Microbial Penetration of Muslin- and Paper-Wrapped Sterile Packs Stored on Open Shelves and in Closed Cabinets

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Microbial penetration of sterile packs was studied using single-wrap (two layers) muslin, double-wrap (four layers) muslin, and two-way crepe paper (single layer) to wrap 20 gauze sponges (2 by 2 inch). These packs were stored in the central sterile supply departments of two hospitals and processed for sterility at predetermined intervals. Microorganisms penetrated single-wrap muslin as early as 3 days and double-wrap muslin and single-wrap two-way crepe paper in 21 to 28 days stored in open shelves. The time required for microbial penetration was at least twice as long when closed cabinets were used. Single-wrap muslin packs stored in sealed, impervious plastic bags remained sterile for at least 9 months. All sterile materials in pervious wrappers should be handled as little as possible and then only with extreme care and caution. Closed cabinets offer more protection than open shelves, and single wrappers are not recommended.

There are reports in the technical literature describing the length of time sterile goods can be stored and still be considered sterile, but the safe storage times reported range from as short as 1 week to indefinitely (10, 11, 14, 15, 17). To add to the confusion, some reports discuss neither the wrapping material used nor conditions for storage in relation to the safe storage periods (10, 17).

Dyer et al. (8) reported the shelf life to be at least 60 days for cotton applicators wrapped in parchment and muslin when stored in closed cabinets. Alder and Alder (1) found that crepe and bleached kraft paper were more effective than calico or balloon cloth. Also, they found that approximately 50 and 100% of the test swabs wrapped in double and single layers of muslin, respectively, were contaminated after 13 to 14 days of storage; 10 and 30%, respectively, were contaminated within 1 to 2 days. These investigators concluded that packs wrapped in two layers of paper and enclosed in cartons should have a shelf life of at least 3 weeks. Nichols (13) found that packs double-wrapped in muslin stored and sealed in plastic bags remained sterile for 18 months.

Fitzwater (9) compared the number of viable microbial particulates settling into open petri dishes on open shelves and in a normally closed cabinet in an operating room during set up and an operative procedure. Doors of the closed cabinet were opened 24 times during the evaluation. Fewer than one-tenth as many viable particulates settled in the closed cabinet than on the open shelves.

The exterior surfaces of sterile packages become contaminated during storage. Several investigators have commented on the probability of transfer of this contamination to the contents when single-wrapped sterile packages are opened (2, 5, 12, 15, 18). Speers and Shooter (16) demonstrated that sequential unwrapping of double-wrapped packages substantially reduces contamination during removal of the sterile contents.

Central supply personnel have no firm scientific data on which to base selection of the most effective wrapping material or establishment of conditions for storing sterile packs; hospital central sterile supply departments (CSSD) use many types of wrappers and storage conditions vary widely. Sterile packs wrapped in muslin are considered by most hospitals in the United States to be safe if used within 1 month after sterilization, an assumption probably based on the findings and recommendations of Perkins (14).

In previous studies on length of uncontaminated storage of sterile packs, the materials in the packs examined for microbial contamination were generally cotton applicators or small metal or
glass objects (1, 4, 8, 13, 14). A deficiency of all of these studies was that only small portions of material inside the sterile packs were assayed for microbial contamination.

The present study was designed to determine how long sterile packs of a size widely used in hospitals (15) would remain sterile when wrapped in single-wrap muslin, double-wrap muslin, and single-wrap two-way crepe paper (4, 15) when stored on open shelves and in closed cabinets.

MATERIALS AND METHODS

Standard packs were used for studies of microbial penetration into the pack. Twenty [2 by 2 inch (5.08 by 5.08 cm)] 12-ply gauze sponges were arranged to form a pack with a surface area of 8 by 10 inches. The packs were wrapped with single-wrap muslin (two layers), double-wrap muslin (each two layers), or single-wrap (single layer) two-way crepe paper. A Kilit Ampule containing resistant bacterial spores was placed inside each pack as a check on the effectiveness of sterilization. All packs were autoclaved in a conventional steam sterilizer for 1 hr at 121 C.

Cloth wrappers used for the standard packs were 140 thread-count muslin, unbleached, dyed green, laundered, and ironed at least 1 to 10 times before use. The paper wrappers used were commercially available two-way crepe paper (Dennison Wrap). Both types of wrappers were approximately 24 by 24 inches (61 by 61 cm).

