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A Simple Method to Reduce *Listeria* in Blast and Holding Chillers

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ABSTRACT

Post-cook blast and holding chillers identified as persistent sources of *Listeria monocytogenes* contamination in a ready-to-eat meal production facility were subjected to a regimen of every 14 days air heating through the use of portable heaters and fans. Each of three blast chillers was sampled for *Listeria* species from Monday to Friday over a two-year period, 12 months pre- and 12 months post-commencement of intervention, amounting to 490 samples per blast chiller per year. Over the two years, a total of 2,940 blast chiller environmental samples were drawn. Similarly, each of two holding chillers was sampled for *Listeria* species from Monday to Friday over a two-year period, 16 months pre- and 8 months post-commencement of intervention, amounting to 919 samples per holding chiller over the 16 months pre-intervention, and 551 samples per holding chiller over 8 months post intervention. Over the two years, there was a total of 2,940 holding chiller environmental samples drawn. Although *Listeria* was not eliminated from chillers, even one year after the intervention, there was a statistically significant ($P < 0.001$) reduction in prevalence of *Listeria* in all chillers. No deleterious effects of heating were noted in wall paneling, seals, synthetic floors, or chilling equipment. The air heating regimen was readily incorporated by sanitation staff into the existing Good Manufacturing Practice program. The application of chiller air heating may result in significant reductions in the prevalence of *Listeria* in chillers.

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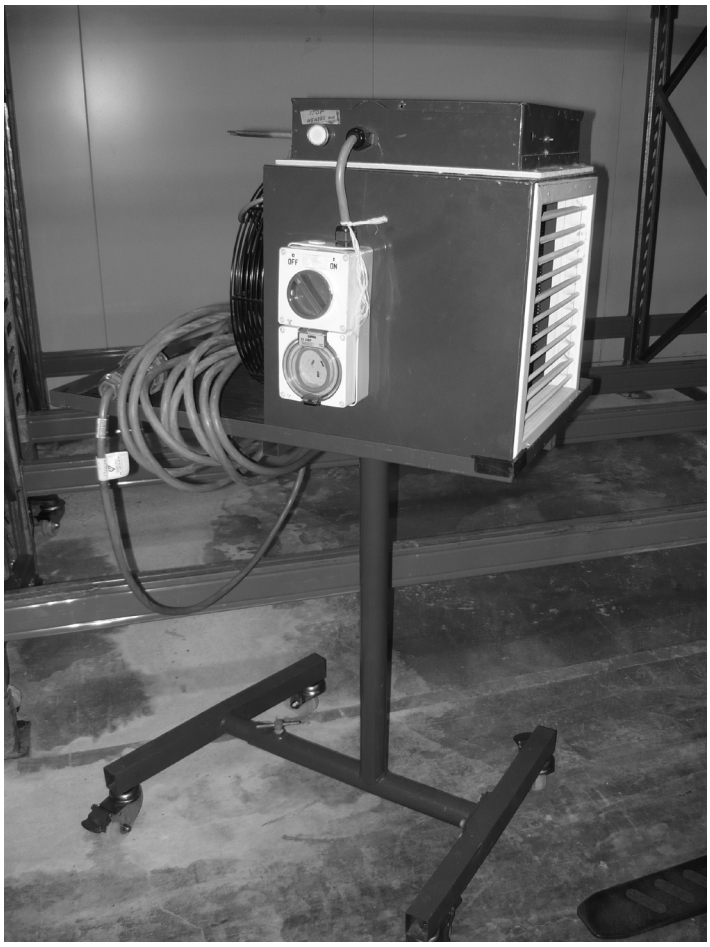
INTRODUCTION

The ubiquitous presence of *Listeria monocytogenes*, coupled with its high long-term survival, growth at low temperatures *(9, 16)* and preference for wet surfaces, results in the common occurrence of this pathogen in refrigerators and chilling units *(9)*. The colonization of post-cook chillers with *L. monocytogenes* may facilitate final product contamination. Recontamination of cooked product is the primary source of *L. monocytogenes* contamination in many commercially produced ready-to-eat (RTE) foods *(10, 15)*.

