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Efficacy of Cleaning and Sanitizing Agents against Attached *Listeria monocytogenes* on Meat Slicer Components

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ABSTRACT

The objective of this study was to evaluate the resistance of surface-attached *Listeria innocua* and *Listeria monocytogenes* to sanitizing agents under laboratory conditions. Six strains of *L. monocytogenes* and one strain of *L. innocua* were attached to stainless steel or aluminum coupons that had been cut from a used deli meat slicer. One cleaner/sanitizer and two sanitizers were tested against the attached cells. No sanitizer caused more than a 1.5 log CFU/cm^2 reduction of *Listeria* when treated and untreated coupons were compared. Many delicatessens are using sanitizing wipes during operating times. Therefore, the best performing sanitizer, sanitizer C, from the first experiment was applied with a variety of cleaning cloths and compared with a commercial sanitation wipe. No cloth produced more than a one log reduction compared to controls.

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INTRODUCTION

Listeria monocytogenes, an intracellular Gram-positive pathogen, caused 0.34 cases of invasive listeriosis per 100,000 persons in 2009, compared with *Salmonella* spp., which caused 15 cases per 100,000 persons *(2)*. However, 89% of listeriosis patients were hospitalized and there was an overall 12.7% case fatality rate (CFR), in contrast to 27.5% hospitalizations and a CFR of 0.34% for salmonellosis *(2)*. Consumption of food contaminated by *L. monocytogenes* is the primary mode of transmission of this pathogen to humans *(7)*. *L. monocytogenes* contaminates food from a variety of environmental sources and food processing facilities. If present in meats or cheese, *L. monocytogenes* can contaminate slicers in delicatessens, and the resulting contaminated food contact surfaces may allow bacterial survival and multiplication and thus become sources of cross-contamination of

TABLE 1. Strains of *Listeria* used in sanitizer evaluation

Strain	Serotype	Origin
LM 27[a]	4b	Philadelphia outbreak, CDC
LM 98[a]	1/2c	Spinal fluid, male, Scotland
LM 187[a]	4b	Cheese outbreak, CDC
LM 189[a]	1/2a	Sliced turkey outbreak
LM 190[a]	1/2a	Human illness isolate
LM 191[a]	1/2a	Human illness isolate
Li 169[a]	Known as M1	Antibiotic resistance to 50 ppm rifampicin & 250 ppm streptomycin

[a]All strains were obtained from the culture collection of the Center for Food Safety at the University of Arkansas-Fayetteville.

foods that are not cooked before consumption. Avoiding cross-contamination between foods and food contact surfaces is thus critical to minimizing the risk of listeriosis.

According to the FDA Food Code *(4)*, food equipment used with potentially hazardous foods must have food contact surfaces cleaned throughout the day, at least every 4 hours. The equipment must be disassembled as necessary, scraped to remove food particulates, and then washed to remove soils, using whatever means are necessary, including application of detergents containing wetting agents and emulsifiers; acid, alkaline, or abrasive cleaners; hot water; brushes; scouring pads; high-pressure sprays, and ultrasonic devices. After washing, the equipment must be rinsed so that any abrasive compounds and cleaning chemicals are removed or effectively diluted. Equipment food contact surfaces must be sanitized after washing and rinsing and again before use by means of hot water or chemicals. Sanitizers are meant to be used on clean surfaces and are not designed to remove organic material or biofilms.

Properly cleaning and sanitizing the meat slicer can reduce the potential for cross-contamination at food service. It is generally agreed that commonly used disinfectants or sanitizers are effective against *L. monocytogenes* in suspension *(1, 3)*; however, cells attached to surfaces may be more resistant to sanitizers than cells in suspension *(5, 9, 12)*.

The objective of this study was to determine the effectiveness of commonly used commercial cleaning and sanitizing agents against surface-attached *L. monocytogenes* on aluminum and stainless steel coupons cut from a used deli meat slicer.

