

## Original Article

# Comparison of the effects of formaldehyde and gaseous ozone on HBV-contaminated hospital quilts

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**Abstract:** Background: Besides being highly infectious, Hepatitis B virus (HBV) is a major cause of liver disease worldwide. In hospital settings, it is easy for the environment and quilts to be contaminated by HBV patient blood and body fluids. Therefore, HBV can be transmitted to other patients via contaminated environmental surfaces or quilts, resulting in an HBV nosocomial infection. Formaldehyde and ozone are commonly used disinfectants that may influence this infectious situation. Objective: To investigate the clinical effectiveness of formaldehyde and gaseous ozone for the terminal cleaning of hospital quilts contaminated by HBV. Methods: Thin cloth and thick cotton soaked with the serum from high HBV copy number patients were prepared and disinfected using formaldehyde fumigation and gaseous ozone at different times. The copy numbers of HBV DNA in the HBV-contaminated cloth and cotton samples were measured quantitatively with fluorescent quantitative polymerase chain reaction (PCR). Results: When gaseous ozone was used to disinfect HBV-contaminated quilts for 23 minutes (min), 36 min, 49 min, and 90 min, the HBV DNA copy number displayed no significant decrease compared with the copy number before disinfection ( $P > 0.05$ ). In comparison, the copy number of the HBV DNA in the cloth group decreased significantly ( $P < 0.05$ ) after formaldehyde fumigation disinfection for 1 hour (h), and there was no difference when longer times and increased concentrations were used. In the thick cotton group, there was also a significant decrease ( $P < 0.05$ ) of the HBV DNA copy numbers, but the decrease was not as dramatic. In addition, in this group, the disinfection effect observed at 4 h was the strongest. Conclusions: The application of ozone to disinfect HBV-contaminated hospital quilts possibly has no effect, whereas, formaldehyde oxide fumigation effectively reduced HBV copy numbers.

**Keywords:** Gaseous ozone, formaldehyde, HBV, disinfection

## Introduction

HBV is a major cause of liver disease worldwide. Infections caused by HBV can become chronic and may lead to liver cirrhosis and carcinoma [1]. Currently, only limited chemotherapy is available. The indirect spread of HBV, although much less common, can occur when objects that are freshly contaminated with tainted blood enter the body or contact damaged skin [2, 3]. Avoiding contact with these materials is the most effective means of protection, but such avoidance is impossible. In addition, HBV is relatively insensitive to ultraviolet rays and far infrared rays. General-purpose environmental disinfection is unlikely to play a significant role in preventing HBV transmission [3]. Numerous alternatives for disinfecting HBV-contaminated sites and quilts have been

described in the literature, such as 40% ethanol, 30% isopropanol, 0.01% peracetic acid, 0.05% glutaraldehyde, 0.7% formaldehyde gas fumigation, 2% glutaraldehyde soaking, high-pressure steam sterilisation technology, and ozone ( $O_3$ ) fumigation [4-6]. The true role and mechanisms of the various applied agents remain undetermined. In addition, the development of medical equipment that cannot adequately be sterilised or disinfected has caused growing concern in recent years. Therefore, it is important to determine which endpoints should be used to judge the success or failure of disinfection and assist in determining how long disinfection should be maintained. The objective of this study was to evaluate the disinfection efficacy of the application of formaldehyde and gaseous ozone in the management of HBV-contaminated hospital quilts. Because a quilt is

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composed of both cloth and cotton, we examined 2 cloth and cotton groups to evaluate and compare the disinfection efficacy of formaldehyde and gaseous ozone.