The definition of persistence in bacteria is that of a strain that is repeatedly isolated from a food-processing environment over an extended period *(14)*. *Listeria monocytogenes* strains are known to persist within the food processing environment for extended periods of time, 10 years or more in some cases *(14)*. The properties that make a bacterial strain persist are not well understood but are thought to be related to properties such as biofilm formation and elevated resistance to sanitizers *(13, 14)*. Our study was precipitated by the persistent isolation of *L. monocytogenes* in blast and holding chillers in a ready-to-eat food production facility over a number of years (data not presented).

FIGURE 1. Hotbox-Axial HBA Fan Blower Heaters HB90415 – 415V 9.0kW used in heat holding chillers



An adjunct to the existing Good Manufacturing Practice (GMP) cleaning regimen was sought to reduce chiller contamination with *L. monocytogenes*. Periodic heat treatment of chillers is an intervention that involves raising the temperature of the chillers to a level which, in combination with the associated drying, may provide multiple stressors and result in a reduction of bacteria present in the chiller. We speculated that periodically holding chillers at an air temperature of 50°C for 2 h may result in reductions in *Listeria* contamination. In this study, we tested this hypothesis by applying this heating and drying regimen to post-cook chillers in an RTE frozen meal production facility, using simple heaters and fans and determining the prevalence of *Listeria* in the chillers before and after implementation of the intervention.

MATERIALS AND METHODS

Post-cook blast and holding chillers

A RTE meal production facility based in Brisbane, Australia was operating an externally audited HACCP food safety system, and was listed for export under the jurisdiction of the Australian Quarantine Inspection Service. The facility had a corporate zero tolerance policy for *Listeria monocytogenes* contamination on finished product. Previous work performed within the facility has demonstrated contamination of finished product with *L. monocytogenes* sub-types that also persistently colonize chillers.

Within the facility, three identical post-cook blast chillers (also known as intensive chillers) were used to chill exposed meal components. These components were then transferred into two holding chillers to await meal assembly and freezing.

Heating of blast chillers

The three blast chillers were each 2 m × 7 m × 4 m. The blast chillers also have a 50 mm sandwich panel mezzanine. Chiller wall panels were 100 mm insulated refrigeration panels consisting of a 1.6 mm sheet over an expanded polystyrene core. These were constructed from Retracom Standard Sandwich Panel 100 (Retracom, Crestmead, Australia) as part of the original building works. Floors were covered in Sikagard 62 (Sika Group, Zurich, Switzerland), a two component high build epoxy resin. Air heating was instituted every 14 days at the end of production and sanitation. The heating of air within blast chillers was achieved by switching ceiling mounted chilling units to heat mode. The blast chiller units were Greenhalgh 16/56-1500 aluminum finned coils (Greenhalgh Asia Pacific, Brisbane, Australia), but these had been changed earlier to stainless finned units because of caustic sanitation chemicals corroding the aluminum fins. The fan motors on these units were sufficient for heating and fans were not used. Heat treatment of blast chillers commenced January 1, 2006. The temperature was maintained at 50°C for a minimum of 2 h at each treatment time.

Heating of holding chillers

The two holding chillers were 3 m × 7 m × 4 m and 7 m × 7 m × 4 m. These were constructed from Retracom Standard Sandwich Panel 100 (Retracom, Crestmead, Australia) as part of the original building works. Wall panels and floors were identical to those described for blast chillers. Product was consolidated into a different holding chiller each week, allowing for an every-14-days heating regimen at the end of production and sanitation. Empty crates were allowed to remain in the chillers. Heaters and fans were wheeled into alternating corners. The holding chillers use Luve Contardo S3HCW 179 N80A (Uboldo–Varese, Italy) forced draft cooler units. Circulating refrigerant valves within these ceiling mounted units were released prior to operation of heaters and

FIGURE 2. Air Boss Pedestal Fan Model #WATPF26 used in holding chillers

fans to minimize heat-induced refrigerant pressure build up. Heaters used were the Hotbox-Axial HBA Fan Blower Heaters HB90415 — 415V 9.0kW and HB15415 415V 15kW (Thermal Electric Elements Pty Ltd, Brisbane, Australia). The fans used were the Air Boss Pedestal Fan Model # WATPF26. Heaters were modified by mounting them onto mobile stands, fitting them with a 10 m × 3 phase cable and installation of a 20A plug top with thermostat/auto cut-off designed to switch the unit off at 50°C. Heat treatment of holding chillers commenced May 1, 2006. The temperature was maintained at 50°C for a minimum of 2 h at each treatment time.

