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Disinfectant Susceptibility Profiling of Glutaraldehyde-Resistant Nontuberculous Mycobacteria

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Abstract

Objective—Activated alkaline glutaraldehyde remains one of the most widely used high-level disinfectant across the world. Yet, several reports have highlighted the potential for nontuberculous mycobacteria to develop high-level resistance to this product. Because aldehyde resistance may lead to cross-resistance to other biocides, we set out to investigate the susceptibility profile of glutaraldehyde-resistant *Mycobacterium chelonae* and *Mycobacterium abscessus* isolates to various disinfectant chemistries.

Methods—High-level disinfectants commonly used in the reprocessing of endoscopes and other heat-sensitive, semicritical medical equipment, including different formulations of aldehyde-based products and oxidizing agents, were tested against ten slow- and fast-growing, glutaraldehyde-susceptible and glutaraldehyde-resistant, *Mycobacterium* isolates in suspension and carrier tests at different temperatures.

Results—While peracetic acid- and hydrogen peroxide-based disinfectants (S40™, Resert™ XL, Reliance™ DG) efficiently killed all of the *Mycobacterium* isolates, glutaraldehyde- and ortho-phthalaldehyde-based products (Cidex®, Aldahol®, Cidex® OPA) showed variable efficacy against glutaraldehyde-resistant strains despite the ability of some formulations (Aldahol®) to overcome the resistance of some of these isolates, especially when the temperature was increased from 20 to 25°C.

Conclusions—Application permitting, oxidizing chemistries may provide a safe alternative to aldehyde-based products, particularly in the case of GTA-resistant mycobacterial outbreaks.

Introduction

The rapidly growing nontuberculous mycobacteria (NTM), *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium abscessus* subsp. *abscessus*, *Mycobacterium abscessus* subsp. *massiliense* and *Mycobacterium abscessus* subsp. *bolletii* have been

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associated with outbreaks and pseudo-outbreaks in health care settings throughout the world.^{1–4} Infections caused by *M. abscessus* complex strains are particularly problematic as these species are not only the most pathogenic rapidly-growing mycobacteria, they are also the most antibiotic resistant NTM with treatment failure rates reported to be as high as 60–70% despite years of combination therapy.⁵

Nosocomial outbreaks of *M. abscessus* subsp. *massiliense* infections that reached unprecedented epidemic proportions have occurred in Brazil between 2004 and 2008 following laparoscopic surgeries and cosmetic procedures. Over 2,000 possible cases involving 15 different states and more than 86 hospital sites were reported and shown to have been caused by one single strain of *M. abscessus* subsp. *massiliense*, named BRA100.^{3–4} The most striking feature of BRA100 isolates was their unusually high level of resistance to glutaraldehyde (GTA), the chemical used for disinfection of surgical instruments in all of the hospital sites that had confirmed cases. Survival times of the BRA100 isolates exceeded 10-hour exposure to 2% GTA. This resistance phenotype was advanced as the most likely reason for the selection and dissemination of BRA100 across the country.⁶ Although by far the largest NTM-associated outbreaks ever reported, sporadic cases of nosocomial infections and pseudo-infections caused by *M. chelonae* and *M. abscessus* isolates resistant to aldehyde-based and other high-level disinfectants have been traced to automated endoscope/bronchoscope washer disinfectors and manual dialyzer reprocessing systems in Japan, the Netherlands, the UK and the US.^{2,7–16}

While the ability of rapidly growing mycobacteria (RGM) to survive and persist within treated medical devices and washers may result from their high intrinsic tolerance to biocides, involvement in biofilms and association with free-living amoebae, it is also sometimes due to their ability to develop high level resistance to disinfectants as illustrated by *M. abscessus* subsp. *massiliense* BRA100 and a recently characterized GTA-resistant *M. chelonae* isolate from the UK.^{3,17} In the latter case, it was shown that defects in porin expression accounted, at least in part, for the resistance phenotype of the isolate, raising concerns that the selection of aldehyde-resistant strains in clinical settings might select for isolates displaying cross-resistances to other disinfectants/antibiotics and increased pathogenicity.¹⁷ Reduced porin expression in the rapidly-growing *Mycobacterium* species, *Mycobacterium smegmatis*, has indeed been associated with increased resistance not only to aldehyde-based products but also quaternary ammonium compounds, isothiazolinones, methylenbisoxazolidine and lipophilic biocides such as polyhexamethylene biguanine and octenidine dihydrochloride, in addition to multiple antibiotics.^{17–19} Furthermore, five studies reported that GTA-resistant *M. chelonae* and *M. abscessus* strains isolated either experimentally or from endoscope washer disinfectors presented an increased resistance to peracetic acid, peroxygen, sodium dichloroisocyanurate or quaternary ammonium compounds.^{13–14,20–22}

