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IITRI Project L6066
IITRI Report L6066-3 (Final Report)
June 1973 to January 1974

# SURVIVAL OF INFECTIOUS MICROORGANISMS IN SPACE CABIN ENVIRONMENTS

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Prepared for:

National Aeronautics and Space Administration
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Medical Support Branch
Houston, Texas 77058

Attn: B. J. Mieszkuc
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TABLE OF CONTENTS



LIST OF TABLES

## FOREWORD

This report, "Survival of Infectious Microorganisms in Space Cabin Environments," describes experimental studies conduced by IIT Research Institute for the Lyndon B. Johnson Space Center, National Aeronautics and Space Administration, under Contract No. NAS9-12778 during the period June 1973 to January 1974. The IITRI Project Number is L6066. This phase of the studies was designed to determine the survival and virulence of airborne bacteria isolates held in the space cabin environment.

Dr. R. Ehrlich served as the Principal Investigator and Mr. S. Vana as the principal technical assistant. Mr. L. Fleming provided the laboratory assistance.

The experimental data are recorded in IITRI Logbooks C21386, C21424, C21466, C21477, C21547 and C21614.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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## SUMMARY

Aerosol survival and virulence of S. aureus and P. aeruginosa cultures isolated during exposure to simulated space cabin environment was studied using the microthread captured aerosol technique. The aerosol survival of P. aeruginosa isolates did not differ significantly from that of the original culture from which the isolates were obtained. The mean death rate of the isolates was 1.03%/min and that of the controls 1.10%/min. Similarly exposure to the 5 psi environment did not affect the virulence of P. aeruginosa.

Both strains of S. aureus (IITRI and NASA) after exposure to 5 psi environment showed some degree of adaptation to this environmental stress. The aerosol death rates of the isolated organisms were 5 to 10-fold lower than of the original cultures (0.04%/min vs 0.30%/min). At the same time the virulence of the isolates ($LD_{50}$ $2.95 \times 10^8$) was approximately 5-fold higher than that of the original culture ($LD_{50}$ $14.9 \times 10^8$).

Thus the data suggest that airborne S. aureus cells surviving in the simulated space cabin environment might become more resistant to subsequent exposures to the same environment.

# I. INTRODUCTION

The overall objective of this program was to study the survival of airborne microorganisms in a space cabin environment. The more immediate objective of the second phase of the program was to determine whether the resistance of the surviving fraction of selected microorganisms as well as their virulence are altered after exposure to a simulated space cabin environment.

Throughout the experiments the microthread technique first described by May and Druett (ref. 1) was used to simulate the airborne state of the microorganisms. The value of this technique for predicting the behavior of microorganisms carried in free airborne particles has been experimentally confirmed (ref. 2). Thus, the results obtained with captive aerosols on microthreads are regarded as closely approximating those of airborne microorganisms disseminated in an aerosol chamber.

# II. MATERIALS AND METHODS

## A. Space Cabin Environment

The space cabin environment, consisting of 70% oxygen and 30% nitrogen atmosphere at 27,000-ft altitude (5 psi), was simulated in the high altitude environmental chamber (ref. 3). The temperature in the chamber was maintained at 24 $\pm$ 2°C and the humidity at 50 or 90 $\pm$ 10% RH. The oxygen concentration was monitored by a $O_2$ analyzer, Beckman Model F3 while carbon dioxide was monitored by a infrared $CO_2$ analyzer, Beckman Model L-B 15A. Humidity was monitored by a wet-dry bulb temperature recorder and a Hygrodynamics electronic humidity sensing monitor.

## B. Microthread Aerosol Technique

The microthread technique used in the experiments was described in detail in Report No. IITRI-L6066-2 (Phase Report) and is only briefly summarized in this report.

The microthreads used in the experiments were obtained from young spiders of the orb-weaving variety whereby they represent the escape thread used by the spider in its normal activity. The diameters of these threads are related to the size of the spider and vary from 0.05 to 2.5μ. Throughout the studies, small spiders were used to assure microthreads of small and uniform diameter.

The stainless steel frames supporting the microthreads were designed to fit into the aerosol exposure chamber and to facilitiate subsequent handling. A mechanical device was used to uniformly wind the microthreads on the frames. The motor of the device was geared down to drive a threaded rod, which advanced approximately 1 mm per revolution. The wound microthreads were visually inspected for uniformity of spacing, sterilized at 60°C for 2 hr, and stored until used. A frame holder consisting of a 35-ml syringe body served as a protective cover for the wound microthreads. To maintain the frame in place and minimize contamination during storage, a vaccine stopper was placed over the handle of the microthread frame and the frame was inserted into the syringe.

