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Survival of *Listeria monocytogenes* in commercial food-processing equipment cleaning solutions and subsequent sensitivity to sanitizers and heat

P.J. Taormina and L.R. Beuchat

Center for Food Safety and Department of Food Science and Technology, University of Georgia, Griffin, GA, USA

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Aims: To determine the ability of *Listeria monocytogenes* to survive exposure to commercial food-processing equipment cleaning solutions and subsequent treatment with sanitizers or heat.

Methods and Results: Cells of five strains of *L. monocytogenes* were suspended in 1% solutions of eight commercial cleaners (pH 7·1–12·5) or in water (control) and incubated at 4°C for 30 min or 48 h before populations were determined by plating on tryptose phosphate agar. After exposure of cells to cleaning solutions for 30 min, populations of the most resistant strain of *L. monocytogenes* were reduced by $\leq 1{\cdot}63$ $\log_{10}$ cfu ml^{-1}. In only three highly alkaline cleaning solutions (pH 11·6–12·4) were populations reduced significantly ($P \leq 0{\cdot}05$) compared with reductions in water. After 48 h, populations were significantly higher in one cleaning solution (pH 10·4) than in water, while populations in six of the other seven cleaning solutions were reduced by $\geq 4{\cdot}72$ $\log_{10}$ cfu ml^{-1}. Cells exposed to cleaning solutions for 30 min became sensitive to 4·0 or 6·0 mg l^{-1} free chlorine and to 50 or 100 mg l^{-1} benzalkonium chloride and cetylpyridinium chloride, common components of quaternary ammonium sanitizers. Cells exposed to four of the five test cleaners had $D_{56°C}$ values less than or equal to those of the control cells.

Conclusions: *Listeria monocytogenes* tolerates exposure to a high concentration of alkaline cleaning solutions but consequently becomes sensitized to sanitizers.

Significance and Impact of the Study: The elimination of *L. monocytogenes* surviving exposure to alkaline cleaning solutions widely used for food-processing equipment is essential and the appropriate use of sanitizers for subsequent application to equipment is important in achieving this goal.

INTRODUCTION

Human listeriosis is characterized by encephalitis, meningitis or sepsis. Pregnant women infected by *Listeria monocytogenes* may exhibit flu-like symptoms, while unborn children may succumb to neonatal septicaemia or meningitis and become spontaneously aborted, typically during or after the third trimester (Rocourt and Cossart 1997). Among neonates and immunocompromised adults, mortality rates often exceed 30%, but the mortality rate among the general population is closer to 20% (Gellin and Broome 1989). Among affected non-pregnant adults, most have some unrelated underlying condition that predisposes them to consequences of infection (Rocourt and Cossart 1997). It is probable that the general public is frequently exposed to low levels of *L. monocytogenes* by consumption of various foods (WHO Working Group 1988; Farber *et al*. 1989; Pinner *et al*. 1992; Beuchat 1996). Mead *et al*. (1999) estimated that *L. monocytogenes* was responsible for 3·8% of the total cases of food-borne illness requiring hospitalization and 27·6% of deaths related to foodborne illness in the USA.

Despite research showing the lethality of sanitizers to *L. monocytogenes* (Mustapha and Liewen 1989; Best *et al*. 1990;

Correspondence to: Dr L.R. Beuchat, Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223–1797, USA (e-mail: lbeuchat@cfs.griffin.peachnet.edu).

Tuncan 1993), the pathogen is occasionally isolated from food-processing environments, even following cleaning and sanitizing of equipment surfaces (Franco *et al*. 1995; Ojeniyi *et al*. 1996; Samelis and Metaxopoulos 1999). The frequency of food product recalls due to contamination with *L. monocytogenes* (Wong *et al*. 2000) attests to the ability of the organism to persist in food-processing environments despite sanitation measures. Chemical cleaners used to remove food residues from these environments are not formulated to sanitize, but may sensitize *L. monocytogenes* to subsequent exposure to sanitizers.

Research has been performed to evaluate sanitizers for their efficacy in killing planktonic and biofilm-associated cells of *L. monocytogenes* (Mustapha and Liewen 1989; Best *et al*. 1990; Sallam and Donnelly 1992; Ren and Frank 1993; Roy *et al*. 1993; Tuncan 1993). However, less work has been performed to define the effects of alkaline cleaners and detergents on biofilm-associated *L. monocytogenes* (Krysinski *et al*. 1992). Studies describing the ability of cleaners and sanitizers to remove cells of *L. monocytogenes* on surfaces typically found in food-processing environments have been reported (Krysinski *et al*. 1992; Mosteller and Bishop 1993; Frank and Chmielewski 1997); however, studies describing the fate of cells dislodged from surfaces are lacking.

We undertook a study to determine the ability of *L. monocytogenes* to survive in 1% solutions of cleaners used in the food-processing industry as well as its sensitivity to sanitizers and heat after exposure to cleaning solutions. The study was performed to assess the ability of *L. monocytogenes* isolated from food-processing environments to survive at refrigeration temperature in cleaning solutions ranging from pH 7·1 to 12·6. Experiments were also performed to compare the efficacy of sanitizers in killing *L. monocytogenes* after exposure to cleaning solutions. An additional study examined the heat tolerance of cells that had been exposed to cleaning solutions. This research was designed to provide information to enable a greater understanding of the ability of *L. monocytogenes* to survive stresses associated with the cleaning and sanitizing of food-processing environments and the effect of exposure to cleaners on subsequent thermotolerance of the pathogen.

