaning room, the storage cabinet for tools and the floor of the carcasses airing room, considered as the areas with the highest contamination level since they had the highest counts. These areas were also separated into a single cluster because they differed significantly ($P < 0.05$) from the rest of the areas, coinciding with the analysis of variance previously obtained between the studied areas. The microbiological analyses indicated that these four zones were not significantly different ($P > 0.05$) among them, grouping them in a subset of values. The rest of the sampled surfaces composed the other conglomerate and were considered as the areas with the lowest degree of microbiological contamination. It can be observed how there are two different associations of areas that end up converging in a larger conglomerate. It should be noted that one of the associations includes surfaces such as the slicing table in processing plant B, the Iberian sausage transportation carts and the side of vacuum machine, which presented the lowest microbial count.

Eisel, Linton, & Muriana (1997) evaluated the microbial load of aerobes, total coliforms and *E. coli* from various food contact surfaces, equipment, walls and floors, determining that the walls and floors represented the most contaminated surfaces. The floors are an important source of microbial contamination, since the resident microorganisms can be transferred to the different areas of an industry, through the footwear of the workers, who circulate inside the establishment spreading them. Drains and soils can provide a favorable environment for microbial growth and, consequently, be an important reservoir, as has been demonstrated for *Pseudomonas* spp. and *Aeromonas* spp. (Hoodt & Zottola, 1997), *Salmonella* spp. (Rivera-Betancourt et al., 2004) or for *L.*

310 *monocytogenes* (Ciccio et al., 2012). Floors can be a direct source of propagation,
311 especially if cleaning is done with high pressure water. This practice can spread
312 microorganisms by suspending them in the air as small drops of water (Barros, Nero,
313 Monteiro, & Beloti, 2007).
314
315 **3.3. Microbial counts obtained from the different surfaces by month**
316 The data were grouped monthly based on the weekly counts obtained to observe
317 graphically the evolution of contamination of the different microbial groups over time.
318 Figure 3, shows the results from some of the surfaces evaluated. Surface point 4,
319 corresponding to the floor of the carcasses airing room, was one of the areas considered
320 to be a major contributor to microbial contamination. The results showed both high
321 aerobic counts and high yeast and moulds counts, with important monthly similarities in
322 the patterns of the two counts. After the slaughterhouse, the carcasess are taken to the
323 airing room where they are stored until being transported to the cutting room. The
324 temperature in this chamber decreases over time, but this cooling process is slow
325 because the carcasses are very hot on arrival. The conventional cooling profile for pig
326 carcasses is generally a gradual, sustained lowering in temperature, requiring a
327 minimum of 60 minutes to go from 10 ºC to 4 ºC (Chang, Mills, & Cutter, 2003). Both
328 this factor and the fact that there is a lot of movement in this area could be the reasons
329 why the microbial counts were so elevated there.
330
331 At surface point 7, corresponding to the floor of the fresh meat carts cleaning room, the
332 microbial contamination was also variable over the months. It is interesting to note that
333 there was a cyclical pattern to the increases and decreases in microbial contamination,
334 although at different counting levels. As this is a designated area for washing the carts
335 and other instruments related to the fresh area, it is considered a potentially dirty place

336 despite being in contact with many cleaning products. Hoses are used to expel water at
337 high pressure, a method that has proven to be effective in removing organic matter,
338 especially when the deposits are found in areas that are difficult to reach and penetrate
339 (Marriott, Schilling, & Gravani, 2018). However, the volume of carts and containers
340 that must be cleaned daily is extremely high and large amounts of waste are generated,
341 which may be one of the factors why high counts in practically all the microbial groups
342 studied were obtained.
343
344 Last, surface points 5, 8 and 10, corresponding to the storage cabinet for tools, the floor
345 of the cured meat carts cleaning room, and the Iberian sausage transportation carts,
346 respectively, were the only three areas where *L. monocytogenes* was detected (Figure 3).
347 On surfaces 5 and 10, *L. monocytogenes* detection was associated with high aerobic
348 counts, showing that there may be ecological interrelations between the microorganisms
349 present on the surfaces, making them able to stimulate biofilm formation from this
350 pathogen through synergistic action. This finding should therefore be considered when
351 searching for new strategies to combat biofilms (Røder et al., 2016). The prevalence of
352 *L. monocytogenes* in food industries is higher when there is a large volume of
353 production and when the processing environment is not controlled (Tompkin, 2002).
354 The same study pointed out that in a smoked fish industry more positive samples of *L.*
355 *monocytogenes* were detected during periods of intensive production, corresponding
356 with the months of November and December, a point that coincides with the results
357 obtained in the present study. In this case, its presence could be due to the fact that as *L.*
358 *monocytogenes* is a poorly competing bacterium, it could have easily developed and
359 been detected for two consecutive weeks. It could also indicate that there may have
360 been inhibitors of the growth of the pathogen among the microorganisms present,

