Aldahol high-level disinfectant

Norman Miner, PhD, Valerie Harris, MPH, Thuy Dung Cao, BS, Towanda Ebron, BS, and Natalie Lukomski, MS
Euless, Texas

Background: Glutaraldehyde and ortho-phthalaldehyde (OPA) are widely used as the active ingredients of high level disinfectants for heat-sensitive, semicritical medical instruments. However, both of these chemicals have limitations in their spectrum of antimicrobial activity. Glutaraldehyde disinfectants are poorly mycobactericidal, and require impractically long exposure times to kill spore-forming bacteria. OPA disinfectants kill many types of mycobacteria in practical exposure times, but require 32 hours to pass the Association of Official Analytical Chemists (AOAC) sporicidal test, and do not claim to be sterilants. These could be serious limitations that contribute to the formation of biofilms in endoscopes, after which the endoscopes are difficult to disinfect. The objective of our research was to discover a disinfectant formulation, based on aldehydes, that killed mycobacteria and spore-forming bacteria in a practical exposure time and temperature.

Methods: Solutions of glutaraldehyde or OPA were prepared with various concentrations of alcohols, sodium and potassium salts, chelating agents, and detergents at alkaline pH values, and tested against cultures of mycobacteria and spore-forming bacteria to find a formulation that would kill these bacteria in practical exposure times at 20°C or 25°C.

Results: Concentrations of ≤ 20% w/w isopropanol and ≤ 8% potassium acetate in combination with ≤ 3.5% w/w glutaraldehyde at alkaline pH values killed 6 $\log_{10}$ of mycobacteria within 10 minutes at 20°C. Similar combinations killed 6 $\log_{10}$ of *Bacillus subtilis* in suspension within 30 minutes at 25°C, and *B subtilis* within 60 minutes at 20°C. The sporicidal activity of OPA was not increased by combination with isopropanol and potassium acetate salts.

Conclusions: Aldahol high-level disinfectant (US FDA K041360), a formulation of 3.5% glutaraldehyde in combination with 20% w/w isopropanol and 8% potassium acetate, kills mycobacteria within 10 minutes at 20°C and kills 6 $\log_{10}$ of cultures of the spore-forming bacteria *B subtilis* within 60 minutes at 20°C.

Key Words: Glutaraldehyde; sporicidal; tuberculocidal; mycobactericidal.

The exposure times for glutaraldehyde high-level disinfectants at 20°C or 25°C generally range from 25 minutes to 45 minutes, and sometimes require as long as 90 minutes.[1] These exposure times are driven by the slow killing of mycobacteria by glutaraldehyde.[2-4] Some glutaraldehyde disinfectants have decreased the high-level disinfection exposure time by increasing the exposure temperature to 35°C, but these disinfectants require some type of heating device and careful recordkeeping regarding exposure temperature. To compensate for these long exposure times, professional organizations have agreed that thoroughly cleaned and rinsed medical devices are not likely to have more than approximately 1000 colony-forming units (CFU) of any type of bacteria, and thus may be exposed to glutaraldehyde disinfectants for 20 minutes at 20°C.[5]

The recommended sporicidal exposure times for glutaraldehyde disinfectants also are impractically long, ranging from 5.75 hours at 35°C to 10.0 hours at 25°C.[6] These exposure times for sterilants are derived from the Association of Official Analytical Chemists (AOAC) Sporicidal Test 966.04, a very stringent test that dries the spore-forming bacteria *B subtilis* and *C sporogenes* as cultured in their organically rich media onto unglazed porcelain penicylinders and braided suture loops.[7]

Formulations of 0.55% OPA, introduced as high-level disinfectants in the late 1990s, kill most species of mycobacteria within 12 minutes at 20°C or within 5 minutes at 25°C.[8] Disinfectants containing OPA are poorly sporicidal, however, requiring 32 hours to pass the AOAC sporicidal test, and these disinfectants do not claim to be sterilants.[9]

Hydrated spores are more likely to be found on freshly used and freshly cleaned and rinsed medical devices than are spores dried in their culture media as used in the AOAC sporicidal test, and thus are a more realistic representation of the spore-forming bacteria that might be found on medical devices, especially gastroscopes and colonoscopes. The killing of hydrated spore-forming bacteria in suspension can be measured by rate-of-kill tests, also known as survivor tests. Such rate-of-kill tests are commonly used in Europe (European

From MicroChem Laboratory, Inc, Euless, TX.

Address correspondence to Norman Miner, PhD, MicroChem Laboratory, Inc, 1107-C South Airport Circle, Euless, TX 76040. E-mail: nminer@microchemlab.net.

The research and manuscript preparation for this manuscript was funded entirely by MicroChem Laboratory, Inc. Norman Miner discloses that he is the inventor of Aldahol High Level Disinfectant (FDA K041360), and has a royalty interest in this disinfectant.

0196-6553/$36.00

doi:10.1016/j.ajic.2009.08.009

suspension test) to determine sterilization exposure times.[10,11] Even with these less-stringent tests, the time required to kill 6 $\log_{10}$ of spore-forming bacteria by solutions of 2.0% w/w alkaline glutaraldehyde ranges from 120 minutes to 240 minutes at 25°C, and OPA disinfectants kill ≤ 1 $\log_{10}$ of *B subtilis* within 4 hours (the longest exposure time tested). Within an exposure time of 20 minutes at 20°C or 25°C, alkaline glutaraldehyde and OPA, do not kill hydrated cells of spore-forming bacteria to any significant degree.

