

1 **Bacterial contamination of lightproof covers for high-calorie infusion solutions in wards**

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3 Kengo Hosomi<sup>1,2</sup>, Yuko Takasu<sup>3</sup>, Yumiko Hisai<sup>1</sup>, Sachiko Komaki<sup>1</sup>, Hiroaki Otsuki<sup>1</sup>, Kyoko

4 Okimoto<sup>4</sup>, Sachiko Omotani<sup>2</sup>, Yasutoshi Hatsuda<sup>2</sup> and Michiaki Myotoku<sup>2</sup>

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6 Department of Pharmacy, Kawanishi City Hospital<sup>1</sup>

7 Faculty of Pharmacy, Osaka Ohtani University<sup>2</sup>

8 Department of Pharmacy, Takarazuka City Hospital<sup>3</sup>

9 Department of Clinical Laboratory, Kawanishi City Hospital<sup>4</sup>

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11 Corresponding author: Michiaki Myotoku

12 Faculty of Pharmacy, Osaka Ohtani University, 3-11-1, Nishikiori-kita, Tondabayashi-shi,

13 Osaka 584-8540, Japan

14 E-mail: myoutom@osaka-ohtani.ac.jp

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1 **Abstract**

2 Deterioration of drugs due to light exposure is one of the major concerns, especially  
3 regarding protection of high-calorie infusion solutions, lightproof covers are used in  
4 hospitals. In the absence of any set standards regarding their usage, they are often reused.  
5 This study aimed to investigate bacterial contamination of lightproof covers used in  
6 hospital wards. For this, lightproof covers which had been used or stored in wards were  
7 collected and bacterial cultures were carried out from them. Examination of the cultures  
8 revealed that bacteria were present in the used lightproof covers. The bacterial species  
9 detected in the used lightproof covers were *Bacillus* species Coagulase-negative  
10 *Staphylococci* (CNS) and Methicillin-resistant *Staphylococcus aureus* (MRSA). *Bacillus*  
11 species and CNS were also detected in lightproof covers stored in wards, whereas MRSA  
12 was not detected. Intestinal bacteria were detected in only one lightproof cover. However,  
13 no bacteria were detected from either inside or outside of the unused lightproof covers that  
14 were stored in the drugs department. After allowing the unused lightproof covers stored in  
15 the drugs department to stand for 24 h, *Bacillus* species and CNS were detected in only one  
16 of the covers, whereas no bacteria was detected in other covers. These results indicate that  
17 there is a risk of bacterial contamination in the reuse of lightproof covers and that they  
18 should either be disposed off properly after usage or hand, finger disinfectants should be  
19 used while handling them to prevent any possible contamination.

20  
21 **Key words:** lightproof covers, high-calorie infusion solutions, bacterial contamination,  
22 *Bacillus* species, Coagulase-negative *staphylococci*, Methicillin-resistant *Staphylococcus*  
23 *aureus*

24 **Introduction**

1        Pharmacists' roles in hospitals include ensuring medical safety, and their involvement in  
2 nosocomial infection control measures is of particular importance. Some pharmaceutical  
3 ingredients are susceptible to light, and lightproof covers are an effective means to prevent  
4 light-induced alterations [1-3]. However, no clear standards have been established for  
5 lightproof covers used for infusion solutions. Lightproof covers are used to cover infusion  
6 preparations requiring protection from light, such as infusion preparations mixed with  
7 multiple vitamin preparations, but reuse is possible due to their nature and so the risk of  
8 bacterial contamination caused by reuse should be considered. However, it was clarified that  
9 lightproof covers are handled differently by ward and the handling method varies among  
10 nurses. Moreover, infusion preparations using lightproof covers are placed mainly at the  
11 bedside of individual patients in hospital rooms, so that patients themselves and their families  
12 have many opportunities to contact them, in addition to healthcare workers, such as nurses  
13 and physicians. Furthermore, healthcare workers may contact the cover during exchanging  
14 transfusion or additional administration through a side tube, creating an environment easily  
15 contaminated by bacteria. However, there are no reports studying bacterial contamination of  
16 lightproof covers during and after use.

