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Environmental meticillin-resistant *Staphylococcus aureus* (MRSA) disinfection using dry-mist-generated hydrogen peroxide☆

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Summary Meticillin-resistant *Staphylococcus aureus* (MRSA) is a major problem in hospitals worldwide. Hand hygiene is recognised as crucial in limiting the spread of MRSA but less is known about the role of MRSA reservoirs in the inanimate hospital environment. We evaluated the effect of hydrogen peroxide vapour diffused by Sterinis® against MRSA in two experimental hospital settings and in two field trials. Dipslides were used for MRSA detection and quantification before and after using the Sterinis disinfection process. In the first experimental hospital setting, four epidemic MRSA strains were placed at five locations and left for one week. All strains survived the week but not the disinfection process. In field trial one 14 upholstered chairs from a department with many MRSA positive patients were left for one month in a closed room prior to disinfection. MRSA was found on the upholstery of four of the 14 chairs. Three chairs became MRSA negative immediately after the disinfection, the fourth 24h later. The second field trial was in the private home of a MRSA positive family of four individuals. One location was found MRSA positive, remaining so after the Sterinis cycles. We found Sterinis to be effective against MRSA in the experimental hospital setting

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and upholstered chairs, but not in the private home of heavily colonised MRSA patients.

Introduction

MRSA can survive in the environment for several months.[1–4] Boyce *et al.* showed that surfaces near MRSA patients often become contaminated and that staff may contaminate their hands or gloves by touching these surfaces.[5] Traditional cleaning methods with detergents might not be sufficient to kill MRSA in the hospital environment.[6]

In Denmark, the prevalence of MRSA is low but community-onset (CO) MRSA has been increasing since 2003.[7] A special problem is elderly people residing in nursing homes as they are frequently hospitalised. Many of them are unrecognised MRSA carriers on admission and they have been associated with several small hospital outbreaks. In order to reduce the risk of MRSA transmission, known MRSA-positive patients are isolated. After their discharge however, there is need for a safe method to eliminate MRSA from the hospital environment.

In this study we tested whether the combination of hydrogen peroxide (H_2O_2) and silver cations (Ag^+) diffused by the dry mist generator Sterinis® (Gloster Santé Europe, Labege Cedex, France) could eliminate MRSA from the hospital environment. In addition, we also tested the system in the private home of MRSA-positive individuals where treatment of the MRSA carrier state had failed. H_2O_2 is a powerful oxidiser and Ag^+ appears to kill micro-organisms instantly, by blocking the respiratory enzyme system as well as altering microbe DNA and the cell wall.[8–10]

Methods

Principles of the dry mist generator Sterinis

Sterinis is a programmable dry mist generator which, combined with the disinfectant Sterusil®, diffuses H_2O_2 and Ag^+ as small droplets (8–12 μm in diameter). The manufacturer claims that these droplets can reach all the areas of a room, including normally difficult-to-reach sites.

Sterusil is composed of 5% H_2O_2, 95% sterile water, <50 ppm Ag^+ and <50 ppm *ortho*-phosphoric acid as a stabiliser. The manufacturer states that it is non-toxic and non-corrosive. Spraying times are calculated according to room size, ranging from 18 min (30 m^3) to 52 min (200 m^3) and the number of cycles can be chosen. We performed three cycles of disinfection except in setting 1b where only one cycle was run. We used 6 mL product per m^3 air and a contact time of 1 h after each cycle. The Sterinis disinfection in experiment 1a (hospital room) took 4 h.

