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THE MYCOBACTERICIDAL EFFICACY OF ORTHO-PHTHALALDEHYDE AND THE COMPARATIVE RESISTANCES OF *MYCOBACTERIUM BOVIS*, *MYCOBACTERIUM TERRAE*, AND *MYCOBACTERIUM CHELONAE*

Adam W. Gregory, BS; G. Bruce Schaalje, PhD; Jonathan D. Smart, BS; Richard A. Robison, PhD

ABSTRACT

OBJECTIVES: To assess the mycobactericidal efficacy of an agent relatively new to disinfection, ortho-phthalaldehyde (OPA) and to compare the resistances of three *Mycobacterium* species. *Mycobacterium bovis* (strain BCG) was compared with *Mycobacterium chelonae* and *Mycobacterium terrae* to investigate the feasibility of using either of the latter two species in tuberculocidal testing. *M chelonae* (a rapid grower) and *M terrae* (an intermediate grower) both grow faster and are less virulent than *M bovis* (a slow grower).

DESIGN: The quantitative suspension protocol specified by the Environmental Protection Agency (EPA), the Tuberculocidal Activity Test Method (EPA test), was used throughout this study. Standard suspensions of all three species were prepared in a similar manner. Two suspensions of *M bovis*, created in different laboratories, were used. These were tested against two concentrations of alkaline glutaraldehyde to provide reference data. Two concentrations of OPA were evaluated

against all mycobacterial test suspensions. Four replicates of each organism-disinfectant combination were performed.

RESULTS: Results were assessed by analysis of variance. *M terrae* was significantly more resistant to 0.05% OPA than either *M bovis* or *M chelonae*. At 0.21% OPA, *M terrae* was slightly more susceptible than one test suspension of *M bovis*, but not significantly different from the other. *M chelonae* was significantly less resistant than the other species at both OPA concentrations. At their respective minimum effective concentration, OPA achieved a 6-log₁₀ reduction of *M bovis* in nearly one sixth the time required by glutaraldehyde (5.5 minutes vs 32 minutes).

CONCLUSIONS: These data, along with other recent studies, lend support to the idea that *M terrae* may be a suitable test organism for use in the tuberculocidal efficacy testing of disinfectants. They also confirm the relatively rapid tuberculocidal activity of OPA (*Infect Control Hosp Epidemiol* 1999;20:324-330).

While airborne transmission of tuberculosis (TB) is the most common route of spread, cases due to indirect contact with semicritical medical instruments (such as bronchoscopes and endoscopes) continue to occur.¹⁻⁴ Contributing to this problem are exaggerated tuberculocidal label claims, which have been discovered even on high-level disinfectants.⁵⁻⁷ Worse yet, some disinfectants have been found to support the presence of contaminating bacteria for extended periods of time.⁸⁻¹⁰ Because mycobacteria generally are more resistant to chemical disinfection than most other vegetative organisms,¹¹ these data raise special concerns with respect to *Mycobacterium tuberculosis*. One way to reduce the incidence of disease caused by inadequately disinfected medical devices is to assure that tuberculocidal assessment of disinfectants is accomplished with accurate and standardized test procedures.

One of the most critical variables in tuberculocidal testing is the selection of the challenge organism (which should possess resistance equivalent to that of *M tuberculosis*). This has been a source of variability in past studies, contributing to the difficulty of establishing a truly standardized protocol. The quantitative suspension procedure currently approved by the Environmental Protection Agency (EPA) was described first in 1987 by Ascenzi et al.⁵ Known as the EPA Tuberculocidal Activity Test Method (EPA test), this quantitative procedure has attempted to control many of the variables involved in tuberculocidal testing. It specifies the use of *Mycobacterium bovis* (strain BCG) as the challenge organism.¹² *M bovis* also is specified in the Association of Official Analytical Chemists tuberculocidal procedure.¹³ It is related closely to *M tuberculosis* and has demonstrated similar levels of resistance to a vari-

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ety of disinfecting agents.¹⁴ However, the use of *M bovis* has drawbacks. In addition to being more virulent than several other known strains, it is slow-growing, requiring at least 3 weeks before final results can be obtained.

