Abstract

Sterile surgical instruments, especially those wrapped with cotton cloth, must be resterilized when they are not used in a certain period of time. The period, called shelf-life, varies depending on each organization; however, it is 14 days in the operating room at Thammasat hospital, Pathumthani. The cotton laundry is a factor that affects this period. This study compares the shelf-lives of numerous laundered cotton cloth wrapped sterile packs kept in a closed cabinet to those on open shelves and identifies the contaminating microorganisms on the sterile packs. The experiments were conducted by preparing autoclaved sterile packs

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with gauzes on a stainless steel tray. The wrapped cotton cloths were laundered 1, 10, 20, 30, 40, or 50 times. The sterile and dry packs were kept in a closed cabinet and an equal number kept on open shelves. They were sampled to determine the contamination at set time periods. The results showed that there was only inconsistent contamination in the 30 and 50 times laundered cotton cloth wrapped packs. Staphylococcus aureus and Bacillus spp. were identified as the contaminants. It is believed that the contamination might occur during handling by the cleaning staff. So, an arrangement and prioritization of use should be implemented to reduce contamination which will extend the shelf-life to at least 60 days. The cotton cloths can be laundered at least 50 times when they are properly prepared after sustaining a tear.

Keywords: Shelf-lives, Sterile Surgical Instrument Packs, Laundered Cotton Cloth

Introduction

Sterile surgical instruments are needed for infection prevention in every surgery (Sharbaugh, 1997). Autoclaving and ethylene oxide fuming are the popular methods used in surgical instrument pack sterilization (Reichert, 1997; Ulhalaykaka and Chala-aim, 2002). Cotton cloth is the most suitable material for wrapping the instruments for sterilization by autoclaving since the steam can pass through the wrapping and eliminate the contaminated microorganisms. The cotton can help maintain sterility for a certain period of time. In addition, cotton cloth can prevent dust, vermin, or microbes from entering the package. Moreover, it is also flexible, cheap, can be labeled and dried, and does not produce dust or conduct electrical current (Fuller and Ness, 2005; Stanewick and Kogut, 1997; Japp, 1997). Unfortunately, there are many small holes in cotton cloth created from the cross over of threads, through which the sterile instruments can be easily contaminated when wrapped with only one ply of cotton cloth. However, the period of sterility will be lengthening 10 times when those instruments are wrapped with double layers of cotton cloth (Standard et al., 1971). This period is called shelf-life (Fuller and Ness, 2005). There are some reports of a difference in shelf-life, (Japp, 1997; Standard et al., 1971) depending on the quality of wrapped materials, environment during storage and transport, and the number of handling occasions (Fuller and Ness, 2005; Stanewick and Kogut, 1997; Japp, 1997). For cotton cloth, the number of times it is laundered may shorten this shelf-life since the thread becomes thinner than its original state.

According to protocol of the operation room at Thammasat hospital, surgical instruments are wrapped with 216 thread single layer green cotton cloth and wrapped again with a double layer of cotton cloth, making three layers in total, before autoclaving (Reichert, 1997). After sterilization the pack is kept in a closed cabinet or on an open shelf. If any pack is kept on the shelf for over 14 days, it will be resterilized. This period is shorter than that in St. Catherine, Garden City, Kansas, USA where the shelf-life was recently lengthened from 28 days to 30 days, which can save more than 12,000 US$ a year (Japp, 1997). A preliminary study revealed that 465 sterile packs or 14.04% of all packs from the operating theater were resterilized in a month.
Whether this shelf-life is suitable or can be lengthened is still in doubt. The objectives of this study are to compare the shelf-lives of a different number of laundered cotton cloth wrapped sterile packs kept in a closed cabinet and on open shelves, and to identify the contaminated microorganisms on the sterile packs.

Materials and Methods

Sample

An experimental design was used in this study. 20 gauzes (2x2 inches) were arranged over a stainless steel tray (8x10 inches). All were wrapped with single layer cotton cloth (36x36 inches) and then wrapped again with double layer cotton cloth of the same size and quality. The cotton cloth was made from no.20 cotton thread with 216 threads per square inch density (80x136 threads). The cloth was laundered and ironed 1, 10, 20, 30, 40, and 50 times, creating six treatment levels. Each level consists of 2 replicates which are 30 packs in total. The packs were sterilized by autoclave at 121°C for 15 min. and vacuum dried and cooled before being arranged either in a closed cabinet or on an open shelf, in a specific room. These packs were rearranged everyday during the experiment. The cabinet and shelf were cleaned every week by wiped with a moist cloth. The floor of the room was cleaned everyday by vacuum cleaner and wiped with a moist cloth. In addition, the channel of air flow from the air conditioner was checked and cleaned monthly.

