

Control of *Listeria* spp. by Competitive-Exclusion Bacteria in Floor Drains of a Poultry Processing Plant

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In previous studies workers determined that two lactic acid bacterium isolates, *Lactococcus lactis* subsp. *lactis* C-1-92 and *Enterococcus durans* 152 (competitive-exclusion bacteria [CE]), which were originally obtained from biofilms in floor drains, are bactericidal to *Listeria monocytogenes* or inhibit the growth of *L. monocytogenes* both in vitro and in biofilms at 4 to 37°C. We evaluated the efficacy of these isolates for reducing *Listeria* spp. contamination of floor drains of a plant in which fresh poultry is processed. Baseline assays revealed that the mean numbers of *Listeria* sp. cells in floor drains sampled on six different dates (at approximately biweekly intervals) were 7.5 log₁₀ CFU/100 cm² for drain 8, 4.9 log₁₀ CFU/100 cm² for drain 3, 4.4 log₁₀ CFU/100 cm² for drain 2, 4.1 log₁₀ CFU/100 cm² for drain 4, 3.7 log₁₀ CFU/100 cm² for drain 1, and 3.6 log₁₀ CFU/100 cm² for drain 6. The drains were then treated with 10⁷ CE/ml in an enzyme-foam-based cleaning agent four times in 1 week and twice a week for the following 3 weeks. In samples collected 1 week after CE treatments were applied *Listeria* sp. cells were not detectable (samples were negative as determined by selective enrichment culture) for drains 4 and 6 (reductions of 4.1 and 3.6 log₁₀ CFU/100 cm², respectively), and the mean numbers of *Listeria* sp. cells were 3.7 log₁₀ CFU/100 cm² for drain 8 (a reduction of 3.8 log₁₀ CFU/100 cm²), <1.7 log₁₀ CFU/100 cm² for drain 1 (detectable only by selective enrichment culture; a reduction of 3.3 log₁₀ CFU/100 cm²), and 2.6 log₁₀ CFU/100 cm² for drain 3 (a reduction of 2.3 log₁₀ CFU/100 cm²). However, the aerobic plate counts for samples collected from floor drains before, during, and after CE treatment remained approximately the same. The results indicate that application of the two CE can greatly reduce the number of *Listeria* sp. cells in floor drains at 3 to 26°C in a facility in which fresh poultry is processed.

Controlling the widely distributed psychrotrophic organism *Listeria monocytogenes* in food processing facilities has been a formidable challenge for the entire food industry, from the smallest food processor to the largest. Besides this pathogen's widespread occurrence in nature, it is nonfastidious, grows at refrigeration temperatures, and can form or coexist in protective biofilms (1, 2, 7, 15, 16). Floor drains in food processing facilities are a particularly important niche for the persistence of listeriae and can be a point of contamination in the processing plant environment and possibly in food products.

Decontaminating floor drains of *Listeria* sp. is especially challenging because when entrapped in a biofilm, *Listeria* sp. is afforded unusual protection against disinfectants and treatments available to control pathogens on environmental surfaces (7, 9, 14, 17, 18). Once attached, the cells may produce multicellular biofilms that are resistant to disinfection and from which cells can become detached and contaminate food products. The establishment of biofilms by pathogenic bacteria in floor drains in food processing plants is believed to protect against effective cleaning regimens and to reduce or minimize the efficacy of bactericidal treatments. Studies have indicated that *L. monocytogenes* growing within mixed-microflora biofilms in food processing environments can be a major source of contamination (1, 9, 20).

Promising in vitro results have been obtained in previous

studies with two competitive-exclusion bacteria (CE), *Lactococcus lactis* subsp. *lactis* C-1-92 and *Enterococcus durans* 152, for inhibition of the growth of *L. monocytogenes* in culture media and in biofilms at temperatures ranging from 4 to 37°C (22). The objective of this study was to evaluate the efficacy of these CE in controlling listerial growth and possibly eliminating the pathogen in floor drains at a wide range of temperatures in a facility in which fresh poultry is processed, including under refrigeration conditions.

MATERIALS AND METHODS

Processing plant. The poultry processing plant had two processing lines; each line processed 140 birds per min, and the plant had the capacity to process 400,200 birds per day. Approximately 268,800 birds were processed during a normal day with two shifts.

Floor drain selection. Based on the direction of flow of the drains, the fluid flow rate, the fluid volume, the drain size, the temperatures of rooms, and the occurrence and persistence of *Listeria* sp., six floor drains for CE treatment and an untreated control were identified. Drains 1 and 2 were open trenches (height, 25 cm; width, 30 cm) and were comprised of concrete with fiberglass covers. These drains were located close to poultry meat cutting lines at mean temperatures of 16.8 and 11.5°C, respectively, and largely contained liquid drippings from meat, including meat that had fallen on the floor. Drains 1 and 2 were typically filled to 10 to 30% of capacity during operation. Drain 3 was an open trench (height, 25 cm; width, 30 cm) comprised of concrete with metal edging and a metal cover. This drain was located in the middle of a high-traffic area at a mean temperature of 15.1°C and was typically 20 to 40% full during operation. Drains 4 and 6 were open trenches (height, 20 cm; width, 30 cm) and were comprised of fiberglass. They were located near poultry meat packing areas at mean temperatures of 2.8 and 3.8°C, respectively, and were typically filled to 5 to 20% of capacity during operation. Drain 8 was an open trench (height, 150 cm; width, 90 cm) and was comprised of concrete with a fiberglass cover. This drain