MATERIALS AND METHODS

Bacterial strains and growth conditions

One strain of *Listeria innocua* and six strains of *L. monocytogenes* were combined into a cocktail for use in this study. See Table 1 for details on strains used. Stock cultures were maintained frozen (–80°C) in tryptic soy broth containing 0.6% yeast extract (TSB-YE; Bacto, Becton Dickinson Co., Sparks, MD) supplemented with 16% glycerol. Each culture was inoculated from frozen stocks onto plates of Bacto tryptic soy agar containing 0.6% yeast extract (TSA-YE; Bacto, Becton Dickinson Co.) and incubated at 37°C for 24 h. Overnight cultures of each strain were prepared by inoculating a colony into 10 ml of TSB-YE and incubating the mixture at 37°C for 18 to 20 h. Cocktails for inoculation were prepared by placing equal aliquots of each overnight culture in a single sterile tube and mixing by use of a vortex mixer.

Preparing deli slicer coupons from components

The stainless steel blade of a Hobart heavy duty slicer (Hobart Food Equipment, Australia) was cut into 2 × 2.5 cm coupons, using a Flow Waterjet Cutting System (Flow International Corporation, Kent, WA). This Waterjet cutting system was used to prevent heat-induced stress that could change the physical properties of the stainless steel. From the blade guard of the same slicer, cast aluminum coupons (2 × 2 × 0.5 cm) were cut, using a Milwaukee Heavy-Duty cold-cutting metal saw (Brookfield, WI) and a Wellsaw metal-cutting band saw (Wells Manufacturing Corporation, Three Rivers, MI). Coupons were washed thoroughly in Micro 90 cleaning solution (International Products Corp., Burlington, NJ) prepared as per directions of the manufacturer and then rinsed in sterile deionized water. Coupons were autoclaved for 15 min at 121°C for sterilization prior to inoculation.

Cell attachment

Sterile coupons were laid individually in alternating sequence, in multiwall flat bottom plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ), and 40 µl (approximately 8 log CFU) of the *Listeria* cocktail was pipetted into the middle of each coupon and carefully spread over the area with a sterile inoculation loop. The inoculum was allowed to adhere during air drying for 2 h before treatment with sanitizer.

Sanitizer evaluation

Sanitizers for testing were recommended to us by the deli managers of

TABLE 2. Cleaners/sanitizers evaluated for inactivation of *Listeria* cocktail surface inoculated on coupons from deli meat slicer

Designation	Ingredients	Per cent by weight
A	Ethyl alcohol	5
	Sodium xlylene sulfonate	5
	Fatty acid alkanolamide	5
	Sodium lauryl ether sulfate	5
	Sodium dodecylbenzene sulfonate	20
	Water	60
B	n-alkyl dimethyl benzyl ammonium chlorides	5
	n-alkyl dimethyl ethylbenzyl ammonium chlorides	5
	Water	90
C	Quaternary ammonium chloride	10
	Ethanol	1
	Water	89
D	n-alkyl dimethyl benzyl ammonium chloride	0.0175
	Isopropyl alcohol	5.48

local retail deli establishments. Sanitizers were prepared at the concentrations recommended by the manufacturer (see Table 2 for composition). Compound A is a cleaner/sanitizer, while compounds B and C are simply sanitizers. Test coupons with attached cells were sprayed with sanitizer and allowed to sit for one minute. One ml of DE Neutralizing broth (Difco, Becton Dickinson Co., Sparks, MD) was added to each test and control coupon and allowed to sit for one minute. Excess DE broth was poured off and coupons swabbed with sterile swabs. Swabs were placed separately into tubes of sterile PBS and mixed with a vortex mixer; 10-fold serial dilutions were then prepared. Dilutions were plated on TSA-YE and plates were incubated at 37°C for 24 hours.

Evaluation of cleaning cloths

Cleaning cloths evaluated included a commercial cloth with sanitizer (D, see Table 2) and three other cloths evaluated with sanitizer C. One cloth evaluated was a 100% terrycloth towel, commonly known as a "bar towel," designated as W. The other two cloths were Textronic Microfibre Cloth (T) and Softronic Microfibre Cloth (S), both manufactured by VERMOP Salmon GmbH, Gilching, Germany. Coupons cut from the slicer were cleaned with the sanitizer-soaked cloths by wiping the coupon 3 times in the vertical direction and 3 times in the horizontal direction. Each coupon was placed in a sterile centrifuge tube containing 10 ml sterile PBS and mixed with a vortex mixer; serial dilutions were made, dilutions were plated on Modified Oxford Agar (MOX; Becton Dickinson Co., Sparks, MD), and the plates were incubated at 37°C for 48 hours.