Several researchers have experimented with faster-growing, less pathogenic strains of mycobacteria in hopes of identifying a more suitable test species. This study involves a comparative analysis of a rapid-growing species (*Mycobacterium chelonae*) and an intermediate-growing species (*Mycobacterium terrae*) with *M bovis* (a slow-growing species).

The comparative resistances of these strains were evaluated using ortho-phthalaldehyde (OPA), a compound relatively new to disinfection. The current standard for high-level disinfection of semicritical instruments is 2% alkaline glutaraldehyde. Although this agent has well-documented bactericidal, sporicidal, and mycobactericidal activity, it is not without deficiencies, including inactivation by spontaneous polymerization and loss of activity with dilution.^{15,16} It also requires a relatively long exposure time to exact a significant tuberculosis kill.¹⁷

Because OPA is a relatively novel disinfectant, few efficacy studies have been performed. However, the limited available data suggest that it has much higher activity than 2% glutaraldehyde, especially with respect to its tuberculocidal and virucidal action. In this study, we evaluated the mycobactericidal effects of two concentrations of OPA on the three mycobacterial species. For comparison purposes, data also were collected on two concentrations of glutaraldehyde using the current tuberculocidal test organism, *M bovis*.

METHODS

Test Organisms

Stock suspensions of *M terrae* (American Type Culture Collection [ATCC] 15755), *M chelonae* (ATCC 35752), and *M bovis* (ATCC 35743) were prepared according to the procedure specified in the EPA test protocol¹² with the following modifications: The final scale-up was performed by adding 50 mL of culture to each of two 2-L plastic roller bottles containing 450 mL of modified Proskauer-Beck medium, prepared in our laboratory, containing 0.1% Tween 80. Cultures were incubated with loose caps at 37°C, rolling slowly, for various times depending on the species.

As specified in the EPA protocol, when the absorbance of the culture at 500 nm reached a minimum of 0.6 ($1-5 \times 10^8$ colony-forming units [CFU]/mL), the cells were harvested. On the day prior to harvest, Tween 80 was added to a final concentration of 0.1% in each bottle. The suspensions were homogenized in a Teflon-on-glass tissue grinder and frozen at -70°C , as specified.

Disinfectants

Four samples of disinfectant were used: two concentrations of alkaline glutaraldehyde (Cidex activated dialdehyde solution, Johnson & Johnson Medical, Inc, Arlington, TX) and two concentrations of OPA (Cidex OPA solution, Johnson & Johnson Medical, Inc). The two glutaraldehyde solutions came from the same lot. One was freshly activated

(2.5%), and one was stressed through simulated reuse, as described below, and diluted to contain 1.5% glutaraldehyde. This latter solution constitutes the manufacturer's minimum effective concentration. The OPA samples originated from a solution stressed through simulated use and diluted to contain 0.21% (the minimum effective concentration) and 0.05% OPA. The 0.05% OPA solution provided longer kill times, for more suitable comparison of the three *Mycobacterium* species. Simulated reuse of the disinfectant solutions consisted of the addition of 5% horse serum and the processing of two sets of anesthesia equipment for 42 cycles over 14 days, according to a standard reuse protocol. A 0.8% phenol standard, the control disinfectant solution specified in the EPA test method, also was assayed with each test suspension.

Test Suspensions

Mycobacterial test suspensions were prepared according to the EPA test protocol. On each test day, the required number of frozen stock vials was allowed to thaw. The contents of each vial were added to a prechilled 30-mL Teflon-on-glass tissue homogenizer with an equal volume of phosphate-buffered gelatin. The mixture was homogenized on ice for 1 to 2 minutes. The homogenized suspension then was diluted using ice-cold physiological saline solution containing 0.1% Tween 80 to approximately 1×10^7 CFU/mL. This suspension was kept on ice until used to inoculate test disinfectants.

