Better environmental survival of outbreak vs. sporadic MRSA isolates


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Summary: Environmental sources have been associated with prolonged epidemics of methicillin-resistant Staphylococcus aureus (MRSA). Our objective was to evaluate whether outbreak MRSA strains differ in their environmental survival from sporadic MRSA strains. The strains were detected in five adult patients unknowingly colonized by MRSA. The MRSA strains from patients No. 1 (phage pattern: III-29) and No. 2 (III-215) caused extensive outbreaks in our hospital. Contact tracing after detection of the different MRSA strains in the three other patients did not reveal further spread.

Suspensions of MRSA with and without added hospital dust were made in sterile PBS. Surviving counts of MRSA were made by culture at two-weekly or longer intervals up to approximately one year. For each MRSA strain the ultimate day of viability was ascertained. The environmental survival patterns of the five MRSA strains showed qualitative and quantitative differences between the two outbreak and three sporadic strains. A gradual decline was noted for all strains. All survived longer than 6 months, but the two outbreak strains survived significantly better (P < 0.01), i.e. in higher quantities (circa 1000-fold) and for a 1–3 months longer period. The survival patterns of the MRSA strains with and without added dust were similar.

Keywords: MRSA; environmental survival.

Introduction

The association between the dispersal of MRSA strains into the hospital environment by colonized patients and MRSA epidemics has been increasingly reported.1–5 The possibility that strains of MRSA might differ in their survival characteristics in inanimate surroundings prompted us to study the long-term survival of different strains isolated from patients in our hospital. In a pilot study the epidemic MRSA strain of phage pattern III-29 survived under ambient hospital conditions for about half a year.6 MRSA strains of phage group III are the most frequent epidemic strains imported into the Netherlands from other European countries. In our hospital, two extensive epidemics with strains of this phage group have occurred on our intensive care unit (ICU) in recent years, while three other MRSA strains detected on patients during hospitalization did not display such behaviour. The objective of the work described here was to assess whether epidemic (outbreak) MRSA strains show different behaviour in survival kinetics to non-epidemic (sporadic) strains.

Patients and epidemiology

MRSA strains 1–5 were isolated from five adult patients unknowingly colonized by MRSA and admitted without infection control precautions. The strains from patients 1 (phage pattern III-29) and 2 (phage pattern III-215) caused extensive epidemics in our ICU and surgical wards, with 32 and 14 patients involved, respectively, in 1992–1993 and
1997. Contact tracing after detection of MRSA in patient 3, who was staying on the ICU (untypable), 4 (phage pattern Z-69) and 5 (phage pattern III-152) – the latter two patients staying on surgical wards – did not reveal any further spread. The strains of patients 1, 4 and 5 originated in Germany, 2 in Belgium and 3 in the former Yugoslavia.

Material and methods

The influence of desiccation on survival of the different MRSA strains was compared. Suspensions containing approximately $10^9$ colony-forming units (cfu) per ml were made in sterile phosphate-buffered saline (PBS; pH 7.2). Hospital dust was collected during cleaning sessions on the ICU and wards. Suspensions of the test bacteria were made with and without dust at a final concentration of 0.1% dust in 100 ml screw-top bottles. Samples of each suspension (1 ml) were transferred to similar bottles and allowed to dry. All bottles were plugged with cotton-wool to allow free communication with the hospital environment in air with indirect light, conventional temperature and relative humidity. The fluid component of this suspension had completely evaporated after 10 days and sampling was begun 4 days later, i.e. 14 days after the suspensions had been prepared.

Survival was measured at 2-weekly intervals. The observations with the last three bottles of each set were prolonged according to predicted decay rates and thus after day 225 cultured with longer and variable intervals of approximately 4, 8 (days 256, 287) and in one episode (strains no. 1 and 2) of 12 weeks (day 318). The scaling in Figure 1 shows different time periods after day 225 on the horizontal axis, indicated by an interruption bar.

