J. Hyg., Camb. (1963), **61**, 265
Printed in Great Britain

The effect of desiccation on the viability of *Staphylococcus aureus*

By PHYLLIS M. ROUNTREE
Fairfax Institute of Pathology, Royal Prince Alfred Hospital, Sydney

(*Received* 16 *February* 1963)

INTRODUCTION

People infected with *Staphylococcus aureus* or carrying this organism can contaminate their bedding and surroundings (Hare & Cooke, 1961; Rountree & Beard, 1962) and pathogenic staphylococci are frequently isolated from air samples collected in hospitals. Lidwell, Noble & Dolphin (1959) found these air-borne staphylococci to be carried on particles of small size, the majority being less than 18 μ. It is presumed that these staphylococci in the environment of patients are in the dried state.

Do these dried organisms retain their ability to infect fresh hosts? And if so, for how long after they have been shed? These are questions of some importance when considering measures for the control of staphylococcal sepsis in hospitals. Some circumstantial evidence (Rountree & Beard, 1962) suggests that cocci deposited on ward blankets by a patient can colonize the nose of the next occupant of the contaminated bed.

Lidwell & Lowbury (1950) examined the death-rates of *Staph. aureus* present in samples of dust collected in three hospitals. The death-rate per day varied with the relative humidity at which the dusts were stored, ranging from 0·030 in a dry atmosphere to 0·53 at 84% relative humidity.

Maltman, Orr & Hinton (1960) studied the effect of drying on glass at room temperature on the survival of the Wood 46 strain of *Staph. aureus*. They found that the viable count was reduced approximately 90% after 6 days. The dried organisms also showed a prolongation of the lag phase which increased with the time of storage, an increase in the time taken to clot plasma and a diminished survival rate in human serum. Further observations (Hinton, Maltman & Orr, 1960) indicated that these dried cells had lost much of their virulence for mice by the intramuscular, intravenous and intracerebral routes.

Wood 46 was isolated many years ago (Burnet, 1929) and its behaviour may not be identical with that of strains of more recent origin. Furthermore, organisms dried on glass may differ from those dried on textiles. The question of the effect of desiccation on the viability of staphylococci has therefore been further studied in a system which attempts to simulate the situation when organisms are shed from the body on to textiles.

METHODS

The strain to be dried was inoculated into broth from an overnight culture and was grown at 37° C. for 3–4 hr. until it contained $1–2 \times 10^8$ cells/ml. Inocula of 0·1 ml. were then deposited on sterile pieces of textile approximately 10 mm. square into which they soaked rapidly. Ten to twenty squares were prepared for each strain and were stored for drying in sterile Petri dishes in a cupboard at room temperature. The temperature and relative humidity in the cupboard were observed daily; the temperature varied from 66 to 70° F., but on a few occasions rose to 80° F. and the R.H. ranged from 42 to 50 %.

For most of the observations, cotton lint of the type used for covering surgical wounds was employed. A few observations were made with woollen blanket and cellular cotton ('Osman') blanket. All textiles were washed and autoclaved before use.

Counting. Viable counts were made by placing a square in 5 ml. of broth in a 1 oz. screw-capped universal container. This was shaken for 2 min. and appropriate tenfold dilutions then made in broth held in an ice-bath. Four or more samples of 0·01 ml. (measured with a calibrated pasteur pipette) were placed on quadrants of blood agar plates and spread with a sterile glass rod until the liquid had adsorbed into the medium. Colonies were counted after overnight incubation at 37° C.

Preliminary experiments showed that all the colony-forming units deposited on a square of textile could be recovered by this method. For example, a broth culture of PS 80 gave a count of $1·6 \times 10^8$ colony-forming units/ml. just before depositing 0·1 ml. volumes on the lint, and $1·5 \times 10^7$ colonies were grown from the shaken material. Similarly, a culture of Wood 46 with a count of $1·05 \times 10^8$/ml. yielded $1·1 \times 10^7$ colonies per square. For reasons of convenience the counts will be expressed as cocci/square without applying any corrections for pairs or larger groups of cocci.

