



Tracking the sources of psychrotrophic bacteria contaminating chicken cuts during processing

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ABSTRACT

The major aim of the study was to establish the routes *via* which spoilage associated psychrotrophic bacteria contaminate poultry products at a large processing plant located in Belgium. Environmental samples were collected consisting of samples of air and swabs of food contact surfaces. Product samples were also collected consisting of modified atmosphere packaged (MAP) chicken wings and legs, which were analyzed microbiologically on the same day they were produced as well as after their sell-by date. Psychrotrophic bacteria from these samples were subsequently clustered and identified by means of MALDI-TOF MS and 16S rRNA gene sequencing. *Carnobacterium maltaromaticum* was determined to dominate the spoilage flora of both wings and legs. Other psychrotrophic bacteria able to grow on MRS which were identified on expired wings and legs included *Carnobacterium divergens*, *Brocothrix thermosphacta*, *Lactobacillus curvatus*, and *Lactobacillus brevis*. These were determined to arise from food contact surfaces such as cutting blades, leg hooks, Ertalon and polyurethane conveyor belts, working tables, and the hands of the operators. Importantly, it was determined that cleaning and disinfection was largely inadequate. Air was also determined to be an important vector of psychrotrophic bacteria in the processing environment, potentially contaminating the products directly or indirectly.

1. Introduction

Poultry is currently the second most important meat consumed worldwide and is expected to be positioned first by 2022 (OECD-FAO, 2013). Global production of broiler meat was estimated at 89 billion tons in 2016 with the largest producers are the United States (20.5%), Brazil (14.5%), China (13.8%) and the European Union (12.9%) (USDA, 2017). Poultry products are generally appreciated due to their low-fat content, relatively high concentration of polyunsaturated fatty acids, excellent quality of protein, and essential amino acids content (Zhang et al., 2016).

During production, the presence of different processes and manipulation of the products, can lead to contamination by pathogens and spoilage associated microorganisms (Vihavainen and Björkroth, 2010). In Europe and Northern America, as much as 21% of food losses are attributed to meat and meat products (Gustavsson et al., 2011). Poultry products, in particular those containing skin, are known to have high initial microbial contamination levels which makes them highly perishable, deteriorating beyond acceptable levels after 4–10 days post-slaughter under chilled storage (Meredith et al., 2014). As a

consequence, poultry meat is usually packaged under modified atmospheres to extend shelf-life under chilled storage conditions. On the one hand, poultry meat is packaged in CO₂/N₂ atmospheres with low residual O₂ levels (Höll et al., 2016) as these atmospheres i) are known inhibit meat spoilage bacteria and ii) because oxymyoglobin is not relevant in white meat (McKee, 2007). On the other hand, poultry processors also use high O₂ concentrations in combination with CO₂ in their packages to achieve shelf-lives comparable to those achieved in packages with CO₂/N₂ atmospheres and low residual O₂ levels (Roussaint et al., 2015).

Psychrotrophic lactic acid bacteria (LAB) have been reported to dominate at the end of shelf-life under MAP and are also associated in several cases with sporadic spoilage and quality fluctuations among production batches (Lyhs and Björkroth, 2008; Nieminen et al., 2011). The LAB most often detected in meat belong to the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc* and *Weissella* (Nychas et al., 2007). LAB show varying potential to cause spoilage (Laursen et al., 2005; Schirmer et al., 2009) including discoloration (Vihavainen and Björkroth, 2007), slime production (Korkeala et al., 1988) or production of off-odorous compounds (Egan, 1983). Hence, characterization of

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LAB communities is needed to understand the microbial spoilage of MAP meat. Some recent studies have also reported non-LAB as part of the dominant flora of MAP poultry products. In a study on the bacterial communities of packaged yellow feather broiler carcasses, Wang et al. (2017) determined that whilst *Aeromonas*, *Acinetobacter*, *Escherichia*, and *Streptococcus* spp. constitute the initial flora, *Lactococcus*, *Serratia*, *Aeromonas* and *Shewanella* dominate during storage under MAP (80% CO₂, rest N₂), whilst *Pseudomonas* spp. dominate under penetrated-air packaging. Rouger et al. (2018) determined that in addition to *Carnobacterium* spp., *Brochothrix thermosphacta* and *Pseudomonas* spp. dominated the flora of chicken legs stored at 4 °C. They also established that in addition to the influence of the composition of packaging atmosphere on the relative abundance of these spoilage microorganisms the slaughterhouse environment can also play an important role in the nature of the microbial contaminants.

Whilst psychrotrophic bacteria have been reported to originate from the feathers and the feet of the birds, the water supply, and the equipment in the processing plant (Capita et al., 2001), very few studies have been performed to verify this. Knowledge of the actual routes via which spoilage microorganisms contaminate food products is essential for appropriate preventive methods to be developed. Whilst this preventive approach has been widely applied with regards to foodborne pathogens, this has not been the case for spoilage associated microorganisms. The majority of the studies that have been performed today on fresh poultry products have focused on the unravelling the microflora of these products and their dynamics during storage under various conditions, as examples see Björkroth et al. (2005), Capita et al. (2001), Doulgeraki et al. (2011), Susiluoto et al. (2003) and Höll et al. (2016).

To date, Vihavainen et al. (2007) are (as far we could establish) the only authors who have attempted to establish in depth the origins of psychrotrophic LAB in MAP broiler products in a processing plant. In their study, Vihavainen et al. (2007) concluded that incoming broiler chickens were not major sources of psychrotrophic spoilage LAB. Air was considered to be the major as these organisms were detected in the air of the processing environment. Whilst offering very important insight, Vihavainen et al. (2007) did not evaluate the entire process. Importantly the study only focused on air and carcass samples and therefore did not establish the role of food contact surfaces. Therefore, this study was performed to determine the routes via which psychrotrophic bacteria contaminate fresh poultry products (wings and legs) during processing (slaughtering till packaging) at a company located in Belgium. The study provides data of importance to fresh poultry meat processors as it enables them to identify areas in their processing plants where appropriate measures could be put in place to reduce contamination by psychrotrophic bacteria.

2. Methods

2.1. Process flow

The process flow diagram is shown in Fig. 1. In brief, live birds (ca. 130000/day) are received in cages and checked for the presence of *Salmonella* serotypes Enteritidis or Typhimurium. They are then off-loaded into a chamber where they are stunned by a mixture of CO₂ and O₂. The birds are then hung upside down by means of Inox leg hooks on an overhead conveyor. They are then bled by manual severing of the carotid artery. The birds are then passed into the scalding room where they are passed through a warm water bath (ca. 50–54 °C) to facilitate removal of the feathers. The birds are then mechanically defeathered at the end of the scalding room. The carcasses are then passed to the evisceration room where the lower legs (feet) and heads are mechanically removed, after which the carcasses are hung again up-side down by means of leg hooks on the overhead conveyor. Thereafter, the carcasses are eviscerated mechanically after which they are washed both on the surface and internally with non-recycled water.