The airborne microorganisms were deposited on the microthread frames in a specially constructed aluminum chamber. Each of the 21 exposure stations in the chamber consisted of a slot cut at right angles to the airflow. The slots supported the body of the frame, thus exposing only the microthreads to the dynamic aerosol. To expose the microthreads to the aerosol, the chamber was inserted into a modified Henderson apparatus located in a microbiological safety cabinet. The dynamic aerosol was generated by a Collison spray fixture which produced a majority of particles of $\leq$5μ mass median diameter.

After deposition of the microbial aerosol the microthreads were removed from the exposure chamber, transferred into storage containers, and placed in the airlock of the high-altitude environmental chamber. The environmental conditions in the airlock were adjusted to coincide with those in the main chamber (5 psi). Upon achieving the equilibrated environmental conditions, the storage containers were transferred to the main chamber and opened to ensure a free flow of the test atmosphere through the microthreads. At 0, 15, 45, 90 and 180 min after initiation of the exposure two frames were transferred from the container into a sterile syringe body. The captured aerosol was resuspended in the collecting fluid contained in a sterile glass vial, which was then passed through an airlock to the ground level for assay.

## C. Microorganisms

The microorganisms used in the studies were Staphylococcus aureus, IITRI, isolated from a rat in our laboratories, S. aureus 52A/79 received from NASA, Pseudomonas aeruginosa ATCC No. 10145, and influenza virus A/PR/8 strain, obtained from Dr. Max Rosenbaum, Naval Medical Research Unit No. 4, North Chicago, Illinois.

To maintain the bacteria trypticase soy agar (BBL) slants were inoculated with the microorganisms biweekly, incubated for 24 hr at 37°C, and stored at 24°C. The growth was harvested, inoculated into 500 ml of trypticase soy broth (BBL) and incubated for 24 hr at 37°C. After incubation, the cultures were centrifuged for 30 min at 2,000 rpm and resuspended in the disseminating fluid consisting of phosphate buffer containing sodium alginate, sucrose, and Dow Corning antifoam. Spores of Bacillus subtilis var. niger (Fort Detrick Lot No. 10-37) serving as a physical decay tracer were mixed with the vegetative bacteria to provide approximately a 10:1 ratio between bacteria and spores.

## D. Bioassay

The captive bacterial aerosol was assayed by placing individual microthread frames in syringes containing 5 ml of gelatin phosphate and glass beads. The contents of the syringe was vigorously agitated for 1 min and transferred to glass vials. Two or three tenfold dilutions were prepared from each suspension and inoculated on duplicate plates of trypticase soy agar (BBL). The agar plates were incubated for 24 to 48 hr at 37°C and the colonies were counted and reported as the number of microorganisms per microthread frame.

## E. Data Analysis

The decay of the microorganisms was measured by first quantitating the numbers of viable bacteria per microthread at the various sampling intervals. The counts were converted to percent recovery, using the count at 0 time as 100% recovery. The slope of the resulting curve, expressed in percent per time unit, defined the death rate of the vegetative microorganisms. For the 180-min exposures, the biological death (decay) rate was expressed in percent per minute (BDR = %/min) values.

The reported geometric mean recoveries and the mean death rates were based on a minimum of six replicate trials conducted for each microorganism. Whenever applicable, the means were compared by the Student's t-test and the significance of the observed differences was reported at the 5% probability level.

## III. RESULTS

The original cultures used in studies of survival of airborne microorganisms in a space cabin environment were utilized to determine if any changes occurred during the exposure that affected the infectivity or viability of the surviving and recovered organisms.

The isolates were obtained by exposing microthreads to an aerosol of the original culture and then exposing the microthreads to 5 psi environment. The bacteria were assayed after up to 30 days of exposure, depending on the survival rate of the test organisms, and individual colonies were isolated. For isolation 0.2 ml of the collecting fluid was plated on duplicate trypticase soy agar plates and after incubation, individual colonies were picked. The isolated colonies were inoculated onto several agar slants of the appropriate media, incubated for 4 to 6 hr, and held overnight at 24 $\pm$ 2°C. To identify the isolates the growth was examined visually, a gram stain performed and the culture was inoculated on differential media. The isolates were held on agar slants at 4°C until used as inoculum for evaluation of their airborne characteristics. The original cultures of the microorganisms were maintained under identical conditions, and served as controls.

