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Sporicidal effects of common disinfectants and their practical application in dental practice in Thailand

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Abstract

Objective Sporicidal activity is a good indicator for testing the efficacy of chemical disinfectants in infection control. We tested the sporicidal effect of common germicides used in Thailand.

Materials and methods The spore-forming bacteria *Bacillus atropheas* and *Geobacillus stearothermophilus* were chosen to test four commercial chemical agents: sodium hypochlorite, 2% glutaraldehyde, 35% hydrogen peroxide, and iodophore. *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi* were also used to ensure compliance with EPA (Environmental Protection Agency) and AOAC (Association of Analytical Communities) laboratory standards for hospital disinfectants.

Results Two percents glutaraldehyde inactivated *B. atropheas* and *G. stearothermophilus* at 60 and 30 min, respectively; while 0.25% sodium hypochlorite (wt/v) killed the spores at 20 and 30 min. Thirty five percents hydrogen peroxide was effective at 5 min, iodophore at 0.007% concentration (w/v) inactivated *G. stearothermophilus* but not *B. atropheas* spores.

Conclusion The commercially available chemical agents: 2% glutaraldehyde, 0.25% sodium hypochlorite and 35% hydrogen peroxide for dental practice in Thailand have sporicidal effects when used as manufacturer suggested; thus, they can be used as high level disinfectants.

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Key words: *dental practice; disinfectants; sporicidal effect*

Introduction

Bacterial spores have long been considered the most difficult microbiological entity to neutralize[1]; therefore, sporicidal activity can be a good indicator of the efficacy of a chemical disinfectant. Given that dental practice is especially exposed to both commensal and pathogenic microorganisms in an operating environment which involves instruments,[2] it is of vital importance that proper disinfecting procedures are applied.[3] In many instances, sterilization is of essence. Improper disinfection/sterilization may be a route in spreading a disease from one individual to others. Another important factor we have chosen to examine is the practical applicability of these disinfectants in Thailand. As dental practice in Thailand is still hindered by accessibility and funding issues,[4] the chemical disinfectants and disinfecting procedures were also evaluated for their applicability.

The spore-forming bacteria *Bacillus atropheas* and *Geobacillus stearothermophilus* were chosen as standard acceptance as indicator organisms[5] representative of the spore-forming families.[6] *Staphylococcus aureus, Pseudomonas aeruginosa* and the pathogenic species of Salmonella: *Salmonella typhi* were also used to ensure compliance with EPA (US Environmental Protection Agency) and AOAC (Association of Analytical Communities) laboratory standards for hospital disinfectants.[7,8]

Chemical disinfectants commonly used in dental practice in Thailand are sodium hypochlorite, iodophore, glutaraldehyde and hydrogen peroxide. Glutaraldehyde at 2% concentration (wt/v) alkalinized with sodium bicarbonate is well documented to have microbicidal, and specifically, sporicidal activity and is in commercial use.[6] Many studies have demonstrated such useful sporicidal effects of 2% (wt/v) glutaraldehyde commonly found in disinfectants required significant exposure time.[1,6,9] Overall sporicidal effects of glutaraldehyde varied greatly according to pH as glutaraldehyde was more effective in alkaline buffers.[10,11] Sagripanti and Bonifacino found that 5% (wt/v) glutaraldehyde at pH 9.3 and 40°C was able to destroy the most spores in the least time.[9] Sodium hypochlorite solutions have very useful disinfectant and sporicidal properties due to the release of chlorine.[6] As chlorine is highly irritable yet highly effective at destroying microorganisms, the efficacy of hypochlorite and other chlorine-releasing is generally based on the amount of chlorine released from such agents.[6,9] Many researches have also identified factors that influence the sporicidal effect of hypochlorite solutions including mixture with 1.5% or 4% (wt/v) sodium hydroxide and buffering hypochlorite solutions to pH 7.6 to 8.1.[9,12] The corrosiveness of hypochlorite has also been investigated to be suitable for use on stainless steel, platinum, glass, teflon, polythene and epoxy resin[13] while another study has also found evidence of sporicidal activity of hypochlorite at low or even sub-zero temperatures.[14] Furthermore, hypochlorite has a high level of efficacy around neutral pH.[9] Hydrogen peroxide is widely used within the food-processing industry as a general disinfectant for many reasons.[15] Of the factors involved in its sporicidal effects, hydrogen peroxide concentration and temperature are the most important.[16] Heat shocking of spores before treatment with hydrogen peroxide reduced their resistance to hydrogen peroxide.[15] Higher temperature and concentration increased the sporicidal rate of hydrogen peroxide.[9]