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TABLE 1. *Listeria* sp. counts for floor drain samples collected before and after CE treatment

Time	Location	<i>Listeria</i> sp. count (log ₁₀ CFU/100 cm ²) for floor drains ^a					
		Drain 1	Drain 3	Drain 4	Drain 6	Drain 8	Drain 2 (control) ^b
Before CE treatment ^c	Bottom side of cover	3.3 ± 0.8	4.2 ± 1.2	4.2 ± 1.1	4.2 ± 1.2	7.6 ± 1.1	4.2 ± 1.1
	Right side of drain	3.9 ± 0.7	5.0 ± 0.9	4.3 ± 0.8	3.5 ± 1.9	7.6 ± 0.8	4.6 ± 0.7
	Left side of drain	4.0 ± 1.0	4.4 ± 1.3	3.4 ± 1.1	3.2 ± 1.5	8.2 ± 0.5	4.1 ± 0.6
	Inside of drain	3.5 ± 0.8	5.4 ± 1.3	4.5 ± 1.1	3.6 ± 1.3	7.8 ± 0.9	5.4 ± 1.4
	Floor within ca. 30 cm of drain	3.6 ± 0.9	5.3 ± 0.6	4.3 ± 1.4	3.3 ± 1.3	6.1 ± 1.9	3.8 ± 1.0
After CE treatment ^d	Bottom side of cover	1.8 ± 0.4 ^e	1.9 ± 0.3 ^e	1.9 ± 0.3 ^e	2.1 ± 0.4 ^e	4.0 ± 2.0 ^e	3.6 ± 0.3 ^f
	Right side of drain	2.0 ± 0.5 ^e	2.1 ± 0.8 ^e	1.7 ± 0.1 ^e	1.9 ± 0.7 ^e	3.9 ± 2.0 ^e	4.0 ± 0.6 ^f
	Left side of drain	2.0 ± 0.6 ^e	2.3 ± 0.6 ^e	2.1 ± 1.2 ^e	1.9 ± 0.4 ^e	3.9 ± 2.2 ^e	4.6 ± 0.7 ^f
	Inside of drain	2.0 ± 0.5 ^e	2.8 ± 1.4 ^e	1.7 ± 0.1 ^e	2.0 ± 0.7 ^e	3.7 ± 1.8 ^e	5.5 ± 1.2 ^f
	Floor within ca. 30 cm of drain	1.9 ± 0.5 ^e	2.1 ± 0.6 ^e	2.1 ± 0.9 ^e	1.9 ± 0.6 ^e	3.5 ± 0.8 ^e	4.1 ± 1.5 ^f

^a The temperatures at drains 1, 3, 4, 6, 8, and 2 were 16.8 ± 2.6, 15.1 ± 2.3, 2.8 ± 1.2, 3.8 ± 1.1, 26.1 ± 1.2, and 11.5 ± 2.7°C (means ± standard deviations), respectively.

^b This drain served as the control and was not treated with CE.

^c Each drain was sampled in four locations and assayed for *Listeria* sp. every 2 weeks for 10 weeks before CE treatment. The values are the means ± standard deviations for six samplings.

^d Each drain was sampled in four locations and assayed for *Listeria* sp. every week for 5 weeks following CE treatment. The values are the means ± standard deviations for five samplings.

^e The *Listeria* sp. counts in drains after CE treatment were significantly different ($P < 0.05$) than the *Listeria* sp. counts in drains before CE treatment.

^f The *Listeria* sp. counts in drains after CE treatment were not significantly different ($P > 0.05$) than the *Listeria* sp. counts in drains before CE treatment.

was near a liver and lung removal line at a mean temperature of 26.1°C and contained blood and meat debris. It was typically filled with liquid to 15 to 20% of capacity.

Sampling. Using sterile gloves, an 18-oz. sterile "speci-sponge" (3.8 by 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) was used to wipe an area that was ca. 10 by 10 cm at each sampling location. Five locations, including the inside (bottom) of the drain, the right side of the drain, the left side of the drain, the bottom side of the trench cover, and the floor within 30 cm of the drain, were sampled for each floor drain. Each sponge was placed in a Whirl-Pak bag and kept at 5°C for 2 to 14 h until the sample was assayed.

