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NOTES

Comparative Mycobactericidal Efficacy of Chemical Disinfectants in Suspension and Carrier Tests

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The efficacy of nine disinfectants on *Mycobacterium smegmatis* was tested in the presence of sputum, using quantitative suspension and carrier tests. Glutaraldehyde, povidone iodine, and chlorhexidine gluconate produced at least a 6-$\log_{10}$ reduction in CFU in all tests. Four disinfectants (sodium dichloroisocyanurate, phenol, ethanol, and sodium hypochlorite) were not as effective in the carrier tests as in the suspension tests; this difference ranged from a 1- to a 5-$\log_{10}$ reduction in CFU. The efficacy of ethanol and sodium hypochlorite was further reduced (3- and 1-$\log_{10}$ reductions in CFU, respectively) in the presence of sputum. The quaternary ammonium compound and iodophor were ineffective in all tests. The findings of this study demonstrate the need for a quantitative carrier test such as the one presented here.

Mycobacteria are generally more resistant to chemical disinfection than other vegetative bacteria (14). Mycobactericidal disinfectants are heavily relied upon despite confusion and concern over their efficacy. Variations in test protocols and the lack of an accurate standard mycobactericidal test have resulted in variable efficacy data.

The Association of Official Analytical Chemists tuberculocidal test (3), currently widely used, has been recently criticized (1, 6–8) and is now generally recognized to be inadequate and unreliable. Several new quantitative tuberculocidal suspension tests have been proposed (2, 10, 16). These tests are useful for screening disinfectants; however, to evaluate disinfectants used on contaminated surfaces, it is necessary to use a carrier test which simulates the in-use practices of general surface and equipment disinfection.

This study was initiated to determine the efficacy of several disinfectants against *Mycobacterium smegmatis*. Precise, reproducible suspension and carrier tests were used. These methods, previously used with viruses (12, 15), were modified for mycobactericidal testing.

M. smegmatis TMC 1515 was obtained from the National Reference Centre for Tuberculosis, Health and Welfare Canada, and was maintained on Lowenstein-Jensen medium. The organisms were inoculated into Middlebrook 7H9 broth (Difco Laboratories) and incubated at 37°C. After 4 days, the culture was homogenized for 2 min with sterile glass beads to obtain 10^9 CFU/ml, dispensed into 2-ml vials, and frozen at −70°C. Sputum, used as the organic load, was a pool of mycobacterium-negative specimens obtained from the National Reference Centre for Tuberculosis. Two test suspensions were thawed at room temperature: one was thoroughly mixed; in the second, the suspending medium was first replaced with the sputum and then mixed. These test suspensions were used as the initial inocula for all tests. Viable counts were carried out on both suspensions by preparing 10-fold dilutions in normal saline containing 0.5% Tween 80 (the Tween 80 prevented macroscopic clumping of cells and was found to be noninhibitory to *M. smegmatis*). Samples (1 ml) from the dilutions were spread on Middlebrook 7H11 agar (Difco).

The disinfectants tested in this study are listed in Table 1. All disinfectants were diluted according to the manufacturer's instructions. Tap water was used as the diluent. In both the suspension and carrier tests, dilution of the reaction mixture (100-fold, followed by a further 10-fold dilution) immediately at the end of the contact time was the method used to terminate disinfectant action. This method of neutralization was found to be effective and allowed the use of a uniform method in the testing of all disinfectant formulations. All disinfectant reactions were carried out in the wells of a 24-well plastic cell culture plate (Falcon; Becton Dickinson Labware).

In the suspension test, 0.1 ml of mycobacterial test suspension (with or without sputum) was added to 0.9 ml of disinfectant. After 1 min of contact, 0.1 ml of the reaction mixture was removed and immediately diluted 100-fold in normal saline containing 0.05% Tween 80. The sample was immediately subjected to further 10-fold dilutions (10^{-3} to 10^{-7}). Controls, using each test suspension, contained 0.9 ml of the diluent instead of disinfectant. Samples (1 ml) from the dilutions were spread on 7H11 agar, in duplicate, and incubated at 37°C for 4 days to determine the level of mycobactericidal activity.