We chose to explore the efficacy of locally available commercial brands according to manufacturer's suggestion. We tested the sporicidal effect of the chosen chemical disinfectants to suggest the use of such agents if any, as liquid sterilizing agent according to the World Health Organization's definition of

"sterilization" as the destruction of all microorganisms, including bacterial spores. Many of dental instruments are expensive and require disinfection/sterilization for re-use. Selecting a disinfectant that requires less operating time to achieve the germicidal effect would be practical in many dental practices. This study should assist dental personnel in Thailand in selecting the proper liquid disinfectants.

Materials and methods

Bacteria

Spores of *B. atropheas* and *G. stearothermophilus* were purchased in strips from Raven Biological laboratories, Inc., USA with a population of 2.0×10^6 and 3.5×10^5 spores, respectively. The percentage of spores versus vegetative cells was observed microscopically to be at least 95%. Spores strips resisted the 2.5 M HCl for 5 min.

Cultures of *S. typhi, S. aureus* and *P. aeruginosa* were used to test the chemical agents according to the suggested US EPA regulation. The bacteria were stored in nutrient broth containing 15% glycerol at -80°C. Bacteria grown in log phase at population of 1.0×10^8 CFU/ml (colony forming units/milliliter) were used in each experiment. All cultures were incubated at 37°C except *G. stearothermophilus* which was carried out at 60°C throughout the study.

Chemical agents

Four different commercial chemical agents were tested. Two percents glutaraldehyde (wt/v) with alkaline activator (Neodex-28D, Neomed, Pathumthanee, Thailand), sodium hypochlorite (wt/v) (Medikleen, Sotiwat, Thailand), Pose-iodophore (Pose Healthcare, Thailand) and 35% hydrogen peroxide (C.U. Dent) were used for the experiments. Each disinfectant was immediately aseptically prepared prior to performing the assay. Glutaraldehyde was mixed with alkaline activator, sodium hypochlorite and iodophore were mixed with sterile distilled water according to manufacturers' instruction to achieve the desired concentrations, and ready-to-use hydrogen peroxide was tested as received.

Kinetic assays

Time and concentrations on bactericidal and sporicidal effect by different disinfectants were determined in 15 ml sterile glass tubes. All experiments were performed at room temperature (28°C). Five milliliters of each chemical agent was added into the tubes. A spore strip or one ml of culture broth was subsequently transferred into each tube and thoroughly vortexed. Each tube was let stand at various times according to each chemical agents and appropriate exposure intervals. The sample was then vortexed vigorously to achieve thorough suspension. One hundred microliters of the mixture was spread on the nutrient agar plates. Results were reported using the colony forming units that were visually formed on agar as + (growth) or - (no growth) value. Gram's stain and colony morphology were used to ensure that no contamination was present. Positive control was performed in each experiment using the sterile distilled water in place of the chemical agent. All experiments were done at least in triplicate, independently.

Results

The positive control showed growth on the agar in all of the chemical agents tested in any given time. All results were read as + (growth) or - (no growth). The no growth was recorded only when there was absolute no growth on any of the samples.

