Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene

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MRSA; Isolation rooms; Environment; Air; Decontamination

**Summary**  
Strategies to control and prevent the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) include early identification of positive patients through screening, patient isolation, hand hygiene, nasal and skin decontamination, and the adequate cleaning and decontamination of clinical areas. However, many national and other guidelines provide few details on environmental decontamination regimens, partly because the role of the environment in the spread of MRSA is not well documented. We prospectively studied the environment of the isolation rooms of 25 MRSA patients for up to four weeks, sampling horizontal surfaces and the air using settle plates as well as an air sampler, while continuing regular daily cleaning according to the hospital protocol. We then typed 20 patient isolates and the corresponding environmental isolates (N=35) to assess the similarity of strains. A high proportion of samples were positive for MRSA; 269/502 (53.6%) surface samples, 70/250 (28%) air samples and 102/251 (40.6%) settle plates. Over half of the surface samples taken from the beds and the mattresses were positive for MRSA. Identical or closely related isolates were recovered from the patient and their environment in 14 (70%) patients, suggesting possible environmental contamination of the isolation rooms, possibly contributing to endemic MRSA. More effective and rigorous use of current approaches to cleaning and decontamination is required as
Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a significant cause of mortality and morbidity in many hospitals throughout the world. Whilst there has been some controversy about the virulence of MRSA, the mortality is no less than that following infection with methicillin-susceptible S. aureus (MSSA). A recent comparison of bloodstream infection caused by MSSA and MRSA showed that the proportion of patients whose death was attributable to S. aureus infection was higher with MRSA than with MSSA, and that death due to disseminated infection was more frequent with MRSA.\(^1\) MRSA bloodstream infection is common in Ireland; the most recent published report of national data showed that 36% of S. aureus isolates recovered from blood cultures were MRSA in the Republic of Ireland.\(^2\) Consequently, the control and prevention of MRSA in Ireland and elsewhere is of considerable significance to the health service.

Recent guidelines on the control of MRSA and other antibiotic-resistant bacteria from North America and guidelines on MRSA from the UK include advice on the cleaning of the environment.\(^3,4\) In the North American guidelines, the section dealing with disinfecting hospital surfaces is relatively vague and does not provide details on frequency, agents to be used or methods.\(^3\) The UK guidelines refer to instruments or equipment that should be decontaminated and side rooms that should be cleaned after the discharge of patients according to the local disinfection policy, but are also vague on details.\(^4\)

There is increasing evidence that the environment may play a significant role in the spread of antibiotic-resistant organisms. Staphylococci, including MRSA, are known to survive in dry conditions and can persist in clinical areas that are inadequately cleaned. Most guidelines emphasize the importance of prudent antibiotic use, screening for high-risk patients, active surveillance and patient isolation, but there has been relatively little emphasis to date on the importance of the environment and ensuring that it is MRSA free.

In a report on the effectiveness of a purpose-built MRSA cohort unit, 60 environmental sites were screened before and after ward opening.\(^5\) After the unit opened, 5/60 and 12/60 sites were positive after six weeks and six months respectively, despite enhanced cleaning.\(^5\) Following a prolonged outbreak of MRSA that lasted for 21 months in another hospital, doubling the domestic cleaning hours led to a decrease in MRSA-positive environment samples from 32% to 0.5%.\(^6\)

It has been suggested that cleaning efficacy should be subjected to internal audit and to a more rigorous scientific assessment that would include not only visual inspection of clinical areas but also microbiological analysis for the presence of indicator organisms and total aerobic colony counts.\(^7\) With increasing concern amongst the public about the risks of acquiring infection in hospital and on the general state of cleanliness in many hospitals, environmental decontamination is an increasing priority. In a prospective study of patients placed in isolation because of MRSA, we surveyed the environment for the level of contamination following admission, and we assessed the similarity of MRSA isolates using pulsed-field gel electrophoresis (PFGE).

