The effect of humidity on the survival of MRSA on hard surfaces

R&D Report B(03)02

2005

STATUS IN WALES

INFORMATION
R&D Project B(03)02: The effect of humidity on the survival of MRSA on hard surfaces
R&D Project B(03)02:
The effect of humidity on the survival of MRSA on hard surfaces

Project Leader: Dr Jillian Swan
Author(s): Catherine Makison
Dr Jillian Swan
Science Group: Health Sciences
Objectives

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been shown to have the ability to survive for long periods of time in healthcare environments. Adjusting the humidity of the environment may inhibit this effect and make it harder for MRSA to persist for long periods. The aim of this study was to measure the effect that different humidities potentially achievable on a hospital ward have on the survival of MRSA on hard surfaces typical of those used in hospitals.

Main findings

Of three relative humidities (RH) tested, EMRSA15 appeared to be most sensitive to an RH of 52%, with the exception of painted wood, where 42% was the most effective in reducing the level of viable bacteria. A higher RH of 65% had less effect on reducing bacterial numbers.

Overall, RH appeared to be less significant than surface type in reducing the numbers of CFU of MRSA.

Of the hard surfaces tested, the rate of decline in numbers of culturable bacteria over time was greatest on Formica and safety vinyl, and least on painted and varnished wood.

Recommendations

This study has shown that, under controlled experimental conditions, increasing the relative humidity to higher than is typical of a hospital ward could reduce the survival of MRSA on hard surfaces to a significant degree. However, in practice, the humidity of hospital environments continuously fluctuates and it would be difficult to maintain a set RH throughout. Previous studies have shown variation of RH between 24 and 47% at temperatures ranging from 20° to 22°C. There is not sufficient justification from these results to recommend humidity changes as a means of reducing MRSA survival in the hospital environment.

This investigation has revealed that surface type has a greater effect on the rate of reduction of MRSA than that of humidity. This complements other recent work where more porous materials with a greater capacity for retaining moisture preserved the greatest numbers of bacterial colonies. Contrary to this, previous work had indicated that survivability was better on glass than on wool or tile. It is therefore suggested that further research into different surface types may be necessary in order to reduce the numbers of bacteria on, in and around hospital furniture.

Further, different types of disinfectant/detergent appear to have different efficacies on different surface/material types. Therefore, investigating the efficacy of a number of different cleaning detergents, disinfectants and even other cleaning methods such as fumigation on various materials may aid the choice and development of more suitable materials, cleaning agents and methods for use in the hospital environment.

HSL has the facilities to carry out further investigations, for example on the types of surface that would be less susceptible to colonisation by MRSA and other microbial contaminants. We would also be able to provide information via experimentation of the efficacy of various disinfectants, detergents, fumigants and fumigation methods used in hospitals to aid the reduction in numbers of potential hospital acquired infections.

Executive summary
Contents

Executive summary

Chapter 1 Introduction
Aim

Chapter 2 Literature review
MRSA survival
Humidity
Other environmental factors
Resistance of MRSA
Hard surfaces
Summary

Chapter 3 Materials and methods
Controlled environments
Culture
Hard surfaces
Experimental set-up
Viability analysis
Scanning electron microscope (SEM)

Chapter 4 Results
Numbers of surviving MRSA over the study period
Percentage decline and variable relative humidity
Percentage decline and surface type
Statistical analysis of data; repeated measures ANOVA
Observation of surface types using scanning electron microscopy

Chapter 5 Discussion
Recommendations

Chapter 6 References

Appendix
Antimicrobial resistant microorganisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), pose a major challenge in hospitals today. The NHS Magazine reports that every year around 100,000 patients pick up infections in hospital, with a further 6000 dying as a direct result. Cases of MRSA, which infects open wounds and can prove fatal, increased from 67 reported cases in 1990 to 3110 in 1999. Recent data published by the National Audit Office (NAO) indicate that at any one time, 1 in 11 patients are affected by healthcare-acquired infection (HCAI) in England, and that this equates to a cost of approximately £1 billion each year for the HCAI cases treated by the National Health Service (NHS) (NAO, Feb 2000). Medical and para-medical hospital staffs are also at a higher risk of acquiring harmful infection at work than in many other occupations (Ross et al 1998). Occurrences of MRSA infection in healthcare settings are therefore a significant concern for patients, staff and the public.