Dipslides, subculture and PCR

Dipslides with neutraliser for detergent and disinfectants (PC2TN, Biotrace International, Bridgend, UK) were used to detect MRSA and the number of colony-forming units (cfu) per cm^2. Dipslides were pressed firmly onto the surface tested and incubated at 37°C. They were inspected for growth after 24 h, 48 h and one week. Inspection after one week was performed as Ag^+ can inhibit *S. aureus* leading to microcolony formation after 4–6 days (in-house results with silver dressings). In the experimental hospital settings, bacterial colonies were counted and the growth was divided into categories (Table I). In the field trials, micro-organisms other than MRSA grew on the dipslides and a definitive MRSA count was not performed. Suspected MRSA colonies were subcultured on 5% blood agar and on MRSA ID plates (Biomérieux, Marcy L'Etoile, France). The presence of the *mecA* and *spa* genes was confirmed by polymerase chain reaction (PCR).[12] Sequencing of the *spa* gene was performed on selected isolates on an ABI Prism 3100 (Applied CE Biosystems, South San Francisco, CA, USA) and the *spa* type designated using Ridom GmBH (Würzburg, Germany) software.

Table I Dipslide density readings*

Growth	Colonies	cfu/cm^2
Very heavy	Confluent	250
Heavy	Semi-confluent	100
Moderate	70–99	40
Slight	40–69	12
Very slight	6–39	2.5
Scant	<6	<2.5
No growth	None	–

* Based on Griffith *et al.*[11]

Experimental hospital room settings

Four MRSA strains were selected. *spa* type t024-sequence type (ST) 8-SCC*mec* IV was related to nursing homes and small hospital outbreaks; t019-ST30-IV and t044-ST80-IV were our most common CO-MRSA and t005-ST22-IV has caused a major outbreak in a Danish hospital and is related to EMRSA-15.[7,13,14] A 0.5 McFarland suspension of the four MRSA strains was diluted in urine to a concentration of 10^5 bacteria/mL urine and the final concentration was confirmed by agar plating. The urine was free from antibiotics and was boiled before MRSA was added. In a hospital room closed for renovation, the ventilation system was switched off and the windows and doors of the rooms were sealed during the Sterinis disinfection process.

Experimental hospital setting 1a

The following five locations for MRSA application were chosen: an upholstered chair, a patient table, an eiderdown, a piece of carpet and a bed railing. The surfaces were divided into squares of $10 \times 10\ cm^2$ with masking tape except on the bed railing, where areas of $2 \times 10\ cm^2$ were marked. Of each MRSA urine solution, 100 μl was replicated onto eight different spots on each of the five locations corresponding to 100 cfu/cm^2. For the bed railing, urine was pipetted onto gauze that was pressed against the railing and removed when the suspensions had penetrated the gauze. The urine was spread on the squares of the table and carpet with a sterile glass spreader and on the chair and eiderdown by pipetting over the area. Sampling for MRSA was performed with dipslides after 30 min drying time and after a further 1 h, one day and seven days. Each sampling was performed on a new spot. After one week, a fresh urine suspension of the same MRSA strain was inoculated in a similar manner on five new spots. Dipslides were used after 30 min to confirm the presence of MRSA. The Sterinis disinfection process was then started. Sampling from the first and second spotting was done 2 h after the final cycle and was repeated after one day, seven days and 14 days to evaluate whether the MRSA was inactivated or killed by the disinfection.

Experimental hospital setting 1b

One month later, we evaluated whether we could reduce the number of Sterinis cycles necessary to kill MRSA from three to one. We performed the experiments in the same hospital room as in the first experiment (1a), using different spots, but the same five location types for MRSA application. MRSA was spotted and left for one week, after which a fresh urine suspension of the same MRSA was inoculated. The Sterinis disinfection process was then started and ran only one cycle. Then dipslides were used on the five locations. All other dipslide testings were performed as in experiment 1a.

Field trial 1: hospital

Fourteen upholstered chairs were involved in an MRSA outbreak caused by t024-ST8-IV. The chairs were stored in a closed room for one month before sampling with dipslides. After the final Sterinis disinfection cycle, dipslides were taken and were repeated after one day.