361 something that should be studied in greater depth. Studying the growth patterns of this
362 pathogen, together with other microorganisms present on food industry surfaces, would
363 be enormously interesting to develop new strategies for their control. To this effect,
364 Heir et al., (2018) indicated that there are currently large variations in the
365 competitiveness of *L. monocytogenes* under multibacterial culture conditions and that
366 this type of relationship should be considered in future studies to understand the
367 persistence of the pathogen in food processing facilities.
368
369 The five isolates of *L. monocytogenes* identified throughout the study were analysed to
370 identify the molecular serotype (Figure 4). The serotypes found were 1/2a (Lm3 and
371 Lm4, both related to the floor of the cured meat carts cleaning room), 1/2b (Lm5,
372 related to the Iberian sausage transportation carts) and 4b (Lm1 and Lm2, the first
373 related to the storage cabinet for tools and the second to the floor of the cured meat carts
374 cleaning room).
375
376 Molecular serotyping permits identification of the most important food-borne strains
377 (1/2a, 1/2b, and 4b) (Doumith et al., 2004). In the studied Iberian pork processing plant,
378 molecular serotype 1/2a was previously found to predominate (68% of all the isolates
379 identified along a 3-year study) followed by 1/2b and 1/2c (Ortiz et al., 2010).
380
381 **3.4. Safety zone based on aerobic count**
382 The results obtained showed what could hypothetically be a relationship between
383 aerobic counts and the presence of *L. monocytogenes* on the food industry surfaces
384 studied. With the goal of establishing a safety zone marking the limits beyond which the
385 surface could be considered as a safety risk, the monthly evolution of the aerobic counts

obtained from the surfaces where *L. monocytogenes* was detected were represented (Figure 5). According to these results, when the aerobic count ranged between 2 and 4 log (CFU cm^{-2}) the pathogen was not present. A relationship between the counts of this microbial group and the presence of *L. monocytogenes* on surfaces was therefore observed, and this relationship varies according to the counts. Three possibilities have consequently emerged to justify this situation.

The first possibility is that there may be microorganisms that inhibit the growth of *L. monocytogenes*. When the aerobic count increases the pathogen cannot grow since there would be an incompatibility in mixed growth. When the count decreases, however, the pathogen can multiply. But if the inhibitors are not present or cannot compete with other microorganisms, *L. monocytogenes* grows in a different ecological environment. This hypothesis could be called the competitive inhibition of growth theory. The second possibility is that there may be microorganisms that facilitate the growth of *L. monocytogenes*. In this case, the pathogen would be detected by increasing the total aerobic count, but if the total aerobic count was inhibited, the pathogen would have greater difficulty growing with other bacteria. Thus, by reducing the total count, the pathogen would not be positively detected. This hypothesis could be called the competitive stimulation theory. The third and last possibility is based on the two possibilities discussed previously occurring simultaneously. Thus, when positive competition occurs, the pathogen could multiply and be further detected on surfaces. Conversely, if negative competition occurs, the pathogen would only be detected when the total aerobic count load is controlled. This hypothesis could be called the variable competition theory.

411 Depending on which hypothesis most closely approaches reality, the consequences for
412 sanitising surfaces could mean stimulating the growth of *L. monocytogenes* with the
413 subsequent safety risk for consumers. If the hypothesis regarding competitive inhibition
414 were true, very efficient sanitation with a significant reduction in the aerobic microbial
415 load would ensure the presence of the pathogen.
416
417 According to the results obtained, two different situations were observed. The first one,
418 associated with the storage cabinet for tools and the Iberian sausage transportation carts,
419 was due to detecting the pathogen when the aerobic counts exceeded 4 log (CFU cm^{-2}).
420 The second, associated with the floor of the cured meat carts cleaning room, was due to
421 detecting *L. monocytogenes* when the count was below 1.5 log (CFU cm^{-2}). Based on
422 the results obtained, the limit values for total aerobic counts can be established between
423 4 log (CFU cm^{-2}) and 1.5 log (CFU cm^{-2}) as the internal control limits on these surfaces.
424 In this regard, establishing this lower limit was given by the fact that *L. monocytogenes*
425 is considered a badly competing bacterium when it is found in multispecies biofilms as
426 recently indicated (Heir et al., 2018; Papaioannou et al., 2018). In the food industry,
427 interactions between the resident background microbiota on the food processing
428 surfaces and *L. monocytogenes* can occur, and these associations can have effects on the
429 survival and subsequent growth of different individuals within the microbial consortia,
430 highlighting among them the antagonistic effect on *L. monocytogenes* (Møretrø &
431 Langsrud, 2017; Rodríguez-López et al., 2018). For that reason, the establishment of an
432 aerobic surface counts as a residential microbiota of food processing environments that
433 does not exceed 4 logs of CFU could imply a constant displacement of poorly
434 competitive pathogens such as *L. monocytogenes*. On this regard, obtaining aerobic
435 counts within this range would be interesting to generate processing environments free

of non-competitive pathogens, especially if potential inhibitors of these pathogenic bacteria are found. Furthermore, the range established between these aerobic counting values did not conditioned the increase of other pathogens studied, such as *Salmonella* spp., *S. aureus* or *E. coli*.

