Biocontrol Science, 2000, Vol. 5, No. 1, 1−7

Original

Development of Disinfectant Mats and Evaluation of Their Effectiveness

SHIN OHTA*[1], KATSUHIRO NAGAI[1], HIROSHI ZENDA[1], HIROYUKI OGATA[2], TETSUYA HAYASHI[3], MASAHIKO OGUCHI[4], YUKIE OKIMURA[5], TAKAYUKI AKAHANE[5], HIROSHI SAITOU[6], AND YOSHIYUKI KAWAKAMI[7]

[1]Department of Pharmacy, [2]Transfusion Service, [4]Radiology and [5]Central Clinical Laboratories, Shinshu University Hospital, [3]Department of Bacteriology, Shinshu University School of Medicine, [7]Department of Medical Technology, School of Allied Medical Sciences, Shinshu University, 3-1-1 Asahi, Matsumoto 390-0802, and [6]Department of Third Internal Medicine, Nagano Red Cross Hospital, 1512-1 Wakasato, Nagano 380-0922, Japan

Received 17 November 1998/Accepted 24 May 1999

One factor causing the spread of bacteria in the hospital environment is transmission of bacteria via shoes. To prevent this, disrupting the spread of bacteria from contaminated areas into clean areas is very important. We experimentally produced the following 3 types of mats. (1) Water retention type mat: The part corresponding to the pile part of commercially available floor mats is made of nylon and acrylic, and polyester is used in the base to improve water retention. In addition, an indicator, which tells the remaining amount of the disinfectant was attached. (2) Paper mat : This is a bag-shaped mat that is composed of nylon and nonwoven fabric, and filled with pulp sheats (tissue-pattern) for better water retention. (3) Tank type mat : This has a water-retaining part in the central area of the mat and parts that absorb water, released from the water-retaining part, on both sides. After spraying 0.2% (w / v) benzalkonium chloride on each mat, the number of viable bacteria on the mat was serially counted. The tank-type mat showed longer term inhibition of bacterial proliferation compared with the other two types. The disinfection rate at which bacteria was eliminated from the soles of footgear (sterile filtration rate) with S.D. was 83 ± 12% for the tank type, 75 ± 9% for the water retention type, and 68 ± 12% for the paper type mat. Since the tank type mat was superior to the other mats in not only the disinfection rate, but also in the water retention rate, it appears to be the most effective device for disrupting the infection routes.

Key words : Disinfectant mat / Methicillin-resistant *Staphylococcus aureus* / Compromised host / Nosocomial infection.

INTRODUCTION

For the prevention of nosocomial infection, an important concern in medical settings, various measures have been proposed. One of them is the disruption of infection routes by means of disinfection of fingers and hands, environmental purification and isolation of compromised hosts (Kitashima et al., 1996 ; Matsuno et al., 1996 ; Sato et al., 1995). To prevent entry of infectious microbes via shoes and wagons, dust- and bacteria-proof mats are placed in clean areas, such as intensive care units and operating rooms (Fukada et al., 1987). We have previously reported that disinfection of the soles of shoes was a preventive measure against methicillin-resistan

Corresponding author. Tel : + 81-263-37-2827, Fax : + 81-263-37-3021.

Staphylococcus aureus (MRSA) infection, and it has been shown that spraying disinfectants on commercially available floor mats placed at the entrance of wards and hospital rooms is effective (Ohta et al., 1991). However, the disinfecting action of these mats were highly affected by their water content. The disinfecting action sharply decreased when the water in the disinfectant remaining on the mat decreased to 30% or less. To maintain the water content in the mat, the disinfectant had to be sprayed repeatedly according to a complicated procedure. To minimize the frequency of spraying and to improve the disinfecting action, we experimentally produced 3 types of mats (water retention type mat, paper type mat, and tank type mat,as tentative names) and compared them on the bases of water retention, difficulty in handling, and disinfecting action.