The effect of desiccation was studied by recovering remaining viable bacteria with the addition of 1 ml of PBS to the bottle. After vigorous vortexing, the suspension was flooded on to a blood agar plate and incubated for 48 h at 37°C. Because of the possibility that clumps of 2–4 S. aureus bacteria may be counted as a single organism,$^7$ colony counts were rounded up to the nearest first decimal place on the log$_{10}$ scale. For all strains, survival counts were made until extinction. Death of the last viable bacteria lay somewhere between this time and the nearest previous time yielding growth. Thus in the absence of a precise determination, the latter point was taken as the end point and defined as the last measured day of viability (DOV) (Table I). Differences in the survival curves were evaluated with the Mann–Whitney–$U$ test.

Results

The relative humidity of the ambient air during the study period showed a range of between 24 and 47%
and temperatures between 20 and 22°C. As shown in Figure 1 (results with dust not shown) and Table I, the environmental survival patterns of the five MRSA strains showed qualitative and quantitative differences between the two outbreak and three sporadic MRSA strains. The two additional time measurement points for strains nos. 3, 4 and 5 at days 256 and 287 are shown in Table I, thus allowing a more precise DOV estimation for these strains. The differences between the DOV endpoint values of the outbreak strains no. 1 and 2 vary between 31 and 107 days. A gradual decline was noted for all strains, but in our experiments the two outbreak strains survived in significantly greater numbers (up to approximately 1000-fold more, \( P < 0.001 \)) and according to the DOV values in Table I approximately one to circa 3 months longer. The survival patterns of the MRSA strains were similar with and without dust. Differences noted between the same strains with or without dust added were not significant (\( P > 0.05 \)).

**Discussion**

The findings that all MRSA strains die off slowly, but that outbreak MRSA strains survive significantly longer and in higher quantities than sporadic strains are likely to be relevant to the clinical epidemiology of such strains, as we experienced ourselves.\(^1\) The environmental behaviour of MRSAs has also drawn attention to the survival characteristics of other persistent hospital pathogens that may contaminate the environment of the patient, such as *Acinetobacter* spp. and *Enterococcus* spp.\(^8\)

One may speculate about reasons for the longer survival of the III-29 strain compared to our pilot study.\(^6\) This may possibly be due to a higher initial inoculum and/or a different influence of western vs. now northern light in a different room. Also the difference in composition and the lower concentration of the dust added gave different results and may reflect the absence of possible ‘toxic’ influences as noted elsewhere.\(^6\) However, it is clearly not possible to draw firm general conclusions from observation of five strains.

The different survival patterns of *S. aureus* under variation of experimental settings such as temperature, light and other circumstances, and the occasionally great variation in survival patterns of *S. aureus* of different origins, have been described by others and summarized elsewhere.\(^6\) Dispersion that results in widespread environmental contamination\(^2,3\) and enhanced strain survival are important prerequisites for persistence in the hospital. The success of such persisting strains can be summarized by Lord Kitchener’s statement during World War I: ‘the outcome of the struggle will be determined by the nation, who has the last remaining million soldiers in the field’.\(^9\)

To deal with organisms that persist in the hospital environment awaiting the opportunity for renewed dissemination and colonization of susceptible patients, standards of hospital cleanliness must be kept at a high level.\(^10\) In a local questionnaire in which patient’s satisfaction with their hospital stay was evaluated, about one-third of patients suggested that cleaning of patients’ rooms could be improved. Hospital hygiene has to make use of simple basic practices as well as of more sophisticated modern methods.

**Table I** Ultimate measured days of viability (DOV) with regard to the environmental survival of two outbreak (III-29, III-215) and three sporadic (UT, Z-69, III-152) MRSA strains with and without added dust.

<table>
<thead>
<tr>
<th>MRSA strain no. phage pattern</th>
<th>Without dust DOV</th>
<th>With dust DOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. III-29</td>
<td>318</td>
<td>318</td>
</tr>
<tr>
<td>2. III-215</td>
<td>318</td>
<td>318</td>
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<tr>
<td>3. UT</td>
<td>287</td>
<td>256</td>
</tr>
<tr>
<td>4. Z-69</td>
<td>225</td>
<td>211</td>
</tr>
<tr>
<td>5. III-152</td>
<td>256</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not done

References


