Microban SilverShield Technology: Reduction of Listeria Monocytogenes Contamination of Refrigeration Units

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Summary

Listeria monocytogenes (Lm) is a bacterial pathogen that causes expensive food recalls, hospitalizations and even death. One of the key differences between Lm and other foodborne bacteria is that it thrives at typical refrigerator temperatures, about 4°C. In examining the effectiveness of an antimicrobial, most industry-standard bacterial testing is performed at 36°C (body temperature) which is not entirely relevant to Lm control. Microban used Silvershield Technology incorporated into a power coat finish on steel shelving units to demonstrate greater than 99.7% reduction in Listeria in 24h in a commercial fridge at 4°C using the industry standard ISO 22196 test protocol.

Objective

Comparative studies were conducted to evaluate the efficacy of Silvershield antimicrobial coating technology in controlling ***Listeria monocytogenes*** contamination on refrigerator shelving in a commercial refrigerator operating at 4°C.

Introduction

Listeria monocytogenes (Lm) is a bacterial foodborne pathogen that causes meningitis, sepsis, and complications during pregnancy. ***Listeria*** became a bacterium of major concern for the agri-food industry in 1981 when an outbreak of listeriosis in Canada, involving 41 cases and 18 deaths mostly in pregnant women and neonates, was epidemiologically linked to the consumption of coleslaw containing cabbage that had been in contact with Lm-contaminated sheep manure. By current estimates ***Listeria*** has caused 1,600 illnesses, more than 1,500 related hospitalizations, and 260 related deaths each year in the United States[1]. Fig.1 shows actual epidemiological data for ***Listeria***-related illnesses, hospitalizations, and deaths for the period 1997-2014.

1 FoodNet conducts laboratory surveys, physician surveys, and population surveys to collect information about each of these steps. This information is used to calculate estimates of the actual number of people who become ill.

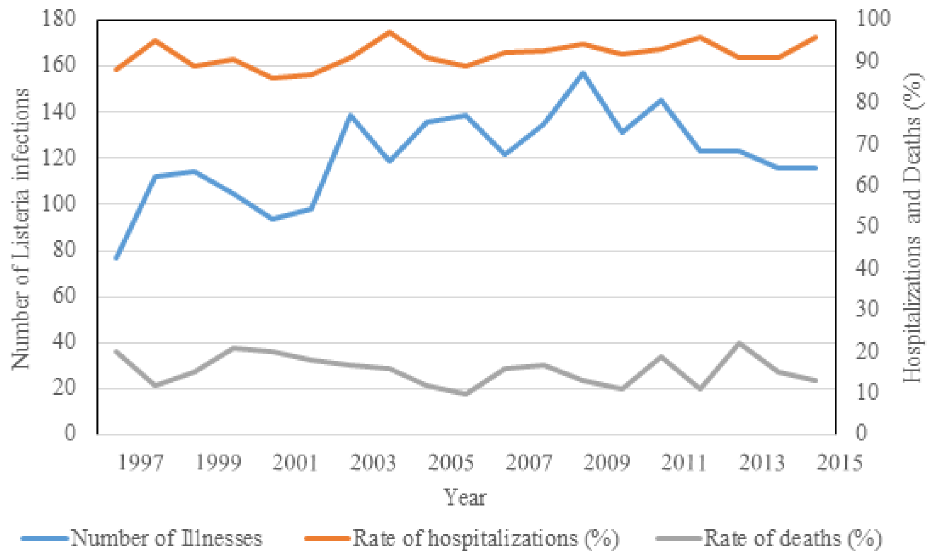


Figure 1. *Listeria* infections, hospitalizations, and deaths - FoodNet Surveillance 1997-2015

According to FoodNet Annual Surveillance Reports for 1997-2014[2], Listeria infections resulted in higher rates of hospitalization than any other tracked pathogen and had the highest annual case fatality rate (Fig. 2). Nearly all cases of listeriosis occurring in persons who were not infants were associated with consumption of food contaminated with Listeria. Newborn infants could develop listeriosis if their mothers ate contaminated food during pregnancy, and incidences of abortions and stillbirths have been attributed to Listeria infection in pregnant women.

