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Survival of wound pathogens under different environmental conditions

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Moisture is known to favour the survival of Gram-negative bacilli in the environment, while dry conditions favour the survival of Gram-positive cocci (Hellat, 1948; Bardsley, 1948; Lidwell & Lowbury, 1950). Lowbury & Fox (1953) found that the proportion of bacteria which died when their suspending medium evaporated to dryness was much greater with a suspension of *Pseudomonas aeruginosa* than with one of *Staphylococcus aureus* or *Streptococcus pyogenes*; the death-rate of the Gram-negative bacilli which had survived drying and which were exposed for a further period under atmospheric conditions was, however, no greater than that of the survivors in dried suspensions of streptococci or staphylococci.

Among the questions left unanswered in the earlier studies were (1) the consistency, on replicate testing, of differences in survival of different strains of *Ps. aeruginosa*, (2) the relative capacity of bacterial cells, which have survived one exposure to drying, to survive a second drying, and (3) the relative capacities of *Ps. aeruginosa* and of *Staph. aureus* to survive in identical moist environments. These questions are considered in this paper. The relevance of the survival of *Ps. aeruginosa* and *Staph. aureus* under different environmental conditions is also discussed.

SURVIVAL OF ORGANISMS ON ATMOSPHERIC DRYING

Methods and materials

Bacterial strains

The Gram-positive cocci tested were three strains of *Staph. aureus* (phage types 52A/80, 3C and 80/81) and two strains of coagulase-negative staphylococci (micrococci). The Gram-negative bacilli included four strains of *Ps. aeruginosa* isolated from burns (phage types 21/68/F7, 119x, 21/68/col.21 and an untypable strain), two strains from wounds (phage types 73/119x/+ and 68/73/119x), two from urine (phage type 7/73/M4 and type unknown), one from faeces (phage type 44/68/109/119x/1214), and six strains isolated from the environment (three from dust in a burns ward, phage types F8, 21/68 and type unknown, and three from ward floor cultures, phage types 7/31/352, 68/73/109/119x/+ and untypable). Two strains of *Escherichia coli* (FE1 and FE2) and a strain of *Klebsiella aerogenes* (FKa) were also studied.

Preparation of suspensions

Cultures of the bacteria in 3 ml. infusion broth incubated at 37° C. for 24 hr. were centrifuged at 2000 rev./min. for 30 min., washed with deionized water and resuspended in deionized water to give a viable count of approximately 2×10^7 organisms/ml.

Exposure to drying

Sterile, grease-free coverslips (16 mm. diameter) were mounted in pairs on glass slides. Approximately 0·03 ml. amounts of the freshly prepared bacterial suspensions were dropped on the coverslips from standard dropping pipettes, and the drops were spread over the surface of the coverslips with a sterile platinum-wire loop. The coverslips, on their slides, were then placed under the lid of a cardboard box and left to dry at room temperature (approximately 25° C.); relative humidity varied from 35 % to 50 %.

With each strain in the first experiment six coverslips were extracted immediately, six after drying for 2 hr. and six at 24 hr. In the second experiment two coverslips were extracted immediately and two at 15 min. or 30 min. intervals for the duration of 4 hr. In an experiment to show long-term survival, one of a set of prepared coverslips was sampled each week over a period of 8 weeks.

Extraction of coverslips and bacterial counts

Coverslips carrying the bacterial suspensions were transferred to 10 ml. quantities of broth in screwcap bottles (Universal containers). After the lids had been screwed on firmly, the bottles were shaken by hand for 1 min.; 1 in 10, 1 in 100 and 1 in 1000 dilutions of the extracts were made in Ringer's solution, and three 0·03 ml. amounts of each dilution and of the undiluted extract were dropped from standard dropping pipettes on nutrient agar or horse-blood agar plates. These were incubated at 37° C. for 24 hr. and the numbers of colonies from each drop were counted. With the suspensions of *Ps. aeruginosa* that had been left after drying for several weeks, the coverslips were transferred to 10 ml. quantities of broth and shaken for 1 min. and the suspensions were incubated for 24 hr. or longer before loopfuls were spread on nutrient agar plates. The plates were incubated for 24 hr. and then, where necessary, growth of *Ps. aeruginosa* was confirmed by growth and fluorescence on subculture to a selective medium (Brown & Lowbury, 1965). Viable counts were not made, since the numbers of survivors were very small; presence or absence of growth only was recorded.

Results

Survival of organisms in films exposed on coverslips for 2 hr. and for 24 hr.