Two packs from the top were sampled from the closed cabinet along with two from the open shelf, and all four were kept in new separate sealed polyethylene bags. These samples were sent for culture analysis in the laboratory. Fifteen samples from each of the six treatment levels were drawn on day 0, 7, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, and 60 (figure 1).

Sterility test

At least 2 packs of each treatment were inserted into a tube of Prospore2 steam (Raven) which contained $10^5$ spores of Geobacillus stearothermophilus (ATCC#7953). After sterilization these tubes were cultured at 55°C for 24 hrs. If the packs were sterile, the medium would still be purple in color. If the medium color changed to yellow, all packs must be resterilized.

Culture method: Each sample was opened in a periodical tested sterile laminar flow cabinet. 10 gauzes were transferred to 100 mL of normal sterile saline in a glass bottle. The bottle was shaken in a vertical manner 50 times before being filtered through a sterile membrane with 0.45 µm diameter pore size. The filtered membrane was then laid on a sterile absorbing pad soaked with 2 mL sterile Trypticase soy broth in a sterile covered Petri dish by aseptic technique, without air bubbles, and incubated at 37°C for 2 days. Every colony grown on the membrane was counted and the total contaminants calculated, and then transferred to Trypticase soy agar slant for purification and identification (Henry, 1992). The last day that the packs were still steriled was determined as the shelf-life of each treatment.

Bacterial identification

Each colony grown on membrane was purified by streak plate technique and identified by Gram’s stain and biochemical tests (Henry, 1992; Koneman et al., 1992; Sneath et al., 1986).

Bacterial count from the air

The number of bacteria in the air of the room was determined by the settle plate method (Pasquarella et al., 2000). Four plates of TSA were laid without cover on the shelf nearby the packs for
an hour. All plates were incubated for 48 hrs. before colony count. The average number of colonies was calculated. Each colony was purified and identified as mentioned above. Bacterial counts from the air after cleaning of the channel of air flow from the air conditioner were also determined weekly.

Results

Shelf-life of cotton wrapped packs in a closed cabinet

The culture from gauze in the cotton wrapped packs in a closed cabinet showed that there was no contamination when the cotton cloth was laundered and ironed 1, 10, 20, and 40 times. However, there was some contamination at day 7 and 28 when the cotton cloth was laundered and ironed 30 times. There was also contaminated at day 7, 24, 28, and 60 when the cotton cloth was laundered and ironed 50 times. All contamination contained only 1 colony each.

Shelf-life of cotton wrapped packs on an open shelf

The results resemble those of the closed cabinet experiment. The packs were contaminated at day 30 when the cotton cloth was laundered and ironed 30 times. There was also contamination amounting to 1 and 3 colonies at day 18 and 60, respectively, when the cotton cloth was laundered and ironed 50 times.

Type of contaminated microorganisms

The contaminants were identified as Staphylococcus aureus 82% and Bacillus spp. 18%.

Bacterial count from the air

The average bacterial counts from the air in the room were $2.33 \pm 0.577$ to $48.0 \pm 7.000$ cfu/plate/hr. (table 1). The Kruskal-Wallis test of bacterial counts showed $\chi^2 (df = 11) = 35.680$, p value <0.001. The multiple comparison showed that p value <0.05 when considering the bacterial count in the open shelf experiment with cloth laundered once and ten times. When comparing the bacterial count of the closed cabinet experiment and open shelf experiment, the analysis showed p value <0.05 in both experiments only with cotton cloth laundered 10 times (table 1).

The average bacterial counts from the air after cleaning of the channel of air flow from the air conditioner were $6.00 \pm 3.366$ to $15.25 \pm 2.872$ cfu/plate/hr. (table 2). The analysis of variance (ANOVA) of these data showed $F(df=22) = 4.438$, p value = 0.009. Turkey HSD showed p value = 0.026 only in the comparison of those data from the closed cabinet and open shelf experiments in week 0. The other experiments showed p value between 0.091 and 1.000.

Type of air microorganisms

All air microorganisms were identified as Staphylococcus aureus 40.5%, Micrococcus lylae 33.3%, M. luteus 21.4%, and Bacillus spp. 4.8%.

Discussion

Shelf-life of cotton wrapped packs

Most of the contaminants were staphylococcus aureus, which, since their natural habitat was human and animal skin, indicates human origin of contamination (Fuller and Ness, 2005; Japp, 1997; Sneath et al., 1986; Dharan and Pittet, 2002).
This contamination might occur through the touch of cleaning staff who cleans the shelf and rearranges the packs everyday. The contamination of *Bacillus* spp. was much fewer than *staphylococcus aureus*. Since the habitat of *Bacillus* spp. is normally in the soil, it might be possible that its spore contaminated the dust in the air and settled on the shelf. When the staff cleaned the shelf, it was moved to the water and staff hands. It was finally transferred to the pack while the packs were rearranged by the staff. To reduce the contamination, newly prepared packs should be arranged in a separate column with a label indicating preparation and expiration date and their priority for use. The oldest prepared packs that have not yet expired should be used as a first priority. The cleaning staff should clean only the vacant area without rearranging the packs. The shelf-life may be extended to 60 days after implement of this management technique. However, there should be determination of the contamination by the microorganisms in the packs, to make sure that the shelf-life can be extended to 60 days. Nosocomial infection in operating theater clients should be monitored after full implementation. The cotton cloth, without lost of contamination prevention, can be laundered and ironed not less than 50 times. If we wish to use this cloth more than that, more research is required. However, after 30 rotations through the laundry process, it was found that some threads were torn off and some small holes resulted which needed repair before reuse.

