MICROBAN SILVERSHIELD TECHNOLOGY: SOLUTION FOR *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 3-log reductions (>99.9%) of *Lm* in 48 h in a commercial fridge at 4°C.

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, bacteremia, 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 of its microbial impact *Listeria* has caused 1,600 illnesses, more than 1,500 related hospitalizations, and 260 related deaths each year in the United States[1].

[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.

Fig.1 shows actual epidemiological data for *Listeria*-related illnesses, hospitalizations, and deaths for the period 1997-2014.

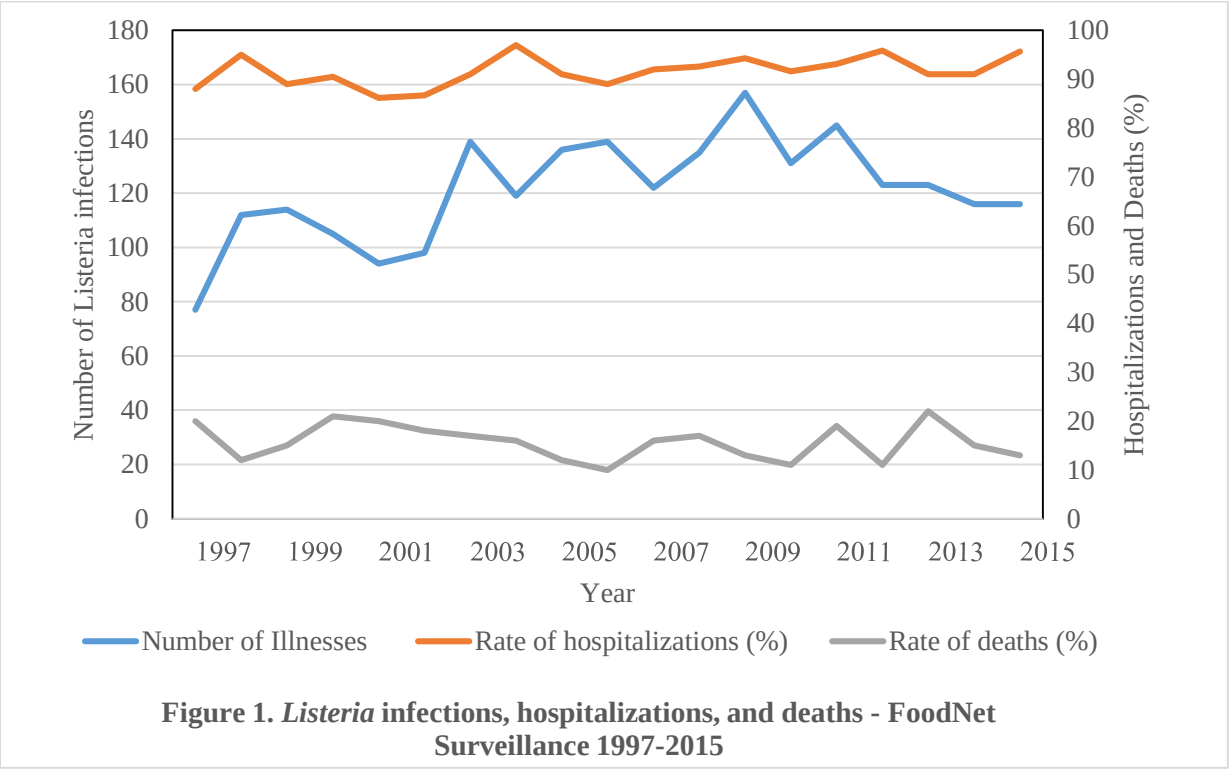


Figure 1. *Listeria* infections, hospitalizations, and deaths - FoodNet Surveillance 1997-2015

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 a *Listeria* microbial infection in pregnant women.

[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 microbial sources of infection, and disseminates information to provide a foundation for food safety policy and prevention efforts.