### Materials and methods

#### *Perspex fumigation cabinets and bed unit ozone sterilizer*

A Perspex fumigation cabinet (0.36×0.40×0.64) m<sup>3</sup> (metres) was acquired (Zhangdian Hardware Doctor Toys Factory, Jiangyan City, Jiangsu province, China). It was separated by 0.14 m<sup>2</sup> glass clapboard into 4 layers. The layers were drilled with 35 holes with a 1 cm diameter. The bottom layer was used to hold the formaldehyde and potassium permanganate (KMnO<sub>4</sub>) powder. A fully computer-controlled bed unit ozone sterilizer (Kz-x-dL1, Guangdong Kangzhen Medical Equipment Co. Ltd., Guangdong province, China) was used. A bed unit ozone sterilizer (LK/CXD) was acquired from Chengdu Laoken Technology Co. Ltd., Chengdu, China.

#### *Preparation of virus carrier and disinfectants*

Sterile cloth (0.5×0.5×0.05 cm<sup>3</sup>) and cotton (0.5×0.5×0.5 cm<sup>3</sup>) were prepared. The serum was collected from HBV-infected people with a HBV DNA copy number of 10<sup>7</sup> copies/ml. The serum was diluted 10-fold with sterile distilled water. We added 0.5 mL of diluted serum to the sterile cotton and cloth followed by drying at 37°C for 30 minutes (mins). Formaldehyde was used as liquid formalin (HCHO) with a concentration of 37%. KMnO<sub>4</sub> was used to oxidase the formaldehyde.

#### *Disinfection assay*

*Formaldehyde oxidization fumigation disinfection of HBV-contaminated cloth:* We divided the HBV-contaminated cloth into 2 groups, and each group contained 2 sets of cloth. For the first group, we placed 4 g of KMnO<sub>4</sub> and 8 ml of 37% formaldehyde (HCHO) on the bottom layer of the Perspex fumigation cabinet for disinfection. For the second group, we placed 8 g of KMnO<sub>4</sub> and 16 ml of 37% HCHO for disinfection. We placed an HBV-contaminated cloth in the same layer. The detection times were 1, 2, and 4 hours (h) after fumigation. The sterilization temperature was set at 55.8°C, according

to the manufacturer's directions. At each end of the detection times, we removed 2 clothes, soaked and eluted them with sterile distilled water, and then tested the HBV DNA copy numbers using the polymerase chain reaction (PCR).

*Formalin oxidization fumigation disinfection of HBV-contaminated cotton:* We divided the HBV-contaminated cotton samples into 2 groups, and each group contained 4 cotton samples. The other procedures were similar to those in the cloth group.

*Fully computer-controlled bed unit ozone sterilizer disinfection of HBV-contaminated cloth:* We divided the HBV-contaminated cloth into 3 groups. Each group was placed in one sterile Petri dish, and each dish contained 5 cloth samples. Cloth samples in the sterile Petri dish were fixed in 5 different positions: top left, top right, bottom left, bottom right, and middle of the sterilizer. Three procedures were performed according to the directions. The bed unit ozone sterilizer (Kz-x-dL1) had 3 working states. The working state for channel 1 was set as follows: preparation vacuum time 5 min, ozone concentration of 300 mg/m<sup>3</sup> and fumigation time 15 min. Then, the cloth samples were soaked and eluted with sterile distilled water, and PCR was used to determine the HBV-DNA copy numbers. The same method was used for channel 2 (preparation vacuum time 5 min and fumigation time 30 min) and channel 3 (preparation vacuum for 5 min and fumigation 60 min) for disinfection. The same experimental method was also used on the bed unit ozone sterilizer (LK/CXD) cloth experiment group, but the disinfection time was increased to 90 min, the preparation vacuum time to 10 min, and the fumigation time to 80 min.

The positive control groups were HBV-DNA-contaminated cotton and cloth samples that were placed outside the disinfection machine for identical lengths of time. The negative control groups were composed of sterile distilled water samples. For each group, the results are representative of 2 independent experiments.