The maximum aerobic counts did not reach these values again either in the storage cabinet for tools or in the Iberian sausage transportation carts, so whether a new increase in the counts of this microbial group had the same response in relation to detecting *L. monocytogenes* could not be observed. It is true that on detecting *L. monocytogenes* in the storage cabinet for tools, the aerobic count increased over the next month and the presence of the pathogen was not detected. Nevertheless, the plant had been informed of the presence of *L. monocytogenes* and associated cleaning and disinfection measures had been taken, which may be why the pathogen was not detected just after the microbial load increased. Regarding the floor of the cured meat carts cleaning room, a low load was not obtained until February 2017 after which there was a progressive increase in the aerobic counts until the end of the study, implying that another study must be designed to verify and corroborate what is occurring.

4. Conclusion

The use of SCH sensors as a real surface sampling system for food industries and their subsequent analysis to determine different microbial groups allows for real-time surface microbial counting, evaluating the resident microbiota and determining the hygienic state of an installation with relative ease. Moreover, the use of a biofilm biodetector and the microbiological analysis of the SCH sensors determined that the areas with the

highest degree of microbial contamination were the floors, both the fresh meat and cured meat carts cleaning room, the floor of the carcasses airing room, as well as the storage cabinet for tools. This may be due to the regular movement of operators and products in these areas, in addition to the very humid environmental conditions. The rest of the evaluated areas, on the other hand, presented significantly lower microbiological levels. Moreover, a relationship between total aerobic counts and the presence of *L. monocytogenes* was observed for food industry surfaces studied. This relationship can be complex as it was observed that the presence of the pathogen could be related to a high load of accompanying bacteria on some surfaces, and on others just the opposite. Therefore, three possibilities have been proposed to justify this situation and new studies are necessary to demonstrate which of them can be confirmed.

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Conflict of interest

The authors declare no conflict of interest.

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Table captions

Table 1. Work surfaces where the SCH sensors were installed.

Table 2. Global average of the microbial counts obtained from the different areas where the SCH sensors were installed as a sampling method throughout the 74 weeks of study in two Iberian pig processing plants (Plant A and B). The data represents the mean in log (CFU cm^{-2}) ± the standard error of the mean.

Figure captions

Figure 1. Design of a SCH surface sensor.

Figure 2. Recombination between the different sampling areas and their subsequent assignment in clusters by similarity based on the total level of contamination per point.

Figure 3. Monthly evolution (1 = January to 12 = December) of the microbial counts obtained from some of the surfaces evaluated in the meat industry under study. The graph shows both the log count (CFU cm^{-2}) for aerobic count, Enterobacteriaceae, coliforms, *E. coli*, positive coagulase *Staphylococcus*, lactic acid bacteria, and yeasts and moulds, and the cumulative detection of *Salmonella* spp. and *L. monocytogenes*, assuming that each presence equalled 1.

Figure 4. Serotypes of *L. monocytogenes* identified from the industrial surfaces, with EF1656 being the isolated Lm1, EF1657 the isolated Lm2, EF1701 the isolated Lm3, EF1702 the isolated Lm4, and EF1718 the isolated Lm5.

Figure 5. Establishment of a safety zone based on the aerobic count as a possible marker for the presence of *L. monocytogenes* in industrial areas. The results are expressed in log (CFU cm^{-2}) throughout the study months (1 = January to 12 = December). The arrows indicate the detections obtained from *L. monocytogenes*.

Highlights

- Environment microbiota is crucial to understand microorganism’s relationships.
- Aerobic microbiota was related to the detection of *L. monocytogenes*.
- Three possible hypotheses were formulated to explain the connection.
- New ways to inhibit *L. monocytogenes* are needed to reduce antimicrobial resistance.
- Competitive inhibition between microbiota and pathogenic bacteria requires study.