As shown in Table 1, the effect of time and concentrations of sodium hypochlorite (NaOCl) on spore

inactivation was determined. *B. atropheas* was inactivated in 20 min at 0.1%, 0.25%, and 10 min at 0.5% concentration of sodium hypochlorite. 0.1% sodium hypochlorite did not inactivate the spore germination of *G. stearothermophilus* within the time that was tested, whereas 0.25% and 0.5% sodium hypochlorite readily inactivated the spores in 15, and 20 min, respectively. Disinfection effect of sodium hypochlorite on *S. typhi, P. aeruginosa,* and *S. aureus* was within 5 min at all concentrations tested, except that 0.1% sodium hypochlorite concentration did not kill *S. typhi.*

Two percents glutaraldehyde inactivated *B. atropheas* and *G. stearothermophilus* spores at 60 and 30 min, respectively (Table 2). Neither *S. typhi, P. aeruginosa* nor *S. aureus* recovered after 10 min exposure time (Table 2). Thirty-five percents hydrogen peroxide was very effective against all bacteria and spores tested in this study as shown in Table 3. Iodophore at concentration recommended by the manufacturer (0.007% w/v) was effective in killing vegetative bacteria and spores of *G. stearothermophilus* in 30 min, but did not kill *B. atropheas* spores within the period of time tested in this study (Table 4).

Table 1 Comparative microbicidal and sporicidal effects of sodium hypochlorite at various concentrations on *B. atropheas, G. stearothermophilus, S. typhi, P. aeruginosa,* and *S. aureus.* The plus sign (+) indicates growth whereas minus sign (-) indicates no growth of the microorganism after exposure to the chemical agent.

Microorganism	Concentration	5 min	10 min	15 min	20 min
B. atropheas	0.1% NaOCl	+	+	+	-
	0.25% NaOCl	+	+	+	-
	0.5% NaOCl	+	-	-	-
G. stearothermophilus	0.1% NaOCl	+	+	+	+
	0.25% NaOCl	+	+	-	-
	0.5% NaOCl	+	+	+	-
S. typhi	0.1% NaOCl	+	+	+	+
	0.25% NaOCl	-	-	-	-
	0.5% NaOCl	-	-	-	-
P. aeruginosa	0.1% NaOCl	-	-	-	-
	0.25% NaOCl	-	-	-	-
	0.5% NaOCl	-	-	-	-
S. aureus	0.1% NaOCl	-	-	-	-
	0.25% NaOCl	-	-	-	-
	0.5% NaOCl	-	-	-	-

Table 2 Comparative effects of 2% glutaraldehyde on various sample organisms versus time. The plus sign (+) indicates growth whereas minus sign (–) indicates no growth of the microorganism after exposure to the chemical agent.

Microorganism	Time				
	10 min	20 min	30 min	1 hr	3 hr
B. atropheas	+	+	+	–	–
G. stearothermophilus	+	+	–	–	–
S. typhi	–	–	–	–	–
P. aeruginosa	–	–	–	–	–
S. aureus	–	–	–	–	–

Table 3 Comparative effects of 35% hydrogen peroxide on various sample organisms versus time. The plus sign (+) indicates growth whereas minus sign (–) indicates no growth of the microorganism after exposure to the chemical agent.

Microorganism	Time			
	5 min	10 min	20 min.	30 min.
B. atropheas	–	–	–	–
G. stearothermophilus	–	–	–	–
S. typhi	–	–	–	–
P. aeruginosa	–	–	–	–
S. aureus	–	–	–	–

Table 4 Comparative effects of iodophore. The plus sign (+) indicates growth whereas minus sign (–) indicates no growth of the microorganism after exposure to the chemical agent.