Considering the prevalence of aldehyde-resistant RGM and concerns about the possible development of cross-resistance to other biocides, we undertook to profile the disinfectant-susceptibility pattern of a panel of aldehyde- susceptible and resistant *Mycobacterium* isolates to explore alternative high-level disinfection strategies in the case of mycobacterial GTA-resistant outbreaks. A variety of high-level disinfectants commonly used in the

reprocessing of endoscopic equipment, including different formulations of aldehyde-based products, oxidizing agents and alcohol, was tested against these isolates in suspension and carrier tests.

Methods

Bacterial Strains and Standard Growth Conditions

The six control (GTA-susceptible) mycobacterial strains used in this study were *M. bovis* BCG (Pasteur strain 1173 P2), *M. avium* 104, *M. terrae* ATCC 15755, *M. chelonae* ATCC 35752, *M. abscessus* subsp. *massiliense* CIP 108297 and *M. abscessus* subsp. *massiliense* CRM-0270.³ The four NTM isolates with known resistance to GTA included *M. abscessus* subsp. *massiliense* CRM-0019,³ *M. chelonae* 9917,¹⁷ *M. chelonae* Harefield and *M. chelonae* Epping.²³ The *M. abscessus* subsp. *massiliense* CRM-0270 isolate is genetically closely related to the GTA-resistant outbreak isolate from Brazil but is GTA susceptible.³ All mycobacterial cultures were grown under agitation in Middlebrook 7H9 (Difco Laboratories, Sparks, MD) medium supplemented with 10% OADC (oleic acid, albumin, dextrose, catalase) enrichment (Becton Dickinson Microbiology Systems) and 0.05% Tween 80 (Sigma Aldrich, St. Louis, MO) at 30°C to an optical density (OD₆₀₀) of 1.0.

Disinfectants

Five commercial disinfectants labeled for high-level disinfection were evaluated along with the quaternary ammonium compound, cetrimonium bromide (10%; Sigma Aldrich). The five products included the GTA-based disinfectants Cidex® (2.4% GTA; Johnson & Johnson) and Aldahol® (3.4% GTA; Alden Medical LLC, West Springfield, MA, USA), the *ortho*-phthalaldehyde-based disinfectant Cidex® OPA (0.55% OPA; Johnson & Johnson), the peracetic acid-based products, Reliance™ DG (Steris, Mentor, OH, USA) and S40™ (Steris), and the hydrogen peroxide-based product Resert™ XL HLD (Steris). To best reflect the use of the products in clinical settings, Minimum Recommended Concentrations (MRC) or Minimum Effective Concentrations (MEC) were chosen for each disinfectant based on the minimum concentration of active disinfectant necessary to create a pass response on the corresponding manufacturer product testing strips. The pass response on Cidex® and Cidex® OPA is based on the MEC, while the pass response for Resert™ XL HLD and Aldahol® test strips is based on the MRC (see Tables 1–3). Prior to each series of tests, Cidex® and Aldahol® were tested for exact concentration of GTA using the hydroxylamine hydrochloride pH titration method and, wherever applicable, diluted to the desired concentration (MEC = 1.5% GTA for Cidex®; MRC = 1.8% GTA for Aldahol®) with sterile double distilled water. Cidex® OPA was similarly diluted to 0.3% OPA (MEC value) with sterile double distilled water. All disinfectants were freshly prepared prior to use. Susceptibility tests were performed at the recommended temperatures.