Figure 1

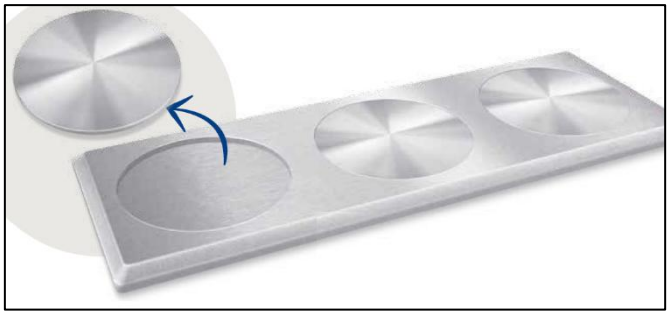
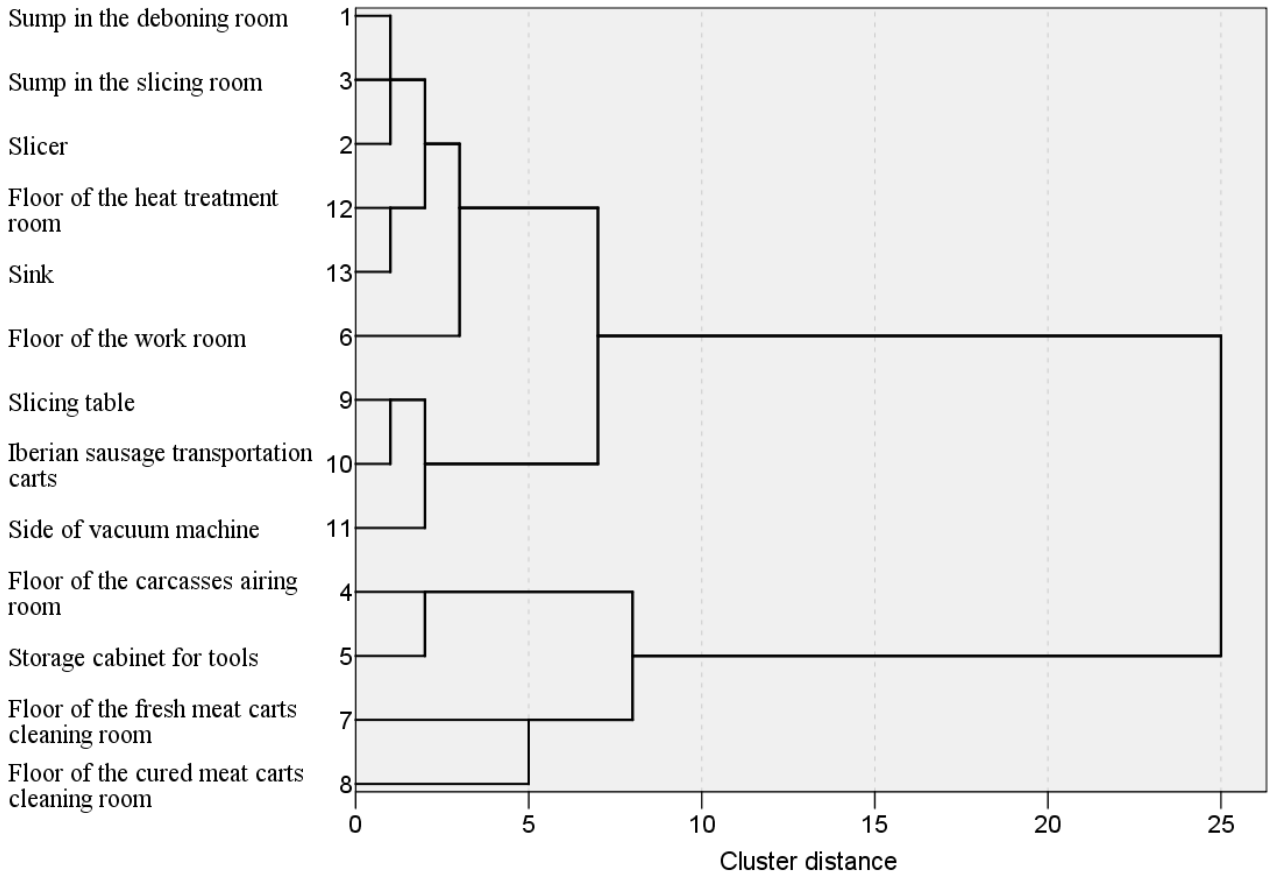


Figure 2


Sump in the deboning room
Sump in the slicing room
Slicer
Floor of the heat treatment room
Sink
Floor of the work room
Slicing table
Iberian sausage transportation carts