Materials and methods

Isolation room and environmental sampling

Beaumont Hospital is a tertiary referral centre for adult patients with national specialist units in neurosurgery and renal/pancreas transplantation. It has 720 beds and two intensive care units (ICUs). Each ward has approximately 30 beds with two to six single rooms. However, single rooms do not have any form of artificial ventilation, e.g. negative pressure ventilation, or anterooms for gloving and gowning. Many of these rooms also do not have ensuite facilities. Therefore, some patients have to leave their room for this purpose but many patients in single rooms are immobile and are unable to leave the room. The inclusion criteria for the study were non-ICU-hospitalized patients who were positive for MRSA on screening, and who had been in an isolation room for no more than 48 h.

Before the patient was placed in isolation, the single room was terminally decontaminated after the discharge of the previous occupant in each
case, using the routine hospital protocol. This consisted of a common detergent and hypochlorite solution (1:1000 ppm; Presept, UK) with particular attention being given to horizontal surfaces and dust-collecting areas. Mattresses, pillows and beds were washed with detergent. Members of staff were instructed on the preparation of the detergent and the hypochlorite, but this was not specifically audited. Bedding and curtains were laundered.

Patients were informed about the study and were given two explanatory leaflets, one about MRSA and the other outlining the nature of the study. No additional samples were taken from participating patients (all study samples were environmental), and all patients were happy to have their room sampled.

Samples were taken during the early morning before the regular daily cleaning took place, i.e. approximately 24 h since the room was last cleaned, twice weekly until the patient was transferred, discharged or died, for a maximum of four weeks. Thorough cleaning of the room and its equipment took place daily. Damp dusting using a detergent and hypochlorite solution (1:1000 ppm) was performed. Floors were vacuumed followed by mopping with hypochlorite. On each occasion when samples were obtained, six horizontal surfaces were sampled by applying a manitol salt agar contact plate using a sweeping motion, unless access to a particular surface was not possible. The surfaces sampled were the bed frame, the mattress, bed linen (using a sweeping motion), the bedside table, the chair and the window ledge. Bed linen consisted of cotton sheets and blankets, and covers were used on mattresses that were changed between patients. Three settle plates were left in the room (on the window ledge, on the bed table and on the floor) for 2 h. Three air samples (each 1000 L) were obtained using a surface air sampler (Super 90, UK).

Mannitol salt agar (Oxoid CM0085, UK) was used for all samples, and the agar plates were incubated at 35 °C for 48 h. We did not assess the level of environmental contamination due to other bacteria, such as enterococci. Presumptive MRSA isolates were confirmed by Gram stain, subculture on Mueller–Hinton agar (MHA, Oxoid CM0337, UK) for 24 h and then coagulase testing using the Staphytec Plus kit (Oxoid DR0850M, UK). Antibiotic susceptibility to methicillin was determined using a methicillin 5 μg disk (Oxoid CT0029, UK) on MHA for 24 h at 30 °C, according to British Society for Antimicrobial Chemotherapy guidelines. All MRSA isolates were then stored pending genotyping on nutrient agar slopes at 4 °C, and also on Protect Bacterial Beads (Technical Service Consultants Ltd, UK) at −85 °C.

**Genotyping**

PFGE was performed on the patient isolate cultured routinely in the diagnostic laboratory and, where possible, on two environmental strains recovered from the patient’s isolation room. When MRSA was isolated from the environment on more than two occasions, we chose an isolate recovered at the beginning and at the end of the patient’s period in isolation. It was not feasible to type all environmental isolates due to limited resources.

Agarose-gel-embedded chromosomal DNA plugs of patient and environmental isolates for PFGE analysis were prepared and digested with the enzyme SmaI (New England Biolabs, USA) using a modification of the ‘HARMONY’ protocol. Macrorestriction fragments were separated on a 1.0% Seakem® Gold agarose gel (Cambrex, USA) by electrophoresis on a CHEF DR 111 apparatus (Bio-Rad Laboratories, Richmond, CA, USA). S. aureus reference strain NCTC 8325 was used as a reference strain in duplicate on all gels analysed. Each patient isolate and its corresponding environmental isolates were placed in adjacent lanes to aid visual evaluation. The gels were stained for 45 min in ethidium bromide (1 μg/mL) and destained for 45 min in distilled water. Gels were viewed under ultraviolet transillumination and photographed.