Sampling and microbiological analysis

Sampling was performed on all chillers from January 2005 to December 2006. All chillers were still used during sampling and still run completely for production. A mix of vegetables, starch (potato mash and rice), sauce and protein (beef and chicken) products were placed in the chillers. The amount of product passing through each chiller varied, but each blast chiller had an approximate 2,000 to 3,000 kg turnover per day. Holding chillers had twice as much turnover. Each of three blast chillers were sampled for *Listeria* species from Monday to Friday over a two-year period, 12 months pre- and 12 months post-commencement of intervention, amounting to 490 samples per blast chiller per year. Over the two years, a total of 2,940 blast chiller environmental samples were drawn. Similarly, each of two holding chillers was sampled for *Listeria* species from Monday to Friday over a two-year period,16 months pre- and 8 months post-commencement of intervention, amounting to 919 samples per holding chiller over the 16 months pre-intervention, and 551 samples per holding chiller over 8 months post intervention. Over the two years, there was a total of 2,940 holding chiller environmental samples drawn. The general areas targeted were internal areas (floors, walls), seals and doors.

Separate polyurethane sponges (Whirl-Pak Speci-Sponge, Nasco, Fort Atkinson, WI), moistened with Butterfield's solution (25 mL; bioMérieux, Hazelwood, MO) were used to sample an area of approximately 25 cm^2. Approximately 2,940 environmental samples were drawn.

Sponge samples were tested for the presence of *Listeria* by use of the *Listeria* BAX Automated System (DuPont Qualicon, Wilmington, DE, USA). Each sponge was enriched in 225 ml of buffered *Listeria* enrichment broth (Amyl Media, Melbourne, Australia) for 24 h at 35°C. One ml of enrichment was inoculated into 10 mL MOPS-buffered *Listeria* enrichment broth (Amyl Media) and incubated at 35°C for 18 – 24 h. Enrichment cultures were analyzed using the automated PCR, following the manufacturer's user's guide for preparing reagents, performing the test, and reading the results. Specifically, enrichment cultures were lysed and the lysate was used to hydrate the PCR reagents contained within a proprietary tablet. Processing in the automated PCR unit took approximately 4 hours, and electronic results appear as positive/negative icons on the unit screen. Presumptive positive samples were confirmed following manufacturer's instructions by streaking retained MOPS-buffered *Listeria* enrichment broth onto Oxford and PALCAM agar (Amyl Media) and incubating at 37°C for 48 h. Colonies surrounded by dark brown or black haloes were confirmed as per the Australian Standard method AS1766.2.16 *(1)*. Results were reported as detected or not detected/25 cm^2.

Statistical analysis

The relationship between *Listeria* prevalence and chiller intervention was analyzed using the CHITEST formula in Microsoft Excel 2003. Significance was indicated when $P < 0.001$.

RESULTS

The prevalence of *Listeria* in chillers pre- and post-chiller interventions is presented in Table 1. Chiller prevalence is the sum of holding and blast chillers

TABLE 1. *Listeria* prevalence in chillers, pre- and post-chiller interventions

	Pre-intervention Blast (Jan. 2005 – Dec. 2005) Holding (Jan. 2005 – April 2006)		Post-intervention Blast (Jan. 2006 – Dec. 2006) Holding (May 2006 – Dec. 2006)	
	n	Detections (%)	n	Detections (%)
Blast Chiller	1470	24 (1.63)	1470	7 (0.48)
Holding Chillers	1838	25 (1.36)	1102	1 (0.09)
All Chillers	3308	49 (1.48)	2572	8 (0.31)

prevalence. Since numbers of detections were low, all samples types were combined for analysis. The percentage of samples with *Listeria* spp. detections for both chillers was 1.48% pre-intervention and 0.31% post-intervention. The decrease in numbers of *Listeria* detections before and after the introduction of chiller intervention was significant ($P < 0.001$) for both blast and holding chillers. The sampling was completed for *Listeria* species only. It would have been possible to look specifically for *L. monocytogenes*, but the additional resources required to perform this testing was not in line with its key outcomes, namely to evaluate the hypothesis that heating chillers reduces the presence of all *Listeria* species.