Death-rates. K, the death-rate per day, was calculated with the following formula:

$$K = 2·3 \frac{B_0 - B_t}{t},$$

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

Strains of staphylococci. The strains examined came from a variety of sources. Two strains, Wood 46 and Bundaberg, isolated many years previously, had been maintained in culture media before reaching this laboratory where they have been dried. Others had been kept as dried cultures. The majority of strains were examined within one subculture of their isolation from air samples or patients. All strains were phage typed using the basic international set of typing phages (Blair & Williams, 1961) plus two phages of local interest, 31B and 47D.

RESULTS

Multiplication of the cocci on textiles. Cultures deposited on the squares and stored in the cupboard appeared to be dry within 24 hr. Counting showed that multiplication had taken place during this period. Squares on which 1×10^7 cocci had been placed gave a count of 1×10^8 or more on the next day. Accordingly, the counts on day 1 and not those of day 0 were used as a base-line. It cannot be excluded that deaths occurred in these first hours of drying but, in view of the slow fall in counts of most of the strains, this is unlikely to be of any significance.

Comparison of Wood 46 *with other strains.* Fig. 1 illustrates the behaviour over a period of 3 months of Wood 46, of PS 80 (the propagating strain of phage 80), of a strain of phage group III showing multiple antibiotic resistance, and of a strain of phage group II. The first three strains were set up on the same day and the group II strain 4 weeks later.

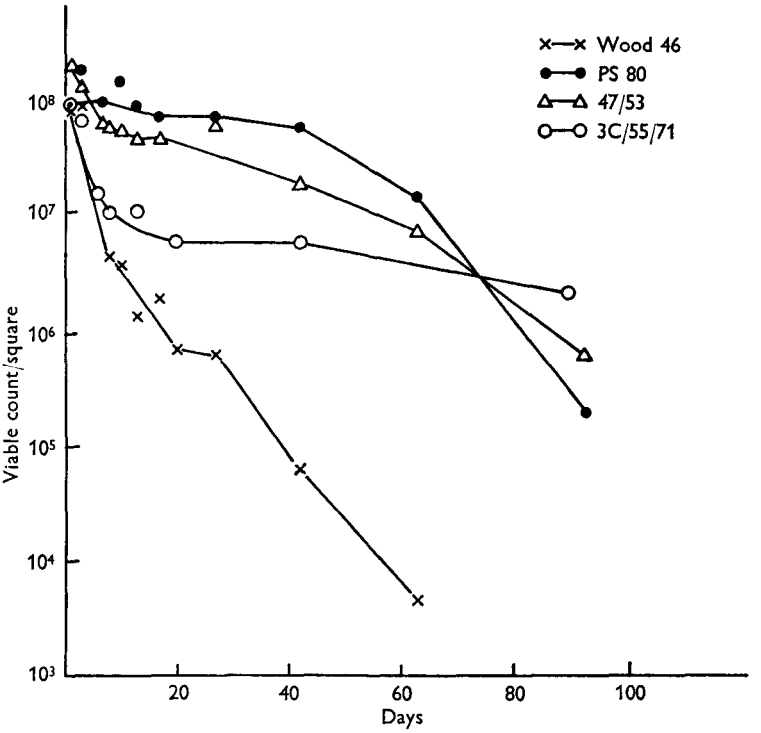

Fig. 1. Loss of viability of four strains of *Staphylococcus aureus* dried on cotton lint.

The count of Wood 46 fell by 2 logs during the first 2 weeks of drying; thereafter, the decline in count was more gradual to the 28th day but at the end of 93 days no viable cells could be recovered. In contrast to this, strain PS 80 showed no significant loss of viability at the 14th day and very little loss between the 14th and 42nd days. The survival curves of the two other strains resembled each other, showing initial loss of viability over the first 14–18 days and then a flattening of the curves. In contrast to Wood 46, all three strains had viable cells at the end of 3 months desiccation.

https://doi.org/10.1017/S0022172400039541 Published online by Cambridge University Press

The question of whether the morphological clumping of the cocci might have any effect on the shape of the curves, particularly during the early period of drying, was considered. PS 80, which showed no apparent loss of viability during the first 14 days, might, in fact, be losing viable cells from pairs or clumps which would still register as a viable unit. Extrapolation of the curve backwards from its slope of the 14th to 42nd day did not however indicate that the count at day 1 was subject to an error of any great magnitude, and it was concluded that there was no significant loss of undetected viability, during the first period of drying.

