Disinfection efficacy of a super-oxidized water with neutral pH,

Medilox™

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Summary Medilox is a new super-oxidized water with a pH of 6.5. Its bactericidal and inactivation effects on the microorganism and HBsAg were evaluated respectively. The bacterial suspension of methicillin resistant staphylococcus aureus (MRSA), acinetobacter baumannii and streptococcus pneumoniae was exposed to Medilox for 1 min, and 100% microbes were killed. When bacillus subtilis spore was subjected to Medilox for 10 min, 100% microbes were killed, when subjected to the stock solution of Medilox at room temperature for 1 min, the S/N value < 2.1 in the test groups, when subjected to 2 fold dilution of Medilox for 5 or 10 min, the S/N value < 2.1, and when subjected to 4 fold dilution of Medilox for 10 min, HBsAg still could not be inactivated. After cleaning according to the routine method, the pathogens could be killed after exposure to Medilox for 1 min. When the bacterial suspension did not contain calf serum, 100% MRSA was killed after 1 min exposure to the disinfectant. When the bacterial suspension contained 25% and 50% calf serum, the killing rates were 100% and 99.98% respectively after exposure to the disinfectant for 15 min. After the disinfection on the object surface, the bacterial log reduction > 1. The mean range was 2.54 on the hands. These results show that Medilox has a quick and highly effective bactericidal action. It can be widely applied to the disinfection in the hospital.

KEYWORDS: Medilox; bacterial suspensions; HBsAg; gastroscope; bactericidal effect

Introduction

MRSA infection is on the rise worldwide and MRSA has become the major pathogenic bacteria. Multidrug resistant A. baumannii is also one of the pathogens causing ICU nosocomial infection. They are resistant to many kinds of antibacterials, the pathogenicity is strong and treatment effectiveness is not good. It can effectively prevent nosocomial infection to eliminate the pathogens on the hands of operators and surface of objects and in the hospital environment.

Gastrosopes can not tolerance high temperature, so disinfection is usually performed with chemical sanitizers. It is the key to choose the proper chemical sanitizer for disinfection. Glutaraldehyde is the most commonly used endoscopic sanitizer at present with the advantages of high bactericidal power, broad antimicrobial spectrum and low corrosivity. However, it is of strong stimulation, high price, and if sterilizing water is not used for washing, it can increase the chance of recontamination. The study reported by Ballantyne et al. showed that the oral LD50 of
2.2% acidic and alkaline glutaraldehyde for mice were 3.45 g/kg and 4.16 g/kg respectively. Skin irritancy test was performed on rabbits, it is found that 5% glutaraldehyde produces moderate stimulation and 2% glutaraldehyde produces slight stimulation. Standard eye irritation test showed that at 2% glutaraldehyde, corneal injury was mild, and at 5% marked. The lowest concentrations producing corneal and conjunctival injury were 1% and 0.2% respectively. At 1% GA, conjunctival hyperemia and chemosis were moderate to marked, and became more severe with higher GA concentrations. Because glutaraldehyde poses an occupational health hazard or risk for staff, UK hospitals are finding an alternative disinfectant to glutaraldehyde. The recent studies reported that the corrosiveness of the super oxidized solution with low pH value and high concentration of chlorine (>650 ppm) is high, and it is highly corrosive for gastroscope.

Medilox has a low concentration of chlorine 68 ppm, with a nearly neutral pH of 6.5 and an ORP 825. We carried on series of experiments to determine its sterilizing effects on the microorganism and inactivation effects on HBsAg. The results showed that Medilox had a quick and highly effective bactericidal action.

Materials and methods

Medilox

The power voltage of the machine (NEC, Korea) was 220 V, power 150 W and water yield 1.5 L/min. After the machine was connected to the power, the water pipe connecting to the machine was opened and the water yield was Medilox. Medilox was composed of 99% HOCL and had strong bactericidal ability and stability.