Suspension Tests

Each mycobacterial strain was prepared for testing by adding 1.0 mL of 0.9% sterile saline (Hospira, Lake Forest, IL) containing 0.1% Tween 80 to 9.0 mL of bacterial suspension (approximately 10¹⁰ Colony Forming Units [CFU]) previously washed three times with sterile double distilled water. The preparation was then dispersed for 1 min using a sterile 15

ml Wheaton pestle to generate a homogeneous bacterial suspension. One mL of bacterial suspension was added to 99 mL of the disinfectant solution maintained at the recommended temperature under continuous mixing conditions. At pre-determined time points, 1.0 mL of test solution was removed and placed in either 9.0 mL of sterile Dey Engley/1% sodium thiosulfate neutralizing broth (BD Difco, Sparks, MD; Sigma Aldrich, St. Louis, MO) for oxidizers or 9.0 mL of sterile Dey Engley/1% sodium bisulfite neutralizing broth (BD Difco, Sparks, MD; Sigma Aldrich, St. Louis, MO) for GTA and OPA, and immediately vortexed for 60 seconds to neutralize the disinfectant solution. The neutralized test solutions were then vacuum-filtered through a sterile 0.45 µm MicroFunnel™ filter unit with a Metrcel® black gridded membrane (Pall Corporation, Ann Arbor, MI). After a final rinse of the filter housing with 30–40 ml 0.9% saline/0.1% Tween 80, the filters were aseptically placed onto 7H11 agar plates supplemented with OADC and incubated at 30° for 7 to 21 days at which point CFU were counted. Control experiments performed in parallel on the GTA-susceptible strain *M. massiliense* CIP 108297 confirmed the neutralizing efficacy of the neutralizing broth with each disinfectant and verified that the neutralizer by itself did not contribute any mycobactericidal activity whether in suspension tests or on carrier disks. No significant loss of bacterial viability was observed in the presence of neutralizing broth in the test solution. The detection limit of the suspension test is 1 CFU/mL. All suspension and carrier tests were performed in duplicate using independent bacterial culture batches and the results of one typical experiment are shown.

Carrier Disk Tests

For the carrier disk test, 10 µL of each of the homogeneous bacterial suspensions described above was placed at the center of a 1 cm stainless steel carrier disk and allowed to air dry for 30–40 min. Each carrier disk was then sterilely placed inoculum side up within a 30 ml straight-side wide-mouth Nalgene jar (Thermo Scientific, Rochester, NY). 50 µL of the freshly prepared disinfectant solutions pre-heated to the recommended temperature was applied to the dried inoculum on each carrier disk and held for the desired contact time. 9.95 mL of sterile neutralizing broth was then added to the jar and vortexed for 60 seconds before vacuum filtration as described above. The carrier disk was then vortexed three more times with 15 mL of 0.9% sterile saline/0.1% Tween 80 with vacuum filtration performed between washes. The Microfunnel filters were aseptically placed onto 7H11 agar plates supplemented with OADC for CFU counting. The detection limit of the carrier disk test is 1 CFU. All suspension and carrier disk tests were performed at least twice.

Results

Approach

Several of the most commonly used disinfectants were tested against ten different rapidly growing and slowly growing mycobacteria, including four *M. chelonae* and *M. abscessus* subsp. *massiliense* isolates that had been reported previously to display resistance to GTA. Each disinfectant was tested using two independent methods, a suspension test and a carrier test, the latter being considered more stringent and more reflective than the former of the disinfection of microorganisms on dried surfaces. Tables 1 and 3 show the results of the suspension tests, while Table 2 shows the results of the carrier tests.

Glutaraldehyde-based disinfectants

All GTA-based high-level disinfectants displayed the expected mycobactericidal efficacy against the GTA-susceptible control strains, *M. bovis* BCG (Pasteur strain 1173 P2), *M. avium* 104, *M. terrae* ATCC 15755, *M. chelonae* ATCC 35752, *M. abscessus* subsp. *massiliense* CIP 108297 and *M. abscessus* subsp. *massiliense* CRM-0270. Under the suspension test, control strains were reduced to no detectable bacterial counts within 10 min of exposure to Cidex® (1.5% GTA) at 25°C, except for the slow-growing species *M. avium*, *M. terrae* and *M. bovis* BCG which required longer contact times of 15 to 30 min [Table 1]. Reduction to no detectable bacterial counts was achieved for all control strains within 5 min of exposure to Aldahol® (1.8% GTA), a GTA-based product containing isopropanol and potassium acetate,²⁴ at both 20°C and 25°C [Table 1]. The carrier disk test yielded similar results with reduction to no viable bacteria for all GTA-susceptible strains and GTA-based products tested [Table 2].