Field trial 2: private home

A family of four had received several treatments for the MRSA carrier state with mupirocin nasal ointment three times daily for five days, combined with chlorhexidine baths daily for five or 10 days. A boy with atopic dermatitis had also received systemic treatment with a combination of fusidic acid and clarithromycin for 10 days. Although atopic dermatitis is known to complicate efforts at decolonisation, recolonisation from the environment was also suspected. Ten dipslides were taken from the following locations: pillow (bed), two different sofa cushions, a favourite upholstered chair, the TV remote control, a computer keyboard and mouse, door handles of bathroom and front doors, refrigerator handle and the handle of the shower cabinet. Two Sterinis robots were used to cover the whole apartment of 100 m^2 with dry mist. After the last Sterinis cycle and again after one week the 10 locations were tested with dipslides.

Results

Experimental hospital room setting 1a

All four *spa* types survived for one week (Figure 1). After the Sterinis disinfection, all one week old and new spots were MRSA negative. No growth of MRSA was found during the 14 days of follow-up.

Experimental hospital room setting 1b

None of the four MRSA inoculated and left for one week prior to the Sterinis disinfection in this experiment had survived. The strains inoculated immediately before the disinfection grew on

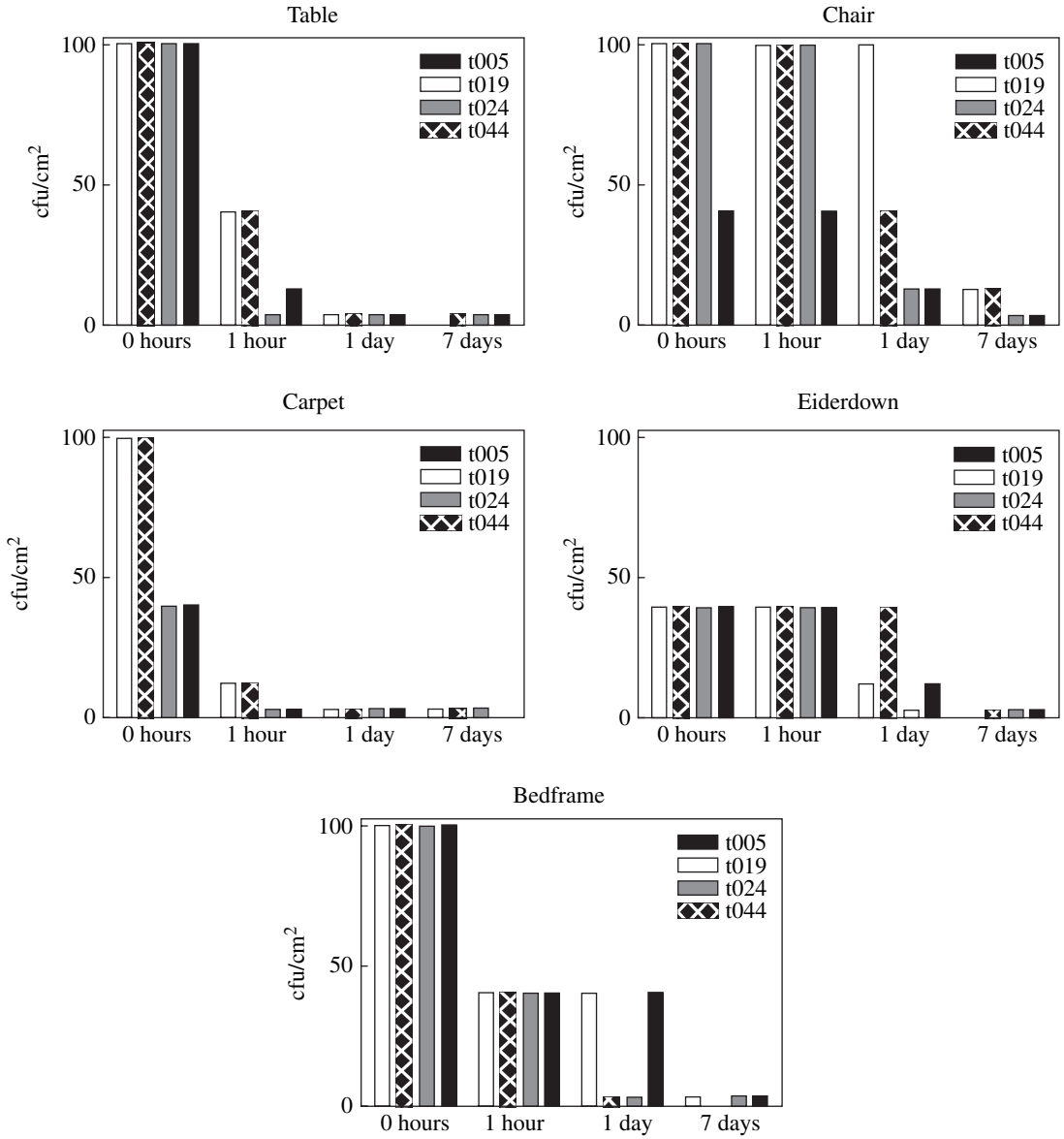

Figure 1 The survival times of four MRSA strains on five locations in a hospital room. *x*-Axis: Dipslide sampling times after inoculation. 0 h: ~30 min after inoculation (after drying). *y*-Axis: The number of colony-forming units (cfu) per cm^2 could only attain the values 100, 40, 12.5, 2.5 and 0. All samplings were performed before the Sterinis disinfection process. The four strains are named by their *spa* (Staphylococcus protein A) types (e.g. t005).

dipslides taken just before the disinfection process but were all killed after one cycle of Sterinis. No MRSA was found during the 14 days of follow-up.

Field trial 1

MRSA was found on four of the 14 upholstered chairs (28.5%). After disinfection MRSA was still found on one chair, but the next day all chairs were MRSA negative. The chairs were then removed.

Field trial 2

An environmental investigation had been performed two months prior to the trial. Three of four locations were MRSA positive. Repeat screening found MRSA only on a covered pillow in the bed. After disinfection the pillow was still MRSA positive. One week later MRSA was found on a pillow in the bed (linen washed and changed, not necessarily the same pillow) and from one upholstered chair. The family had started a 10-day treatment of the MRSA carrier state four days