### A. Aerosol Survival

The aerosol survival of individual isolates and a pool of the isolates was evaluated. In addition a control, consisting of the original culture from which the isolates were obtained, was included in all experimental trials. In each trial two microthreads were assayed at 0, 15, 45, 90 and 180 min of exposure to the simulated space cabin environment. The humidity in the space cabin environment was maintained at 90% and 50% RH when S. aureus and P. aeruginosa were used, respectively. The aerosol survival

and aerosol decay rates were determined for 12 isolates of P. aeruginosa, ATCC No. 10145, 6 isolates of S. aureus, IITRI strain, and 18 isolates of S. aureus 52A/79, NASA strain.

Figure 1 shows the mean percent recovery of P. aeruginosa at various aerosol ages when maintained in 5 psi environment at 50% RH. The isolate cultures represented microorganisms surviving a 24-hr exposure to the 5 psi environment. It is apparent that the differences in recovery between the isolates and original culture were small. This was further confirmed when the mean decay rates were calculated, which were 1.10%/min for the control culture and 1.03%/min for the isolates (Table 1). The decay rates were very similar to those observed during the initial aerosol characterization studies conducted at 50% RH: (1.35%/min; Report IITRI-L6066-2).

Figure 2 shows mean aerosol recovery of six isolates and the control culture of S. aureus IITRI strain when held for 180 min in the 5 psi environment at 90% RH. The isolates were obtained from microthreads of the original S. aureus culture held in the 5 psi environment for 30 days. The corresponding decay rates are shown in Table 1. The data indicate some increase in the resistance of the isolates. The decay rate of the original culture was 0.30%/min but it decreased to 0.04%/min in the isolates, the differences being significant at $P \leq 5\%$. The mean decay rate of S. aureus culture used to obtain the isolates (0.30%/min) was similar to that of the original culture (0.53%/min at 90% RH) used in studies to characterize the behavior of airborne S. aureus at various humidity conditions (Report IITRI-L6066-2).

Table 1

DEATH RATES OF AIRBORNE MICROORGANISMS DURING A 3-hr EXPOSURE TO 5 psi ENVIRONMENT

| Microorganism | Death Rate (%/min) Mean | 95% C.L. |
|---|---|---|
| *P. aeruginosa* (50% RH) | | |
| Isolate control culture | 1.10 | 0.14 - 2.33 |
| Isolates | 1.03 | 0.90 - 1.16 |
| *S. aureus* - IITRI (90% RH) | | |
| Isolate control culture | 0.30 | 0.05 - 0.54 |
| Isolates | 0.04 | -0.03 - 0.12 |