The percentage survival on exposure to atmospheric drying of suspensions of *Staph. aureus*, coagulase negative staphylococci and *E. coli* is shown in Table 1. The survival of the Gram-positive cocci was found to be variable, but all showed a higher percentage survival at 2 and 24 hr. than the strain of *E. coli*, or the strains of *Ps. aeruginosa* shown in Table 2. One strain of coagulase negative

Table 1. *Survival of* Staphylococcus aureus, *micrococci and* Escherichia coli *on drying of suspensions*

Organism	Antibiotic resistance*	Phage type	Viable counts of bacteria on coverslips† 2 hr. (*a*)	2 hr. (*b*)	24 hr. (*a*)	24 hr. (*b*)	Survivors (%) 2 hr.	Survivors (%) 24 hr.
Staph. aureus FS 1	P.T	52A/80	$8{\cdot}9 \times 10^4$	$8{\cdot}6 \times 10^4$	$2{\cdot}0 \times 10^4$	$2{\cdot}5 \times 10^4$	31·5	7·70
Staph. aureus FS 2	Sens.	3c	$1{\cdot}5 \times 10^5$	$1{\cdot}6 \times 10^5$	$5{\cdot}7 \times 10^3$	$6{\cdot}1 \times 10^3$	24·7	1·08
Staph. aureus FS 3	T.N.Ne	80/81 (1000 R.T.D.)	$2{\cdot}9 \times 10^5$	$3{\cdot}0 \times 10^5$	$5{\cdot}7 \times 10^4$	$5{\cdot}9 \times 10^4$	100	34·5
Staph. aureus FS 3‡	T.N	80/81 (1000 R.T.D.)	$1{\cdot}8 \times 10^5$	$2{\cdot}2 \times 10^5$	$4{\cdot}1 \times 10^4$	$3{\cdot}9 \times 10^4$	100	38·0
Micrococcus (white)	.	.	$1{\cdot}4 \times 10^5$	$1{\cdot}1 \times 10^5$	$8{\cdot}1 \times 10^4$	$7{\cdot}9 \times 10^4$	53·4	32·3
Micrococcus (yellow)	.	.	$4{\cdot}9 \times 10^5$	$5{\cdot}0 \times 10^5$	$4{\cdot}5 \times 10^5$	$4{\cdot}1 \times 10^5$	99·0	87·0
E. coli FE 1	.	.	$2{\cdot}6 \times 10^4$	$2{\cdot}3 \times 10^4$	$1{\cdot}3 \times 10^3$	$1{\cdot}4 \times 10^3$	2·2	0·1

* P = Penicillin, T = tetracycline, N = novobiocin, Ne = Neomycin, Sens. = sensitive to all antibiotics tested.

† (*a*) and (*b*) show the mean results from coverslips 1, 2 and 3 and from coverslips 4, 5 and 6 respectively, with each strain tested; they are presented as simultaneous replicate experiments.

‡ A neomycin-sensitive variant isolated after storage of the culture that was resistant to T, N and Ne.

Table 2. *Survival of* Pseudomonas aeruginosa *from patients and from dry environment on drying of suspensions*

Strain	Source	Phage type	Viable counts of bacteria on coverslips* 2 hr. (*a*)	2 hr. (*b*)	24 hr. (*a*)	24 hr. (*b*)	Survivors (%) 2 hr.	24 hr.
F 1	Burn	21/68/F7	$0{\cdot}8 \times 10^4$	$1{\cdot}3 \times 10^4$	$1{\cdot}0 \times 10^2$	$1{\cdot}0 \times 10^2$	1·74	0·020
F 2	Burn	119x	$2{\cdot}4 \times 10^4$	$2{\cdot}2 \times 10^4$	$3{\cdot}9 \times 10^2$	$4{\cdot}3 \times 10^2$	5·30	0·097
F 3	Burn	Unknown	$6{\cdot}3 \times 10^4$	$6{\cdot}1 \times 10^4$	$5{\cdot}6 \times 10^2$	$5{\cdot}9 \times 10^2$	9·40	0·089
F 4	Burn	21/68/col. 21	$2{\cdot}7 \times 10^4$	$3{\cdot}1 \times 10^4$	$5{\cdot}9 \times 10$	$6{\cdot}6 \times 10$	3·00	0·006
F 5	Wound A	73/119x/+	$2{\cdot}2 \times 10^4$	$2{\cdot}3 \times 10^4$	$2{\cdot}1 \times 10^3$	$1{\cdot}8 \times 10^3$	3·06	0·251
F 6	Wound B	68/73/109/119x	$4{\cdot}0 \times 10^4$	$4{\cdot}3 \times 10^4$	$3{\cdot}6 \times 10^3$	$3{\cdot}0 \times 10^3$	3·90	0·300
F 7	Urine	7/73/M4	$1{\cdot}5 \times 10^4$	$2{\cdot}1 \times 10^4$	$2{\cdot}7 \times 10^3$	$2{\cdot}4 \times 10^3$	3·20	0·480
F 8	Urine	Unknown	$3{\cdot}4 \times 10^4$	$3{\cdot}1 \times 10^4$	$1{\cdot}3 \times 10^2$	$1{\cdot}5 \times 10^2$	15·00	0·060
F 9	Faeces	44/68/109/119x/1214	$1{\cdot}0 \times 10^4$	$1{\cdot}2 \times 10^4$	.	.	0·77	.
F 10	Dust (Burns Ward)	Unknown	$2{\cdot}7 \times 10^5$	$3{\cdot}0 \times 10^5$	$5{\cdot}1 \times 10^3$	$4{\cdot}5 \times 10^3$	19·80	0·315
F 11	Dust (Burns Ward)	F 8	$3{\cdot}8 \times 10^4$	$3{\cdot}9 \times 10^4$	$2{\cdot}8 \times 10^3$	$2{\cdot}9 \times 10^3$	10.20	0·760
F 12	Dust (Burns Ward)	21/68	$3{\cdot}3 \times 10^4$	$3{\cdot}8 \times 10^4$	$1{\cdot}6 \times 10^2$	$1{\cdot}8 \times 10^2$	5·80	0·016
F 13	Ward floor	7/31/352	$3{\cdot}2 \times 10^5$	$3{\cdot}0 \times 10^5$	$3{\cdot}2 \times 10^2$	$3{\cdot}3 \times 10^2$	26·80	0·110
F 14	Ward floor	Not typable	$6{\cdot}0 \times 10^4$	$6{\cdot}3 \times 10^4$	$1{\cdot}4 \times 10^2$	$2{\cdot}0 \times 10^2$	4·20	0·012
F 15	Ward floor	68/73/109/119x/+	$1{\cdot}9 \times 10^4$	$2{\cdot}1 \times 10^4$	.	.	5·80	.