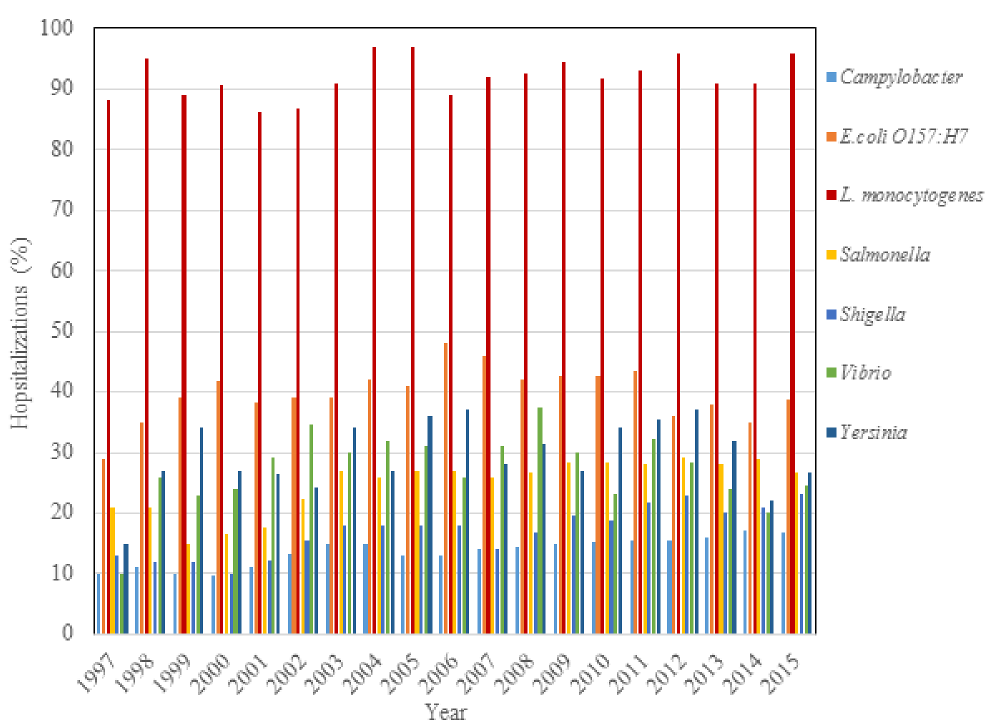


Figure 2. Percentage of Hospitalizations, by Selected Pathogens - FoodNet 1997-2015

2 The Foodborne Diseases Active Surveillance Network (FoodNet) is a collaborative program of the Centers for Disease Control and Prevention (CDC), 10 state health departments, the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), and the Food and Drug Administration (FDA). FoodNet determines the number of laboratory-confirmed infections caused by selected pathogens transmitted commonly by food, monitors changes in their incidence, collects information about the sources of infection, and disseminates information to provide a foundation for food safety policy and prevention efforts.

Listeria has been isolated from a variety of foods: uncooked meats and vegetables, unpasteurized (raw) milk and cheeses as well as other foods made from unpasteurized milk, cooked or processed foods, including certain soft cheeses, processed (or ready-to-eat) meats, and smoked seafood. However, active surveillance and epidemiologic investigation of listeriosis outbreaks indicated that certain Ready-To-Eat (RTE) processed foods were high-risk vehicles for transmitting listeriosis in susceptible populations. Post-processing contamination was the most likely route of ***Listeria*** contamination of processed foods. While heat processing steps such as cooking and pasteurization are able to kill bacteria, Listeria contamination issues might still occur after factory cooking but before packaging. Packaging and the deli counter, with RTE meats, are potential points for post-processing contamination. These RTE foods, which were common vehicles for ***Listeria***, are usually preserved by refrigeration.