The carcasses are then conveyed to the chilling room for cooling by

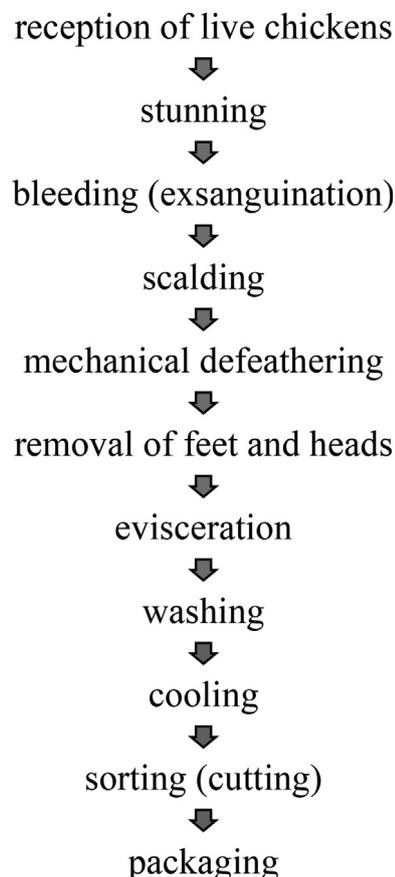


Fig. 1. Process flow diagram for the production of fresh poultry products.

means of a forced air system. After cooling the chickens are conveyed to the sorting room where they are first sorted according to size. The birds are then placed on lines where cutting is done mechanically into various portions such as legs and wings. The chicken portions are then packaged in an atmosphere containing 35% O₂, 45% CO₂ (rest N₂). Thereafter, the packaged products are sent for a second cooling again by means of cold forced air. Thereafter, they are palletized and sent to the expedition area which is maintained at 0–2 °C.

2.2. Sample collection and analysis

Samples were collected in November 2016. These consisted of air samples, swabs of food contact surfaces and packaged fresh poultry. The samples were collected and analyzed as follows.

800L air samples were collected on de Man Rogosa Sharpe (MRS) agar (Oxoid, Hampshire, UK) by means of a Spin Air sampler (IUL, Barcelona, Spain) in all the processing rooms every 2–3 h. The air sampler was sterilized with 70% ethanol in between measurements. The contamination levels on food contact surfaces in the scalding, evisceration, chilling, sorting and packaging rooms were determined by means of swabs (where possible on ca. 25 cm² areas). These surfaces included leg hooks on which the carcasses are hung as from bleeding up to sorting, rubber defeathering fingers in the defeathering and evisceration room, blades of the cutting machines in the sorting room, conveyor belts in the sorting room, and working tables and hands of the operators in these rooms. The swabs were wetted in 5 ml of sterile (autoclaved) de Man Rogosa Sharpe broth (MRS broth, Oxoid, Hampshire, UK). Serial decimal dilutions of the swabs were then prepared in tubes containing 9 ml of peptone physiological saline [PPS, 1 g neutralized bacteriological peptone (Oxoid, Hampshire, UK) + 8.5 g NaCl per liter] after which the decimal dilutions were pour-plated on MRS agar. The plates were incubated anaerobically in an anaerobic jar

Table 1
MRS and total mesophilic aerobic plate counts (\log_{10} CFUs/g or ml) of poultry and water samples.

Sample	Sample #	MRS counts (\log_{10} CFU/g or ml)		Total mesophilic aerobic counts (\log_{10} CFU/g)
		psychrotrophic	mesophilic	
Fresh wings (day 0)	1	3.7	4.3	4.6
	2	4.5	4.6	5.1
	average \pm stdev.	4.1 \pm 0.6	4.4 \pm 0.2	4.9 \pm 0.3
Fresh legs (day 0)	1	2.6	4.4	3.7
	2	2.7	3.7	4.0
	average \pm stdev.	2.7 \pm 0.1	4.1 \pm 0.5	3.9 \pm 0.2
Expired wings	1	8.7	8.3	8.9
	2	8.5	9.3	9.9
	average \pm stdev.	8.6 \pm 0.2	8.8 \pm 0.7	9.4 \pm 0.7
Expired legs	1	7.8	7.5	7.7
	2	8.2	7.0	8.1
	3	8.5	8.0	8.3
	4	8.9	7.6	8.1
	5	8.4	7.8	8.3
	6	8.2	7.2	7.7
	average \pm stdev.	8.3 \pm 0.4	7.5 \pm 0.4	8.0 \pm 0.3
Water (carcass cleaning)	1	< 1	nd ^a	nd
	2	1	nd	nd

^a nd = not determined.

containing an Anaerogen Compact sachet (Thermo Scientific-Oxoid, Hampshire, UK). The swabs were then incubated at 4 °C for 20 days to establish visually if growth (development of turbidity) occurred or not. Tubes were growth took place indicated the presence of psychrotrophic/psychrotolerant bacteria at counts below the limit of detection (< 5 CFU/swab).

Samples of fresh chicken wings and legs were collected immediately after packaging. Half of these samples were analyzed on the same day they were collected whilst the rest were kept at 7 °C and analyzed 2–3 days after the sell-by date had passed. The wings and legs were analyzed as follows. 15–20 g samples, representative of the products, were aseptically collected and transferred to sterile stomacher bags. Primary decimal dilutions of the samples of the products were prepared by adding an appropriate amount of PPS. The samples were then thoroughly homogenized in a stomacher. Thereafter, serial decimal dilutions were prepared in tubes containing 9 ml of sterile PPS. The serial decimal dilutions were then spread plated on MRS agar after which the plates were placed in anaerobic jars containing Anaerogen™ 2.5L sachets (Oxoid, Hampshire, UK). The sealed jars were incubated at 30 and 22 °C for up to 1–2 and 3–5 days, respectively, to enumerate the mesophilic and psychrotrophic MRS counts, respectively. The total aerobic (mesophilic) counts were determined by spread plating of the same decimal dilutions on plate count agar (PCA, Oxoid, Hampshire, UK) after which the plates were incubated at 30 °C and enumerated after 1–2 days. Water used to clean the carcasses in the evisceration room was also collected in sterile falcon tubes (50 ml). Serial decimal dilutions of the water samples were prepared in PPS, after which they were pour plated on MRS agar. The plates were incubated anaerobically at 22 °C as described above before the arising colonies were enumerated.

The contamination levels in the air samples were reported as colony forming units (CFU)/m³ of air. The contamination levels of LAB on food contact surfaces were reported as \log_{10} CFU/swab. The microbial contamination counts of the fresh and expired poultry samples were reported as \log_{10} CFU/g whereas those in the water were reported as \log_{10} CFU/ml.

2.3. Identification of psychrotrophic bacteria (source tracking)

Clustering of the isolates was done by means of Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) at LMG-UGent. MALDI-TOF allows for a high through-put due to the 'minimal' sample preparation and has already shown to be powerful tool for the assessing the dynamics of cultured meat spoiling bacteria on poultry meat in detail below the species level (Höll et al., 2016). For each sample, 10–15 colonies were picked from the MRS plates used for enumeration and purified by means of successive 4 × 4 streak plates. Purity was confirmed microscopically. Morphologically atypical isolates were also identified during the microscopic examination and removed from the collection of isolates to be identified by means of MALDI-TOF MS. To prepare the isolates for identification by MALDI-TOF MS, they were cultured three consecutive times at 22 °C in MRS broth. The third-generation axenic cultures were then dereplicated by MALDI-TOF MS followed by curve-based data analysis (Ghyselinck et al., 2011) using BioNumerics 7.0 software (Applied Maths, Sint-Martens-Latem, Belgium). This enabled the isolates to be grouped into clusters with identical chromatographs. Direct identification of some of the clusters to species level was possible using the Applied Biosystems database.