#### *Detection methods*

Fluorescent quantitative PCR was performed using a kit according to the manufacturer's instructions to assess the ability of ozone and

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**Table 1.** HBV DNA copy numbers (copies/ml) in cloth groups disinfected using formaldehyde oxidization fumigation

| Time (h) | Disinfection group                                |       |  |       | Non-disinfection group |
|----------|---|-------|--|-------|------------------------|
|          | 8 ml of formaldehyde and 4 g of KMnO <sub>4</sub> |       | 16 ml of formaldehyde and 8 g of KMnO <sub>4</sub> |       |                        |
| 1        | 438.8   | 433.7 | 661.8  | 1972  | 1.22×10 <sup>7</sup>   |
| 2        | 937.7   | 2436  | 652.8  | 318.1 | 1.16×10 <sup>7</sup>   |
| 4        | 796.8   | 2642  | 313.8  | 340.2 | 1.17×10 <sup>7</sup>   |

formaldehyde to disinfect HBV-contaminated hospital quilts. Fluorescent quantitative PCR detection was performed using an MJ Opticon Monitor Fluorescent PCR detector and an HBV-DNA quantitative detection kit (Both are from Shenzhen Piji Bioengineering Co., Ltd.; lot number: S20020033).

### Statistical analysis

All statistical calculations were performed using SAS version 3.1 for windows (SAS, Institute, United States of America). The results are expressed as the mean values. Differences between subgroups were tested with a t-test, and *p* values of < 0.05 were considered significant.

### Results

#### Disinfection effect of formalin oxidization fumigation

As shown in **Table 1**, after 1 h of disinfection, the HBV-DNA copy number in the non-disinfection cloth group was 1.22×10<sup>7</sup>/ml, whereas the average HBV-DNA copy numbers in the 8 ml of formalin/4 g of KMnO<sub>4</sub> and 16 ml of formalin/8 g of KMnO<sub>4</sub> disinfection groups were 435.85 and 1316.9/ml, respectively, which was significantly lower than the levels in the non-disinfection group (*P* < 0.05). There was no difference between the 8 ml of formalin/4 g of KMnO<sub>4</sub> and 16 ml of formalin/8 g of KMnO<sub>4</sub> disinfection groups. Furthermore, the 1 h, 2 h and 4 h formaldehyde/KMnO<sub>4</sub> disinfection groups displayed no differences in the HBV-DNA copy number (*P* > 0.05).

In the cotton groups, after 1 h of disinfection, the HBV-DNA copy number in the non-disinfection group was 1.28×10<sup>7</sup>/ml, whereas the average HBV-DNA copy numbers in the 8 ml of formalin/4 g of KMnO<sub>4</sub> and 16 ml of formalde-

hyde/8 g of KMnO<sub>4</sub> disinfection groups were 1.76×10<sup>5</sup> and 3.95×10<sup>5</sup>/ml, respectively, which was significantly lower than the copy number in the non-disinfection group (*P* < 0.05). After 4 h disinfection, a significant decrease in the copies of HBV DNA could be observed (*P* < 0.05). There

was no difference between the 8 ml of formaldehyde/4 g of KMnO<sub>4</sub> and 16 ml of formaldehyde/8 g of KMnO<sub>4</sub> disinfection groups (**Table 2**).

#### Disinfection effect of bed unit ozone sterilizer

After 23 min of disinfecting the cloth using a Kz-x-dL1 bed unit ozone sterilizer, the HBV-DNA copy number in the disinfection groups was no different from the non-disinfection groups. After 23 min, 36 min and 46 min of disinfection using a Kz-x-dL1 bed unit ozone sterilizer, the HBV-DNA copy number displayed no significant differences among the disinfection groups (*P* > 0.05). After 90 min of disinfection using an LK/CXD bed unit ozone sterilizer, there was no significant difference in HBV-DNA copy number among the in disinfection groups, and there was no significant decrease compared with the non-disinfection groups (*P* > 0.05) (**Table 3**).