MATERIALS AND METHODS

Bacterial strain and medium

Twelve MRSA strains of coagulase type II, isolated from the hospital environment at Shinshu University Hospital between January 1996 and December 1997, were used for the disinfection experiments of the mats. Antibiotic Medium 3 (AM3, Difco) was used as a liquid medium and AM3 supplemented with 1.5% (w/v) agar (Difco) as a solid medium.

Drugs

Benzalkonium chloride (Osvan®, 10%. w/v, Nihon Seiyaku Co., Ltd.) was used as the disinfectant. For inactivation of benzalkonium chloride, the LP diluent "Daigo"® (Wako Pure Chemical Industries, Co., Ltd.) was used.

Materials and structure of each disinfectant mat

Water retention type : This mat has a structure similar to commercially available floor mats. To improve water retention, polyester filament fibers (polyester long fibers) and acrylic spun fibers (acrylic spun yarn) were used in the water retention part (Fig. 1a).

Paper type : This mat has a bag shape and is composed of nylon and nonwoven fabric (Fig. 1 b). The bag contains 6 sheets of pulp, and this particular structure helps to retain water.

Tank type : This mat has a water retention part (tank) in the center of the mat and also has water absorption parts on both sides, which absorb the water released from the water retention part (Fig. 1 c). Polyester filament fibers were used in the tank and the water absorption parts. To prolong the water retention time, the tank was filled with plastic nylon that inhibits

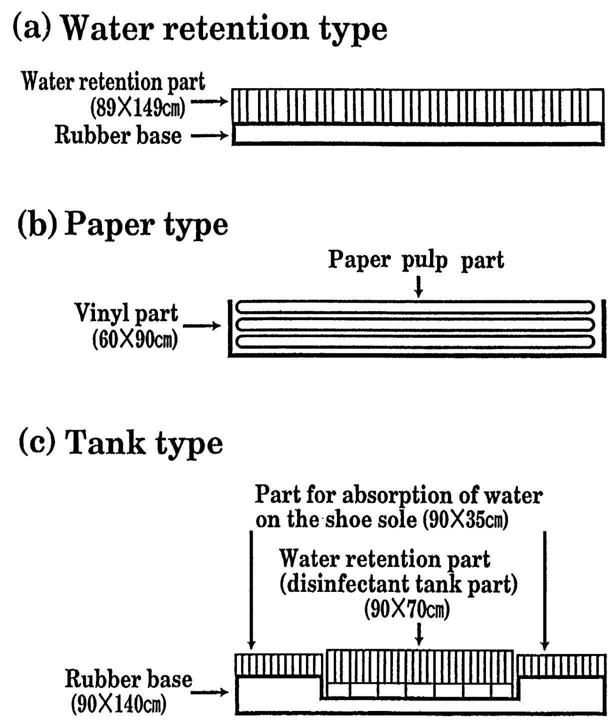

FIG. 1. Structure of disinfectant mats. (a) Water retention type. To increase water retention, the water retention part is composed of polyester filament fibers (polyester long filaments) and acrylic spun fibers (acrylic spun yarn). (b) Paper type. The bag-shaped external part is composed of nylon and nonwoven fabric and filled with pulp sheets. (c) Tank type. There is a water retention part (tank) that stores the disinfectant in the center of the mat and water absorption parts on both sides that absorb water released from the tank.

evaporation of the disinfectant solution and that has cushioning effects as well.

Counting of viable bacteria on mats

Bacteria were collected by pressing plates (Repliplate®, Takara Shuzo Co., Ltd.) onto 5 sites of each mat placed at the entrance of the mixture ward (RP method) and then pressing then onto AM3 agar medium containing a neutralizer. After incubation at 32℃ for 24 h, the numbers of colonies were counted. This experiment was performed twice for each mat at the same sites every week. Mats disinfected with formalin gas, mats sprayed with 0.2% (w / v) benzalkonium chloride (2,500 ml) and control mats not sprayed with the disinfectant were evaluated. The disinfectant was sprayed only once, bacteria were collected by the RP method, and colonies were counted at 24 h intervals for 6 d for the water retention type mats and tank type mats and for 3 d for the paper type. The colony count was expressed as the mean value per plate plus or minus the standard deviation value.