Disinfectant Test Procedure

All disinfectants were assayed according to the EPA test protocol. Each test consisted of inoculating the disinfectant at time zero with a standardized test organism suspension and sampling this mixture at predetermined time intervals. In all cases, tubes containing 9 mL of the appropriate test disinfectant were equilibrated in a 20°C water bath. At time zero, a 1-mL aliquot of the prepared test suspension was added to the disinfectant and mixed by vortexing. For the tests involving glutaraldehyde, sampling times were 2, 4, 8, and 16 minutes for the 2.5% freshly activated solution and 4, 8, 16, and 32 minutes for the 1.5% minimum effective concentration solution. In each case, 2.0-mL samples were taken and added to 2.0 mL of a freshly prepared sodium bisulfite neutralizer. The concentration of sodium bisulfite was 2.2 times the assayed concentration of glutaraldehyde. For all tests involving OPA, 1-mL samples were taken at 1, 2, 4, 8, and 16 minutes for the 0.05% solution and at 0.5, 1, 2, 4, and 8 minutes for the 0.21% solution. Samples were added to 9-mL tubes of Lethen broth containing 1% fresh sodium bisulfite (neutralizer solution). All remaining procedures in the testing of both disinfectants were identical. After approximately 1 minute, serial dilutions (1:10) of neutralized samples were carried out in physiological saline solution to a predetermined point (dependent upon expected counts for each sample).

Phenol control evaluations of each prepared test suspension were performed as specified in the EPA test protocol. Tubes containing 9 mL of 0.8% phenol were equilibrated in a 25°C water bath and inoculated with 1 mL of test sus-

pension. After 20 minutes, 1-mL samples were taken and mixed with 9 mL of Lethen neutralizer solution. Serial dilutions (1:10) were carried out in physiological saline solution.

For all samples, selected dilution tubes (of neutralized suspensions) were assayed in duplicate for the number of viable organisms by membrane filtration. A 1-mL aliquot of the chosen dilution was added to approximately 50 mL of physiological saline solution in a disposable plastic funnel (Microfil system, Millipore, Bedford, MA). Standard cellulose filters (Millipore, 0.45- μ m pore size, 47-mm diameter) were used. After applying vacuum, filters were washed with an additional 50 to 100 mL of physiological saline solution and placed on Middlebrook 7H11 agar supplemented with 10% BACTO-OADC enrichment (Difco, Detroit, MI). The agar plates were placed in 1-gallon polyethylene bags, sealed with twist ties, and incubated until colonies could be counted readily. This was approximately 20 to 24 days for both *M terrae* and *M bovis*, and 3 to 4 days for *M chelonae*. While small colonies of *M terrae* were counted after 14 days, plates were reincubated and assessed again at the same time as the *M bovis* samples to ensure reliable colony counts. *M chelonae* was incubated at 32°C, whereas both *M terrae* and *M bovis* were incubated at 37°C.

Results of the individual colony counts for the various assays are available on request.

Disinfectant Neutralization Controls

For all disinfectant samples, a neutralization verification was conducted with each organism on every test day. During the glutaraldehyde tests, this procedure consisted of inoculating a mixture of 2 mL of disinfectant and 2 mL of neutralizer with 400 organisms in 40 μ L. In the OPA tests, 100 μ L containing 1,000 organisms was added to a mixture of 1 mL of disinfectant and 9 mL of neutralizer. This resulted in an inoculum of approximately 100 CFU/mL in each tube. In all cases, these suspensions were allowed to sit for at least 20 minutes prior to assay to detect any residual killing activity from unneutralized disinfectant. They then were assayed for viable organisms in a manner identical to the procedure described above. The percent inhibition for each control was determined as follows: percent inhibition (PI)=[(Expected # of Colonies)-Observed # of Colonies]/(Expected # of Colonies) $\times$ 100. The Expected # of Colonies was obtained from the viable count assay for each test suspension.

Statistical Methods

Data for this study were collected over a total of 12 test days, 4 for glutaraldehyde, and 8 for OPA. Data for each disinfectant were analyzed separately. The response variable for both analyses was the time to a 6-log₁₀ reduction for each test organism. The times were calculated for each replication of every organism-disinfectant combination by linear regression of log(S/S₀) versus time, where S₀ is the baseline count and S is the concentration of viable organisms at a particular sample time. The SAS (SAS Institute, Cary, NC) procedure REG was used for these regressions.