### Discussion

HBV DNA can be found in different body fluids from HBV-infected patients [7]. It has been reported that the sera from certain HBV carriers, even at dilutions of 1:100,000,000, are still able to infect chimpanzees when administered intravenously [8], which indicates that HBV viruses possess potent contagious ability. Reports in the literature indicate that hepatitis B viruses can live for several days in dried blood on table surfaces, needles, syringes, and razors [9]. In hospital settings, many patients, particularly surgical patients, have wounds that are at high risk of HBV infection. Franka E. et al. examined the prevalence of HBV in 300 medical waste handlers (MWHs) and 300 non-medical waste handlers (NMWHs). The HBV detection rates were 7 (2.3%) and 1 (0.3%) among the MWHs and NMWHs, respectively. Significant differences were observed in the detection rates of HBV (odds ratio [OR], 7.14; *P* < 0.04) in

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**Table 2.** HBV DNA copy numbers (copies/ml) in the cotton groups disinfected using formaldehyde oxidization fumigation

| Groups<br>Time (h) | Disinfection groups                             |                    |                    |                    |  |                    |                    |                    | Non-disinfection group |
|--------------------|---|--------------------|--------------------|--------------------|--|--------------------|--------------------|--------------------|------------------------|
|                    | 8 ml of formaldehyde and 4 g of $\text{KMnO}_4$ |                    |                    |                    | 16 ml of formaldehyde and 8 g of $\text{KMnO}_4$ |                    |                    |                    |                        |
|                    | 1   | 2                  | 3                  | 4                  | 1  | 2                  | 3                  | 4                  |                        |
| 1                  | $2.26 \times 10^5$                              | $1.05 \times 10^5$ | $1.61 \times 10^5$ | $1.77 \times 10^5$ | $3.68 \times 10^5$                               | $4.67 \times 10^5$ | $4.27 \times 10^5$ | $4.27 \times 10^5$ | $1.28 \times 10^7$     |
| 2                  | $2.85 \times 10^5$                              | $3.47 \times 10^5$ | $1.45 \times 10^5$ | $2.12 \times 10^5$ | $0.95 \times 10^5$                               | $1.35 \times 10^5$ | $1.62 \times 10^5$ | $1.03 \times 10^5$ | $1.13 \times 10^7$     |
| 4                  | $8.31 \times 10^4$                              | $7.30 \times 10^4$ | $5.83 \times 10^4$ | $8.32 \times 10^4$ | $8.94 \times 10^4$                               | $2.70 \times 10^4$ | $4.51 \times 10^4$ | $4.46 \times 10^4$ | $1.06 \times 10^7$     |

**Table 3.** HBV DNA copy numbers (copies/ml) in the cloth groups disinfected using Kz-x-dL1 and LK/CXD bed unit ozone sterilizers

| Groups<br>Time (min) | Disinfection directions |                    |                    |                    |                    | Non-disinfection group |
|----------------------|-------------------------|--------------------|--------------------|--------------------|--------------------|------------------------|
|                      | Top left                | Bottom left        | Top right          | Bottom right       | Middle             |                        |
| 23                   | $1.20 \times 10^7$      | $1.17 \times 10^7$ | $0.92 \times 10^7$ | $0.82 \times 10^7$ | $1.22 \times 10^7$ | $1.01 \times 10^7$     |
| 36                   | $0.40 \times 10^7$      | $1.74 \times 10^7$ | $2.88 \times 10^7$ | $0.48 \times 10^7$ | $1.85 \times 10^7$ | $0.80 \times 10^7$     |
| 46                   | $1.01 \times 10^7$      | $0.81 \times 10^7$ | $0.56 \times 10^7$ | $0.72 \times 10^7$ | $0.19 \times 10^7$ | $1.01 \times 10^7$     |
| Average              | $0.84 \times 10^7$      | $1.24 \times 10^7$ | $1.45 \times 10^7$ | $0.65 \times 10^7$ | $1.09 \times 10^7$ | $0.94 \times 10^7$     |
| 90                   | $0.87 \times 10^7$      | $0.96 \times 10^7$ | $0.76 \times 10^7$ | $0.88 \times 10^7$ | $1.18 \times 10^7$ | $1.37 \times 10^7$     |

the MWHs compared with the NMWHs [2]. These studies suggest that in HBV-contaminated hospital settings, the environment and quilts can contain sufficient virions to transmit HBV infection. MWHs and patients with wounds are at high risk of exposure to HBV infection. Therefore, establishing an efficient disinfection method that can decrease the likelihood of HBV infection is imperative.

