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Prevalence of *Listeria* spp. at a poultry processing plant in Brazil and a phage test for rapid confirmation of suspect colonies

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Abstract

Sites and occurrence of *Listeria* contamination in an industrial poultry processing plant were investigated by sampling carcasses at varying stages of processing and testing the hands and gloves of food handlers as well as the chilling water used in the process. In the course of nine visits to a local processing plant we collected a total of 121 samples: 66 from carcasses, 37 from workers' hands and gloves and 18 from the water used for chilling. Except for the water samples *Listeria* was isolated at all sampling sites. The species most often isolated was *Listeria innocua*, which accounted for 28 of the 31 (90.3%) isolates. The frequency of *Listeria* in the chicken carcasses was similar at bleeding, defeathering and end of evisceration stages (33.3%), reduced during scalding (16.7%), and rose immediately after initial evisceration stage (50%) to peak after packaging (76.2%). The carcasses were contaminated by *L. monocytogenes* serotypes 1b and 1c only during packaging. The prevalence of *Listeria* spp. on workers' hands and gloves was 46% mostly with *L. innocua* (40.5%) followed by *L. monocytogenes* 1b (11.8%). Chilling water presented more than 100 ppm of chlorine, which could explain why the samples were negative to *Listeria*. As the contamination by *Listeria* in the carcasses progressively rose both in number, species and strains during processing it seems reasonable to conclude that those carcasses become contaminated at the processing level. Improvement and innovation measures to control bacteria in general at the processing plant level are necessary to effectively reduce final product contamination by *L. monocytogenes*. In the course of this work we introduced a bacteriophage susceptibility test to confirm suspected *Listeria* colonies which was able to reduce the time of analysis to a minimum of 30 h depending on the isolation technique employed.

Keywords: *Listeria monocytogenes*; Industrial poultry processing; Carcass

1. Introduction

Foodborne listeriosis, caused by *Listeria monocytogenes*, continues to be of major concern to the food industry and the general public because of its rate of lethality (at more than 25%) and its economic impact (McLauchlin, Hall, Velani, & Gilbert, 1991).

Listeria monocytogenes have been isolated from raw poultry in many countries including more industrialized ones, such as USA, UK, and Germany (Rijpens, Jannes, & Herman, 1997; Tobia, Mengoni, & Pellon, 1997; Wilson, 1995).

The high incidence of *L. monocytogenes* in raw chicken is a problem because of cross-contamination to other foods at home and the possibility of the microorganism surviving in processed chicken. Studies indicate that *Listeria* may survive marginal thermal processing of such products. In terms of survival in cooked chicken, *L. monocytogenes* has been isolated from prepared chicken sandwiches (Lieval, Tache, & Poumeyrol, 1989) and from samples of ready-to-eat, precooked chicken (Kerr, Rotowa, Hawkey, & Lacey, 1990).

Two cases of listeriosis attributed to the consumption of precooked refrigerated chicken purchased at a supermarket in the UK (Kerr, Dealler, & Lacey, 1988),

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involved a pregnant woman, and the other was related to consumption of turkey by a cancer patient (Anonymous, 1989). These sporadic cases have changed the thinking about the role of cooked meat and poultry in human listeriosis.

It is particularly important to develop sensitive and rapid methods since conventional methods are time-consuming and laborious. Despite the introduction of new methods gene based ones in particular, and of course, the improvements in the traditional microbiological methods, there remains a real need for a *L. monocytogenes* detection method adapted to industrial processing (Almeida & Almeida, 2000).

A511 is a wild virus that specifically infects cells of the genus *Listeria*. It lyses approximately 95% of *L. monocytogenes* strains belonging to serovars 1/2 and 4 (Loessner, 1991; Wendlinger, Loessner, & Scherer, 1996), which were found to be responsible for most, if not all, cases of human listeriosis.

This study was set up to establish the prevalence of *Listeria* spp. and possible contamination routes of *L. monocytogenes* at an industrial poultry processing plant in Bahia, Brazil, as well as to propose a rapid protocol to confirm the presence of *Listeria* species.