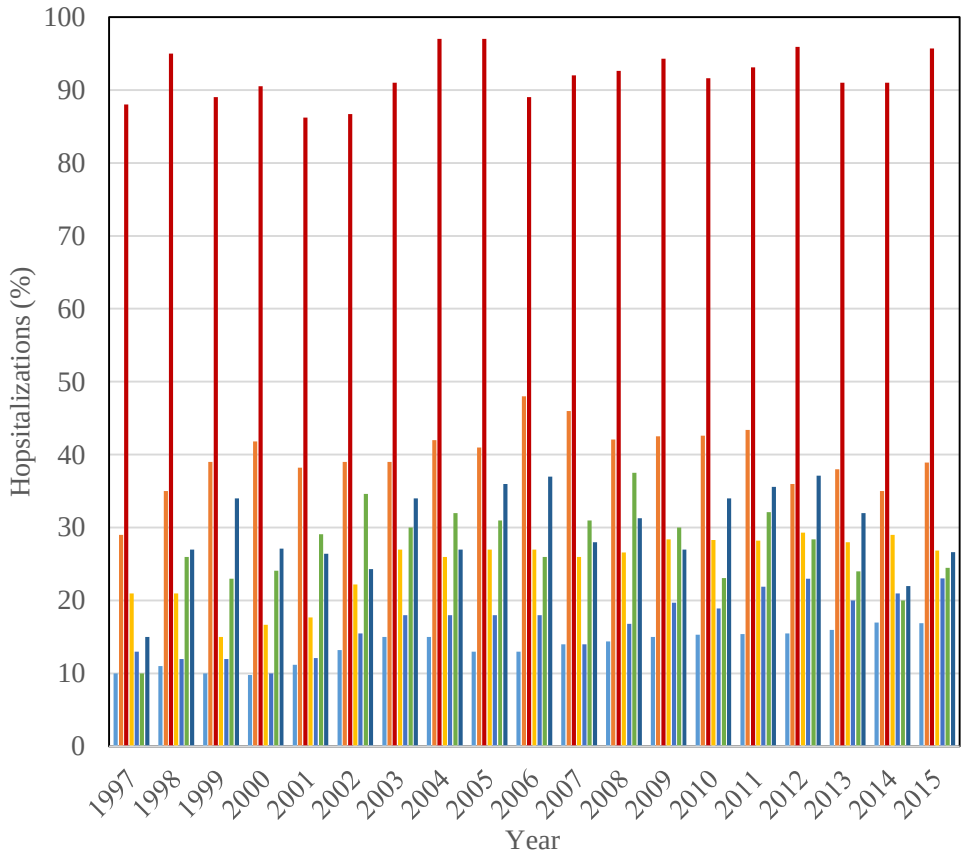


Figure 2. Percentage of Hospitalizations, by Selected Pathogens - FoodNet 1997-2015

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 microbial 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 were able to kill *Listeria* cells, contamination issues might still occur after factory cooking but before packaging. Contamination could even occur at the deli counter

with some RTE meats, such as hot dogs or deli meats. These RTE foods, which were common microbial vehicles for *Listeria,* were usually preserved by refrigeration.

Unlike most bacteria and microbial growth, *Listeria* can grow and multiply in RTE foods stored in the refrigerator. Also, 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 microbial 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 these microbial 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.

[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

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

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 microbial 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 a key component of a 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 microbial and 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.

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 acts as an antimicrobial to inhibit 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 silver antimicrobial properties 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].

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

[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.

silver ions whenever there is moisture present. However, 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.