Loss of viability by various strains. A total of thirty-eight strains was examined for their loss of viability, when dried on lint.

The strains could be divided into two categories, those whose viable count fell from the beginning of the drying period and those that showed no significant loss of viability until after the 14th day or later.

Table 1 gives details of the strains that fell into the first category. The death-rates varied from 0·031 to 0·278/day. It was notable that the two old laboratory strains, Bundaberg and Wood 46, had the highest values for K.

Table 1. *Death-rates of strains of* Staphylococcus aureus *showing loss of viability within* 15 *days of drying*

Strain no.	Phage group	Phage pattern	Source	Log loss at day 15	Death-rate, K/day at day 15
Bundaberg	I	52/52A/80/81		1·1092	0·17
Haines		29/52/52A/79/80	Nasal swab	0·2876	0·043
M4		52/52A/80/81	Air sample	0·2382	0·039
3085		29	Nasal swab	0·3736	0·057
3095		52	Nasal swab	0·2001	0·031
3100		29/52	Nasal swab	0·3688	0·057
136	II	3C/55/71	Air sample	0·9208	0·15
672		3A/3B/3C	Infected ulcer	0·9488	0·145
AR		55/71	Air sample	0·7090	0·109
3028		55/71	Surgical wound	0·4327	0·066
2807		3B/3C/55/71	Osteomyelitis	0·4463	0·068
2812	III	7/31B/47D	Abscess	0·4994	0·077
1002		53	Empyema	0·6632	0·101
2502		53	Blood culture	0·6637	0·101
SS6/1	N.C.	Groups I–III	Air sample	0·4046	0·062
Wood 46	—	N.T.	—	1·8110	0·278
814	—	N.T.	Nasal swab	1·000	0·153
815	—	N.T.	Nasal swab	0·3553	0·055
817	—	N.T.	Surgical wound	0·7959	0·122

For those strains that showed no significant loss during the first part of the drying period, the death-rates were calculated over that period of time when their counts were falling. Details of these strains are shown in Table 2. The death-rates/day ranged from 0·018 to 0·137.

Effect of sunlight on the death-rate. A culture of PS 80 was dried on squares of lint, half of which were stored in the dark and the other half exposed to the effect

Table 2. *Death-rates at later times of strains of* Staphylococcus aureus *showing no loss of viability during first* 15 *days of drying*

Strain no.	Phage pattern	Source	Days of drying	Log loss	Death-rate, K/day
PS 80	80/81	Epidemic of newborn, Sydney (1953)	13–63	0·7696	0·035
PS 80/1*a*	52/52A/80/81	Mutant of PS 80	15–50	1·2083	0·079
PS 80 (*a*)	52/52A/80/81	'Converted' PS 80	21–65	0·6989	0·038
PS 80 (*n*)	52/52A/80/81	'Converted' PS 80	15–65	1·1498	0·055
U 9	80/81	Epidemic of newborn, U.S.A. (1954)	15–50	1·6173	0·106
9684	52/52A/80/81	Epidemic of newborn, Victoria (1954)	15–50	2·0792	0·137
Allen	52/52A/80	Epidemic, Atlanta, U.S.A.	22–50	1·6600	0·136
Bellville	52/52A/80	Epidemic, Atlanta, U.S.A.	15–50	1·7233	0·113
594B	52/52A/80/81	Empyema	29–65	0·8171	0·052
3082	52A/79	Nasal swab	15–28	0·4102	0·072
	52A/79		28–64	1·2323	0·080
3023	6/54	Infected surgical wound	15–29	0·5376	0·088
820	7/47D	Sputum	15–38	1·3310	0·133
	7/47D		38–91	1·9313	0·084
2994	42E	Infected surgical wound	29–92	3·2821	0·120
PP 7/1	42E	Air sample	36–84	0·5878	0·029
1009	47	Infected surgical wound	15–65	1·6990	0·078
8503	53	Cross-infected empyema	15–66	1·3388	0·060
2825	77	Sputum	15–38	0·1841	0·018
3027	77	Infected ulcer	15–92	3·6484	0·108
670	42D	Nasal swab	15–64	0·8921	0·042

