THE RESISTANCE OF LISTERIA SPP. TO DESINFECTANTS

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Abstract

This study was realized to determine the efficacy of some disinfectants on surfaces contaminated with Listeria innocua and L. monocytogenes by using a quantitative test. There was studied the efficacy of 10 disinfectants against Listeria innocua and two strains of Listeria monocytogenes in the presence of organic matter.
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). Tests were carried out in the presence of serum and milk to determine any effect that such organic loads may have on disinfectant efficacy. 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 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.
***Keywords**: Listeria innocua, L. monocytogenes, disinfectants, surfaces*

Introduction

Listeria monocytogenes is a pathogen of humans and animals which has been implicated in several outbreaks and sporadic cases of listeriosis, resulting in numerous food product recalls. Listeria monocytogenes is a gram-positive, catalase-positive, aerobic to facultative anaerobic bacterium.

*Listeria monocyto*genes is widespread in the environment and has been isolated by enrichment techniques from various sources, including sewage, vegetables, soil, straw, silage, dust, milk, cheeses, and feces of

healthy animals and humans. The organism can grow well in foods at refrigeration temperatures.

Although these organisms are 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. The organism was isolated from the surface of a gown of a professional exposed at work. Precautions, including appropriate disinfection, are necessary to avoid possible cross-infections in plants and restaurants.

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 pathogens performed by this method concluded that disinfectants which showed low activity on contaminated surfaces did not necessarily do so in suspension. 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.

Experimental

The organisms were isolated from a specimen of contaminated milk. The detection of the strains was realized according SR ISO 11 290-1/2000. Three test suspensions were prepared: one with tryptic soy broth (TSB), 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 105 CFU/ml. These test suspensions were used as the initial inocula for all tests.

Ten disinfectants (table 1) were selected to represent commonly used disinfectants. All disinfectants were diluted 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 disinfectants were tested against L. innocua and L.monocytogenes 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). Selected disinfectants (table 1) were also tested against L. monocytogenes 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 cell culture plate as needed. In the test, 0,002 ml of each test suspension was placed on the carrier surface and allowed to air dry for 2h in a safety cabinet. The contaminated area (not all of the disk surface was contaminated) was then covered with 0,002 ml of disinfectant. Controls for each test suspension were covered with 0,002 ml of normal saline instead of disinfectant. After 1 min of contact, 0,980 ml of diluent (normal saline) was added to each sample to dilute the disinfectant and elute the bacteria from the steel carrier disk.

Table 1: Concentrations of disinfectants used

Disinfectant	Conc.used
1. Ethanol (95%)	70%
2. Sodium dichloroisocyanurate (tablets)	0,006mg/ml
3. Chloramine-T (67%)	0,4%
4. Sodium hypochlorite	0,006mg/ml
5. Phosphoric acid (18%)	0,45%
6. Povidone-iodine	1%
7. Iodophor (1.0% titratable 12)	0,008%
8. Chlorhexidine gluconate (4%)	4%
9. Glutaraldehyde	2%
10.Formaldehyde	3,7%

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 and Discussions

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 (0,001mg/ml]). Disinfectant activities are usually expressed as $\log_{10}$ reductions of whole values. 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 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 (0,006 mg/ml), sodium dichloroisocyanurate (0,006 mg/ml), sodium hypochloritea were ineffective in the carrier test with serum. When the concentrations of the sodium hypochlorite and sodium dichloroisocyanurate solutions were reduced to 0,001 mg/ml, their efficacies were further reduced; these solutions were found to be ineffective in all of the carrier tests, regardless of the organic load. 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 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 disinfectants were virtually ineffective (<3-$\log_{10}$ reduction) in the carrier test in the presence of milk.

Conclusions

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