2. Materials and methods

2.1. Bacteria strains and bacteriophage

Listeria monocytogenes strain Scott A (serovar 4b), *Listeria innocua* HPB 124, *Listeria grayi* HPB 29, *Listeria seeligeri* HPB 62, *Listeria welshimeri* HPB 32, provided by Farber, from Health Products and Food Branch of Health Canada. *Staphylococcus aureus* ATTC 25923, *Rhodococcus equi* ATCC 33701 provided by Kabuki from University of Campinas, Campinas, SP, Brazil. Bacteriophage A511 was a gift from Dr. Loeesner, from University of Munich, Munich, Germany.

2.2. Sampling procedure

Nine visits over a period of nine months in 2001 were made to a poultry processing plant located in Bahia, in the Northeast of Brazil. The plant uses conventional slaughtering and processing techniques and processes approximately 12,000 carcasses daily. The scalding process was conducted at 55 °C for 90 s. Potable water is used in the scalding tank and feather pickers. The temperature of processing environment ranged between 27 and 32 °C in the bleeding, scalding and defeathering areas and 20–25 °C in the evisceration and packaging areas. The equipment and environment were cleaned at midday and at the end of the day using quaternary ammonium compound.

Sites were sampled at alternate month intervals, resulting in 121 samples of which 66 were from carcasses, 37 from workers' hands and gloves, nine from the pre-chilling water and nine from chilling water. A total of 24 carcass samples at bleeding, scalding, defeathering and at initial evisceration steps (six samples per site), and 42 carcass samples at end of evisceration and packaging steps (21 samples per site) were taken and put in sterile bags for individual samples and care was taken to prevent cross-contamination. Sampling was performed during working hours in the morning, right at the beginning of the processing.

After the samples of carcass were taken, University of Vermont modified *Listeria* enrichment broth (UVM, Difco) (Donnelly, Brackett, Doores, Lee, & Lovett, 1992) was added to each bag, 300 ml to the carcass after the bleeding step and before defeathering, 100 ml to the carcass after defeathering, at initial evisceration, and the end of evisceration and after packaging. The bags were shaken for 2 min to wash the carcass evenly, then the carcass was removed and the resultant broth used for analysis (Surkiewcz, Johnston, Moran, & Krumm, 1969).

Samples from workers' hands and gloves were taken with polyurethane sponges (5×5 cm) (3M^{TM}) moistened with 10 ml phosphate buffered saline (PBS) with 0.5% sodium thiosulfate to neutralize any residual chlorine (Sveum, Moberg, Rude, & Frank, 1992). The sponges were placed into sterile whirlpak bags (capacity 2 l) and 100 ml of UVM was added. Sponges were tested for inhibitory properties against *Listeria* species (Daley, Pagotto, & Farber, 1995).

Approximately 100 ml of immersion pre and chilling water, in the steps before packaging, were collected in 250 ml screw cap bottles on each visit.

All samples collected at the processing plant were placed in an insulated cold box filled with ice and brought to the laboratory for analysis within 24 h of collection (Messer, Midura, & Peeler, 1992).

In the laboratory samples of water were filtered under a laminar flow hood in a Millipore module, using a cellulose acetate membrane, pore size 0.2 μm, and then the membrane was transferred to a 50 ml of UVM plus sodium thiosulfate 0.5%.

2.3. Isolation and identification of Listeria spp.

For all samples, a two stage enrichment procedure was used (Donnelly et al., 1992) involving UVM and Fraser broth. UVM was incubated at 30 °C for 24 h and Fraser broth at 35 °C for 48 h. After enrichment, those broth cultures that changed color from straw to black were streaked on Oxford modified selective medium (Difco), supplemented with antibiotics, and incubated for 48 h at 35 °C. Five typical dark esculin-positive-

colonies from each plate were streaked on trypticase soy agar plus yeast extract 0.6% (TSA-YE) for purification and then transferred to TSA-YE slants for further biochemical characterization. Tests included Gram stain, morphology and arrangement, catalase reaction, motility, β-hemolysis on 5% horse blood agar plates, CAMP reaction with *S. aureus* ATCC 25923 and *R. equi* ATCC 33701, carbohydrate fermentation (glucose, maltose, rhamnose, mannitol and xylose) and nitrate reduction. Motility was determined inoculating a column of SIM medium (Difco) and incubating at 25 °C. *L. monocytogenes* Scott A, serovar 4b, *L. innocua* (HPB 124), *L. grayi* (HPB 29), *L. welshimeri* (HPB 32) and *L. seeligeri* (HPB 62) were used in control positive tests. Serological slide agglutination tests were done according to Seeliger and Hohne (1979) on all isolates presumed to be *Listeria*, using commercially prepared antisera (Difco).