MATERIALS AND METHODS

Strains and culture conditions

Five isolates of *L. monocytogenes* were used. Four were serotype 4b with flagellar antigen ABC (strains CFS 1, 3, 4 and 5) and one was serotype 1/2a with flagellar antigen AB (strain CFS 2). All were isolates from a food-processing environment. Stock cultures were prepared from subcultures grown in tryptose phosphate broth (TPB; pH 7·3; Difco Laboratories, Detroit, MI, USA). Cultures were incubated at 37°C for 24 h, supplemented (15%, v/v) with glycerol and stored in cryogenic vials (Nalgene, Rochester, NY, USA) at –20°C until use.

Prior to each experiment, a stock culture was thawed and loop inocula transferred to Erlenmeyer flasks (250 ml) containing 100 ml sterile TPB. Flasks were incubated for 18 h in an incubator shaker (New Brunswick Scientific, New Brunswick, NJ, USA) set at 37°C and 200 rev min^{-1}. Cultures (10 ml) were dispensed into 15-ml conical polystyrene centrifuge tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and centrifuged (5000 *g*, 4°C) for 10 min (brake 3·5 min) in a precooled benchtop refrigerated centrifuge (Marathon 12KBR; Fisher Scientific, Pittsburgh, PA, USA). Pellets washed twice in sterile distilled water (water) were used as inocula for test cleaning solutions.

Exposure to cleaner solutions

Eight commercially available food-processing equipment cleaners were selected for evaluation based on their range of pH, intended usage in food-processing environments, differences in chemical composition and variability of recommended application procedures (Table 1). Cleaners 1–4 were supplied by manufacturer A as 1% solutions and cleaners 5–8 were supplied by manufacturer B as concentrated liquids that were diluted with sterile deionized water to 1% (v/v) in our laboratory. Each product has a range of manufacturer-recommended use concentrations. The recommended use concentrations of cleaners 4 and 7 vary, depending on the manner of application. Some of the cleaning products were evaluated at concentrations higher or lower than those recommended by the manufacturer for the purpose of simulating misuse in a food-processing environment.

Washed pellets were suspended in 10 ml 1% solutions of cleaners or water (control) at 4°C to give a population of *ca* 9·4 $\log_{10}$ cfu ml^{-1} and suspensions stored in tubes at 4°C. After 30 min or 48 h, the contents of each test tube were thoroughly mixed using a vortex mixer and undiluted suspensions (0·25 ml in quadruplicate or 0·1 ml in duplicate) or suspensions serially diluted in sterile 0·1% peptone water (0·1 ml in duplicate) surface plated on tryptose phosphate agar (TPA; pH 7·3; Difco) and on TPA supplemented with 4% NaCl (TPAS) in order to assess total and uninjured populations of *L. monocytogenes*, respectively. All plates were incubated at 37°C for 48 h prior to counting colony-forming units (cfu) using an automated colony counter (Countermat; Cogent Technologies, Cincinnati, OH, USA) and a manufacturer-recommended calibration of the standard plate count file. Suspensions of the five isolates (strains CFS 1–CFS 5) were combined to form an inoculum for further experiments.

Table 1 Properties of alkaline cleaners

Manufacturer	Cleaner no.	Product description	Properties*	Components†	Concentrations‡	
					Recommended (%)	At-use ratio
A	1	General purpose cleaner and foam additive	pH 7·8 at 100% pH 7·6 (7·1)* at 1%	5–20% anionic surfactants	0·13% in warm water	7·69
	2	Heavy-duty liquid alkaline detergent for CIP, bottlewashing and boil out applications	pH 12·5 (12·4)* at 1% pH 11·7 at 0·1%	NaOH (caustic soda) 49%	Variable with application 0·25–1·5%	4·00–0·667
	3	Alkaline detergent for manual cleaning of bulk raw and pasteurized tanks, transfer lines, pumps and associated equipment	pH 9·5 (8·4)* at 1%	Sodium tripolyphosphate 55%, sodium dodecylbenzene-sulphonate 10%	0·19–0·75% in 21–88°C water	5·26–1·33
	4	Low alkaline foaming cleaner with solvents for manual or foam cleaning of floors and walls or maintenance areas	pH 10·8 at 2% pH 10·4 at 1%*	Non-ionic surfactants 8%, sodium linear alkyl benezene sulphonate 3%, propylene glycol monomethyl ether 1–5%, dipropylene glycol methyl ether 1–5%, diethylene glycol methyl ether 3%, unspecified sequestering agent 1–5%	Foam cleaning: 2·34–4·68% Manual cleaning: 0·39–1·56% Spray cleaning: 0·78–2·34%	Foam cleaning: 0·427–0·214 Manual cleaning: 2·56–0·641 Spray cleaning: 1·28–0·427
B	5	Chlorinated, foaming, highly alkaline cleaner, many surfaces in food-processing environments	pH 13·4 concentrated pH 12·2 at 1%*	KOH, NaOH, NaOCl	2·34–3·14%	0·427–0·318
	6	Non-butyl alkaline cleaner for many surfaces in food-processing and preparation environments	pH 13·2 concentrated pH 11·6 at 1%*	KOH, sodium metasilicate	1·57–3·14%	0·637–0·318
	7	Non-butyl heavy-duty alkaline cleaner and degreaser	pH 13·2 concentrated pH 11·6 at 1%*	KOH, sodium metasilicate, NaOCl	General cleaning: 0·78–3·14% Foam cleaning: 1·57–3·14% Pressure cleaning: 1·57–3·14%	General cleaning: 1·28–0·318 Foam cleaning: 6·37–0·318 Pressure cleaning: 6·37–0·318
	8	Heavy-duty foaming alkaline cleaner	pH 13·4 concentrated pH 12·6 at 1%*	NaOH	5%–10%	0·2–0·1