Determination of the residual water rate in mats

Each mat was sprayed with 0.2% (w / v) benzalkonium (2,500 ml) and weighed at 24 h intervals for 5 d in the case the water retention type and tank type mats and for 2 d in the case of the paper type mat . From the decrease in weight, the water decrease rate was calculated.

Determination of the disinfection rate on shoes after use of mats

Thirty subject nurses were divided into 3 groups (10 subjects each) according to the water retention type, paper type, and tank type mats to be tested. Bacteria on the backs of the shoes of each pair were collected by the RP method from each subject. Subsequently, each subject stepped once on the mat sprayed with 0.2% (w/v) benzalkonium chloride, and bacteria were collected again by the RP method from each pair of shoes by using sites from which collection had not yet been made. After incubation at 32 ℃ for 24 h, the number of colonies were counted. Analysis of variance was then performed.

Evaluation of MRSA bactericidal effects at a water retention rate of 50%

Disinfection effects of each type of mat at a water retention rate of 50% were evaluated. After inoculation of 1ml (10^6-10^7 CFU/ml) of each of the 12 MRSA strains onto the 3 types of mats containing 0.2% benzalkonium chloride by the RP method, samples were serially obtained and transferred to AM3 agar medium containing a neutralizer by the RP method. After incubation at 32℃ for 48 h, the presence or absence of bacterial growth was determined.

Production of an indicator which shows changes in the water content

For visual confirmation of the residual water content, an indicator was produced. A mixture of finely powdered silicic acid and deep red pearl pigments was applied to nonwoven fabric composed of synthetic fibers and was heated at 110℃ for 10 min, producing a water-sensitive indicator. This indicator was attached to the corner of the water retention type mat. This indicator is deep red in a wet state and turns white in a dry state.

Detection of MRSA from hospital environments based on whether the tank type mat was used or not.

Between January 1996 and December 1997 (24 mo), we evaluated the level of MRSA contamination in the following three wards by random sampling: a ward furnished with the tank type mat, a ward not furnished with the tank type mat and a ward furnished with the tank type mat at 1-mo intervals. These three wards were located in the Department of Internal Medicine, and an almost the identical number of inpatients were being cared for in each of these wards. To prevent dust-mediated contamination, each mat was washed once a week then sprayed with 2,500 ml of 0.2% (w/v) benzalkonium chloride solution. Using RP, samples were collected once a month from 10 sites (5 sites on handrails and 5 sites on the passage floor) of the respective wards to detect MRSA.

RESULTS

Viable bacterial count on mats

The mean colony counts on the mats sprayed and mats not sprayed with the disinfectant are shown in