Statistical analysis

Mean number of colonies per ml survivors was converted to log CFU/cm^2 and means were calculated. Differences were determined by student's *t*-test, with significance assigned at $P < 0.05$.

RESULTS AND DISCUSSION

To the best of our knowledge, no research specifically on survival or persistence of *L. monocytogenes* on aluminum has been published. The primary food contact surface for the deli slicer is the stainless steel blade, but the cast aluminum guard and other components of the slicer housing could also serve as a niche for survival of *Listeria* and thus could lead to cross contamination. *L. monocytogenes* cells attached to the deli slicer may detach and contaminate food products, and there is some indication that these detached cells could survive stressful conditions, even if they are older or have been injured in some fashion *(10)*.

We were able to recover a high number of attached bacteria, approximately 7 log/cm^2, when inocula were spotted on coupons and allowed to dry. This result is comparable to those of Kastbjerg and Gram *(6)* and Kim and others *(8)*, who recovered similar numbers of bacteria. This arrangement simulates situations where insufficient cleaning and disinfection allows *L. monocytogenes* to survive through protection by organic residues. Coupons were inoculated with an average of 8 log CFU of *Listeria* cocktail. Recovery from non-treated coupons (controls) was approximately 90%.

FIGURE 1. Log CFU/cm^2 survivors of *Listeria* cocktail on aluminum and stainless steel coupons from a deli meat slicer after use of cleaners and sanitizers. Survival data for controls are the mean of three samples; survival data for tests are the mean of 12 samples. [a-d]Stainless steel bars not having the same letter are significantly different by student's *t*-test ($P < 0.05$). [x-z]Aluminum bars not having the same letter are significantly different by student's *t*-test ($P < 0.05$).

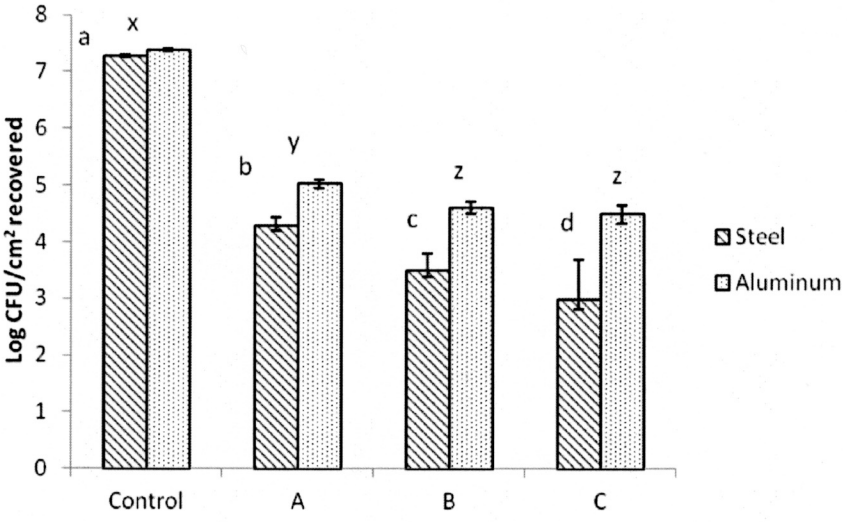
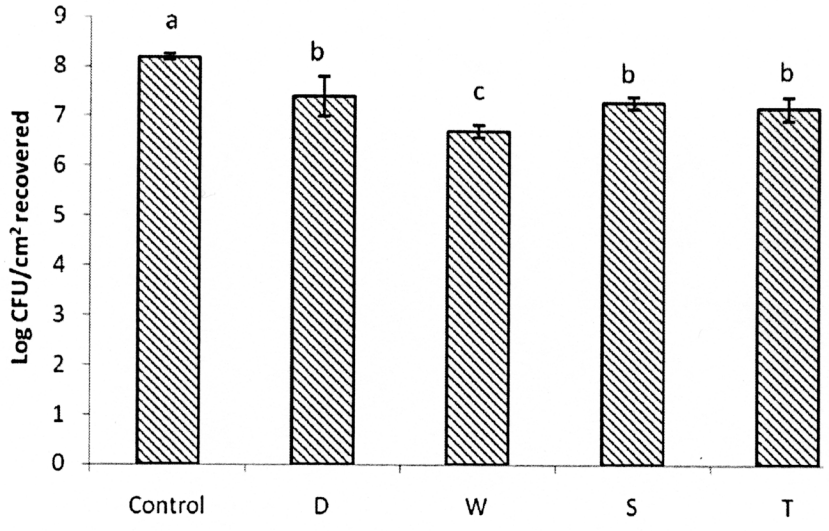


