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Evaluation of Bactericidal Activity of Certain Gluteraldehyde Free Disinfectants Used in the Disinfection of Endoscopes and Surgical Devices by a Quantitative Suspension Test

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Introduction

Endoscopes have been used widely for the diagnosis and treatment of several diseases and have been used increasingly in laparoscopic surgery. Since the endoscopes generally have close contact with mucosal membranes, they invariably become contaminated with microorganisms. Thus, high-level disinfection procedure is required after each patient use (1). Appropriate cleaning, disinfection and sterilization play major role in preventing nosocomial outbreaks and serious life-threatening infections. Specifically elastic and fiber optic endoscopes are not available for routine use for their relatively high cost. Thus, in the intervals between patients, the application of a rapid and effective high level disinfection procedure is of great importance. Since those instruments are highly sensitive to heat, they can not be sterilized with heating. Consequently, today chemical disinfectants that perform high-level disinfection such as gluteraldehyde, hydrogen peroxide, peracetic acid and ortho-phthalaldehyde are used. Among these, gluteraldehyde, a saturated dialdehyde, is a widely used chemical for endoscopes[1-3]. However, gluteraldehyde has several adverse effects to health care staff; it fixes proteins and is inactivated by organic

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material. Further research on new disinfectant formulations has been stimulated by those disadvantages of gluteraldehyde[4]. The purpose of this paper was to investigate bactericidal activity of some gluteraldehyde free disinfectants against standard disinfectant test strains. The effects of organic contamination and standard hard water on biocidal activity of these disinfectants were also evaluated.

Materials And Methods

Microorganisms: In this study, *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538 were used. These strains are recommended for testing of bactericidal activities of disinfectant[5].

Disinfectants: The agents tested were succinic dialdehyde/dimethoxy hydrofuran (Gigasept FF, Schulke and Mayr GmbH, Hamburg), guanidine/phenoxypropanol/benzalkonium chloride (Lysetol AF, Schulke and Mayr GmbH, Hamburg), bis (3-aminopropyl)dodecylamin/quaternary ammonium compounds (Actosed Endo, Acto GmbH, Germany), guanidine diacetate/quaternary ammonium compounds (Perfectan Endo, Dr. Schumacher GmbH, Germany). The disinfectant concentrations and their contact times have been selected according to the producers' instructions and test procedures. Three dilutions should be used in recommended concentrations and at least two of them have to be in the active range[6]. Furthermore, since it was important to carry out the disinfection of endoscope and surgical devices in short period of time, shorter contact times were also studied. The concentrations of disinfectants and exposure times tested as 5 %, 7.5 % and 10 % for Gigasept FF; 5 % and 10 % for Actosed Endo; 2.5 % and 5 % for Lysetol AF for 5 and 10 minutes, 3 % Perfectan Endo for 10 and 15 minutes. These gluteraldehyde free disinfectants are especially used for endoscope and surgical devices disinfection. All disinfectants were freshly prepared prior to testing. Sterile hard water (342 ppm) was used as a diluent and disinfectant control. The disinfectants were stored in the dark at room temperature. Neutralizers were also tested to determine whether it was appropriate to inactivate each of the chemicals.

Neutralizers are listed in Table I. In order to determine the effect of dirty conditions, bovine serum albumin (Cohn Fraction V, Sigma) was used.

Detection of Bactericidal Activity: In this study quantitative suspension test procedure was used[5, 6]. The methods for preparing bacterial inoculum and performing the suspension test were described in detail

TABLE I

Neutralizers used in this study

Disinfectant	Neutralizer substances
Gigasept FF, Perfectan Endo	Sodium thiosulphat (2%)
Lysetol AF	L-histidin, L-sistein, Saponin and Tween 80 (0,1%: 0,1%: 3%: 3%)
Actosed Endo	Tween 80 and Lesitin (5%: 0,75%)

earlier[7]. Briefly, bacterial strains were inoculated in tryptic soy broth (TSB, Acumedia, MD) for 24 hours at 37 °C. After incubation, bacterial suspension was centrifuged for 15 minutes at 2000 rpm with a rotor centrifuge (Nuve NF 615, Ankara, Turkey). The cell pellets were washed with sterile distilled water for 3 times. Then bacterial suspensions in distilled water were adjusted to McFarland 0.5 standard.

In the test, bacterial suspension was added to the disinfectant solutions (1:10) at room temperature and then 1 ml was removed to neutralizing solution after contact times in order to terminate the activity of the disinfectant (Neutralizing solutions used in this study are shown in Table I). After 5 minutes dilutions were prepared. Inoculations were performed from each dilution tube onto tryptic soy agar (TSA, Difco) plates by the spread-plate technique and incubated for 24 hours at 37 °C. The samples were studied in triplicate. Then colonies were enumerated and expressed as colony-forming units per milliliter. Plates that have colony count between 30 and 300 were taken into account. The reduction factor was calculated as the expression of the disinfectant efficacy, according to the following formula[5]:

RF= log Nc – log Nd

RF: Logarithmic reduction factor

Nc: Bacterial colony number from control plates

Nd: Bacterial colony number after contact with disinfectant

Log_{10} reductions of 5 or more were taken as an indication of satisfactory microbicidal activity.