before the disinfection. They were still MRSA carriers one month later.

Discussion

MRSA has been found to survive for long periods on many different surfaces in the hospital environment and in private homes.[2,15–19] A study by Wagenvoort *et al.*[4] showed that outbreak strains survived for a longer period in the surroundings than sporadic MRSA strains. In our study we found that MRSA strains of different *spa* types were able to survive on hospital furniture and fabrics for at least one week. We also found that MRSA could survive for at least one month on upholstered chairs.

We believe that environmental sampling should be performed with standardised, validated and sensitive methods. Swabbing has been found to have a very low sensitivity, especially in the recovery of dried cells, and dipslides and contact plates have been reported to have a high sampling efficiency and sensitivity.[20–22] In our study we used dipslides to achieve a standardised and sensitive method of detecting MRSA. Dipslides are most suitable for flat surfaces, but there was no difficulty in detecting MRSA from the curved bed railing.[22] We recommend using dipslides that neutralise detergents and disinfectants; in addition, we also recommend examining slides for growth for at least one week due to the possible inhibition of growth from Ag^+ ions.

The inoculum we chose was 100 cfu/cm^2. This is in the range used in studies regarding the detection limit of sampling methods.[20–22] In a study by Wagenvoort *et al.* looking at survival of MRSA, a starting concentration of 10^9 cfu/mL was chosen.[4] This amount is much higher than our starting concentration of 10^5 cfu/mL. We have not found any reports describing the viable counts of MRSA found from environmental sampling, but the amount of MRSA in the surroundings could be influenced by several factors such as infection or colonisation, skin disease and period of time since last cleaning. In the private home we found <40 cfu/cm^2. This suggests that ~100 cfu/cm^2 is closer to the quantity found in the environment of MRSA-colonised patients, but infected MRSA patients might disperse larger amounts of MRSA. Future studies should quantify the number of cfu/cm^2 when sampling for MRSA in hospital rooms and evaluate this against the efficiency of the Sterinis system.