17        In this study, to clarify the criteria for proper use of lightproof covers, we conducted a study  
18 on bacterial contamination of lightproof covers collected after use or stored without use in the  
19 wards of Kawanishi City Hospital. Following this, bacterial culture tests were performed on the  
20 collected covers.

21

## 22 **Methods**

### 23 1. Lightproof covers studied

24        In total, 23 lightproof covers collected from 4 wards were included in the bacterial

1 contamination study: 9 lightproof covers used in 2 wards (SU (U = used) covers), 8 used  
2 lightproof covers stored in 4 wards (SK (K = keep) covers), and control groups comprising 3  
3 unused lightproof covers stored in the pharmacy department (CP (P = Pharmacy) covers) and 3  
4 unused lightproof covers stored in the pharmacy department and placed in a ward for 24 hours  
5 (CW (W = ward) covers). A lightproof cover applied for high-calorie infusion preparation,  
6 FULCALIQ<sup>®</sup> (Terumo Co., Ltd., Tokyo, Japan), was used. Lightproof covers were primarily  
7 made from polyethylene terephthalate and polyethylene. The temperature of the lightproof  
8 cover storage place was set at room temperature on both the ward and in the pharmacy  
9 department. The lightproof covers were stored in a drawer exclusive for them on the ward and  
10 in an exclusive corrugated cardboard boxes with protection from light in the pharmacy  
11 department. The number of uses was unknown for the lightproof covers used or stored in the  
12 wards. The CW covers were installed in the hospital rooms by hanging only the lightproof  
13 covers on the infusion stands in the hospital rooms.

14

## 15 2. Collection and bacterial culture testing methods

16 The swab method [4] was used for collecting bacteria. After collecting the lightproof covers  
17 from the ward and pharmacy department, the following procedure was performed at a clean  
18 bench in a bacteriological examination room. The outside of the lightproof covers was evenly  
19 rubbed with sterilized gauze moistened with sterilized purified water for sampling. Then, the  
20 cover was cut open with sterilized scissors and the same procedure was applied to the inside.

21 The swab samples were subjected to enrichment culture under aerobic conditions at 36°C for  
22 18 hours, followed by isolation culture at 36°C for 24 hours. Cell colonies were stained using  
23 Gram stain, and were observed microscopically to identify bacterial species based on the  
24 staining patterns and morphology. Brain heart infusion broth for general bacteria (Nippon

1 Becton Dickinson Co., Ltd., Tokyo) was used as the enrichment culture medium, and sheep  
2 blood agar (Nissui Pharmaceutical Co., Ltd., Tokyo) and MacConkey agar (Nippon Becton  
3 Dickinson Co., Ltd., Tokyo) were used as the isolation culture media. MS-CFX agar (Nissui  
4 Pharmaceutical Co., Ltd., Tokyo) was used for isolating methicillin-resistant *Staphylococcus*  
5 *aureus* (MRSA).

6

## 7 **Results**

8 The bacterial culture results for SU covers (1–9) are shown in Table 1. Bacteria were detected  
9 from the outer surface of all SU covers. Bacterial species detected included *Bacillus* species,  
10 coagulase-negative staphylococci (CNS), and MRSA; these bacteria were detected from the  
11 outer surface of 9, 4, and 7 covers, respectively, and from the inner surface of 7, 6, and 4 covers,  
12 respectively, out of the 9 lightproof covers. The same bacterial species were detected from the  
13 same used covers' outer and inner surfaces, with the exception of 4 covers, in which different  
14 bacteria were detected. The usage duration (days) of the lightproof covers was unknown.

15 The bacterial culture results for SK covers (1–8) are shown in Table 2. Within the same  
16 covers, the same *Bacillus* species and CNS were detected on the inner and outer surface, with  
17 the exception of MRSA, and the growth of enteric bacteria was observed in one cover.

18 The bacterial culture results for CP covers (1–3) and CW covers (1–3) are shown in Table 3.  
19 No bacteria were detected from the inner and outer surfaces of the CP covers. *Bacillus* species  
20 and CNS were detected from the outer surface of only one CW cover, and no bacteria were  
21 detected from the inner and outer surfaces of the other CW covers.