Unlike most bacteria, ***Listeria*** can grow and multiply in RTE foods stored in the refrigerator. In addition, implementation of effective sanitation procedures is particularly difficult because ***Listeria*** adheres to food contact surfaces and forms biofilms. ***Listeria*** can persistently survive on food contact surfaces such as stainless steel surfaces of dicing machines and repeatedly contaminate RTE meats. Refrigerated moist environments in food processing plants and refrigerated units provide a good growth environment for ***Listeria*** because as a psychrophilic bacterium it survives and grows in very low temperature (0- 4°C) environments, on foods, and in food-processing plants for prolonged periods of time. ***Listeria*** differs from most other foodborne pathogens in that it is widely distributed, enters the food-processing plants in various ways, and is resistant to diverse environmental conditions including low temperature.

Food recalls represent high health and economic costs. According to estimates from the Department of Agriculture Economic Research Service (USDA/ERS), the total annual cost of illness from ***Listeria monocytogenes*** in 2013 dollars exceeded $2.8million. Although economic costs were expensive to manufacturers, wholesalers, retailers, and consumers, calculated estimates did not include food industry costs, loss of consumer confidence in a brand or a business, associated recall expenses, or charges stemming from litigation, nor did they include the cost to taxpayers for local, state, and federal health agencies that responded to outbreaks. Table 1 lists Class I food recalls associated with ***Listeria*** contamination[3]. For the decade 2005-2015, contamination associated with ***L. monocytogenes*** accounted for 18% all 127 food recalls and more than 9 million pounds of food.

Table 1: Food recalls associated with Listeria contamination, 2005-2015

Year	Number of Recalls	Percent of All Recalls	Pounds of Food Recalled
2005	30	57	3,450,947
2006	6	18	48,346
2007	11	19	2,996,628
2008	15	28	349,661
2009	8	12	47,341
2010	8	11	384,135
2011	11	11	525,998
2012	16	20	439,848
2013	9	12	784,350
2014	7	7	270,926
2015	6	4	82,547

3 A food recall is a voluntary action by a manufacturer or distributor to protect the public from products that may cause health problems or possible death. A recall is intended to remove food products from commerce when there is reason to believe the products may be adulterated or misbranded. Recalls are initiated by the manufacturer or distributor sometimes at the request of FSIS. All recalls are voluntary. However, if a company refuses to recall its products, then FSIS has the legal authority to detain and seize those products in commerce. http://www.fsis.usda.gov/wps/portal/fsis/home

The significance of ***Listeria*** as a foodborne pathogen is complex. The severity and case-fatality rate of the disease require appropriate preventative measures, but the characteristics of the microorganisms present a challenge to remove ***Listeria*** from food-processing establishments and prevent contamination of RTE foods. Many contact surface areas of possible post-processing product contamination with ***Listeria*** have been identified: cutting tools, containers, shelves and racks. Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) plans are often implemented to ensure safety during the manufacturing process; however, many unforeseen events can and do occur in the long chain from farm to fork. Through food handling and spills, ***Listeria*** on contaminated refrigerated food can spread to non-contaminated foods. The FDA recommends implementing steps to minimize opportunities for ***Listeria*** to contaminate refrigerator surfaces: wipe spills immediately, clean refrigerator regularly, and pay special attention to the inside walls and shelves with hot water and a mild liquid dishwashing detergent, rinsing, and then wiping dry with a clean cloth or paper towel. However, experience has shown that it is very challenging to restore a ***Listeria***-free refrigeration unit environment subsequent to an initial contamination event.

MICROBAN® Silvershield® Technology

Sanitary equipment design (SED) has been identified as one of the key components of any food safety program. In 2014, the American Meat Institute (AMI) outlined ten SED principles to guide food equipment design, construction, and use. The AMI recommends that food equipment be designed to prevent bacterial ingress, survival, and growth on both product and non-product contact surfaces of the equipment as well as constructed to ensure effective and efficient cleaning over the life of the equipment. Construction materials used for equipment must be completely compatible with the product, environment, cleaning and sanitizing chemicals and the methods of cleaning and sanitation. Product contact surfaces must be made with materials which are corrosion resistant, non-toxic, and non-absorbent.