Enumeration of *Listeria* sp. and *L. monocytogenes*. Ten milliliters of 0.1% peptone was added to each bag, and the sponge and peptone were blended with a stomacher blender (Seward Medical, London, United Kingdom) at 150 rpm for 1 min. The fluid was serially diluted (1:10) in 0.1% peptone (Becton Dickinson Microbiology Systems, Sparks, MD), and 0.1 ml from each dilution tube was plated in duplicate on the surface of modified Oxford medium (MOX) (Oxoid, Ogdensburg, NY) plates. When *Listeria* sp. was not detected by the direct plating method, the broth plus the sponge was selectively enriched in 225 ml Fraser broth (Becton Dickinson Microbiology Systems) for 24 h at 37°C. Then 1 ml of the broth was serially diluted (1:10) in 0.1% peptone to obtain 10⁻⁸ CFU/ml, and 0.1 ml of each dilution was surface plated on MOX plates in duplicate. The MOX plates were incubated at 37°C for 48 h, and typical black colonies were counted as presumptive *Listeria* sp. colonies. Colonies counted as *Listeria* sp. colonies were randomly selected and transferred to MOX plates, and they were confirmed to be *Listeria* sp. by biochemical tests (API 20I miniaturized diagnostic test; bioMérieux Vitek, Hazelwood, MO) and a lateral flow latex agglutination assay (*Listeria* Rapid Test; Oxoid, Ogdensburg, NY) and to be *L. monocytogenes* by an enzyme-linked fluorescent immunoassay with an automated VIDAS instrument (miniVIDAS; bioMérieux Vitek, Hazelwood, MO) and by a PCR assay (Qualicon BAX system; DuPont, Wilmington, DE).

Determination of aerobic plate counts (APCs). Serial dilutions (duplicate 0.1-ml portions of each dilution) of the samples described above were surface plated on plate count agar plates (Becton Dickinson Microbiology Systems) and incubated at 30°C for 72 h for enumeration.

Preparation of competitive-exclusion bacteria. Each CE strain, *L. lactis* subsp. *lactis* C-1-92 or *E. durans* 152, was grown individually in 250 ml of *Lactobacillus* MRS broth (Becton Dickinson Microbiology Systems) at 32°C for 24 h. Cells were sedimented by centrifugation at 10,000 × *g* for 20 min at 4°C. The bacteria were resuspended in 25 ml of MRS broth at a density of ca. 10⁹ CFU/ml, serially diluted (1:10) in 0.1% peptone, and plated on MRS agar and tryptic soy agar in duplicate for bacterial counting.

Application of competitive-exclusion bacteria to floor drains. The CE treatment was performed within 20 min after routine sanitation procedures in the plant were completed. Two enzyme-based cleaning products, DY-GEST I (Ecolab, St. Paul, MN) and DY-GEST II (Ecolab), were used to apply the CE

treatment. This treatment was routinely used with the cleaning products as part of the plant's routine sanitation procedures; however, the drains were not included as part of the daily routine sanitation procedures. When used under pressure, the cleaning agents formed a foam that remained for about 15 min. The DY-GEST preparation was prepared by mixing in a bottle 20 ml of each product with 3.78 liters of tap water. The two CE isolates (25 ml each) were added to a tank foaming dispenser (model 925916; Lafferty Equipment Manufacturing Co., North Little Rock, AR), followed by the ca. 4 liters of diluted DY-GEST preparation. The tank foaming dispenser was connected to pressurized air, and foam was applied to each floor drain. Approximately 10⁷ CE/ml in the foam formula was applied in order to cover all locations in the floor drain following sanitation four times during the first week of application (Monday through Thursday). After this, the CE treatment was applied twice a week (Tuesday and Thursday) for the following 3 weeks. Samples were collected once a week during the 5 weeks after the CE treatment was initiated. After this, samples were collected every other week or once a month for up to 18 weeks following the last CE treatment.

Statistical analysis. The least-square means of *Listeria* sp. counts and APCs at each sampling location before and after CE treatment in each floor drain were determined using the general linear model of the Statistical Analyses System procedure (SAS Institute, Cary, NC). The values used for statistical analysis when bacteria were detected and not detected by selective enrichment in each floor drain were 1.6 and 0 log₁₀ CFU/100 cm², respectively. The criterion used for significance for differences was $P < 0.05$ for all assays.

RESULTS

Baseline determinations revealed that the mean *Listeria* sp. counts in floor drains sampled at six different times (2-week intervals) before treatment with CE were 7.5 log₁₀ CFU/100 cm² for drain 8, 4.9 log₁₀ CFU/100 cm² for drain 3, 4.4 log₁₀ CFU/100 cm² for drain 2, 4.1 log₁₀ CFU/100 cm² for drain 4, 3.7 log₁₀ CFU/100 cm² for drain 1, and 3.6 log₁₀ CFU/100 cm² for drain 6 (Table 1). This included *Listeria* sp. counts in drains after routine cleaning and sanitation treatment of processing equipment and facilities were performed (10-week sample) (Fig. 1 to 5). The results revealed that *Listeria* sp. counts for drain samples collected after plant sanitation treatments were not significantly different from *Listeria* sp. counts for drains before processing equipment and facilities were cleaned and sanitized.

