

Brief Report

Inactivation of *Clostridium difficile* Spores by Disinfectants

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ABSTRACT

OBJECTIVE: The current study was designed to evaluate the activity of glutaraldehyde-based disinfectants against *Clostridium difficile* using the Association of Official Analytical Chemists' (AOAC) sporicidal test. This study was undertaken because gastrointestinal endoscopes that may be contaminated with *C difficile* spores are most commonly disinfected between patients using glutaraldehyde-based disinfectants.

DESIGN: Using the AOAC test, the following disinfectants were tested: 2% alkaline glutaraldehyde, 2% acid glutaraldehyde, a 1:16 dilution of a 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate, and a 1:20 dilution of a 10% glutaraldehyde-0.5% phenylphenol-0.1% amyphenol.

RESULTS: Test results of the four disinfectants against *C difficile* spores at exposure times of 10, 20, and 60 minutes were as follows

(number of positive penicylinders per 30 replicates): 0, 0, and 0 for 2% alkaline glutaraldehyde; 6, 3, and 0 for 2% acid glutaraldehyde; 30, 29, and 30 for a 1:16 dilution of glutaraldehyde-7.05% phenol-1.20% sodium phenate; and 30, 30, and 30 for a 1:20 dilution of glutaraldehyde-0.5% phenylphenol-0.1% amyphenol.

CONCLUSIONS: *C difficile* spores are more susceptible to inactivation by glutaraldehyde-based disinfectants than the spore-forming organisms recommended in the AOAC sporicidal test (i.e., *Bacillus subtilis* and *Clostridium sporogenes*). Diluting glutaraldehyde-based disinfectants below 2% led to the inability to inactivate spores of *C difficile* using exposure times commonly employed to disinfect semicritical items such as gastrointestinal endoscopes. (*Infect Control Hosp Epidemiol.* 1993;14:36-39.)

INTRODUCTION

Current studies suggest that *Clostridium difficile* is the causative agent in 15% to 25% of antibiotic-associated diarrhea and 70% to 95% of antibiotic-associated colitis. Clustering of cases within hospitals

is well described.^{1,2} Recent studies using molecular analysis of *C difficile* strains indicate that unique organisms have been responsible for both epidemic and endemic nosocomial *C difficile* disease and carriage.^{3,4} This suggests that many cases of *C difficile*

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TABLE

ACTIVITY OF GLUTARALDEHYDE-BASED DISINFECTANTS AGAINST *C DIFFICILE* SPORES USING THE AOAC SPORICIDAL TEST*

Disinfectant	No. of Positive Penicylinders/30 Replicates		
	Exposure Time (Minutes) at °		
	10	20	60
2% alkaline glutaraldehyde	0	0	0
2% acid glutaraldehyde	6†	3	0
0.13% glutaraldehyde-0.44% phenol-0.08% sodium phenate	30†	29†	30†
0.5% glutaraldehyde-0.025% phenylphenol 0.005% amyphenol	30†	30†	30†

*Mean number of *C difficile* spores per penicylinder was 2.3×10^4 .
†Statistically significantly ($p < .05$) by two-sided Fisher's exact test when compared with 0 positive penicylinder per 30 replicates (complete inactivation).

disease are due to exogenous acquisition of organisms (from the environment or human contacts) with resultant diarrhea in hosts predisposed by antimicrobial therapy.

Endoscopy is a widely used diagnostic and therapeutic procedure. Endoscopes are routinely contaminated during use, and high-level disinfection following each patient use is recommended to prevent indirect contact transmission of infectious agents.^{5,6} Inappropriate disinfection has led to cross-transmission of infection between patients with hepatitis B, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Salmonella* species, *Strongyloides*, and other microorganisms.^{1,6} Most hospitals choose to employ glutaraldehyde-based agents to achieve high-level disinfection of endoscopes.⁷

Endoscopy may be performed in patients with suspected *C difficile* colitis to identify pseudomembrane formation. In infected patients, such use will lead to contamination of the endoscope with *C difficile*. Because *C difficile* is a spore-forming organism, it is likely to be relatively resistant to chemical disinfectants.^{5,6} We therefore undertook this study to assess the efficacy of glutaraldehyde-based disinfectants against *C difficile* spores. Both 2% glutaraldehydes and diluted glutaraldehyde-based formulations were tested.