*pH of 1% solutions determined in laboratory.

† Components listed are only those which are proprietarily insensitive or are disclosed as hazardous components on Material Safety Data Sheets.

‡ For consistency, concentrations are listed as percentages based on recommendations by manufacturers, which may refer to dilution of concentrated liquid or granulated powder in water. At-use ratio concentrations indicate test concentration (1%) divided by recommended concentrations.

CIP, Clean-in-place.

Treatment with sanitizers

The selection of sanitizers for evaluation in this experiment was based upon their frequency of use in food-processing environments and lack of published information on their effects on the viability of *L. monocytogenes* after exposure to cleaning solutions. Sodium hypochlorite (NaOCl) solution (minimum 4% available chlorine; Aldrich Chemical, Milwaukee, WI, USA) was used to prepare solutions with 4·0 and 6·0 mg l^{-1} available chlorine by diluting with 0·05 mol l^{-1} potassium phosphate buffer at pH 7·00 ± 0·05 (PB). Concentrations were verified using a Digital Titrator (model 16900; Hach, Loveland, CO, USA) fitted with a 0·0451 N phenylarsine oxide titration cartridge, an Amperometric Digital Titrator (model 19300) and a TitraStir™ stir plate following a forward titration procedure for determining concentrations of free chlorine ranging from 0 to 10 mg l^{-1}.

Benzalkonium chloride (Sigma Chemical, St. Louis, MO, USA) and cetylpyridinium chloride (Sigma) were selected as chemicals to treat cells after exposure to cleaning solutions because they are major components of some quaternary ammonium sanitizers commonly applied to surfaces in food-processing environments after cleaning. Stock solutions were prepared by dissolving 100 mg in 10 ml water. Solutions containing 50 (pH 6·6) and 100 (pH 6·7) mg l^{-1} benzalkonium chloride and 50 (pH 6·6) and 100 (pH 6·4) mg l^{-1} cetylpyridinium chloride were used to treat *L. monocytogenes* cells. All chemical treatment solutions and PB used to prepare chlorine solutions were prepared using chlorine demand-free glassware and from filtered (0·45 μm), laboratory-grade, sterile water with a total hardness < 10 mg l^{-1} $CaCO_3$. Solutions were protected from light, held at 21 ± 2°C and used within 1 h of preparation.

Eighteen-hour TPB cultures (2 ml) of each of the five *L. monocytogenes* isolates were combined and centrifuged at 5000 *g* and 4°C for 10 min with a 3·5-min brake time. Each cell pellet was resuspended in 10 ml water (control) or cleaner solution and incubated for 30 min at 4°C. After incubation, cells were centrifuged, washed and resuspended in water prior to treatment with sanitizers. A volume (1 ml) of each washed cell suspension was dispensed into 99 ml of each sanitizer treatment solution at 25°C and thoroughly mixed by vortexing. After 30 s, 1 ml treated cell suspension was dispensed into 9 ml sterile Dey-Engley neutralizing broth (Difco) and mixed for 10 s. Undiluted, neutralized treatment suspensions (0·25 ml in quadruplicate or 0·1 ml in duplicate) or suspensions serially diluted in sterile 0·1% peptone water (0·1 ml in duplicate) were surface plated on TPA. The TPA plates were incubated at 37°C for 48 h before counting colonies as described above. Log populations of cells recovered on TPA after exposure to water (control) or cleaning solutions for 30 min and treatment with either PB or water (sanitizer treatment controls) for 30 s were subtracted from log populations recovered following exposure to water (control) or cleaning solutions and treatment with chlorine, benzalkonium chloride or cetylpyridinium chloride to generate population reduction values.

Treatment with heat

Suspensions (50 μl) of cells previously exposed to cleaning solutions were injected into capillary tubes (Kimax-51; 0·8–1·0 mm I.D. × 90 mm long; no. 34507–99; Kimble, Vineland, NJ, USA) and the ends flame sealed. Capillary tubes were brought to 21 ± 2°C before subjecting to heat treatment in a water-bath at 56°C for 0, 1, 2, 5, 10, 20 or 25 min. The come-up time for a tempered fluid-filled capillary tube in the water-bath, as measured with a Microprocessor Thermometer (model HH23; Omega, Stamford, CT, USA) connected with a type J thermocouple, was 2 s. Capillary tubes were immediately cooled and sanitized by immersing, in succession, in an ice bath, 70% ethanol and sterile water before aseptically transferring to screw-capped test tubes (16 mm I.D. × 125 mm long) containing 5 ml sterile 0·1% peptone water. The capillary tube containing the heated cell suspension was crushed using a sterile glass rod and thoroughly mixed with the peptone water. Undiluted suspensions (0·25 ml in quadruplicate or 0·1 ml in duplicate) or suspensions serially diluted in sterile 0·1% peptone water (0·1 ml in duplicate) were surface plated on TPA. The TPA plates were incubated at 37°C for 48 h before colonies were counted as described above.