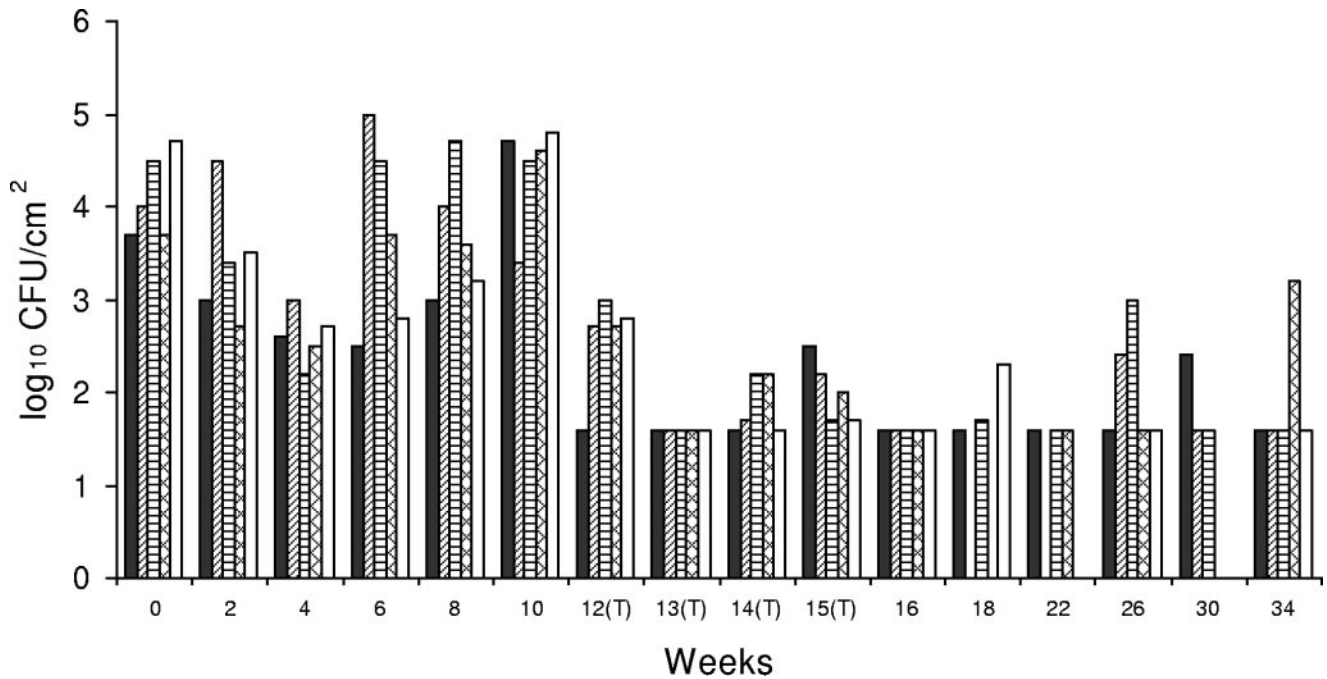


FIG. 1. *Listeria* sp. counts in concrete, open-trench floor drain 1, which was located near poultry meat cutting lines and contained liquid drippings from meat, including meat that had fallen on the floor. The drain was typically filled to 10 to 30% of capacity during operation. ■, bottom side of cover; ▨, right side of drain; ▩, left side of drain; ▤, inside of drain; □, floor within ca. 30 cm of the drain. (T), CE treatment applied.

Drain 8 had considerably higher initial *Listeria* sp. counts than the other drains, and there was only a modest reduction in the *Listeria* sp. counts following the first week of treatment. However, *Listeria* sp. counts were dramatically reduced in all locations following the second and third weeks of treatment. The mean *Listeria* sp. count following CE treatment at weeks 12 to 16 was $3.8 \log_{10}$ CFU/100 cm² (Fig. 5), which indicated

that there was a $3.7\text{-}\log_{10}$ CFU/100 cm² reduction after CE treatment ($P < 0.05$) (Table 1).

A significant reduction in *Listeria* sp. counts was also observed for drain 3 in the second week after CE treatment, and *Listeria* sp. was not detectable by a direct plating method at all sites. The mean *Listeria* sp. count at weeks 12 to 16 was $2.2 \log_{10}$ CFU/100 cm², which indicated that there was a $2.7\text{-}\log_{10}$