MATERIALS AND METHODS

The Association of Official Analytical Chemists' (AOAC) sporicidal test⁸ was used to assess the sporicidal activity of glutaraldehyde-based disinfectants. The *C difficile* isolate was obtained from a 45-year-old white male transferred to the University of North Carolina (UNC) Hospitals for fever, headache, and mental status changes despite six days of antibiotic therapy. At UNC Hospitals, the patient was treated for presumed Rocky Mountain Spotted Fever with doxycycline with resolution of all symptoms. A toxin-producing strain of *C difficile* was isolated from

In brief, the AOAC sporicidal test was conducted as follows. The *C difficile* isolate was grown in egg-meat media (Difco Laboratories, Detroit, Michigan) at 37°C for 72 hours. The media was then sterilely filtered through a funnel containing glass wool into sterile glass tubes. Hollow porcelain cylinders (i.e., penicylinders or carriers) were inoculated by being immersed for 10 to 15 minutes in the glass tubes containing the filtered *C difficile*. After removal and vacuum drying for 24 hours at room temperature, the inoculated penicylinders were placed into the disinfectant solutions and exposed for 10, 20, or 60 minutes at 20°C. After exposure to the disinfectant, each penicylinder was removed and placed into a 10 ml tube containing thioglycollate broth, and then removed and transferred to a second 10 ml tube containing thioglycollate broth. Both tubes were then tightly capped and incubated at 37°C for 21 days and examined for growth. The presence of *C difficile* spores was monitored by exposing inoculated penicylinders to 2.5 N hydrochloric acid as specified in the AOAC sporicidal test. Test spores resisted hydrochloric acid for 2 minutes or longer.

The number of organisms per penicylinder (i.e., microbial load) was determined as follows: an inoculated penicylinder was placed into a tube containing Tween 80-saline, exposed to ultrasound, and vortexed. The number of colony forming units (CFU) per ml was quantitated by a spread plate method.

All products were tested using 30 inoculated penicylinders. The number of *C difficile* spores on inoculated penicylinders averaged 2.3×10^4 . The number of spores on inoculated penicylinders did not vary significantly (<0.5 log) during a test day. All disinfectants were purchased for the study and used within their specified use-life. Sterile distilled water (USP, Travenol Laboratories, Deerfield, Illinois) was used for disinfectant dilution, and all disinfectants were used according to the manufacturers' instruc-

ents before dilution, activated shelf life, use-dilution, and sporicidal label claims were: 2% alkaline glutaraldehyde, 14 days, undiluted, and 10 hours at 25°C; 2% acid glutaraldehyde, 2 years, undiluted, and 10 hours at 21°C; 10% glutaraldehyde with 0.5% ortho-phenylphenol and 0.1% para-tertiary amylphenol, 30 days, 1:5 dilution for 6 hours at 20°C or 1:20 dilution for 12 hours at 20°C; and 2% glutaraldehyde with 7.05% phenol and 1.2% sodium phenate, 30 days, undiluted, and 6.75 hours at 20°C. Because the latter product is recommended to be used at a 1:16 dilution as a high-level disinfectant and because this is the concentration used for endoscope disinfection,⁷ this concentration was tested.

RESULTS

Test results of the four disinfectants against *C difficile* spores are displayed in the Table. The 2% alkaline glutaraldehyde demonstrated excellent sporicidal activity even at brief exposure times. In contrast, the diluted glutaraldehyde-based disinfectants demonstrated no measurable activity in the AOAC sporicidal test, even with a 60-minute exposure time. The 2% acid glutaraldehyde demonstrated some breakthrough at exposure times of 10 and 20 minutes.

When the 2% acid and alkaline glutaraldehydes were tested against *C difficile* spores using the manufacturers' recommended sporicidal label claim (10-hour exposure), both demonstrated complete inactivation of *C difficile* spores (0 positive penicylinders/30 replicates). A 1:16 dilution of 2% glutaraldehyde-7.05% phenol-1.2% sodium phenate also demonstrated complete inactivation of *C difficile* spores with an exposure time of 12 hours (0 positive penicylinders/30 replicates). However, a 1:20 dilution of 10% glutaraldehyde-0.5% phenylphenol-0.1% amylphenol demonstrated no sporicidal activity at the manufacturer's recommended label claim of 12 hours (30 positive penicylinders/30 replicates).