FIGURE 2. Comparison of a commercial sanitation wipe to various cloths and a sanitizer for cleaning stainless steel with surface-attached *Listeria*. D = commercial sanitizing wipe, W = white bar towel, S = Softronic Microfibre Cloth and T = Textronic Microfibre Cloth. [a-c]Different superscripts between treatments denote significant difference of means ($P < 0.05$).



On stainless steel coupons, all three sanitizers were effective against the attached *Listeria*, removing 2 to 3 log CFU/cm^2 of inoculated cocktail, a statistically significant reduction compared with untreated controls. Sanitizer C was significantly better ($P < 0.05$) than A and B, effectiveness significantly better ($P < 0.05$) in the order C, then B, then A (Fig. 1). Kastbjerg and Gram *(6)* also found that quaternary ammonium sanitizers were effective against 14 strains of *L. monocytogenes* tested on stainless steel coupons. The description of ingredients in sanitizer A indicates that it is intended primarily for cleaning rather than for disinfection. Taormina and Beuchat *(13)* found that *L. monocytogenes* could survive suspended in cleaning solutions commonly used in food processing plants. However, they also found that cells that survived in the cleaning solutions were still susceptible to sanitizers and heat.

Sanitizers B and C were significantly more effective ($P < 0.05$) in reducing *Listeria* recovery than was sanitizer A on the cast aluminum coupons (Fig. 1), although sanitizer A significantly reduced recovery compared to the control ($P < 0.05$). Comparison of sanitizer treatments on coupons made from the stainless steel blade and cast aluminum guard of a deli slicer revealed no significant differences ($P > 0.05$) in recovery of *Listeria* from control (untreated) coupons or for sanitizer A. However, sanitizers B and C performed significantly better ($P < 0.05$) on stainless steel than on cast aluminum.

Sanitizer C, because it was the most effective in the study just completed, was selected for further study using different cleaning cloths on stainless steel coupons from the blade. The commercial sanitation wipe (D) produced an approximate 1 log reduction of *Listeria*, as did sanitizer C used with the two microfiber cloths (S and T). These results are shown in Fig. 2. The most successful combination was the white bar towel with sanitizer C. These results are significant, because retail foodservice workers commonly wipe the slicer blade with a sanitation wipe or towel that was stored in sanitizer between products. In this scenario, if there was heavy *L. monocytogenes* contamination from a previously sliced food or cross-contamination, the practice of just using a sanitation wipe would not be adequate to prevent cross-contamination.

CONCLUSIONS

In most foodservice operations, the retail meat slicer is used randomly and sporadically throughout the day while being kept at room temperature, unlike other equipment such as food processors or mixers that are used and cleaned after each use. This study revealed that all three sanitizers were effective against attached *Listeria* on stainless steel and

cast aluminum coupons, with sanitizer C being the most effective in both cases. Sanitizers B and C were found to be more effective on stainless steel than on aluminum coupons. Of the various cloths tested, the common white bar towel was the most effective. Often the operators prefer using wiping cloths or sanitary wipes in place of disassembling and cleaning *(11)*, which this research indicates may not be adequate to insure safety.

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