As expected, the GTA-resistant *M. abscessus* subsp. *massiliense* and *M. chelonae* isolates, CRM-0019, 9917, Harefield and Epping, showed significant resistance to the GTA-based products with less than a half log₁₀ reduction in CFUs after 10 min exposure to Cidex® (1.5% GTA) in suspension tests (less than 3.2 log₁₀ reduction in CFUs after the recommended time of exposure of 45 min), and less than a 3.6 log₁₀ CFU reduction after 45 min in the carrier test [Tables 1 and 2]. For Aldahol® (1.8% GTA), the suspension test results for the GTA-resistant strains at the recommended exposure time of 10 min and temperature of 20°C showed significant resistance to this disinfectant as well [Table 1]. The greatest CFU reduction at the 10 min time point was seen for the GTA-resistant *M. abscessus* subsp. *massiliense* strain CRM-0019 (3.33 log₁₀ CFU reduction), while the GTA-resistant *M. chelonae* strains 9917, Harefield and Epping showed less than a 1.0 log reduction in CFU counts. When tested at additional time points out to 30 min, the three GTA-resistant *M. chelonae* strains continued to show greater resistance with a maximum 1.0 log₁₀ reduction in CFUs while *M. abscessus* subsp. *massiliense* CRM-0019 was reduced to no detectable CFU by 30 min. The carrier disk testing similarly pointed to the significant resistance of *M. chelonae* 9917, Harefield and Epping with no more than a 4.42 log₁₀ CFU reduction after 20 min, while *M. abscessus* subsp. *massiliense* CRM-0019 again showed susceptibility to Aldahol® at the extended 20 min time point with a greater than 7.0 log₁₀ CFU reduction [Table 2]. When the testing temperature for Aldahol® was increased from 20 to 25°C, the suspension testing results showed greater bactericidal efficacy. All GTA-resistant strains but one were reduced to no detectable CFU within the recommended 10 min. However, *M. chelonae* Harefield only achieved a 0.68 log₁₀ reduction after 10 min and, even at the extended 30 min time point, CFU counts were only reduced by a meager 1.62 log₁₀ [Table 1]. Since the 1.8% GTA Aldahol® preparation contains isopropanol,²⁴ 15% isopropanol alone was tested for mycobactericidal activity. Neither *M. abscessus* subsp. *massiliense* CIP108297 nor the GTA-resistant *M. abscessus* subsp. *massiliense* strain CRM-0019 showed any significant reduction in CFU counts upon 30 min exposure to this compound [Table 1].

ortho-phthalaldehyde

Suspension test results for the Cidex® OPA (0.3% OPA) at 25°C achieved reduction to no detectable CFU counts at the recommended time point of 5 min for all *M. chelonae* strains except for *M. chelonae* Harefield. However, this strain was reduced to no detectable CFU by the 10 min time point at 25°C, and to no detectable CFU by the recommended 12 min time point at 20°C [Table 1]. When 0.3% OPA was tested under the carrier disk method, all test strains were reduced to no viable CFU within 5 min [Table 2].

Oxidizing agents and other disinfectant products

For the two disinfectants requiring usage in automated endoscope washers at 50°C, S40™ and Reliance™ DG (two peracetic acid-based products), CFU counts for all tested strains were reduced below detection levels within 2 min of exposure under the suspension testing method, and by the 6 min time point under the carrier disk method [Tables 2 and 3]. To assess the impact of the 50°C temperature on the viability of mycobacteria, two GTA-resistant strains, *M. abscessus* subsp. *massiliense* CRM-0019 and *M. chelonae* Harefield, were exposed to sterile water at 50°C for up to 6 min; in both cases, no significant loss of viability was detected [Table 3]. For Resert™ XL (a hydrogen peroxide-based product), viable bacterial counts were reduced greater than 7 log₁₀ within 8 min under both the suspension and carrier disk methods [Tables 2 and 3]. Finally, testing of a quaternary ammonium compound found in many disinfectants and antiseptics (10% CTAB) in the suspension test demonstrated, as expected,²² very modest bactericidal activity against rapidly growing mycobacteria, with less than 4 log₁₀ reduction in CFU for all tested strains after 24 hours [Table 3].

Discussion

Whereas OPA is now the leading high-level disinfectant in the USA, activated alkaline GTA at ambient temperature remains one of the most widely used high-level disinfectant across the world for its wide spectrum microbicidal activity, relatively low cost, ease of use and its non-damaging effects on medical equipment and reprocessors. Concerns over the emergence of globally-circulating virulent clones of *M. abscessus* complex species²⁵ coupled to reports of rapidly growing mycobacteria presenting resistance to aldehyde-based products, including recently, during the largest outbreak ever reported of *M. abscessus* complex infections in Brazil,^{3-4,6} and the finding that some of the mechanisms of resistance to aldehydes evolved by NTM may confer cross-resistance to other disinfectants and antibiotics,^{17,19} led us to systematically investigate the susceptibility profile of a collection of GTA-resistant *M. chelonae* and *M. abscessus* subsp. *massiliense* isolates to various disinfectant chemistries using two different testing methods.

