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Evaluation of bedmaking-related airborne and surface methicillin-resistant *Staphylococcus aureus* contamination

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Summary: The number of airborne methicillin-resistant *Staphylococcus aureus* (MRSA) before, during and after bedmaking was investigated. Air was sampled with an Andersen air sampler in the rooms of 13 inpatients with MRSA infection or colonization. Sampling of surfaces, including floors and bedsheets, was performed by stamp methods. MRSA-containing particles were isolated on all the sampler stages—stage 1 (>7 μm diameter) to stage 6 (0.65–1.1 μm). The MRSA-containing particles were mostly 2–3 μm diameter before bedmaking and >5 μm during bedmaking. The number was significantly higher 15 min after bedmaking than during the resting period, but the differences in counts after 30 and 60 min were not significant. MRSA was detected on many surfaces. The results suggest that MRSA was recirculated in the air, especially after movement. To prevent airborne transmission, healthcare staff should exercise great care to disinfect inanimate environments. Further studies will be needed to confirm the level of MRSA contamination of air during bedmaking and establish measures for prevention of airborne transmission.

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Keywords: MRSA; air contamination; bedmaking; Andersen air sampler.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has frequently been reported as a major hospital-acquired pathogen in community hospitals, long-term care facilities and tertiary care hospitals.[1] The principal mode of transmission is from patient to patient via the transiently colonized hands of health-care staff, who acquire the organism from patient contact or by handling contaminated materials.[2,3] Since MRSA has been recovered from many sites, including floors, linen, medical equipment, and hospital furnishings, transmission via inanimate environments may also be important.[2–4] Airborne transmission is generally considered to occur at lower frequency than transmission via direct contact,[3,5–7] but MRSA in the form of a bioaerosol can contaminate air and cause airborne infection.[3,5,6]

Bedmaking generates dust and airborne micro-organisms.[8] Increased bacterial counts in air resulting from bedmaking were first noticed in the early 1940s.[8,9] Although several studies on air sampling of MRSA have been reported,[10,11] few have included timed studies, or studied the numbers and size of particles released during bedmaking using an Andersen air sampler (as in our previous study[12]).

To investigate airborne MRSA contamination further, we used an Andersen air sampler to detect the MRSA-containing particles before, during and after bedmaking, in the single rooms of patients at

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the University Hospital of Occupational and Environmental Health in Kitakyushu, Japan. We also investigated surface contamination. Based on our findings, we discuss the dynamic state of airborne MRSA during bedmaking and the dispersal of MRSA in relation to air and surfaces.

Materials and methods

Patients

Thirteen patients infected (10) or colonized (three) with MRSA during their stay in the hospital were studied (Table I). All were identified by cultures obtained for clinical purposes. MRSA was isolated from the sputum of eight patients (pneumonia) and the wounds of two. Nose swabs from seven of the infected patients were positive for MRSA, as were those from the three carriers. All patients were hospitalized for more than three months and had harboured MRSA for more than one month. They were nursed in closed, single-patient rooms connected to a central air-conditioning plant (Fig. 1). The 'bedsheets' were made from 100% cellular cotton. The numbers of MRSA isolates in the clinical samples were $>10^6$ colony-forming units (CFU) per specimen; no change in this number was observed during the study.

Table I *Characteristics of patients with MRSA infection or colonization*

Patient no.	Sex/ age (years)	Underlying disease	Infection	Nasal carriage
1	M/70	Parapharyngeal Ca[1]	Pneumonia	Positive
2	M/73	Hypopharynx Ca[1]	Pneumonia	Positive
3	M/67	Acute renal failure	Pneumonia	Negative
4	F/69	DCM[2]	Pneumonia	Positive
5	F/75	Lung Ca[1]	Pneumonia	Positive
6	F/8	Cerebral palsy	Pneumonia	Positive
7	M/9	WH disease[3]	Pneumonia	Negative
8	M/5	HE[4]	Pneumonia	Positive
9	M/71	Oesophageal Ca[1]	Wound	Positive
10	M/65	Skin Ca[1]	Wound	Negative
11	F/52	Tongue Ca[1]	Colonization	Positive
12	M/56	Diabetes	Colonization	Positive
13	F/64	Larynx Ca[1]	Colonization	Positive

[1]Ca: cartinoma; [2]DCM: dilated cardiomyopathy;
[3]WH disease: Werdnig–Hoffmann disease;
[4]HE: Hypoxic encephalopathy.