Neutralizer

The PBS containing 0.3% sodium thiosulfate and 1% tween 80 was used in this study.

Preparation of bacterial suspension

This study was carried out using clinical isolated strains of MRSA, A. baumannii and S. pneumoniae in our hospital and staphylococcus aureus (ATCC 6538) and B. subtilis spore (ATCC25922) purchased from the China General Microbiological Culture Collection Center. After the separation and purification, typical bacterial colonies were selected and inoculated in agar medium at 37°C for 24 h. All the test organisms were washed using PBS containing peptone 10 g/L and then diluted to $1 \times 10^7$ CFU/mL-$5 \times 10^7$ CFU/mL.

Neutralizer appraisal test

S.aureus (ATCC 6538) was selected as the test organism. The suspension quantitative germicidal test method was used for the neutralizer appraisal test. The experiment was performed in 6 groups. In group A, there was no test organism or colony appeared in only a few organisms. In group B, bacterial growth was present and the number was bigger than that in group A. In group C, D and E, there was similar number of bacterial growth and the error rate between them $\leq 15\%$. In group F,
the organisms had no growth of bacteria. The results were in agreement with after 3 times of experiments, showing that both the neutralizer and its concentration were proper.

**Bacterial suspension quantitative germicidal test**

The bacterial suspension 0.5 mL and neutral ion water 4.5 mL were mixed together, and the mixture was then gently shaken to interact for 10 min. 0.5 mL of this mixture was added to the tube of 4.5 mL neutralizer. After 10 min neutralization, the solution 1.0 mL was taken and inoculated. The number of viable organisms was counted and the mean kill rate was calculated. The experiment was carried out in triplicate.

**HBsAg destruction test**

This test was performed using the suspension quantitative germicidal test method described in “Disinfection Technical Guidelines”, 3rd edition. A calibrated time was commenced immediately once a suspension of one of the above HBsAg suspensions was added to 1 mL of Medilox. At the desired times (0.5, 1, 5, 10 and 15 min), the suspension of HBsAg and Medilox was gently shaken to obtain a homogeneous suspension, and this suspension was mixed with 1 mL neutralizer. After 10 min neutralization, the antigenicity of HBsAg was detected using ELISA (sensitivity of the reagent 1.0ng/mL). Appropriate positive controls for each micro-organism were always run in parallel using sterile, deionized water (control). The experiment was carried out in triplicate. The result was defined as negative if S/N value <2.1, which showed that the antigenic destruction was qualified.

**Detection of disinfection efficacy of gastroscope**

Samples from the surface of gastroscope before and after disinfection were collected using sterile cotton swabs immersed PBS and neutralizer. Sterilized distilled water 10 mL was given through the biopsy hole. Irrigating solution 1 mL was added to the control tube and PBS tube containing neutralizer and both were treated by well mixing. 1 mL of this mixture was plated on to a plate and spread evenly over the entire plate surface. The nutrient agar was pored into the plate and mixed well. The plate was then incubated at 37°C for 24 h. The number of resulting colonies was counted and bactericidal rate calculated.

**Field experiment of disinfection of the object surface**

The experiment was performed according to the method described in “Disinfection Technical Guidelines”. The surfaces of objects (table, door, etc.) were randomly chosen. Two block area of 25 cm² was calibrated using the standard boards. One block was for the sampling before disinfection and the other for the sampling after disinfection. Before disinfection, sterile cotton swab was moistened in the test tube containing 5 mL of diluent to obtain samples from one block through 8 times of horizontal and vertical smearing. After sampling, the sample end of the swab was cut and put into the original test tube in the way of sterile operation. The diluent was shaken to interact for 20 s. After proper dilution, it was served as positive control sample. The disinfectant
was used to sterilize the object surfaces through spraying or inunction according to the prescriptive dosage. After disinfection, sterile cotton swab was moistened in the test tube containing 5 mL of neutralizer to obtain samples from the other block through 8 times of horizontal and vertical smearing. After sampling, the sample end of the swab was cut and put into the original test tube in the way of sterile operation. The diluent was shaken to interact for 20 s. It was served as disinfection group sample. After the experiment, diluent 1.0 mL and neutralizer 1.0 mL of the same batch unused were inoculated in the culture media, serving as the negative control samples. Three portions of 1.0 mL samples from positive control group, negative control group and disinfection group were taken and incubated using the falt plate method of agar touring. Two plates per condition were incubated at 37°C for 48 h. The results were observed.