Representative isolates from each cluster were conclusively identified by means of 16S rRNA sequencing. Partial 16S rRNA sequences were obtained as described by De Bruyne et al. (2007). In brief, DNA was extracted according to the method of Pitcher et al. (1989), as modified for Gram positive bacteria by Björkroth and Korkeala (1996). The PCR products were purified and commercially sequenced as described by Kostinek et al. (2005). The universal primers, BKL 1 (5'-GTA-TTA-CCG-CGG-CTG-CTG-GCA-3', reverse) and Gamma (5'-CTC-CTA-CGG-GAG-GCA-GCA-GT-3', forward) were used. Identification of the consensus sequences was performed in EzTaxon-e according to Kim et al. (2012). Editing, alignment and creation of the consensus sequences was performed in BioNumerics. In those cases where the isolates selected from each cluster were determined to be different species or strains by 16S rRNA gene sequencing, the clusters from which these

Table 2
Contamination levels (MRS counts) on various surfaces in the poultry processing environment.

Location	Sample #	Time	CFU/swab	Growth after 20 days at 4 °C
Scalding room				
Leg hook	26	2:20	25	+
Rubber defeathering fingers	51	2:25	< 5	+
	55	5:00	115	+
Evisceration room				
Leg hooks	36	2:55	1325	+
	21	5:17	285	+
	50	7:18	935	+
	18	9:40	2560	+
Conveyor belt (polyurethane-coated)	29	2:50	80	+
Inox container ^a	58	7:15	500	+
	17	9:35	115	+
Drain (cover)	13	9:45	880	+
	2	9:45	135	+
Operator - left hand	28	5:10	545	-
Operator - right hand	46	5:10	140	-
Operator - left hand	53	7:10	150	+
Operator - right hand	31	7:13	415	+
Operator - left hand	16	9:35	145	+
Operator - right hand	19	9:36	10	+
Chilling room				
Wall (with condensate)	57	5:30	> 3000	+
Wall (with condensate)	59	5:40	600	+
Wall (middle)	30	5:30	10	+
Wall (near door)	20	5:40	35	+
Sorting room				
Line A				
Leg hook	52	2:42	3940	+
Cutting blade	24	2:40	1725	+
Polyurethane conveyor belt	35	2:40	< 5	+
	38	7:42	950	+
	7	10:02	300	+
Ertalon conveyor belt	32	2:45	2905	+
	37	7:40	450	+
	5	10:05	420	+
Working table (Inox)	60	2:44	< 5	+
	44	7:45	< 5	+
	11	9:58	65	+
Line B				
Cutting blade	33	2:35	110	+
Polyurethane conveyor belt	39	2:30	85	+
	47	7:35	255	+
	15	9:57	250	+
Working table (Inox)	34	2:32	5	-
	49	7:33	< 5	+
	12	9:55	< 5	-
	48	10:00	3000	+
Operator - left hand	3	10:06	310	+
Operator - right hand	8	10:06	140	+

Table 2 (continued)

Location	Sample #	Time	CFU/swab	Growth after 20 days at 4 °C
Line C				
Cutting blade	22	2:35	5	+
	43	7:30	10	+
	14	9:55	205	+
Polyurethane conveyor belt	40	2:30	40	+
	25	7:35	15	+
	6	9:58	280	+
Working table (Inox)	42	2:32	< 5	+
	54	7:40	< 5	+
Operator - right hand	41	7:40	385	+
Operator - left hand	23	7:40	245	+
Operator - right hand	10	10:03	265	+
Operator - left hand	9	10:05	550	+
Packaging room				
Operator - left hand	27	8:45	410	+
Operator - right hand	45	8:47	125	+
Operator - left hand	1	10:15	240	+
Operator - right hand	4	10:15	255	+

^a Container for chickens which were unsuccessfully hooked after the legs are cut off.

isolates arose were analyzed again and sub-divided into smaller (and smaller) clusters until the cluster consisted of the same isolate.

3. Results and discussion

3.1. Microbial contamination profiles

The MRS and total aerobic counts on fresh and expired chicken wings and legs are shown in Table 1. The MRS counts on the food contact surfaces and in the air in various sections of the processing environment are shown in Tables 2 and 3, respectively. These results are discussed below.

3.1.1. Contamination profiles of fresh and expired chicken wings and legs

As can be seen in Table 1, the psychrotrophic and mesophilic MRS counts on fresh wings analyzed on day 0 (= the day they were packaged) were similar, on average 4.1 and 4.4 log₁₀ CFU/g, respectively. The total aerobic plate counts (TAPCs) were slightly higher at 4.9 log₁₀ CFU/g. After the sell-by date, a similar trend was observed as the psychrotrophic and mesophilic MRS counts on the expired wings were on average 8.6 and 8.8 log₁₀ CFU/g, respectively, whilst the total aerobic plate counts were higher at 9.4 log₁₀ CFU/g.

The average psychrotrophic MRS counts on fresh legs analyzed on day 0 (2.7 log₁₀ CFU/g) were much lower than the corresponding mesophilic MRS counts (4.1 log CFU/g), whilst the mesophilic MRS counts were similar to the TAPCs (3.9 log₁₀ CFU/g). In difference to the expired wings, a different trend was observed for the expired legs, whereby the psychrotrophic MRS counts of the expired legs were almost 1 log₁₀ CFU/g higher than the mesophilic MRS counts. This indicated that cold adapted bacteria dominated the spoilage flora of expired legs. In this case the use mesophilic enumeration techniques such as ISO 15241:1998 (ISO, 2015) would underestimate the true numbers of bacteria able to grow on MRS agar on chicken legs. These results indicate that although psychrotrophic bacteria able to grow on MRS agar are not dominant in the initial microflora of fresh poultry products, they become an important part of the spoilage flora of fresh poultry.

Table 3
MRS counts of the air samples collected in the processing environment.

Location	Sample #	Time	CFU/m ³
Slaughtering room	13	2:25	69
	15	4:50	1625
	18	7:07	1510
Scalding room	12	2:20	11
Evisceration room (near entrance)	3	2:50	28
	6	5:18	25
	7	7:20	70
	5	9:42	80
Evisceration room (back of the room)	2	5:40	1225
	4	7:20	150
	8	9:37	100
Cooling room	9	5:40	19
Sorting (middle of the room)	1	2:40	5
Line A	17	7:50	10
	14	10:03	4
Line B	10	7:35	10
	18	10:02	6
Packaging room	11	8:50	11
	16	10:25	4

This is supported by Bjorkroth et al. (2005) who determined that enterococci (35.7% of all LAB isolates) dominate the initial spoilage LAB flora of fresh MA-packaged marinated broiler legs stored at 6 °C, whilst carnobacteria dominate the spoilage flora (59.7% if all LAB isolates); indicating a shift from homofermentative cocci towards heterofermentative rods.