The 4 days of glutaraldehyde testing consisted of two suspensions of *M bovis* (a and b, each prepared in different

laboratories) exposed to two concentrations of glutaraldehyde (1.5% minimum effective concentration and 2.5% [fresh] solution). On every test day, each *M bovis* suspension was tested against both concentrations of glutaraldehyde. Six-log₁₀-reduction times were analyzed using a mixed-effects analysis of variance model. "Technician" and "test day" were deemed random factors, whereas "suspension" and disinfectant "concentration" were considered to be fixed factors. The variance components associated with the random factors were estimated using restricted maximum likelihood and were tested using an approximate Z test. The main effects and the interaction of the fixed factors were evaluated with F statistics. All calculations were done using the MIXED procedure of SAS.

The 8 days of testing with OPA originally were designed as a balanced two-factor split-unit experiment, with days as whole units. The whole-unit factor was disinfectant "concentration" (0.05% and 0.21%) and the sub-unit factor was "organism" (*M chelonae*, *M terrae*, and *M bovis*). Concentrations were assigned to days, and every organism was tested on every day. Thus, every organism-concentration combination was replicated four times. As in the glutaraldehyde testing, two different suspensions of *M bovis* (a and b) were used. This time, one was used for the first 4 days and the other for the last 4 days. In this analysis, it was decided to treat these suspensions as different organisms. Therefore, "organism" was partially a whole-unit factor and partially a split-unit factor. Due to variance heterogeneity, the 6-log₁₀ reduction times for each organism-concentration combination were transformed to the logarithmic scale for analysis. Because the design was an unbalanced split-unit design, statistical analysis was done using a maximum likelihood analysis of variance procedure. The SAS procedure MIXED was used for the analysis. "Test day" was treated as a random factor, whereas "organism," "concentration," and their interaction were treated as fixed factors. Adjusted means, along with their standard errors, were calculated for all organism-concentration combinations.

These were converted back to the original scale for presentation in figures and tables. Results expressed as time to achieve a 6-log₁₀ reduction are those determined by the described statistical regression procedures and do not necessarily represent an actual measurement at that point. Linear contrasts were used to investigate prespecified comparisons of means, such as the mean for *M bovis* versus the mean for *M terrae* at each of the disinfectant concentrations. *P* values were adjusted for the multiplicity of contrasts examined.

RESULTS

Figure 1 shows the mean times (in minutes) required for a 6-log₁₀ reduction for each suspension of *M bovis* tested against both concentrations of glutaraldehyde. Table 1 shows the results of the statistical analysis of these data. The *M bovis* "a" suspension was significantly more resistant, rendering longer 6-log₁₀ reduction times across both glutaraldehyde concentrations. As expected, there was a significant difference between disinfectant concen-

FIGURE 1. Mean times required to effect a 6- $\log_{10}$ reduction of *Mycobacterium bovis* suspensions from two different laboratories (*M bovis-a* and *M bovis-b*). Two glutaraldehyde concentrations were tested: 2.5% fresh solution (grey bars) and 1.5% minimum effective concentration (white bars). The precise result is shown above each column. The bars show the standard error of the mean.

TABLE 1
STATISTICAL ANALYSIS OF GLUTARALDEHYDE DATA

Variance Components Estimates*				
Parameter	Estimate			
Technician	0.00			
Test day	2.87			
Error	5.58			
Tests of Fixed Effects				
Source	Degrees of Freedom		F Statistic	P>F
	Numerator	Denominator		
Suspension†	1	8	22.9	.001
Concentration‡	1	8	183	<.001
Interaction	1	8	1.61	.240

* Restricted maximum likelihood.
† Suspension A or suspension B.
‡ 1.5% (minimum effective) or 2.5% (fresh).

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‡ 1.5% (minimum effective) or 2.5% (fresh).

trations. The 1.5% minimum effective concentration produced times approximately twice those of the 2.5% fresh solution, across both *M bovis* suspensions. Variance components for “technician” and “test day” were both small. The “suspension” by “concentration” interaction was non-significant, indicating that the effect of one factor on the 6- $\log_{10}$ reduction times was not affected by a change in the other factor. In other words, both *M bovis* suspensions gave the same results relative to each of the two concentrations of glutaraldehyde.

