

Survival of Bacteria on Metal Surfaces

T. R. WILKINSON

U.S. Naval Medical Research Unit No. 1 and Naval Biological Laboratory, School of Public Health,
University of California, Berkeley, California

Received for publication 13 August 1965

ABSTRACT

WILKINSON, T. R. (University of California, Berkeley). Survival of bacteria on metal surfaces. *Appl. Microbiol.* 14:303-307. 1966.—Survivor curves were determined for *Serratia marcescens*, *Sarcina lutea*, *Pasteurella tularensis*, and *P. pestis* deposited from the airborne state onto metallic surfaces and subsequently stored at various humidities and temperatures. Cells of all species tested remained alive longest in a dry atmosphere, except that cells of *S. marcescens* survived best in a saturated atmosphere. Survival decreased most rapidly at the intermediate humidity level for three of the test organisms, yet *P. tularensis* died most rapidly in a saturated atmosphere. An increase in temperature decreased survival of *P. pestis* and *P. tularensis*.

The ability of bacteria to survive on various surfaces is important to studies of sanitation, sterilization, food handling and production, and epidemiology. Survival patterns of microorganisms deposited from the airborne state onto metal surfaces, a more natural form of deposition, have not been studied. To date, only test suspensions applied directly to surfaces have been considered.

McDade and Hall (13) demonstrated that 10^8 cells of several gram-negative organisms stored on a variety of surfaces survived 48 hr at 25 C in an intermediate or high humidity range. Surfaces inoculated with *Staphylococcus aureus* (11, 12) exhibited optimal survival at 25 and 50 C in a relatively dry state. Common wound bacteria, dried on glass slides and exposed to indoor atmospheric conditions, indicated a sharp decline in survival in 100 min, followed by a slower death rate (10). Cotton, glass, wood, paper, and metal were exposed to *Shigella sonnei* at a variety of temperatures at ambient relative humidities; longest survival occurred at -20 C, shortest at 45 C (15). Lidwell and Lowbury (9) found that certain pathogens would multiply in dust when the humidity was near saturation.

The gravitational deposition of microorganisms onto surfaces closely approximates nature's way of contamination. By use of an aerosol chamber, defined surfaces were contaminated with a high concentration of viable cells; subsequently, their viability was measured in relation to relative humidity (RH) and temperature.

MATERIALS AND METHODS

Organisms. *Serratia marcescens*, 8 UK; *Sarcina lutea*, Fort Detrick strain; avirulent *Pasteurella pestis*, strain A-1122 (7); and *P. tularensis*, strain LVS (5), were used in this investigation.

Media. *S. marcescens* and *S. lutea* were cultivated in Difco Heart Infusion Broth. These species were diluted in physiological saline containing 1% gelatin and were assayed for viability on Difco Blood Agar Base. *P. pestis* was cultured and assayed for survival in the above Difco media, but the diluent was 1% peptone. *P. tularensis* was grown in SB broth (18) and assayed for viability on SB agar containing 1% human blood. The diluent contained 2.6% sucrose, 2.7% *D*-inositol, 0.38% K_2HPO_4 , 0.1% L-cysteine-HCl, 0.11% KH_2PO_4 , 0.05% L-histidine-HCl, 0.1% thiourea, and 0.002% spermidine- PO_4 in distilled water (pH 7.0).

Cultivation. A second-pass 24-hr shake culture was used in all experiments. All organisms were grown at 28 C except *P. tularensis*, which was incubated at 37 C.

Cell deposition. A 20-ml amount of the unwashed 24-hr culture was aerosolized into a 40-ft³ settling chamber (3) by means of a peripheral air direct-spray atomizer with an output of 8.6 ml/min at 20 psi. Microorganisms were not washed free of their growth media before aerosolization, since Kethley, Cown, and Fincher (8) pointed out that naturally occurring airborne cells would possess an outer layer of nonliving material. The atomizing fluid was Difco Heart Infusion Broth for three of the test organisms, and SB broth for *P. tularensis*. Atmospheric condition in the chamber before bacterial aerosolization was 100% RH and 21 C. At all times during aerosolization, the cloud was kept circulating by means of a 6-inch screw-type fan at the base of the chamber. After aerosoliza-

tion, particles were allowed to settle on 1-inch² stainless-steel strips (20 gauge, type 304, no. 4 finish) for a period of time necessary to recover a maximal number of viable cells. The collection period was 1 hr for *S. marcescens* and *S. lutea*, 15 min for *P. pestis*, and 5 min for *P. tularensis*.