Stainless steel, polypropylene, and glass were selected for the carrier test. Stainless-steel and polypropylene sheets (0.75 mm thick) were obtained locally and 1-cm-diameter disks were cut from them. Glass cover slips (1-cm diameter) were obtained from a scientific supply company (Chance Propper Co.). The disks were placed in the wells of the cell culture plate as needed.

In the carrier test, 20 μl of mycobacterial test suspension (with or without sputum) was placed on the carrier surface and allowed to air dry for 2 h in a class II biological safety cabinet: the drying process was found not to reduce the CFU

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TABLE 1. Disinfectants tested and in-use concentration

Disinfectant	In-use concn
Glutaraldehyde	2%
Sodium hypochlorite (600 μg of available chloride [Av Cl] per ml)	100 μg/ml (Av Cl) 60 μg/ml (Av Cl) 6 μg/ml (Av Cl)
Sodium dichloroisocyanurate (tablets)	60 μg/ml (Av Cl)
Ethanol (95%)	70% (vol/vol)
Phenol (crystals)	5% (wt/vol)
Quaternary ammonium compound (10% dimethyl benzylammonium chloride)	0.04%
Povidone iodine (1.0% titratable I_2)	1.0% I_2
Iodophor {9.10% polyethoxypolypropoxy-polyethoxyethanol-iodine complex and 8.74% nonylphenoxypoly(ethyleneoxy)ethanol-iodine complex [1.6% titratable I_2]}	0.008% I_2
Chlorhexidine gluconate (4%)	4%

of the test suspension. The contaminated area was then covered with 20 μl of disinfectant. After 1 min of contact, 980 μl of the diluent was added to each well to dilute the disinfectant and elute the bacteria from the carrier. Subsequently, the eluates were serially diluted and plated as in the suspension test. Controls were similar to those in the suspension test.

Tests were carried out in at least triplicate, and in most cases six replicates were done. Each disinfectant was tested for its capacity to cause up to a 6-$\log_{10}$ (99.9999%) reduction in CFU of the test bacterium.

The results of the suspension and carrier tests are summarized in Table 2. In all tests, control reactions containing no disinfectant resulted in complete recovery (10^9 CFU/ml) of the initial inocula. The tests with glutaraldehyde and the three concentrations of sodium hypochlorite were conducted on all three carrier types. Since no variation was observed

TABLE 2. Comparison of mycobactericidal activity of selected disinfectants in suspension and carrier tests

Disinfectant	$\log_{10}$ reduction in CFU			
	Suspension test	Carrier test		
		Stainless steel	Polypropylene	Glass
Glutaraldehyde				
Without sputum	>6	>6	>6	>6
With sputum	>6	>6	>6	>6
Sodium hypochlorite (100 μg of available chlorine per ml)				
Without sputum	>6	>6	>6	>6
With sputum	>6	>5 and <6	>5 and <6	>5 and <6
Sodium hypochlorite (60 μg of available chlorine per ml)				
Without sputum	>6	>5 and <6	>5 and <6	>5 and <6
With sputum	>6	>4 and <5	>4 and <5	>4 and <5
Sodium hypochlorite (6 μg of available chlorine per ml)				
Without sputum	>6	>2 and <3	>2 and <3	>2 and <3
With sputum	>5 and <6	>2 and <3	>2 and <3	>2 and <3
Sodium dichloroisocyanurate				
Without sputum	>6	>4 and <5		
With sputum	>6	>4 and <5		
Ethanol				
Without sputum	>6	<1		
With sputum	>3 and <4	<1		
Phenol				
Without sputum	>6	>1 and <2		
With sputum	>6	>1 and <2		
Quaternary ammonium compound				
Without sputum	>2 and <3	<1		
With sputum	<1	ND[a]		
Providone iodine				
Without sputum	>6	ND		
With sputum	>6	>6		
Iodophor				
Without sputum	>1 and <2	<1		
With sputum	<1	<1		
Chlorhexidine gluconate				
Without sputum	>6	>6		
With sputum	>6	>6		

[a] ND, Not done.

for the efficacy of disinfectants and the controls on each of the three surfaces, all further tests were carried out on stainless-steel disks only.