In order to determine the effect of dirty conditions, each of the disinfectant concentration were also tested with albumin. For this purpose, bovine serum albumin was added to disinfectant solution before bacterial suspension.

Results

Bactericidal activities of several gluteraldehyde free disinfectants used for disinfection of endoscopes and surgical devices were evaluated by quantitative suspension test. Logarithmic reduction values obtained from different concentrations and contact times of tested disinfectants are shown in Table II and Table III.

There was no difference between the values of bactericidal activity test with and without albumin.

Gigasept FF caused logarithmic reduction factor above 5 at all tested concentrations and contact times against *S. aureus* ATCC 6538. Same efficiency was obtained at concentrations of 5 % and 7.5 % at 10 minutes against *P. aeruginosa* ATCC 15442. On the other hand Gigasept FF showed bactericidal activity at concentration of 10 % at 5 minutes.

Lysetol AF was found to be effective at concentration of 2.5 % and in 5 minutes against both of the bacteria.

Actosed Endo was shown to be effective against both of the bacteria at concentrations of 5 % and 10 % at 5 and 10 minutes contact times.

Perfectan Endo was tested only at the concentration of 3 %. This disinfectant achieved the required effect at 10 minutes contact time.

TABLE II

Logarithmic reduction factors of *Pseudomonas aeruginosa* ATCC 15442 after contact to tested disinfectants.

		Logarithmic reduction factors (RF) at different contact times		
Disinfectant	Concentrations (%)	5 (min)	10 (min)	15 (min)
Actosed Endo	5,0	7,96	7,96	-
	10	7,96	7,96	-
Gigasept FF	5,0	0,30	8,22	-
	7,5	0,86	8,22	-
	10	8,22	8,22	-
Lysetol AF	2,5	7,56	7,56	-
	5,0	7,56	7,56	-
Perfectan Endo	3,0	-	7,97	7,97

TABLE III

Logarithmic reduction factors of *S. aureus* ATCC 6538 after contact to tested disinfectants.

		Logarithmic reduction factors (RF) at different contact times		
Disinfectant	Concentration (%)	5 (min)	10 (min)	15 (min)
Actosed Endo	5,0	7,80	7,80	-
	10	7,80	7,80	-
Gigasept FF	5,0	8,14	8,14	-
	7,5	8,14	8,14	-
	10	8,14	8,14	-
Lysetol AF	2,5	8,13	8,13	-
	5,0	8,13	8,13	-
Perfectan Endo	3,0	-	7,43	7,43

In this study, we have not seen any negative effect of the protein source (bovine serum albumin) used for the detection of the effect of dirty conditions on disinfectant activity (Data not shown). Also, it was clear that neutralizers did not have any bactericidal activity against control strains. Consequently, it is possible to conclude that these disinfectants were not affected by dirty conditions.

Discussion

Processing of reusable medical devices remains as a major concern for the health care institutions and the public opinion. Endoscopes are classified as critical devices for their contact with mucous membranes, and for that reason they have to be subjected to high level of disinfection[1]. This disinfection procedure is performed by immersion of the endoscopes into the approved disinfectant solutions. This period should be extended with the inclusion of rinsing, further extending the interval until it can be used for the next patient. That should be particularly disadvantageous for crowded clinics. Thus, the disinfectants with shorter contact period and higher levels of disinfection are needed[8].

Gluteraldehyde, a saturated dialdehyde, has been the most widely used chemical for high-level disinfection of endoscopes[1]. Exposure to glu-

teraldehyde fumes is known to cause irritation to eyes, nose, and throat and cause dermatitis in healthcare staff. For this reason endoscopy units are required to install expensive ventilation system[8]. Alternative gluteraldehyde-free disinfectants can replace those with aldehyde-containing products. They appear to be more compatible with the skin with smaller risk of causing allergy. They have lower level of inhalation toxicity and lower room air load. Their use does not cause alterations of color in the skin and textile products[1].

Alternative disinfectants such as peracetic acid and chlorine dioxide have been evaluated to be successful. However these disinfectants are expensive and they have weak material compatibility. Chlorine-containing compounds as another alternative disinfectant do not have those disadvantages but their activity is reduced substantially in the presence of heavy organic load[8]. Our tested disinfectants are claimed to be non-corrosive by the manufacturers and they are also non-irritant to skin and mucous membranes. One of their advantages is that they were not affected by the organic load.