* (*a*) and (*b*) show the mean results from coverslips 1, 2 and 3 and from coverslips 4, 5 and 6 respectively, with each strain tested; they are presented as simultaneous replicate experiments.

staphylococcus showed a particularly high survival rate at 24 hr. (87%); in contrast one strain of *Staph. aureus* (FS2) showed only 1·08% survivors at 24 hr. A neomycin-sensitive variant of *Staph. aureus*, strain FS3, showed a survival rate similar to that of the neomycin-resistant parent strain. Replicate coverslips showed similar survival patterns for the same strains.

Table 2 shows the survival of strains of *Ps. aeruginosa* isolated from patients and from the environment. The mean percentage survival at 2 hr. of strains isolated from patients ($5{\cdot}04 \pm 2{\cdot}22$) was lower than the mean percentage survival at 2 hr. of strains isolated from the environment ($12{\cdot}1 \pm 3{\cdot}98$) ($t = 3{\cdot}83$, $P < 0{\cdot}01$, $> 0{\cdot}001$). Strains F5 and F6, apparently of the same phage type but isolated from two different wounds (A and B), showed similar survival rates at 2 hr. Environmental strain F15 was of the same phage type as strains F5 and F6 and showed approximately the same survival rate (5·8%). Strains F6 and F15 were excluded from the analysis because their types duplicated those of other strains in the series.

The percentage survival of all strains on exposure for 24 hr. was very low ($< 1\%$).

Replicate tests of strains F3, F8 and F13 made on successive days showed similar results (see Table 3).

Table 3. *Survival of three strains of* Pseudomonas aeruginosa *on drying: replicate tests on successive days*

			Survivors (%)			
			Test 1		Test 2	
		Phage type	2 hr.	24 hr.	2 hr	24 hr.
F 3	Burn	.	9·4	0·089	8·8	0·082
F 8	Urine	.	15·0	0·060	19·2	0·082
F 13	Ward floor	7/31/352	26·8	0·110	21·5	0·037

Survival of organisms at intervals during periods of drying (4 *hr.*)

Figure 1 shows that the number of survivors rapidly decreased during the period of drying; this occurred 30–90 min. from the start of the experiment, when the suspensions became visibly dry, after which there was little or no further death of bacteria. The percentage survival of *Staph. aureus* (phage type 52A/80) and of the strain of coagulase-negative staphylococcus tested was much higher than that of the strains of *E. coli* and *Klebsiella aerogenes*.

Figure 2 shows the survival during 4 hr. of two strains of *Ps. aeruginosa*, one of which showed a high percentage survival at 2 hr. (26·8%) while the other (strain F15) showed a lower survival rate (5·8% survivors at 2 hr.) in the previous experiment (see Table 2). The curves confirm the difference between the two organisms in survival on drying.

