

Observations on the resistance to drying of staphylococcal strains

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Summary. Death rates have been determined for staphylococcal strains dried on cotton blanket material and stored at room temperature in the dark and in the light. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains that produced a golden pigment and had a wide distribution within the hospital survived for longer periods than MRSA strains that produced little pigment and had a restricted local distribution. Death rates of methicillin-sensitive strains of *S. aureus* at day 7 were similar to those of the general epidemic MRSA strains, and there was no significant difference between the death rates at day 7 of the local epidemic MRSA strains and the coagulase-negative strains.

Introduction

Strains of staphylococci can survive for varying periods in samples of dust. Lidwell and Lowbury (1950) reported that the daily death rate of strains of *Staphylococcus aureus* varied with the relative humidity. Rountree (1963) studied the survival of staphylococci on various textiles in common use in hospitals and showed that some epidemic strains of *S. aureus* belonging to group-I phage-typing patterns survived for longer on cotton lint than non-epidemic strains with group-II phage-typing patterns. She also observed that cultures dried on pieces of woollen blanket survived for a longer time than when they were dried on cotton. It has been reported that the survival of staphylococci dried on glass was associated with pigment production and it was also noted that there was no difference in the survival rate of strains of pigmented methicillin-resistant *S. aureus* (MRSA) when compared with the survival rates of pigmented methicillin-sensitive staphylococci after drying for 5 h (Lacey and Grinsted, 1973).

Since 1981, strains of MRSA causing severe infections have been isolated from hospital patients in many countries including Australia (McDonald *et al.*, 1981), Ireland (Cafferkey *et al.*, 1983), England (Bradley *et al.*, 1985) and the USA (Schaeffler *et al.*, 1981). In Royal Prince Alfred Hospital (RPAH) five prevalent strains of MRSA have been identified by phage typing over this period (Vickery *et al.*, 1983, 1986). Epidemiological

studies have shown that strains of two of these phage-typing patterns have been consistently isolated from general epidemics in many wards in RPAH and from several other hospitals in Sydney and NSW (Vickery and Beard-Pegler, 1986).

Strains belonging to the three other phage-typing patterns have been isolated from individual outbreaks in some hospitals but not in others and have also been restricted to specific areas in RPAH. Their distribution would appear, therefore, to be localised, in contrast to the wide-spread distribution of the other MRSA strains.

To investigate why MRSA strains vary in their ability to survive and spread in the ward environment, their resistance to drying on a cotton-blanket material was studied. The survival rates of various MRSA strains were compared with each other, with strains of methicillin-sensitive *S. aureus* and with strains of coagulase-negative staphylococci (CNS).

Materials and methods

Strains of staphylococci

The strains of *S. aureus* examined included 16 methicillin-resistant and nine methicillin-sensitive isolates from RPAH. The strains were selected to include several representative examples of each of the major phage-typing patterns identified since 1980 (Vickery *et al.*, 1983, 1986). Twelve strains of CNS, recently isolated at RPAH and identified by the API Staph system, were also studied. These strains were isolated from blood cultures and cerebrospinal fluid (CSF) infections and were considered clinically significant on the basis of the

following criteria: isolates from blood cultures were regarded as significant if a CNS was isolated from sequential blood cultures from a febrile patient with an intravenous line (peripheral or central) or another vascular prosthetic device such as a prosthetic heart valve; CNS isolates from CSF specimens were regarded as significant if the patient had a CSF shunt, a fever and a raised CSF neutrophil count. All strains were stored on nutrient-agar slopes at room temperature. Media, antibiotic-sensitivity-testing and phage-typing methods have been described previously (Vickery *et al.*, 1983).

Drying

The method of drying and the calculation of the death rates were those used by Rountree (1963). The strain to be dried was inoculated into broth from an overnight culture and grown at 37°C for 4 h. Between 10 and 20 squares of cotton-blanket material, 20 mm × 20 mm, were placed in glass petri dishes, inoculated with 0.1 ml of the test strain, and placed either in a dark cupboard or on an open shelf in a laboratory that had good natural lighting. Temperature and humidity were not controlled but readings were taken daily and the temperature ranged from 22°C to 29°C, with a relative humidity of 50–67% for the period of the study.