In this study, in-vitro bactericidal efficiencies of certain gluteraldehyde-free chemicals that used for high-level disinfection against standard microorganisms are evaluated for different concentrations and contact times. In order to assess the effect of dirty conditions, bovine serum albumin was added to disinfectants before bacterial inoculum. Our results show that the potent biocidal activity of these disinfectants is not reduced in the presence of organic matter contamination. In that case, the cleaning process which is used prior to the disinfection can be shortened in the whole process. That reduces the total amount of time needed for the disinfection. Furthermore, the use of standard hard water as disinfectant diluent did not reduce the activity of the products.

We found that all disinfectants were effective against two control strains at 5 minutes of exposure. However Gigasept FF at 5 % and 7.5 % concentrations was not effective at 5 minutes of contact time. That should not be considered as a negative finding or a failure, because recommended concentration for this disinfectant was 10 % for 15 minutes of contact time.

Similar findings have been reported by others for these agents[3, 8]. One of the study indicated that Gigasept FF at 4 % concentration with a contact time of 30 min. effective against control strains. And this study also showed that at the same concentration of Gigasept FF solution was effective after 14 days standing time of the use solution[9].

In conclusion, Gigasept FF, Lysetol AF, Actosed Endo and Perfektan Endo were found to be have high bactericidal activity. These disinfectants may be considered as an alternative to gluteraldehyde in the disinfection of endoscopes and surgical devices. However, it appears that practical in use test still needed further study.

Abstract

Evaluation of Bactericidal Activity of Certain Gluteraldehyde Free Disinfectants Used in the Disinfection of Endoscopes and Surgical Devices by a Quantitative Suspension Test

In this study, bactericidal efficiency of Gigasept FF and Lysetol AF (Schülke and Mayr GmbH, Hamburg), Perfektan Endo (Dr. Schumacher GmbH, Germany) and Actosed Endo (Acto GmbH, Germany) which do not contain gluteraldehyde and being used in the disinfection of endoscopes and surgical instruments were evaluated against *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 by quantitative suspension test. The concentrations of disinfectants and exposure times tested as 5 %, 7.5 % and 10 % for Gigasept FF; 5 % and 10 % for Actosed Endo; 2.5 % and 5 % for Lysetol AF for 5 and 10 minutes, 3 % Perfectan Endo for 10 and 15 minutes. Furthermore, in order to assess the effect of dirty condition on disinfectant activity, bovine serum albumin was used. Consequently, all of the tested disinfectants were found to be effective against *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 15442 at studied concentrations and exposure times. Results were similar in cases with and without albumin. The test used in this study is a basic bactericidal activity test. Practical in use tests on endoscopes and the surfaces of instruments still need further study.

Key words: Endoscope, gluteraldehyde-free disinfectants, quantitative suspension test

Özet

Endoskop ve Cerrahi Aletlerin Dezenfeksiyonunda Kullanılan Gluteraldehit İçermeyen Bazı Dezenfektanların Bakterisidal Etkinliğinin Kantitatif Süspansiyon Testi ile Değerlendirilmesi

Bu çalışmada endoskop ve cerrahi aletlerin dezenfeksiyonu amacıyla kullanılan, gluteraldehit içermeyen Gigasept FF ve Lysetol AF (Schülke and Mayr GmbH, Hamburg), Perfektan Endo (Dr. Schumacher GmbH, Almanya) ve Actosed Endo (Acto GmbH, Almanya) isimli dezenfektanların bakterisidal etkinlikleri kantitatif süspansiyon testi

kullanılarak *Staphylococcus aureus* ATCC 6538 ve *Pseudomonas aeruginosa* ATCC 15442 suşlarına karşı araştırılmıştır. Dezenfektanların kullanılan konsantrasyonları ve temas süreleri, Gigasept FF için % 5, % 7.5 ve %10; Actosed Endo için % 5, % 10; Lysetol AF için % 2.5 ve % 5'lik konsantrasyonlarda temas süreleri 5 ve 10 dakika olacak şekilde, Perfektan Endo için ise %3'lük konsantrasyonda 10 ve 15 dakika olmak üzere test edilmiştir. Çalışmada ayrıca kirli ortamın dezenfektanların aktiviteleri üzerine etkisini araştırmak amacıyla sığır serum albümini kullanılmıştır. Sonuç olarak tüm dezenfektanlar test edilen konsantrasyonlarda ve temas sürelerinde çalışılan standart suşlara karşı etkili bulunmuştur. Albüminli ve albüminsiz uygulamalarda aynı sonuçlar alınmıştır. Bu çalışmada uygulanan test, temel bir bakterisidal aktivite testi olup, endoskoplar ve alet yüzeyleri üzerinde pratik kullanım testlerinin de yapılması uygun olacaktır.

Anahtar sözcükler: Endoskop, gluteraldehit içermeyen dezenfektanlar, kantitatif süspansiyon testi.

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