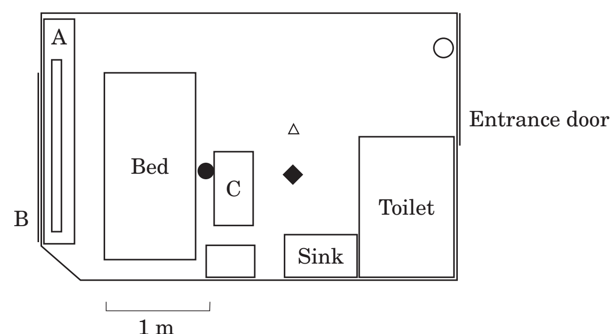

Figure 1 Schematic of a single inpatient room. Floor sample, taken at a distance of 0 m (●), 1 m (△), and 3m (○) from bed. ◆, airsampler; A, air conditioner; B, window; C, overbed table.

Air sampling

From August 2000 to July 2001, air samples were collected using a six-stage Andersen air sampler[12] set to collect 28.3 L/min for 10 min (total 283 L).[13] The Andersen is a sieve-type cascade impactor, which separates micro-organism-carrying particles into the following six stages according to diameter; stage 1: >7 μm; 2: 4.7–7 μm; 3: 3.3–4.7 μm; 4: 2.1–3.3 μm; 5: 1.1–2.1 μm; 6: 0.65–1.1 μm.[13,14] The sampler was placed on a rack, 1 m above the floor, and 1 m from the bedside (Fig. 1).

Three sets of air samples were carried out in each room between 9 and 11 am at weekly intervals in the same month. Each set was as follows: before (resting period), during, 15, 30 and 60 min after bedmaking. There were no other activities by personnel and no visitors for an hour before or during bedmaking. Samples during the resting period were collected on Trypto-soya Agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) followed by a second set of samples collected on MSO Agar (salt egg-yolk agar containing 6 mg/L of oxacillin, Nissui Pharmaceutical Co.) for detecting total bacteria and MRSA, respectively. Samples taken during and after bedmaking were collected on MSO agar to detect MRSA. After sampling, culture media were incubated at 37°C for 48 h, colonies were counted and the results expressed as colony-forming units per cubic metre (cfu/m^3).

Environmental samples

Samples were collected from areas of 10 cm^2 in four sites (floors, sheets, overbed table and patient's clothing) in the rooms of each patient. Floors were sampled at three locations, directly beside the bed (F0), and 1 m (F1) and 3 m (F3) away. The stamp method was chosen to perform sampling using MSO agar plates; agar stamps are rotated several times on the test surface. Three sets of samples were taken.

Each sample was from a different spot in the area. This was done before bedmaking. Further sampling was carried out on the bedsheet and on the floor directly beside the bed 60 min after bedmaking. Agar plates were incubated at 37°C for 48 h, and the numbers of cfus counted.

Isolation of bacteria from the patients' nares and hands

At times of air sampling, swabs from the nasal cavities of the patients were taken, using sterile cotton swabs moistened with sterile phosphate-buffered saline.[15] The swabs were inoculated on MSO agar, and incubated at 37°C for 48 h. Samples were also taken from the patients' hands using the stamp method and MSO agar plates. Samples were obtained from three separate spots on the hands on three occasions.

Identification of MRSA

Staphylococcus aureus was identified by Gram-staining, growth on salt egg yolk agar, agglutination by protein A and clumping factor, and the coagulase test. Methicillin resistance was determined after 48 h incubation at 37°C on MSO agar supplemented with 6 mg/L oxacillin. Three isolates from growth on MSO agar from each sample were tested for the *mec*A gene by PCR using primers as previously described.[16] Fifty nanograms of bacterial DNA was used as a template. DNA amplification was carried out for 40 cycles in 50 µl of reaction mixture as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min with a final extension of 5 min at 72°C. Ten microlitres of the PCR products were analysed by 2% agarose gel electrophoresis. The presence of a 533 basepair amplimer was taken to indicate the *mec*A gene.