Statistical analysis

Three replicates of each experiment were performed. Population means were calculated and analysed using the general linear model procedure of SAS (Cary, NC, USA) and separated by significance ($P \leq 0{\cdot}05$) using the Duncan's multiple range test. Populations of *L. monocytogenes* surviving heat treatment were analysed using appropriate forms of the logistic equation (Pruitt and Kamau 1993) applied by the non-linear regression procedure of SAS. The log-transformed equations used to analyse data were as follows:

$$\log S = \log 2 - \log[1 + \mathrm{e}(\beta t)] \quad (1)$$

where $\log S$ is the log (cfu/cfu_0) at any given time (t) and β is the maximum specific death rate.

For survival curves with no initial lag in killing but having two distinct killing phases (biphasic), data were fitted to the following two-term exponential form of eqn (1):

$$\log S = \log(\{2f_1/[1 + \mathrm{e}(\beta_1 t)]\} + \{2(1 - f_1)/[1 + \mathrm{e}(\beta_2 t)]\}) \quad (2)$$

where f_1 and $(1 - f_1)$ represent two fractions of cells (differing with respect to heat resistance) and β_1 and β_2 are the specific killing rates for the two fractions, respectively. The assumption of this model is that two fractions (subpopulations) are killed exponentially but at different, independent rates.

Curves which included a lag in killing (shoulder) and biphasic inactivation were fitted to the following two-term exponential form of eqn (1):

$$\log S = \log(f_1\{1 + e[-\beta_1 t_1]\}/\{1 + e[\beta_1(t - t_1)]\}) + \log([1 - f_1]\{1 + e[-\beta_2 t_1]\}/\{1 + e[\beta_2(t - t_1)]\}) \quad (3)$$

where t_1 is the lag period.

For eqn (1), logistic D-values (Pruitt and Kamau 1993) were calculated as $D = 2{\cdot}94/\beta$ and, for eqns (2) and (3), $D = \ln(19)/\beta_2$.

RESULTS

Populations of *Listeria monocytogenes* surviving exposure to cleaner solutions

The survival of *L. monocytogenes* isolates (CFS 1–CFS 5) suspended in solutions of 1% cleaners or water (control) for 30 min at 4°C was compared (Fig. 1). Cleaners 2 and 5 (pH 12·4 and 12·2 at 1%, respectively) were consistently more lethal than the other cleaners to isolates CFS 1–CFS 4, although isolate CFS 1 survived better than other isolates after exposure to cleaner 5. With the exceptions of cleaners 2 and 5, the exposure of isolate CFS 2 (the only 1/2a serotype) to cleaners reduced populations of *L. monocytogenes* by $\leq 1{\cdot}63$ $\log_{10}$ cfu ml^{-1}; in only three cleaning solutions (2, 5 and 7 at pH 11·6–12·4) were populations significantly ($P \leq 0{\cdot}05$) less than those recovered from the control (water). Populations of isolates CFS 1–CFS 5 surviving exposure to cleaning solutions 4 and 6 (pH 10·4 and 11·6, respectively) were not significantly less than those surviving exposure to water. Significantly lower numbers of *L. monocytogenes* were recovered on TPAS compared with TPA following exposure to cleaners 2, 5 and 7, indicating some degree of cell injury.

After 48 h at 4°C, populations of isolates CFS 2–CFS 5 were significantly higher in a 1% solution of cleaner 4 (pH 10·4) than in water, while populations in 1% solutions of all other cleaners except cleaner 6 (pH 11·6) were reduced by at least 4·72 $\log_{10}$ cfu ml^{-1} (Fig. 2). A large percentage of cells of these isolates was injured following exposure to cleaner 6. The survival of isolate CFS 1 was slightly different from that of isolates CFS 2–CFS 5; populations of CFS 1 were significantly reduced by exposure to cleaner 4 and injury was not evident after exposure to cleaner 6. However, the response of isolate CFS 1 to cleaners 2, 5, 7 and 8 was similar to that of the other isolates. The magnitude of the reduction in the population of *L. monocytogenes* was, overall, not correlated with at-use ratio concentrations relative to the concentrations recommended by the manufacturers (Table 1). Since 1% solutions of cleaners 2, 5 and 7 caused large reductions in the populations of individual isolates of *L. monocytogenes* exposed at 4°C for 30 min, relative to other solutions of other cleaners, subsequent sensitivity to sanitizers or thermotolerance of those cells was not determined.