Fears about MRSA have prompted the Department of Health (DH) to introduce a mandatory MRSA bacteraemia surveillance scheme, and between April 2001 and March 2002, 187 acute trusts participated in the first year of this scheme. The scheme will allow detailed analyses of MRSA bacteraemia rates and trends to be made, and should provide important information for those undertaking evaluations of intervention strategies.

Environmental conditions can affect the survival and persistence of hazardous microorganisms; for instance, humidity levels are known to influence the ability of some bacteria to persist in the indoor environment (Jawad et al 1996). Humidity in buildings can vary widely; the acceptable range quoted by the Department of Trade and Industry is 40–70% relative humidity (RH) (quoted from the CIBSE guide to ventilation and air-conditioning). There is very little information available on humidity levels likely to promote the long-term survival and persistence of MRSA in the indoor environment. MRSA has been shown to persist for long periods of time on surfaces and on furnishings in healthcare settings (Wagenvoort et al 2000), therefore measures that reduce its survival time on surfaces are worthy of consideration in the development of risk control strategies. Information on how humidity can affect the survival of MRSA on hard surfaces is extremely limited, and further data may offer further options for the control of this microorganism within the hospital environment. Low-cost adjustments to ventilation systems are capable of large-scale internal air quality effects. Modification of the indoor environment of healthcare facilities may be a cost-effective method of decreasing the risk of MRSA persistence. This study will investigate the possibility of reducing the ability of MRSA to persist on hard surfaces by adjusting the humidity of the environment.

**Aim**

To measure the effect that different humidities have on the survival of MRSA on hard surfaces. MRSA has been shown to have the ability to survive for long periods of time in healthcare environments. The study sought to establish whether adjusting the humidity of the environment inhibits this effect and makes it harder for MRSA to persist for long periods.
2 Literature review

A preliminary literature search was carried out in order to identify any recent developments in the field of research prior to undertaking practical work.

The literature search has revealed that there is very little information available on the effect of humidity on the survival of MRSA or Staphylococcus aureus (S. aureus) in general. However, it has been shown in numerous studies, including those by Wagenvoort et al (2000) and Jawad et al (1996), that MRSA can survive on dry surfaces for prolonged periods of time. It has been requested that this study use humidities between 40 and 60% relative humidity (RH), as this is the acceptable patient comfort range. Only two studies have looked at the effects of humidity on the survival of MRSA on hard surfaces, and both of these used humidities outside this range (Jawad et al 1996 and Kryswicka et al 1990).

**MRSA survival**

Wagenvoort et al (2000) investigated the survival of MRSA in a Dutch hospital. They made suspensions of MRSA with and without added hospital dust in sterile PBS. The samples were plugged with cotton wool to allow free communication with the hospital environment, temperature and humidity etc. The liquid had evaporated off completely by day 10. Relative humidity (RH) ranged between 24 and 47%, and temperature between 20° and 22°C. Surviving counts of MRSA were made by culture at two-weekly or longer intervals up to approximately one year. All strains survived longer than six months; two strains that had caused major hospital outbreaks survived significantly better and for 1–3 months longer. The survival patterns of MRSA strains with and without added dust were similar.

Jawad et al (1996) investigated the influence of humidity on the survival of Acinetobacter and S. aureus on dry surfaces. However, the humidity levels used here are outside the range that is acceptable for patient comfort. Bacterial cells were suspended in distilled water or bovine serum albumin (BSA) and dried onto glass coverslips and kept at different relative humidities. The survival time was prolonged when they were suspended in BSA rather than distilled water. The survival times of the strains at higher relative humidity (31% or 93%) were longer than those strains kept at 10% RH (11 days at 31% RH and 4 days at 10% RH). They concluded that Acinetobacter and S.aureus can survive on dry surfaces and be transferred not only by moist factors but also in dry conditions in the hospital environment during nosocomial infection outbreaks. Freshly isolated, clinically important Acinetobacter strains were found to survive longer than American type culture collection strains. Jawad et al also classed their S. aureus strain as “desiccation resistant” as it survived for longer than most of the other bacteria in the study. Strains of Acinetobacter isolated from the hospital environment or clinical materials generally survive better than laboratory culture collection strains that had been sub-cultured for some time in artificial medium.

Dietze et al (2001) investigated the survival of MRSA on sterile goods packaging, paper and foil. MRSA survived on the packaging for more than 38 weeks; no MRSA was recovered after 50 weeks.