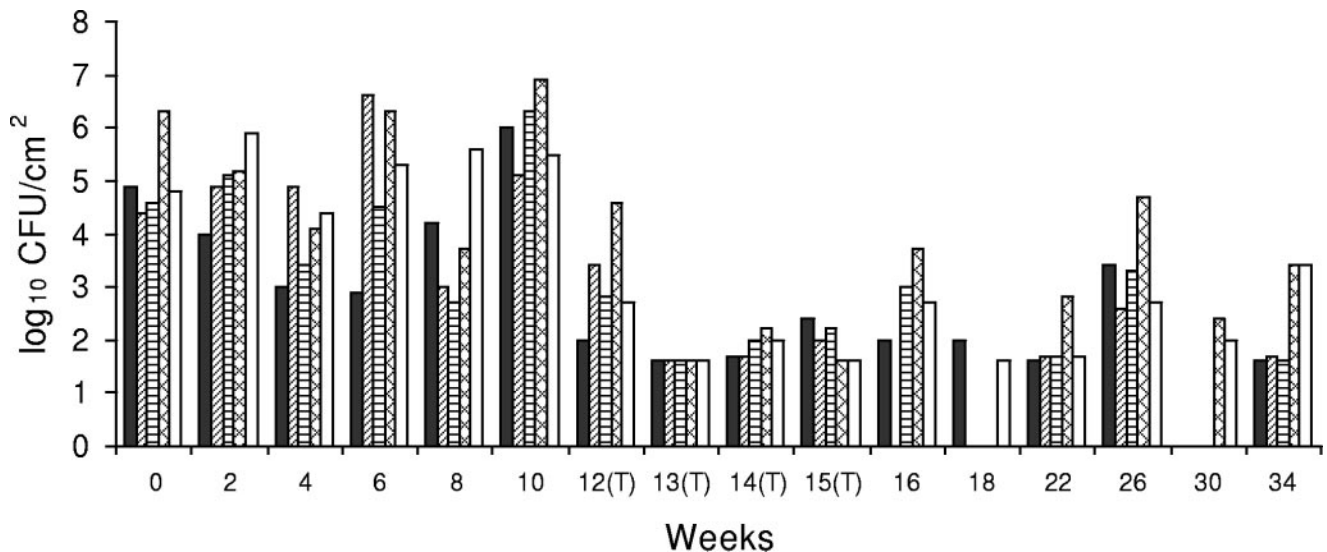


FIG. 2. *Listeria* sp. counts in concrete, open-trench floor drain 3, which was located in the middle of a high-traffic area and was typically filled to 20 to 40% of capacity during operation. ■, bottom side of cover; ▨, right side of drain; ▩, left side of drain; ▤, inside of drain; □, floor within ca. 30 cm of the drain. (T), CE treatment applied.

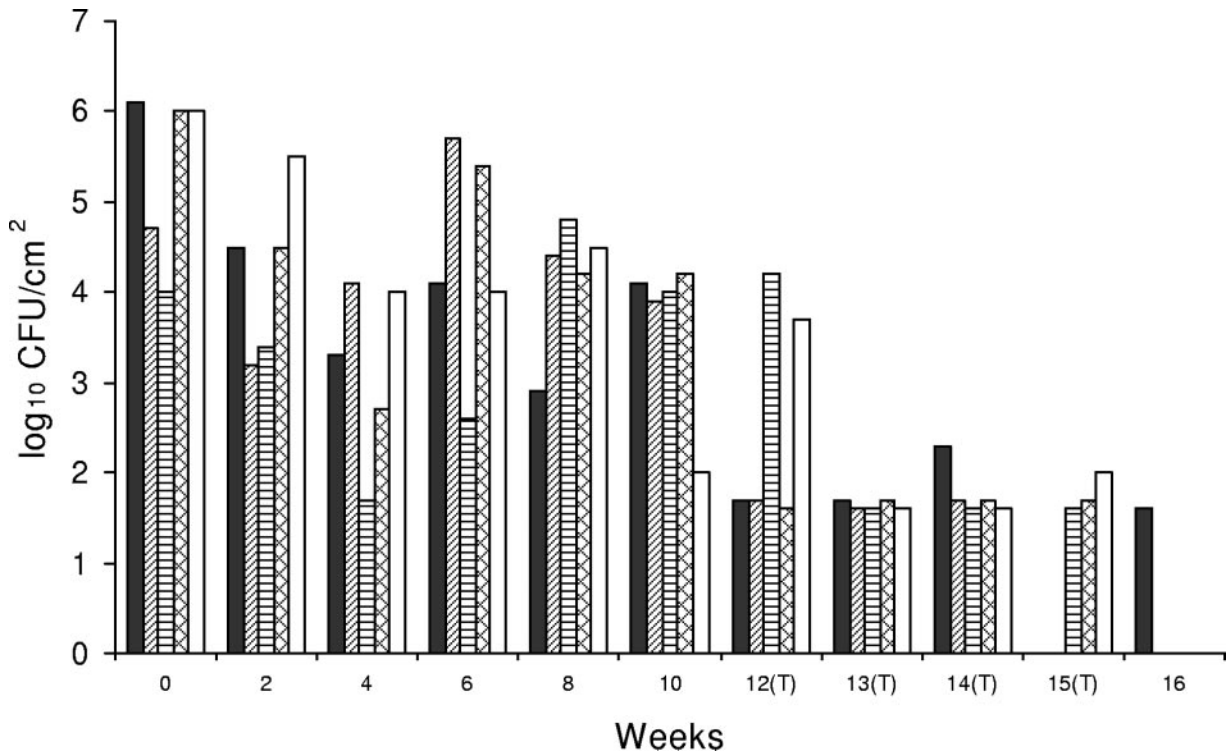


FIG. 3. *Listeria* sp. counts in fiberglass, open-trench floor drain 4, which was located near a poultry meat packing area and was typically filled to 5 to 20% of capacity during operation. ■, bottom side of cover; ▨, right side of drain; ▩, left side of drain; ▤, inside of drain; □, floor within ca. 30 cm of the drain. (T), CE treatment applied.

CFU/100 cm² reduction after the CE treatments ($P < 0.05$) (Table 1). However, the *Listeria* sp. counts in some locations of drain 3 were highly variable at later sampling times (Fig. 2).