Statistical analysis

All of the values from sampling were compared by Student's *t*-test. Two-tailed tests were performed and a *P*-value less than 5% was considered statistically significant.

Results

Air sampling

During the resting period the mean (± SD) cfu/m^3 air counts were 163.0 ± 23.7 for the rooms containing the 10 MRSA infected patients and 159.0 ± 18.8 for those of the three carriers. Most particles were collected on stages 4 and 5 of the air sampler; 53% were within the respirable range (<4 µm). Mean counts of MRSA were 4.7 cfu/m^3 for infected patients, and 0.7 cfu/m^3 for carriers. The *mec*A gene of *S. aureus* on MSO agar was positive.

During bedmaking MRSA counts rose to 116.0 ± 43.7 in rooms of infected patients, and 18.3 ± 3.6 in those of carriers (Fig. 2), counts of 25 and 26 times the baseline counts, statistically significant differences (*P*, 0.01). MRSA were present on all stages of the air sampler, but most (>80%) particles were in the non-respirable range of >5 µm diameter (Fig. 3). Fifteen minutes after bedmaking, MRSA particle counts were 29.6 ± 18.9 (infection), and 5.3 ± 3.7 (carrier), also significantly higher than those during the resting period ($P < 0.01$). Mean counts of MRSA at 30 min and 60 min after bedmaking were 4.9 ± 3.4 (30 min, infection), 0.7 (30 min, carrier), and 4.7 (60 min, infection), 0.4 (60 min, carrier), respectively. These were not significantly different to the pre-bedmaking count.

Sampling of surfaces and patients' hands

The results are shown in Table II. The mean ± SD total cfus/10 cm^2 MRSA on the floors at 0 m, 1 m and 3 m from the beds in the patients' rooms before bedmaking were 5.9 ± 3.4, 4.9 ± 3.2, and 4.4 ± 3.2 and for carriers were 22 ± 1.1, 2.0 ± 1.3, 2.0 ± 1.8. MRSA were isolated from bedsheets, overbed tables, and patients' clothing. After bedmaking the mean total cfus/10 cm^2 on the floors at 0 m from the bed were 6.1 (infection) and 2.3 (carrier), and from the bedsheet, 0.4 (infection), and 0.1 (carrier). These did not differ significantly from pre-bedmaking levels. The counts of MRSA on bedsheets 60 min after bedmaking were lower than those before bedmaking [$P < 0.01$ (infection), $P < 0.01$ (carrier)], although numbers were small. The mean ± SD total cfus/10 cm^2 MRSA on the patients' hands before bedmaking, were 4.3 (infection) and 0.9 (carrier), respectively.

Discussion

We demonstrated that MRSA-containing particles were liberated into the air during bedmaking. MRSA was mainly carried on larger particles, 4–8 µm in size but some were on <4 µm particles, so were respirable; able to reach the lung and possibly cause infection.[12]