The high morbidity and mortality associated with listeriosis coupled with the frequency of high profile outbreaks have demonstrated the need for a new approach to controlling the survival and growth of ***Listeria*** on refrigerator surfaces. The traditional measures of surface decontamination that are used to minimize opportunities for ***Listeria*** to contaminate food products have been less than successful because experience has shown that it is very challenging to restore a ***Listeria***-free refrigeration unit environment subsequent to an initial contamination event. Incorporating antimicrobial silver technology in surface coatings as a sanitary equipment design measure to create an inhospitable environment for ***Listeria*** survival is a powerful tool for maintaining refrigerator surfaces subsequent to Listeria contamination.

One of the ways to combat bacterial contaminations is with silver ion technologies. For many centuries, cultures worldwide have been dependent on the antimicrobial properties of silver to discourage microbial contamination of food and water. In 1884, it became a common practice to administer drops of aqueous silver nitrate to newborn's eyes to prevent the transmission of Neisseria gonorrhoeae from infected mothers to children during childbirth. Nevertheless, the mechanisms by which silver inhibits bacterial growth have only been recently studied and elucidated. Most of the proposed mechanisms involved silver entering the cell in order to cause damage. A plethora of research findings indicate that the antimicrobial properties of silver were due to its ionized form, Ag^+, and its ability to cause damage to cells by interacting with thiol-containing proteins and DNA. Fig. 3 shows that treatment of Gram positive (Staphylococcus aureus) and Gram negative (E. coli) cells with silver ions (Ag^+) results in irreparable cell damage[4].

4 Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., Kim, J.O. ***A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus.*** Journal of Biomedical Materials Research Part A. 2000. Volume 52, issue 4. p. 662-668.