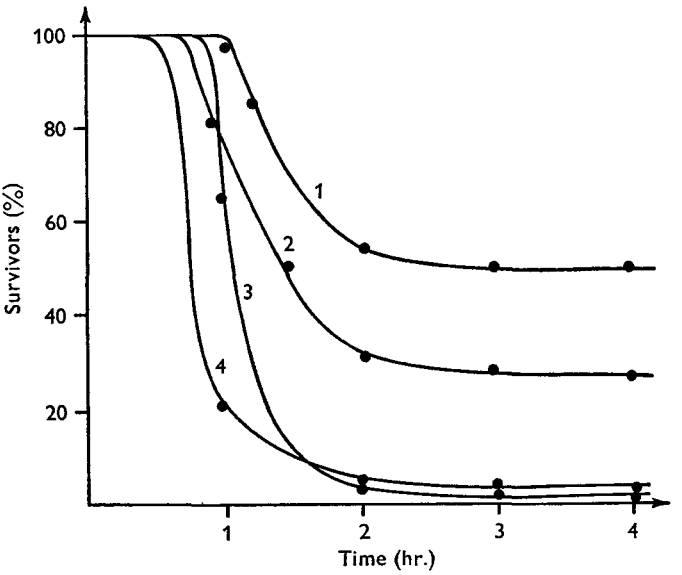

Fig. 1. Percentage of bacteria surviving on coverslips at intervals during and after the evaporation of suspending fluid (deionized water). 1, Micrococcus; 2, *Staphylococcus aureus*; 3, *Escherichia coli*; 4, *Klebsiella aerogenes*.

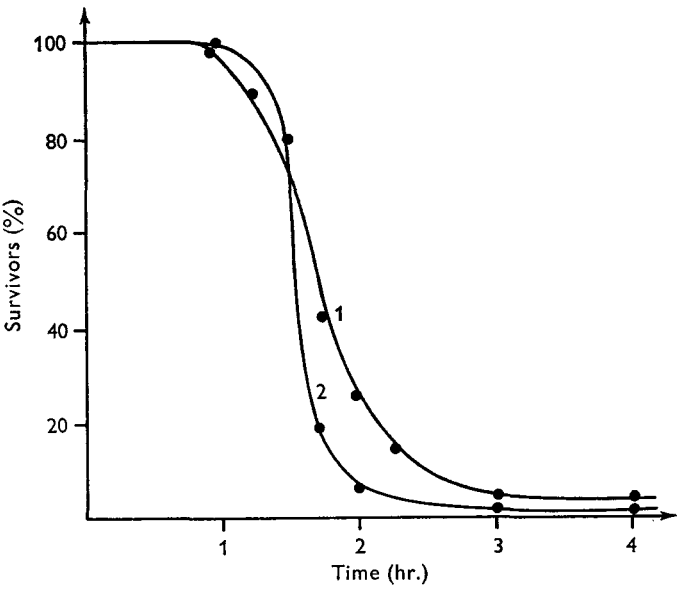

Fig. 2. Survival curves on drying (cf. Fig. 1) showing different proportions of survivors in two strains of *Pseudomonas aeruginosa*. 1, Survival curve for strain F 13; 2, survival curve for strain F 15.

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Survival of Ps. aeruginosa *after drying, during periods of* 8 *weeks*

Of the eight strains examined, four showed survivors after 8 weeks' exposure on coverslips. These results were not related to place of isolation, phage type, or survival at 2 hr. In a repeat experiment, three out of the eight strains showed survivors, but only one strain showed survivors in both experiments after this period of exposure.

Survival of Ps. aeruginosa *at* 37° *and at* 4° *C.*

In the test made at 37° C. and relative humidity 20–25 %, the percentage of survivors was 0·24 %; at 4° C. and relative humidity 35–45 % the coverslips were still moist at 2 hr. and 35·5 % of the organisms remained viable. After 24 hr. there were 0·003 % survivors at 37° C. and 0·58 % survivors at 4° C.

THE SURVIVAL ON A SECOND DRYING OF BACTERIAL CELLS WHICH HAVE SURVIVED ONE DRYING

Materials and Methods

Bacterial strain

Ps. aeruginosa (strain F 3) isolated from a human burn was used.

Preparation of suspensions and exposure to drying

A 20 ml. infusion broth culture of *Ps. aeruginosa* (strain F 3) incubated at 37° C. for 24 hr. was centrifuged at 2000 rev./min. for 30 min., washed and resuspended in deionized water (approximately 3 ml.) to provide a concentrated suspension. Of this suspension, 0·03 ml. was then pipetted on to each of sixty coverslips; the drops were spread over the surface of the coverslips, left to dry and kept for 6 days in a closed cardboard box.

Estimation of the number of organisms surviving exposure on coverslips

After 6 days, organisms were extracted from ten coverslips by shaking with 5 ml. of deionized water in a Universal container, as described above, and a viable count was made.