Counting

Counts were calculated by placing a square of textile in 5 ml of broth which was then vortex mixed for 1 min and ten-fold dilutions were made; 0.1-ml samples were placed on to quadrants of blood-agar plates, spread with a sterile glass rod and incubated overnight at 37°C.

Death rates

K, the death rate per day, was calculated as follows (Rountree, 1963):

$$K = 2.3 \times \frac{B_0 - B_t}{t}$$

when t = time of drying in days, B_0 = \log_{10} count at time 0, B_t = \log_{10} count at time t .

Pigment

All strains were tested for pigment production on milk agar (Lacey *et al.*, 1970).

Statistical analysis

Death rates/day (K) were compared by Student's t test.

Results

Recovery of staphylococci deposited on samples

All inoculated squares had dried within 24 h. Counts at this time showed that multiplication had

taken place. Squares inoculated with 5.0×10^7 staphylococci gave counts of 1.0×10^8 after 24 h; therefore, counts made on day 1 were used as baseline data. No strains showed a fall in count at this time. All counts recorded are the average of quadruplicate 0.1-ml samples and, when duplicate textile squares were sampled, there were no significant differences between counts. Strains were tested on two separate occasions and gave reproducible results.

Loss of viability of MRSA

Sixteen MRSA strains were examined. All strains showed loss of viability at day 7, and their death rates (K) at day 7, fall into two groups (table I). Strains of phage types 83A/85/95/90/88 and 83A/85/95/88 had K values (death rates/day) of 0.09–0.13 with a mean of 0.12; strains of phage types 77/83A/84/85/95/90/88, 77/85/88 and 29/53/85/90/88 had K values of 0.32–0.46/day with a mean of 0.38/day. This difference was statistically significant ($p < 0.01$).

Loss of viability of other staphylococcal strains

K values at day 7 of the nine methicillin-sensitive *S. aureus* strains of varying phage types and antibiotic sensitivities were 0.07–0.22/day with a mean of 0.12/day (table II). There was no obvious relationship between phage-typing group or antibiotic sensitivity and behaviour on desiccation. Though the strain with the highest death rate, 0.22/day, belonged to phage group II (type 3C/55/71) and was sensitive to all antibiotics, the other group-II strain (of phage type 71) was resistant to three antibiotics and had a death rate of 0.13/day. The 12 coagulase-negative strains identified by API Staph were 10 *S. epidermidis*, one *S. haemolyticus* and one *S. hominis* (table III). Their antibiotic-sensitivity-patterns varied and the range of their K values at day 7 was wide, 0.13–0.52 with a mean of 0.25/day. There was no relationship between resistance to antibiotics and resistance to desiccation in these strains either.

Effect of sunlight on loss of viability of staphylococcal strains

There was no significant difference between K values for strains when the textile samples were stored in the dark or in natural light (table IV). The death rates for MRSA strains remained constant over 28 days. Those strains belonging to the local epidemic group showed high K values at day 7 and continued to do so at day 28, whereas the death

Table I. Death rates of methicillin-resistant *S. aureus*

| Type of epidemic spread | Phage-typing pattern | Death rate (K) at day 7 | Mean K (range) |
|-------------------------|-----------------------|-------------------------|----------------------|
| General | 83A/85/95/90/88 | 0.13 | 0.12* (0.09–0.13) |
| | " | 0.09 | |
| | " | 0.13 | |
| | 83A/85/95/88 | 0.13 | |
| | " | 0.11 | |
| | " | 0.13 | |
| Local | " | 0.13 | 0.38* (0.32–0.46) |
| | 77/83A/84/85/95/90/88 | 0.39 | |
| | " | 0.46 | |
| | " | 0.42 | |
| | " | 0.41 | |
| | 77/85/88 | 0.32 | |
| | " | 0.42 | |
| | 29/53/85/90/88 | 0.36 | |
| | " | 0.34 | |
| | " | 0.32 | |