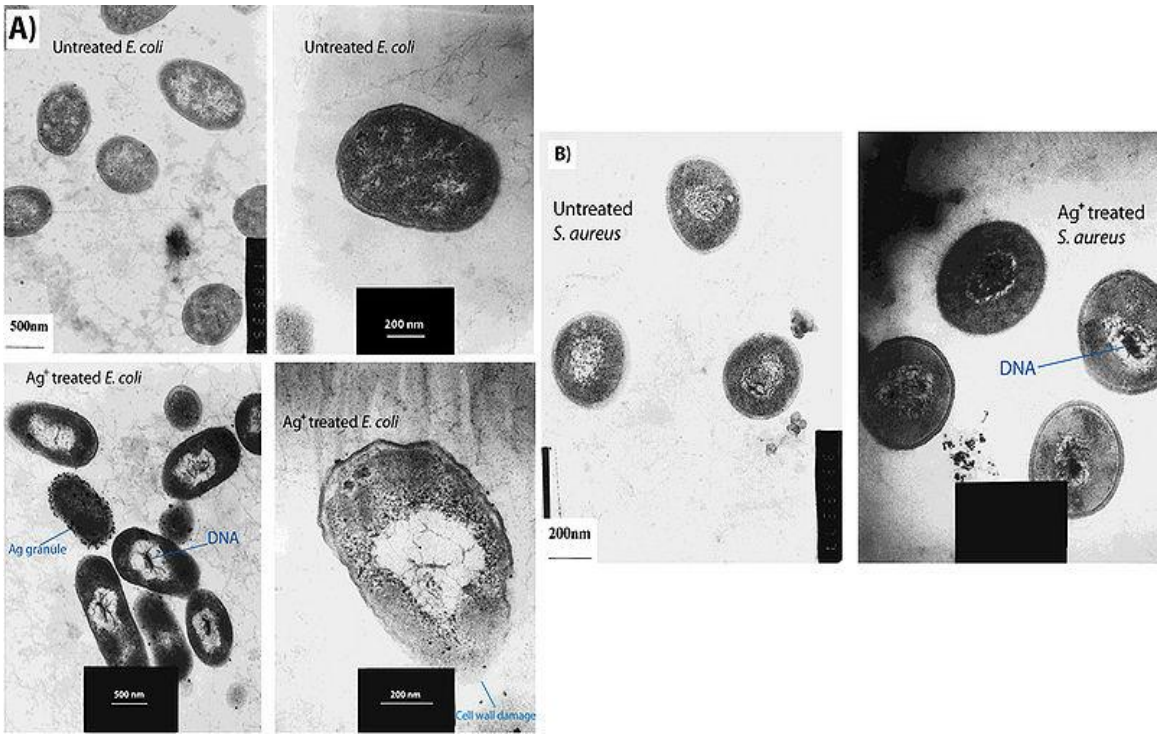


Figure 3: Treatment of cells with Ag^+ results in DNA condensation, cell wall damage, and silver granule formation. (A) *E. coli* and (B) *S. aureus* cells with and without Ag^+ treatment was observed with transmission electron microscopy (Feng *et al.*, 2000).

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 microbial growth 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.

Testing Equipment and Setup

To create an antimicrobial coated shelving surface, Microban SilverShield Technology was powder-blended with the standard polyester powder coat material used to coat refrigerator shelves. This powder coat blend was sprayed and cured onto shelving units at the manufacturer; both treated and untreated shelving units were manufactured. No processing, finish or aesthetic effects 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.

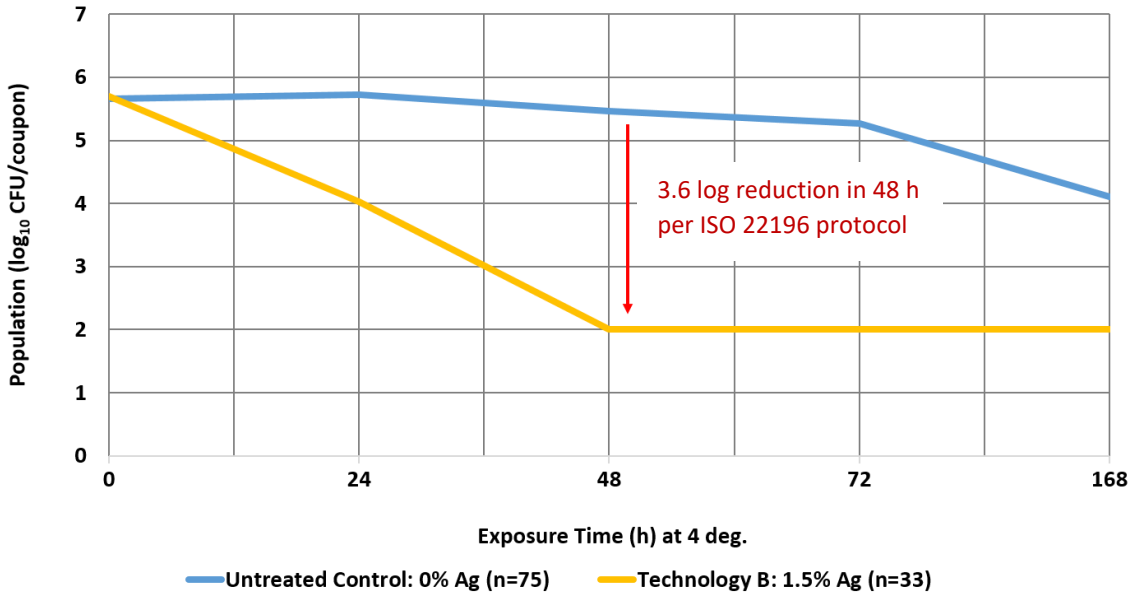
For the study, a 4-feet wide commercial refrigeration case equipped with five shelves was installed and commissioned. Based on the manufacturer's instructions, the unit was loaded and operated at 4°C. The temperature at multiple locations within the unit was monitored for the duration of the study, found to be within ±2°C for the duration of the testing cycle. Five groups of powder-coated coupons (2″ x 2″) representing untreated and two silver incorporated antimicrobial technologies were used to simulate the surface of refrigeration shelves. The test coupons were placed in irrigated dishes to maintain high humidity and support *Listeria* survival and microbial growth. These were 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 and stocked with inoculated powder-coated coupons

The acclimated coupons were inoculated with 10^5 CFU/mL of *L. monocytogenes* (ATCC #43256 – CDC strain designation F2380). The irrigated 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.

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 data shows that *Listeria* can survive on untreated powder-coated refrigerator surface for more than 72 h after inoculation before the population begins to decrease. The Microban SilverShield Technology was evaluated at two concentrations (0.75% and 1.5% Ag). At 1.5% Ag, *Listeria* population recovered from the coupons was reduced by > 90% within 24 h and > 99.9% reduction within 48h.



[a] Detection limit = 100 CFU/mL

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

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* forms 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 a silver antimicrobial solution, among them is powder coated surfaces (2).

The comparative data gathered from these studies show that the Microban SilverShield Technology is effective in limiting the survival and growth of *Listeria* that was inoculated onto the shelving of refrigerator units.

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