3.1.2. Contamination profile of carcass wash water

One of the two samples of the water used to wash the carcasses were determined to be contaminated with psychrotrophic bacteria able to grow on MRS agar. Although the counts were low and the water is not recycled, the volume of water used to wash the carcasses is large and contact is made with both the interior and exterior surfaces of the carcass, implying that wash water could be a route *via* which psychrotrophic bacteria able to grow on MRS agar contaminate fresh poultry products. This observation is supported by Capita et al. (2001) who reported that the water used in a poultry processing plant could be a source of psychrotrophic bacteria.

3.1.3. Contamination profiles on food contact surfaces after cleaning and disinfection

The contamination profiles on the various food contact surfaces analyzed in the study are shown in Table 2. Swabs of selected food contact surfaces were collected periodically during the course of a shift to establish if the contamination levels evolved during processing. The samples highlighted in bold letter type depict swabs which were taken of selected food contact surfaces after cleaning and disinfection (= shortly before production started). This analysis was performed to assess the efficacy of the current cleaning and disinfection protocols (strictly) with regards to bacteria which are capable of growing on MRS.

The food contact surfaces swabbed after cleaning and disinfection were i) leg hooks on which the carcasses are hung upside-down and conveyed overhead throughout the processing environment, ii) the cutting blades on three sorting lines (A, B and C) which were in use on the day of sampling, iii) the working tables on these sorting lines, and iv) Ertalon and polyurethane conveyor belts on these sorting lines. As can be seen in Table 2, bacteria capable of growing on MRS agar were

recovered from all 15 surfaces sampled after cleaning and disinfection. On 12 of the 15 surfaces, the MRS counts were above the limit of detection (LOD, < 5 CFU/swab) and ranged from 5 to as high as 3940 CFU/swab. Whilst the MRS counts were below the LOD on three of the surfaces, it was determined that psychrotrophic bacteria capable of growing on MRS agar were present on these surfaces after enrichment of the swabs (= 20 days at 4 °C).

In more detail, swabs taken of leg hooks in the scalding, evisceration and sorting rooms had MRS counts ranging from 25 to 3940 CFU/swab. The leg hooks were made of either plastic (Ertalon) or Inox and had several corners which make it difficult to clean and disinfect all surfaces thoroughly, especially given the fact they were cleaned *in situ* (without removal from the overhead conveyor system). The cutting blades on sorting lines A, B and C had MRS counts ranging from 5 to 1725 CFU/swab after cleaning and disinfection. As for the leg hooks, the cutting blades were also cleaned *in situ*, which could compromise access to all surfaces as evidenced by these results. Swabs taken after cleaning and disinfection of the polyurethane conveyor belts in the evisceration room, and on lines A, B and C of the sorting room had MRS counts of 80, < LOD, 85 and 40 CFU/swab, respectively. These counts were in general lower than those observed on the leg hooks and was attributed to their flat, smooth surfaces which are easier to clean and disinfect. In difference, the Ertalon conveyor belt on sorting line A had a very high MRS count of 2905 CFU/swab after cleaning and disinfection. Unlike the polyurethane conveyor belts which were smooth and flat, the Ertalon conveyor belt had large holes and open linkages which are not only potential sites where bacteria may be harboured but are also make cleaning and disinfection difficult. Psychrotrophic bacteria capable of growing on MRS agar were present on all conveyor belts sampled as growth was evident in all swabs after enrichment at 4 °C.

The Inox working tables on lines A, B and C of the sorting room had low MRS counts of ≤ 5 CFU/swab. As for the polyurethane conveyor belts, the low contamination levels on the Inox working tables was attributed to the fact that they are flat and smooth which facilitated cleaning and disinfection. In addition, these tables are regularly disinfected with an alcohol-based disinfectant during processing, which limits the accumulation of bacteria on their surfaces. The rubber fingers of the defeathering machine located in the scalding room had < 5 CFU/swab, which indicated that despite their complex structure they can be effectively cleaned and disinfected. However, it can be seen in Table 2 that psychrotrophic bacteria were present on the rubber fingers as growth was observed after enrichment at 4 °C.

These results show that the current cleaning and disinfection protocols are not effective as rather high levels of bacteria capable of growing on MRS agar were found on some of the food contact equipment sampled and psychrotrophic bacteria were recovered after enrichment from almost all swabs (11/12) taken after cleaning and disinfection. It should also be taken into account that food contact surfaces such as leg hooks, Ertalon conveyor belts and cutting blades come into direct contact with the carcasses, whereby they are contaminated by blood and other organic matter from the carcasses. This organic matter could support growth of present bacteria to high levels and/or the formation of biofilms on those parts of these food contact surfaces which are difficult to clean and disinfect. Additional contamination of these food contact surfaces could occur by airborne bacteria in the processing environment (see §3.1.5).

3.1.4. Evolution of contamination levels on food contact surfaces during processing

As expected, the MRS counts on the food contact surfaces generally increased during processing (see Table 2). The trends observed for each food contact surface were as follows.

The MRS counts on rubber fingers on the defeathering machine in the scalding room increased from < 5 after cleaning and disinfection to 115 CFU/swab after approximately 2 h of processing. Psychrotrophic bacteria were recovered from both swabs after enrichment at 4 °C,

indicating that cross-contamination of the carcasses with psychrotrophic bacteria can occur via the rubber fingers. The MRS counts on the leg hooks in the evisceration room initially decreased from 1325 CFU/swab before processing started to 285 CFU/swab after approximately 2 h of processing. Thereafter, the MRS counts increased to 2560 CFU/swab after approximately 8 h of processing. The initial decrease attributed to the fact that during start-up the hooks were passed through the scalding water bath (ca. 55–58 °C) for a number of cycles to ensure that the system was working properly. During this process some of the bacteria present on the hooks was inactivated. *Lactobacillus sakei*, *Leuconostoc mesenteroides* and *Lactobacillus curvatus*, which are common meat spoilage LAB, have been determined to have $D_{57^\circ\text{C}}$ values of 52, 34.9 and 22.5s (Franz and von Holy, 1996). During processing the temperature of the scalding water dropped to 50–54 °C as result of the passage of the carcasses, which implied that in comparison to the start-up period, the rate of inactivation during scalding was reduced once processing had started. As the scalding water itself is not recycled, it is only topped up to compensate for water lost due to splashing or water which remains on the carcasses when they exit the water bath. As a result of the transfer of bacteria from contaminated parts of the carcasses to the water (i.e. feathers, legs and faecal matter), the MRS counts in the water and (by extension) on the leg hooks increase steadily during production. Psychrotrophic bacteria were recovered from all swabs of the leg hooks, indicating that the carcasses could be cross-contaminated during conveyance.

