Microbial Contamination of Antiseptic-Soaked Cotton Balls

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We investigated microbial contamination of in-use antiseptics at a hospital. No microbial contamination was observed in 70 samples of 0.02% benzalkonium chloride solution (500-ml volume), 70 samples of 1% titratable I₂ povidone-iodine solution (250-ml volume), or 15 samples of 0.1% ethacridine lactate solution (500-ml volume) during use in reduced amounts. Nor was any microbial contamination observed in 70 samples of cotton balls soaked in 1% titratable I₂ povidone-iodine solution in canisters or cotton gauze soaked in 70% (w/v) ethanol solution in canisters. However, among 70 samples of cotton balls soaked in 0.02% benzalkonium chloride solution in canisters, 6 (8.6%) were contaminated with 10⁴ to 10⁶ viable cells/ml. The microbial species detected were glucose non-fermentative bacilli such as Alcaligenes xylosoxidans and Pseudomonas putida. The contaminants obtained from cotton balls soaked in 0.02% benzalkonium chloride solution did not proliferate in that solution or in distilled water but showed rapid growth in the cotton balls soaked in either of these liquids. These findings suggested that benzalkonium chloride solution tends to become contaminated when cotton balls are immersed. Therefore, cotton balls soaked in benzalkonium chloride solution are not recommended as an antiseptic. When no other choice is available, the cotton balls should be soaked in benzalkonium chloride solution at the time of use.

Key words antiseptics; benzalkonium chloride; cotton ball; microbial contamination

The use of dilute benzalkonium chloride and chlorhexidine has been associated with numerous outbreaks of infection and pseudoinfections, and the microbial contamination of these antiseptics has been recognized for more than 30 years.¹ ³ Despite the fact that such contamination is still being reported, preventive measures remain inadequate.⁴ ⁶ We evaluated the present status of microbial contamination of antiseptics and possible means of prevention at our hospital.

MATERIALS AND METHODS

Microbial Contamination of Antiseptics We investigated the microbial contamination of in-use antiseptics for patient skin and other superficial tissues at 16 departments of Yamaguchi University Hospital from November 1995 to July 1996. The analyzed materials were 70 samples of 0.02% benzalkonium chloride solution prepared in a sterilized 500-ml volume in the hospital, 70 samples of 1% titratable I₂ povidone-iodine solution (Meiji Seika Co., Tokyo, Japan, 250-ml volume), and 15 samples of 0.1% ethacridine lactate solution (Maruishi Pharm. Co., Osaka, Japan, 500-ml volume), all of which were used in divided amounts (ca. 10—30 ml). Seventy samples each of cotton balls (Kawamoto Co., Tokyo, Japan; sterile 100% cotton fiber) soaked in 0.02% benzalkonium chloride solution in canisters, cotton balls soaked in 1% titratable I₂ povidone-iodine solution in canisters, and cotton gauze (Kawamoto Co.; sterile 100% cotton fiber) soaked in 70% (w/v) ethanol (Kenei Pharm. Co., Tokyo, Japan) were also analyzed. Microbial contamination was also evaluated in 16 samples each of cotton balls and cotton gauze unssealed immediately prior to examination. Antiseptic-soaked cotton balls or gauze in canisters were freshly prepared using sterilized canisters, cotton balls or gauze, and antiseptic solution at 1-week intervals in the wards. Examination of the antiseptic-soaked cotton balls or gauze was therefore performed after one week of use in each ward.

The samples were diluted 10⁻¹, 10⁻², 10⁻³, and 10⁻⁵-fold in nutrient broth containing 0.5% Tween 80, 0.5% Lubrol W and 0.25% soya lecithin for benzalkonium chloride and containing 0.5% thiosulphate for povidone-iodine as inactivators.⁷ Cotton balls or gauze soaked in antiseptic was squeezed, and the obtained solution was used as samples. For cotton balls or cotton gauze, physiological saline (20 ml per 2 g) was added, and the solution obtained by squeezing was used as samples. Undiluted and diluted samples (0.2 ml) were transferred to tryptcine soy agar or Sabouraud dextrose agar both containing the above inactivators, streaked with a glass hockey stick and incubated at 30°C for 24—72 h (tryptcine soy agar) or at 25°C for 2—7 d (Sabouraud dextrose agar). Ethanol was inactivated by diluting it 1:100 with tryptcine soy broth. No inactivator was used for ethacridine lactate. Colonies were counted on each plate to determine colony-forming units (CFU) and were identified by Gram staining, morphological examination, the oxidation-fermentation test, the cytochrome-oxidase test, and the API system (Analytab Products, Plainview, NY, U.S.A.).