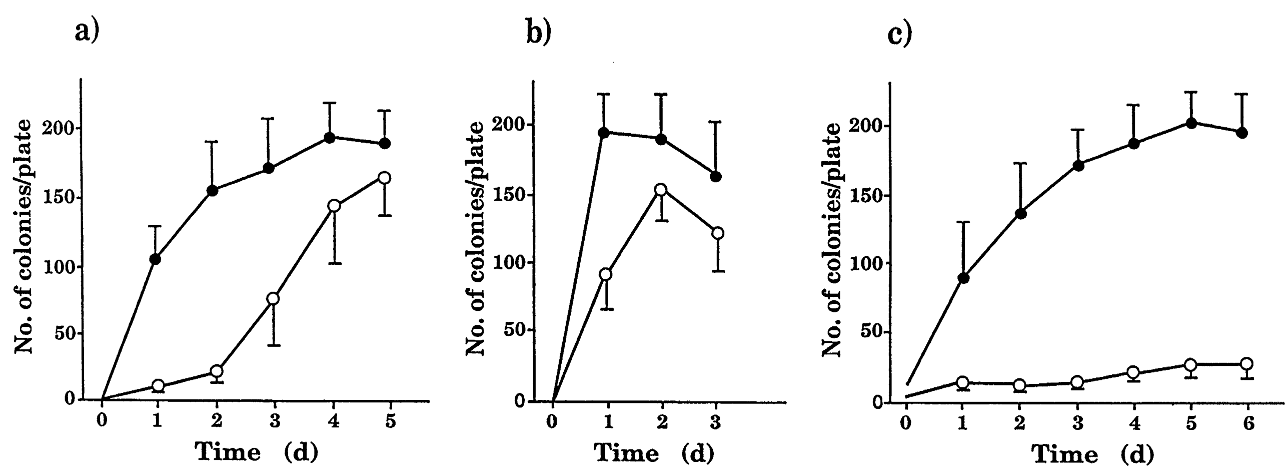

FIG. 2. Bacterial colony count on each type of mat. The colony count was compared between mats sprayed (○) and not sprayed (●) with 0.2% (w/v) benzalkonium chloride (2,500 ml) for a) the water retention type, b) the paper type, and c) the tank type mats.

Fig. 2.

On the water retention type mats sprayed with the disinfectant, the mean colony count with S.D. was 18.0 ± 7.6/plate after 2 d, but it rapidly increased after 3 d up to 168.8 ± 36.8/plate after 5 d. On the mats without disinfectant spraying, the colony count was high even after 1 d.

On the paper type mats, the colony count after 1 d was 88.5 ± 26.8/plate with disinfectant spraying and 103.0 ± 33.8/plate without disinfectant spraying. The increase was, however, inhibited after 3 d on both the mats.

On the tank type mats sprayed with the disinfectant, the colony count was only 9.3 ± 3.2 - 11.8 ± 3.6/plate after 1 -3 d and 26.5 ±11.4 /plate even after 6 d, showing negligible changes. In the tank type mats not sprayed with the disinfectant, the colony count continued to increase until after 5 d and slightly decreased to 199.2 ± 52.2/plate after 6 d.

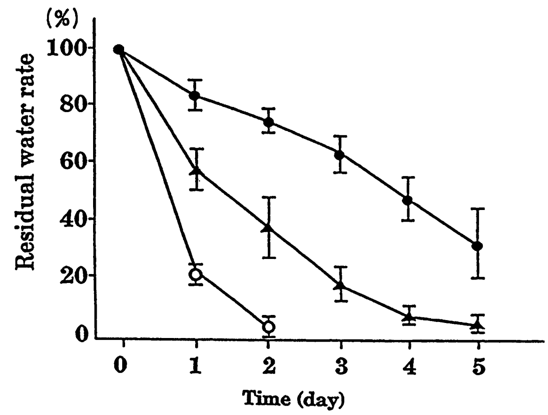

FIG. 3. Residual water rate on each type of mats. The water retention type mat (▲), paper type mat (○), and tank type mat (●) mats were sprayed with 0.2% (w / v) benzalkonium chloride (2,500 ml) and weighed at 24 h intervals. The water decrease rate was calculated from the decrease in weight.

Residual water rate on mats

The relationship between the results of the above viable bacterial count on the mats and the residual water rates were evaluated, and the water retention rates were compared among the 3 types of mats in Fig. 3.

In the paper type mats, the residual water rate rapidly decreasedto about 20% after 1 d and was 3 ± 7% after 2 d, indicating a negligible amount of residual water.

In the water retention type mats, the residual water rate was 58.6 ± 8.9% after 1 d and 38.0 ± 11.4% after 2 d, being more than 30% for two d, but gradually decreased and dropped to 7.3 ± 2.4% after 5 d.

In the tank type mats, the residual water rate was 64.3 ± 8.7% after 3 d, being higher than the value of the water retention mat after 1 d, and was 32.3 ± 10.5% even after 5 d.