The MRS counts on the polyurethane conveyor belts located in the sorting room also increased during production. As an example, the MRS counts on the polyurethane conveyor belt on sorting line A increased from < 5 CFU/swab after cleaning and disinfection to 950 and 300 CFU/swab after approximately five and 7 h of processing. In contrast, the MRS counts on the Ertalon conveyor belt decreased from 2905 CFU/swab after cleaning and disinfection to 450 and 420 CFU/swab after five and 8 h of processing, respectively. This apparent reduction could simply reflect variation in contamination levels on different sections of the conveyor belt. Before production, swabs were taken when the conveyor belt was stationary. These swabs included the holes on the Ertalon plates and the spaces in between the linkages connecting the plates. These areas are much more difficult to clean and disinfect and are therefore expected to harbour more bacteria. These areas could not be sampled during processing as the conveyor belt was operated continuously. Only the flat surfaces of the belt, which are easier to clean and disinfect, were accessible. Psychrotrophic bacteria were recovered from all swabs of both polyurethane and Ertalon conveyor belts after enrichment, which implies that cross-contamination of fresh-cut poultry products can occur on the conveyor belts.

Although psychrotrophic bacteria were recovered from nearly all (7/9) swabs collected of the Inox working tables in the sorting room, only three of the nine swabs had counts \geq LOD. The working tables on line A and C which were contaminated by 65 and 3000 CFU/swab after 7 h of processing, respectively. As mentioned previously, the low MRS counts on most of the working tables were attributed to the fact that they are regularly disinfected with an alcohol-based disinfectant by the sorting operators. The high MRS count on the working table of line C could have been due to a swab that was taken before the table was periodically disinfected. This, however, implies that products can be cross-contaminated with psychrotrophic bacteria on these tables either directly when some cut poultry products are temporarily placed on the them or via sorting operators who come into contact with both the working tables and the poultry products. As observed for the other food contact surfaces, the MRS counts on the cutting blade of sorting line C increased during processing from 5 CFU/swab after cleaning and disinfection to 205 CFU/swab, after approximately 7 h of processing. Psychrotrophic bacteria were recovered from all swabs of the cutting blades after enrichment of the swabs at 4 °C. This also indicates that cross-contamination by psychrotrophic bacteria can take place during cutting. In addition, it has to be noted that due to the nature of the

process, cutting also distributes psychrotrophic bacteria in the normally sterile flesh.

The operator's hands in the evisceration room had MRS counts which ranged from 10 to 545 CFU/swab. These were similar to those on the hands of the operators in the sorting and packaging rooms. Psychrotrophic bacteria were recovered from almost all (14/16) swabs taken of the hands of the operators. As these operators frequently came into contact with the poultry carcasses or the cut poultry portions, they were expected to contribute to cross-contamination of the products by psychrotrophic bacteria. Bacteria capable of growing on MRS agar were found on all three walls swabbed in the chilling room at counts ranging from 10 to > 3000 CFU/swab. As can be seen in Table 2, the wall in the chilling room which had condensate was contaminated by bacteria capable of growing on MRS agar at high levels (\geq 600 CFU/swab). The other two other walls swabbed in the chilling room (which did not have condensate on them) had much lower MRS counts (\leq 35 CFU/swab). This indicates that condensate supports the growth of psychrotrophic LAB on the walls of the chilling room and may also facilitate their dislodgment from the walls. Contaminated condensate could be a source of contamination by psychrotrophic bacteria in the chilling room as the forced cold air system blows air at a high rate into the room which could create aerosols of the contaminated condensate. Once airborne, the bacteria would then be able to contaminate the carcasses in the chilling room as well as carcasses and cut products in the other processing rooms.

3.1.5. Contamination profiles of air in the processing environment

The counts of airborne bacteria capable of growing on MRS agar in various locations of the processing environment during processing are shown in Table 3. In general, the highest counts were observed in the slaughter room followed by the evisceration room. The lowest counts of airborne bacteria were observed in the chilling, sorting and packaging rooms. The trends observed during production were as follows.

The air in the slaughter room was determined to have airborne MRS counts of 69 CFU/m³ before slaughtering started. The counts increased sharply to 1625 and 1510 CFU/m³ after approximately two and 4 h of slaughtering, respectively. It was observed that waste in the form of faecal matter, feathers and blood accumulated rapidly once slaughtering has started. Psychrotrophic bacteria have been reported to originate from the feathers and the feet of the birds (Capita et al., 2001). Additionally, LAB, including *Lactobacillus*, *Enterococcus* and *Streptococcus* spp. are known to be present in their faeces as well (Gertzou et al., 2017). In difference to Capita et al. (2001), Vihavainen et al. (2007) reported that, with the exception of *Lactobacillus sakei*, the birds (= feathers, skin and mucous membranes) were not major sources of psychrotrophic LAB recovered from broiler products analyzed at the sell-by date. Non-psychrotrophic species such as *Enterococcus faecalis* and *Enterococcus faecium* dominated the carcass samples analyzed by Vihavainen et al. (2007). It should also be noted that differences between Capita et al. (2001) and Vihavainen et al. (2007) could have been due to the fact that the latter only analyzed the shaft of the feather after discarding the vanes, which could potentially harbour LAB.

In order to minimize the accumulation of waste, the floor of the slaughter room was cleaned every 30–40 min with high pressure water jet cleaners. This creates bio-aerosols and a very humid environment via which the bacteria present on the floor (and feathers and faeces thereon) not only became airborne but also remain viable. In this way the airborne bacteria can cross-contaminate the carcasses during slaughtering and food contact surfaces and products in the rest of the processing rooms. This is very likely as flow of air in the processing environment was not controlled. In addition, some of the bacteria on the feathers, legs and faeces are expected to become airborne as result of the activities (physical handling) that takes place in the steps leading to and after slaughtering. Whilst the majority of the airborne bacteria in the slaughter room were expected to originate from the birds and the waste generated during slaughter, the floors (which were always wet)

could potentially harbour 'in-house' bacteria which are adapted to the low temperatures ($\leq 10^{\circ}\text{C}$) maintained in the processing environment.

In the evisceration room, counts of airborne bacteria capable of growing on MRS of 28 CFU/m³ were determined near the entrance before processing started. The MRS counts subsequently increased to 70 and 80 CFU/m³ after approximately four and 7 h of processing, respectively. Air samples were also collected at the back of the evisceration room near a large opening in the wall separating the evisceration room from the slaughter room. It is through this opening that the overhead conveyor belt on which slaughtered birds were transferred on leg hooks to the evisceration room. The counts of airborne MRS counts were generally higher in this section of the evisceration room, ranging from 100 to 1225 CFU/m³. The higher counts of airborne bacteria observed in this section of the room compared to the entrance were a direct result of the passage of contaminated air from the slaughter room into this area. Additionally, the carcasses and the floor in this section of the evisceration room were washed with high pressure water jets in this section of the room, which could also create aerosols resulting in higher levels of viable airborne bacteria. The air in the cooling and packaging rooms had lower airborne MRS counts (≤ 19 CFU/m³). It is important to note that in these sections cleaning of the floors with high pressure water jets was performed well before processing started.

In difference to our findings, Vihavainen et al. (2007) determined that the highest counts of airborne LAB in the air of the chilling room (> 344 CFU/m³) of a broiler slaughter and processing facility in Finland. However, it should be noted that in difference to the present study, Vihavainen et al. (2007) did not collect air samples in the slaughterhouse and evisceration rooms. In agreement with our findings, Vihavainen et al. (2007) reported that the counts of airborne LAB were generally very low (< 30 CFU/m³) to not detectable in the processing areas downstream of the chilling room.