The period from the first use of antiseptics (0.02% benzalkonium chloride solution, 1% titratable I₂ povidone-iodine, 0.1% ethacridine lactate solution) and the period of use after preparation of antiseptic-soaked cotton balls or gauze were ascertained in interviews with the nurses in charge.

Viability of Contaminants in Diluted Antiseptics and in Cotton Balls Soaked in Diluted Antiseptics Contaminated solutions were obtained from cotton balls soaked in 0.02% benzalkonium chloride solution contaminated with 10⁶ CFU/ml of Pseudomonas putida or Alcaligenes xylosoxidans; 0.05 ml of each was added to: 20 ml of 0.02% sterile benzalkonium chloride solution; 20 ml of sterile distilled water; 2.0 g of sterile cotton balls soaked in 20 ml of 0.02% sterile benzalkonium chloride solution; 2.0 g of sterile

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cotton balls soaked in 20ml of sterile distilled water. The samples were incubated at 30 °C. Plate counts were performed at 24 and 48h, and 3, 4, and 7 d. Organisms were counted and identified by the previously described method. This experiment was performed 3 times, and the mean viable count was calculated.

RESULTS

Microbial Contamination of Antiseptics The period from the first use of the 0.02% benzalkonium chloride solution, 1% titratable I₂ povidone-iodine solution, and 0.1% ethacridine lactate solution examined in this study was 7 d—5 years, 7 d—1 month, and 1 month—2 years, respectively. None of these antiseptics showed microbial contamination (Table 1). The period of use after preparation of the antiseptic-soaked cotton balls or gauze in canisters was 7 d for 65 of the 70 samples of cotton balls soaked in 0.02% benzalkonium chloride solution and more than 2 months for the other 5 samples, 7 d for 68 of the 70 samples of cotton balls soaked in 1% titratable I₂ povidone-iodine solution and 14 d for the other 2 samples, and 7 d for all 70 samples of cotton gauze soaked in 70% ethanol. No sample of cotton balls soaked in 1% titratable I₂ povidone-iodine solution or cotton gauze soaked in 70% ethanol showed microbial contamination, but 6 (8.6%) of the 70 samples of cotton balls soaked in 0.02% benzalkonium chloride solution did show the contamination (Table 1). No microbial contamination was observed in the 16 samples each of cotton balls and cotton gauze unsealed immediately before examination. The viable cell count, major contaminant, and the period after preparation of the 6 contaminated samples of cotton balls soaked in 0.02% benzalkonium chloride are shown in Table 2. Each of these 6 samples was obtained from one of 6 of the 16 departments examined at the hospital. Samples No. 2—70 were handled by nurses, and sample No. 1 was commonly handled by patients. The purpose of use was disinfection of the genital area at the time of self-catheterization for sample No. 1, disinfection of the skin at the injection site in patients with alcohol hypersensitivity for samples No. 2—6, and disinfection of a wound or mucosa for samples No. 7—70.

Viability of Contaminants in Diluted Antiseptics and in Cotton Balls Soaked in Diluted Antiseptics Figures 1 and 2 show the viability profiles of P. putida and A.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Number of contaminated samples</th>
<th>Number of examined samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02% benzalkonium chloride solution</td>
<td>0/70</td>
<td>0/70</td>
</tr>
<tr>
<td>1% titratable I₂ povidone-iodine solution</td>
<td>0/70</td>
<td>0/70</td>
</tr>
<tr>
<td>0.1% ethacridine lactate solution</td>
<td>0/15</td>
<td>6/70</td>
</tr>
<tr>
<td>Cotton balls soaked in 0.02% benzalkonium chloride solution</td>
<td>0/70</td>
<td>0/70</td>
</tr>
<tr>
<td>Cotton balls soaked in 1% titratable I₂ povidone-iodine solution</td>
<td>0/70</td>
<td>0/70</td>
</tr>
<tr>
<td>Cotton gauze soaked in 70% ethanol</td>
<td>0/70</td>
<td>0/70</td>
</tr>
</tbody>
</table>

Fig. 1. Viability of Pseudomonas putida at 30 °C in 0.02% Benzalkonium Chloride (○), Cotton Balls Soaked in 0.02% Benzalkonium Chloride (●), Distilled Water (△), and Cotton Balls Soaked in Distilled Water (▲).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Colony counts (CFU/ml)</th>
<th>Contaminants</th>
<th>Period of use after preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5 x 10⁴</td>
<td>GNGB</td>
<td>7 d</td>
</tr>
<tr>
<td>2</td>
<td>4.9 x 10⁷</td>
<td>Pseudomonas putida</td>
<td>&gt; 2 months</td>
</tr>
<tr>
<td>3</td>
<td>1.2 x 10⁶</td>
<td>Alocigenes xylosoxidans</td>
<td>&gt; 2 months</td>
</tr>
<tr>
<td>4</td>
<td>2.5 x 10⁵</td>
<td>GNGB</td>
<td>&gt; 2 months</td>
</tr>
<tr>
<td>5</td>
<td>2.6 x 10⁴</td>
<td>Pseudomonas chlororaphis</td>
<td>&gt; 2 months</td>
</tr>
<tr>
<td>6</td>
<td>2.0 x 10⁴</td>
<td>GNGB</td>
<td>&gt; 2 months</td>
</tr>
<tr>
<td>7</td>
<td>&lt; 5</td>
<td>—</td>
<td>7 d</td>
</tr>
</tbody>
</table>

