Efficacy of Disinfectants and Hot Water against Biofilm Cells of *Burkholderia cepacia*

Naoyuki MIYANO, a Shigebaru OIE, b and Akira KAMIYA*. b

*Department of Pharmacy, Yamaguchi Rosai Hospital; Minaminakagawa-cho, Onoda 756–0095, Japan; and b Department of Pharmacy, Yamaguchi University Hospital; 1–1–1 Minamikogushi, Ube 755–8505, Japan.

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The effects of various disinfectants and hot water on planktonic cells and biofilm cells of *Burkholderia cepacia* were investigated. The survival rate of viable *B. cepacia* cells in suspension decreased to 0.001% or lower within 15 s of exposure to 0.5% benzalkonium chloride, within 30 s of exposure to 0.5% alkyltrimethylammonium chloride or benzalkonium chloride, or within 1 min of exposure to 0.1% alkyltrimethylammonium chloride, and decreased to about 0.1% with 60 min of exposure to 0.1% benzalkonium chloride or 0.5% chlorhexidine gluconate, but did not decrease to 1% or less with 60 min of exposure to 0.1% chlorhexidine gluconate. There were no effects of 0.1% and 0.2% chlorhexidine gluconate and 0.1% benzalkonium chloride against biofilm cells of *B. cepacia*, and 0.5% chlorhexidine gluconate, 0.5% benzalkonium chloride and 0.1% alkyltrimethylammonium chloride were barely effective against biofilm cells even after 60-min exposure. On the other hand, both planktonic cells and biofilm cells of *B. cepacia* were eradicated within 15 s by sodium hypochlorite, povidone-iodine, 80% v/v ethanol, and hot water at 65°C or higher.

**Key words** *Burkholderia cepacia*; biofilm; disinfectant; hot water; silicone surface

*Burkholderia cepacia* is an important causative agent of opportunistic infection. This bacteria has been implicated in various hospital outbreaks (bloodstream infections, urinary tract infections, and respiratory tract infections) associated with contaminated disinfectants, aqueous solutions, and pharmaceuticals.1–4) The presence of biofilm has been implicated as a cause of infections in implanted devices and catheters, and an outbreak caused by biofilm cells of *B. cepacia* on the inner surface of a subclavian catheter was reported. 5) Although the effects of disinfectants are reported on planktonic cells of *B. cepacia* in suspension, the effects of disinfectants and hot water on planktonic cells in suspension and biofilm cells of *B. cepacia* have not been reported to the best of our knowledge. In this study, we compared the effects of various disinfectants and hot water on planktonic cells in suspension and biofilm cells of *B. cepacia*.

**MATERIALS AND METHODS**

**Bacterial Strains Used** The suspension test was performed using six isolates of *B. cepacia* from disinfectants and aerosol solution containing antibiotics at Yamaguchi University Hospital. 6, 7) The strain that showed the lowest sensitivity was subjected to biofilm formation and this strain was detected in a 0.02% benzalkonium chloride solution.

**Disinfectants Used** Chlorhexidine gluconate (Astra Zeneca, Osaka, Japan), benzalkonium chloride (Nihon Pharmaceuticals, Tokyo, Japan), alkyltrimethylammonium chloride (Azwell Inc., Osaka, Japan), sodium hypochlorite (Procter and Gamble, U.S.A.), povidone–iodine (1% titratable I2; Meiji Seika, Tokyo, Japan), and 80% v/v ethanol (Dainippon Pharmaceutical, Osaka, Japan) were used. These agents were diluted with sterilized distilled water.

**Effects of Various Disinfectants on Planktonic Cells of *B. cepacia* in Suspension** Bacteria cultured in trypticase soy agar at 30°C for 48 h were scraped and suspended in 5 ml of sterile physiological saline, and a bacterial suspension with about 10^8 cells/ml was prepared. This suspension (0.05 ml) was added to a test tube containing 4.95 ml of each disinfectant at 22±2°C and vortexed for about 10 s. From these test tubes, 0.5 ml was sampled after 15 and 30 s, and 1, 2, 5, 10, 30, and 60 min, added to 4.5 ml of trypticase soy broth containing an inactivator, and vortexed. The following inactivators were used: 0.5% Tween 80, 0.5% Lubrol W, and 0.25% soya lecithin for chlorhexidine gluconate, benzalkonium chloride, and alkyltrimethylammonium chloride; and 0.5% sodium thiosulfate for sodium hypochlorite and povidone–iodine. Ethanol (80% v/v) was inactivated by diluting it 1:100 with trypticase soy broth. To determine viable cells, bacteria were serially diluted 10-fold with sterilized physiological saline and cultured in trypticase soy agar at 30°C for 48 h. The experiment was repeated three times for each strain, and the mean and standard deviation for six strains were calculated.