Microorganism	Time				
	10 min	20 min	30 min	45 min	60 min
B. atropheas	+	+	+	+	+
G. stearothermophilus	+	+	–	–	–
S. typhi	–	–	–	–	–
P. aeruginosa	+	+	–	–	–
S. aureus	–	–	–	–	–

Sporicidal effects of liquid chemical disinfectants

Tables 1-4 displayed the exposure time versus concentration of disinfectants and its subsequent effect on both spore samples and vegetative bacteria. Spores of *B. atropheas* and *G. stearothermophilus* were generally more difficult to eliminate than vegetative bacteria; however, with prolonged exposure most disinfectants showed an increase in effectiveness. Of those disinfectants tested, the efficacy of 35% hydrogen peroxide stood out but the corrosive and concentrated nature of the substance creates a number of drawbacks from practical application. The iodophore was least effective of the disinfectants tested, as shown in Table 4.

Discussion

Glutaraldehyde at alkaline pH has been shown to be lethal to all microorganisms when exposed at long period of time.[6,7] We observed that 2% glutaraldehyde added with alkaline activator as manufacturer suggested achieved pH 8 at room temperature. It also showed sporicidal effect within 60 min. Glutaraldehyde is toxic, has unpleasant odor, and is irritating to human tissue. To avoid this, one needs to thoroughly rinse the glutaraldehyde-soaked instruments with sterile distilled water to wash out any remaining of the chemical agent and still retain the sterility. Iodophore has the ability to inactivate the *G. stearothermophilus,* but not *B. atropheas* because spores of *G. stearothermophilus* are tolerant to high temperature, thus they are more vulnerable to chemical disinfectants than *B. atropheas* spores. We interpreted our result as positive if any colony forming units were visualized. Identification of the bacteria was based on colony morphology and Gram stain. We found that 35% hydrogen peroxide at room temperature is effective in inactivating bacterial spores; however, hydrogen peroxide at this high concentration could be irritating if brought into contact with human skin. Sodium hypochlorite has been used as a disinfectant at much lower concentration than 0.25%. We tested the efficacy of this disinfectant at various concentrations and found that the concentrations of 0.25% or 0.5% effectively inactivated spores. However we suggest using 0.25%, which is the less concentrated one for at least 20 min, as it causes less damage to dental instruments due to its corrosiveness. It is noteworthy to discuss the point from the result in Table 1 that the 0.25% sodium hypochlorite can kill the *G. stearothermophilus* spores faster than the 0.5% concentration (15 versus 20 min). The possible explanation behind 0.5% sodium hypochlorite requiring more exposure time to inactivate spores could be the result of other factor that was not controlled in our study such as pH or temperature, which seems to be factors affecting chlorination and therefore disinfection. Further research is needed on this point to see if it is merely a chemical occurrence that decreases the potency of hypochlorite or whether it is a biological response from the bacteria.

All disinfectants were tested at room temperature and prepared according to manufacturer's instruction to mimic the procedures that are regularly practiced in dental clinics. We used *S. aureus, P. aeruginosa,* and *S. typhi* to ensure compliance with EPA and AOAC laboratory standards for hospital disinfectants. We tested the sporicidal effect of *B. atropheas* and *G. stearothermophilus.* The number of spores used in this experiment were far more excessive than those that exist naturally in dental practice. In addition, not many spore-forming bacteria cause infectious diseases in dental setting. The disinfectants used in this study were products available locally. In order to evaluate manufacturers' claims concerning the effectiveness of a product, one needs to be skeptical as some companies might overestimate their products' efficacy.

Our results demonstrated that 35% hydrogen peroxide, 0.25% sodium hypochlorite, and 2% glutaraldehyde at the minimum time of 5 min, 20 min, and 60 min, respectively have the sporicidal effects. We concluded that either 35% hydrogen peroxide, 0.25% hypochlorite, or 2% glutaraldehyde can be used as a sporicide. Iodophore is a widely used and acceptable disinfectant. It possesses only selective sporicidal activity, thus, is not recommended for use in cold sterilization. There may not be perfect liquid chemical germicide. Some are better than others; it depends on the application. As most liquid disinfectants do not have the penetrating ability, we suggest a thorough sanitary cleaning and proper drying of any instrument, equipment or working surface before disinfection.