After sterilizing and drying, the packs were held overnight in the autoclave with the steam supply turned off to allow the packs to cool. The packs were then removed and placed in sealed, sterile 8-mil polyvinyl chloride (PVC) bags [25 by 35 inches (63 by 89 cm)]; transported to the CSSD of the two hospitals; removed from the PVC bags; and placed on shelves in the same areas that the hospitals' sterile supplies were stored. On the same day, three packs wrapped in each type of wrapper were chosen at random and transported back from the hospitals to the laboratory for an initial control assay to confirm that the packs were not contaminated during transportation. The remaining test packs were picked up in pairs at selected time intervals and transported back to the laboratory for microbiological assay. All packs returned from the hospitals were transported in sealed, sterile, 3-mil polyethylene bags [9.5 by 18 inches (20 by 46 cm)].

Relative humidity and temperature were monitored in the two CSSD throughout the study by using 7-day recording hygrothermographs. Calibration for accuracy of these instruments was checked at weekly intervals by using a sling psychrometer.

Hospitals were used for this study to provide locations for storage of test packs under actual institutional rather than laboratory conditions. Hospital no. 1 is a 100-bed pediatric hospital; the CSSD of the hospital has four employees on the day shift on weekdays and one on each of the other shifts. Hospital no. 2 is a 350-bed hospital; the CSSD had 14 employees on the day shift on weekdays and at least one on all other shifts.

Storage shelves with enclosed backs and sides, but with the fronts open, were used in hospital no. 1. The distance between shelves ranged from 11 to 14 inches (28 by 36 cm). No packs were less than 16 inches (31 cm) from the floor.

Some shelving completely open on all sides and some shelving with enclosed backs were used in hospital no. 2. The shelves were spaced 10 to 13 inches (25 by 33 cm) apart, and no packs were less than 24 inches (61 cm) from the floor. In addition, closed metal cabinets measuring 36 by 78 by 18 inches (91 by 198 by 46; width by height by depth) also were set up in hospital no. 2 for this study. The shelves were arranged 9 inches (23 cm) apart; no packs were less than 13 inches (33 cm) to the floor. A silent electrical counter was installed to record the number of times the cabinets were opened and closed.

Test packs were picked up at various times during the first week of the study: days 1, 2, 3, and 4 for study series 1 (hospital 1); days 1 and 6 for study series 2 and 3 (hospital 2); days 1 and 3 for study series 4 (hospital 1); and day 3 for study series 5 (hospital 2). Thereafter, weekly pick-ups were made in all study series. The first pick-up for study series 6 (hospital 2) was on day 7. At each pick-up, two sterile packs used as transportation controls were wrapped in single-wrap (two layers) muslin, transported to the hospital in sterile plastic bags, and then returned to the laboratory along with and in the same manner as the test packs. Series 1, 2, and 3 were done during cold months, and series 4, 5, and 6 were done during hot months.

Immediately on arrival at the laboratory, all packs were processed inside a closed laminar-flow hood (40 by 20 by 40 inch [102 by 51 by 102 cm]). The hood was decontaminated before each daily use with 70% ethanol and purged and dried for at least 30 min at a rate of three air changes per minute (ac/min) through an ultra-high-efficiency air filter (6). While packs were being processed, the airflow was set at a rate of 1 ac/min. The hood was purged for at least 2 min at a rate of 3 ac/min between processes of each pack.

After the packs were opened, seven of the sponges were placed in each of two screw-cap serum bottles containing 100 ml of Trypticase soy broth (TSB, BBL) and incubated aerobically at 37 C. The remaining six sponges were placed into another serum bottle containing 100 ml of TSB and incubated under anaerobic conditions in a Brewer jar at 37 C. All cultures were incubated for 21 days before being considered negative for viable microorganisms.