DISCUSSION

C difficile is carried in the gastrointestinal tract of 2% to 4% of the normal adult population, and has been isolated from 15% of asymptomatic medical patients and from 30% to 75% of asymptomatic neonates.¹ It is the most important etiology of antibiotic-associated colitis.⁹ Both endemic and epidemic nosocomial infections have been described. Gerding has summarized the two basic hypotheses regarding the origin of *C difficile* diarrhea: the first is endogenous activation (through antimicrobial or antineoplastic drug use) of asymptotically carried pathogens; the second is exogenous acquisition of organisms (from the environment or human contacts) with resultant diarrhea

The latter hypothesis is supported by recent studies using molecular analysis of *C difficile* strains that indicate unique organisms are responsible for multiple cases of infection.

Because endoscopes have been demonstrated to serve as a vehicle for cross-contamination of enteric pathogens, it is recommended that they be meticulously cleaned and then disinfected with a high-level disinfectant between patients.^{5,6} A recent survey of all hospitals in North Carolina revealed that the most common means of high-level disinfection was immersion in 2% glutaraldehyde.⁷ Immersion in 0.13% glutaraldehyde-0.44% phenol-0.08% sodium phenate also was used. Most hospitals employed short immersion times: 44% for less than or equal to 10 minutes and 49% for 15 to 20 minutes.

Environmental contamination with *C difficile* is common.¹¹ Cases of cross-transmission commonly occur via contamination of the hands of medical personnel.¹² However, contaminated endoscopes such as colonoscopes can serve as a vehicle of transmission. For this reason, we studied commonly used disinfectants and exposure times to assess whether current practices may be placing patients at risk. Our data demonstrate that 2% alkaline glutaraldehyde reliably kills *C difficile* spores even using short exposure times. It is likely that 2% acid glutaraldehyde is also highly effective in the clinical setting where the spore burden is likely to be lower than in our test system. Diluting glutaraldehyde-based disinfectants below 2% led to the inability to inactivate *C difficile* spores using exposure times commonly employed to disinfect endoscopes.

In December 1991, the Environmental Protection Agency (EPA) issued an order stopping the sale of a diluted glutaraldehyde (a 1:16 dilution of 2% glutaraldehyde-7.05% phenol-1.2% sodium phenate) because of lack of efficacy against spores and possibly other microorganisms.¹³ A 10% glutaraldehyde-0.5% phenylphenol-0.1% amylphenol registered with the EPA for use as a sterilant at 1:5 dilution has been claimed by its manufacturer to be an effective high-level disinfectant at a 1:20 dilution.¹⁴ The Centers for Disease Control and Prevention (CDC) specifically recommends that only liquid chemical germicides registered by the EPA as "sterilant/disinfectants" be used for high-level disinfection. Germicides that do not meet EPA criteria for a liquid chemical sterilant also do not meet the CDC criteria for a high-level disinfectant. Thus, this latter product should be used as a high-level disinfectant at a 1:5 dilution. The EPA issued an order stopping the sale of one batch of this product on May 15, 1992.¹⁵ This latter EPA product recall was challenged in US District Court, and this action resulted in

ing, disseminating, or releasing these test data to the public. Our data support the EPA's action and the CDC's recommendation.

Investigators have demonstrated that 2% alkaline glutaraldehyde resulted in 99% or greater killing of *C difficile* spores using a suspension test with an exposure time of 5 minutes.^{16,17} Our data confirm the sporicidal activity of 2% alkaline glutaraldehyde using a more conservative test because spores dried on penicylinders are more difficult to inactivate than spores suspended in a liquid media.¹⁸

C difficile spores are more readily inactivated by 2% glutaraldehydes than the spores of other spore-forming organisms, such as *Bacillus subtilis* and *Clostridium sporogenes*, recommended in the AOAC test.¹⁷ Our data therefore should not be interpreted to assure that the exposure times used in our study would reliably inactivate other sporeforming organisms.

Current guidelines recommend that endoscopes should undergo thorough cleaning prior to high-level disinfection between patients. By definition, high-level disinfection should inactivate all microbes with the exception of high numbers of bacterial spores. Thus, complete killing of spores from spore-forming organisms such as *C difficile* would not be assured by using disinfectants at exposure times recommended for high-level disinfection. However, our data suggest that immersion in 2% glutaraldehydes for 20 minutes as recommended⁶ is likely to be successful in eradicating *C difficile* spores from contaminated endoscopes. Twenty minute exposure times are required to reliably inactivate other resistant microorganisms such as *Cryptosporidium* cysts and *Mycobacterium* species. Diluting glutaraldehydes below 2% may lead to an inability to reliably inactivate *C difficile* spores using exposure times commonly employed in clinical practice.

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