Figure 2 shows the mean times (in minutes) required by each concentration of OPA to effect a 6- $\log_{10}$ reduction of each of the three *Mycobacterium* species involved in this study. Table 2 shows the statistical analysis of these data. The variance component for “test day” was small. There was a significant difference between test

FIGURE 2. Mean times required to effect a 6- $\log_{10}$ reduction for *Mycobacterium chelonae*, *Mycobacterium terrae*, and *Mycobacterium bovis*, using two different ortho-phthalaldehyde concentrations: 0.21% minimum effective concentration (grey bars) and 0.05% (white bars). *M bovis* suspensions from two different laboratories (*M bovis-a* and *M bovis-b*) were used. The precise result is shown above each column. The bars show the standard error of the mean.

TABLE 2
STATISTICAL ANALYSIS OF ORTHO-PHTHALALDEHYDE DATA

Variance Components Estimates*				
Parameter	Estimate			
Test day	0.003			
Error	0.010			
Tests of Fixed Effects				
Source	Degrees of Freedom		F Statistic	P>F
	Numerator	Denominator		
Organism†	3	10	54.4	<.001
Concentration‡	1	6	869	<.001
Interaction	1	10	37.9	<.001

* Restricted maximum likelihood.
† *Mycobacterium bovis* (A or B), *Mycobacterium terrae*, and *Mycobacterium chelonae*.
‡ 0.21% (minimum effective) or 0.05%.

* Restricted maximum likelihood.

† *Mycobacterium bovis* (A or B), *Mycobacterium terrae*, and *Mycobacterium chelonae*.

‡ 0.21% (minimum effective) or 0.05%.

“organisms” used. Disinfectant “concentrations” also differed significantly in activity, as expected. The “organism” by “concentration” interaction was also significant, indicating that the different species responded differently from one disinfectant concentration to the other. To examine these differences in more detail, specific contrasts were performed. These contrasts are detailed in Table 3 and, together with Figure 2, show the following:

- Both *M bovis* suspensions (a and b) react in a similar manner to changes in OPA concentration (Table 3, line 1).
- M bovis* and *M chelonae* do not react in a similar way to changes in OPA concentration (Table 3, line 2).
- M bovis* and *M terrae* do not react in a similar way to changes in OPA concentration (Table 3, line 3).

TABLE 3

DETAILED STATISTICAL ANALYSIS OF ORTHO-PHTHALALDEHYDE DATA: SELECTED INDIVIDUAL CONTRASTS AND THEIR CORRESPONDING SIGNIFICANCE

Parameter	Estimate	SE	P
Interactions with OPA concentrations			
1. <i>Mycobacterium bovis</i> : a vs b	0.050	0.157	.755
2. <i>M bovis</i> vs <i>Mycobacterium chelonae</i>	0.872	0.102	<.001
3. <i>M bovis</i> vs <i>Mycobacterium terrae</i>	1.00	0.102	<.001
4. <i>M chelonae</i> vs <i>M terrae</i>	-0.130	0.102	.232
Comparisons over OPA concentrations			
5. <i>M bovis</i> : a vs b	-0.294	0.078	.004
6. <i>M chelonae</i> vs <i>M terrae</i>	-0.611	0.051	<.001
0.05% OPA			
7. <i>M bovis</i> vs <i>M terrae</i>	0.693	0.072	<.001
8. <i>M bovis</i> -a vs <i>M terrae</i>	0.559	0.091	<.001
9. <i>M bovis</i> -b vs <i>M terrae</i>	0.827	0.091	<.001
0.21% OPA			
10. <i>M bovis</i> vs <i>M terrae</i>	-0.309	0.072	.002
11. <i>M bovis</i> -a vs <i>M terrae</i>	-0.468	0.091	<.001
12. <i>M bovis</i> -b vs <i>M terrae</i>	-0.150	0.091	.131

Abbreviations: OPA, ortho-phthalaldehyde; SE, standard error.

4. *M chelonae* and *M terrae* react in a similar way to changes in OPA concentration (Table 3, line 4).