* $p < 0.01$.**Table II.** Death rates of methicillin-sensitive *S. aureus*

| Phage-typing pattern | Antibiotic resistance | Death rate (K) at day 7 | Colour on 40% milk agar |
|----------------------|-----------------------|-------------------------|-------------------------|
| 29 | Pc | 0.16 | Gold-orange |
| 29/52/80 | PcTc | 0.08 | Gold-orange |
| 71 | PcEmTc | 0.13 | White |
| 3C/55/71 | O | 0.22 | White |
| 53 | Pc | 0.07 | Gold-orange |
| 53/83A/85/90 | O | 0.07 | Gold-orange |
| 42E/47/53/77/88 | Pc | 0.10 | Gold-orange |
| 29/52A/52A/79/80/ | | | |
| 6/47/75/83A/85 | PcEm | 0.08 | Buff |
| 96 | Pc | 0.13 | Buff |

O = no resistance; Pc = penicillin; Em = erythromycin; Tc = tetracycline.

rates of those strains of the general epidemic group remained lower. However some methicillin-sensitive strains showed higher K values at day 28 than at day 7, whereas the results obtained with the coagulase-negative strains were varied. The results for three representative strains of CNS are shown in detail. Strain one showed a relatively consistent death rate over 28 days, strain two showed an increase in the rate, whereas the death rate for strain three had decreased at day 28.

The viability of staphylococcal strains after longer periods of desiccation also varied. Viable cells could be recovered from 5 of the 12 coagulase-negative strains after 12 weeks. Although the K

values for 2 of the 9 methicillin-sensitive strains were low these values did increase with time and no viable cells were recovered from any methicillin-sensitive strains after desiccation for 9 weeks. Viable cells were recovered from the MRSA strains belonging to the general epidemic group after 9 weeks but no viable cells from those MRSA strains of the local epidemic group were recovered after 6 weeks.

Pigment production of staphylococcal strains

All seven MRSA strains of phage types 83A/85/95/90/88 and 83A/85/95/88 produced gold-orange

Table III. Death rates of coagulase-negative staphylococci

| Species | Antibiotic* resistance | Death rate (K) at day 7 |
|------------------------|---------------------------|----------------------------|
| <i>S. epidermidis</i> | 0 | 0.13 |
| | Pc | 0.27 |
| | Pc | 0.19 |
| | Pc | 0.17 |
| | PcTc | 0.29 |
| | PcTc | 0.16 |
| | PcMc | 0.52 |
| | PcTcCm | 0.46 |
| | PcMcTc | 0.23 |
| <i>S. haemolyticus</i> | PcMcEmTcCm | 0.13 |
| | PcMc | 0.23 |
| <i>S. hominis</i> | PcMcCm | 0.23 |

* O = no resistance; Pc = penicillin; Mc = methicillin; Em = erythromycin; Tc = tetracycline; Cm = chloramphenicol.

pigment on milk agar; the remaining nine MRSA strains produced buff-coloured colonies (table V). The pigment production of the methicillin-sensitive

strains varied (table II) but the coagulase-negative strains produced no pigment.

Discussion

The ability of MRSA strains to survive in atmospheric conditions, similar to those found in hospital wards in our climate, varied. Lidwell and Lowbury (1950) showed that there was a correlation between atmospheric humidity and the death rate of bacteria. The temperature and relative humidity during this study showed a range experienced during many months in Sydney.

The interesting observation made from this study of MRSA strains was that strains belonging to those phage types isolated consistently from many hospitals in Sydney and distributed within RPAH showed a low death rate over 28 days, whereas those strains isolated from certain hospitals and from certain wards only within RPAH showed a higher death rate. Variation in resistance to desiccation was also found because viable cells of general epidemic strains were recovered after 9