In an attempt to discover a high-level disinfectant formulation with practical exposure times and temperatures to kill mycobacteria and hydrated cells of spore-forming bacteria, as well as gram-positive and gram-negative bacteria, fungi, and the major virus surrogates, we evaluated various combinations of glutaraldehyde and other aldehydes with different types and concentrations of alcohols, detergents, monovalent cations, chelating agents, and buffer salts against several species of mycobacteria and spore-forming bacteria in rate-of-kill suspension tests at temperatures of 20°C to 25°C.

MATERIALS AND METHODS

Tests for rate of kill of mycobacteria

Mycobacterium bovis var BCG, obtained from Organon Teknika, Schering-Plough, *M terrae* ATCC 15755, obtained from the American Type Culture Collection, and glutaraldehyde-resistant *M chelonae* var abscessus ATCC 14472,[12] obtained from the American Type Culture Collection, were cultured quiescently in 25 × 150-mm screw-capped test tubes for 28 to 35 days (7 to 10 days for *M chelonae*) in Middlebrook 7H9 medium (M7H9) (BD Diagnostic Systems, Sparks, MD) fortified with 5% OADC medium (BD Diagnostic Systems) at 35 ± 2°C. The mature cultures were mixed for 30 seconds on a vortex mixer, and then homogenized with 5 to 10 slow strokes in a 40-mL Tenbroeck tissue homogenizer (Corning Life Sciences, Lowell, MA) to break up clumps of cells. Bovine calf serum (HyClone, Logan, UT) was added to the homogenized cultures to 5% v/v. For *M bovis* and *M terrae*, 1.0 mL of the homogenized culture was added to 9.0 mL of the various test disinfectant solutions in test tubes (the reaction test tube), briefly mixed in a vortex mixer, and then held at 20°C or 25°C in a water bath. After various exposure times (ie, 2.5, 5, 7.5, 10, 15, 20, and 30 minutes), a 1-mL aliquot was removed from the reaction test tube and serially diluted 10-fold in Dey-Engley neutralizing recovery medium (BD Diagnostic Systems). Each dilution was filtered through a 0.45-μm membrane filter, the filter rinsed with sterile water, and the membrane placed onto M7H9 agar (BD Diagnostics) fortified with 10% OADC medium in petri plates. The petri plates were incubated inverted at 35 ± 2°C for 12 to 15 days for *M terrae* and 25 to 30 days for *M bovis* var BCG, and then evaluated for colonies of surviving mycobacteria.

For *M chelonae*, sterile polyester suture loops were soaked in the culture of *M chelonae* and then dried for 30 minutes at 35 ± 2°C in a petri plate. The inoculated and dried loops were placed into 5 mL of the various test disinfectant solutions in test tubes, and held at 20°C or 25°C in a water bath for 5, 10, and 15 minutes. After the prespecified exposure time, 15 mL of Dey-Engley neutralizing recovery medium was added to each tube, and the contents were mixed for 1 minute on a vortex mixer to release the cells into suspension. The surviving mycobacteria were counted by making serial 10-fold dilutions into M7H9 broth, placing 0.5-mL portions of each dilution onto M7H9 agar fortified with 10% OADC in petri plates, incubating the plates inverted for 7 to 10 days at 35 ± 2°C, and then counting the colonies. The entire contents of the reaction test tube also were evaluated for surviving mycobacteria by filtering the contents through a membrane filter and counting the surviving colonies as described above.

The surviving colonies were counted and multiplied by the appropriate dilution factor to determine the surviving colonies in the reaction tube at any given exposure time. The fraction of surviving cells was calculated as the number of survivors at any given exposure time (S) divided by the original number of cells in the reaction test tube (S_0). This value was plotted on a semilogarithmic graph as a function of exposure time to generate the rate-of-kill survivor curves/lines.

Tests for rate of kill of spore-forming bacteria

B subtilis ATCC 19659 was cultured according to the methods specified in AOAC Sporicidal Test 966.04. The titer of this *B subtilis* culture was 10^7 CFU/mL. In this procedure, 1 mL of this culture of *B subtilis* was added to 9.0 mL of the various test disinfectants, held in the reaction test tube in a water bath at 20°C or 25°C, and then sampled for surviving colonies at various time intervals (ie, 15, 30, 45, 60, 90, 120, 150, and 240 minutes). After serial 10-fold dilutions into Dey-Engley neutralizing recovery medium, 0.5 mL was added to the surface of nutrient agar in petri plates, the plates were incubated for 48 to 72 hours at 35 ± 2°C, and then colonies were counted and multiplied by the appropriate dilution factor to determine the surviving colonies in the reaction test tube. The number of surviving colonies at any given exposure time (S) was divided by the original number of cells in the reaction test tube (S_0), and this fraction (S/S_0) was plotted as a function of the exposure time to give the rate-of-kill curve/line for these spore-forming bacteria.