On the seventh day the fifty remaining coverslips were extracted in deionized water. The suspension was concentrated by centrifugation to give approximately 2×10^7 viable organisms/ml., and this was used for a 4 hr. drying experiment as described above.

Results

Figure 3 shows the survival curve on drying of previously dried, resuspended bacteria, compared with that for suspensions of the same strain prepared from a fresh culture and from a culture of the once-dried organisms. Although all these suspensions show a rapid decrease in numbers followed by a period of more gradual decreases, these decreases were smaller and less rapid with the pre-dried organisms than with the suspensions prepared from cultures. A considerably higher proportion (approximately 15 % more) of the pre-dried organisms than of the organisms resuspended from culture remained viable when the numbers of survivors reached their constant level at the end of the experiment. When

organisms which had been left to dry for a period of time, as described above, were harvested and grown overnight in broth, this new population tested for survival on drying showed a survival curve which was similar to that of the fresh suspensions of organisms.

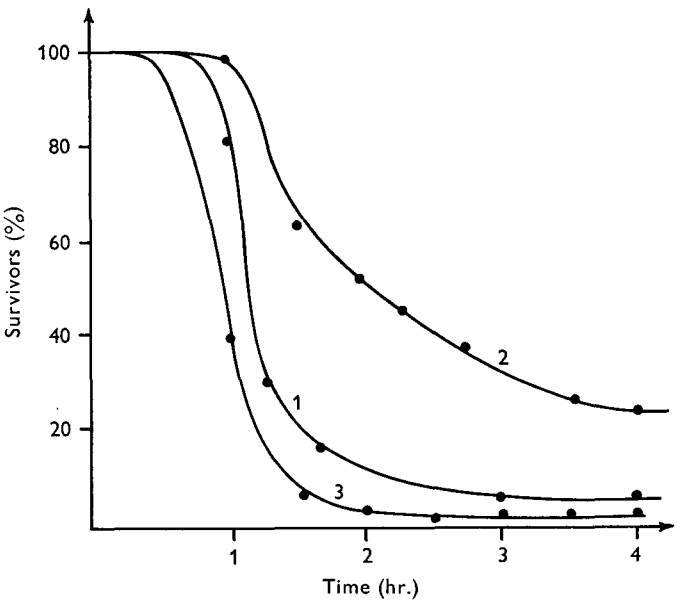

Fig. 3. Survival curves on drying of *Pseudomonas aeruginosa* cells before and after they had survived one exposure to drying, and of a culture of dried cells. 1, Survival curve for fresh suspensions of organisms; 2, survival curve for organisms previously dried; 3, survival curve, after subculture, for organisms previously dried.

SURVIVAL OF *PS. AERUGINOSA* AND *STAPH. AUREUS* IN WATER AND IN SOLUTIONS

Materials and Methods

Bacterial strains

Ps. aeruginosa (strains F3 and F16) and *Staph. aureus* (strains FS2 and FS6) were used to test survival in suspension for a limited period of time (6 hr.). Seven strains of *Ps. aeruginosa* (F1, F3, F6, F11, F12, F13, and F15) and seven strains of *Staph. aureus* (FS1–7) were selected for tests on survival in suspension during a period of several weeks.

Preparation of suspensions

Suspensions were prepared as described in the previous section, after washing with deionized water. Two drops (0·03 ml./drop) of freshly prepared suspensions were then added to 20 ml. amounts of sterile deionized water, physiological saline solution and Ringer's solution for tests of survival during 5 hr., and to the same amounts of deionized water, tap water and Ringer's solution for tests of survival during several weeks. The diluted suspensions were briefly agitated to disperse the bacteria.

Sampling and bacterial counts

In the first experiment 0·5 ml. samples were removed in duplicate immediately after the suspending fluids were inoculated, and from then at 15 min. or 30 min. intervals for the duration of the 5 hr. Viable counts were made on each of these samples.

In the second experiment, viable counts were made on 0·5 ml. samples removed at the start and at 24 hr. From then, loopfuls of the suspensions were removed twice weekly for 3 weeks, and at weekly intervals during periods of up to 25 weeks. These were spread on the surface of nutrient-agar plates and the presence or absence of growth recorded after a minimum of 24 hr. incubation at 37° C.