Side of vacuum machine
Floor of the carcasses airing room
Storage cabinet for tools
Floor of the fresh meat carts cleaning room
Floor of the cured meat carts cleaning room
1
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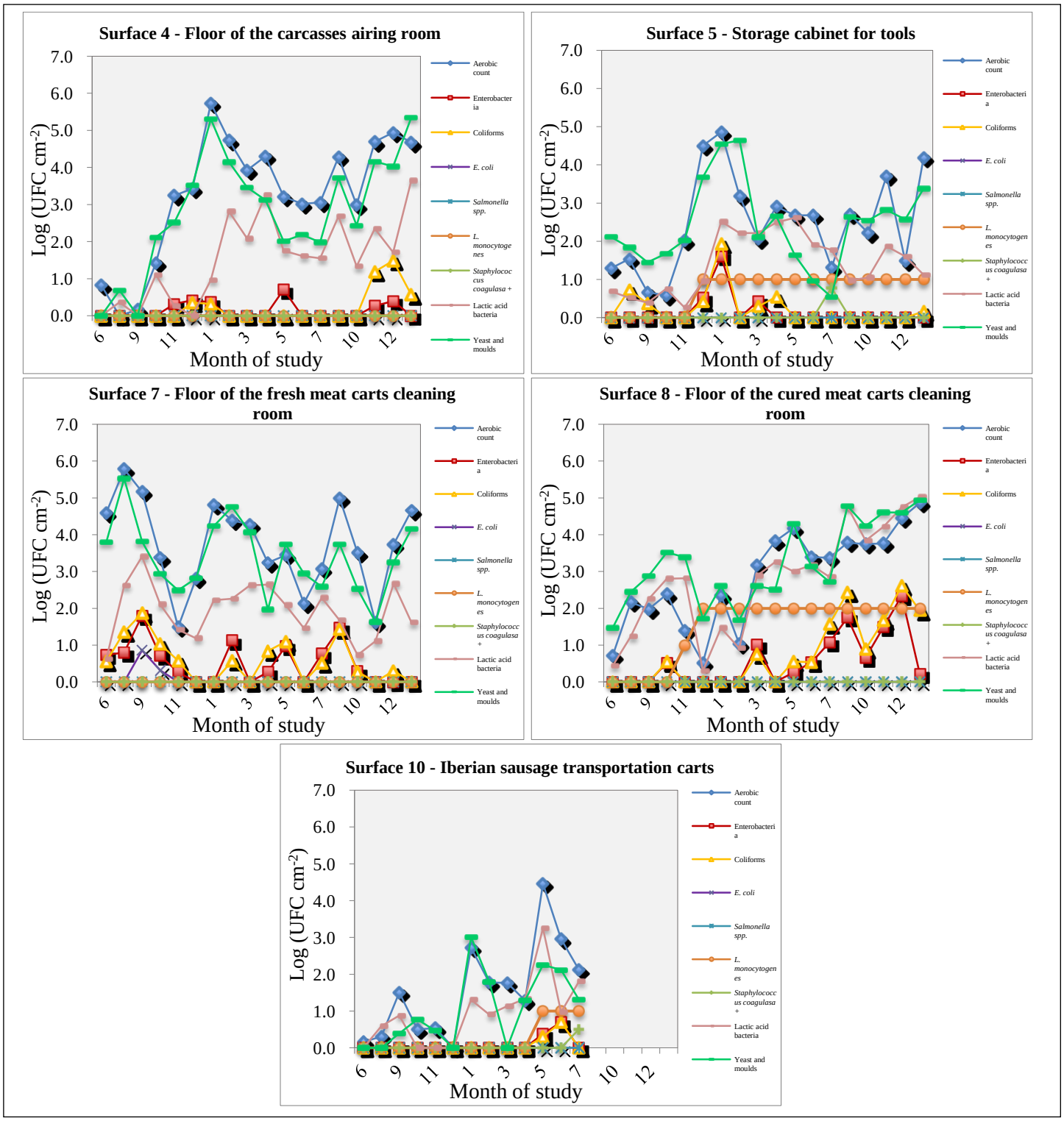
Cluster distance