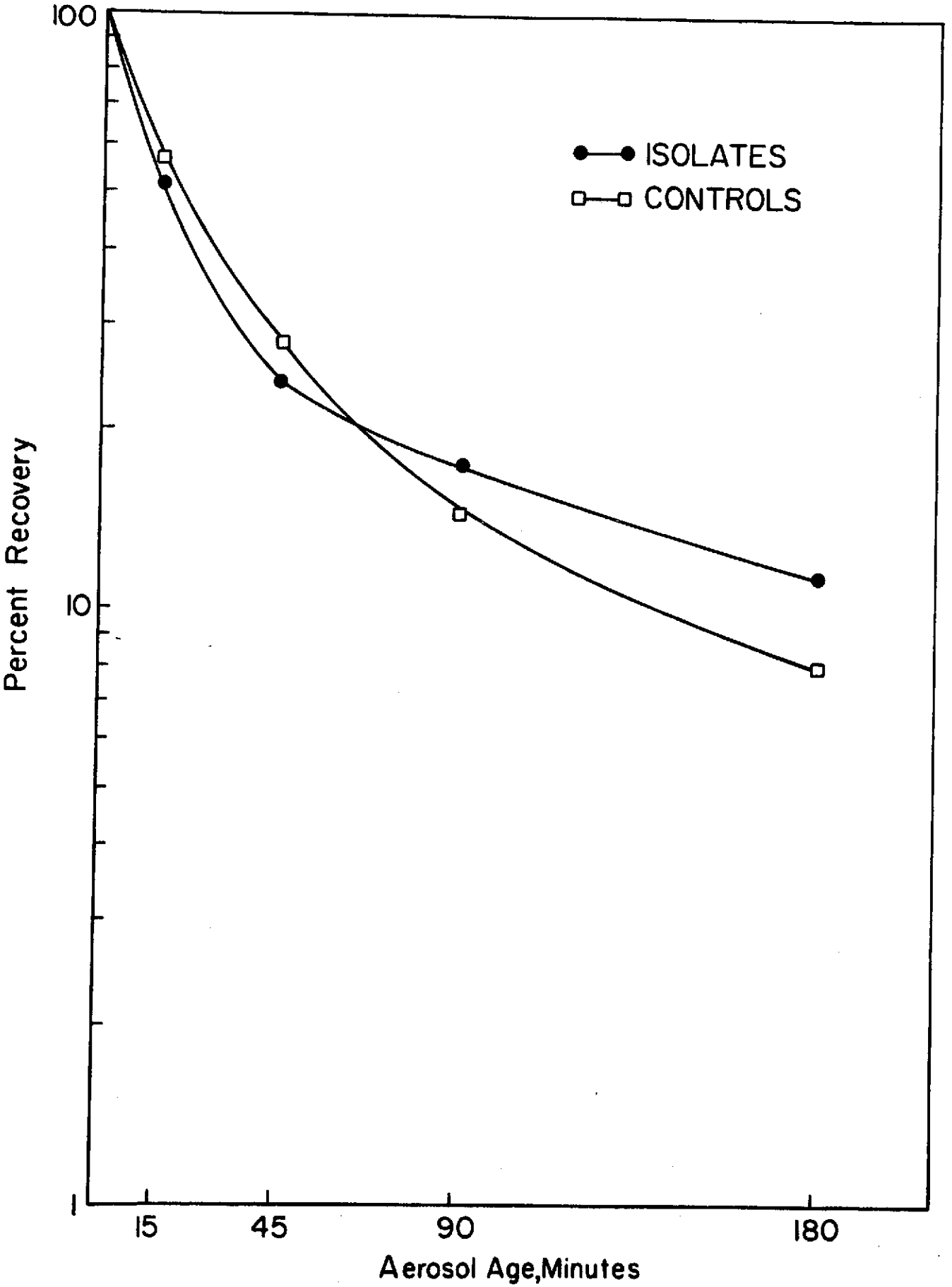


Fig. 1 AEROSOL SURVIVAL OF P. aeruginosa ISOLATES DURING A 3-HR. EXPOSURE TO 5 P.S.I. ENVIRONMENT AT 50% RH

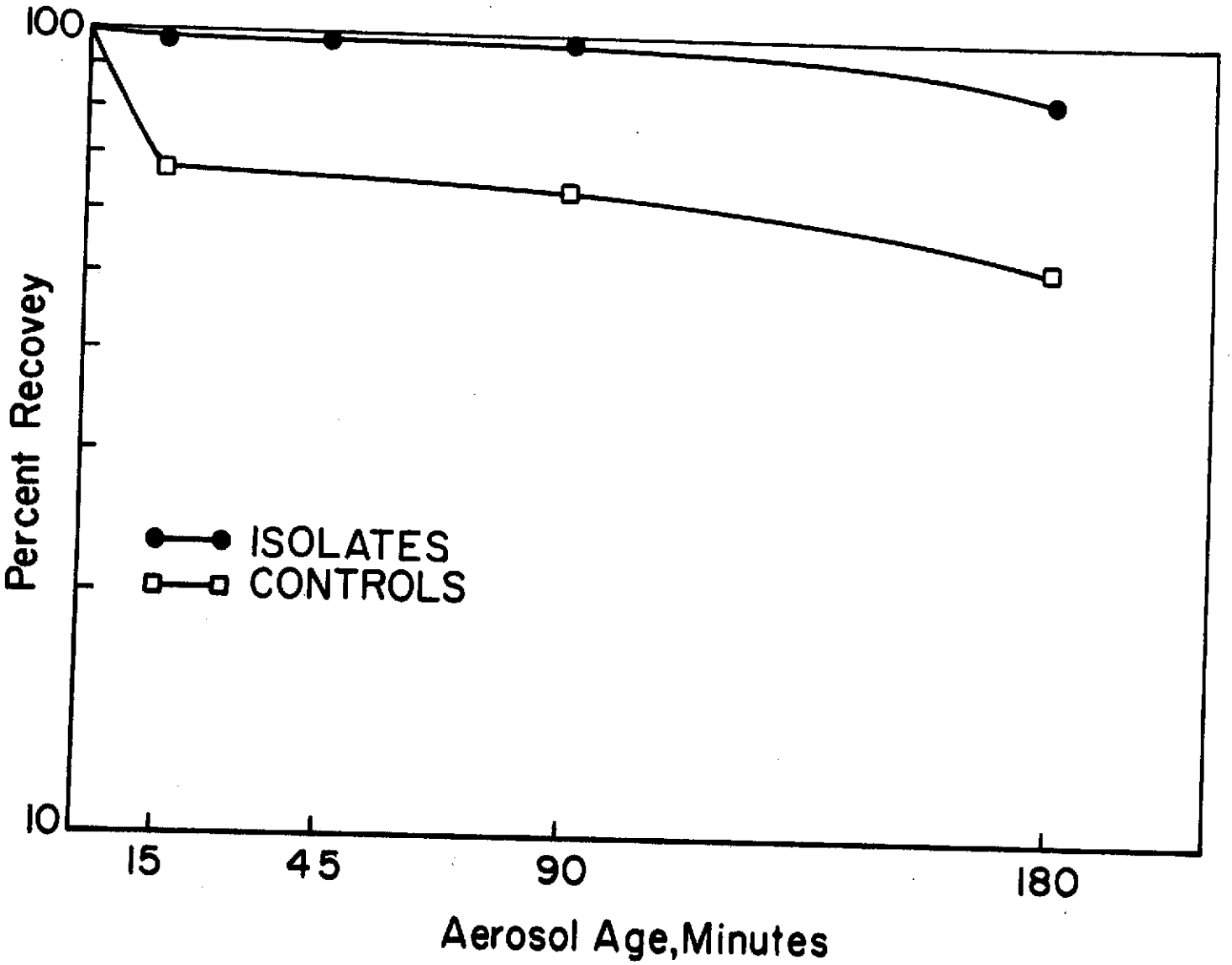


Fig. 2 AEROSOL SURVIVAL OF S. aureus (IITRI) ISOLATES DURING A 3-HR EXPOSURE TO 5 P.S.I. ENVIRONMENT AT 90% RH

The original culture of S. aureus 52A/79 was evaluated thoroughly inasmuch as this organism was not available for the previously conducted characterization studies. Table 2 summarizes the aerosol characteristics of this strain of S. aureus. The 1st group of isolates represents colonies of S. aureus surviving a 30 day exposure to 5 psi environment. The 2nd and 3rd group of isolates represent similar survivors obtained from the 1st and 2nd growth, respectively. Thus the third group of isolates included microorganisms surviving a 90 day long exposure to the 5 psi environment. Only small differences were seen in the percent recoveries at the various sampling points between the isolates, isolate controls and the original culture. Similarly the decay rates were not markedly different; furthermore the decay rates were similar to those estimated for S. aureus IITRI strain.

Of some interest is the observation that the death rates decreased somewhat in the 3rd group of isolates when compared to controls as well as previous isolates. Similar decrease of decay rates was observed in S. aureus IITRI strain. This could suggest some degree of adaptation to the 5 psi environment by S. aureus resulting in increased resistance to this environmental stress.