**Humidity**

Jawad et al (1996) used controlled RH chambers (10% ± 1%, 31% ± 3% and 93% ± 3%) established and maintained using saturated salt solutions or dried silica gel. The chambers were maintained at a temperature of 22°C ± 2°C that is representative of most hospital environments.

Kryswicka et al (1990) investigated the survival of S. aureus at 37°C for 24 hours on glass, tile and woollen material at 95% and 35% RH. At 95% RH, ability to survive is reduced, but resistance to disinfectants was increased. Best survival was on glass (71% survived at 21°C at 35% RH, 38% at 95% RH). However, again these humidities are outside the range that it has been requested this study use.

Siau et al (1996) studied Acinetobacter in a hospital in Hong Kong and found that they did not observe seasonal variations in isolation and infection rates in an intensive care unit fitted with an air-conditioning system compared with the rates in the other wards without air-conditioning systems.
Other environmental factors

Often, bacteria are shed from the human body in droplets. Viability increases if the bacterial cells are dried in the presence of sugars, blood, serum or complex bacteriological media, and survival times increase if the dry cells are kept in the dark (Jawad et al. 1996). Cells are not typically found in isolation; they are usually associated with organic residues, especially in environments such as hospital settings. Beard-Pegler et al. (1988) investigated the survival of staphylococcal strains dried on cotton blanket material and stored in glass petri dishes at room temperature in the dark or light. They found no significant difference in survival between the strains in the dark and the strains in the natural light.

An ever-increasing demand for beds forces additional beds into bays. Respiratory tract droplets, desquamating skin scales, increased patient load, and decreased space for nursing and patient activities each aid in increasing the risk of cross-contamination and infection via airborne and contact routes (Kibbler et al. 1998).

Resistance of MRSA

*S. aureus* strains have been shown to have different abilities to survive in the environment. MRSA has been shown to be able to survive better than methicillin-sensitive strains of *S. aureus* (MSSA). Sasatsu et al. (1993) found that when 108 cells of each of five strains of MRSA and five strains of MSSA were inoculated onto absorbent cotton and incubated at 30% RH at 25°C, MSSA strains decreased significantly to well below those of MRSA after four weeks; MRSA strains remained above 10^3 after 14 weeks.

Hard surfaces

There is plenty of information in the literature on the hard surfaces that can become contaminated by MRSA in the hospital environment.

Oie and Kamiya (1998) studied ten hospital wards containing ten patients with endemic MRSA. They found no MRSA on the walls; however, 45% of the 67 environmental surfaces yielded MRSA. They found MRSA on 100% of the floors and 70% of the over-bed tables as well on the bedside rails (38%) and the room door handles (30%). In addition, Boyce et al. (1997) established that over the bed tables are frequently contaminated with MRSA.

MRSA on the floor can be re-circulated by air turbulence. Patient-related items can fall onto floors. Patients may have increased contact with the floors through falling, crawling, bare feet back into bed etc (Dancer 2004).

French et al. (2004) carried out a study in surgical wards of a London teaching hospital affected by MRSA. MRSA contamination, measured by surface swabbing, was compared before and after cleaning. All isolation rooms, ward bays and bathrooms tested were contaminated with MRSA. MRSA was isolated from areas used by non-MRSA patients. 74% of 359 swabs taken before cleaning yielded MRSA. After cleaning, all areas remained contaminated; 66% of 124 swabs yielded MRSA. The hospital environment can become extensively contaminated with MRSA that is not eliminated by standard cleaning methods.

Fujii et al. (1996) investigated the distribution of MRSA on and around six patients with MRSA infections. High counts of MRSA were detected on horizontal planes such as floors, side tables and chairs, but MRSA was not detected on vertical planes such as walls.

Lemmen et al. (2004) investigated the distribution of multi-resistant bacteria within the hospital environment. They demonstrated that the extent of contamination with multi-resistant Gram-positive organisms was far more widespread than that of Gram-negative pathogens. They confirmed that the extent of contamination of the inanimate environment with MRSA was sufficient to contaminate the gloves of personnel who had contact with the inanimate environment of the patient’s room, but had no direct contact with the patient. Their data also established that patients colonised with multi-resistant Gram-positive bacteria frequently contaminate the inanimate environment and, therefore, surfaces and objects may serve as secondary reservoirs for cross-contamination.