Tests with the GTA-based products Cidex® and Aldahol® confirmed the aldehyde resistance phenotype of the four *M. abscessus* subsp. *massiliense* and *M. chelonae* isolates from the UK and Brazil. Interestingly, increasing the incubation temperature of Aldahol® from 20 to 25°C overcame this resistance in the case of all isolates but one, *M. chelonae* Harefield, probably reflecting differences in the physiology and mechanisms of aldehyde-resistance evolved by these isolates. Similarly, Cidex® OPA at 25°C showed variable

efficacy against the GTA-resistant strains achieving only partial killing of *M. chelonae* Harefield after the recommended 5 min time point in the suspension test at the minimum effective concentration of 0.3% OPA; however, although CFU counts for all isolates were reduced to 0 upon exposure to 0.3% OPA for 12 min at 20°C. Under the testing conditions used herein that lacked organic load in the suspensions and, therefore, favored disinfectant activity, all of the *Mycobacterium* isolates tested were fully susceptible to the peracetic acid- and hydrogen peroxide-based disinfectants, independent of their level of resistance to GTA.

In summary, all of the oxidizing disinfectants tested in this study effectively killed GTA-resistant mycobacteria while aldehyde-based products (GTA and OPA) showed variable results despite evidence that changing the products formulation (i.e., Aldahol®) and increasing the temperature might improved their efficacy against some isolates. Although more isolates would need to be tested in the presence and absence of organic load to reach definitive conclusions, it appears that the use of oxidizing chemistry (compatibility with the medical devices permitting) provides a safe alternative to aldehyde-based products, especially when GTA resistance is suspected. Alternatively, increasing the temperature and/or formulation of aldehyde-based products may increase their efficacy but this option should carefully be evaluated on a case-to-case basis.

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Table 1

Mycobactericidal effects of aldehyde-based products against GTA-susceptible and GTA-resistant *Mycobacterium* isolates in suspension tests.

Product tested	Mmas CIP 108297 (GTA ^S)	Mmas CRM-0270 (GTA ^S)	Mmas CRM-0019 (GTA ^R)	Mch ATCC 35752 (GTA ^S)	Mch 9917 (GTA ^R)	Mch Hardfield (GTA ^R)	Mch Epping (GTA ^R)	M. bovis BCG (Pasteur) (GTA ^S)	M. avium 104 (GTA ^S)	M. terrae ATCC 15755 (GTA ^S)
Cidex® (1.5% GTA) 25°C										
0 min	8.20	8.29	7.79	7.11	7.06	7.60	7.65	6.29	8.04	7.97
5 min	5.27±0.08	0	7.47±0.04	0	7.55±0.05	7.96±0.00	8.02±0.11	4.33±0.26	nd	4.70±0.18
10 min	0	0	7.48±0.13	0	7.49	7.87±0.06	7.95±0.02	3.30±2.55	5.97±0.03	3.81±0.14
15 min	0	0	7.48±0.05	0	6.95±0.21	7.70±0.05	7.91±0.04	0	5.10±0.35	3.18±0.21
30 min	0	0	7.23±0.03	0	7.23±0.03	7.50±0.09	7.73±0.04	0	0	0
0 min	nd	nd	7.39	nd	8.83	8.33	8.38	nd	nd	nd
45 min	nd	nd	6.76±0.02	nd	5.63±0.38	6.85±0.15	7.20±0.01	nd	nd	nd
Aldahol® (1.8% GTA) 20°C										
0 min	7.25	8.29	6.69	7.11	7.06	7.60	7.65	6.29	8.16	7.97
5 min	0	0	4.54±0.13	0	7.39±0.09	7.78±0.00	7.45±0.04	0	0	0
10 min	0	0	3.36±0.01	0	7.27	7.64±0.01	7.15±0.17	0	0	0
15 min	0	0	2.49±0.71	0	6.97	7.44±0.08	6.97±0.04	0	0	0
30 min	0	0	0	0	6.13	7.17±0.03	6.65±0.00	0	0	0
Aldahol® (1.8% GTA) 25°C										
0 min	7.25	8.29	6.69	7.11	8.13	7.60	7.65	6.29	8.32	7.97
5 min	0	0	0	0	0	7.30±0.02	4.70±0	0	0	0
10 min	0	0	0	0	0	6.92±0.25	0	0	0	0
15 min	0	0	0	0	0	6.58±0.17	0	0	0	0
30 min	0	0	0	0	0	5.98±0.17	0	0	0	0
15% Isopropanol 25°C										
0 min	8.31	nd	8.23	nd	nd	nd	nd	nd	nd	nd
5 min	8.36±0.07	nd	9.02±0.03	nd	nd	nd	nd	nd	nd	nd
10 min	8.53±0.15	nd	8.86±0.08	nd	nd	nd	nd	nd	nd	nd
15 min	8.43±0.03	nd	8.87±0.00	nd	nd	nd	nd	nd	nd	nd