5. When both concentrations of OPA are considered, there is a significant difference in the resistances of the two *M bovis* suspensions (Table 3, line 5).

6. When both concentrations of OPA are considered, *M terrae* is significantly more resistant than *M chelonae* (Table 3, line 6).

7. *M terrae* is significantly more resistant to 0.05% OPA than either suspension of *M bovis* (Table 3, lines 7-9).

8. *M terrae* is significantly more susceptible to 0.21% OPA than *M bovis*-a," but not significantly different than *M bovis*-b" (Table 3, lines 10-12).

On every test day, each organism suspension was exposed to 0.8% phenol, as specified in the EPA protocol. Log₁₀ reductions were calculated for every exposure, and, after completing all test days, mean values were determined for each organism. As shown in Figure 3, both *M chelonae* and *M terrae* were significantly more resistant to 0.8% phenol than was *M bovis*.

An analysis of the percent inhibition data to confirm neutralization efficacy indicated that all disinfectants were neutralized completely following their specific contact period (data not shown).

DISCUSSION

The use of a standardized mycobacterial test suspension is an issue central to effective and meaningful tuberculocidal testing. Although *M bovis* has been the standard for tuberculocidal testing in the United States, other species may prove ultimately to be more suitable. Rapid-growing, less virulent saprophytic species of mycobacteria, such as *M smegmatis*, have been considered for tubercu-

locidal testing in the past, but it has since been demonstrated that such species are more susceptible to disinfection than *M tuberculosis*.¹⁸⁻²¹ *M chelonae* was included in the present study, to evaluate the performance of a representative rapid-growing species against OPA. As expected, this organism demonstrated significantly less resistance to OPA than either of the other species tested. Clearly, estimates of tuberculocidal activity based on data from such rapid-growing species are not predictive of activity against either *M bovis* or clinical tuberculosis isolates.¹⁴

M terrae, an intermediate grower, may represent a more reasonable alternative. This study showed *M terrae* to be either similar or more resistant to OPA than *M bovis*. Although relatively unexplored in the United States, European researchers have shown this organism to have resistance comparable to, or greater than, *M tuberculosis*. In 1978, Sonntag conducted a comparative analysis of *M terrae* and *M tuberculosis* against several disinfectants.²² It was found that the two species exhibited approximately equal resistance to aldehydes, phenols, amphotensides, and peracetic acid. Seven years later, Sonntag and Hingst presented a comparison of *M terrae* and *M tuberculosis* using six different disinfectants.²³ Their findings again revealed very similar resistance profiles between the two organisms, this time with *M terrae* showing slightly greater resistance to all of the disinfectants tested. Also of possible significance was their finding of high levels of common membrane antigens shared by these two species. Similar results were achieved by vanKlinger and Pullen.²⁴ After finding *M terrae* and *M tuberculosis* to have similar resistance to 11 disinfectants, they recommended the use of *M terrae* for quantitative suspension tuberculocidal testing. A 1998 study by Griffiths et al provided recent confirmation

of these findings.²⁵ They reported *M terrae* either to equal or exceed *M tuberculosis* in resistance to six disinfectants. In addition, the German Society for Hygiene and Microbiology already has declared *M terrae* its official tuberculocidal test species.²⁶

Obviously, the most important consideration when selecting an organism to assess tuberculocidal activity is the way it behaves in relation to *M tuberculosis* or a similar documented standard. This study involved a comparative analysis of *M chelonae* and *M terrae* against the current EPA-approved test organism, *M bovis*. Previous work done in our laboratory has shown that standard *M bovis* suspensions often differ from laboratory to laboratory.⁷ For this reason, two different *M bovis* suspensions (each prepared in different laboratories) were used. Not surprisingly, the two suspensions gave slightly different results. Nonetheless, *M terrae* showed similar resistance to *M bovis* when subjected to a new, highly effective chemical disinfectant not evaluated in any of the aforementioned studies. These results support the conclusions of other researchers who have proposed using *M terrae* in tuberculocidal testing of aldehydes. Along with possessing a resistance profile similar to *M tuberculosis* (and in this case, *M bovis*), the lower virulence and more rapid growth of *M terrae* make it an attractive alternative to the current *M bovis* standard. Interestingly, the resistances of *M bovis*, *M chelonae*, and *M terrae* to the 0.8% phenol standard were very different (Figure 3). *M chelonae*, although the organism most sensitive to OPA, was by far the most resistant to phenol. These results raise old doubts concerning the validity of the phenol resistance window that all *M bovis* suspensions used for tuberculocidal testing currently are required to meet. They reinforce the fact that the phenol resistance of a particular organism does not necessarily correlate with its resistance to other disinfectants.