An estimate of the amount of viable surface contamination that may have collected on the outside of the packs was made by using stainless-steel strips [1 by 2 inches (2.54 by 5.08 cm)]. Using these strips, two evaluations were made of open shelves versus closed cabinets in hospital 2: one was carried out in the cold months (series 2 and 3), and the other was carried out during the hot months (series 5 and 6). Strips were placed on a stainless-steel tray wrapped in double-thick aluminum foil and sterilized in a hot-air oven at 150 C for 3 hr. The strips with strips were transported to the hospital with the test packs. The strips were placed on the same shelves with the packs and opened. Five randomly selected strips were collected for microbial assay when each set of packs was picked
up for examination. Each strip was aseptically placed into a sterile 4-oz specimen jar and returned to the laboratory. Upon return to the laboratory, 50 ml of TSB was aseptically added to each jar, and the jars were vigorously shaken for 5 min on a wrist-action shaker. Just before assay, each jar also was hand-shaken 50 times. Sets of two pour plates each for aerobic and for anaerobic incubation were prepared by using 5-ml sample amounts mixed with 10 to 15 ml of Trypticase soy agar (TSA). The remaining sample in the jars was then heat-shocked for 15 min at 80 °C, and four additional pour plates were prepared, as described above, again for aerobic and anaerobic incubation. Aerobic incubation was carried out in a water-jacketed incubator at 37 °C, and anaerobic incubation was carried out in Brewer jars at 37 °C. Colonies on the plates were enumerated after 48 hr and after 7 days of aerobic incubation and after 7 days of anaerobic incubation.

Concurrent with these studies, single-wrap (two layers) muslin packs prepared and sterilized as previously described were sealed in sterile 3-ml polyethylene bags (0.5 by 18 inch). Eight packs were stored in each hospital CSSD on shelves that collected the most dust. Two packs were examined at 1, 3, 6, and 9 months for assay as previously described.

In an attempt to determine depth of penetration of contamination, six single-wrap (two layers) muslin packs containing nine stacks of 15 sponges per stack, sterilized as previously described, were placed on open shelves with other sterile supplies in the CSSD of hospital no. 2 for a period of 5 weeks. The first nine sponges were placed in separate 2-oz screw-cap bottles containing 25 ml of TSB. These specimens were incubated aerobically at 37 °C for 21 days before being considered negative for viable microorganisms.

RESULTS

Microbial contamination was determined from single-wrap (two layers) muslin packs, double-wrap (each two layers) muslin packs, and single-wrap (single layer) two-way crepe paper packs. Table 1 shows the first time in days at which each type of pack was found contaminated in the open-shelf study in both hospitals; contamination occurred as early as 3 days after initiation of storage with single-wrap muslin and in 21 and 28 days (first noted at 28 days) with double-wrap muslin or single-wrap two-way crepe paper.

Results from the closed-cabinet studies (series 3 and 6) are shown in Table 2. Contamination first became apparent at 14 days for single-wrap muslin and at 56 days for double-wrap muslin, and no contamination was found for at least 63 days with two-way crepe paper.

Series 1, 2, and 3 were done during cold months, and series 4, 5, and 6 were done during hot months. Series 1 and 4 were done in hospital no. 1; 2, 3, 5, and 6 were done in hospital no. 2. The time interval in which contamination first became apparent in single-wrap muslin packs was shorter in hot months than in cold months; however, the double-wrap muslin and single-wrap two-way crepe did not appear to follow this pattern. Averages of weekly average indoor temperatures in the CSSD did not vary significantly between the hot (25 °C) and the cold (25.5 °C) months and only slightly between hospital 1 (27.2 °C) and hospital 2 (24.4 °C). The CSSD in hospital 1 was more humid in the summer and less humid in the winter than the CSSD of hospital 2. Averages of weekly average indoor relative humidities were about 35% in the studies during the cold months and about 48% in the studies during the hot months.

Table 3 shows the total microbial counts from stainless-steel strips exposed in two evaluations of open shelves versus closed cabinets in hospital 2. The microbial counts were calculated on the basis of an area measuring 80 square inches, equal to the surface area of the packs used in the study. The doors on the closed cabinets were opened (and closed) an average of 28 times per day during the investigation. The results of these evaluations showed that only about one-tenth as much viable microbial contamination settled onto horizontal surfaces in the closed cabinets as on the open shelves. About 50% of the settled microorganisms were aerobic and not heat-shocked, about 20% were anaerobic and unshocked, over 10% were molds, and less than 10% each were aerobic and anaerobic, heat-shocked organisms.

| Type of wrap                | Time (days) |
|-----------------------------|-------------|
|                             | Series 3    | Series 6    |
| Single-wrap muslin...........| 21          | 14          |
| Double-wrap muslin...........| 56          | 77          |
| Single-wrap two-way crepe...| ---.a       | ---.b       |

* No contamination in 63 days.

