APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1990, p. 377–380
0099-2240/90/020377-04$02.00/0

Vol. 56, No. 2

Efficacy of a Variety of Disinfectants against *Listeria* spp.

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Received 24 August 1989/Accepted 13 November 1989

The efficacy of 14 disinfectants against *Listeria innocua* and two strains of *Listeria monocytogenes* in the presence of organic matter was studied. Quantitative efficacy tests were used. Many of the disinfectants tested were not as effective on *Listeria* spp. when the test organisms were dried onto the surface of steel disks (carrier tests) as they were when the organisms were placed in suspension (suspension test). The presence of whole serum and milk (2% fat) further reduced the disinfectant capacities of most of the formulations studied. Only three disinfectants (povidone-iodine, chlorhexidine gluconate, and glutaraldehyde) were effective in the carrier test in the presence of serum; however, all three were ineffective when challenged with milk (2% fat). Only one solution, sodium dichloroisocyanurate, was effective in the presence of milk. All but four formulations (chloramine-T, phosphoric acid, an iodophor, and formaldehyde) were effective in the suspension tests, regardless of the organic load. *L. monocytogenes* was observed to be slightly more resistant to disinfection than *L. innocua* was. There was no difference in disinfectant susceptibility between the two strains of *L. monocytogenes*. These findings emphasize the need for caution in selecting an appropriate disinfectant for use on contaminated surfaces, particularly in the presence of organic material.

Listeriosis, caused by the environment contaminant *Listeria monocytogenes*, is an emerging public health problem. The disease frequently occurs in immunocompromised or elderly individuals, pregnant women, and neonates; the consequences of this disease to these risk groups are serious and often fatal. At least four major outbreaks of listeriosis have been associated with food within the last 7 years (7). Its incidence appears to be increasing worldwide, and the evidence of food-borne transmission in humans is now quite significant (7, 17). The increased attention to listeriosis has resulted in a rapid growth of basic research and clinical studies on *L. monocytogenes* and other *Listeria* spp.

While these organisms are now becoming common in many laboratories, there is a paucity of information concerning the efficacy of disinfectants on listeriae. Such information is of value for the selection of appropriate disinfectants, since *L. monocytogenes* survives well on surfaces and it has been suggested that listeriosis is transmitted via contaminated objects (3, 5, 11, 14). The organism was isolated from the surface of a gown of a professional exposed at work (6). Precautions, including appropriate disinfection, are necessary to avoid possible cross-infections in hospitals, especially in neonatal units, where this organism is regarded as a nosocomial pathogen (3, 5, 14).

Studies to evaluate dairy and food plant sanitizers against *L. monocytogenes* have been undertaken (10, 12). Researchers in both investigations concluded that many commonly used sanitizers are effective at the recommended concentrations, and several guidelines for controlling *Listeria* spp. in dairy plants have been published (4, 15, 16). However, the need to examine the efficacy of disinfectants used in a clinical or research setting remains.

This study was initiated to determine the efficacy of a variety of disinfectants on surfaces contaminated with *Listeria innocua* and *L. monocytogenes* by using a quantitative test that simulates actual practices for general equipment and surface disinfection (carrier test). Previous studies with mycobacteria performed by this method concluded that disinfectants which showed low activity on contaminated surfaces did not necessarily do so in suspension (1). For this reason, disinfectants that were not effective in this test were also tested on *L. innocua* and *L. monocytogenes* in a suspension (suspension test). Tests were carried out in the presence of serum and milk to determine any effect that such organic loads may have on disinfectant efficacy.

MATERIALS AND METHODS

L. innocua LCDC 86-417, *L. monocytogenes* LCDC 88-702, and *L. monocytogenes* LCDC 81-682 were obtained from the National Laboratory for Bacteriology, Laboratory Centre for Disease Control, Health and Welfare Canada. Stock cultures were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) at 4°C. Three test suspensions were prepared: one with tryptic soy broth (TSB; Difco), one with whole pooled human serum, and one with pasteurized milk (2% fat). Aerobic plate counts of the serum and milk revealed no initial microbial load in these samples. The organisms were inoculated onto tryptic soy agar and incubated at 37°C. The organisms were harvested after 24 h of growth, and the cells were suspended in TSB, milk, or serum to obtain 10^9 CFU/ml. These test suspensions were used as the initial inocula for all tests.