Figure 3


Surface 4 - Floor of the carcasses airing room
Surface 5 - Storage cabinet for tools
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Log (UFC cm-2)
Month of study
Aerobic count
Enterobacteria
Coliforms
E. coli
Salmonella spp.
L. monocytogenes
Staphylococcus coagulasa +
Lactic acid bacteria
Yeast and moulds

Figure 4

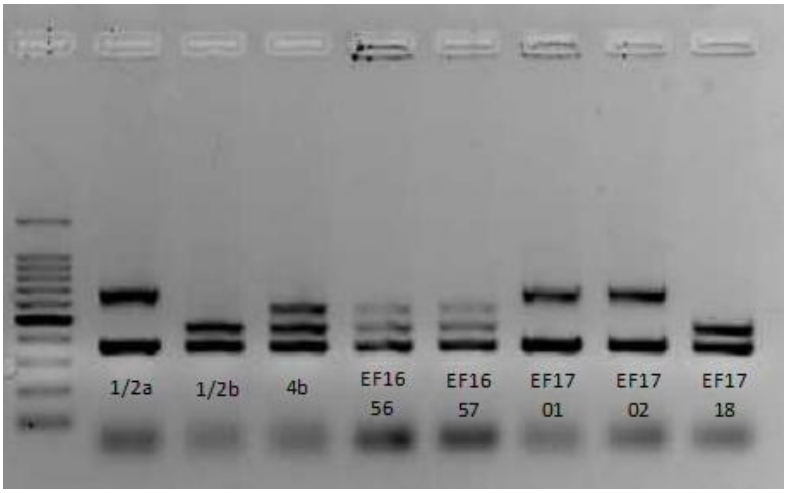
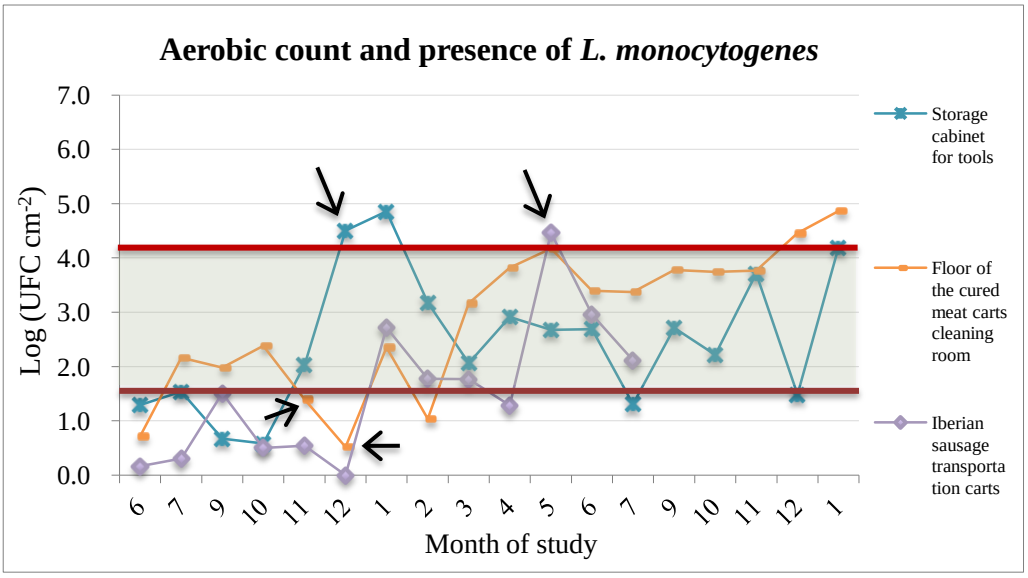