2.4. Confirmation of the presence of Listeria using bacteriophage A511

The same morphologically typical colonies used for biochemical characterization were streaked onto brain heart infusion agar containing yeast extract 0.6% and $CaCl_2$ 0.01% for bacteriophage susceptibility test. After the surface plate dried, aliquots of 10 μl containing ca 10^8 A511 listeriophage particles were deposited on the area of the suspected *Listeria* spreading. After incubation at 30 °C for 8 h, plates were inspected for areas of confluent lyses. Cultures showing susceptibility to A511 were considered positive to *Listeria* spp. *L. monocytogenes* Scott A was used as positive control and *S. aureus* ATTC 25923 as negative control.

2.5. Determination of available chlorine in water used for chilling

Iodometric method (APHA, 1989) was used for total available chlorine in immersion pre and chilling water.

3. Results and discussion

3.1. Prevalence of Listeria spp. in carcass samples

A total of 66 samples representing individual carcasses were obtained from the processing plant and analyzed for the prevalence of *Listeria* spp.

The species most often isolated was *L. innocua*, which accounted for 28 of the 31 (90.3%) isolates. Three (9.7%) of the isolates were *L. monocytogenes* and two (6.5%) were *L. grayi*.

Listeria innocua was isolated from carcass samples at all steps of the processing line, including bleeding, scalding, defeathering, initial and end of evisceration, and packaging. *L. monocytogenes* were isolated only from carcasses packaging ready to go to the market and *L. grayi* was isolated in the bleeding and end of the evisceration steps (Table 1). The strains of *L. monocytogenes* isolated belong to serotypes 1b and 1c, *L. innocua* to serotypes 6a, 6b and not typeable.

At the bleeding stage, two carcasses (33.3%) were found positive for *Listeria* spp. and the strains isolated were identified as *L. grayi* and *L. innocua* 6a (Table 1). Hudson and Mead (1989) investigated carcass samples after bleeding at three abattoirs and failed to find *Listeria* at this stage in processing.

At the scalding stage, only one carcass sample (16.7%) was contaminated by *Listeria* and the strain identified was *L. innocua* 6a (Table 1). This result is similar to that presented by Varabioff (1990) and Skovgaard and Morgen (1988).

In the defeathering step, *Listeria* spp. were isolated in 33.3% of the samples analyzed in this work, and the strains identified were *L. innocua* 6a and 6b (Table 1). Varabioff (1990) did not found positive samples of *Listeria* at this stage in the four abattoirs he investigated.

At the initial evisceration stage, we found three (50%) carcass samples contaminated with *L. innocua*, serotypes 6a and 6b (Table 1) and at the end of evisceration both serotypes of *L. innocua* were presented, including one non-typeable, in seven (33.3%) carcass samples and

Table 1
Number and percentage of carcass at different stages of poultry processing, workers' hands and gloves, and pre-chiller and chiller waters samples contaminated by *Listeria* spp. and serotypes identified

Samples	*Listeria* spp.	*L. innocua* (serotype)	*L. grayi*	*L. monocytogenes* (serotype)
Carcass at bleeding (6)	2/33.3	1/16.7 (6a)	1/16.7	0/0.0
Carcass at scalding (6)	1/16.7	1/16.7 (6a)	0/0.0	0/0.0
Carcass at defeathering (6)	2/33.3	2/33.3 (6a, 6b)	0/0.0	0/0.0
Carcass at initial evisceration (6)	3/50.0	3/50.0 (6a, 6b)	0/0.0	0/0.0
Carcass at end of evisceration (21)	7/33.3	7/33.3 (6a, 6b, NT)	1/4.8	0/0.0
Carcass at packaging (21)	16/76.2	14/66.7 (6a)	0/0.0	3/14.3 (1b, 1c)
Hands and gloves (37)	17/46.0	15/40.5 (6a, 6b, NT)	0/0.0	4/11.8 (1b)
Pre-chill water (9)	0/0.0	0/0.0	0/0.0	0/0.0
Chill water (9)	0/0.0	0/0.0	0/0.0	0/0.0

( ) no. of samples investigated. NT—not typeable.