Fourteen disinfectants (Table 1) were selected to represent commonly used disinfectants. All disinfectants were diluted according to the instructions of the manufacturers, with tap water as the diluent. In all tests, the method used to terminate disinfectant action was dilution of the reaction mixture immediately at the end of the contact time. All disinfectant reactions were carried out in the wells of a 24-well plastic cell culture plate (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.) as previously described (1).

All disinfectants were tested against *L. innocua* and *L. monocytogenes* LCDC 88-702 on contaminated surfaces in the presence of TSB and serum (carrier test). Disinfectants that were not effective in this test were subsequently tested in suspension (suspension test). The effect of milk on the activity of a selected number of disinfectants (Table 1) was

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TABLE 1. Concentrations of disinfectants used

Disinfectant (original concn or form)	Concn used
Ethanol (95%)	70% (vol/vol)[a]
Sodium hypochlorite (600 μg of Av Cl[b] per ml)	60 μg/ml (Av Cl)[a]
	10 μg/ml (Av Cl)
Sodium dichloroisocyanurate (tablets)	60 μg/ml (Av Cl)[a]
	10 μg/ml (Av Cl)
Chloramine-T (67%)	0.4% (wt/vol)
Sodium hypochlorite (0.5% with 5% methylethanol)	0.5%
Phosphoric acid (18%)	0.45% (vol/vol)
Povidone-iodine (1.0% titratable I_2)	1.0% I_2[a]
Iodophor (1.0% titratable I_2)	0.008% I_2
Chlorhexidine gluconate (4%)	4%[a]
Glutaraldehyde (2%)	2%[a]
Glutaraldehyde-phenate (2%)	0.125%
Formaldehyde (37% in 15% methanol)	3.7%
Quaternary ammonium compound (10% dimethyl benzylammonium chloride)	0.04%
Quaternary ammonium compound (3.88% dimethyl benzylammonium chloride)	0.05%[a]

[a] Disinfectants tested in the presence of pasteurized milk (2% fat) and tested against *L. monocytogenes* LCDC 81-682.
[b] Av Cl, Available chloride.

also studied with *L. monocytogenes* LCDC 88-702. Selected disinfectants (Table 1) were also tested against *L. monocytogenes* LCDC 81-682 in the presence of TSB (carrier test) to observe potential variation in disinfectant susceptibility between the two strains.

For the carrier test, stainless steel sheets (0.75 mm thick) were obtained locally, and 1-cm-diameter disks were cut from them. The disks were placed in the wells of the cell culture plate as needed. In the test, 20 μl of each test suspension was placed on the carrier surface and allowed to air dry for 2 h in a class II biological safety cabinet. The contaminated area (not all of the disk surface was contaminated) was then covered with 20 μl of disinfectant. Controls for each test suspension were covered with 20 μl of normal saline instead of disinfectant. After 1 min of contact, 980 μl of diluent (normal saline) was added to each well to dilute the disinfectant and elute the bacteria from the steel carrier disk. The sample was immediately subjected to further 10-fold dilutions (10^{-3} to 10^{-7}) to bring the organisms to a countable range. Samples (1 ml) from the dilutions were spread on tryptic soy agar in duplicate and incubated at 37°C for 24 h. The plates were incubated for 48 h if no growth was observed after 24 h.

In the suspension test, 0.1 ml of each test suspension was added to 0.9 ml of disinfectant. Controls for each suspension contained 0.9 ml of the diluent instead of the disinfectant. After 1 min of contact, 0.1 ml of the reaction mixture was removed and immediately diluted 100-fold in diluent. Subsequently, the eluates were serially diluted and plated as in the carrier test.

Tests were carried out at least in triplicate, with two batches for each disinfectant (six replicates). Disinfectant activity was determined by comparing growth on the control and disinfectant plates and was measured in log reductions in CFU per milliliter. Each disinfectant was tested for its capacity to cause up to a 6-$\log_{10}$ (99.9999%) reduction in CFU.

RESULTS

In all tests, control reactions containing no disinfectant resulted in complete recovery (10^9 CFU/ml) of the initial inocula. There were no significant differences for the most variable disinfectant replicates (which were obtained with sodium hypochlorite [10 μg/ml]). Disinfectant activities are usually expressed as $\log_{10}$ reductions of whole values. For comparison, a log reduction of >3 and <4 (99.9% to 99.99% reduction in CFU) is considered a minimal effective value. All disinfectant efficacies are discussed according to their effectiveness or ineffectiveness compared with a >3 and <4-$\log_{10}$ reduction in CFU.