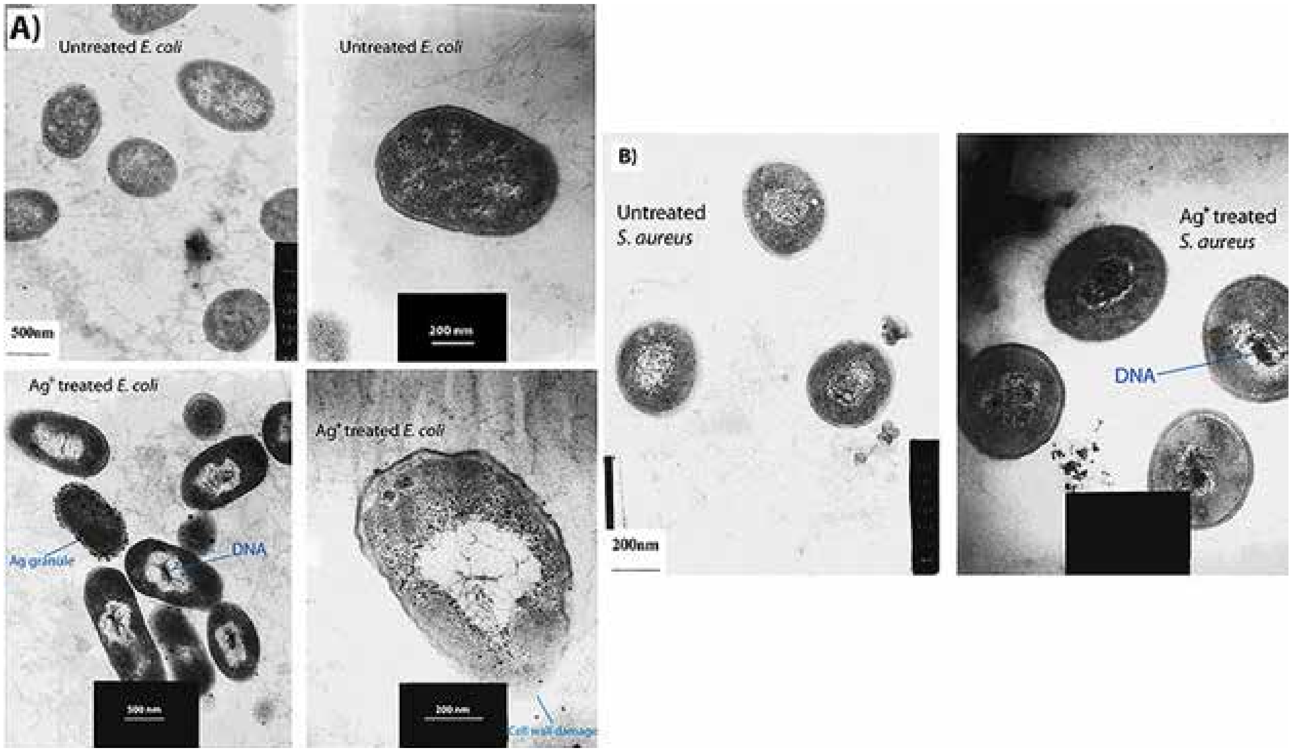


Figure 3: Treatment of cells with Ag+ results in DNA condensation, cell wall damage, and silver granule formation. (A) E. *coli* and (B) S. aureus cells with and without Ag+ treatment was observed with transmission electron microscopy (Feng *et* al., 2000).

Microban Silvershield Technology is a unique glass-based silver technology. Silver ions are infused into an amorphous glass matrix which is ground into fine particles. These particles can be incorporated into surfaces or surface coatings at very low levels and are designed to release silver ions whenever there is moisture present. The release rate is highly regulated by the unique amorphous glass structure giving extraordinary control and longevity compared to other silver technologies. The highly inert glass particles are also very temperature resistant (>600°C) and so are suitable for incorporation into most polymer processes.

Testing Equipment and Setup

To create an antimicrobial coated shelving surface, Microban Silvershield was powder-powder blended with a standard polyester powder coat material used to coat refrigerator shelves. This powder coat blend was sprayed and cured onto steel shelving units at the manufacturer; both treated and untreated shelving units were manufactured with the only difference in the coating materials being the level of Silvershield. No processing, finish or aesthetic differences were observed between samples; the treated and untreated shelves appeared identical. These units were shipped to Microban where they were cut into 2” by 2” squares configured for the microbiological testing protocol. Samples with several different grades and levels of Silvershield were made, all with additive levels appropriate to the guidelines of the specific additive’s EPA product registration use label.

For this study, a 4-feet wide commercial refrigeration case equipped with five shelves was installed and commissioned in the Microban microbiology lab. Based on the manufacturer’s instructions, the unit was loaded and operated at 4°C. Water jugs were used to add thermal mass and simulate food products being stored. The temperature at multiple locations within the unit was monitored for the duration of the study and found to be within ±2°C for the duration of the testing cycle. Five groups of powder-coated coupons (2” x 2”) representing untreated and silver incorporated antimicrobial technologies were used to simulate the surface of refrigeration shelves. The test coupons were placed in petri dishes. These dishes were then placed on the refrigerator shelves based on a statistically randomized design to minimize any local temperature variation effects, and allowed to acclimate for 48 h prior to inoculation (Fig. 4).

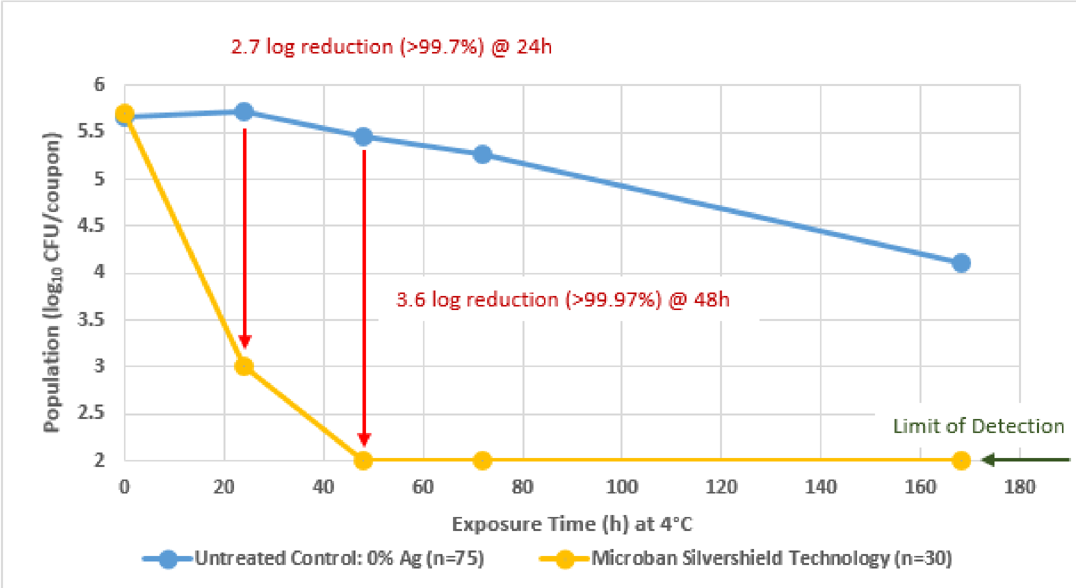
Figure 4. Commercial refrigeration unit loaded as per manufacturer's instruction using water jugs for thermal mass and stocked with inoculated powder-coated coupons