Disinfection rates on the sole of shoes after use of the mats

Table 1 shows the colony counts from the soles of the right and left shoes after 10 subjects in each group stepped on the disinfectant-sprayed (2500ml: at 100% water retention rate) mats once. The disinfection rate was 83 ± 12% ($p < 0.005$) for the tank type, 75 ± 9% ($p < 0.005$) for the water retention type, and 68 ± 12% ($p < 0.005$) for the paper type mat, showing effective disinfection after the use of each type of mat. No significant difference was observed between the tank type and water retention type or between the water retention type and paper type mats, but a significant difference was noted between the tank type and paper type mats ($p < 0.05$).

MRSA disinfecting action with a water retention rate of 50% in mats

Disinfecting action against MRSA was evaluated at a water retention rate of 50% in each mat. After inoculation of 12 MRSA strains onto each mat containing 0.2% (w/v) benzalkonium chloride, samples were serially collected and analyzed. As shown in Table 2, no bactericidal effects were observed in any mat after 0.5 min, but all strains on the tank type and water re-

TABLE 1. Bacteial colony count on the shoe sole before and after uses of each type of mats.

Treatment type of mat	No. of colonies (mean ±S.D.) (n=10)					
	Tank type		Water retention type		Paoer type	
	Left	Right	Left	Right	Left	Right
Before use	137 ± 68	171 ± 128	153 ± 58	109 ± 37	120 ± 51	91 ± 38
After use	23 ± 12	20 ± 13	31 ± 16	33 ± 15	35 ± 21	27 ± 15
Disinfection rate (%)[a]	83 ± 12		75 ± 9		68 ± 12	

[a]The rate of removing bacteria from the solo of footgear.

TABLE 2. Bactericidal effects of each type of mat on MRSA at a water retention rate of 50%.

Type of mat	Reaction time (min)			
	1	2	5	10≦
Control	0	0	0	12[a]
Tank type	8	4	0	0
Water retention type	7	5	0	0
Paper type	4	6	2	0

[a]Number of MRSA strains.

tention type mats were killed after 2 min. On the paper mat type, 10 strains were killed after 2 min, and the remaining 2 after 5 min.

Performance of the indicator for the residual water rate

An indicator was produced for the visual estimation of the residual water rates and ready determination of the remaining amount of disinfectant. The indicator device in this study gradually turned white when the residual water rate decreased below about 50%. Since the degree of dryness of the mat was proportional to the decrease in the disinfection rate, this indicator was useful.

Detection of MRSA from hospital environments based on whether the tank type mat was used or not

The results of MRSA detection in three wards (a ward furnished with the tank type mat, a ward not furnished with the tank mat and a ward furnished with the tank mat at 1-mo intervals) are shown in the Table 3. When samples obtained from 5 sites on handrails and 5 sites on the passage floor of the respective wards were investigated once a month for 24 mo (24 times), the frequency of detecting MRSA on the floor was significantly lower in the wards with the tank type mat than that in the ward without the tank type mat ($p < 0.05$).

DISCUSSION

The spread of hospital infection occurs in various ways. As a method of restricting transmission of bacteria from the floor in a contaminated area to a clean area, we evaluated the usefulness of disinfectant mats by using the level of MRSA contamination on the floor as an index. Although contaminated fingers, and hands are thought to be largely responsible for cross infection by MRSA, there have been some reports suggesting the possibility of MRSA transmission via dust. (Allen et al., 1997 ; Kihara et al., 1998 ; Perdreau-Remington et al., 1995 ; Satoh et al., 1992 ; Watabe et al., 1992).

In our studies, although it remains unclear whether MRSA detected on the floor adhere to patients and health-care professionals, identical strains of MRSA have been detected on the floor around the bed of an MRSA carrier as well as on other parts of the sickroom floor (data not shown). Therefore, it may be considered that the incidence of MRSA carriers is proportionate to the level of floor contamination, and the environment in a carrier's sphere of action may be the source of infection, resulting in an increased possibility of spreading the MRSA contamination.