Oie et al. (2005) investigated hospital environmental surfaces of a dermatological ward contaminated with MRSA and MSSA before and after disinfection. It was found that levels of contamination of between 100 and 105 colony-forming units (CFU) of MRSA or MSSA existed on a number of surfaces prior to disinfection. After disinfection, no *S. aureus* was detected on smooth surfaces such as immersion bathtubs and foot washbowls. *S. aureus* was, however, detected after disinfection on porous surfaces such as stretchers for immersion baths and shower chairs. Scanning electron microscopy of these surfaces identified large concentrations of coccus and bacillus biofilms on the walls of pores of the porous materials.
Summary

- MRSA has the ability to survive for months after drying onto hard surfaces.
- The survival period can be affected by humidity, but no information is available on the effects of humidities between 40 and 60% RH.
- Saturated salt solutions can be used to control the humidity during studies on the survival of MRSA.
- There is some evidence that light/dark conditions have limited effect.
- Suspension of the bacteria in a salt solution or serum first increases the survival period and also mimics the condition of bacteria released in droplets of bodily fluids from patients.
- MRSA cannot be replaced by MSSA in these studies, as MRSA has been shown to survive better than MSSA.
- MRSA is frequently found on hard horizontal surfaces in hospitals as well as soft furnishings and hand-touch areas such as door handles.
3 Materials and methods

The materials and methods we used in this study were selected using information from the literature review and after discussions with NHS Estates.

**Controlled environments**

The controlled environments were set up at a constant "room" temperature of 23°C; this has been selected as representative of the temperature on wards containing patients who may be vulnerable to MRSA infection such as the elderly and babies. The humidity levels used were as close as possible to the normally acceptable range of 40–60% humidity recommended in Heath Technical Memorandum 2025 – ‘Ventilation in healthcare premises’.

Humidity was maintained using saturated aqueous solutions in contact with an excess of the solutes (Merck Index, Merck and Co Inc, Rahway, New Jersey, USA) – see Table 1. Conditions in each environment were monitored throughout the experiment using a calibrated humidity and temperature probe.

**Table 1 Humidity levels maintained using saturated salt solutions**

<table>
<thead>
<tr>
<th>Target humidity level (± 3%)</th>
<th>Saturated salt solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>42%</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>52%</td>
<td>Magnesium nitrate</td>
</tr>
<tr>
<td>65%</td>
<td>Ammonium nitrate</td>
</tr>
</tbody>
</table>

**Culture**

A control culture of MRSA was obtained from the Health Protection Agency. *S. aureus* strains have been shown to have different abilities to survive in the environment. MRSA has been shown to be able to survive better than methicillin-sensitive strains of *S. aureus* (MSSA; Sasatsu et al 1993). We selected MRSA strain type 15 to use in this study, as it is a common cause of HCAI in the UK.

**Hard surfaces**

There have been several studies into the survival of MRSA on hard surfaces and the contamination with MRSA of surfaces in hospitals; from these we have selected the following hard surfaces to be used in this investigation:

- painted wood;
- varnished wood;
- Formica;
- standard vinyl flooring;
- safety vinyl flooring.

**Experimental set-up**

A nutrient broth was inoculated with EMRSA15 and incubated in a shaking incubator at 37°C for 18 hours to allow exponential growth to be established. The broth culture was then removed from the incubator, poured into a 50 ml Falcon tube and centrifuged at 4500 rpm for 3 minutes. The supernatant was removed and the cells were re-suspended in 30 ml of PBS (phosphate buffered saline) containing 1% BSA (bovine serum albumin). The number of cells contained within the suspension was then determined via haemocytometer. The cell suspension was then altered accordingly to ensure a cell concentration of $5 \times 10^6 – 5 \times 10^7$ cells/ml.

Each surface type was cut into 315 tiles measuring 1 cm², allocating 105 tile samples to each of the three humidity-controlled environments. 105 tile samples of each surface type were inoculated with 100 µl of EMRSA15-PBS/BSA cell suspension. The tiles were then incubated at 23°C and 42% relative humidity (RH). This was repeated for incubation at 52% and 65% RH. Note that samples of the painted wood surface type were set up one week earlier to ensure that experimental conditions were optimum before repeating the tests with the other four surface types.

**Viability analysis**

At each sampling time, three tiles of each surface type were removed from all three humidity-controlled environments. Each tile sample was placed in a 15 ml Falcon tube containing 10 ml of ¼ Ringers reagent and vortex mixed for 30 seconds to dislodge the cells from the
surface of the tile. To obtain countable colonies of between 30 and 300 CFU (colony forming units), the cell suspensions were diluted with ¼ Ringers reagent accordingly before spreading 100 µl of the final suspensions onto nutrient agar plates. These plates were then incubated over night at 37°C. Subsequently, the numbers of CFU per plate were counted and CFU/ml (from the 10 ml suspension) calculated, taking into consideration any dilution factors that were made. Samples were taken on a daily basis for the first two weeks, after which samples were taken once a week for eight weeks and subsequently every two weeks.