## B. Antibiotic Resistance

Cultures of the isolates were prepared by inoculating 10 ml of trypticase soy broth (BBL) with the agar slant growth. After 4-6 hours incubation at 37°C, 0.1 ml of the broth was streaked on trypticase soy agar. Antibiotic sensitivity disks (Wilson Diagnostics) were placed on the agar surface by means of sterile forceps and the plates were inverted after 10 min, and incubated for 18-24 hours at 37°C. The results were recorded as antibiotic resistant (R) or sensitive (S) and inhibition zone was measured

Table 2

PERCENT RECOVERY AT VARIOUS AEROSOL AGES AND DEATH RATES OF *S. aureus* 52A/79 AND ITS ISOLATES DURING A 3-hr EXPOSURE TO 5 psi ENVIRONMENT AT 90% RH

| S. aureus | Aerosol Age, min | | | | Death Rate, %/min | |
|---|---|---|---|---|---|---|
| | 15 | 45 | 90 | 180 | Mean | 95% C.L. |
| Original culture | 87.3[a] | 87.9 | 80.2 | 67.9 | 0.19 | 0.13 - 0.25 |
| 1st group of 6 isolates | 84.8 | 80.1 | 84.9 | 67.6 | 0.19 | 0.06 - 0.32 |
| 1st isolate controls | 87.9 | 113 | 76.7 | 57.6 | 0.33 | 0.11 - 0.56 |
| 2nd group of 6 isolates | 88.9 | 83.6 | 78.8 | 60.9 | 0.25 | 0.17 - 0.33 |
| 2nd isolate controls | 98.6 | 83.8 | 73.1 | 71.5 | 0.25 | 0.05 - 0.45 |
| 3rd group of 6 isolates | 83.1 | 74.1 | 88.0 | 67.0 | 0.06 | -0.16 - 0.25 |
| 3rd isolate control | 85.4 | 85.8 | 56.4 | 65.6 | 0.26 | 0.01 - 0.50 |

[a] Mean percent survival based on 100% survival at 0 min aerosol age.

and reported when the diameter was ≤30 mm. The isolates were evaluated individually and compared to the original culture which served as a control.