Field test of the hand disinfection

The field use operators were randomly selected as subjects. Before disinfection, the subjects were told to hold fingers on the left hand together after adequate wiping of both hands. Sterile cotton swab was moistened in the test tube containing 10 mL of diluent. After the diluent was squeezed out on the wall of the tube, the swab was used to wipe the flexor surface of 5 fingers from the finger tip to the finger root in the way of round trip for 2 times and it was turned when each wiping was finished. After sampling, the sample end of the swab was cut and put into the tube of neutralizer in the way of sterile operation and it was served as the positive control sample. After 1 min disinfection of the right hand, neutralizer was then used instead of diluent. Sampling was carried out as the above method in the positive control group and it was served as the sample in the test group. One or two portions of the swab samples obtained from the homologous diluent 1.0 mL and neutralizer 1.0 mL unused were served as the samples in the negative control group. Three portions of 1.0 mL sample from the test group, positive control group, and negative control group were incubated using the falt plate method of agar touring. Two plates per condition were incubated at 37°C for 48 h. The results were observed and all log reduction values were calculated.

Calculation formula of log reduction value

Log reduction value (LR) = log value of mean live bacteria concentration in control group (No) - log value of live bacteria concentration in test group (Nx)

Results

Qualification test of neutralizer

The test showed that that use of PBS containing 0.3% sodium thiosulfate and 1% tween 80 as the neutralizer could effectively eliminate the residual effect of Medilox on the test bacteria (Table I).

Disinfection test of bacterial suspension

The bacterial suspension of MRSA, A. baumannii, and S. pneumoniae was exposed to Medilox for 1 min and the killing rate could reach 100% (Table II). B. subtilis spore was exposed to Medilox for 10 min and the killing rate could also reach 100% (Table III).
Destruction test of HBsAg

When HBsAg was subjected to the stock solution of Medilox at room temperature for 1, 5 or 10 min, the S/N value in each group < 2.1; when subjected to 2 fold dilution of Medilox for 5 or 10 min, the S/N value < 2.1; when subjected to 4 fold dilution of Medilox for 10 min, the S/N value > 2.1 (Table IV).

Disinfection efficiency of 40 gastroscopes

Bacteria determined by gastroscope before disinfection mainly included conditioned pathogens: alpha streptococcus, neisseria, enterobacteria, pseudomonas aeruginosa, etc. After cleaning according to the routine method, all the bacteria could be killed after exposure to Medilox for 1 min (Table V).

Effect of organisms on disinfection of Medilox

The experiment was repeated for 3 times. When the bacterial suspension did not contain calf serum, 100% MRSA was killed after 1 min exposure to the disinfectant. When the bacterial suspension contained 25% and 50% calf serum, the killing rates were 100% and 99.98% respectively after exposure to the disinfectant for 15 min (Table VI).

Field experiment of disinfection of the object surface

Before disinfection, the bacterial number of positive control was 368.2, 279.5 and 426.7 CFU/cm2 in the 3 experiments respectively. After disinfection, all the bacterial number <8 CFU/cm2 and log reduction >1. The specimens in the negative control group were cultured in the test media and had no growth of bacteria. The object surface met the standard of hygenics and disinfection (Table VII).

Field test of the hand disinfection

The hands of the 30 operators were checked, the bacterial colony ranges in positive control and test specimens were 36-530 CFU/cm2 and 0-8 CFU/cm2 respectively. The range of log reduction value was 2.38-3.27 and the mean range was 2.54. The specimens in the positive control group were cultured in the test media and had no growth of bacteria. The subjects were free of symptoms during the test and no adverse reaction was found on the hand skin.