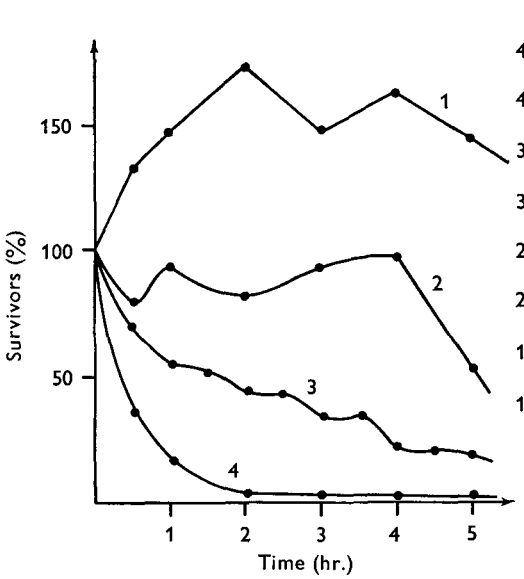

Fig. 4

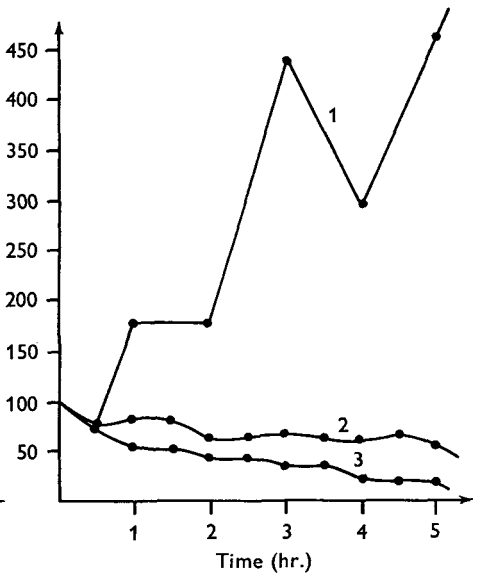

Fig. 5

Fig. 4. Survival curves for 5 hr. of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in deionized water. 1, *Staph. aureus*, strain FS 6; 2, *Staph. aureus* strain FS 2; 3, *Ps. aeruginosa* strain F 3; 4, *Ps. aeruginosa* strain F 16.

Fig. 5. Survival curves for 5 hr. of a strain of *Pseudomonas aeruginosa* in deionized water, physiological saline and Ringer's solution. 1, In Ringer's solution; 2, in saline; 3, in deionized water.

Results

Figure 4 shows the survival curves over a period of 5 hr. for *Staph. aureus* (strains FS 2 and FS 6) and *Ps. aeruginosa* (strains F 3 and F 16) suspended in deionized water. Both strains of *Staph. aureus* show a higher percentage survival than the strains of *Ps. aeruginosa*. During the first 2 hr. *Staph. aureus* (strain FS 6) showed an increase (approximately 70 %) over the number of organisms present initially, followed by a fluctuating decline in numbers. FS 2, after an initial drop of 20 % in the number of viable organisms present, showed a pattern of increase and decrease similar to that shown by FS 6, though the number of viable organisms

present during the experiment never exceeded the numbers present at the beginning; at 5 hr. approximately 52% were still shown to be viable. *Ps. aeruginosa* (strain F3) showed a steady decrease in the number of viable organisms, 18% of those initially present still being found viable at 5 hr.; a more rapid decline in the number of survivors of strain F16 occurred during the first 2 hr., but the numbers then stayed constant at 1·5% of those initially present.

Figure 5 shows the survival curves over a period of 5 hr. for *P. aeruginosa* (strain F3) suspended in saline, deionized water and Ringer's solution. Organisms suspended in saline showed an initial decline in numbers of viable organisms, followed by a more gradual fluctuating decline, to approximately 54% of the organisms initially present at the end of the 5 hr. period. In deionized water the pattern of decline was similar, but with greater loss, approximately 18% of the organisms initially present surviving after 5 hr. By contrast, in Ringer's solution the number of surviving organisms fell during the first 30 min., but then rose by irregular stages to approximately 460% of the initial counts after 5 hr.

In the experiment with strains left in suspension for several weeks (see Tables 4 and 5) viable counts of *Ps. aeruginosa* were considerably reduced after 24 hr. in deionized water and tap water, but much greater reductions occurred with *Staph. aureus*; in deionized water, strains of *Ps. aeruginosa* showed between 1·9 and 12% of survivors, but with strains of *Staph. aureus* the proportion of survivors in deionized water was between 0·003% and 0·5%. In tap water, survivors of all strains of *Ps. aeruginosa* were fewer than 1%; no strain of *Staph. aureus* showed any survivors after 24 hr. in tap water, or on subsequent samplings from tap water or deionized water.

In Ringer's solution, at the 24 hr. and 48 hr. samplings all strains of *Ps. aeruginosa* gave confluent growth from the dilution used for making viable counts on the initial sample, indicating an increase in numbers. With *Staph. aureus*, fewer than 1% of the numbers present at the start were present after 24 hr., and after 48 hr. viable organisms could only be detected in three out of the seven strains. No viable organisms were found on subsequent samplings.

After 25 weeks, viable organisms were found in suspensions of all strains of *Ps. aeruginosa* in Ringer's solution and tap water; viable organisms could be detected in four out of the seven strains in deionized water.