Effectiveness of sanitizers on cells previously exposed to cleaning solutions

Treatment of cells previously exposed to 1% solutions of cleaners 1, 3, 4, 6 and 8 with 4·0 or 6·0 mg l^{-1} free chlorine was lethal to all cells (Table 2). The numbers of cells previously exposed to water before treating with 4·0 or 6·0 mg l^{-1} chlorine were significantly reduced by 6·95 and 6·66 $\log_{10}$ cfu ml^{-1}, respectively. Treatment with buffer resulted in a 1·07 $\log_{10}$ cfu ml^{-1} reduction in the population of cells previously exposed to a 1% solution of cleaner 8. Cells previously exposed to cleaner solutions were also highly sensitive to treatment with 50 and 100 mg l^{-1} benzalkonium chloride and cetylpyridinium chloride; treatment with these compounds caused significantly greater reductions than treatment with water. Populations of cells not previously exposed to cleaning solutions (control cells) were reduced by treatment with 50 and 100 mg l^{-1} benzalkonium chloride by 3·48 and 4·80 $\log_{10}$ cfu ml^{-1}, respectively, while treatment with 50 and 100 mg l^{-1} cetylpyridinium chloride reduced populations by 5·64 and 7·23 $\log_{10}$ cfu ml^{-1}, respectively. Performing this experiment using water of standard hardness (200 mg l^{-1} $CaCO_3$) to prepare sanitizer solutions gave similar results.

Thermotolerance of cells previously exposed to cleaning solutions

Logistic $D_{56°C}$ values indicate that previous exposure of cells of *L. monocytogenes* to cleaning solutions altered tolerance to heat treatment at 56°C (Table 3). $D_{56°C}$ values of cells previously exposed to cleaners 3 and 4 were 6·67 and 6·97 min, respectively, and were significantly lower than the $D_{56°C}$ value of control cells or cells previously exposed to cleaners 6 or 8. $D_{56°C}$ values of cells exposed to cleaners 1 and 6 were not significantly different from the $D_{56°C}$ value of control cells. Although the $D_{56°C}$ value of cells exposed to cleaner 8 was significantly higher than that of the control, r^2 indicates a less than optimal fit of the equation to the survival curve.

DISCUSSION

Typical cleaning processes in food-processing environments involve a series of steps, each with a specific purpose