3.2. Identification and source tracking

As mentioned previously, (where possible) 10–15 isolates (capable of growing on MRS agar) were selected from each for clustering and identification by means of MALDI-TOF MS and 16S rRNA gene sequencing, respectively. The later was performed on at least two representative isolates from each definitive cluster. Overall diversity of the psychrotrophic bacteria capable of growing on MRS agar in the fresh and expired wings and legs is shown in Table 4.

Table 4
Overall diversity of psychrotrophic bacteria in fresh and expired chicken wings and legs.

Cluster	N# of isolates in cluster	Product type	Fresh	Expired
<i>B. thermosphacta</i> 1	11	Legs	–*	5, 6**
<i>B. thermosphacta</i> 2	2	Legs	–	3, 5
<i>C. divergens</i>	2	Legs	–	4
<i>C. maltaromaticum</i> 1	72	Legs	1, 2	3, 4, 5
		Wings	1, 2	1
<i>C. maltaromaticum</i> 2	49	Wings	2	1, 2
		Legs	–	5
<i>C. maltaromaticum</i> 3	4	Legs	–	3, 5
<i>L. brevis</i>	2	Legs	–	3
<i>L. curvatus</i>	8	Wings	–	2
<i>L. mesenteroides</i> subsp. <i>dextranicum</i>	3	Wings	–	2
<i>L. graminis</i>	1	Legs	–	6
<i>L. paraplantarum</i>	11	Wings	2	1, 2
<i>L. gelidium</i>	2	Wings	–	1
<i>L. helveticus</i>	5	Wings	2	1
<i>L. paracasei</i>	1	Legs	–	4

* Not recovered from the sample, ** = sample number.

3.2.1. Diversity of psychrotrophic bacteria in fresh and expired chicken wings and legs

As can be seen in Table 4, 173 isolates recovered from expired wing and leg samples were grouped into 14 species specific clusters using a combination of MALDI-TOF MS and 16S rRNA gene sequencing. All 14 clusters were assigned a definitive species epithet.

The clear majority (71%, 125/177) of these isolates were determined to belong to one of three *Carnobacterium maltaromaticum* clusters designated as *C. maltaromaticum* 1 (72 isolates), *C. maltaromaticum* 2 (49 isolates), and *C. maltaromaticum* 3 (4 isolates). These findings are in agreement with Vihavainen et al. (2007) who determined that 64% of isolates recovered from MAP broiler products were clustered together with either *Carnobacterium divergens* or *C. maltaromaticum* type strains. Leisner et al. (2007) also stated that *Carnobacterium* spp., in particular *C. maltaromaticum* and *C. divergens*, were predominant spoilage microorganisms in raw poultry meat regardless of the packaging conditions. Rouger et al. (2018) have also reported that *Carnobacterium* spp. (in addition to *B. thermosphacta* and *Pseudomonas* spp.) dominated the flora of MAP chicken legs stored at 4 °C. The other clusters that were identified to the species level in the present study were *B. thermosphacta* (two distinct clusters), *C. divergens*, *L. mesenteroides* subsp. *dextranicum*, *Lactobacillus brevis*, *L. curvatus*, *Lactobacillus graminis*, *Lactobacillus paraplantarum*, *Lactobacillus helveticus* and *Leuconostoc gelidium*. *B. thermosphacta* has been reported to be an important spoilage associated non-LAB in aerobically or MAP packaged meat products (Doulgeraki et al., 2012; Ercolini et al., 2006, Rouger et al., 2018). As mentioned above, *C. divergens* is well established as a predominant spoilage species in raw poultry products (Leisner et al., 2007). In difference to our findings, Susiluoto et al. (2003) reported that *Leuconostoc gasicomitatum* dominated the LAB spoilage flora of marinated broiler meat strips after the end of the sell-by date. However, in agreement with our findings they also determined that *C. divergens* and *L. curvatus* were important members of the spoilage flora after the sell-by date.

Isolates were recovered from both expired and fresh products in only four of the 14 clusters (*C. maltaromaticum* 1, *C. maltaromaticum* 2, *Lactobacillus paraplantarum* and *Lactobacillus helveticus*. This confirms the results of the enumeration (see Table 1) whereby it was concluded that bacteria which are potentially associated spoilage of chicken cuts (= bacteria which dominate the microflora of expired chill-stored MAP wings and legs) initially constitute a minor part of the microflora of the fresh products. This is also in agreement with Björkroth et al. (2005) who reported that whilst *Enterococcus* spp. dominate the microflora of fresh MAP marinated broiler legs, they are overgrown by *Carnobacterium* and *Lactobacillus* spp. during storage at 6 °C. It was noted that in six of the 14 clusters more than one sample of a expired (and/or fresh) product were contaminated by the same isolate. As an example, *C. maltaromaticum* 1 was recovered from samples 1 and 2 of fresh legs and also from samples 3, 4 and 5 of expired legs. In addition to this, the same isolate was recovered from samples of both wings and legs in two of the 14 clusters (= *C. maltaromaticum* 1 and *C. maltaromaticum* 2). These results imply that cross-contamination can occur along and across the processing lines. This was expected as the carcasses are for the most processed along the same lines and are only processed on specialized lines after they have been cut.

3.2.2. Source tracking

Of primary importance for the source tracking where those clusters containing isolates which were recovered from either fresh or expired wings or legs (= analyzed after the sell-by date) and were also recovered from either i) fresh legs or wings sampled on the same day as they were processed and packaged, and/or ii) swabs taken of food contact surfaces, and/or iii) air in the processing environment. These clusters are shown in Table 5. The clusters consisting of isolates which were not recovered from the fresh or expired poultry products are shown in Table 6. In addition to these, some of the isolates recovered

Table 5
Clusters of product linked strains which were identified by means of MALDI-TOF and gene sequencing.