Due to depleting cell numbers, tile samples were re-suspended in a reduced volume of 5 ml of ¼ Ringers reagent at day 43 (50 for painted wood samples). At day 78 (85 for painted wood) this was further reduced to 3 ml. These suspension concentrations were necessary to achieve countable colonies during the latter experimental testing and were taken into account when calculating the CFU/ml.

**Scanning Electron Microscope (SEM)**

Based on the results emerging from survival on different surfaces, additional work was done to observe directly the different materials tested. A tile sample of each of the surface types was coated with gold and viewed under the SEM to determine microscopic variations between them and, therefore, offer reasoning behind any differences found with cell culture experiments. Interaction between bacteria and the surfaces was also examined.
4 Results

Numbers of surviving MRSA over the study period

Total numbers of EMRSA15 retrieved from tiles at each sample time are summarised graphically in the Appendix, showing decline in numbers with time for each humidity and surface type tested. From starting inoculums of 10^7 colony forming units (CFU) per tile, numbers generally showed a relatively steep decline before levelling off. On Formica and standard vinyl, numbers were reduced to 10^3 CFU by about 50–100 days, and to 102 by 140 days (Appendix, Figures 14 and 15). On varnished wood, numbers were reduced to 10^3 CFU by about 40–60 days, and to 102 by 140 days (Appendix, Figure 15). On painted wood, numbers were reduced to 10^3 CFU by about 50 days, and to 102 by 140 days at 42% RH and by 90 days at other RH (Appendix, Figure 17). On safety vinyl, numbers were reduced to 10^3 CFU by about 35 days, and to 102 by 60 days (Appendix, Figure 16).

Percentage decline and variable relative humidity

The collected data have been displayed in a graphical format illustrating the percentage decline in CFU for each type of surface and humidity. The graphs themselves are bar charts with a scatter graph of the same data superimposed. This was done to demonstrate the flow of reduction in CFU.

Figure 1 shows the percentage decline in CFU on a Formica surface. The graphical data illustrates a rapid decline by day three at 42% and 52% RH (relative humidity). CFU continued to decrease thereafter at a much slower rate. The numbers of CFU also declined at 65% RH, but at a slower rate compared to samples incubated at 42% and 52% RH. Overall, on a Formica surface EMRSA15 was most susceptible to a loss of viability at relative humidity of 52%, and most persistent to 65% RH.

Figure 2 represents the percentage decline of CFU on a standard vinyl surface. On this graph, standard deviation bars have not been added to day three or week three, as they exceeded the scale. The standard deviation at day three was 129.90% and 45.72% at week three. These two data points are therefore anomalous, and if they were removed, incubation at 65% RH would be more efficient in decreasing the numbers of CFU on this surface type than at 42% RH. As it stands, 52% RH proved to be most effective at reducing the numbers of EMRSA15.

Figure 3 indicates the percentage reduction in CFU on a varnished wood surface. These data present a slower decline in CFU over time compared with that of Formica and standard vinyl. Again, large deviation at day two with incubation at 42% RH indicates an anomalous data point; however, even excluding this data point, CFU depletion at 42% RH does not drop significantly until week two. As with Formica and standard vinyl, 52% RH appears to reduce CFU numbers most effectively, followed by 65% and 42% respectively.

Figure 4 presents the percentage decline in CFU on safety vinyl. Numbers of CFU are reduced to below 25% of the starting total by week two, as was also observed with the previous surface types. Again, EMRSA15 CFU was most susceptible to a relative humidity of 52%.

Figure 5 shows the percentage decline in CFU on a painted wood surface. The data indicate a much slower decrease in CFU numbers over time compared with all of the above surface types. In addition, it also contradicts the 52% RH efficacy trend observed with the other surface types. Rather, EMRSA15 appears to be marginally more susceptible to incubation at 42% RH than incubation at 52% or 65%. However, remaining CFU were depleted to below 20% of the starting total by week three.
R&D Project B(03)02: The effect of humidity on the survival of MRSA on hard surfaces

**Figure 1** Percentage decline in CFU on Formica

**Figure 2** Percentage decline in CFU on standard vinyl
Figure 3  Percentage decline in CFU on varnished wood

Figure 4  Percentage decline in CFU on safety vinyl
Figure 5 Percentage decline in CFU on painted wood

Figure 6 represents the percentage decline in CFU of all surface types at 42% RH. EMRSA15 proved most resistant to painted wood, closely followed by varnished wood. It declined in numbers quickest on the surface of Formica and safety vinyl, closely followed by standard vinyl.