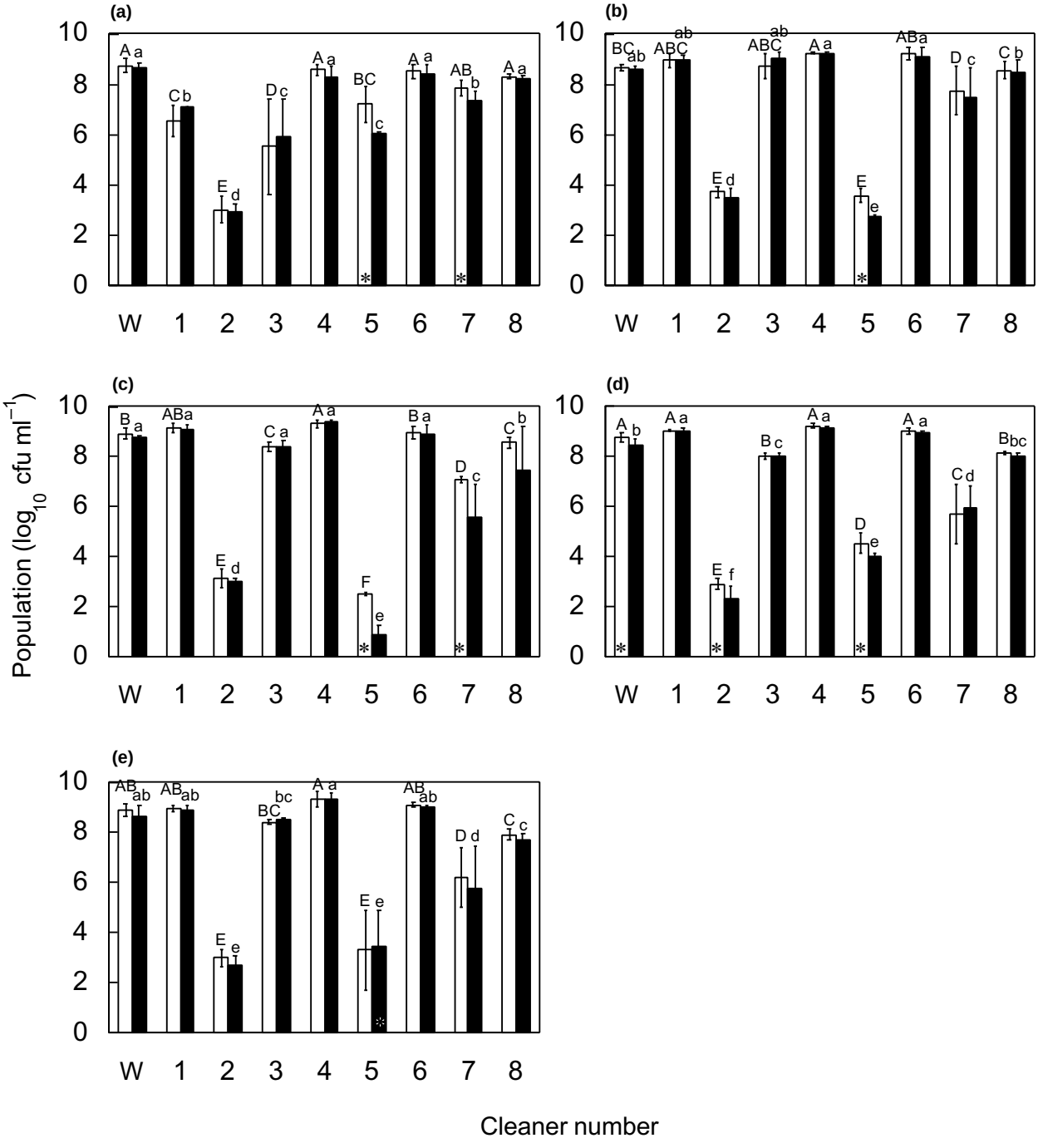

Fig. 1 Populations of isolates ((a) CFS 1; (b) CFS 2; (c) CFS 3; (d) CFS 4 and (e) CFS 5) of *Listeria monocytogenes* from a food-processing environment surviving incubation in 1% solutions of eight food-processing equipment cleaners (described in Table 1) and water (W; control) at 4°C for 30 min as determined by plating inoculated solutions on tryptose phosphate agar (TPA; □) and TPA supplemented with 4% NaCl (■). Bars denote S.D. Within strain and medium, bars not noted with the same letter are significantly ($P \leq 0{\cdot}05$) different. Within strain and cleaner, bars noted by an asterisk are significantly greater

(Tamplin 1980). A pre-rinse is performed using water to remove gross, loose soil, followed by a cleaning step using suitable detergents (often alkaline) for removal of residual soils. An inter-rinse then removes all detergent and soil and sanitizing is performed by application of antimicrobial chemicals, either with or without heat. Finally, a post-rinse,