The rapid tuberculocidal activity of OPA is a separate important finding of this study. Although glutaraldehyde has long been the standard for high-level disinfection among chemical germicides, its tuberculocidal activity has been documented to be relatively slow. In a 1995 analysis of three glutaraldehyde-based disinfectants, Jette et al concluded that "the search for a safer, non-damaging and 'user-friendly' alternative to glutaraldehyde should continue as safer more dilute solutions require long, impractical exposure times for high level disinfection."²⁷ A comparison of the tuberculocidal activities of glutaraldehyde and OPA can be made by contrasting Figures 1 and 2. At the minimum effective concentration of 1.5%, glutaraldehyde required a mean of 32 minutes to achieve a 6-log₁₀ reduction of *M bovis*. In contrast, OPA at its minimum effective concentration of 0.21% required a mean of only 5.5 minutes to achieve the same level of kill, making OPA nearly six times faster than alkaline glutaraldehyde by this measure.

OPA has been shown to have other advantages over glutaraldehyde as a hospital-level disinfectant. In 1993, Alfa and Sitter conducted a thorough in-hospital assessment of the effectiveness of 0.5% OPA on bronchoscopes, gastroscopes, and colonoscopes.²⁸ Instruments were checked for the presence of residual bacteria, viruses, parasites, and

FIGURE 3. Mean log₁₀ reductions for *Mycobacterium chelonae*, *Mycobacterium terrae*, and *Mycobacterium bovis* produced by exposure to 0.8% phenol at 25°C for 20 minutes. The precise result is shown above each column. The bars show the standard error of the mean.

fungi after a 5-minute exposure to OPA at room temperature. Of the 100 endoscopes disinfected following use, none of these organisms could be detected (the detection limit being 10 CFU/mL). Data from microbial load assays prior to disinfection indicated that OPA effected greater than a 5-log₁₀ reduction of organisms (protected by extensive organic material) within 5 minutes. They further confirmed that the concentration of OPA remained >0.45% over the entire 14-day test period for all 19 batches tested. These data are consistent with a study by Robison et al in which both 0.5% OPA and 2% alkaline glutaraldehyde were compared in a clinical-use crossover study involving four dental operators.²⁹ This study compared the antimicrobial and clinical characteristics of both disinfectants. OPA was found to have a significantly higher antimicrobial activity than glutaraldehyde against *M bovis*, polio virus, and *Candida albicans* under all phases of clinical use. The mean tuberculocidal times of the 0.5% OPA were roughly one tenth those of the glutaraldehyde. Even after 3 weeks of storage and loading of the OPA solution with 10% human whole blood, its tuberculocidal activity remained at least two times faster than that of the fresh glutaraldehyde solution. Because OPA does not polymerize, the concentration of active ingredient did not decrease with age alone. Also, consistent with the results of Alfa and Sitter, the detrimental effects of dilution caused by clinical use were minimal for the OPA solutions. Clinically, OPA was preferred by most users because it was less corrosive than glutaraldehyde and had a much less objectionable odor.

In summary, this study has shown that *M terrae* and *M bovis* have similar resistances to OPA. The rapid grower, *M chelonae*, was significantly more susceptible. This, viewed with similar studies using other disinfectants, lends support to the consideration of *M terrae* as a replacement for *M bovis* in tuberculocidal testing of aldehydes. In addition, this work confirms the efficacy and celerity of OPA as a tuberculocidal disinfectant, acting with nearly six times

the speed of glutaraldehyde at their respective minimum effective concentrations.

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