* No contamination in 91 days.

| Table 2. Days required for first packs to become contaminated during closed-cabinet storage |
|-----------------------------------------------|-----|
| Type of wrap                | Time (days) |
|-------------------------------|-------------|
|                               | Series 3    | Series 6    |
| Single-wrap muslin........... | 21          | 14          |
| Double-wrap muslin........... | 56          | 77          |
| Single-wrap two-way crepe... | ---.a       | ---.b       |

* No contamination in 63 days.

* No contamination in 91 days.

Table 1. Days required for first packs to become contaminated during open-shelf storage

| Type of wrap                | Time (days) |
|-------------------------------|-------------|
|                               | Series 1    | Series 4    | Series 2    | Series 5    |
| Single-wrap muslin........... | 14          | 7           | 14          | 3           |
| Double-wrap muslin........... | 28          | 56          | 28          | 35          |
| Single-wrap two-way crepe... | ---.a       | 49          | 28          | 28          |

* No contamination in 63 days.
maintain data muslin VOL. 22, available packs the Two single-wrap penetration examined contaminated. nationafter sealed and organisms the of area

TABLE 3. Comparisons of number of microorganisms* isolated from stainless-steel strips on open shelves and in closed cabinets during series 2 and 3 (cold months) and series 5 and 6 (hot months)

| Time strips exposed (days) | Counts of settled organisms per pack area |
|---------------------------|------------------------------------------|
|                           | Series 2, open shelves | Series 3, closed cabinet | Series 5, open shelves | Series 6, closed cabinet |
| 1                         | 440                      | 80                       | —                        | —                        |
| 3                         | 960                      | 0                        | 4,300                    | —                        |
| 7                         | 1,100                    | 200                      | 12,000                   | 1,000                    |
| 14                        | 2,900                    | 240                      | 7,200                    | 360                      |
| 21                        | 3,600                    | 160                      | 6,100                    | 480                      |
| 28                        | 3,000                    | 80                       | 16,000                   | 1,100                    |
| 35                        | 6,300                    | 120                      | 11,000                   | 880                      |
| 42                        | 5,200                    | 250                      | 10,000                   | 1,200                    |
| 49                        | 6,400                    | 400                      | 8,400                    | 1,300                    |
| 56                        | 4,700                    | 530                      | 12,000                   | 1,300                    |
| 63                        | 11,000                   | 400                      | 10,000                   | 1,600                    |
| 70                        | —                       | —                        | 10,000                   | 1,900                    |
| 77                        | —                       | —                        | —                        | 1,700                    |
| 84                        | —                       | —                        | —                        | 1,800                    |
| 91                        | —                       | —                        | —                        | 2,000                    |

* Expressed as number per 80 square inches, the area of the test packs used in studies on length of sterile storage.

No packs or strips processed.

Table 4 shows the types and frequencies of organisms isolated from contaminated packs. About half of the organisms isolated were gram-positive rods; the next most frequently isolated organisms were staphylococci, *Aspergillus* spp., and *Micrococcus* spp.

The single-wrap (two layers) muslin packs sealed in 3-mil polyethylene bags remained sterile throughout the 9-month study.

Examination of the six single-wrap muslin packs used to study depth of microbial contamination after 5 weeks of open-shelf storage showed penetration down to the fifth sponge in one pack. Two to seven of the nine surface sponges per pack examined were found to be contaminated.

A total of 195 control packs were utilized throughout the study. Only two (1.0%), both single-wrap (two layers) muslin, were found to be contaminated. No growth was found in any of the ampoules of resistant spores placed in the packs to check the effectiveness of sterilization.

**DISCUSSION**

Many wrapping materials are commercially available to hospital CSSD today, but almost no data are available on how well these materials maintain sterility. Basically, bacteria penetrate muslin draping material much more rapidly than

they penetrate water-repellent paper drape fabrics (7). It has been shown that bacteria penetrate a surgeon's intact gown more readily and in greater numbers during surgery requiring unusual physical effort than during simple procedures (3). The same may be true of the more porous materials used to wrap sterile supplies. That is, the less the materials are handled and moved, the less likely it is for contamination to occur. It has been suggested that a change in atmospheric conditions may cause a breathing effect in packs and thus contribute to penetration of microorganisms (14). In the present study, neither the temperature nor the relative humidity varied greatly, and there were few rapid changes in either of these variables; thus, the frequency of contamination could not