DISCUSSION

As in the earlier studies of Lowbury & Fox (1953), the work reported here showed that a smaller proportion of Gram-negative bacilli than of Gram-positive cocci survived after the atmospheric drying of suspensions. Variations in the pattern of survival were found, on replicate testing of strains with different death rates, to be consistent. It was also found that strains of *Ps. aeruginosa* isolated from the dust of floors had, in most cases, a lower death-rate on drying than strains isolated from patients. An even larger difference was found between the death-rate on drying of *Ps. aeruginosa* cells from natural sources and the much reduced death-rate of *Ps. aeruginosa* cells resuspended in distilled water

Table 4. *Survival of* Staphylococcus aureus *in suspension*

(+ = presence of growth; − = absence of growth.)

Strain	Phage type	Antibiotic resistance*	Survival in: Ringer's solution 24 hr.	Ringer's solution 48 hr.	Ringer's solution 96 hr.	Deionized water 24 hr.	Deionized water 48 hr.	Deionized water 96 hr.	Tap water 24 hr.	Tap water 48 hr.	Tap water 96 hr.
FS 1	52A/80	P.T	0·6%	+	−	−	−	−	−	−	−
FS 2	3C	Sens.	−	−	−	−	−	−	−	−	−
FS 3	80/81 (1000 × R.T.D.)	T.N.Ne	0·9%	+	−	0·1%	−	−	−	−	−
FS 4	29/52/52A/80	P	0·15%	−	−	−	−	−	−	−	−
FS 5	29/52	Sens.	0·8%	+	−	0·003%	−	−	−	−	−
FS 6	29/77	P.S.T.C.E.N.Ne	0·25%	−	−	0·5%	−	−	−	−	−
FS 7	75/77	S.T.E.Ne	−	−	−	0·005%	−	−	−	−	−

* P = penicillin, S = streptomycin, T = tetracycline, C = chloramphenicol, E = erythromycin, N = novobiocin, Ne = neomycin, sens. = sensitive to all antibiotics tested.

Table 5. *Survival of* Pseudomonas aeruginosa *in suspension*

(+ = presence of growth; − = absence of growth.)

Strain	Phage type	Source	Survival in: Ringer's solution 24 hr.	Ringer's solution 25 weeks	Deionized water 24 hr.	Deionized water 5 weeks	Deionized water 6 weeks	Deionized water 16 weeks	Deionized water 17 weeks	Deionized water 25 weeks	Tap water 24 hr.	Tap water 25 weeks
F 1	21/68/F7	Burn	C.G.*	+	6·2%	+	+	+	+	+	0·68%	+
F 3	.	Burn	C.G.	+	8·5%	+	+	+	+	+	0·19%	+
F 6	68/73/109/119x	Wound B	C.G.	+	11·2%	+	−	−	−	−	0·27%	+
F 11	F 8	Dust (Burns Ward)	C.G.	+	12·0%	+	+	+	+	+	0·2%	+
F 12	21/68	Dust (Burns Ward)	C.G.	+	5·7%	+	+	+	−	−	0·2%	+
F 13	7/31/352	Ward floor	C.G.	+	2·2%	+	−	−	−	−	0·12%	+
F 15	68/73/109/119x/+	Ward floor	C.G.	+	1·9%	+	+	+	+	+	0·57%	+

* Confluent growth.

after they had survived one drying. These dried organisms, however, reverted to their original survival pattern on subculture; the reduced death-rate of the cells which had survived one drying was, presumably, an aspect of the greater viability of the survivors than of the great majority of cells in the original suspensions.

Differences in viability on drying of strains from infections in a ward would be expected to lead to some selection in dust of the strains which survive better after exposure to drying. This was supported to some extent by the different phage types of *Ps. aeruginosa* found on the floor in a burns ward and in the burns of patients occupying the ward. Though *Ps. aeruginosa* is not commonly found in air samples, it is usually found in the dust of burn wards, and dust-borne or air-borne *Ps. aeruginosa* can be an important source of infection in burns (Lowbury & Fox, 1954; Lowbury, 1954; Hurst & Sutter, 1966).

The contrasted pattern of survival of *Staph. aureus* on the one hand and of *Ps. aeruginosa* on the other are well illustrated in this study. The staphylococci not only survived drying better than the strains of pseudomonas, but they were also shown to survive less well than pseudomonas in deionized water. There were some unexpected observations. *Staph. aureus* showed better survival in deionized water during the first 5 hr. than *Ps. aeruginosa*, which showed a large reduction in numbers during that period, but then, unlike the staphylococcus, persisted in small numbers for several weeks. In Ringer's solution, on the other hand, a strain of *Ps. aeruginosa* multiplied appreciably during the first 5 hr. and also survived for many weeks.