The acclimated coupons were inoculated with 106 CFU/mL of L. monocytogenes (ATCC #43256 – CDC strain designation F2380). The dishes containing the inoculated coupons were incubated in the refrigerator at 4°C and the surviving population was recovered at 0 h, 2 h, 24 h, 48 h, 72 h, and 1 week after inoculation. Recovery and enumeration of surviving Listeria population at each time point was conducted according to the ISO 22196 protocol (excepting that the incubation temperatures were 4°C rather than 36°C and the incubation times are as noted rather than fixed at 24h). As a quantitative measure of Microban Silvershield Technology's efficacy for controlling Listeria, populations recovered from the treated coupons were compared to those recovered from untreated coupons at each recovery interval (Fig. 5). The results for the optimal Silvershield formulation are discussed below.

The data plotted in Fig. 5 is the accumulated results of two trial cycles, with each treated data point run in triplicate and untreated controls run in quintuplicate. There are two main points of importance:

1. Listeria can survive on untreated powder-coated refrigerator surfaces for extended time periods. Under these particular test conditions, the initial inoculation was still viable at the end of the week long test period.
2. For the Silvershield treated shelving, the Listeria population recovered from the shelving coupons indicated organism population reductions of
    a. > 99.7% within 24h
    b. > 99.97% within 48h

when compared against the growth on the untreated shelving coupons.



[a] Equipment detection limit for counting organisms = 100 CFU/mL

Figure 5. Survival of Listeria on Untreated and Treated Powder-Coated Refrigerator Surface

Results generated using an ISO 22196 protocol run at 4°C.

Conclusions

Listeria contamination of food contact surfaces in the food processing service environment is costly to consumers, producers, and retailers. The traditional measures of decontamination have not been successful in restoring environments and surfaces to Listeria-free status because Listeria can form biofilms on surfaces. Model studies have demonstrated that L. monocytogenes, when encapsulated in a multispecies biofilm, is resistant to certain sanitizing agents, such as hypochlorite. Normally efficient sanitizers are inefficient against L. monocytogenes when the organism is embedded in an organic biofilm (1). Incorporation of sanitary equipment design into processing, storage, and display equipment needs to be considered as part of routine cleaning and sanitation protocol. Numerous systems have been identified for delivery of silver as an antimicrobial, among them is powder coated surfaces (2). The comparative data gathered from these studies show that when the Microban Silvershield Technology is incorporated into a powder coated surface, it is effective in limiting the survival and growth of Listeria, in this case on the shelving of refrigerator units. Organism reductions of >99.7% in 24h were obtained when comparing Silvershield-treated shelving samples with untreated samples using the ISO 22196 test protocol.

1. Bagge-Ravn, D., Gardshodn, K., Gram, L., and B.F. Vogel. 2003. Comparison of Sodium Hypochlorite–Based Foam and Peroxyacetic Acid–Based Fog Sanitizing Procedures in a Salmon Smokehouse: Survival of the General Microflora and Listeria monocytogenes. J. Food Protection, 66: 592–598

2. Campoccia, D., Montanaro, L. and C.R. Arciola. 2013. Review A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34: 8533-8554 http://dx.doi.org/10.1016/j.biomaterials.2013.07.089