Discussion

Medilox was a colorless, odorless and non-irritative liquid. Its pH value was 4-7, closing to neutrality. It had a slight concentration of chlorine 50-80 ppm with an ORP 800-1000 mV. Medilox had a shelf life of one year and remained effective if it was stored for 15 days after the seal was removed. After determination, Medilox used in this studied had a low concentration of chlorine 68 ppm, with a pH of 6.5 and an oxidation-reduction potential (ORP) 825. The results of
the test showed that if clinical isolated strains of MRSA, A.baumannii and S.pneumoniae were exposed to Medilox for 0.5 min, the killing rate was 99.99%, and if for 1 min, 100%. Therefore, it had a quick and highly effective bactericidal action. The results of our test showed that Medilox could be used to treat the disinfection and external infection of the clinical isolated strains. When bacillus subtilis spore was exposed to Medilox for 10 min, 100% microbes were killed, and when exposed to the stock solution of Medilox at room temperature for 1 min, the S/N value < 2.1 in the test groups, showing that neutral-pH Medilox could quickly inactivated HbsAg and could be applied to hepatitis B virus disinfection. When B.subtilis spore was subjected to 2 fold dilution of Medilox and the action time was prolonged to 5 min, HBsAg remained inactivated, but when subjected to 4 fold dilution of Medilox, even if the action time was prolonged to 10 min, HBsAg still could not be inactivated, showing that Medilox was similar to other disinfectants, it must reach certain concentration to achieve the effect. Otherwise, no matter how long the action time was prolonged, it still could not achieve the aim of disinfection. The test of the effect of organisms on disinfection of Medilox showed that organisms, such as calf serum, had obvious effect on the bactericidal effect of the stock solution of Medilox, indicating that to ensure the bactericidal effect in the practical use, organisms on objects should be eliminated as much as possible, and medical equipments contaminated by blood or body fluids should be cleaned repeatedly before disinfection.

With the wide spread use of endoscopy and further understanding about nosocomial infection, hospital infections continue to be studied and reported. In addition, improper use of disinfection could also cause chemical injury to patients and staff. Therefore, it was important for them to prevent and control cross infection and other harms caused by endoscopy. Spach et al. analyzed 377 infections related to gastrointestinal endoscopy, including 201 pseudomonas aeruginosa infections, 84 salmonella specie infections, 80 mycobacterium infections, 4 Hp infections, 1 hepatitis B virus infection. Cheung et al. investigated 294 gastrointestinal endoscopy centers in the United States and reported 22 cases of endoscopic cross infection, including 7 of pseudomonas aeruginosa, 1 one of hepatitis C virus. Nelson et al. reported many bacteremia and infection cases after gastrointestinal endoscopy. The findings performed by Sugiyama et al. provided direct evidence that postendoscopic acute gastritis in two cases can be caused by cross-infection with H. pylori via endoscopy through the DNA fingerprinting. The attention must be paid on endoscope disinfection. The choice of endoscope was the key to achieve the bactericidal effect and ensure the normal useful life of endoscope. Ideal disinfectants should have the following properties: highly effective, safe for use, harmLess and non-irritating to human, structure stable and easy to store, no damage to equipments, compatible with some washer/disinfectors, and cheap. After cleaning the 40 gastroscopes according to the routine method, all the viruses could be killed after exposure to Medilox for 1 min, showing that Medilox could be conveniently and quickly applied to endoscope disinfection. The study reported by C. Landa-Solis et al. shows that super-oxidized water (Microcyn) exerts a wider antimicrobial spectrum than acidic SOWs.