The *Listeria* sp. counts at most locations in drain 4 were reduced after CE treatment was initiated (first week), and the mean *Listeria* count was 1.9 log₁₀ CFU/100 cm², which indicated that there was a 2.2-log₁₀ CFU/100 cm² reduction after

CE treatments ($P < 0.05$) (Table 1). One week following the conclusion of CE treatments (week 16) *Listeria* sp. was detectable (at the minimum level of detection) in only one drain location and was not detectable at the other three sampling sites (Fig. 3). Sampling in this drain was discontinued after this because of construction at this location of the plant.

At all sites in drain 1 the *Listeria* sp. counts were greatly

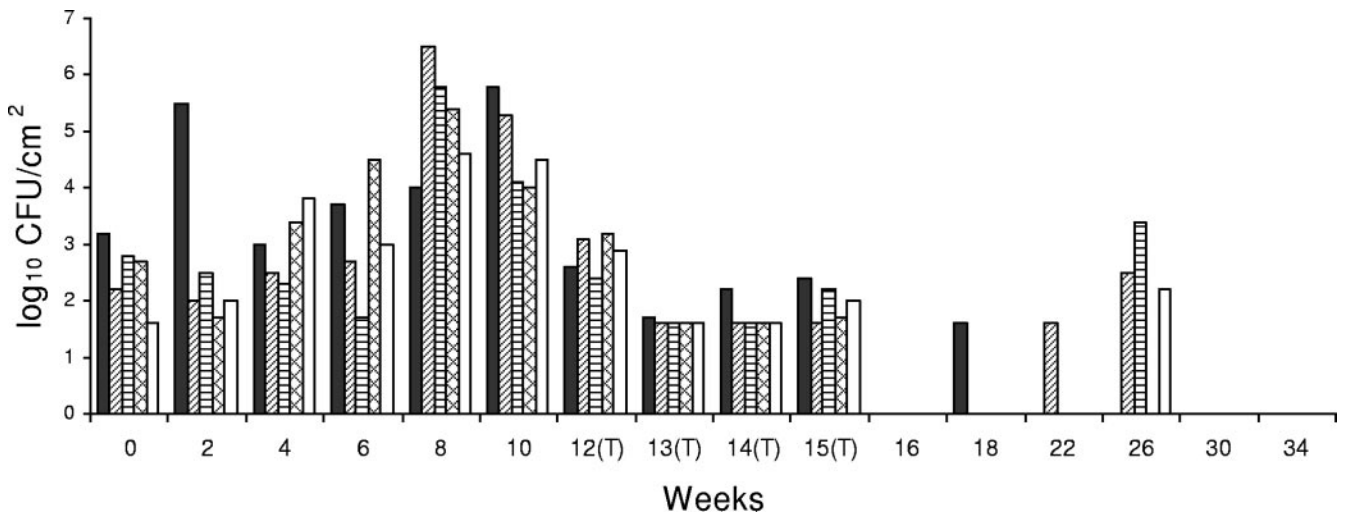


FIG. 4. *Listeria* sp. counts in fiberglass, open-trench floor drain 6, which was located near a poultry meat packing area and was typically filled to 5 to 20% of capacity during operation. ■, bottom side of cover; ▨, right side of drain; ▩, left side of drain; ▤, inside of drain; □, floor within ca. 30 cm of the drain. (T), CE treatment applied.