Product tested	<i>Mmas</i> CIP 108297 (GTA ^S)	<i>Mmas</i> CRM-0270 (GTA ^S)	<i>Mmas</i> CRM-0019 (GTA ^R)	<i>Mch</i> ATCC 35752 (GTA ^S)	<i>Mch</i> 9917 (GTA ^R)	<i>Mch</i> Harefield (GTA ^R)	<i>Mch</i> Epping (GTA ^R)	<i>M. bovis</i> BCG (Pasteur) (GTA ^S)	<i>M. avium</i> 104 (GTA ^S)	<i>M. terrae</i> ATCC 15755 (GTA ^S)
30 min	8.32±0.05	nd	8.88±0.10	nd	nd	nd	nd	nd	nd	nd
Cidex® OPA (0.3% OPA) 25°C										
0 min	7.25	8.29	7.32	7.11	8.13	7.63	7.65	6.29	8.32	7.97
5 min	0	0	0	0	0	3.92±0.21	0	0	0	0
10 min	0	0	0	0	0	0	0	0	0	0
15 min	0	0	0	0	0	0	0	0	0	0
30 min	0	0	0	0	0	0	0	0	0	0
Cidex® OPA (0.3% OPA) 20°C										
0 min	nd	5.13	7.64	7.69	8.27	7.65	7.80	nd	nd	nd
12 min	nd	0	0	0	0	0	0	nd	nd	nd
30 min	nd	0	0	0	0	0	0	nd	nd	nd

Shown are the mean log₁₀ CFU counts and standard deviations of duplicate tests for each strain and test product. The 0 min time point (enumeration of CFU in the bacterial suspension prior to disinfectant exposure) is the result of a single serial dilution plating. GTA^R, GTA-resistant; GTA^S, GTA-susceptible. nd, not determined. Control suspension tests using the GTA-susceptible isolate, *M. massiliense* CIP 108297, confirmed the neutralizing efficacy of the neutralizing broth with each disinfectant product. The detection limit of the suspension tests is 1 CFU/mL.

Table 2

Mycobactericidal effects of aldehyde-based and oxidizing high-level disinfectants against GTA-susceptible and GTA-resistant *Mycobacterium* isolates in carrier tests.