In a word, the most significant feature of Medilox is odorless, tasteless and non-irritant, has a quick and highly effective bactericidal action, restores to ordinary water at room temperature after disinfection, is environmentally safe, and also avoids the recontamination caused by washing with the running water after disinfection. Therefore, it has a wide application range.
### Table I  Results of Medilox neutralizer test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of bacterial colony in each experiment (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disinfectant + bacterial suspension(Groups A)</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Disinfectant+bacterial suspension+neutralizer(Groups B)</td>
<td>465 510 486</td>
</tr>
<tr>
<td>Neutralizer + bacterial suspension(Groups C)</td>
<td>2230000 2350000 1940000</td>
</tr>
<tr>
<td>Disinfectant + neutralizer +bacterial suspension(Groups D)</td>
<td>2080000 2130000 1910000</td>
</tr>
<tr>
<td>PBS+ bacterial suspension(Groups E)</td>
<td>2102000 2372000 2051000</td>
</tr>
<tr>
<td>PBS+ neutralizer + culture medium(Groups F)</td>
<td>2216000 2286000 2014000</td>
</tr>
</tbody>
</table>

Temperature of the test (20 ±1) ℃, exposure to the stock solution of Medilox for 1 min, exposure to the neutralizer for 10 min, experiment carried out in triplicate.

### Table II  Bactericidal test of clinical isolated strains

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Mean number of bacterial colony in positive control (CFU/mL)</th>
<th>Killing rate at different time points (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 min 1 min 3 min</td>
</tr>
<tr>
<td>MRSA</td>
<td>1432000</td>
<td>99.99 100.00 100.00</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1230000</td>
<td>99.99 100.00 100.00</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1360000</td>
<td>99.99 100.00 100.00</td>
</tr>
</tbody>
</table>

### Table III  Killing effect of bacillus subtilis (ATCC25922)

<table>
<thead>
<tr>
<th>Experimental No</th>
<th>Killing rates after exposure for different time periods (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min 5 min 1 0 min</td>
</tr>
<tr>
<td>One</td>
<td>46.70 99.52 100.00</td>
</tr>
</tbody>
</table>
Two  49.30  99.71  100.00  
Three  43.50  98.43  100.00  
Mean value  46.50  99.22  100.00

Table IV  Destruction effect of Medilox on HBsAg

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean S/N value after exposure for different time periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 min</td>
</tr>
<tr>
<td>Stock solution</td>
<td>7.56</td>
</tr>
<tr>
<td>2 fold dilution</td>
<td>16.37</td>
</tr>
<tr>
<td>4 fold dilution</td>
<td>45.21</td>
</tr>
</tbody>
</table>


Table V  Disinfection efficiency of Medilox on gastroscopes

<table>
<thead>
<tr>
<th>Disinfection place</th>
<th>Mean number of bacterial colony before disinfection (CFU/cm²)</th>
<th>Mean number of bacterial colony after disinfection (CFU/cm²)</th>
<th>Killing rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>External surface area</td>
<td>126</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Internal surface area</td>
<td>45</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Whole</td>
<td>171</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table VI  Influence of organisms on effect of Medilox on MRSA

<table>
<thead>
<tr>
<th>Concentration of calf serum (%)</th>
<th>Mean killing rate after exposure for different time periods (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 min</td>
</tr>
<tr>
<td>25</td>
<td>98.51</td>
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<tr>
<td>50</td>
<td>97.64</td>
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Acknowledgements

Thanks to the key projects of the medical science of Hebei province for funding the whole program.

References


Table VII  Mean value and range of viable organism number in field experiment of disinfection for object surface (CFU/cm²)

<table>
<thead>
<tr>
<th>Experimental No</th>
<th>In positive control group</th>
<th>Disinfection group</th>
<th>Mean value of log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
<td>Range</td>
<td>Mean value</td>
</tr>
<tr>
<td>1</td>
<td>368.2</td>
<td>60-1576</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>279.5</td>
<td>45-1340</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>426.7</td>
<td>58-2073</td>
<td>1.6</td>
</tr>
</tbody>
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