The criteria as defined by Tenover et al. were used to analyse the PFGE patterns. Isolates with identical PFGE banding or profiles were considered to be indistinguishable, and isolates that had similar profiles (i.e. a three band difference or less) were defined as being clonally related and subtypes of each other. However, isolates that differed by more than three bands were considered to be different strains. Two individuals examined each gel independently and, in addition, each image was analysed using the Phoretix 1D advanced (v5.2) and 1D Database (v2.0) software packages (Phoretix International, Newcastle upon Tyne, UK) using a modification of the method described by Bennett et al. Briefly, the position of each band was interpreted as a binary code and entered into a spreadsheet (Microsoft Excel) by comparing its position with that of each and every other band in every other profile. The binary code for each isolate was then converted to a gel image by using the Scored Tiff (v1.0) software (Nonlinear Dynamics, Newcastle Upon Tyne, UK).
upon Tyne, UK), which was then read as an 'ALF' gel by the Phoenix ID advanced software (vr 5.01) to generate a dendogram.

**Results**

Isolates were collected from 25 patients with a mean age of 59.7 years, almost all of whom were on general surgical (13 patients) or general medical (11 patients) wards. All patients were in isolation for a week or longer. Sixteen of the patients were colonized but not infected, i.e. MRSA was recovered from nasal swabs taken during screening or from non-infected surgical sites, e.g. abdominal, neurosurgical. Nine patients had MRSA infections including two with bloodstream infection.

A single site (e.g. nose) was positive for MRSA in nine patients, two sites were positive in eight patients and three sites were positive in four patients. A skin swab or surgical site specimen was positive for MRSA in 13 patients (for 10 patients, this was the only site positive), and a nose swab was positive in eight patients (for five patients, this was the only site positive). There were no patients with underlying skin conditions such as eczema, and there was only one patient with cystic fibrosis.

The number of samples obtained, the proportion and percentage of samples positive for MRSA, and the week obtained are shown in Tables I and II. A high proportion of samples were positive: 269/502 (53.6%) surface samples, 70/250 (28%) air samples and 102/251 (40.6%) settle plates. The percentage of positive samples was higher in weeks 3 and 4 than weeks 1 and 2, but the number of samples taken during the latter two weeks of isolation were fewer as many patients had been transferred or discharged home by then. Over half of the surface samples taken from the beds and the mattresses were positive (Table I). A higher proportion of settle plate samples were positive for MRSA compared with air samples (Table II).

For five patients, there was only one corresponding environmental isolate because either the patient was in isolation for a short period of time, e.g. less than two weeks, or the environmental samples were not positive on more than one occasion. Although environmental samples were taken from the isolation rooms of 25 patients, PFGE could only be performed on 20 patient isolates and their corresponding environmental isolates because five patient isolates could not be recovered from stored stocks. Therefore, PFGE was performed on a total of 55 isolates, i.e. 20 patient isolates and 35 linked isolates recovered from the patient’s environment.

For 14 of the 20 (70%) linked patient and environmental isolates, identical or closely related isolates were found amongst at least two isolates in the patient and environmental subgroups tested. For seven of these patients (35% of the total), the environmental isolates were indistinguishable from the patient isolate. One paired patient and environmental collection of isolates had similar PFGE profiles and were therefore considered to be clonally related. Six paired isolates (30%) had more than three band differences and the isolates were therefore considered to be unrelated. Of the remaining six paired patient and environmental isolates, the two environmental isolates were

<table>
<thead>
<tr>
<th>Week</th>
<th>Bed</th>
<th>Mattress</th>
<th>Linen</th>
<th>Table</th>
<th>Chair</th>
<th>Window ledge</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25/42 (59.5)</td>
<td>22/42 (52.4)</td>
<td>18/42 (42.9)</td>
<td>28/42 (66.7)</td>
<td>24/41 (58.5)</td>
<td>16/42 (38.1)</td>
<td>133/251 (53)</td>
</tr>
<tr>
<td>2</td>
<td>11/25 (44)</td>
<td>11/25 (44)</td>
<td>11/25 (44)</td>
<td>16/25 (64)</td>
<td>11/25 (44)</td>
<td>8/25 (32)</td>
<td>68/150 (45.3)</td>
</tr>
<tr>
<td>3</td>
<td>6/10 (60)</td>
<td>7/10 (70)</td>
<td>4/9 (44.4)</td>
<td>6/10 (60)</td>
<td>6/10 (60)</td>
<td>5/10 (50)</td>
<td>34/59 (57.6)</td>
</tr>
<tr>
<td>4</td>
<td>7/7 (100)</td>
<td>5/7 (71.4)</td>
<td>4/7 (57.1)</td>
<td>6/7 (85.7)</td>
<td>5/7 (71.4)</td>
<td>7/7 (100)</td>
<td>34/42 (81)</td>
</tr>
</tbody>
</table>