Table 3 lists the antibiotic discs and their concentrations used in the evaluation. Tables 4 and 5 show the inhibition zones obtained with P. aeruginosa and S. aureus 52A/79, respectively. The sensitivity of P. aeruginosa to the antibiotics was not affected by the exposure to the 5 psi environment. Some, although not significant, changes were observed in the sensitivity of S. aureus to the antibiotics. In general the changes suggested increase in susceptibility to the antibiotics, with the exception of somewhat higher resistance of the second group of isolates to chloramphenicol, chlortetracycline and tetracycline at 30 mcg. The ability of S. aureus 52A/79 isolates to ferment mannitol was evaluated in this series of experiments by growing the organisms on coangulase mannitol agar. After 24-hr incubation all culture showed positive mannitol fermentation and were coagulase positive.

## C. Infectivity

The infectivity of P. aeruginosa and S. aureus recovered after exposure to 5 psi was compared by challenging groups of Swiss albino mice either with the original culture or with the isolates. Inoculum for the challenge was obtained from growth used for dissemination.

To determine the virulence of P. aeruginosa three tenfold dilutions of a 24-hr culture were prepared in phosphate buffered saline and 0.2 ml quantities were injected into mice by the intraperitoneal route. Mortalities were observed during a 14 day holding period at ambient environmental conditions. The $LD_{50}$ values for two groups of isolates and the corresponding controls were as follows:

Table 3

ANTIBIOTICS USED IN MICROBIAL SENSITIVITY PLATE DISC TESTING

| Antibiotic | Code | Concentration |
|---|---|---|
| Ampicillin (Polycillin) | AM + | 10 mcg |
| Chloramphenicol (Chloromycetin) | C + | 30 mcg |
| | C | 5 mcg |
| Chlortetracycline (Aureomycin) | A + | 30 mcg |
| | A | 5 mcg |
| Colistin | CS + | 10 mcg |
| Dihydrostreptomycin | S + | 10 mcg |
| | S | 2 mcg |
| Erythromycin | E + | 15 mcg |
| | E | 2 mcg |
| Kanamycin | K + | 30 mcg |
| Novobiocin (Albamycin) | AL + | 30 mcg |
| | AL | 5 mcg |
| Oxytetracycline (Terramycin) | T + | 30 mcg |
| | T | 5 mcg |
| Penicillin G | P + | 10 units |
| | P | 2 units |
| Polymixin B | PB + | 300 units |
| Tetracycline | TE + | 30 mcg |
| | TE | 5 mcg |

Table 4

ANTIBIOTIC RESISTANCE OF *P. aeruginosa* ISOLATES

| Antibiotic Code | Control | Isolates 1 | 2 | 3 | 4 | 5 | 6 | Control | Isolates 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| C + | 13 | 13 | 15 | 14 | 12 | 12 | 12 | 12 | 12 | 12 | 13 | 12 | 12 | 13 |
| C | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| A + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| A | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| CS + | 14 | 14 | 14 | 14 | 14 | 13 | 14 | 15 | 15 | 15 | 14 | 15 | 15 | 15 |
| S + | R-T | R-T | R-T | R-T | R-T | 11 | R-T | 11 | 11 | R-T | 12 | R-T | R-T | 13 |
| S | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| E + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| E | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| K + | 13 | 12 | 12 | 12 | 12 | 11 | 13 | 12 | 12 | 12 | 12 | 12 | 11 | 11 |
| AL + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| AL | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| T + | R | R-T | R | R-T | R-T | R-T | R-T | 11 | 11 | R-T | 11 | R-T | R | R-T |
| T | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| P + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| P | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| PB + | 16 | 16 | 16 | 16 | 16 | 15 | 16 | 16 | 17 | 16 | 16 | 17 | 16 | 16 |
| TE + | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| TE | R | R | R | R | R | R | R | R | R | R | R | R | R | R |

R = Resistant (no inhibition zone)

S = Sensitive (zone of inhibition >30 mm)