**TABLE 4. Types and number of microorganisms isolated from contaminated test packs**

| Type of organism | No. of microorganisms isolated |
|------------------|--------------------------------|
|                  | Series 1 | Series 2 | Series 3 | Series 4 | Series 5 | Series 6 |
| *Staphylococcus epidermidis* | 1       | 8       | 0       | 4       | 13      | 1       |
| *S. aureus* | 0       | 0       | 0       | 0       | 3       | 1       |
| *Micrococcus* spp. | 2       | 5       | 1       | 1       | 5       | 6       |
| Gram-positive NSF rods* | 10      | 10      | 3       | 4       | 12      | 8       |
| *Clostridium perfringens* | 0      | 0       | 0       | 1       | 0       | 0       |
| Gram-positive SF rods** | 5       | 20      | 0       | 5       | 17      | 3       |
| Gram-negative rods | 0       | 0       | 0       | 2*      | 1*      | 2*      |
| *Aspergillus* spp. | 3       | 3       | 0       | 5       | 17      | 5       |
| *Streptomyces* spp. | 3       | 2       | 1       | 3       | 1       | 2       |
| *Trichoderma* spp. | 2       | 0       | 0       | 0       | 0       | 0       |
| *Neurospera sitophila* | 1       | 0       | 0       | 0       | 0       | 0       |
| *Penicillium* spp. | 1       | 8       | 1       | 3       | 1       | 0       |
| *Fusarium* spp. | 0       | 0       | 0       | 1       | 0       | 0       |
| *Paecilomyces* spp. | 0       | 0       | 0       | 0       | 1       | 1       |
| *Cephalosporum* spp. | 0       | 0       | 0       | 0       | 0       | 0       |
| Unidentifiable fungus | 0       | 1       | 1       | 0       | 0       | 0       |

* Mannitol salt-positive, coagulase-negative.
* NSF, nonsporeforming rods.
** SF, sporeforming rods.
* Herbicola lothrii.
* Pseudomonas group IV d.
* Pseudomonas group V e.

...
be shown to be directly related to atmospheric conditions. Gradual increases in settled contamination on the outside of packs together with handling or vibration and possibly atmospheric changes were responsible for ultimate contamination of packs. This study utilized the entire contents of the sterile package as the assay system. Using this system, single-wrap (two layers) muslin packages become contaminated as early as 3 days, and double-wrap (each two layers) muslin and single-wrap (single layer) two-way crepe paper maintained sterility for at least 3 weeks (contamination was first found at 4 weeks) stored on open shelves. Packs stored in closed cabinets remained sterile for at least twice as long as those held on open shelves; however, even in closed cabinets, the time of sterile storage for single-wrap muslin (less than 14 days) was too short to be practical.

In an effort to show a visual comparison of a single thickness of muslin and a single thickness of two-way crepe paper, a photograph at approximately 40× magnification was made of each. Figure 1 shows the muslin; there is a visible opening through the material at almost every thread junction. Figure 2 shows the two-way crepe paper; in this figure, three dark areas near the center indicate very thin areas in the paper fiber. These photographs help demonstrate the possibility of rapid recontamination of sterile objects wrapped in a single wrap of muslin.

The results of the study on the amount of microbial contamination settling on stainless-steel strips corroborate the findings of Fitzwater (9); only about one-tenth as many viable microorganisms settled in the closed cabinets as on the open shelves. Thus, the results of our studies clearly show the advantages of closed-cabinet over open-shelf storage of sterile packs. Of course, cabinet doors must be kept closed except for replacement or removal of contents.

Although a single thickness of two-way crepe paper was observed to maintain sterility as long as double-wrap muslin, it is not recommended that any sterile pack be wrapped with only a single thickness of material. With a single wrapper, the possibilities for contamination are greatly increased via contamination from the outside surface of the pack (16).

Impervious plastic wrappers have been observed to maintain sterility for as long as 18 months (13). The 9-month period of sterile storage obtained from this study of materials sealed in polyethylene bags at least in part confirms these earlier observations. With proper rotation of stocks, no material should be held for as long as 9 months.

All sterile materials in pervious wrappers should be handled as little as possible and then only with extreme care and caution. Closed cabinets offer more protection than open shelves, and single wrappers are not recommended.

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