Based on bacterial counts after cleaning and disinfection in 29 ward locations, Griffith *et al.* have suggested a benchmark value of 2.5 cfu/cm^2 for staphylococci on surfaces.[11] Although there are different opinions on whether MRSA in the environment is connected with MRSA infection rates in hospitals, we aimed for total elimination of MRSA from the inanimate environment.[23–25] Whereas some studies have identified MRSA in the environment as the cause of an outbreak, others have not demonstrated a connection.[15,17,24,25] Further controlled studies are needed to determine whether MRSA in the environment has any influence on the rate of nosocomial MRSA infection.

The amount of bacteria spread on each location in the hospital room was calculated to correspond to 100 cfu/cm^2. However, on the chair, carpet and eiderdown the number of cfu/cm^2 was in some cases only 40 cfu/cm^2 (see Figure 1). We suspect that in these places, the urine suspension containing MRSA penetrated deeply into the materials. Therefore we believe that these surfaces are more difficult to clean with traditional cleaning methods and could be an important reservoir of MRSA.

The MRSA strains in the hospital room were not naturally dispersed but urine was chosen as a matrix to imitate a natural situation. Nevertheless, we cannot be sure that the survival rates would be the same if MRSA patients had been in the room and daily cleaning had been performed. MRSA on the upholstered chairs was shed directly from patients, however, and had a month-long survival in a closed room.

Based on a previous study we chose to run three cycles of disinfection.[26] We also tested whether one cycle was sufficient to eliminate MRSA. All procedures were identical to the initial experimental setting in the room. Surprisingly, we found that none of the MRSA strains survived for one week and the strains inoculated just prior to the disinfection were eliminated after just one cycle of Sterinis. The hospital room had been dormant between the two experimental settings and had not been cleaned. We suggest that a residual effect of the Sterinis disinfection from the Ag^+ ions explains the difference between the results from the two experiments. In experiment 1a, MRSA was cultivable after one week from 17 of 20 spots; in experiment 1b none was cultivable. Although the residual effect may be removed by cleaning, the residual disinfectant effect will probably remain in areas that are not reached by cleaning.

One of four MRSA-positive chairs was still positive after 2 h but MRSA negative the next day. This could be due either to a prolonged effect of the disinfectant or to a sampling error.

In the private home, the MRSA found on a pillow was not eliminated by the Sterinis disinfection.

This pillow was deeply buried in the bed and may not have been reached by the dry mist. MRSA has been successfully eliminated from private homes using gaseous ozone, and the use of general-purpose detergent and steam-cleaning.[18,19] If Sterinis is used in a private home we recommend that all areas be easily reached, bed areas stripped down and closets opened. We suspect that it is much more complicated to eliminate MRSA in a private home than a hospital room.

Recently Hardy *et al*. found hydrogen peroxide vapour to be very effective in eliminating MRSA from the environment.[27] However, rapid recontamination from new MRSA patients was seen and it was concluded that hydrogen peroxide vapour could not maintain a low level of environmental contamination in an open-plan intensive care unit where MRSA is endemic. In Denmark and other countries with a low prevalence of MRSA, the situation is quite different. MRSA patients are isolated in single rooms and on discharge these rooms could be disinfected with Sterinis in order to protect the next patient from acquiring MRSA. Currently these rooms are cleaned with chlorine-releasing agents.

In conclusion, we have shown that MRSA can survive on hospital furniture and fabrics for at least one month and that Sterinis is effective in eliminating experimental MRSA from hospital furniture and MRSA from upholstered chairs contaminated by patients. We suggest that Sterinis could be used as a supplement to final cleaning of MRSA isolation rooms. The disinfection of private homes is more complicated and we do not recommend Sterinis in this setting.

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Conflict of interest statement
None declared.

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