L. grayi in one (4.8%) of the same sample, concomitantly. The latter results were similar to those showed by Varabioff (1990), who reported the occurrence of two (66.7%) post-evisceration carcass samples positive to *L. innocua*. According to Ojeniyi, Wegener, Jensen, and Bisgaard (1996), these stages play an important role in the dissemination of *Listeria* spp. on the production lines.

At the packaging stage, three carcass samples (14.3%) ready to go to market were found to be contaminated by *L. monocytogenes* and fourteen with *L. innocua* (66.7%), however two of which harbors both species, concomitantly (Table 1). The presence of a pathogenic strain at this stage was also observed by Genigeorgis, Dutulescu, and Garayzabal (1989), Hudson and Mead (1989), Ojeniyi et al. (1996), Skovgaard and Morgen (1988), and Varabioff (1990), who found *L. monocytogenes* in 8.3%, 50.0%, 59.0%, 50.0% and 41.9%, respectively.

According to Miettinen, Palmu, Bjorkroth, and Korkeala (2001), *L. monocytogenes* contamination of broiler carcasses probably occurred during or after the chilling step in the skin-removing machine, because no positive samples were found prior to these steps.

As our research progressed, it became increasingly evident that *Listeria* contamination rose during the packaging step. The fact that the contamination was greater in the latter stages of processing coincides with data in Genigeorgis et al. (1989) and Skovgaard and Morgen (1988).

This study shows that three strains of *L. monocytogenes* isolated from carcass samples belonged to serotype 1b (2) and 1c (1) (Table 1). According to Johnson, Doyle, and Cassens (1990) the high percentage of serogroup 1/2 *L. monocytogenes* strains isolated from meat and poultry in the US, the presence of numerous serogroup 3 strains, and the relatively infrequent isolation of serogroup 4 are in direct contrast to the predominance of serogroup 4 strains that were responsible for reported food-borne outbreaks of listeriosis. It is not known whether this reflects an actual difference in serotype distribution between animal/meat sources and human *L. monocytogenes* isolates, or if it is merely an artificial relationship resulting from a small number of samples.

In contrast, Hofer, Ribeiro, and Feitosa (2000) investigated species and serovars of Genus *Listeria* isolated from varying sources in Brazil between 1971 and 1997 and demonstrated that the most prevalent serovars of *L. monocytogenes* in meat products were 4b, 1/2a and 1/2b.

3.2. Contamination of workers' hands and gloves with Listeria

Poultry slaughter and processing have many routes for cross-contamination. An evaluation of the *Listeria* species present on workers' hands and gloves samples in this study revealed that 17 (46%) carried *Listeria* spp. Four of them (11.8%) carried *L. monocytogenes* and 15 (40.5%) individuals carried *L. innocua* (Table 1). Strains of *L. monocytogenes* isolated from these samples belong to serotype 1b and *L. innocua* to serotypes 6a, 6b and not typeable. The employees who showed contamination with *Listeria* spp. tended to work in more than one area of the plant, which could contribute to the poultry carcasses contamination.

The prevalence of *L. monocytogenes* on the hands of people at a Federal inspected California turkey processing plant was 10%, according Genigeorgis, Oanca, and Dutulescu (1990), a percentage which is very similar to our results. Kerr, Birkenhead, Seale, Major, and Hawkey (1993) investigated 99 workers' hands at 44 establishments and found that 12 (12%) food workers carried *Listeria* spp. and seven (7%) carried *L. monocytogenes*.

The above data has demonstrated beyond any doubt the potential contribution of handlers to cross-contamination of products by *Listeria* spp. This also applies to our study because the serotypes identified on hands and gloves were the same as those found in the packaging carcasses ready to go to supermarket.