T = Trace (trace of inhibition)

Table 5

ANTIBIOTIC RESISTANCE OF S. aureus (52A/79) ISOLATES

| Antibiotic Code | 1st Isolates | | | | | | | 2nd Isolates | | | | | | | 3rd Isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | 1 | 2 | 3 | 4 | 5 | 6 | C | 7 | 8 | 9 | 10 | 11 | 12 | C | 13 | 14 | 15 | 16 | 17 | 18 |
| AM + | 28 | 27 | 28 | 30 | 27 | 30 | 29 | 28 | 30 | 30 | 30 | 30 | 28 | 28 | 28 | 29 | 27 | 28 | 28 | 25 | 29 |
| C + | S | S | S | S | S | S | S | 15 | S | 16 | 16 | 17 | 15 | 19 | S | S | S | S | S | S | S |
| C | 18 | 16 | 16 | 17 | 20 | 17 | 18 | S | S | S | S | S | S | S | 18 | S | S | S | S | 18 | 20 |
| A + | S | S | S | S | 23 | S | S | 15 | 15 | 15 | 17 | 15 | 13 | 15 | S | S | S | 23 | S | S | S |
| A | 17 | 14 | 16 | 17 | 16 | 18 | 17 | 19 | S | S | 21 | 20 | 23 | 18 | 14 | 18 | 17 | 16 | 15 | 17 | 16 |
| CS + | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| S + | 16 | 18 | S | 20 | 16 | 16 | 15 | 12 | 11 | 11 | 12 | 11 | 11 | 12 | 15 | 15 | S | 15 | 20 | 16 | S |
| S | 12 | 12 | 13 | R | 12 | 12 | 12 | 16 | 19 | 16 | 18 | 16 | 17 | 15 | R | R | 11 | R | 11 | R | R |
| E + | S | S | S | S | S | S | S | 23 | S | S | S | S | S | 21 | S | S | S | S | S | S | S |
| E | 18 | S | 21 | 20 | S | 20 | 20 | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| K + | 22 | 23 | 22 | 23 | 23 | 22 | 22 | 23 | 24 | 26 | 25 | 25 | 23 | 24 | - | 23 | 25 | 26 | 25 | 21 | 23 |
| AL + | S | S | S | S | S | S | S | S | S | S | S | 22 | S | 21 | S | S | S | S | S | S | S |
| AL | 23 | S | S | S | S | 23 | S | S | S | S | S | S | S | S | 20 | S | S | S | S | S | S |
| T + | 22 | S | S | S | 21 | 22 | 23 | 18 | 19 | 19 | 20 | 19 | 20 | 19 | S | S | S | 21 | S | 18 | S |
| T | 21 | 20 | 20 | 20 | 20 | 21 | 20 | 23 | 22 | 22 | 22 | 22 | 20 | 22 | 20 | 19 | 22 | 21 | 21 | 19 | 22 |
| P + | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| P | S | S | S | 26 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| PB + | 13 | 12 | 14 | 13 | 14 | 14 | 14 | 13 | 13 | 13 | 14 | 13 | 13 | 13 | - | 13 | 13 | 13 | 12 | 12 | 13 |
| TE + | S | S | S | S | 22 | S | S | 16 | 16 | 14 | 16 | 15 | 17 | 15 | S | S | S | 21 | S | 22 | S |
| TE | 18 | 15 | 16 | 15 | 16 | 17 | 17 | 21 | 21 | 22 | 22 | 23 | 22 | 21 | 18 | 20 | 20 | 18 | 17 | 18 | 18 |

R = Resistant (no inhibition zone)

S = Sensitive (zone of inhibition >30 mm)

T = Trace (trace of inhibition)

| | $LD_{50}$ | S.D. |
|---|---|---|
| 1st group of P. aeruginosa isolates | $1.13 \times 10^8$ | $0.24 \times 10^8$ |
| 1st group controls | $0.54 \times 10^8$ | $0.34 \times 10^8$ |
| 2nd group of P. aeruginosa isolates | $2.27 \times 10^8$ | $0.74 \times 10^8$ |
| 2nd group controls | $1.90 \times 10^8$ | $0.96 \times 10^8$ |

Although a 1.5 to 2 fold increase in the dose of isolates required to produce 50% mortality in the mice was observed when compared to controls this difference was not significant.