Other studies have evaluated bacterial disinfecting effects of adhesive mats or disinfection mats for disruption of the MRSA infection routes (Ayliffe et al., 1967 ; Dragas et al., 1983 ; Meddik et al., 1977).

The usefulness of cloth immersed in disinfectants has also been suggested for eliminating MRSA at-

TABLE 3. Detection of MRSA from hospital environments based on the whether the tank type mat was used or not.

Ward	Presence of tank type mat	Duration (mo)	No. of sites where MRSA was detected (spots)	
			Handrails	Floors
A	+	24	5	10
B	−	0	16	56
C	+	12	13	42

Three internal medicine wards were selected by random sampling. The first ward was furnished with the tank type mat for 24 mo, the second ward was not furnished with the tank type mat and the third ward was furnished with the tank type mat at 1 mo intervals (12 mo).

tached to the soles of shoes (Hayazaki et al., 1991 ; Watabe et al., 1994). In our previous study (Ohta et al., 1991), about 76% of bacteria attached to the shoe sole were killed by using commercially available floor mats sprayed with a disinfectant. However, since drying of the mats affected their disinfecting action, frequent supplements of the disinfectant were required to keep mats at an appropriate moisture level. Therefore, we prepared 3 different types of disinfectant mats on an experimental basis. These mats were developed to remove bacteria from the soles of footgear as well as to prevent contamination of the mat itself. The tank type mat, water retention type mat and the paper type mat differed in materials and structures.

The tank type mat, which was characterized by the presence of a tank to store the disinfectant at the center of the mat, retained a larger amount of water than the other two types of mats. In addition, the tank type mat has water absorption parts on both sides, which absorb the water released from the water storage part. Therefore, these absorption parts can prevent the surrounding floor from being wet and slippery with the disinfectant solution . The highest sterile filtration rate was observed in the tank type mat (about 82%), and the difference compared with the water retention type mat (about 75%) was not statiscally significant, suggesting adequate contact of the disinfectant with the sole of shoes on the tank type mats. The residual water rate in the tank type mat was also higher than those in the other two types, which appears to be associated with the material and the structure of the mat which prevent the evaporation of water.

The water retention type mat showed a lower residual water rate than that of the tank type. However, to overcome this disadvantage, an indicator was devised with a special ink, which allowed visual determination of the remaining amount of disinfectant. At a water rate of about 50%, this indicator began to lose its deep red color and became white at a dry state. Using this indicator, it will be possible to know the remaining amount of disinfectant, although the relationship between the water rate and color changes has to be evaluated in more detail.

The paper type mat is very light weight and is made of nonwoven fabric, pulp and nylon. This type of mat is placed on a rubber base or directly attached to the floor, and disinfectants are sprayed on it. Two days after spraying the disinfectant, however, drying progressed and water retention levels became very low. Thus, it requires frequent disinfectant applications. In addition, this type of mat is susceptible to mechanical damage because its surface is made of nonwoven fabric. However one advantage of this type of mat is that it can be discarded or burned when physical contamination progresses. Thus it may prove to be useful as a disposable mat.

Since the count of bacterial colonies was low at a residual water rate of higher than 30-50%, the disinfectant action of these mats on MRSA, isolated from the hospital environment, were evaluated using mats adjusted at a water retention rate of 50%. The bactericidal effects were similar among the 3 types of mats. While all 12 tested MRSA strains were killed in 2 min with the water retention and tank type mats,only the paper type mat needed 5 min to kill the last 2 strains. This may be associated with the material on the mat surface. The paper type mat is made of nonwoven fabric and the other two types are made of polyester and acrylic.

In summary, we produced 3 types of mats with good disinfection capability using commercially available mats as references. They are useful for preventing dust contannation and for killing bacteria that are normally spread in the hospital environment via shoes. In particular, in the room environment of patients highly susceptible to infection, disinfection by these mats will be effective in disrupting the spread of bacteria from the contaminated sections. In this study, benzalkonium chloride-resistant MRSA was not detected. However, considering the possibility of contamination with disinfectant-resistant bacteria, it is necessary that each mat be either exchanged or washed. Designing a disposable mat was considered necessary. Frequent washing is also needed to prevent physical contamination by dust.