Table 3. *Loss of viability of two strains of* Staphylococcus aureus *dried on cotton cellular blanket and on woollen blanket*

	Cotton			Wool		
Strain no.	Day	Log loss	Death-rate, K/day	Day	Log loss	Death-rate, K/day
PS 80	7	0·7634	0·25	7	0·1677	0·055
	15	1·4847	0·227	15	0·2156	0·033
	21	1·7392	0·190	—	—	—
	30	2·5873	0·198	28	0·3406	0·028
	51	4·6700	0·210	51	1·1577	0·052
8503	7	0·2853	0·093	7	0·0969	0·031
	15	0·6072	0·093	15	0·1426	0·022
	21	0·7633	0·084	21	0·2218	0·024
	28	1·2304	0·104	28	0·1938	0·016
	51	2·1512	0·097	51	1·0555	0·048

of indirect light on a ledge behind the laboratory window. Counting over a period of 21 days showed that the death-rate/day in the light was 0·432 compared with 0·029 in the dark.

Effect of different textiles on the death-rate. Two strains were examined for their death-rates on cotton and on woollen blanket. The strains, PS 80 and 8503, were set up on both textiles on the same day, so that conditions during the drying period were identical. The counts (Table 3) showed that survival on the woollen material was prolonged for both strains, little or no loss occurring until the end of 28 days. (The difference in counts before this are within the range of experimental error.) By contrast, on the cotton blanket, PS 80 died off quickly, the mean value of K being 0·21. Strain 8503 was more resistant to drying on this material, the mean value of K being 0·095 . It was concluded that the nature of the textile on which the staphylococci were dried had a considerable effect on their survival.

Relationship of survival on drying to mercury resistance

Moore (1960) reported that 'epidemic' strains of staphylococci were more resistant to mercury salts than non-epidemic strains. For example, in phage group I, practically all strains lysed by phage 80, and in group III, a large proportion of those frequently associated with epidemics of wound infection, were mercury resistant. The mechanism of the mercury resistance is unknown but the possibility that the strains showing no loss of viability in the early part of the drying period might also be mercury resistant was discussed with Dr Moore. Some of the strains used in the drying experiments had been tested for mercury resistance and, although strains lysed by phage 80 were also mercury resistant and resistant to drying, many exceptions to this association were found. For example, Wood 46 was mercury resistant.

The death-rates of three related strains received from Dr Moore were determined after drying on lint. Strain 75 AY was penicillin and Hg resistant, and the death-rate at 13 days was $K = 0{\cdot}101$. A penicillin-resistant, Hg sensitive strain, 75B, also had a value for K of 0·101 while a Hg-sensitive, penicillin-sensitive strain, 75 C, gave $K = 0{\cdot}130$.

DISCUSSION

The conditions in which the death-rates of these staphylococci were measured were arranged to be comparable to those in hospital wards in this climate. The work of Lidwell & Lowbury (1950) on the death-rates of staphylococci in stored hospital dust showed that the relative humidity had an important influence on the survival of the organisms. The present experiments were carried out for the most part over a fairly narrow range of temperatures and relative humidities. It was noted, however, that on several occasions during the summer when temperature and humidity rose there was an accelerated death-rate in the strains then being studied. Such results have been excluded from this report. It would be desirable for this study to be repeated under controlled climatic conditions.

The strains that showed no significant loss of viability for the first fortnight of storage are of particular interest. Most were strains isolated from epidemics of infection in this hospital or elsewhere, or were of phage types implicated in such infection in other parts of the world, e.g. 52A/79. Mutants of PS 80 whose phage pattern had been altered by loss of their defective prophage (Rountree & Asheshov, 1961) retained the resistance to drying of the original wild type. Among the group III strains were two, viz. 1009 and 8503, showing resistance to many antibiotics and representative of strains that have caused numerous infections in this hospital. These strains showed resistance to drying but, on the other hand, two strains of similar character, 1002 and 2502, did show significant loss of viability in 15 days. Evidently, the correlation between resistance to drying and ability to cause epidemics is not complete.