Table 2 outlines the results of the suspension and carrier tests with *L. innocua* and *L. monocytogenes* LCDC 88-702 suspended in TSB and serum. Two of the disinfectants tested (povidone-iodine and chlorhexidine gluconate) produced at least a 6-$\log_{10}$ reduction in CFU in all tests; glutaraldehyde was also efficacious in all tests, although it was not as effective in the carrier tests. Ethanol, sodium hypochlorite (60 μg/ml), sodium dichloroisocyanurate (60 μg/ml), sodium hypochlorite with 5% methylethanol, and a quaternary ammonium compound (3.88%) were ineffective in the carrier test with serum. When the concentrations of the sodium hypochlorite and sodium dichloroisocyanurate solutions were reduced to 10 μg/ml, their efficacies were further reduced; these solutions were found to be ineffective in all of the carrier tests, regardless of the organic load. The second quaternary ammonium compound tested was also ineffective in all the carrier tests. Glutaraldehyde-phenate was able to effectively reduce the number only of *L. innocua* organisms in the presence of TSB. The chloramine-T solution was effective only in the suspension tests with a minimal organic load (TSB). Three solutions were ineffective in all tests: phosphoric acid, an iodophor, and formaldehyde.

L. monocytogenes was found to be slightly more resistant to the action of disinfectants than *L. innocua* was; the difference in reductions in CFU ranged from 1 to 3 $\log_{10}$ and was especially noticeable in the presence of TSB, in which case effective reductions could be compared.

All the disinfectants tested on *L. monocytogenes* LCDC 88-702 in pasteurized milk (2% fat) were effective (>5-$\log_{10}$ reduction in CFU) in the suspension test. The results are similar (a variation of only a 1-$\log_{10}$ reduction in CFU) to those observed when the test organism was suspended in either TSB or serum. However, in the carrier test, sodium dichloroisocyanurate was the only formulation tested that was effective (>4- and <5-$\log_{10}$ reduction in CFU). The remaining six disinfectants were virtually ineffective (<3-$\log_{10}$ reduction) in the carrier test in the presence of milk.

The second strain of *L. monocytogenes* tested (LCDC 81-682) was isolated in 1981 from an outbreak of food-borne listeriosis in Nova Scotia, Canada. The activities of selected disinfectants against this strain were identical to those obtained with *L. monocytogenes* LCDC 88-702.

DISCUSSION

In this study, *Listeria* spp. suspended in TSB, serum, or milk were used to simulate natural conditions under which disinfection could occur. Environmental contamination is effectively reduced only when disinfectants are capable of inactivating microorganisms on surfaces in the presence of organic loads. Our finding that microorganisms dried onto surfaces were more resistant to disinfectants than those in suspension agrees with previous findings and may be explained by the interference of physical properties of the surface with contact between the microorganism and the disinfectant (1, 8). *L. monocytogenes* has been shown to adhere to stainless steel surfaces at various temperatures

TABLE 2. Activities of disinfectants on *L. innocua* LCDC 86-417 and *L. monocytogenes* LCDC 88-702

Disinfectant (concn)	Organic matter	Log_{10} reduction in CFU of:			
		L. innocua		*L. monocytogenes*	
		Suspension test	Carrier test	Suspension test	Carrier test
Ethanol	TSB	ND[a]	>4 and <5	>5 and <6	>3 and <4
	Serum	>5 and <6	>1 and <2	>5 and <6	<1
Sodium hypochlorite (60 μg/ml)	TSB	ND	>6	ND	>5 and <6
	Serum	>6	>1 and <2	>6	<1
Sodium hypochlorite (10 μg/ml)	TSB	>5 and <6	<1	>6	>1 and <2
	Serum	>3 and <4	<1	>3 and <4	<1
Sodium dichloroisocyanurate (60 μg/ml)	TSB	ND	>6	ND	>4 and <5
	Serum	>6	>1 and <2	>6	>1 and <2
Sodium dichloroisocyanurate (10 μg/ml)	TSB	>5 and <6	<1	>5 and <6	<1
	Serum	>3 and <4	<1	>3 and <4	<1
Chloramine-T	TSB	>3 and <4	>1 and <2	>3 and <4	>1 and <2
	Serum	<1	<1	<1	<1
Sodium hypochlorite with 5% methylethanol	TSB	>6	>3 and <4	>6	>3 and <4
	Serum	>5 and <6	>1 and <2	>5 and <6	>1 and <2
Phosphoric acid	TSB	>2 and <3	>1 and <2	>1 and <2	<1
	Serum	>1 and <2	<1	<1	<1
Povidone-iodine	TSB	ND	>6	ND	>6
	Serum	ND	>6	ND	>6
Iodophor	TSB	<1	<1	>1 and <2	<1
	Serum	<1	<1	<1	<1
Chlorhexidine gluconate	TSB	ND	>6	ND	>6
	Serum	ND	>6	ND	>6
Glutaraldehyde	TSB	ND	>6	>6	>3 and <4
	Serum	>6	>3 and <4	>6	>3 and <4
Glutaraldehyde-phenate	TSB	>5 and <6	>3 and <4	>4 and <5	>1 and <2
	Serum	>4 and <5	>1 and <2	>3 and <4	>1 and <2
Formaldehyde	TSB	>1 and <2	>1 and <2	<1	<1
	Serum	>1 and <2	>1 and <2	<1	<1
Quaternary ammonium compound (10%)	TSB	>6	>1 and <2	>5 and <6	>1 and <2
	Serum	>4 and <5	<1	>4 and <5	<1
Quaternary ammonium compound (3.88%)	TSB	ND	>6	ND	>5 and <6
	Serum	>4 and <5	<1	>4 and <5	<1