Atmospheric control. Contaminated strips were removed from the exposure chamber and were placed in an airtight chamber (1 ft³). The latter was located inside an incubator where humidity was controlled by CaCl₂ beds or by atomized sterile water. The chamber was opened to the incubator air to establish atmospheric conditions and was then sealed. The contaminated surfaces were tested in low (below 20%), intermediate or medium (20 to 80%), and high (above 80%) humidity ranges. RH was measured by means of wet-dry thermometers and an Abbeon humidity indicator (Abbeon Supply Co., New York).

Viable assay. To determine the number of viable cells, a strip was removed from the controlled environment and put into a round, wide-mouth screw-cap jar (84-ml size) containing 10 ml of the prescribed diluent. After vigorously swirling the contents for 15 sec, the solution was serially diluted and then plated on appropriate medium. Viability at any given time was determined by averaging colony numbers from four strips.

RESULTS

Variation in viable cell counts from 24 replicate samples taken immediately after surfaces were exposed to a bacterial aerosol in a saturated atmosphere at 21 C was determined for each species. The 95% confidence intervals were $\pm 30\%$

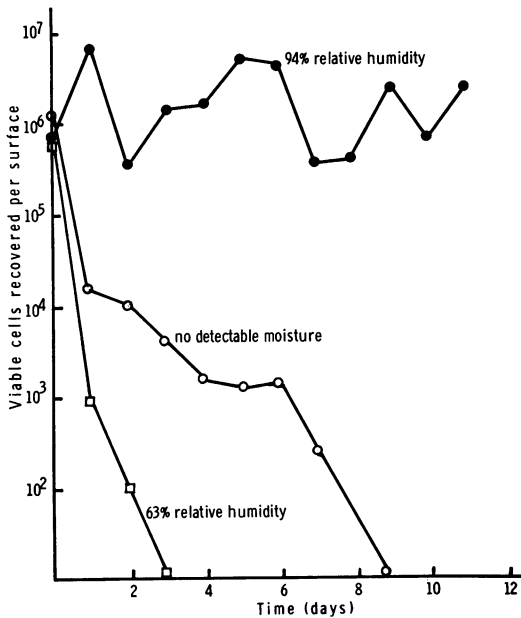


FIG. 1. Viability of *Serratia marcescens* on metal surfaces at 30 C.

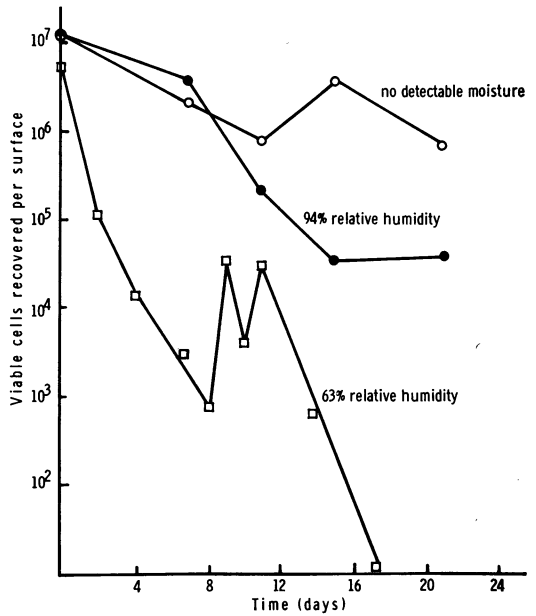


FIG. 2. Viability of *Sarcina lutea* on metal surfaces at 30 C.

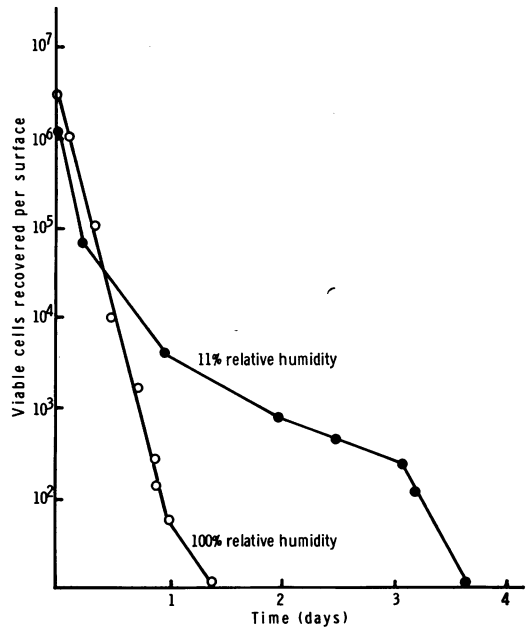


FIG. 3. Viability of *Pasteurella pestis* on metal surfaces at 30 C.