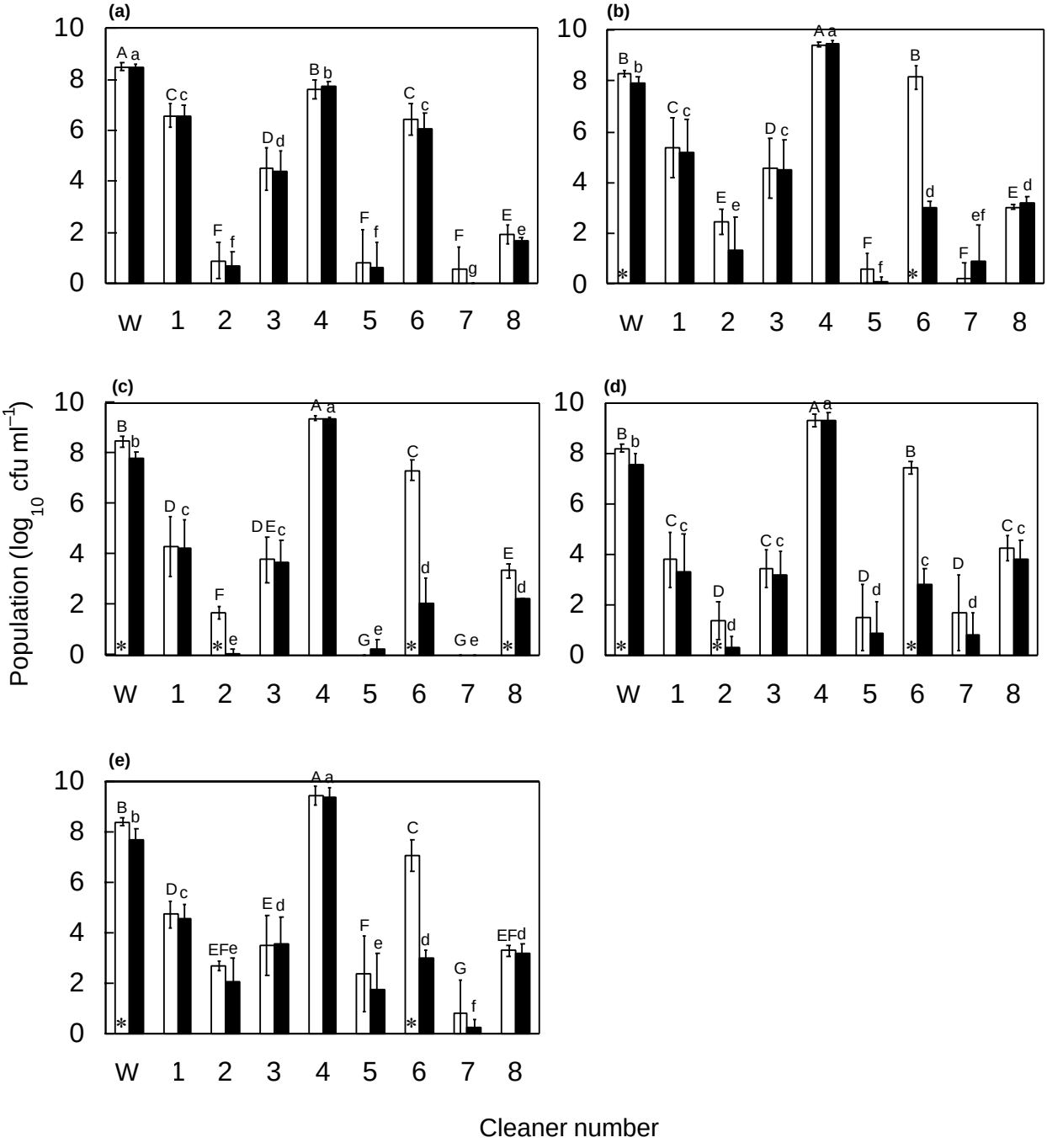

Fig. 2 Populations of isolates ((a) CFS 1; (b) CFS 2; (c) CFS 3; (d) CFS 4 and (e) CFS 5) of *Listeria monocytogenes* from a food-processing environment surviving incubation in 1% solutions of eight food-processing equipment cleaners (described in Table 1) and water (W; control) at 4°C for 48 h as determined by plating inoculated solutions on tryptose phosphate agar (TPA; □) and TPA supplemented with 4% NaCl (■). Bars denote S.D. Within strain and medium, bars not noted with the same letters are significantly ($P \leq 0{\cdot}05$) different. Within strain and cleaner, bars noted by an asterisk are significantly greater

using potable water, removes the antimicrobial chemicals. The effectiveness of sanitizers may be influenced by numerous factors, including the presence of residuals from cleaners not properly removed before application of antimicrobials.

The ability of *L. monocytogenes* to survive exposure to alkaline pH cleaner solutions for 30 min at 4°C and, to a lesser extent, for 48 h was demonstrated. Other studies have also revealed that *L. monocytogenes* can survive rather well in

Table 2 Effectiveness of sanitizer treatments on *Listeria monocytogenes* cells surviving exposure to 1% solutions of various commercial cleaning solutions*

Treatment†		Population reduction ($\log_{10}$ cfu ml^{-1})‡					
			Cleaner no.				
Solution	Conc. (mg l^{-1})	Water (control)	1	3	4	6	8
Phosphate buffer (control)		e 0·00 e	b 0·33 c	b 0·82 b	b 0·10 de	c 0·24 cd	b 1·07 a
Chlorine	4·0	a 6·95 b	a 7·28 a	a 7·28 a	a 7·28 a	a 7·28 a	a 7·28 a
	6·0	a 6·66 b	a 7·28 a	a 7·28 a	a 7·28 a	a 7·28 a	a 7·28 a
Water (control)		e 0·00 d	b 0·53 c	b 0·96 b	b 0·33 c	b 0·45 c	b 1·26 a
Benzalkonium chloride	50	d 3·48 b	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a
	100	c 4·80 b	a 7·23 a	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a
Cetylpyridinium chloride	50	b 5·64 b	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a
	100	a 7·23 b	a 7·23 b	a 7·56 a	a 7·56 a	a 7·56 a	a 7·56 a

*Cells were suspended in water (control) or 1% solutions of cleaners (described in Table 1) and incubated for 30 min at 4°C prior to exposure to treatments.

† Treatment consisted of dispensing 1 ml of a cell suspension previously exposed to cleaning solutions into 99 ml treatment solution at 25°C, incubating for 30 s, transferring 1 ml to 9 ml neutralizing broth and then surface plating on tryptose phosphate agar.

‡ Values in the same column not preceded by the same letter are significantly ($P \leq 0{\cdot}05$) different. Values in the same row not followed by the same letter are significantly different.

Table 3 Heat (56°C) survival parameter estimates for *Listeria monocytogenes* cells previously incubated at 4°C for 30 min in 1% cleaner solutions

Cleaner*	Eqn†	(1 – f) least heat-sensitive fraction	β_2 – least heat-sensitive fraction (min^{-1})	β_1 – most heat-sensitive fraction (min^{-1})	β (min^{-1})	Pseudo r^2‡	Logistic $D_{56°C}$ value (min)§
Water (control)	1	–	–	–	0·278 ± 0·0145	0·970	10·58 b
1	2	0·13	0·322 ± 0·0115	4·24 ± 2·13	–	0·990	9·13 bc
3	2	0·13	0·441 ± 0·0147	2·74 ± 1·042	–	0·992	6·67 c
4	3	0·40	0·422 ± 0·0461	– 1·19 ± 0·375	–	0·956	6·97 c
6	1	–	–	–	0·282 ± 0·0120	0·978	10·43 b
8	2	0·50	0·191 ± 0·130	0·0939 ± 0·0235	–	0·884	15·40 a

*See Table 1 for description of cleaners.

† Parameter estimates were obtained by fitting survival data to the appropriate logistic equation by non-linear regression. See text for equations.

‡ Calculated from 1 – (residual sum of squares/corrected total).

§ Calculated per replicate from $D = 2{\cdot}94/\beta$ for eqn (1) or $D = (\ln 19)/\beta_2$ for eqn (2). Means not followed by the same letter are significantly ($P \leq 0{\cdot}05$) different.

high pH environments (Rossmoore and Drenzek 1990; Laird *et al.* 1991; Cheroutre-Vialette *et al.* 1998; Taormina and Beuchat 2001). These survival capabilities may help to explain why the pathogen is difficult to eradicate from food-processing environments treated with alkaline pH cleaning products. Although commercial food-processing equipment cleaners are not intended to be lethal to *L. monocytogenes* cells, they may, inadvertently through detergency, relocate cells from equipment to other areas within the processing environment that do not receive sanitization. These cells may survive exposure to high pH cleaners and be transferred to sanitized surfaces where they may contaminate food.