Isolate (cluster)	Sample type	Sample	Sampling location	Time
<i>B. thermosphacta</i> 1	Legs (expired)	6* (2**)	Packaging room	–
	Legs (expired)	5 (3)	Packaging room	–
	Swab	52	Leg hook - sorting line A	2:42
	Swab	24 (6)	Cutting blade - sorting line A	2:40
<i>B. thermosphacta</i> 2	Legs (expired)	3	Packaging room	–
	Legs (expired)	5	Packaging room	–
<i>C. divergens</i>	Legs (expired)	4	Packaging room	–
	Swab	33	Slicing blades - sorting line B	2:35
<i>C. maltaromaticum</i> 1	Wings (fresh)	2 (3)	Packaging room	–
	Wings (fresh)	1 (2)	Packaging room	–
	Wings (expired)	1	Packaging room	–
	Legs (fresh)	2 (4)	Packaging room	–
	Legs (fresh)	1 (3)	Packaging room	–
	Legs (expired)	3 (4)	Packaging room	–
	Legs (expired)	4 (8)	Packaging room	–
	Legs (expired)	5 (4)	Packaging room	–
	Swab	8	Operator - sorting line B (right hand)	9:06
	Swab	23 (7)	Operator - sorting line C (left hand)	7:40
	Swab	10	Operator - sorting line C (right hand)	10:03
	Swab	4 (7)	Operator – packaging room (right hand)	10:15
	Swab	27 (4)	Operator - packaging (left hand)	8:45
	Swab	32 (6)	Ertalon conveyor belt - sorting line A	2:45
	Swab	38 (6)	Ertalon conveyor belt - sorting line A	7:42
	Swab	7 (5)	Ertalon conveyor belt – sorting line A	10:02
Swab	5	Ertalon conveyor belt - sorting line A	10:05	
Swab	52 (3)	Leg hooks - sorting line A	2:42	
<i>C. maltaromaticum</i> 2	Wings (fresh)	2	Packaging room	–
	Wings (expired)	1 (2)	Packaging room	–
	Wings (expired)	2	Packaging room	–
	Legs (expired)	5	Packaging room	–
	Swab	32 (5)	Ertalon conveyor belt – sorting line A	2:45
	Swab	38	Ertalon conveyor belt - sorting line A	7:42
	Swab	7 (2)	Ertalon conveyor belt – sorting line A	10:02
	Swab	39 (3)	Polyurethane conveyor belt - sorting line B	2:30
	Swab	15	Polyurethane conveyor belt - sorting line B	9:57
	Swab	52 (3)	Leg hook - sorting line A	2:42
	Swab	11 (2)	Working table - sorting line A	9:58
	Swab	33 (2)	Cutting blade - sorting line B	2:35
	Swab	14	Cutting blade - sorting line C	9:55
	Swab	47 (8)	Inox container - evisceration room	9:35
	Swab	41 (6)	Inox container - evisceration room	9:35
	Swab	45 (3)	Operator packaging (right hand)	8:47
Swab	1 (2)	Operator packaging (left hand)	10:15	
<i>C. maltaromaticum</i> 3	Legs (expired)	3	Packaging room	–
	Legs (expired)	5	Packaging room	–
	Swab	27 (2)	Operator packaging (left hand)	8:45
<i>L. brevis</i>	Legs (expired)	3	Packaging room	–
	Swab	40	Polyurethane conveyor belt - sorting line C	2:30
<i>L. curvatus</i>	Wings (expired)	2	Packaging room	–
	Air	15 (4)	Slaughter floor	4:50
	Air	13	Slaughter floor	2:25
	Air	12	Scalding room	2:20
	Swab	33	Cutting blades - sorting line B	2:35
<i>L. paraplantarum</i>	Wings (fresh)	2	Packaging room	–
	Wings (expired)	1	Packaging room	–
	Wings (expired)	2 (2)	Packaging room	–
	Air	7	Evisceration room	7:20
	Swab	14	Cutting blade – sorting line C	9:55
	Swab	10 (2)	Operator - sorting line C (right hand)	10:03
	Swab	9	Operator - sorting line C (left hand)	11:05
Swab	45 (2)	Operator - packaging (right hand)	8:47	
<i>L. gelidium</i>	Wings (expired)	1	Packaging room	–
	Swab	6	Polyurethane conveyor belt - sorting line C	9:58

(continued on next page)

Table 5 (continued)

Isolate (cluster)	Sample type	Sample	Sampling location	Time
<i>L. helveticus</i>	Wings (fresh)	2	Packaging room	–
	Wings (expired)	1	Packaging room	–
	Swab	33 (2)	Slicing blade - sorting line B	2:35
	Swab	45	Operator - packaging (right hand)	8:47

* = sample number, ** = number of isolates.

from swabs of food contact surfaces or the air were not clustered (= had unique MALDI TOF chromatograms), these results are not shown. These clusters show were along the processing line products are potentially contaminated by psychrotrophic bacteria. The most important locations were i) the cutting (slicing) blades in the sorting room ii) the Ertalon and polyurethane conveyor belts in the sorting room iii) leg hooks iv) air v) hands of the sorting and packaging operators and vi) Inox working tables in the sorting room. These results are discussed below.

3.2.3. Cutting (slicing) blades

As can be seen in Table 5, *B. thermosphacta* 1, *C. maltaromaticum* 2, *C. divergens*, *L. curvatus*, *L. paraplantarum* and *L. helveticus* which were recovered from expired and/or fresh wings and legs, were also recovered from the cutting blades used on all three sorting lines (A, B and C). With the exception of *L. paraplantarum*, these isolates were also recovered from the cutting blades on lines A and B before production

had started (= after cleaning and disinfection). The cutting blades sampled on lines A and B after cleaning and disinfection had MRS counts of 1725 to 110 CFU/swab, respectively. *C. maltaromaticum* 2 was also recovered from a swab of the cutting blade on line C after approximately 3 h of processing whilst *L. paraplantarum* was also recovered from the same swab. These results indicate viable bacteria which remain attached to the surfaces of the cutting blades after cleaning and disinfection are indeed transferred to the poultry products during cutting, after which they become important with regards to the spoilage of these products. Once contaminated the products themselves can contaminate other food contact surfaces, which would facilitate cross contamination. As mentioned previously, the cutting blades are cleaned *in situ* (whilst they are still mounted) which could limit the efficacy of the cleaning and disinfection performed. The high counts on some of the cutting blades indicate the possibility of biofilms occurring on the blades.

Table 6

Clusters of non-product linked strains which were identified by means of MALDI-TOF and gene sequencing.

Isolate	Sample type	Sample	Sampling location	Time
<i>E. gilvus</i>	Air	15* (2**)	Slaughter room	4:50
	Air	14	Slaughter room	7:07
	Air	7	Evisceration room	7:20
	Air	8	Evisceration room	9:37
	Air	19	Sorting room - line A	10:03
	Swab	13	Drain cover - evisceration room	9:45
	Swab	16	Operator - evisceration room (left hand)	9:35
	Swab	8	Operator - sorting line B (right hand)	9:06
<i>Lactobacillus raffinolactis</i>	Air	12	Scalding room	2:20
	Air	6 (2)	Evisceration room	5:18
	Air	3	Evisceration room	2:50
	Air	4	Evisceration room	7:20
	Swab	13	Drain cover - evisceration room	9:45
	Swab	5	Ertalon conveyor - line A	10:05
<i>L. sakei</i> subsp. <i>sakei</i>	Air	9 (2)	Evisceration room	5:40
	Air	6	Evisceration room	5:18
	Air	5 (2)	Evisceration room	9:42
	Swab	20	Chilling room - wall with condensate	5:40
	Swab	17 (2)	Slicing blade - sorting line A	2:40
<i>E. hirae</i>	Air	13	Slaughter room	2:25
	Air	8	Evisceration room	9:37
	Swab	13	Drain cover - evisceration room	9:45
	Swab	5	Ertalon conveyor belt - sorting line A	10:05
	Swab	6	Polyurethane conveyor belt - sorting line C	9:58
	Swab	26	Leg hooks - scalding room	2:20
	Swab	4	Operator packaging (right hand)	10:15
	Swab	10	Operator - sorting line C (right hand)	10:03
<i>L. lactis</i>	Air	14 (2)	Slaughter room	7:07
	Air	3	Evisceration room	2:50
	Air	2 (5)	Chilling room	5:40
	Swab	50	Leg hooks - evisceration room	7:18
	Swab	18	Leg hooks - evisceration room	9:40
	Swab	55	Cutting blade - sorting line A	2:40
	Swab	17	Cutting blade - sorting line A	2:40
	Swab	16 (3)	Operator - evisceration room (left hand)	9:35
	Swab	1	Operator - packaging room (left hand)	10:15

* = sample number, ** = number of isolates, + = growth after enrichment at 4 °C.