for *S. marcescens*, $\pm 14\%$ for *S. lutea*, $\pm 12\%$ for *P. pestis*, and $\pm 15\%$ for *P. tularensis*. These percentages include a minimal variation due to biological stress, and a maximal variation attrib-

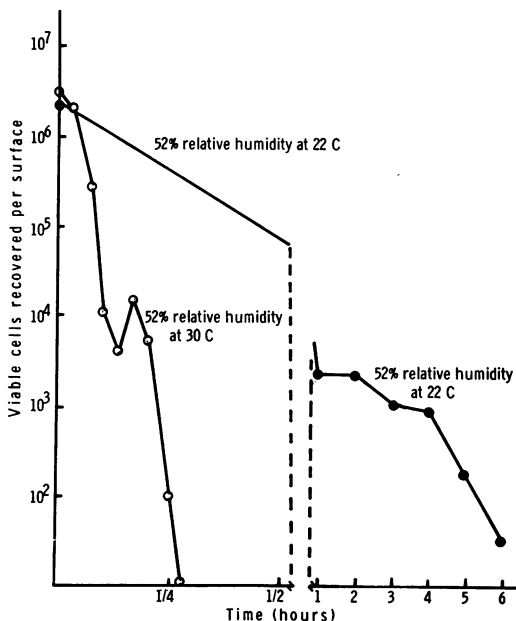


FIG. 4. Viability of *Pasteurella pestis* on metal surfaces.

uted to the efficiency of removing living cells from a metal surface to a growth medium. When contaminated surfaces were sampled after a period of time in a controlled atmosphere, the number of living cells from the four strips usually showed less than a twofold spread from their mean, but sometimes isolated points varied as much as 100-fold without correlation with time, temperature, or humidity. This variation beyond the 95% confidence limits set above cannot be attributed to the mechanics of the experiment, but must be arising from environmental stress on individual cells within the population. Nevertheless, the general survival curve of each species at the prescribed temperature and relative humidity was repeatedly observed.

Figure 1 represents typical results obtained from experiments with strips held at 30 C at high, medium, and low humidity. When contaminated strips were held at 94% RH, there was essentially no decline in number of viable cells after 11 days. A 4-log decline in viable organisms resulted after they were stored 7.5 days in a dry atmosphere. Shortest survival was noted at the intermediate humidity level (63% RH), in which a 4-log decline had occurred in 2 days.

The influence of 0, 63, and 94% RH at 30 C on the viability of approximately 10^7 cells of *S. lutea* per surface is shown in Fig. 2. Over a 21-day period, viable cells per surface decreased approximately 1 log in a dry atmosphere, and 3

logs in a saturated RH. However, in an intermediate humidity, a 4-log drop was evident in 15 days. During this latter experiment, an increased number of viable cells was repeatedly observed after 1 week's holding period, but, because of biological variation, the exact percentage increase occurring after 6 to 8 days at this hu-

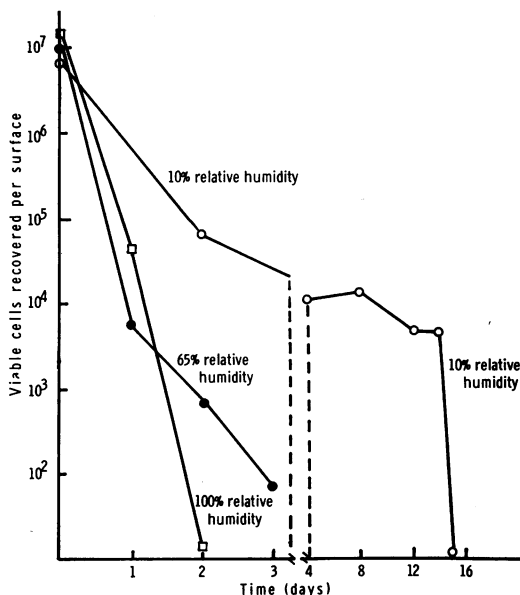


FIG. 5. Viability of *Pasteurella tularensis* on metal surfaces at 25 C.