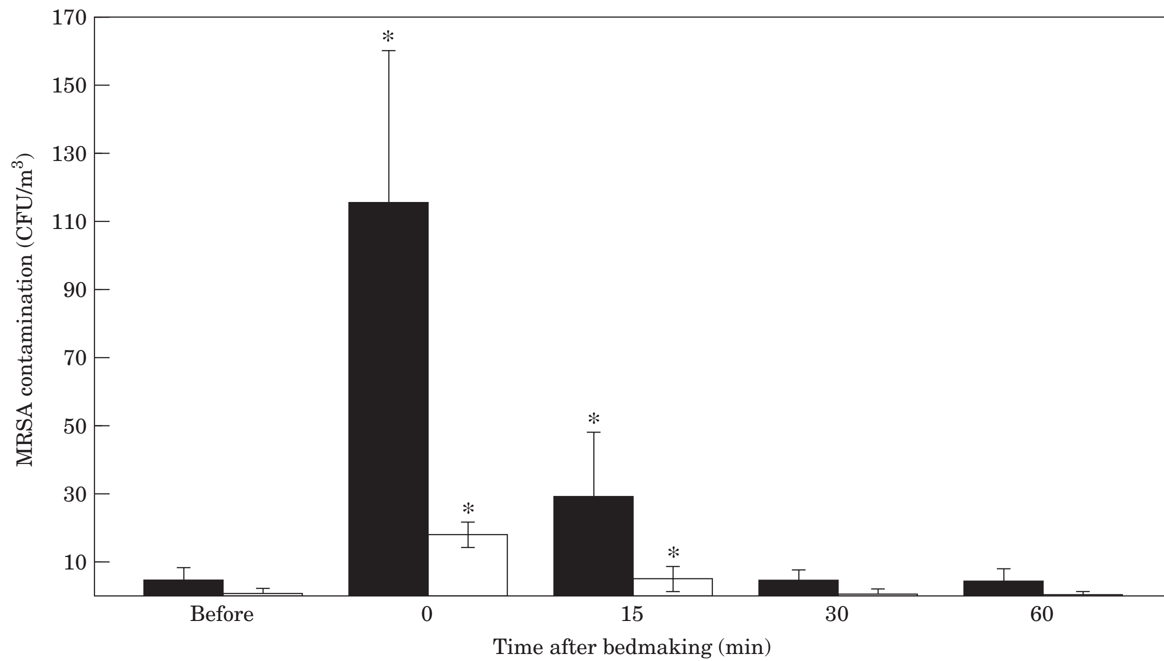

Figure 2 Air contamination with MRSA in the single rooms of inpatients with MRSA infection and colonization before, during and after bedmaking. ■, Inpatients with MRSA infection ($N = 10$). □, Inpatients with MRSA colonization ($N = 3$). *, $P < 0.01$.

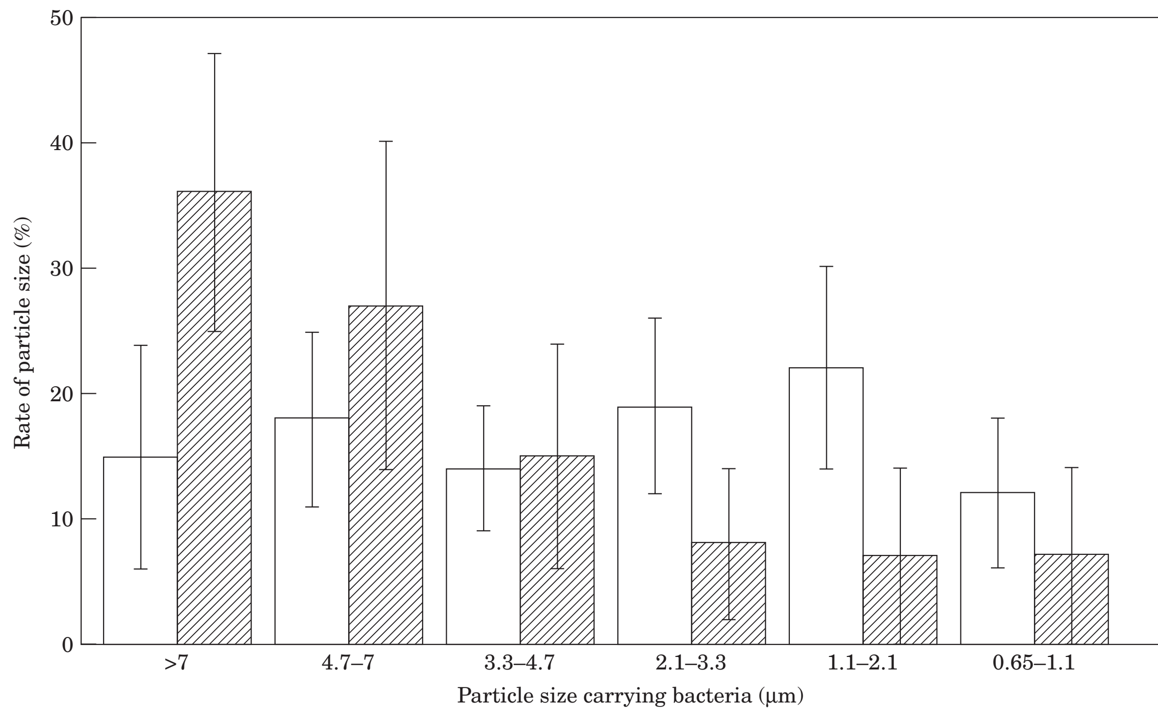

Figure 3 Percentage of bacteria-carrying particles by aerodynamic size. □, General bacteria during resting period. ■, MRSA during bedmaking.