*Sampling was carried out twice weekly where possible and the results from both sets of samples have been aggregated.*

<table>
<thead>
<tr>
<th>Week</th>
<th>Air samples</th>
<th>Settle plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38/124 (30.6)</td>
<td>51/125 (40.8)</td>
</tr>
<tr>
<td>2</td>
<td>13/75 (17.3)</td>
<td>23/75 (30.7)</td>
</tr>
<tr>
<td>3</td>
<td>12/30 (40)</td>
<td>16/30 (53.3)</td>
</tr>
<tr>
<td>4</td>
<td>7/21 (33.3)</td>
<td>12/21 (57.1)</td>
</tr>
</tbody>
</table>

*Sampling was carried out twice weekly where possible and the results from both sets were aggregated.*

*b Three samples (1 L) were obtained on each occasion.

*c Plates were placed on the window ledge, locker (bedside table) and floor for 2 h.*
identical but the patient’s isolate represented a different strain in three of the sets of paired isolates. For the other three sets of paired isolates, there were identical PFGE profiles for the patient and one of the two environmental isolates but the second environmental strain represented a different strain. Figure 1 shows a representative PFGE gel and a dendrogram.

**Discussion**

The objective of this study was to assess the degree of environmental contamination in isolation rooms with patients colonized or infected with MRSA. Over half of the surface samples, including those taken from beds and mattresses, were positive, and these strains were similar to those isolated from patients,
as determined by PFGE fingerprinting. This suggests that MRSA patients contaminate the environment. Although this does not confirm that the environment is the source of the patient’s MRSA, positive environmental sites represent a potential source for patients or even healthcare workers.

However, there are a number of limitations to our study which include the relatively small number of patients studied, the absence of sampling before the patient was placed in isolation to serve as a control (indistinguishable isolates may have been shed by a previous patient and persisted despite terminal cleaning, i.e. possible carryover of MRSA), and the possibility that other patients on the ward may have dispersed MRSA into the patient’s isolation room when the doors may have been open. We did not audit the cleaning/decontamination practices to confirm that this was done appropriately, and it is possible that other patients or staff who were MRSA carriers but who were not in isolation may have contaminated the isolation room environment with MRSA. Furthermore, the absence of ensuite toilet facilities in many of our isolation rooms means that mobile patients had to leave the room and might, in consequence, have brought back isolates of MRSA from other clinical areas. Finally, we only used PFGE to characterize our isolates because we were comparing epidemiologically related isolates and not determining definitive typing characteristics for each isolate.

Thirty-eight consecutive patients were studied in a similar study in a university affiliated teaching hospital in the USA, where the surfaces in the room were sampled once; 27% of surfaces were positive for MRSA.\textsuperscript{12} There was a higher degree of environmental contamination in rooms occupied by patients who were infected compared with those rooms occupied by patients who were colonized but not infected. Also in this study, environmental isolates were indistinguishable from paired patient isolates.\textsuperscript{12} However, air samples or settle plates were not included in their assessment of environmental contamination. Furthermore, surface samples were only taken on one occasion, unlike in this study where samples were taken up to twice weekly for four weeks.

Environmental sources or environmental reservoirs contributing to persistent MRSA have been described in the past. Such reservoirs include mattresses\textsuperscript{13} and nebulizers.\textsuperscript{14} In the case of an outbreak caused by contaminated nebulizers in the Netherlands, intense cleaning contributed to bringing the outbreak to an end.\textsuperscript{14}

A recent study from Germany that assessed the level of environmental contamination of the inanimate hospital environment found that samples were more likely to be positive for antibiotic-resistant Gram-positive bacteria, including MRSA, than antibiotic-resistant Gram-negative bacteria (25% vs 5%).\textsuperscript{15} The authors concluded that isolation of patients with MRSA should be a priority. The isolates from 12 patients and isolates from the environment of a long-term care facility were assessed using PFGE in a Japanese study.\textsuperscript{16} Forty of 90 (44%) environmental surfaces were contaminated with MRSA, and patient and environmental isolates were closely related. There was no correlation between environmental and patient MSSA isolates.