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อำนาจในการทำลายสปอร์ของน้ำยาฆ่าเชื้อที่ใช้งานในคลินิกทันตกรรมในประเทศไทย

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บทคัดย่อ

วัตถุประสงค์ เนื่องจากความสามารถในการทำลายสปอร์ของแบคทีเรียเป็นคุณสมบัติที่บ่งชี้ถึงประสิทธิภาพของน้ำยาฆ่าเชื้อ การศึกษานี้เพื่อทดสอบความสามารถในการทำลายสปอร์ของแบคทีเรียของน้ำยาฆ่าเชื้อที่ใช้กับเครื่องมือทันตกรรมซึ่งมีขายในท้องตลาดของไทย

วัสดุและวิธีการ เลือกใช้สปอร์ของเบสิลัสอโทรเฟียส และจีโอเบสิลัสสเตียโรเทอร์โมฟิลัส มาทดสอบน้ำยาฆ่าเชื้อ 4 ชนิดคือ น้ำยาโซเดียมไฮโปคลอไรท์ กลูตาอัลดีไฮด์ความเข้มข้นร้อยละ 2 ไฮโดรเจนเปอร์ออกไซด์ความเข้มข้นร้อยละ 35 และไอโอโดฟอร์ โดยมีแบคทีเรียอีก 3 ชนิด ซึ่งใช้เป็นมาตรฐานในการทดสอบประสิทธิภาพของน้ำยาฆ่าเชื้อตามที่องค์การพิทักษ์สิ่งแวดล้อมแห่งสหรัฐอเมริกาและสมาคมนักวิทยาศาสตร์เคมีเชิงวิเคราะห์นานาชาติแนะนำ ได้แก่ สแตปฟิโลค็อคคัสออเรียส ซูโดโมแนสแอรูจิโนซา และแซลโมเนลลาไทฟี

ผลการศึกษา พบว่ากลูตาราลดีไฮด์ความเข้มข้นร้อยละ 2 สามารถทำลายสปอร์ของเบสิลัสอโทรเฟียส และจีโอเบสิลัสสเตียโรเทอร์โมฟิลัส ได้ในระยะเวลา 60 และ 30 นาทีตามลำดับ ส่วนโซเดียมไฮโปคลอไรท์ที่ความเข้มข้นร้อยละ 0.25 สามารถทำลายสปอร์ของเบสิลัส อโทรเฟียส และจีโอเบสิลัส สเตียโรเทอร์โมฟิลัสทั้งสองชนิดได้ในระยะเวลา 20 และ 30 นาที ไฮโดรเจนเปอร์ออกไซด์ ความเข้มข้นร้อยละ 35 ฆ่าสปอร์ทั้งสองชนิดได้หมดใน 5 นาที ไอโอโดฟอร์ที่ความเข้มข้นร้อยละ 0.007 ฆ่าสปอร์ของจีโอเบสิลัสสเตียโรเทอร์โมฟิลัส แต่ไม่สามารถทำลายสปอร์ของเบสิลัสอโทรเฟียสได้

สรุป เมื่อปฏิบัติตามคำแนะนำของบริษัทผู้ผลิตสามารถใช้กลูตาราลดีไฮด์ความเข้มข้นร้อยละ 2 โซเดียมไฮโปคลอไรท์ที่ความเข้มข้นร้อยละ 0.25 และไฮโดรเจนเปอร์ออกไซด์ความเข้มข้นร้อยละ 35 ที่ใช้ในคลินิกทันตกรรมในประเทศไทยเป็นน้ำยาฆ่าเชื้อระดับสูงสำหรับทำลายแบคทีเรียและสปอร์ของแบคทีเรียได้

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คำสำคัญ: *คลินิกทันตกรรม; น้ำยาฆ่าเชื้อ; ฤทธิ์การทำลายสปอร์ของแบคทีเรีย*