Table II *MRSA environmental contamination in the single rooms of inpatients with MRSA infection or colonization*

	Level of MRSA contamination, cfu/10cm^2	
	Infection ($N = 10$)	Carrier ($N = 3$)
Floor 0	5.9 ± 3.4	2.2 ± 1.1
Floor 1	4.9 ± 3.2	2.0 ± 1.3
Floor 3	4.4 ± 3.2	2.0 ± 1.8
Bedsheet	3.7 ± 2.9	1.2 ± 1.2
Overbed Table	3.4 ± 2.4	1.2 ± 1.3
Clothing	3.5 ± 1.3	0.7 ± 0.8
Hands	4.3 ± 2.9	0.9 ± 0.9
Floor 0′	6.1 ± 3.2	2.3 ± 1.7
Bedsheet′	0.4 ± 0.6	0.1 ± 0.3

Data are given as mean ± SD. cfu; colony-forming unit. Floor 0′; floor at a distance of 1 m from the bed at 1 hour after bedmaking. Bedsheet′; bedsheet at 1 hour after bedmaking.

The number of MRSA-containing particles in the air of MRSA-infected patient rooms during bedmaking was higher than that observed during the resting period, or in the rooms of the MRSA-colonized patients. Counts decreased 15 min after bedmaking, and by 30 min were comparable to those obtained before bedmaking. These findings suggest that increase of airborne MRSA during bedmaking can contaminate the hospital environment and may play a role in MRSA colonization in the nasal cavity, or sequentially, in respiratory MRSA infections. Thus, it is crucial to design an efficient control system to limit the accumulation of MRSA in environments in which air is recirculated. Laminar unidirectional airflow, air ventilation, and air filtration could also be beneficial in hospital environments, and should be considered for isolation rooms.

When healthcare staff were present, the cfus of MRSA increased, indicating that movements such as bedmaking can spread MRSA on surfaces. There is also a potential danger of staff acquiring the epidemic strain from a patient by direct contact, and transmitting it to other patients.[2,3] Moreover, MRSA may be transferred from patient to patient by airborne transmission. To prevent spread, gloves should be worn by all personnel entering the rooms of patients with MRSA.[18] Although measures for prevention and control of hospital-acquired infection with MRSA include hand disinfection, wearing a gown, gloves and a mask, and removing MRSA from the nasal vestibule,[2,18] few measures are aimed at control of airborne bacteria.

The number of MRSA on the bedsheets was significantly decreased after bedmaking, becoming hardly detectable. To control airborne MRSA dispersal, it is important to minimize the contamination of bedsheets and avoid dispersing the MRSA adhering to the bedsheets for at least 30 min after bedmaking. The greater the contamination of the bedsheet, the greater was the increase in airborne MRSA during bedmaking. Thus, it is important to consider the frequency of bedmaking for each patient.

To control airborne MRSA during bedmaking, it is first necessary to examine the materials of bedsheets and the techniques by which they are handled. Carpet-cleaning tape could be applied to the sheet, or the sheets could be made of an antiseptic fabric. A plastic apron must be worn to protect clothing during bedmaking, and hands should be washed afterwards. The numbers of MRSA on the floor and the bedsheet correlated with those in the air in the room. Therefore, careful disinfection of inanimate environments is also required to prevent airborne transmission of MRSA.

In conclusion, MRSA may be spread via respirable-sized aerosols, such as those generated by bedmaking. Inhalation of such particles is likely to play a role in MRSA colonization of the nares or in respiratory infection. In light of these findings, measures including continuing education on bedmaking for all medical and nursing staff should be taken to prevent airborne transmission.

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