22

## 23 **Discussion**

24 Lightproof covers are essential for securing the efficacy of drugs. Particularly, the use of

1 lightproof covers effectively prevents decomposition of multivitamins added to frequently used  
2 high-calorie infusion solutions (FULCALIQ® infusion solution pharmaceutical interview form  
3 (the fourth edition), Terumo, revised in April 2019) [5].

4 In this survey, the adhesion of bacterial species, such as *Bacillus species*, CNS, and MRSA,  
5 to the lightproof covers was frequently detected. Various studies on the occurrence of  
6 nosocomial infection due to these species have been reported [6-9]. In some cases, different  
7 bacteria were found on the same lightproof cover's inner and outer surfaces, suggesting that  
8 bacteria were attached to the respective surfaces in different conditions. More specifically,  
9 bacterial contamination of the outer surface was primarily mediated by the hands of patients  
10 and healthcare professionals, such as nurses. A previous study has reported that MRSA was  
11 isolated from 42% of the gloves worn by nurses who only had contact with the surrounding  
12 environment and no contact with MRSA carriers [10]. Therefore, healthcare professionals  
13 should thoroughly wash their hands before preparing infusion solutions. Additionally, infusion  
14 solution containers on the patient's bedside should be disinfected after being touched by  
15 healthcare professionals. Measures undertaken with the assumption of bacteria being present  
16 on lightproof covers could prevent the spread of infection.

17 Furthermore, the inner surface of lightproof covers may be contaminated with bacteria during  
18 the preparation of infusion mixtures. At our hospital, mixtures of high-calorie infusion solutions  
19 are prepared by nurses in the wards. Hence, bacteria are likely to get attached to the infusion  
20 container's surface during the preparation of infusion mixtures and then spread to the inner  
21 surface of the lightproof cover when the infusion container is covered by the lightproof cover.  
22 However, it is challenging to prevent bacterial contamination during the preparation of infusion  
23 mixtures in wards. Hence, the entire operating procedures for the preparation of infusion  
24 mixtures in wards should be reviewed, including the adequacy of disinfection steps, such as

1 those used for infusion solution containers, gloves, and workbenches. Based on the  
2 contamination status of the lightproof covers in this study, the method of handling was  
3 discussed by the infection control committee of our hospital, and it was decided to use disposal  
4 gloves for handling infusion preparations, such as mixing formulation, and thorough hand  
5 hygiene was promoted. Also, high-calorie infusion solutions are to be prepared in the pharmacy  
6 department and then delivered to the ward. Furthermore, used lightproof covers cannot be  
7 returned to the shelf for spares. Following strict implementation of these measures, the use of  
8 lightproof covers without detectable bacteria, similar to the ones stored in the pharmacy  
9 department, would be possible, and the risk of bacterial contamination would be minimized.

10 The bacterial strains adhering to the lightproof covers were identified, but the bacteria were  
11 not quantitated because of the small number of samples. **We did not examine the infection status  
12 of patients for whom we used the lightproof covers, nor perform antibiotic susceptibility tests  
13 for bacterial species that were detected.** As MRSA and CNS were not detected in the direct  
14 culture (data not shown), these bacteria are likely to be present in small numbers. However,  
15 they still pose a risk for infection, which cannot be overlooked. Although bacteria were detected  
16 regardless of the usage duration, the risk of spreading bacterial contamination to the  
17 environment likely increases with a lightproof cover being reused for a longer duration [11-12].  
18 In particular, *Bacillus* species and CNS, frequently detected in this study, are known to be  
19 resident bacteria; however, they have also been reported to cause catheter infection [13-14],  
20 suggesting that contaminated lightproof covers can mediate nosocomial infections. Therefore,  
21 hands and fingers should be thoroughly disinfected, particularly before infusion fluid exchange.  
22 Intestinal bacteria were detected only in one sample in this survey, but when infection is caused  
23 by intestinal bacteria, such as extended spectrum  $\beta$ -lactamases and carbapenem-resistant  
24 *Enterobacteriaceae*, the effect of antibacterial agents may not be acquired and a serious problem

1 may occur.