REFERENCES

Allen K. D., Anson J. J., Parsons L. A., and Frost N. G.(1997) Staff carriage of methicillin-resistant *Staphylococcus aureus* (MRSA 15) and the home environment: a case report. *J. Hosp. Infect.*, **35**, 307-311.

Ayliffe G. A., Collins B. J., and Lowbury E. J. L. (1967) Ward floors and other surfaces as reservoirs of hospital infection. *J. Hyg. Camb*, **65**, 515-536.

Dragas A. Z., Susec-Michieli M., Kornhauser P., and Skerl M. (1983) The bactericidal mats in the pediatric intensive care unit. *Zbl. Bakt. Hyg.*, **177**, 406-411.

Fukada T., Shimizu K., Kumada T., Katahira J., Kikuchi K., Enari T., and Ooki Y. (1987) Antimicrobial adhesive mats and bacterial contamination (in Japanese). *Ikigaku*, **57**, 272-276.

Hayazaki T., Kato M., Asao T., and Shimizu S. (1991) The sterilizing effects of bacteria for wiping hospital footwear with towel mats soaked with various antiseptics and absorbent mat (in Japanese). *Byoin Yakugaku*, **17**, 435-439.

Kihara M., Kojima M., and Takahashi Y. (1998) Classification of patients with methicillin-resistant

Staphylococcus aureus (MRSA) based on their environmental contamination level and MRSA status (in Japanese). *Bokin Bobai*, **26**, 169-176.

Kitashima H., Hanazono J., Kasuno K., Urata H., Tashiro T., Matuda J., Hirakata Y., and Kamihira S. (1996) Investigation and elimination of methicillin-resistant *Staphylococcus aureus* in medical ward[5] (in Japanese). *Kankyokansen*, **11**, 176-182.

Meddik, H.M. (1977) Bacterial contamination control mats : a comparative study. *J. Hyg. Camb*, **79**, 133-140.

Matsuno Y., Mizuno H., and Daitoku Y. (1996) A consideration of bacterial transmission between fingers and push buttons of hospital elevators (in Japanese). *Kankyokansen*, **11**, 123-127.

Perdreau-Remington F., Stefanik D., Peters G., Ruckdesche G., Haas F. Wenzel R., and Pulverer G. (1995) Methicillin-resistant *Staphylococcus haemolyticus* on the hands of health care workers: a route of transmission or a source?. *J. Hosp. Infect.*, **31**, 195-203.

Ohta S., Zenda., Kawakami Y., Nakayama J., Saito H., Furuta S. Kanamaru K., and Matsumoto H.. (1991) Disinfective effect of the "New mat" for methicillin resistant *staphylococcus aureus* (MRSA) (in Japanese). *Byoin Yakugaku*, **17**, 229-235.

Satoh T., Hirose T., Koroku M., Kumamoto Y., Kobayashi N., Urasawa S., Uehara N., and Ohmizu Y. (1992) Study on the route of the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in nosocomial infection (in Japanese). *Kankyokansen*, **7**,1-7.

Sato S., Miura T., Saitoh Y., and Kudo H. (1995) Study on caracteristics of *Staphylococcus aureus* isolated from medical personnel and environment (in Japanese). *Kankyokansen*, **10**, 8-14.

Watabe S., Toda S., Yamamoto M., Odagiri s., and Okuda K. (1992) Incidences and soil of methicillin-resistant *Staphylococcus aureus* in floors of wards (in Japanese). *Kankyokansen*, **7**, 47-51.

Watabe S., Ihara I., and Okuda K. (1994) Eliminating MRSA from the soles of nurses' shoes (in Japanese). *Kankyokansen*, **9**, 44-48