Three of the disinfectants tested (glutaraldehyde, povidone iodine, and chlorhexidine gluconate) produced at least a 6-$\log_{10}$ reduction in CFU in both suspension and carrier tests, even in the presence of sputum. This high susceptibility of *M. smegmatis* to alkaline glutaraldehyde is in accordance with previous studies (9). The high efficacy of povidone iodine contrasted greatly with the iodophor, which was virtually ineffective in all tests. This may be because the povidone iodine was used undiluted in accordance with the manufacturer's recommendation, resulting in a much higher concentration of available iodine. Chlorhexidine gluconate was also found to be extremely efficacious on the inanimate carrier; as this chemical is used mainly as an antiseptic and hand wash, it would be useful to determine its efficacy on human skin as well.

The sodium hypochlorite and sodium dichloroisocyanurate solutions were not as effective in the carrier tests as in the suspension tests, and, in the case of sodium hypochlorite, this difference was magnified as the concentration of the available chlorine decreased. Sodium dichloroisocyanurate has been reported to have several advantages over sodium hypochlorite: tablets are stable and compact, use dilutions can be prepared simply (however, they are not stable), and it has a greater resistance to neutralization by organic matter (4, 5). This study also found no noticeable effect of sputum on sodium dichloroisocyanurate, whereas the efficacy of sodium hypochlorite was slightly reduced in the presence of sputum.

Ethanol and phenol also produced a lower reduction in CFU in the carrier test as compared with the suspension test. This difference was marked, as these disinfectants were virtually ineffective in the carrier test. The disinfectant capacity of ethanol was reduced in the presence of sputum.

The quaternary ammonium compound produced a slight reduction in CFU in the suspension test but was completely ineffective in the presence of sputum and in the carrier test.

Log_{10} reductions in CFU was used as a measure of disinfectant efficacy in this study. Various minimum acceptable $\log_{10}$ reductions have been suggested (11–13, 15, 16). This study does not define a minimum acceptable level of efficacy but presents a comparison of mycobactericidal activity of different disinfectant types.

The contact time between a disinfectant and an infectious agent can vary from <1 min for hand and surface disinfection to several hours for instrument soaks. It is therefore desirable that a disinfectant produce its effect after a minimal contact time, and 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.

The mycobactericidal tests used in this study are reproducible and accurate, and the carrier test closely simulates actual conditions of disinfectant use. As indicated by the results, disinfectants showing high activity in suspension tests did not necessarily do so on contaminated surfaces. It is therefore clear that a carrier test should be used to test surface disinfectants. In the Association of Official Analytical Chemists carrier test (3), the carrier is dipped into the test suspension to contaminate the surface and then into the disinfectant under test. This results in considerable variation in the bacterial loading of the carrier as well as the washing off and loss of organisms in the disinfectant solution (1, 6–8). The carrier method presented here reduces variability in bacterial load from carrier to carrier, and since the carrier and disinfectant are both immersed in the eluent, all viable bacteria can be accounted for.

We have demonstrated the mycobactericidal activity of various disinfectants with *M. smegmatis* as a test organism. This nonpathogenic strain of mycobacteria is relatively fast growing and easy to work with. The efficacy of disinfectants on *M. tuberculosis*, using the described suspension and carrier tests, is currently under investigation to provide a comprehensive study of the tuberculocidal activity of disinfectants.

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