The relevance of these findings to the epidemiology of infection appears obvious, and they are consistent with the observed tendency of *Ps. aeruginosa* to be transferred in solutions and in moist vectors, while *Staph. aureus* is more likely than *Ps. aeruginosa* to be transferred by air. These generalizations must, however, be qualified in the light of other evidence from laboratory and field study. For example, strains of staphylococci vary greatly in their death-rate subsequent to drying, and some strains die out when dry almost as quickly as the Gram-negative rods; this is consistent with the rapid elimination of a *Staph. aureus* from a ward after the departure of a patient carrying staphylococci of the same type (Skaliy & Sciple, 1964), and from a wall contaminated by the fingers of a heavy carrier (Ayliffe, Collins & Lowbury, 1967).

Not only the viability but also the numbers of bacteria in the environment and their access to the patient will determine the role of environmental contaminants in causing infection. For example, *Staph. aureus* is very common in floor dust, but the small amount of redispersal of these organisms from the floor into the air makes their transmission by this route improbable (Ayliffe *et al.* 1967); the more likely sources of *Staph. aureus* are by direct contact transfer from carriers or by direct airborne contamination from heavy dispersers of the organisms. *Ps. aeruginosa*, unlike *Staph. aureus*, is rarely carried in large numbers by normal subjects, but in fluids it not only survives better than the staphylococcus but may multiply in water with few nutrient additives. This capacity may be important not only in making contaminated eyedrops and other solutions an important source of infection, but also, perhaps, in allowing the multiplication of *Ps. aeruginosa* from

very small inocula on sloughs, in the aqueous humour (see Crompton, 1962), and in the cerebrospinal fluid. The same potentiality of growth from small inocula makes it possible for *Ps. aeruginosa* to be transferred in airborne dust and on the hands of nurses, where the numbers of the organism, if present, are usually very small, as well as in the more commonly recognized fluid vectors.

SUMMARY

Suspensions of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes* and micrococci were tested for survival on drying and after drying in the atmosphere. The proportion of Gram-negative bacilli that died during drying was greater than that of the Gram-positive cocci, but survival after drying was similar in the two groups of organisms. There were considerable differences in the death-rates on drying of different strains of bacteria, which were consistent on replicate sampling.

A suspension of *Ps. aeruginosa* prepared from cells which had survived one drying showed a considerably higher proportion of survivors on a second drying; suspensions prepared from subcultures of the dried cells, however, showed a death-rate on drying which was similar to that of the original culture.

Strains of *Ps. aeruginosa* isolated from floor dust showed a significantly higher proportion of survivors immediately after drying than strains from patients, but the proportion of survivors after 24 hr. of exposure to the atmosphere was approximately the same in the two groups.

Tests were made for survival of *Ps. aeruginosa* and *Staph. aureus* in deionized water, tap water, physiological saline and Ringer's solution. In deionized water, *Ps. aeruginosa* showed a rapid initial loss but some survival for several weeks; *Staph. aureus*, on the other hand, showed an initial increase in numbers but no survivors after 48 hr. In Ringer's solution all strains of *Ps. aeruginosa* multiplied rapidly and survived for many weeks; *Staph. aureus* died rapidly and no strain could be detected after 4 days.

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REFERENCES

Ayliffe, G. A. J., Collins, B. J. & Lowbury, E. J. L. (1967). Ward floors and other surfaces as reservoirs of hospital infection. *J. Hyg., Camb.* **65**, 515.

Bardsley, D. A. (1948). A study of coliform organisms in the Melbourne water supply and in animal faeces, with observations on their longevity in faeces and in soil. *J. Hyg., Camb.* **46**, 269.

Brown, V. I. & Lowbury, E. J. L. (1965). Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. *J. clin. Path.* **18**, 752.

Crompton, D. O. (1962). Ophthalmic prescribing. *Australas. J. Pharm.* 30 Oct., p. 1020.

Hellat, A. (1948). Studies on the self-disinfecting power of the skin. *Annls Med. exp. Biol. Fenn.* **26**, supplement 8.

Hurst, V. & Sutter, V. L. (1966). Survival of *Pseudomonas aeruginosa* in the hospital environment. *J. infect. Dis.* **116**, 151.

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

LOWBURY, E. J. L. (1954). Air conditioning with filtered air for dressing burns. *Lancet.* i, 292.

LOWBURY, E. J. L. & FOX, J. (1953). The influence of atmospheric drying on the survival of wound flora. *J. Hyg., Camb.* **51**, 203.

LOWBURY, E. J. L. & FOX, J. (1954). The epidemiology of infection with *Pseudomonas pyocyanea* in a burns unit. *J. Hyg., Camb.* **52**, 403.

SKALIY, P. & SCIPLE, G. W. (1964). Survival of staphylococci on hospital surfaces. *Archs envir. Hlth* **8**, 636.