**Effects of Hot Water on Planktonic Cells of *B. cepacia* in Suspension** The strain of *B. cepacia* that was the most resistant in the suspension test was used to determine the effects of hot water on planktonic cells. A bacterial suspension with about 10^8 cells/ml was prepared as described above. This suspension (0.05 ml) was added to a test tube containing 4.95 ml of sterile distilled water prewarmed to a specific temperature in a water bath. From these test tubes, 0.5 ml was sampled after 15 and 30 s, and 1, 2, 5, and 10 min, added to 4.5 ml of sterile physiological saline (22±2°C) and vortexed for about 10 s. Viable cells were counted as described above. The experiment was performed three times for each strain.

**Effects of Various Disinfectants on Biofilm Cells of *B. cepacia*** The strain of *B. cepacia* that was the most resistant in the suspension test was used to determine the effects of disinfectants on biofilm cells. Silicone discs (1 mm thick, 8 mm in diameter) were purchased from Nichiden Rika Shoshi K.K (Itami, Japan). A 20-μl aliquot of the 10^8 cells/ml suspension prepared as described above was dripped onto a sterilized silicone disc and dried on a clean bench for 2 h. The silicone disc was then
washed with sterile physiological saline, placed in a Petri dish containing 20 ml of physiological saline and 0.2 ml of trypticase soy broth, and cultured at 30 °C for 5 d. Biofilm formation of *B. cepacia* was confirmed using scanning electron microscopy. After rinsing again with sterile physiological saline, the test discs were placed in a Petri dish containing 20 ml of each disinfectant at 22 ± 2 °C. The disc was removed from the dish after a specified period and immediately transferred to 5 ml of trypticase soy broth containing the inactivator and vortexed for 10 s. It was then sonicated at 36 kHz for 10 min and vortexed again for 10 s. This procedure releases viable bacteria adhering to the disc into the physiological saline. The bacteria in the physiological saline were counted and the number of bacteria on the disc was calculated. Viable cells were counted as described above. At least three discs were used. The viable bacteria on the control discs were counted after the discs had been stored in sterile physiological saline for 60 min.

**Effects of Hot Water on Biofilm Cells of *B. cepacia***

Discs with bacteria adhering to them, prepared as described above, were washed with sterile physiological saline and transferred into sterile water prewarmed to a specific temperature in a water bath. The discs were removed over time, immediately transferred into 5 ml of sterile physiological saline at 22±2 °C, and vortexed for 10 s. Each disc was sonicated at 36 kHz for 10 min and vortexed again for 10 s. The discs were processed as described above, and the number of viable bacteria on each disc was obtained.

**RESULTS**

**Effects of Disinfectants on Planktonic Cells of *B. cepacia***

The survival curves of planktonic cells of *B. cepacia* in suspension after exposure to various disinfectants are shown in Fig. 1. The survival rate of viable cells decreased to 0.001% or lower within 15 s of exposure to povidone–iodine, 0.01% (100 ppm) sodium hypochlorite, and 0.5% benzalkonium chloride (quantification limit: 0.0005%). As a rapid reduction to 0.01% or lower was recorded within 15 s of exposure to 80% v/v ethanol (quantification limit: 0.005%), it was presented as 0.001% or lower. The survival rate of viable cells was below the detection limit after 30-s exposure to 0.5% alkyldiaminoethyl glycine or 1-min exposure to 0.1% alkyldiaminoethyl glycine. However, the survival rate of viable cells did not decrease to 1% or less even after 60-min exposure to 0.1% chlorhexidine gluconate. The survival rate decreased to 0.1% or less after 5-min exposure to 0.5% chlorhexidine gluconate and decreased to 0.1% or less after 60-min exposure to it. Although the survival rate decreased to 0.1% or less after 5-min exposure to 0.1% benzalkonium chloride, the survival rate changed little after 60-min exposure to it.