[a] ND, Not done.

and pH values, with adherence possibly mediated by any exopolymer surrounding the cells (8).

That relatively few disinfectant formulations were effective on steel surfaces in the presence of high amounts of organic material is noteworthy. Only povidone-iodine, chlorhexidine gluconate, and glutaraldehyde were effective in the presence of serum; however, all three were ineffective when challenged with milk. *L. monocytogenes* has been reported to be very susceptible to chlorhexidine gluconate (13). Sodium dichloroisocyanurate was not inactivated by milk, whereas the efficacy of sodium hypochlorite was reduced in the presence of milk, despite similar concentrations of available chlorine. This greater resistance of sodium dichloroisocyanurate to neutralization by organic matter has been demonstrated previously (2).

Reducing the organic load did not always improve the capacities of the disinfectants in the carrier test. However, most formulations were effective in the suspension test, regardless of the organic load. For example, sodium hypochlorite (at concentrations of both 60 and 10 μg/ml) was effective in all the suspension tests. This is in accordance with suspension studies by Lopes (12) and Knight et al. (10), who found that sodium hypochlorite at similar concentrations was effective against *L. monocytogenes*.

Chloramine-T, phosphoric acid, an iodophor, and formaldehyde were ineffective in all tests. Higher concentrations of chloramine-T may be necessary for disinfection, even though it is associated with greater stability under temperature changes and sunlight and has a less powerful odor than hypochlorites. The active ingredients of the iodophor may also have been at too low a concentration, as this solution was diluted according to the recommendation of the manufacturer, resulting in a low concentration of available iodine. The phosphoric acid tested was also ineffective, in contrast with results obtained by Lopes, although the active ingredients of the product we tested differed from those of the product tested by Lopes (12). Formaldehyde did not produce an effective result after 1 min, and it may require longer contact times to inactivate *Listeria* spp.

The contact time between a disinfectant and an infectious agent can vary from less than 1 min for surface disinfection to several hours for instrument soaks. It is therefore desirable that a disinfectant produce its effect after minimal contact time. The selection of a 1-min contact time gave a reproducible time interval and a realistic picture of the usual practices of routine surface disinfection.

Various *Listeria* spp. differ considerably in their pathogenicity. Hof and Hefner report that all strains of *L. innocua* tested so far are avirulent and that there are definite differences in virulence and that there are definite differences in virulence between strains of *L. monocytogenes* (9). There were slight differences in susceptibility to disinfectants between these two species. These variations emphasize the difficulties in extrapolating from disinfectant efficacy against one species to efficacy against another. However, no variation in disinfectant susceptibility was observed with the two

strains of *L. monocytogenes*, the species of greatest concern.

This investigation outlines the bactericidal efficacy of a variety of disinfectants on *Listeria* spp. The results presented generate much-needed information on the selection of appropriate disinfectants for research facilities, hospitals, and dairy and food plants. The application of adequate disinfection practices in these and other settings is a widely recognized and needed control measure against listeriosis.

ACKNOWLEDGMENTS

We thank N. C. Irvine (Ontario Ministry of Health, Public Health Laboratory, Ottawa, Canada) for provision of the serum and are grateful to F. Ashton (National Laboratory for Bacteriology, Laboratory Centre for Disease Control, Health and Welfare Canada) for his assistance with this project.

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