Table IV. Comparison of K values of staphylococcal strains over 28 days

| Strain | Phage-typing pattern | Environmental condition | Death rate (K) on day | | | |
|--|---------------------------|----------------------------|-----------------------|-------------|-------------|-------------|
| | | | 7 | 14 | 21 | 28 |
| MRSA general epidemic (7 strains) | 83A/85/95/ 90/88 | Dark | 0.13 | 0.16 | 0.15 | 0.17 |
| | | Light | 0.20 | 0.22 | 0.16 | 0.18 |
| | | mean* | 0.12 | 0.15 | 0.16 | 0.14 |
| | | range | (0.09-0.20) | (0.12-0.23) | (0.11-0.23) | (0.10-0.21) |
| | | | | | | |
| local epidemic (9 strains) | 77/83A/84/85/ 95/90/88 | Dark | 0.39 | 0.43 | 0.44 | 0.30 |
| | | Light | 0.39 | 0.38 | 0.23 | 0.28 |
| | | mean | 0.38 | 0.38 | 0.31 | 0.30 |
| | | range | (0.32-0.46) | (0.30-0.49) | (0.20-0.46) | (0.20-0.43) |
| | | | | | | |
| Methicillin-sensitive <i>S. aureus</i> (9 strains) | 29 | Dark | 0.13 | 0.16 | 0.24 | 0.27 |
| | | Light | 0.15 | 0.18 | 0.19 | 0.24 |
| | 3C/55/71 | Dark | 0.22 | 0.21 | 0.22 | 0.22 |
| | | Light | 0.20 | 0.27 | 0.23 | 0.29 |
| | 53 | Dark | 0.07 | 0.08 | 0.09 | 0.13 |
| | | Light | 0.07 | 0.07 | 0.11 | 0.16 |
| | | mean | 0.12 | 0.12 | 0.15 | 0.20 |
| | | range | (0.07-0.22) | (0.05-0.27) | (0.09-0.25) | (0.13-0.29) |
| | | | | | | |
| | | | | | | |
| Coagulase-negative staphylococci (12 strains) | NA | Dark | 0.13 | 0.14 | 0.10 | 0.11 |
| | | Light | 0.23 | 0.19 | 0.17 | 0.13 |
| | NA | Dark | 0.04 | 0.14 | 0.18 | 0.23 |
| | | Light | 0.09 | 0.09 | 0.10 | 0.21 |
| | NA | Dark | 0.46 | 0.23 | 0.17 | 0.21 |
| | | Light | 0.42 | 0.21 | 0.15 | 0.22 |
| | | mean | 0.25 | 0.18 | 0.16 | 0.20 |
| | | range | (0.13-0.52) | (0.09-0.26) | (0.10-0.28) | (0.10-0.30) |
| | | | | | | |
| | | | | | | |

* Results for dark and light conditions are included in average K values and ranges.

NA = not available.

Table V. Pigment production of methicillin-resistant *S. aureus*

| Epidemic spread | Phage-typing pattern | Number of strains | Mean death rate* (K) at day 7 | Colour on 40% milk agar |
|-----------------|-----------------------|-------------------|-------------------------------|-------------------------|
| General | 83A/85/95/90/88 | 7 | 0.12 | Gold-orange |
| | 83A/85/95/88 | | | |
| Local | 77/83A/84/85/95/90/88 | 9 | 0.38 | Buff |
| | 77/85/88 | | | |
| | 29/53/85/90/88 | | | |

* See table I for range.

weeks of drying, whereas no viable cells of the local epidemic strains were recovered after 6 or 7 weeks.

No difference in survival was observed when MRSA strains and methicillin-sensitive strains were dried on glass (Lacey and Grinsted, 1973) and in our study the death rates of the methicillin-sensitive strains at day 7 were low, similar to those of the general epidemic MRSA strains. However, over the longer period of 7 weeks the methicillin-sensitive strains did not survive as long as the general epidemic MRSA strains. There was no significant difference between the survival of the local epidemic MRSA strains and that of the coagulase-negative strains at day 7.

Resistance to drying has been reported to be associated with production of pigment (Grinsted and Lacey, 1973; Gedney and Lacey, 1982) and the present observations support those findings. The MRSA strains most resistant to drying produced a gold-orange pigment; the less resistant strains produced little pigment and were buff-coloured.

This correlation was not as clearly defined with the methicillin-sensitive strains, although the strain with the highest death rate was non-pigmented and sensitive to all antibiotics.

Some correlation between resistance to drying and involvement of staphylococcal strains in epidemics was observed by Rountree (1963) who suggested that the ability to survive for long periods on textiles could be an advantage in the hospital environment. This study shows that some MRSA strains do have a greater ability to withstand drying and this may confer on them a selective advantage over strains that die more quickly. This may explain the variation in epidemiological spread of MRSA strains which we have observed over the last 7 years at RPAH and other Sydney hospitals.

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