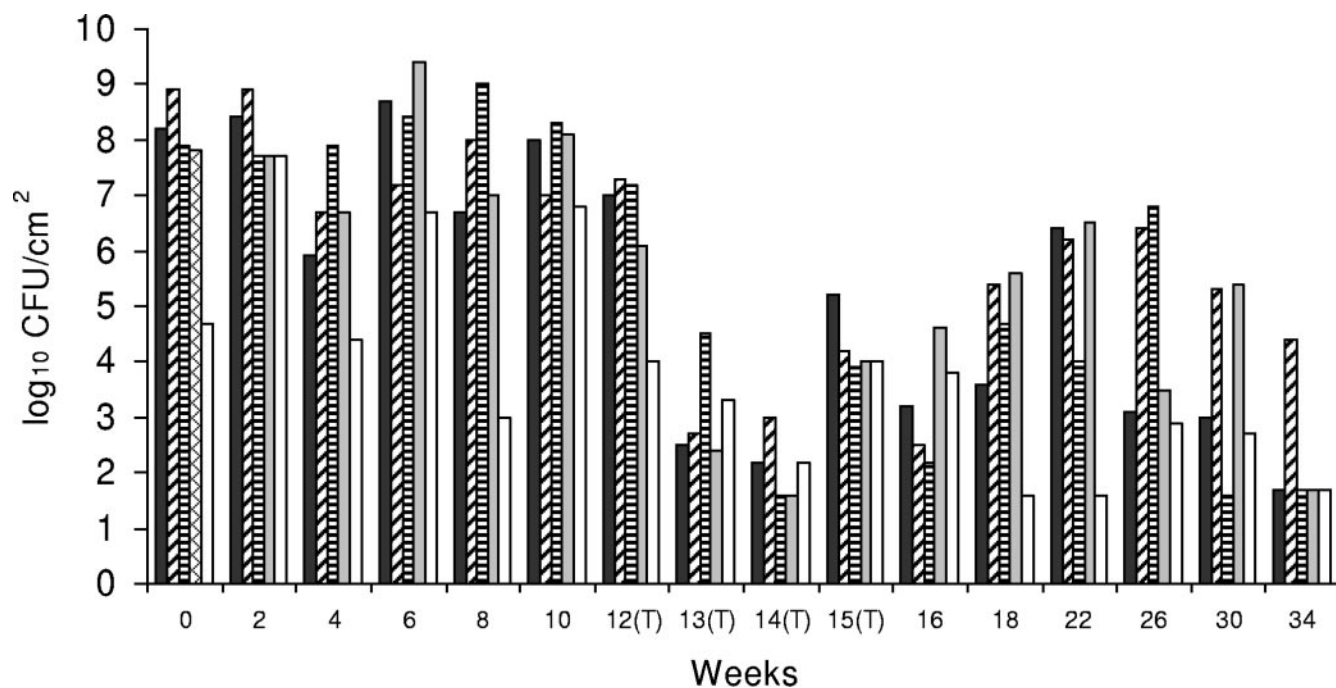


FIG. 5. *Listeria* sp. counts in concrete, open-trench floor drain 8, which was located near a liver and lung removal line at a mean temperature of 26.1°C and contained blood and meat debris. This drain was typically filled with liquid to 15 to 20% of capacity. ■, bottom side of cover; ▨, right side of drain; ▩, left side of drain; ▤, inside of drain; □, floor within ca. 30 cm of the drain. (T), CE treatment applied.

reduced the second week following the sixth CE treatment, and *Listeria* sp. was undetectable by a direct plating method. The mean *Listeria* sp. count at weeks 12 (first week of CE treatments) to 16 (fourth week of CE treatments) was 1.9 log₁₀ CFU/100 cm² (Fig. 1), which indicated that there was a 1.8-

log₁₀ CFU/100 cm² reduction after CE treatments ($P < 0.05$) (Table 1). This trend was consistent for most locations in drain 1 until the study was terminated (week 34) (Fig. 1).

The mean *Listeria* sp. count for drain 6 at weeks 12 to 16 was 2.0 log₁₀ CFU/100 cm² after CE treatment, which indicated

TABLE 2. Aerobic plate counts for drain samples collected before, during, and after CE treatment

Time	Location	Aerobic plate count (log ₁₀ CFU/100 cm ²) for drains				
		Drain 1	Drain 3	Drain 4	Drain 6	Drain 8
Before CE treatment ^a	Bottom side of cover	8.4 ± 1.0	8.3 ± 0.6	8.7 ± 1.0	9.1 ± 0.6	10.0 ± 0.8
	Right side of drain	8.5 ± 0.5	8.6 ± 0.6	8.6 ± 0.3	9.3 ± 0.6	9.6 ± 0.4
	Left side of drain	8.7 ± 0.3	8.5 ± 0.6	8.3 ± 0.7	9.1 ± 0.2	9.6 ± 1.0
	Inside of drain	8.7 ± 0.8	9.4 ± 0.3	8.8 ± 0.6	8.4 ± 0.4	9.9 ± 0.4
	Floor within ca. 30 cm of drain	8.4 ± 0.7	9.0 ± 0.8	8.1 ± 1.2	9.5 ± 0.6	9.0 ± 0.7
During CE treatment ^b	Bottom side of cover	8.4 ± 0.9 ^c	7.3 ± 0.7 ^d	9.1 ± 0.6 ^e	9.9 ± 0.6 ^e	9.3 ± 0.4 ^e
	Right side of drain	8.4 ± 1.4 ^c	8.0 ± 0.6 ^e	8.8 ± 0.9 ^e	9.5 ± 0.8 ^e	8.8 ± 0.5 ^d
	Left side of drain	8.0 ± 0.7 ^e	7.4 ± 0.7 ^d	8.5 ± 1.5 ^e	9.4 ± 0.8 ^e	9.1 ± 0.5 ^e
	Inside of drain	8.1 ± 0.8 ^c	7.4 ± 0.8 ^d	8.6 ± 0.7 ^e	9.0 ± 1.2 ^e	9.0 ± 0.9 ^e
	Floor within ca. 30 cm of drain	7.8 ± 0.3 ^c	8.1 ± 0.6 ^d	9.2 ± 0.5 ^e	9.2 ± 0.7 ^e	8.5 ± 0.4 ^e
After CE treatment ^c	Bottom side of cover	8.8 ± 1.1 ^c	8.0 ± 0.9 ^e	9.6 ± 0.5 ^e	8.2 ± 0.4 ^d	9.6 ± 1.3 ^e
	Right side of drain	8.3 ± 1.3 ^c	8.5 ± 1.3 ^e	7.1 ± 0.3 ^d	8.7 ± 0.9 ^c	9.2 ± 1.4 ^e
	Left side of drain	8.9 ± 1.4 ^c	8.3 ± 1.6 ^e	6.7 ± 0.4 ^d	7.9 ± 0.9 ^d	8.2 ± 1.3 ^d
	Inside of drain	8.7 ± 1.5 ^c	8.2 ± 1.0 ^d	7.6 ± 0.5 ^d	8.4 ± 0.8 ^e	9.8 ± 0.7 ^e
	Floor within ca. 30 cm of drain	8.3 ± 1.3 ^c	9.0 ± 0.8 ^e	7.7 ± 0.2 ^c	8.4 ± 1.2 ^d	9.0 ± 1.6 ^e

^a Each drain was sampled in four locations and assayed for APCs every 2 weeks for 10 weeks before CE treatment. The values are the means ± standard deviations for six samplings.