All the strains of group II that were tested died off relatively rapidly. In general, strains of this group are rarely implicated nowadays in hospital epidemics.

The two strains with the highest death-rates were the old laboratory strains, Wood 46 and Bundaberg. Whether these death-rates are the result of their maintenance in laboratory media for many years cannot now be determined. On the basis of their results with Wood 46, Hinton *et al.* (1960) suggested that staphylococci shed from the body and surviving in the hospital environment had suffered damage which decreased their potential for infection. The present results indicate that Wood 46 behaves differently from more recently isolated strains and cannot be regarded as a typical 'hospital' strain. From the data in Table 2 of the paper by Maltman *et al.* (1960), K for Wood 46 dried on glass was calculated as 0·18 at 14 days; this value was not as high as that obtained in the present investigation with drying on lint.

The ability to survive for prolonged periods on textiles could be a character that gives a strain a selective advantage in its occupation of the ecological niche of the modern hospital in a temperate climate. The fact that the majority of strains implicated in recent hospital epidemics survived for at least a fortnight with undiminished viability might be sufficient to give them a slight selective advantage over strains that died more quickly. Their survival could allow them greater chances to come in contact with new hosts who need not necessarily develop clinical infection; establishment of the carrier state would be sufficient to perpetuate the strain. The question of whether the dried and stored staphylococci can initiate lesions is now being re-examined.

Recent discussion on the respective merits of various types of textile used in the composition of hospital blankets have been concerned chiefly with their ease of laundering. The present studies indicated that on material with an open weave such as cellular cotton, death-rates of staphylococci were faster than on woollen blanket material. This might be an additional factor to be considered in choosing which type of blanket to use in hospitals.

SUMMARY

The death-rates have been determined of strains of *Staphylococcus arueus* dried on lint and other textiles and stored at room temperature in the dark.

Two categories of strains were distinguished, those that showed no significant loss of viability after 15 days storage and those whose death commenced from the beginning of the drying period.

There was some degree of correlation between survival for 15 days and implication in epidemics of hospital infection.

REFERENCES

BLAIR, J. E. & WILLIAMS, R. E. O. (1961). Phage typing of staphylococci. *Bull. World Hlth Org.* **24**, 771.

BURNET, F. M. (1929). The exotoxins of *Staph. pyogenes aureus*. *J. Path. Bact.* **32**, 717.

HARE, R. & COOKE, E. M. (1961). Self-contamination of patients with staphylococcal infection. *Brit. med. J.* ii, 333.

HINTON, N. A., MALTMAN, J. R. & ORR, J. H. (1960). The effect of desiccation on the ability of *Staphylococcus pyogenes* to produce disease in mice. *Amer. J. Hyg.* **72**, 343.

LIDWELL, O. M. & LOWBURY, E. J. (1950). The survival of bacteria in dust. II. The effect of atmospheric humidity on the survival of bacteria in dust. *J. Hyg., Camb.*, **48**, 21.

LIDWELL, O. M., NOBLE, W. C. & DOLPHIN, G. W. (1959). The use of radiation to estimate the numbers of micro-organisms in air-borne particles. *J. Hyg., Camb.*, **57**, 299.

MALTMAN, J. R., ORR, J. H. & HINTON, N. A. (1960). The effect of desiccation on *Staphylococcus pyogenes* with special reference to implications concerning virulence. *Amer. J. Hyg.* **72**, 335.

MOORE, B. (1960). A new screen test and selective medium for the rapid detection of epidemic strains of *Staph. aureus*. *Lancet*, ii, 453.

ROUNTREE, P. M. & ASHESHOV, E. H. (1961). Further observations on changes in the phage-typing pattern of phage type 80/81 staphylococci. *J. gen. Microbiol.* **26**, 111.

ROUNTREE, P. M. & BEARD, M. A. (1962). Observations on the distribution of *Staphylococcus aureus* in the atmosphere of a surgical ward. *J. Hyg., Camb.*, **60**, 387.