Given the fact that workers under investigation in this study might be expected to employ more thorough hand washing techniques, it is of concern that 46% of samples yielding positive cultures for *Listeria* spp. were identified. We believed that most of them did not follow appropriate personal hygiene routines.

3.3. Evaluation of water used for chilling for Listeria spp. presence and total available chlorine

No *Listeria* spp. was isolated from any of the samples of the pre-chilling and chilling waters. These results coincide with those demonstrated by Hudson and Mead (1989). In contrast Genigeorgis et al. (1989) isolated *L. monocytogenes* in 12.5% from water samples, with chlorine at a concentration of 20–25 ppm.

Varabioff (1990) in an investigation at a small chicken processing plant in Brisbane, Australia, also isolated the microorganism in one sample of water chilling at a temperature of 9 °C. According to the author the temperature of chilled water tanks should be less than 5 °C, preferably 0–2 °C, and the chlorine level should be maintained at an appropriate level (e.g., 40 ppm of sodium hypochloride).

In this study the concentrations of chlorine utilized in pre-chilling and chilling waters used for the carcass before packaging were on average 102 and 156 ppm, respectively. These high chlorine concentrations explain the absence of *Listeria* spp. in the samples. Unfortunately, the use of high chlorine concentration is a dangerous practice because of the probability of the presence of trihalomethans, carcinogenic substances

resulting from the reaction between inorganic chlorine and organic matter.

3.4. Confirmation of Listeria using the bacteriophage A511

The results obtained using the bacteriophage A511 as a screening method for rapid confirmation of *Listeria* species showed that all 239 suspected colonies confirmed as *Listeria* through conventional methods demonstrated susceptible to the phage (Fig. 1). The use of this methodology could be a powerful tool for screening *Listeria* species on processing lines in the poultry industry, as it is not necessary to perform the biochemical tests. Despite the fact that phage typing must be performed on isolated strains, the time for confirmation of *Listeria* species is reduced from 4 days to approximately 8 h, facilitating the monitoring and the adoption of corrective actions at those stages where *Listeria* is present. This method also eliminates the occurrence of false positive results which can occur when conventional techniques are used.

This work detected contamination sites by sampling of carcasses and workers' hands and gloves at different production stages for species of *Listeria* and by serotyping the isolates. Given the fact that raw and processed poultry and workers' hands and gloves harbor listeriae, the poultry industry has to restrain contaminating *L. monocytogenes* during processing and finally eliminate it from carcasses before it is ready to go to the market.

Although the poultry industry has made great progress in recent years towards the establishing of good manufacturing and sanitation practices and the development of effective quality assurance systems, such as HACCP, strict attention must be paid to cleaning and disinfecting to control the level of *L. monocytogenes* and to avoid in-plant colonization by this organism.

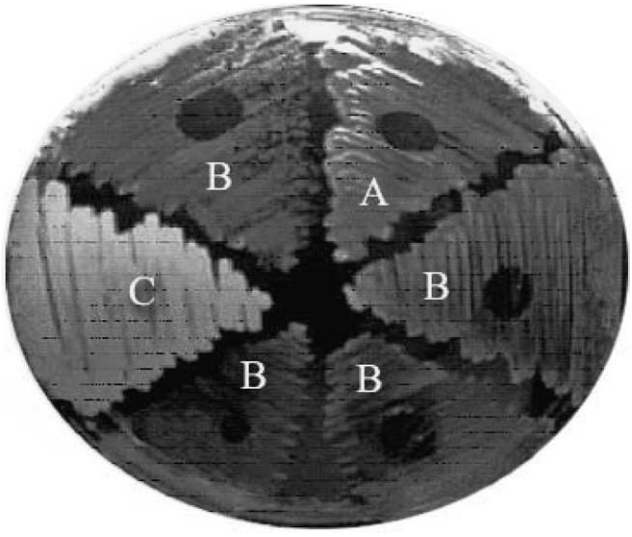

Fig. 1. Confirmation of *Listeria* spp. using the bacteriophage A511. (A) *Listeria monocytogenes* Scott A; (B) *Listeria* spp. isolated from carcass; (C) *Staphylococcus aureus*.

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