**Effects of Disinfectants on Biofilm Cells of *B. cepacia***

Table 1 shows the effects of the disinfectants on biofilm cells of *B. cepacia*. The number of viable cells is presented as a logarithm, and the control value of 5.79±0.27 was obtained from 17 samples stored in physiological saline for 60 min. Bacteria were not detected after exposure to 0.01% (100 ppm) and 0.1% (1000 ppm) sodium hypochlorite, povidone–iodine, or 80% v/v ethanol for 15 s (detection limit: 1.40). There were no effects of 0.1% and 0.2% chlorhexidine gluconate or 0.1% benzalkonium chloride. The numbers of viable cells decreased after 60-min exposure to 0.5% chlorhexidine gluconate and 0.5% benzalkonium chloride, but the bacteria on all of the discs were still viable. Although the numbers of viable cells decreased earlier after exposure to 0.1% or 0.5% alkyldiaminoethyl glycine than to chlorhexidine gluconate and benzalkonium chloride, the bacteria on some of the discs were viable even after exposure for 60 min. The numbers of viable cells after exposure to 0.5% alkyldiaminoethyl glycine were less than after exposure to 0.1% alkyldiaminoethyl glycine.

**Effects of Hot Water on Biofilm Cells and Planktonic Cells of *B. cepacia***

Table 2 shows the effects of hot water on planktonic cells of *B. cepacia*. The survival rates are expressed as percentages of initial counts. The survival rate was below the detection limit after the cells had been stored at 60, 65, and 70 °C for 15 s. The survival rate also fell below...
the detection limit after the cells were stored at 55 °C for 5 min.

Table 3 shows the effects of hot water on biofilm cells of *B. cepacia*. The number of viable cells is presented as a logarithm, and the control value of 5.84 ± 0.21 was obtained from 12 samples stored in physiological saline for 60 min. The number was below the detection limit after the cells had been stored at 65 °C and 70 °C for 15 s. The number also fell below the detection limit after the cells were stored at 55 °C for 1, 2, and 5 min, respectively.

The effects of hot water on biofilm cells of *B. cepacia* were similar to those on planktonic cells of *B. cepacia* and eradicated by hot water at 65 °C or higher.

DISCUSSION

*B. cepacia* is a nonauxotrophic aerobic bacteria and the optimal temperature for its growth is low (28—30 °C). This bacteria is reported to have certain properties enabling it to grow in hospital environments, such as the use of many organic compounds as a source of carbon, and reported to be able to survive for long periods on environmental surfaces. Although the colonization of biofilm cells of *B. cepacia* has been reported, there have been few data on methods of disinfection for this bacteria. Therefore, we studied the effects of various disinfectants and hot water on biofilm cells of *B. cepacia*.

The effects of disinfectants are usually investigated using the suspension test. However, test bacteria are easily eradicated in the test because they are readily exposed to disinfectants. In this study, the resistance to disinfectants (chlorhexidine gluconate, benzalkonium chloride, alkyldiaminoethyl glycine) was increased in the biofilm cells compared to the planktonic cells in suspension. In experiments...
on biofilm cells of *Pseudomonas aeruginosa* and *Staphylococcus aureus* reported previously,\(^1\) the effects of these disinfectants increased as the concentrations increased, but the effects of chlorhexidine gluconate and benzalkonium chloride on biofilm cells of *B. cepacia* increased only slightly at higher concentrations. The effect of alkylidiaminoethyl glycine on biofilm cells increased as the concentration increased, but was insufficient.

These findings suggest that among the disinfectants examined, agents other than sodium hypochlorite, povidone–iodine, and 80% v/v ethanol cannot be expected to have an antibacterial effect on biofilm cells of *B. cepacia*. However, sodium hypochlorite is corrosive to metal and therefore inappropriate for the disinfection of instruments. Povidone–iodine is also inappropriate because it causes coloring, although it is useful for local disinfection of the human body. Moreover, 80% v/v ethanol can be used for humans but may be inappropriate for dipping disinfection due to its price and flammability.

Biofilm cells of *B. cepacia* were eradicated by hot water at 50°, 55°, 60°, and 65 °C within 5 min, 2 min, 1 min, and 15 s, respectively. Based on these results, hot water at 65 °C or higher exhibits a bactericidal effect comparable to that of sodium hypochlorite, povidone–iodine and 80% v/v ethanol.

This study showed that both biofilm and planktonic cells of *B. cepacia* are eradicated by hot water at 65 °C or higher within 15 s and conditions that eradicate the hepatitis B virus (80 °C for 1 min or longer, or 71 °C for 3 min or longer) may also eradicate the bacteria. Hot water also has advantages in terms of cost and environmental considerations. These results indicate that hot water at 65 °C or higher is useful for the disinfection of medical instruments and apparatus contaminated by *B. cepacia*.

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**REFERENCES**