Gaseous ozone ( $\text{O}_3$ ) is a strong oxidizing agent that can directly affect the survival of pathogens. It has been reported that  $\text{O}_3$  can be employed in various forms, such as ozonized saline solution [10], ozonized water [11], ozonized oil [12], ozone associated with other substances [13], and, more frequently, a gaseous  $\text{O}_3$ /oxygen ( $\text{O}_2$ ) mixture [14]. It is widely used as a disinfectant in drinking water treatment plants and food processing plants in many European countries [15-17]. Gaseous ozone has also been potentially considered for disinfecting the hospital environment, a source of microorganism infection for patients [18]. Previously, a number of ozone disinfection studies of viruses such as adenoviruses, enteroviruses, hepatitis A virus, norovirus, and poliovirus have indicated that  $\text{O}_3$  is a strong disinfectant and is able to effectively inactivate these viruses [19-23]. However, few studies have reported the effect of HBV disinfection by gaseous ozone.

A study demonstrated that ozone can destroy hepatitis B surface antigen (HBsAg) within 20 min at a concentration of 56.7 mg/m [3, 24], but the disappearance of HBsAg does not always correlate with the infection of HBV, and HBsAg itself has no infectivity. Thus, it is improper to evaluate HBV activity based on HBsAg detection. In the current study, we observed that the application of gaseous ozone could not inactivate HBV, even when the disinfection time was prolonged to 90 min. We only used thin cloth (not thick cotton) for the ozone experiments. Previous research has demonstrated that ozone can inactivate HBV DNA [24].

Formaldehyde oxidization fumigation has been in widespread use for over a century as an effective disinfectant in hospital rooms to reduce microbial agents on hospital surfaces and to control nosocomial infections. Heat- and pressure-sensitive medical devices can be sterilized with saturated water steam in combination with formaldehyde [25]. Our findings indicate that formalin oxidization fumigation can be used for disinfecting HBV-contaminated cotton and cloth. In the cloth group, the HBV DNA copy numbers decreased to their lowest point after 1 h of disinfection with 8 ml of formalin/4 g of  $\text{KMnO}_4$ , and increasing the time or raising the concentration had no effect. These results indicate that for thin items, a short dis-

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infection time and low concentration of formaldehyde fumigation can effectively reduce HBV infection. With the cotton groups, the alteration was not as dramatic because the thick material is more difficult to penetrate, but a significant decrease of the HBV DNA copy numbers (compared with the non-disinfection group) ( $P < 0.05$ ) was still observed. With the cotton groups, we observed that prolonging the disinfection time to 4 h could further significantly decrease the HBV copy numbers, but the values were still far higher than those in the cloth group. These results indicate that the disinfecting effect on thick material is relatively low, and a concentration increase may have little influence on the final disinfection effectiveness.

Previous investigations have demonstrated that formaldehyde can cause eye or skin irritation, and high levels of formaldehyde can induce squamous cell carcinomas in the nasal passages of rats [26, 27]. Therefore, for safety reasons, shorter disinfection times and lower concentrations of formaldehyde would be more suitable for hospital disinfection. Certainly, other measures, such as strong ammonia water solutions, can be used to absorb residue formaldehyde, but they could cause second contamination [28].

In conclusion, our findings may provide useful information on disinfecting HBV-contaminated hospital quilts that will help reduce the spread of HBV. The application of ozone to disinfect HBV-contaminated hospital quilts was ineffective. The formaldehyde oxide fumigation did produce a significant HBV copy-number reduction, but it also had unpleasant side effects. Thus, the identification of highly effective and safe disinfectants that can be used for sterilizing HBV-contaminated hospital quilts and other such materials is urgently needed.

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### Disclosure of conflict of interest

None.

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