3.2.4. Leg hooks

As can be seen in Table 5, *B. thermosphacta* 1, *C. maltaromaticum* 1 and *C. maltaromaticum* 2 which were recovered from expired or fresh wings and legs were also recovered from swabs taken of the leg hooks used on sorting line A. All three isolates originated from a swab that was collected after cleaning and disinfection had been done which had very high MRS counts of 3940 CFU/swab. As noted above for the cutting blades, these results confirm that psychrotrophic bacteria on the leg hooks are transferred to carcasses. As mentioned previously, leg hooks are very difficult to clean and disinfect thoroughly due to the fact that they are cleaned *in situ* (whilst they are on the overhead conveyor system) and their complex structure whereby bacteria could be harboured in one of the many hooks. As mentioned above, once contaminated, the products themselves can contaminate other food contact surfaces, which would facilitate cross contamination.

3.2.5. Conveyor belts

C. maltaromaticum 1, *C. maltaromaticum* 2, *L. brevis* and *L. gelidium*, which were recovered expired or fresh wings and legs, were also recovered from swabs taken of the conveyor belts on sorting lines A, B and C. *C. maltaromaticum* 1 and *C. maltaromaticum* 2 were both recovered from swabs of the Ertalon conveyor belt on sorting line A after cleaning and disinfection had been as well as after approximately three and 5 h of processing. In addition to this, *C. maltaromaticum* 2 was also recovered from a swab of the polyurethane conveyor belt on line B after cleaning and disinfection had been done and after 5 h of processing. *L. brevis* was recovered from a swab of the polyurethane conveyor belt on line C after cleaning and disinfection had been done. *L. gelidium* was also recovered from the polyurethane conveyor belt on line C after approximately 5 h of processing. These results also indicated that residual bacteria on the conveyor belts after cleaning and disinfection are transferred to carcasses after which they become important with regards to the spoilage of these products.

3.2.6. Operators hands and working tables

C. maltaromaticum 1, *C. maltaromaticum* 2, *C. maltaromaticum* 3, *L. paraplantarum* and *L. gelidium*, which were recovered from expired or fresh chicken wings and legs, were also recovered from swabs of the operator's hands in the sorting and packaging rooms. *C. maltaromaticum* 2 was also recovered from a swab of the working table on sorting line A during processing. These results confirm that poultry products are indeed (cross) contaminated with psychrotrophic bacteria as a result of coming into contact with the operators and the working tables.

3.2.7. Air

Air was also determined to a vector *via* which bacteria could contaminate poultry products during processing. As discussed previously, very high levels of airborne bacteria capable of growing on MRS agar were observed in the slaughter room when processing had started, with the numbers in the rest of processing rooms decreasing the further the room was located from the slaughter room. *L. curvatus* and *L. paraplantarum*, which were recovered from expired or fresh wings, were also recovered from samples of air collected in the slaughter, scalding and evisceration rooms. As the flow of air in the processing environment was not controlled, air contaminated with aerosols of viable bacteria from the slaughter, scalding or evisceration rooms could directly contaminate the carcasses in these rooms or moved to the other rooms where it would have either directly contaminated the products during chilling, sorting or packaging or alternatively contaminated important vectors of psychrotrophic bacteria such as cutting blades, operators hands and working tables. This later route is very important as both *L. curvatus* and *L. paraplantarum* were also recovered from the cutting blades on sorting lines B and C, respectively.

The important role of air as vector of psychrotrophic bacteria in the processing environment was also confirmed in the clusters shown in Table 6. These clusters, consisted of isolates which were not recovered

from either fresh or expired wings or legs, but nonetheless provide valuable data with regards to the source tracking. In these cluster it can be seen that some airborne LAB isolates arising from the slaughter and/or evisceration rooms were recovered in other processing rooms, in either the air or on food contact surfaces. As an example, *Enterococcus gilvus*, which was recovered from the air in the slaughter and evisceration rooms, was also recovered from air in sorting room and from swabs taken of the cover of the drain in the evisceration room and from the hands of the operators in the evisceration and sorting rooms. *Enterococcus hirae*, which was recovered from the air in the slaughter and evisceration rooms, was also recovered from swabs taken of the cover of the drain in the evisceration room, leg hooks in the scalding room, cover of the drain in the evisceration room, Ertalon and polyurethane conveyor belts on sorting lines A and C, respectively, and the hands of operators in the sorting and packaging rooms. Although the isolates shown in Table 6 were not recovered from either fresh or expired wings and legs, it is very clear that once airborne, bacteria capable of growing on MRS agar have the potential to contaminate products and/or food contact surfaces throughout the processing environment.

4. Conclusions

The sources of psychrotrophic bacteria associated with fresh and expired chicken cuts were established in the study by means of collecting and analyzing environmental and product samples at a large poultry processing plant located in Belgium. The results of the study also enabled for the dynamics of the contamination profiles to be established as the samples were collected during the course of a normal production shift.

This study showed that the cleaning and disinfection protocols employed on important food contact surfaces such as the cutting blades, conveyor belts and leg hooks were not adequate with regards towards psychrotrophic bacteria which are capable of growing on MRS agar. The importance of psychrotrophic bacteria which remained on these surfaces after cleaning and disinfection was established when it was determined that they could contaminate the carcasses or cut products (legs and wings) during processing and subsequently become predominant in the flora of the products after the sell-by date. The rather high MRS counts on some of the food contact surfaces after cleaning and disinfection indicate the potential presence of biofilms on these surfaces. Additional contamination of the food contact surfaces could also occur post cleaning and disinfection *via* airborne bacteria. During processing the MRS counts on the food contact surfaces generally increased indicating that they are important locations at which cross-contamination takes place.

The study also showed that air is a very important vector by which bacteria can contaminate fresh chicken cuts during processing. Firstly, the levels of airborne bacteria in the slaughter room increased steeply when production started as a result of liberation of bacteria from feathers, feet and faeces (physically) and bio-aerosol formation due to the frequent cleaning of the floors with high pressure water jets. This was also expected to continue during defeathering and evisceration. Airborne bacteria recovered from the slaughter, scalding and evisceration rooms were in some cases also recovered in the air on food contact surfaces in other processing rooms. This showed that once airborne, the bacteria were highly mobile and able to cause both direct and indirect contamination in the processing rooms.

These results are of importance to poultry processors as they establish areas of attention with regards to contamination of their products by psychrotrophic bacteria. Certainly, effective cleaning and disinfection of all food contact surfaces should be a priority. Whilst fresh poultry processing plants operate under GMP conditions, it is also advised to consider the use of over-pressure to separate the poultry processing plants into two zones, the first being a 'low care' zone consisting of the slaughter, scalding and evisceration rooms and a 'high care' zone consisting of the rest of the processing rooms. This would

reduce the role of air as a vector of not only psychrotrophic bacteria but other relevant spoilage and pathogenic bacteria.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.fm.2018.06.003>.

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