2 MRSA was detected from SU covers, but not from SK covers, in this study. The survival rate  
3 of MRSA varies depending on the bacterial strain and various conditions, such as the material  
4 to which the bacterium is attached, temperature, and humidity [15-17]. Notably, MRSA attached  
5 to covers may survive differently depending on the storage conditions. Therefore, SK covers  
6 contaminated with MRSA are predicted to be found if the number of samples is increased.

7 At our hospital, methods of handling and storing lightproof covers after human contact have  
8 not been examined previously. Based on this study's findings, we formulated the standards for  
9 the use of lightproof covers and enforced adherence to the disinfection manual. In the future,  
10 continuous examinations of and measures against bacterial contamination of lightproof covers,  
11 in cooperation with Infection Control Team, are required to determine the validity of the  
12 measures. Furthermore, pharmacists are expected to play additional roles in the management of  
13 steps preceding drug administration to patients in wards or at bedside. Moreover, development  
14 of lightproof covers made from new materials that prevent bacterial attachment to infusion bags  
15 and infusion preparations in bags is warranted.

16

## 17 **Conflicts of interest**

18 The authors have no conflicts of interest to disclose.

19

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Fig 1. The lightproof covers



Table 1. Bacterial culturing result of used lightproof covers in ward

No.	Ward	Outside	Inside	Period of use
SU1	5N	<i>Bacillus species</i>	(-)	Half day
SU2	5N	<i>Bacillus species</i> , CNS, MRSA	<i>Bacillus species</i> , CNS, MRSA	Half day
SU3	3S	<i>Bacillus species</i> , CNS, MRSA	<i>Bacillus species</i> , MRSA	1 day
SU4	3S	<i>Bacillus species</i> , CNS, MRSA	<i>Bacillus species</i> , CNS, MRSA	2 day
SU5	3S	<i>Bacillus species</i> , CNS, MRSA	<i>Bacillus species</i> , CNS, MRSA	4 day
SU6	3S	<i>Bacillus species</i> , CNS	<i>Bacillus species</i> , CNS	number of days unknown
SU7	3S	<i>Bacillus species</i>	CNS	number of days unknown
SU8	3S	<i>Bacillus species</i> , CNS	<i>Bacillus species</i> , CNS	number of days unknown
SU9	3S	<i>Bacillus species</i> , CNS	<i>Bacillus species</i>	number of days unknown

(-): No detection; CNS : Coagulase-negative Staphylococci; MRSA: Methicillin-resistant *Staphylococcus aureus*

3S : medicine ward, pediatric ward, ophthalmology ward; 5N : medicine ward

Table 2. Bacterial culturing results of lightproof covers stored in the ward

No.	Ward	Outside	Inside
SK1	5S	<i>Bacillus species</i>	<i>Bacillus species</i>
SK2	5S	<i>Bacillus species</i> , CNS	<i>Bacillus species</i> , Intestinal bacteria
SK3	5N	(-)	<i>Bacillus species</i>
SK4	5N	<i>Bacillus species</i>	<i>Bacillus species</i>
SK5	4S	CNS	CNS
SK6	4S	<i>Bacillus species</i> , CNS	CNS
SK7	3S	<i>Bacillus species</i>	<i>Bacillus species</i>
SK8	3S	<i>Bacillus species</i>	CNS

(-): No detection; CNS: Coagulase-negative Staphylococci  
 5S, 5N: medicine ward; 4S: Surgical ward·Urology ward, 3S: Medicine ward, Pediatric ward,  
 Ophthalmology ward

Table 3. Bacterial culturing results of lightproof covers in the comparison group

	No.	Outside	Inside
Unused lightproof covers for pharmacy storage	CP1	(-)	(-)
	CP2	(-)	(-)
	CP3	(-)	(-)
Unused portion stored in the pharmacy department lightproof covers installed in the ward for 24 hours	CW1	(-)	(-)
	CW2	(-)	(-)
	CW3	<i>Bacillus species</i> , CNS	(-)

(-) : No detection; CNS : Coagulase-negative Staphylococci