Figure 7 indicates the percentage decline in CFU of the five surfaces at 52% RH. EMRSA15 was most sensitive to Formica and safety vinyl. Painted wood showed the slowest decline in CFU, closely followed by varnished wood.

Figure 8 shows the percentage decline in CFU of the five surfaces at 65% RH. EMRSA15 was most sensitive to Formica and safety vinyl. Painted wood showed the slowest decline in CFU, closely followed by varnished wood.

Percentage decline and surface type

Figure 6 depicts the percentage decline in CFU for each of the five surfaces at 42% RH. At this humidity, varnished wood retained the highest number of CFU over the time period. Safety vinyl and painted wood retained similar CFU numbers, which depleted faster than that of varnished wood. The number of CFU on standard vinyl dropped steadily over time at a faster rate than each of the above surfaces. Numbers of EMRSA15 declined quickest on Formica at this humidity.
In order to determine significant differences in the decline of CFU over time between 42%, 52% and 65% RH, the decline of CFU over time between the five surface types, and the significance of each humidity and surface type collectively, a repeated measures ANOVA (analysis of variance) was carried out using SPSS statistics package version 13.

The applicable results from this analysis can be observed in Table 2. In order to accept the null hypothesis (that is, treatments have no effect on the depletion of CFU over time), the significance (Sig) requires a value of 0.05 or
greater. In addition, the F-value or variation ratio is required to be 0.05 or below in order to accept the null hypothesis. As the significance values for differences in the rates of decrease as a result of humidity, surface type, and humidity and surface type combined are all below 0.05, the null hypothesis can be rejected.

The extent of the significance between each treatment can be examined through the F-value. The F-value calculated by the statistics programme can be calculated manually using the following formula:

\[
F = \frac{\text{Between Samples Variance}}{\text{Within Samples Variance}}
\]

The F-value for differences in the decline of CFU over time due to humidity is 11.493, which is greatly above the required 0.05, suggesting that variable humidity, in this case mainly 52% RH, has a significant impact on MRSA survivability. This value is, however, much lower than the F-value of 44.954 observed with the type of surface sample used. This indicates that the type of surface is more important in reducing MRSA viability over long periods than the humidity of the environment. Humidity and surface type combined have little significance in comparison to humidity and surface type alone with an F-value of 2.575.

It should be noted that due to a large volume of sample types being analysed at any one time point, the number of replications was limited to \( n = 3 \). Although this represents a minimum for statistical analysis, this generally causes greater deviation than would usually be seen where \( n = \geq 6 \). As such, the results of statistical analyses may appear significant to a greater extent than would be seen with increased experimental replication. For a truer statistical outcome, further experimental data should be obtained.

### Observation of surface types using scanning electron microscopy

Figure 9 illustrates the five different surface tile samples. At the macroscopic level, both Formica and standard vinyl had very smooth surfaces. Painted wood appeared slightly rougher on the surface than varnished wood. Safety vinyl had the roughest plane, with crystalline structures protruding from the surface.

These surfaces were observed more closely using scanning electron microscopy (Figure 10). Each surface was examined at 300× magnification. Painted and varnished wood looked very similar, as might be expected. Both these surfaces look reasonably smooth, but have many small, spot projections throughout the surface. Standard vinyl looks moderately smooth, although widely distributed flaky regions were clearly seen. As with the wood samples, standard vinyl had small protrusions over its surface. The Formica sample appeared very fibrous throughout the surface. Safety vinyl was very smooth over the entire surface of the sample and had large crystalline structures extending out from the surface.

Figure 11 exemplifies a sample that has been inoculated with a suspension of EMRSA15 and PBS/1% BSA (phosphate buffered saline/1% bovine serum albumin). This sample had been incubated at 65% RH for 10 weeks. The 100× photograph illustrates how serum/albumin has broken up like an eggshell and has even started to peel away from the sample surface.