Over 25% of air samples and approximately 40% of settle plates were positive in our study, indicating the potential for airborne transmission as well as spread through direct contact. Shiomori \textit{et al.} carried out a sequence of air samples before, during and after bed making, and demonstrated that MRSA counts remained elevated for up to 15 min after bed making was complete.\textsuperscript{17} They argued that this may justify the need for some form of air ventilation and filtration to minimize airborne transmission. Interestingly, in the study reported here, 40-57% of samples taken from bed linen were MRSA positive.

The contribution of bed making to the shedding of MRSA from colonized or infected patients may also be exacerbated by inadequate ward or patient space. In an 18-month prospective survey that was carried out to examine the effect of adding a fifth bed to what was previously a four-bedded bay, the relative risk of colonization in five-bedded bays was 3.15 compared with the four-bedded bays.\textsuperscript{18} Clearly, where airborne transmission is possible, the greater the number of patients in a relatively small confined space, the greater the likelihood of increased colonization or infection.

Strategies to control and prevent MRSA have been reviewed recently.\textsuperscript{19} Whilst good infection control practices, such as compliance with hand hygiene recommendations, enhanced screening, patient isolation, decolonization and the prudent use of antibiotics, are all important strategies in the control and prevention of MRSA, there is increasing emphasis on the need for better environmental decontamination to prevent MRSA and other nosocomial pathogens. Bhalla \textit{et al.} showed that hands regularly acquire bacterial pathogens, including \textit{S. aureus}, MRSA and vancomycin-resistant enterococci (VRE), even after contact with decontaminated surfaces.\textsuperscript{20} Although effective hand hygiene may minimize this, patient areas need to be cleaned and decontaminated more effectively.

Conventional decontamination may be inadequate because the contact between surfaces...
MRSA in isolation rooms

and detergent or disinfectant is inadequate. Compared with conventional disinfection using a dampened cloth, a clean cloth dipped into a bucket containing disinfectant and then used immediately to drench all surfaces was found to be more effective at eradicating VRE; it took a second or third attempt at disinfection to render surfaces VRE negative using the conventional damp cloth alone. We also experienced difficulties in eradicating VRE from the environment and mattresses during an outbreak, despite enhanced cleaning regimens that used a conventional approach. Dancer has argued that we need a more rigorous and scientific approach to assessing hospital cleaning, and she advocates cleaning standards that include inspection and bacteriological sampling. Bacteriological standards would incorporate an assessment of whether certain ‘indicator’ organisms were present, including S. aureus, VRE, Gram-negative bacilli and Clostridium difficile.

Hospital patients are increasingly vulnerable to infection and many bacteria may persist for prolonged periods on inanimate surfaces; in the case of S. aureus, for up to 20 days. New technologies are emerging that may offer some alternatives in those areas of our hospital where either particularly vulnerable patients are housed or where MRSA and other nosocomial pathogens are endemic. French et al. have shown that after exposing six rooms to hydrogen peroxide vapour, only 1.2% of swabs yielded MRSA compared with 66% of swabs from rooms that were cleaned conventionally. This and other new approaches need to be assessed further, especially to determine if there is a corresponding reduction in the number of patients colonized and infected as well as a fall in environmental sites positive for MRSA. However, this is likely to be more expensive and will not replace conventional decontamination practices that are performed appropriately. The doors of isolation rooms also need to be kept closed whenever possible to minimize airborne spread.

In conclusion, we have shown that the environment of isolation rooms with patients who are colonized or infected with MRSA is often positive for MRSA, and that patient and environmental isolates are usually indistinguishable. Environmental reservoirs may therefore be a significant contribution to endemic MRSA, but larger prospective studies are needed to assess the correlation between environmental MRSA and the acquisition of MRSA by patients. Interventions to reduce potential environmental sources may then be shown to be effective in reducing the clinical burden of MRSA.

Acknowledgements

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References