The alkaline cleaning solutions used in this study exhibited various degrees of lethality against *L. monocytogenes* within 30 min of treatment. This observation is useful when seeking to add yet another hurdle to control *L. monocytogenes* in food-processing environments. Cleaners 4 and 6 permitted survival of significantly higher populations of individual isolates of *L. monocytogenes* after 48 h compared with the other six cleaners. This indicates that any potentially lethal component that may be present in solutions prepared from these cleaners was at an ineffective concentration in solutions of cleaners 4 and 6. All cleaners were evaluated at a concentration of 1% because some of the products were provided to our laboratory in liquid form at that concentration. While some of the cleaners are recommended for use at higher or lower concentrations, a uniform at-use concentration of 1% was selected regardless of the concentration of the active component (Table 1). Cleaners 1 and 3 are recommended for use by the manufacturer at

concentrations lower than 1%, but exposure of *L. monocytogenes* to these cleaners at 1% in practice could result from evaporation of water after application, egregious mixing or dispensing errors. Cleaners 5, 6 and 8 are recommended for use by the manufacturer at concentrations higher than 1%, and are more likely to occur in food-processing environments at 1% as a result of dilution in the sanitizing process. The at-use concentration (1%) of cleaners 2, 4 and 7 was within the range of the manufacturer-recommended concentrations for use for at least one intended application and was otherwise lower than the recommended concentration for other applications. The effectiveness of treatment with 1% cleaning solutions must be assessed relative to concentrations recommended by manufacturers. If all of the cleaners had been tested at recommended concentrations, the results may have been different. Nevertheless, no overall relationship was evident between higher or lower at-use ratio concentrations and reduction in populations of *L. monocytogenes* exposed to the test cleaners. Cleaning solutions which were not lethal or had minimal lethality to most of the isolates of *L. monocytogenes* within 30 min of exposure were used to further assess the behaviour of cleaner-exposed cells upon treatment with sanitizers or challenge with heat.

Cells exposed to cleaning solutions that had minimal effects on viability were sensitive to subsequent treatment with sanitizers. Similar observations on sanitizer sensitivity of chemically shocked *L. monocytogenes* cells were reported by Pickett and Murano (1996). Since treatment with 4·0 or 6·0 mg l^{-1} chlorine and 50 or 100 mg l^{-1} cetylpyridinium chloride reduced populations of control cells by $\geq$ 6·66 and $\geq$ 5·64 $\log_{10}$ cfu ml^{-1}, respectively, the independent influence of prior exposure of cells to cleaning solutions on viability when subsequently treated with those particular sanitizers has not been determined. Although cells exposed to cleaning solutions were at least as sensitive as control cells to chlorine and cetylpyridinium chloride, the degree to which they were sensitized is masked by the high lethality of these sanitizers. Populations of control cells were so greatly reduced by treatment with these sanitizers that a comparison of sensitivity of control and cleaner-exposed cells upon subsequent exposure to the sanitizers is confounded. The increased sensitivity of *L. monocytogenes* to benzalkonium chloride after exposure to cleaning solutions is clearer since the reduction in the population of control cells (treated with water) was less pronounced than that observed in cells treated with chlorine or the same concentrations of cetylpyridinium chloride.

Previous studies in our laboratory revealed that *L. monocytogenes* can survive for 6 d at 4°C in TPB adjusted to pH 9·0, 10·0 or 11·0 with NaOH, while cells stored for 2 or 6 d at 4°C in TPB at pH 10 or shocked for 45 min at 37°C in TPB at pH 12·0 had increased tolerance to mild heating at 56°C and 56 or 59°C, respectively (Taormina and Beuchat, 2001). In the present study, heating of *L. monocytogenes* cells previously exposed for 30 min to commercial cleaning solutions (pH 7·1–12·6) resulted in significant differences among logistic $D_{56°C}$ values. $D_{56°C}$ values of cells exposed to cleaning solutions 3 and 4 were significantly lower than the $D_{56°C}$ value of control cells, while cells previously exposed to a 1% solution of cleaner 8 had a significantly higher $D_{56°C}$ value. Interestingly, cleaner 8 is the only test cleaner containing only NaOH listed as a hazardous component.

Future research to determine the survival characteristics of *L. monocytogenes* in manufacturer-recommended concentrations of cleaners at a range of temperatures and upon subsequent exposure to sanitizers in the presence and absence of organic matter at refrigeration temperatures would be of interest. Also, studies on *L. monocytogenes* in biofilms, preferably mixed-species biofilms, would more accurately reflect actual conditions in food-processing environments. The observations reported here provide important information on the potential effects of sanitation regimens on *L. monocytogenes* cells isolated from a food-processing environment and underscore the need for strict adherence to cleaning and standard sanitation operating procedures to ensure that foods do not become contaminated in post-processing environments. Further investigations to characterize the effects of exposure of *L. monocytogenes* to alkaline cleaners and other alkaline environments are warranted. Of particular interest are the survival and growth characteristics of alkaline-stressed *L. monocytogenes* in ready-to-eat foods during refrigerated storage.

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