Figure 5


Aerobic count and presence of L. monocytogenes
Log (UFC cm-2)
7.0
6.0
5.0
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3.0
2.0
1.0
0.0
6 7 9 10 11 12 1 2 3 4 5 6 7 9 10 11 12 1
Month of study
Storage cabinet for tools
Floor of the cured meat carts cleaning room
Iberian sausage transportation carts

Table 1

Processing plant	ID surface	Surface
A	1	Sump in the deboning room
	2	Slicer
	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	Slicing table
	10	Iberian sausage transportation carts
	11	Side of vacuum machine
	12	Floor of the heat treatment room
	13	Sink

* Plant A is where raw meat products and RTE are sliced and packaged. Plant B consists of a slaughterhouse and a processing room where cured meat products are prepared.

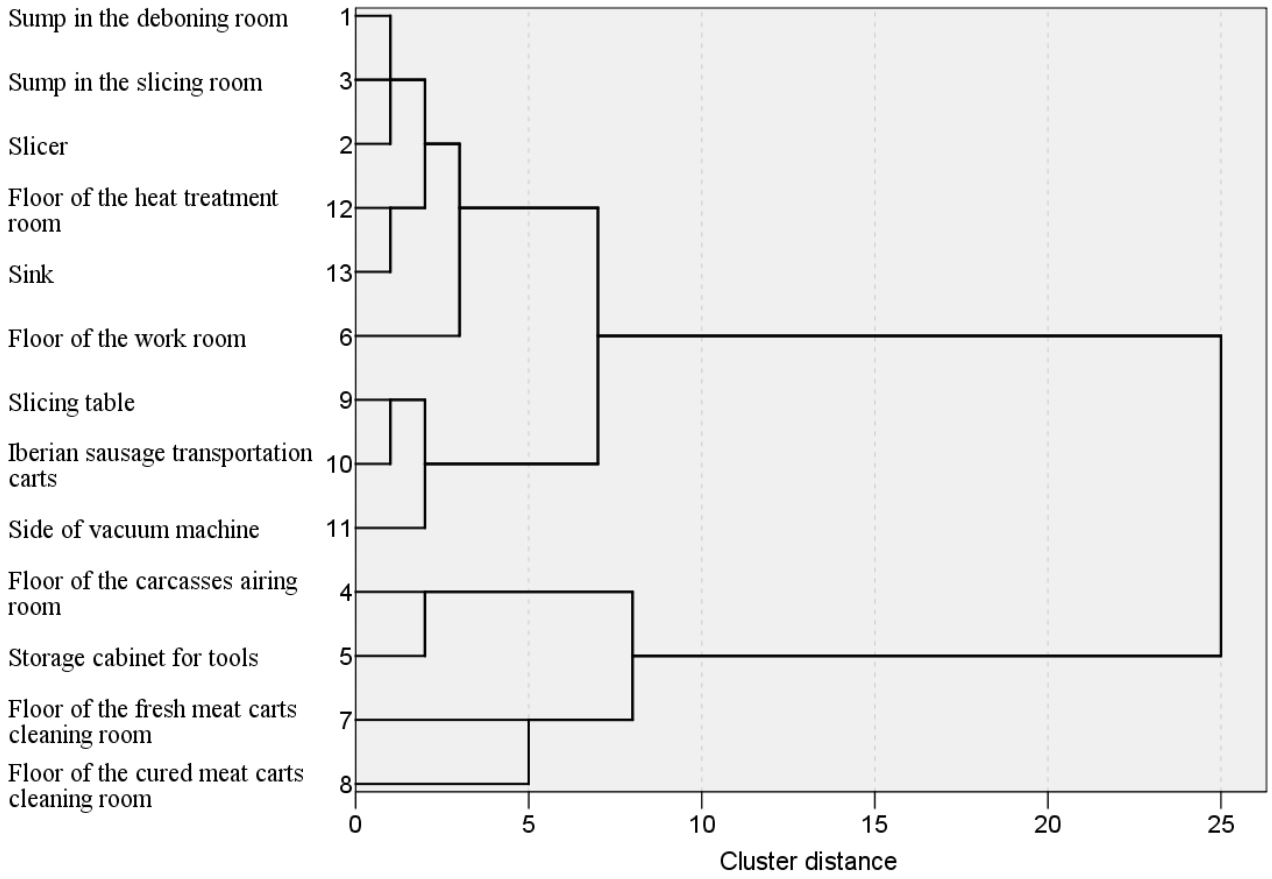
Table 2

Table 2

		Microbial counts log (UFC cm^{-2})							Detection (absence/presence)	
Processing plant	**Surface**	**Aerobic count**	**Enterobacteria**	**Coliforms**	***E. coli***	***Staphylococcus* coagulase positive**	**Lactic acid bacteria**	**Yeast and moulds**	***Salmonella* spp.**	***L. monocytogenes***
A	Sump in the deboning room	$1,97±0,19^{bcd}$	$0,25±0,08^{a}$	$0,21±0,08^{ab}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$1,27±0,23^{abc}$	$1,23±0,32^{a}$	0	0
	Slicer	$2,07±0,19^{bcde}$	$0,11±0,08^{a}$	$0,11±0,11^{a}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$1,49±0,28^{bc}$	$1,67±0,29^{abc}$	0	0
	Sump in the slicing room	$1,72±0,20^{abcd}$	$0,03±0,02^{a}$	$0,10±0,05^{a}$	$0,00±0,00^{a}$	$0,04±0,00^{a}$	$1,15±0,04^{abc}$	$1,17±0,20^{a}$	0	0
B	Floor of the carcasses airing room	$3,14±0,39^{ef}$	$0,13±0,05^{a}$	$0,21±0,10^{ab}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$1,45±0,27^{abc}$	$2,71±0,37^{cd}$	0	0
	Storage cabinet for tools	$2,48±0,28^{cdef}$	$0,13±0,09^{a}$	$0,23±0,11^{ab}$	$0,00±0,00^{a}$	$0,04±0,04^{a}$	$1,45±0,17^{abc}$	$2,45±0,25^{bcd}$	0	1
	Floor of the work room	$2,41±0,22^{cde}$	$0,26±0,14^{a}$	$0,34±0,18^{ab}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$0,92±0,10^{abc}$	$1,70±0,29^{abc}$	0	0
	Floor of the fresh meat carts cleaning room	$3,58±0,31^{f}$	$0,48±0,13^{a}$	$0,55±0,13^{ab}$	$0,07±0,05^{a}$	$0,00±0,00^{a}$	$1,89±0,17^{cd}$	$3,28±0,25^{c}$	0	0
	Floor of the cured meat carts cleaning room	$2,70±0,32^{def}$	$0,52±0,16^{a}$	$0,71±0,21^{b}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$2,63±0,36^{d}$	$3,12±0,27^{c}$	0	2
	Slicing table	$1,13±0,16^{ab}$	$0,07±0,04^{a}$	$0,03±0,03^{a}$	$0,00±0,00^{a}$	$0,04±0,04^{a}$	$0,69±0,15^{ab}$	$0,67±0,22^{a}$	0	0
	Iberian sausage transportation carts	$1,47±0,34^{abc}$	$0,08±0,05^{a}$	$0,07±0,05^{a}$	$0,00±0,00^{a}$	$0,04±0,04^{a}$	$0,87±0,24^{ab}$	$0,96±0,27^{a}$	0	1
	Side of vacuum machine	$0,63±0,12^{a}$	$0,02±0,02^{a}$	$0,04±0,04^{a}$	$0,00±0,00^{a}$	$0,03±0,03^{a}$	$0,49±0,12^{a}$	$0,71±0,16^{a}$	0	0
	Floor of the heat treatment room	$1,44±0,18^{abc}$	$0,31±0,13^{a}$	$0,42±0,14^{ab}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$1,10±0,17^{abc}$	$1,82±0,26^{abc}$	0	0
	Floor of the heat treatment room	$1,74±0,39^{abcd}$	$0,36±0,22^{a}$	$0,39±0,27^{ab}$	$0,00±0,00^{a}$	$0,00±0,00^{a}$	$0,77±0,11^{ab}$	$1,51±0,32^{ab}$	0	0

$^{a-f}$ Means within a column lacking a common superscript differ significantly ($P < 0,05$)