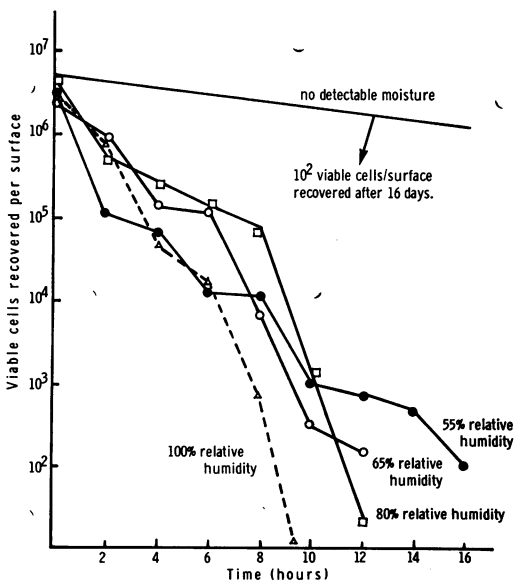


FIG. 6. Viability of *Pasteurella tularensis* on metal surfaces at 37 C.

midity and temperature could not be established. A typical example of this phenomenon is illustrated in the figure.

The survival of *P. pestis* on metal surfaces under the influence of saturated, intermediate, and low humidity at 30 C, along with viability at 22 C and intermediate RH, is illustrated in Fig. 3 and 4. At 30 C, in an atmosphere of 11% RH, 10^6 viable organisms decreased 4 logs in 78 hr; in a saturated environment, a 4-log drop was evident in 24 hr. Cells died most rapidly at the intermediate humidity (52%) level: a 4-log decline in viability in 14 min. Similarly, a decrease in temperature (22 C) caused an increase in survival time to 5.3 hr at 52% RH. Repeated observations showed a slight increase in cell recovery after approximately 8 min of holding in the intermediate humidity range at 30 C, but not at 22 C.

The influence of various humidities (10, 65, and 100% RH) on the survival of *P. tularensis* on metal surfaces held at 25 C is illustrated in Fig. 5. At an initial concentration of 10^7 cells per surface, a 4-log drop in viability was evident after 14.5 days at 10% RH, after 2.5 days at 65%, and after 1.5 days at 100% RH.

Figure 6 illustrates typical data on the survival of *P. tularensis* on metal surfaces stored at 37 C under various humidity conditions (0, 55, 65, 80, and 100% RH). A 4-log drop in viability was observed over a 16-day period, when 10^8 viable cells per surface were held in a dry environment. A 4-log decrease from the initial 10^8 cell concentration per strip was apparent after 15 hr at 55% RH and after 11 hr at 65% RH. When RH was increased to 80%, the initial viable cell concentration dropped 4 logs in 10.5 hr; under saturated conditions, a similar drop was revealed in 8.3 hr.

DISCUSSION

Aerosol deposition, as described in this work, proved to be very successful, since it provided a sufficiently high and replicable initial bacterial recovery to permit determination of the survival of the population for extended times. The method most commonly employed to investigate bacterial survival on surfaces was to contaminate the surface with a liquid suspension. Investigators, such as McDade and Hall (11, 12, 13) and Nakamura (15), demonstrated much lower initial contamination of their surfaces (10^8 organisms per surface) with use of a liquid suspension, as compared with the aerosol deposition method (10^6 to 10^7 organisms per surface).

Intermediate humidity caused the most damaging effect on cells of *S. marcescens* held on metal surfaces. Others have reported varied

results. For example, Monk and McCaffrey (14) and Bateman et al. (1) found that 33% RH was most detrimental to these cells. Webb (17) showed a decrease in viability of airborne *S. marcescens* as the humidity increased. Hemmes (6) demonstrated the shortest survival of this airborne organism at 50% RH.

Intermediate humidity was found to be lethal also to *S. lutea* and *P. pestis* on metal surfaces. However, sensitivity to intermediate RH was not apparent in the survival of *P. tularensis* on metal surfaces, even though Beebe (2) and Schlamm (16) showed greatest aerosol decay of this organism at the intermediate range. Viability of this organism on metal surfaces decreased with an increase in humidity and temperature. It is evident that the method of testing or the location of the test organism will cause a shift in the observed survival pattern as related to humidity.