It was found that only bacteria were contaminants since the environment i.e. low humidity and cool temperature was not suitable for fungal growth (Barer et al., 1993). Only *S. aureus* and *Bacillus* spp. were contaminants. This was less than the types of contaminants found in the air, which also contained *Micrococcus* spp.. It is still unclear why *Micrococcus* spp. was not found to be a contaminant in spite of its mammal skin habitat (Sneath et al., 1986) and its size being much smaller than the cotton cloth hole size. Its diameter is about 2 µm bigger than *S. aureus*, which is about 1 µm in diameter, and *Bacillus* spp., which is around 0.5 µm wide (Sneath et al., 1986). It may adhere with cotton thread better than the others. In this experiment a greater bacterial type was found than in previous reports, which found only *S. epidermidis* (Kudo et al., 2004; Bland et al., 1992; Ezzedine et al., 1991; Schwieger et al., 1989). The other studies were conducted in the operating theater of other countries while the operating theater was available for cases. Operating theaters have a HEPA filter to trap microorganisms and limit the number of personnel that have access. Alternatively, this experiment was done in a store room without a HEPA filter and with an unlimited number of personnel likely to visit. Because of the difference in environment, the type of microorganisms is different.

There was only one significant statistical difference and that was the bacterial number in air from the closed cabinet and in air from the open shelf experiment. Although the number of bacteria in the air in the closed cabinet was less than that from the open shelf, it was recommended that a closed cabinet should be better than an open shelf for sterile pack storage (Japp, 1997; Standard et al., 1971). However, the bacterial count might be less than the real number since there were some bacteria that could not grow on TSA (Barer et al., 1993; Grimes et al., 2000). The bacterial counts after cleaning of the air flow channel from the air conditioner showed the same results. It also showed that there were no significant differences among the weekly number of bacterium after cleaning. This indicated the suitable cleaning period, monthly.
It can be concluded that the shelf-life of cotton wrapped packs may be extended to 60 days if the management of the pack is changed. The cotton cloth could be laundered and ironed at least 50 times when the thread tears have already been repaired. The contaminants were Staphylococcus aureus and Bacillus spp., while the bacteria in the air were Micrococcus spp., Staphylococcus aureus and Bacillus spp. To reduce contamination, newly prepared packs should be arranged in a separate column with a label indicating preparation and expiration date and their priority for use. The oldest prepared packs that have not yet expired should be used as a first priority. The cleaning staff should clean only the vacant area without rearranging the packs. It is recommended that a closed cabinet is better than an open shelf for sterile pack storage. In addition, the shelf-life of sterile cotton wrapped packs with cloth laundered and ironed more than 50 times should be further studied. The shelf-life of other material wrapped packs should be also studied. The number of microorganisms in each operating room and in other hospitals should be compared.

Acknowledgements

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References


Table 1. The average of bacterial counts from the air during the experiment.

<table>
<thead>
<tr>
<th>Times of laundry</th>
<th>Closed cabinet (cfu/plate/hr)</th>
<th>Opened shelf (cfu/plate/hr)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.75±0.957</td>
<td>37.67±9.504</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>10</td>
<td>2.33±0.577</td>
<td>5.50±0.577</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>2.75±1.708</td>
<td>8.25±8.539</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>30</td>
<td>13.50±11.790</td>
<td>48.00±7.000</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>40</td>
<td>5.33±0.577</td>
<td>13.00±4.761</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>50</td>
<td>10.50±3.317</td>
<td>13.25±0.957</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 2. The weekly average bacteria counts from the air after cleaned the channel of air flow from the air conditioner.

<table>
<thead>
<tr>
<th>Week</th>
<th>Closed cabinet (cfu/plate/hr)</th>
<th>Opened shelf (cfu/plate/hr)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.00±3.366</td>
<td>15.25±2.872</td>
<td>0.026</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>7.00±2.943</td>
<td>12.25±3.862</td>
<td>0.374</td>
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<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>6.25±2.986</td>
<td>12.00±4.582</td>
<td>0.269</td>
</tr>
</tbody>
</table>
Figure 1. Microbiological contamination determination method of sterile surgical instrument packs