Product tested	<i>Mmas</i> CIP 108297 (GTA ^S)	<i>Mmas</i> CRM-0270 (GTA ^S)	<i>Mmas</i> CRM-0019 (GTA ^R)	<i>Mch</i> ATCC 35752 (GTA ^S)	<i>Mch</i> 9917 (GTA ^R)	<i>Mch</i> Hardfield (GTA ^R)	<i>Mch</i> Epping (GTA ^R)	<i>M. bovis</i> BCG (Pasteur) (GTA ^S)	<i>M. avium</i> 104 (GTA ^S)	<i>M. terrae</i> ATCC 15755 (GTA ^S)
Cidex® (1.5% GTA) 25°C										
0 min	7.55±0.03	7.30±0.06	7.52±0.00	7.59±0.05	6.91±0.11	7.34±0.01	7.44±0.11	nd	nd	nd
45 min	0	0	6.14±0.06	0	4.59±0.28	3.74±0.00	5.77±1.3	nd	nd	nd
Aldahol® (1.8% GTA) 20°C										
0 min	7.55±0.03	7.30±0.06	7.52±0.00	7.59±0.05	6.91±0.11	7.38±0.12	7.44±0.11	nd	nd	nd
20 min	0	0	0	0	4.57±0.17	2.96±0.09	5.94±0.35	nd	nd	nd
15% Isopropanol 25°C										
0 min	7.55±0.03	7.30±0.06	nd	7.59±0.05	6.91±0.11	7.38±0.12	7.44±0.11	nd	8.43	nd
20 min	7.67±0.34	7.77±0.00	nd	7.91±0.05	6.89±0.02	7.45±0.14	7.52±0.17	nd	6.33±0.00	nd
Cidex® OPA (0.3% OPA) 25°C										
0 min	7.55±0.03	7.30±0.06	7.52±0.00	7.59±0.05	6.91±0.11	7.38±0.12	7.44±0.11	nd	nd	nd
5 min	0	0	0	0	0	0	0	nd	nd	nd
Reliance™ DG 50°C										
0 min	7.45±0.01	7.59±0.06	8.31±0.00	7.63±0.03	6.49±0.00	7.63±0.06	7.46±0.00	nd	8.43±0.03	7.00±0.08
6 min	0	0	0	0	0	0	0	nd	0	0
S40™ 50°C										
0 min	7.45±0.01	7.59±0.06	8.31±0.00	7.63±0.03	6.49±0.00	7.63±0.06	7.46±0.00	nd	8.43±0.03	7.00±0.08
6 min	0	0	0	0	0	0	0	nd	0	0
Resert™ XL 25°C										
0 min	7.55±0.03	7.30±0.06	nd	7.59±0.05	6.91±0.11	7.38±0.12	7.44±0.11	nd	nd	nd
8 min	0	0	nd	0	0	0	0	nd	nd	nd

See Table 1 for legend. Control carrier tests using the GTA-susceptible isolate, *M. massiliense* CIP 108297, confirmed the neutralizing efficacy of the neutralizing broth with each disinfectant product. The detection limit of the carrier disk tests is 1 CFU.

Table 3

Mycobactericidal effects of oxidizing high-level disinfectants and a quaternary ammonium compound against GTA-susceptible and GTA-resistant *Mycobacterium* isolates in suspension tests.

Product tested	Mmas CIP 108297 (GTA ^S)	Mmas CRM-0270 (GTA ^S)	Mmas CRM-0019 (GTA ^R)	Mch ATCC 35752 (GTA ^S)	Mch 9917 (GTA ^R)	Mch Harefield (GTA ^R)	Mch Epping (GTA ^R)	M. bovis BCG (Pasteur) (GTA ^S)	M. avium 104 (GTA ^S)	M. terrae ATCC 15755 (GTA ^S)
Reliance™ DG 50°C										
0 min	8.39	8.42	9.00	8.79	7.54	8.13	7.05	6.92	9.15	7.92
2 min	0	0	0	0	0	0	0	0	0	0
6 min	0	0	0	0	0	0	0	0	0	0
S40™ 50°C										
0 min	8.63	8.73	9.11	8.58	7.75	7.92	7.91	6.92	9.15	7.92
2 min	0	0	0	0	0	0	0	0	0	0
6 min	0	0	0	0	0	0	0	0	0	0
Resert™ XL 25°C										
0 min	8.63	8.73	9.11	8.58	7.89	7.92	8.40	nd	9.15	nd
4 min	0	3.12±0.18	3.51±0.22	0	0	4.17±0.11	0	nd	0	nd
8 min	0	0	0	0	0	0	0	nd	0	nd
CTAB 25°C										
0 min	8.39	8.42	9.00	8.79	7.89	8.13	8.40	nd	nd	nd
60 min	5.97±0.01	6.15±0.02	nd	8.33±0.02	7.61±0.03	7.97±0.04	nd	nd	nd	nd
1440 min	6.27	5.55±0.12	7.16±0.05	5.05±0.90	6.46±0.16	7.43±0.01	6.35±0.03	nd	nd	nd
Sterile Water 50°C										
0 min	nd	nd	8.80	nd	nd	8.20	nd	nd	nd	nd
2 min	nd	nd	8.27±0.11	nd	nd	7.96±0.02	nd	nd	nd	nd
6 min	nd	nd	8.24±0.13	nd	nd	8.00±0.12	nd	nd	nd	nd

See Table 1 for legend. Control suspension tests using the GTA-susceptible isolate, *M. massiliense* CIP 108297, confirmed the neutralizing efficacy of the neutralizing broth with each disinfectant product. The detection limit of the suspension tests is 1 CFU/mL.