Figure 1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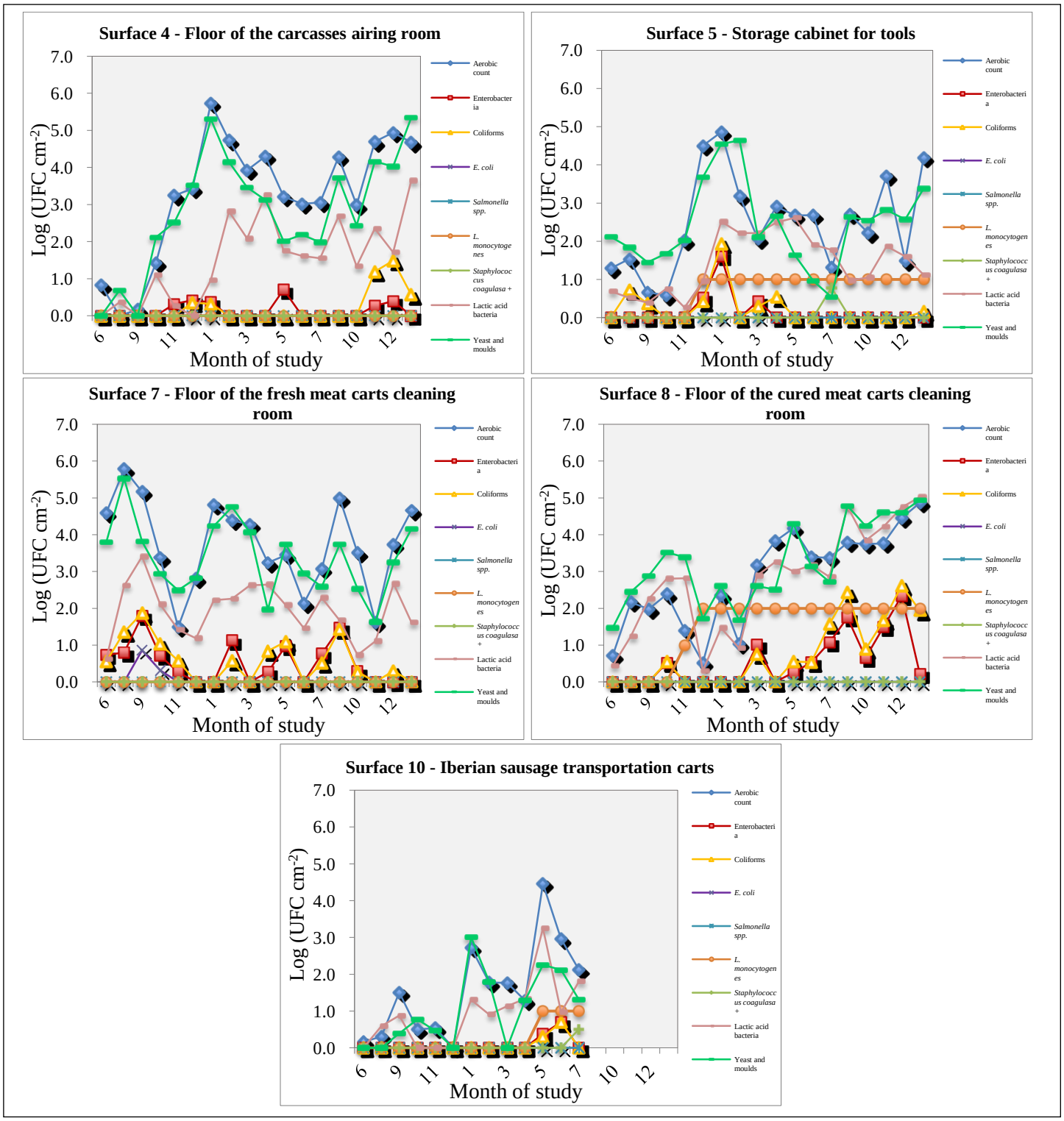
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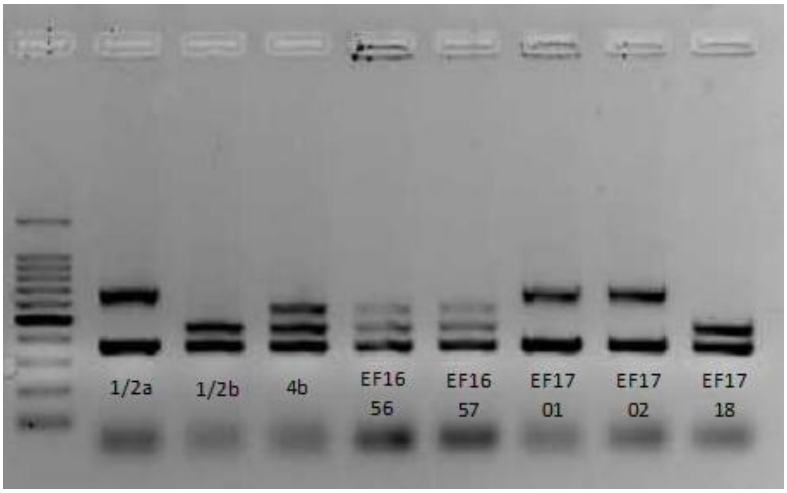

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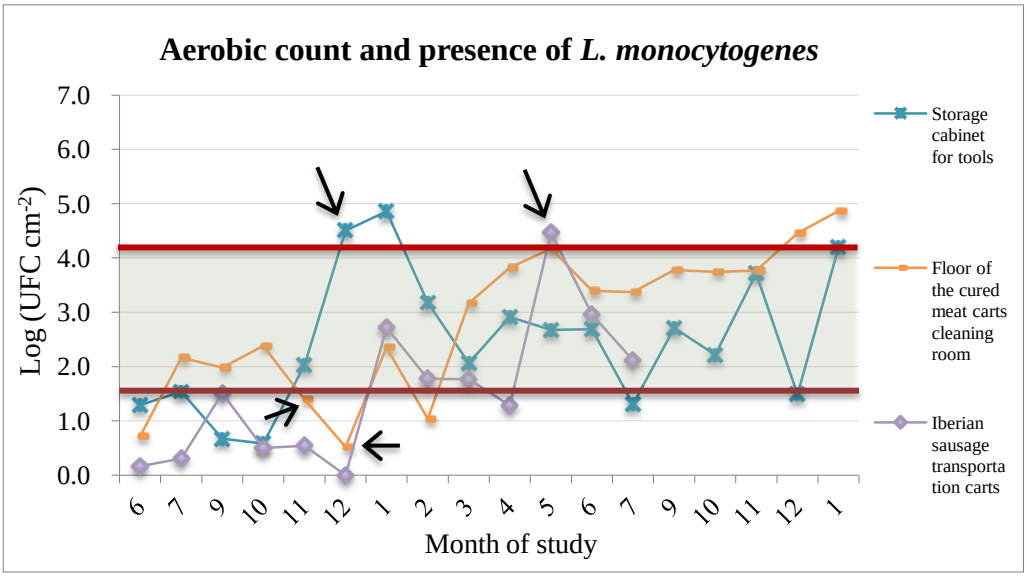
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