a) GNGB: glucose non-fermentative gram-negative bacilli. b) Additional cotton balls and 0.02% benzalkonium chloride solution had been added to the canisters as needed.

Fig. 2. Viability of Alocigenes xylosoxidans at 30 °C in 0.02% Benzalkonium Chloride (○), Cotton Balls Soaked in 0.02% Benzalkonium Chloride (●), Distilled Water (△), and Cotton Balls Soaked in Distilled Water (▲).
**DISCUSSION**

We examined microbial contamination in in-use antiseptics for patient skin and other superficial tissues and found contamination in 6 of 70 samples of cotton balls soaked in 0.02% benzalkonium chloride solution. The detected microorganisms were glucose-nonfermentative gram-negative bacilli such as *A. xylosoxidans* and *P. putida*. There have been reports of various infections and pseudoinfections due to antiseptics contaminated with *A. xylosoxidans* and *Pseudomonas* spp. 

Our questionnaire survey carried out in 1996 at hospitals in Yamaguchi Prefecture showed that 8 (80%) of 10 hospitals use methods of preparation and use of cotton balls soaked in antiseptics similar to those at our hospital. Many hospitals in Japan may use methods similar to ours for the preparation and use of cotton balls soaked in 0.02% benzalkonium chloride solution. The results of this study thus suggest there is probably microbial contamination in cotton balls soaked in this solution at hospitals other than ours and the risk of nosocomial infection due to the use of such balls.

We determined the maximum use period after preparation of cotton balls in 0.02% benzalkonium chloride to be 1 week in our hospital. There were, however, cases of contamination due to use of these balls for longer than 1 week (Table 2, sample No. 2—6) or due to handling these balls by patients though the use period was 1 week (Table 2, sample No. 1). Therefore, we do not recommend the use of cotton balls or gauze soaked in 0.02% benzalkonium chloride solution for any period. When there is no choice but to use them instead of povidone-iodine or 70% ethanol, preparation should be done immediately prior to use. Chlorhexidine was not examined in this study because one-dose packages (25 ml) are used at our hospital. When cotton balls and cotton gauze soaked in chlorhexidine solution are used, attention similar to that for benzalkonium chloride solution may be necessary.

Cotton balls or gauze soaked in cationic surface-active antiseptics such as benzalkonium chloride are known to be susceptible to microbial contamination. The suggested cause is that cotton actively adsorbs these antiseptics and markedly reduces their bactericidal properties. However, our results indicate that microbial contamination of cotton balls soaked in a cationic surface-active antiseptic is the result of the presence of cotton balls rather than inactivation of the antiseptic. Contaminants obtained from cotton balls soaked in 0.02% benzalkonium chloride solution did not proliferate in this solution or in distilled water but did show rapid growth in the balls themselves which had been soaked in these liquids (Figs. 1 and 2). We previously reported that ethacridine lactate, an acridine derivative, becomes susceptible to microbial contamination after addition of cotton gauze. 

Though samples of benzalkonium chloride solution and ethacridine lactate solution used in reduced amounts have been used for long periods, no microbial contamination has been observed. These antiseptics thus may not be susceptible to microbial contamination in the absence of cotton balls or gauze. Since there is a possibility of microbial contamination of these antiseptics, however, attention should not be relaxed. In using either of these antiseptics in a reduced amount, contact of the hands and fingers with the mouthpiece of the container should be avoided, and the maximum period of use should be 1 month. Antiseptics with marked antimicrobial action such as 1% titratable I₂, povidone-iodine, or 70% ethanol are not likely to become contaminated during use.

A recommendation was issued in the U.S. not to use benzalkonium chloride products as antiseptics at hospitals because of their possible microbial contamination. In Japan, however, this agent is still widely used, and in the U.S. it was also still being used in 12% of the hospitals investigated in 1991. It should be recognized that benzalkonium chloride-soaked cotton balls or gauze tend to be contaminated with a relatively high concentration of microorganisms (10⁵⁻⁰⁵ viable counts/ml).

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**REFERENCES**