Figure 12 takes a closer look (500×) at the flakes of serum/albumin and the adhered cells. Here it can be seen that MRSA cells aggregate not only on the surface of the serum/albumin flakes, but on the sides as well. It was not possible to determine whether cells aggregate on the undersides of the flakes.

At magnification of 3000× a single EMRSA15 cell measuring approximately 4 µm in diameter can be noted (Figure 13).

### Table 2 Summary of statistical treatment of data

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1157.125</td>
<td>1</td>
<td>1157.125</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>Humidity</td>
<td>27017161387.631</td>
<td>2</td>
<td>13508580693.816</td>
<td>11.493</td>
<td>.000</td>
</tr>
<tr>
<td>SurfaceType</td>
<td>52839748279.557</td>
<td>4</td>
<td>52839748279.557</td>
<td>44.954</td>
<td>.000</td>
</tr>
<tr>
<td>Humidity * SurfaceType</td>
<td>24209177592.883</td>
<td>8</td>
<td>3026147199.111</td>
<td>2.575</td>
<td>.029</td>
</tr>
<tr>
<td>Error</td>
<td>352626957974.865</td>
<td>30</td>
<td>1175423199.162</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 9

Painted wood  Standard vinyl  Varnished wood

Safety vinyl  Formica

Figure 10

Painted wood surface 300 x  Standard vinyl surface 300 x  Varnished wood surface 300 x

Safety vinyl surface 300 x  Formica surface 300 x
Figure 11  Cells present on flakes of BSA at magnification 100×

Colonies of MRSA on BSA surface

Flakes of bovine serum albumin (BSA)

Figure 12  Cells around the edges of the BSA flakes at magnification 500×

MRSA on the edge of BSA flakes
Figure 13  A single cell on the surface of BSA at magnification 3000×
Discussion

This study has provided valuable data on the survival of methicillin-resistant Staphylococcus aureus (MRSA) bacteria on hard surfaces typical in a hospital environment. It is important to note the length of time that significant numbers of the bacteria could survive. Although long-term survival of live bacteria represented only a small percentage of the 10 million cells used per tile as a typical initial inoculum, as many as 1000 bacteria remained culturable for at least five weeks after, and as long as 14 weeks irrespective of the type of surface and humidity. In most treatments up to 100 of those bacteria remained culturable for at least 20 weeks after inoculation. This emphasises the challenge posed to hospitals in trying to deal with MRSA contamination.

On each of the five hard surface types studied, the numbers of culturable bacteria had declined to 20% or below the original inocula after two to three weeks’ incubation. EMRSA15, the strain of MRSA chosen for the experiments as the most commonly encountered in UK hospitals (personal communication, City Hospital Infection Control Research Lab), appeared to be most sensitive to a relative humidity of 52% for most of the hard surfaces tested. The exception was painted wood, where 42% was the most effective. The highest RH tested, 65%, proved to be least efficient for reducing bacterial viability, but in many cases this was closely followed by 42% (52% for painted wood). However, statistical analysis of the data has shown that relative humidity appears to be less significant than surface type in reducing the numbers of CFU of MRSA. Indeed, the statistics demonstrate that the type of surface is much more relevant in controlling the rate of decline of MRSA.

The rate of decline in numbers of CFU was greatest on Formica and safety vinyl and slowest on painted and varnished wood. Observation of the surface materials by scanning electron microscopy (SEM) provides a possible explanation. Safety vinyl appears at the macroscopic scale to be a very rough surface but is in fact very smooth when viewed under the SEM. Potentially, this provides less protection for the cells because they are less capable of becoming embedded in the material and, therefore, more open to dessication. This in turn may have caused the decrease in CFU to be more rapid than on rougher surfaces such as painted and varnished wood. In contrast, Formica, on which MRSA numbers declined as quickly as on safety vinyl, looked very smooth to the naked eye but had an especially fibrous texture when examined under SEM. However, this texture may have limited the ability of the cells to be embedded in the surface. Although painted and varnished wood and standard vinyl do not appear as smooth as safety vinyl under SEM they do not appear to provide as much refuge for the bacteria as the Formica surface. The results of our study complement recent work (Oie et al 2005) where more porous materials with a greater capacity for retaining moisture preserved the greatest numbers of bacterial colonies. By contrast, earlier work (Kryzwicka et al 1990) indicated that percentage survivability was better on glass than on wool or tile.