DISCUSSION

Heat treatment of the food processing environment to manage *Listeria.* The main focus when managing *L. monocytogenes* contamination of cooked meals is on preventing contamination by the post-cook factory environment. Heat can be used to manage *L. monocytogenes* in the post-cook factory environment, as this pathogen is not unusually heat resistant among vegetative Gram positive bacteria *(12)*. The maximum growth temperature of *L. monocytogenes* is 45°C *(12)* and heat inactivation takes place above that limit, with the rate of inactivation being a function of both time and temperature. Heat has been used, for example, to surface pasteurize and reduce *L. monocytogenes* on vacuum-sealed precooked ready-to-eat meat products *(11)*. Heat can also be applied as steam directly onto surfaces and equipment that need to be sanitized. Of course, the potential of "caking-on" of product needs to be considered individually, based on the particular food matrix. The application of steam onto equipment can be optimized for complex machinery by covering the equipment to be treated with a tarpaulin so as to maximize steam contact time and penetration. Cookrooms also manage environmental *Listeria* by "pasteurizing" mobile equipment capable of surviving such a heat treatment *(17)*. It has been observed that heating air within a room can be effective for removing moisture at the end of cleaning sanitation *(17)*. A noteworthy observation from staff at the facility we worked in was how the wet chillers were transformed to dry via the heat treatment. Chmielewski and coworkers *(6)* used predictive modeling to suggest that with proper control of time and temperature, hot water sanitation of stainless steel surfaces could serve as an efficient method for elimination of *L. monocytogenes* in biofilms.

Listeria biofilms in the food processing environment

The persistence of *L. monocytogenes* in food processing facilities has been ascribed to the ability of this pathogen to live in biofilms. A biofilm may be defined as "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interphase, or to each other, are embedded in a matrix of extracellular polymeric substance that they have produced, and exhibit an altered phenotype" *(13)*. The *Listeria* present in chillers, targeted by this intervention, are likely to be in this biofilm state and would be expected to be more resistant to both heat and sanitizers than their suspended counterparts *(13)*; if they are in the biofilm strata where nutrients are depleted, cell growth is slow and may induce stress response and clustering. Dense clustering of cells and production of extracellular polymers effectively changes the heating menstruum, providing additional heat tolerance *(5)*. In this survey, the presence of all *Listeria* spp. was monitored. The presence of any *Listeria* species in food may indicate poor hygiene *(12)*. Previous biofilm formation by one species (e.g., a non-pathogenic species) may provide a niche for another species *(2)*. It may be possible for a non-pathogenic bacterial species (*Listeria* or another genus) to take residence and develop a biofilm, and a pathogenic species such as *L. monocytogenes* may then establish residence in the pre-existing biofilm *(5)*. Indeed, many *Listeria* species can exist within the same environmental site *(8)*. Biofilms are more difficult to remove when formed in the presence of food residues *(3, 4)* as soil can have a protective effect on the heat inactivation of planktonic or sessile microorganisms. High fat substrates increase heat resistance of *L. monocytogenes (3, 4)*. Food residues may also promote bacterial growth, subsequently influencing heat inactivation *(7)*. There may be a degree of synergy between chiller heat treatment and desiccation-related stress. It has been suggested that simultaneous stressors may achieve an antimicrobial effect greater than the individual sum of each individually.

Application of heat treatment of chillers

In the intervention described here, a multi-discipline approach was taken at

the facility level. Engineering, operations and quality assurance teams were all involved in the chiller heat treatment planning as well as the ongoing treatment. The minimum effective parameters that completely dried chillers without impacting internal floor, walls and chilling equipment was found to be 50°C for 2 hours. The engineering team was given the responsibility of preparing chilling units for weekend heating, and no chiller is excluded from the heat treatment for more than 2 weeks. The operations team consolidates product into other chillers, ensuring that a gap around the chiller walls to be treated is maintained to allow airflow and passage of heaters and fans. The quality assurance team verifies temperature graphs displaying each of the chillers treated. As these protocols were developed by all the teams involved, standard operating procedures were readily taken into the GMP program.

We have described a simple way to potentially reduce *Listeria* contamination in the post processing chiller environment. Certainly a limitation of this technique is the required redundancy of chillers, and it is recognized that many facilities do not operate with such a redundancy. Although this protocol is unable to completely eliminate *Listeria,* it does dry chillers, is easily taken up into the GMP program, produces no deleterious effects to the treated chillers and has significantly reduced environmental post cook chiller *Listeria* contamination.

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