Survival patterns of the test organisms on metal surfaces were established, but the nature of the observed data precluded calculation of decay constants. However, these results do show that one cannot always extrapolate survival curves to estimate "sterility" times, especially at concentrations below the 0.01% level. To obtain realistic information, viability assays should be continued beyond the holding period normally considered adequate. *S. lutea* demonstrated a 6-day stationary stage or possibly an increase in viable cells recovered (recuperation) after a 99.9999% reduction, which most certainly would have been unobserved if one had ceased sampling prior to this point and had extrapolated to zero recovery. Dimmick (4) observed a recuperation phenomenon with *S. marcescens* and pointed out that a cell is not necessarily dead because it does not reproduce itself at a specified time and on a particular medium. This theory could explain the variation that was observed from strip to strip. Cells in the system were under various degrees of physical stress due to aerosolization, cell clumping, trapped moisture, residual media, etc., which would certainly upset the cellular mechanisms and thus influence replication. Before one can firmly fix the decay rates of organisms on metal surfaces as influenced by temperature and humidity, one must be able to control, if not understand, this biological variation.

ACKNOWLEDGMENTS

I thank W. R. Leif for his guidance and stimulation on this project. I am also indebted to R. C. Altis, H. A. Correa, and J. E. Hebert for their technical assistance.

This investigation was sponsored by the Office of Naval Research under a contract with the Regents of the University of California.

LITERATURE CITED

1. BATEMAN, J. B., P. A. MCCAFFREY, R. J. O'CONNOR, AND G. W. MONK. 1961. Relative humidity and the killing of bacteria. The survival of damp *Serratia marcescens* in air. *Appl. Microbiol.* **9**:567-571.
2. BEEBE, J. M. 1959. Stability of disseminated aerosols of *Pasteurella tularensis* subjected to stimulated solar radiations at various humidities. *J. Bacteriol.* **78**:18-24.
3. DIMMICK, R. L. 1964. A study of airborne microorganisms. *Naval Res. Rev.* **17**:9-13.
4. DIMMICK, R. L. 1965. Rhythmic response of *Serratia marcescens* to elevated temperature. *J. Bacteriol.* **89**:791-798.
5. EIGELSBACH, H. T., AND C. M. DOWNS. 1961. Prophylactic effectiveness of live and killed tularemia vaccines. I. Production of vaccine and evaluation in the white mouse and guinea pig. *J. Immunol.* **87**:415-425.
6. HEMMES, J. H. 1959. De overleving van micro-organismen in lucht. *Drukkeris en Uitgeverij Labor, Utrecht.*
7. JAWETZ, E., AND K. F. MEYER. 1943. Avirulent strains of *Pasteurella pestis*. *J. Infect. Diseases* **73**:124-143.
8. KETHLEY, T. W., W. B. COWN, AND E. L. FINCHER. 1957. The nature and composition of experimental bacterial aerosols. *Appl. Microbiol.* **5**:1-8.
9. LIDWELL, O. M., AND E. J. LOWBURY. 1950. The survival of bacteria in dust. II. The effect of atmospheric humidity on the survival of bacteria in dust. *J. Hyg.* **48**:21-27.
10. LOWBURY, E. J., AND J. FOX. 1953. The influence of atmospheric drying on the survival of wound flora. *J. Hyg.* **51**:203-214.
11. MCDADE, J. J., AND L. B. HALL. 1963. Survival of *Staphylococcus aureus* in the environment. I. Exposure on surfaces. *Am. J. Hyg.* **78**:330-337.
12. MCDADE, J. J., AND L. B. HALL. 1964. Survival of *Staphylococcus aureus* in the environment. II. Effect of elevated temperature on surface-exposed staphylococci. *Am. J. Hyg.* **80**:184-191.
13. MCDADE, J. J., AND L. B. HALL. 1964. Survival of gram-negative bacteria in the environment. I. Effect of relative humidity on surface-exposed organisms. *Am. J. Hyg.* **80**:192-204.
14. MONK, G. W., AND P. A. MCCAFFREY. 1957. Effect of sorbed water on the death rate of washed *Serratia marcescens*. *J. Bacteriol.* **73**:85-88.
15. NAKAMURA, N. 1962. The survival of *Shigella sonnei* on cotton, glass, wood, paper, and metal at various temperatures. *J. Hyg.* **60**:35-39.
16. SCHLAMM, N. A. 1960. Detection of viability in aged or injured *Pasteurella tularensis*. *J. Bacteriol.* **80**:818-822.
17. WEBB, S. J. 1960. Factors affecting the viability of air-borne bacteria. III. The role of bonded water and protein structure in the death of air-borne cells. *Can. J. Microbiol.* **6**:89-105.
18. WON, W. D. 1958. New medium for the cultivation of *Pasteurella tularensis*. *J. Bacteriol.* **75**:237-239.