The physical contours of the hard surfaces may be one of a number of factors affecting adhesion and survival of bacteria, and it may be an important consideration for future work to control survival of MRSA on hospital surfaces. Also likely to have an influence is the ability of the biological matrices (for example body fluids) containing bacteria to adhere to the surfaces. The SEM images clearly demonstrated the aggregation of the bacterial cells within the bovine serum albumin in which the cells were suspended to inoculate the tiles. Cells were not only dispersed throughout the dried flakes of BSA, but also, as the drying process occurred, they aggregated at the edges, and this could influence cell survival and adhesion. From a practical point of view, dried material on hard surfaces may be relatively easily removed (for example by wiping) at the edges where it is becoming detached from the surface, but may be less easy to remove where the dried liquid is bound to the surface. In addition, the biological matrix increases bacterial survival (Jawad et al 1996), so a surface type that allows for greater adhesion of biologically derived liquids is also more likely to enhance surviving numbers of bacteria. Resins, glues and various other chemicals used in the manufacture of man-made materials may adversely affect bacterial survival, as may coatings applied to natural materials. Further work is recommended to explore these factors.
The results from this study suggest important practical considerations for the hospital environment. At the outset, the evidence from previous studies was that changes in relative humidity affected MRSA survival. Extremely low and extremely high RH have both been shown to reduce survival, but these extremes were outside the conditions that would have practical application from a patient and staff comfort viewpoint in the hospital ward. If modest changes to ward humidity could achieve reduction in MRSA survival, this would have been a practical measure that could be applied by building management at reasonable cost. However, the data suggested that although some changes in the rate of survival could be achieved, it probably does not justify changes to building management. Of greater significance was the effect of hard surface materials on MRSA survival. Consideration given to the material types used within hospital wards at the design/building/refurbishment stage could significantly affect the ability to control MRSA at minimal cost. There is justification in further work to examine the influence of hard surface materials on MRSA survival, incorporating a wider variety of construction materials.

**Recommendations**

- This study has shown that a 52% relative humidity increases the rate of decline of EMRSA15 on all surface types with the exception of painted wood, where 42% RH was most effective. However, only a limited number of replicates could practicably be used within the experimental design, therefore the statistical data may have exaggerated the significance of variable data sets. Further experimental replications would be necessary in order to obtain a truer representation of the differences in the rate of decline of EMRSA15 between the relative humidity ranges used in this project.

- The humidity of hospital environments continuously fluctuates, and it would be difficult to maintain a set relative humidity throughout. For example, Wagenvoort et al (2000) showed variation of relative humidity between 24% and 47% at temperatures ranging from 20 to 22°C. Taking this into consideration, changing the humidity in a hospital ward is probably not a justifiable practical option for MRSA control. This investigation has revealed that surface type has a greater effect on the rate of reduction of MRSA than humidity. This complements recent work (Oie et al 2005) where more porous materials with a greater capacity for retaining moisture preserved the greatest numbers of bacterial colonies, but by contrast, earlier work (Kryzwicka et al 1990) indicated that percentage survivability was better on glass than on wool or tile. It is therefore suggested that further research into different surface types may be necessary in order to reduce the numbers of bacteria on, in and around hospital furniture. Other materials used in the manufacture and preservation of hospital surfaces and furniture may also need to be studied as they may have antimicrobial properties or, conversely, properties that aid microbial survival. The choice of which materials to study might logically relate to those materials most associated with the highest levels of skin contact from staff and patients.

- Further, different types of disinfectant and other cleaning agent appear to have different efficacies on different surface/material types (Oie et al 2005; Kryzwicka et al 1990). Therefore, investigating the compatibility between different cleaning agents/disinfectants and the surfaces on which they are used may aid the choice and development of more suitable materials for use in the hospital environment.
6 References


Appendix

Figure 14  Differentiation in the decline of EMRSA15 at three different humidity-controlled environments (RH) on a Formica surface

Figure 15  Differentiation in the decline of EMRSA15 at three different humidity-controlled environments (RH) on a standard vinyl surface
Figure 16  Differentiation in the decline of EMRSA15 at three different humidity-controlled environments (RH) on a varnished wood surface

Figure 17  Differentiation in the decline of EMRSA15 at three different humidity-controlled environments (RH) on a safety vinyl surface
Figure 18  Differentiation in the decline of EMRSA15 at three different humidity-controlled environments (RH) on a painted wood surface

Figure 19  Variations in cell number between surface types at 42% relative humidity
Figure 20  Variations in cell number between surface types at 52% relative humidity

Figure 21  Variations in cell number between surface types at 65% relative humidity