^b Each drain was sampled in four locations and assayed for APCs every week for 4 weeks during CE treatment. The values are the means ± standard deviations for four samplings.

^c Each drain was sampled in four locations and assayed for APCs six times at 2- to 4-week intervals for up to 19 weeks after the last CE treatment was applied. The values are the means ± standard deviations for six samplings.

^d The APCs were significantly different ($P < 0.05$) from the APCs before CE treatment.

^e The APCs were not significantly different ($P > 0.05$) from the APCs before CE treatment.

that there was a 1.6- \log_{10} CFU/100 cm^2 reduction after CE treatment ($P < 0.05$) (Table 1). Following CE treatment either the *Listeria* sp. counts at most locations in the drain were substantially reduced or *Listeria* sp. was undetectable, and this response continued for at least 18 weeks after the last treatment (Fig. 4).

The listerial counts in control floor drain 2 that was not treated with CE were not significantly different throughout the study ($P > 0.05$) (Table 1). Most of the mean APCs for the drains before, during, and after CE treatment were not significantly different ($P > 0.05$) (Table 2); the few exceptions included drain 3 during CE treatment and drain 4 after the CE treatment regimen was completed.

A total of 184 *Listeria* sp. isolates from drains were tested further for identification of *L. monocytogenes*. The results revealed that 89 of the 184 isolates (48.4%) were *L. monocytogenes* isolates.

DISCUSSION

Our results revealed that the *Listeria* sp. counts in all five drains in rooms at different temperatures were substantially reduced following CE treatment. The efficacy of the treatment appeared to be influenced by room temperature, foot traffic, the composition of the drain material, and the amount of fluid flowing through the drain. The greatest reduction in *Listeria* sp. counts occurred in drain 6 ($>3.6\text{-}\log_{10}$ CFU/100 cm^2 reduction to an undetectable level at week 16), which was comprised of a fiberglass material, was located in a low-temperature room (3.8°C), usually contained less than 20% liquid, and was not in a high-traffic area. Substantial reductions in *Listeria* sp. counts occurred within 1 week after only four CE treatments were applied. In contrast, drain 8, which was in a warm room (mean temperature, 26.1°C) and a dirty environment with frequent inflow of blood and poultry debris and which was comprised of concrete, required ca. 3 weeks of CE treatments until there was a substantial reduction (3.7 \log_{10} CFU/100 cm^2) in the *Listeria* sp. counts.

We used an enzyme-foam-based cleaner to apply the CE to the drains. The advantage of the foam cleaner was that it provided more exposure time (minimum, 15 min) for CE to be in contact with the drain surface. Data obtained from inoculation studies revealed that CE strains survived well in the foam cleaner and that there was no change in CE counts during 24 h of exposure (data not shown).

Our results revealed that application of the two CE strains in the floor drains did not substantially change the total countable bacterial population during or after the treatment compared with the APCs of samples collected before CE treatment. However in some locations (especially drains 3 and 4) the APCs were less during and after treatment than before treatment. CE may have colonized the drains and not only reduced *Listeria* sp. populations but also subsequently formed their own biofilms and controlled bacterial populations that were sensitive to the antagonistic metabolites of the CE (1, 2, 6, 19).

The mechanism by which the CE reduce *Listeria* sp. counts has not been elucidated; however, we have previously reported that both CE isolates produce antimicrobials that are active against *L. monocytogenes* (22). *L. lactis* subsp. *lactis* C-1-92 possesses genes for nisin expression. Nisin is approved as a

food preservative in many countries and has antimicrobial activity against *L. monocytogenes*. Direct use of nisin in food products for control of *Listeria* may increase the likelihood of *Listeria* adaptation to nisin and subsequent tolerance to this bacteriocin. Although there is a possibility that listeriae in floor drains could develop tolerance to nisin following repeated exposure to *L. lactis* C-1-92, the likelihood is reduced by the inclusion of *E. durans* 152, which produces a different antimicrobial(s) than nisin (22).

The antimicrobial activity of lactic acid bacteria and their metabolites against *L. monocytogenes* has been well documented (3–5, 8, 10–13, 21). However, the application of these bacteria to floor drains in food processing facilities to control *L. monocytogenes* has not been reported previously. Our study revealed that application of two CE isolates can greatly reduce the numbers of *Listeria* sp. and *L. monocytogenes* cells in floor drains at 3 to 26°C in a facility in which fresh poultry is processed.

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