The virulence of S. aureus 52A/79 isolates and control culture was evaluated in a similar manner as that of P. aeruginosa. However, because of the low infectivity observed in the initial experiments only undiluted cultures were used for the subcutaneous inoculation. Although this procedure resulted in mortalities it made the determination of the dose response and thus estimation of $LD_{50}$ difficult. The mean $LD_{50}$ estimated for the 18 isolates was $2.95 \times 10^8 \pm 10^8$ (S.D.), while the $LD_{50}$ for the corresponding controls was $14.9 \times 10^8 \pm 1.9 \times 10^8$ (S.D.). The $LD_{50}$ for the 1st group of six isolates was $1.56 \times 10^8$ ($\pm 0.69 \times 10^8$) and for the second group of six isolates it was $4.66 \times 10^8$ ($\pm 2.23 \times 10^8$) organisms. Thus, the exposure to 5 psi appeared to increase the virulence of S. aureus. However, because of the before mentioned difficulties in estimating the $LD_{50}$, this must be considered as only a tentative conclusion and should be more thoroughly investigated.

To evaluate the infectivity of influenza A/PR/8 virus two microthreads at each sampling period were pooled in 2 ml of PBS and tenfold dilutions of the suspension were prepared in PBS and used for intranasal inoculation in mice. In two replicate trials groups of 6 mice were inoculated with 0.05 ml of each dilution and the undiluted pooled suspension of the virus. The death rates were observed during a 14 day holding period at ambient environmental condition.

The data shown in Table 6 indicate that the mortality rates did not exceed the 50% point, thus the $LD_{50}$ could not be estimated. Nevertheless, it is apparent that the virulence of the virus was not affected by exposure to the 5 psi environment.

## D. Effects of Temperature on S. aureus 52A/79

Limited exploratory experiments were conducted to determine the effect of temperature at ambient environmental condition on the survival of S. aureus 52A/79 aerosols suspended on microthreads.

The methodology in this experiment was essentially the same as previously outlined. For each temperature twenty microthread frames were exposed to an aerosol of S. aureus. After exposure, the frames were divided at random into two groups of ten each. One group of the microthreads was held at the specified test temperature and the control group at ambient room temperature ($24^{\circ}C$). Two frames were assayed from each group at 0, 15, 45, 90 and 180 min of exposure. The test temperature investigated were 0, 37, 60, 80, and $100^{\circ}C$.

Table 6

INFECTIVITY OF INFLUENZA VIRUS EXPOSED TO 5 psi ENVIRONMENT AT 90% RH

| Aerosol Age, min | Virus Dilution | Mortality 5 psi D/T | 5 psi % | Ambient D/T | Ambient % |
|---|---|---|---|---|---|
| 0 | $10^0$ | 5/12 | 42 | 5/12 | 42 |
| | $10^{-1}$ | 2/12 | 17 | 3/12 | 25 |
| | $10^{-2}$ | 0/12 | 0 | 1/12 | 8 |
| 15 | $10^0$ | 3/12 | 25 | 4/12 | 33 |
| | $10^{-1}$ | 1/12 | 8 | 2/12 | 17 |
| | $10^{-2}$ | 0/12 | 0 | 1/12 | 8 |
| 45 | $10^0$ | 2/12 | 17 | 0/12 | 0 |
| | $10^{-1}$ | 0/12 | 0 | 1/12 | 8 |
| | $10^{-2}$ | 0/12 | 0 | 1/12 | 8 |
| 90 | $10^0$ | 3/12 | 25 | 1/12 | 8 |
| | $10^{-1}$ | 0/12 | 0 | 0/12 | 0 |
| | $10^{-2}$ | 1/12 | 8 | 0/12 | 0 |
| 180 | $10^0$ | 0/12 | 0 | 0/12 | 0 |
| | $10^{-1}$ | 1/12 | 8 | 1/12 | 8 |
| | $10^{-2}$ | 1/12 | 8 | 0/12 | 0 |

Figure 3 summarizes the data obtained in a single experiment. The corresponding decay rates were as follows:

| °C | BDR, %/min |
|---|---|
| 0 | 0.18 |
| 24 | 0.15 |
| 37 | +0.08 |
| 60 | 0.58 |
| 80 | 2.54 |
| 100 | 4.13 |

The data suggest a close relationship between environmental temperature and survival of S. aureus. Within the temperature range of 0° to 24°C decay of S. aureus did not vary significantly. At 37°C apparently some growth took place resulting in a positive decay rate. Within the range of 60° to 100°C the decay rates increased progressively, while the recoveries decreased. The effect of temperature was not apparent during the first 45 min of exposure but it markedly affected the viability at the 90 and 180 min aerosol sampling periods.

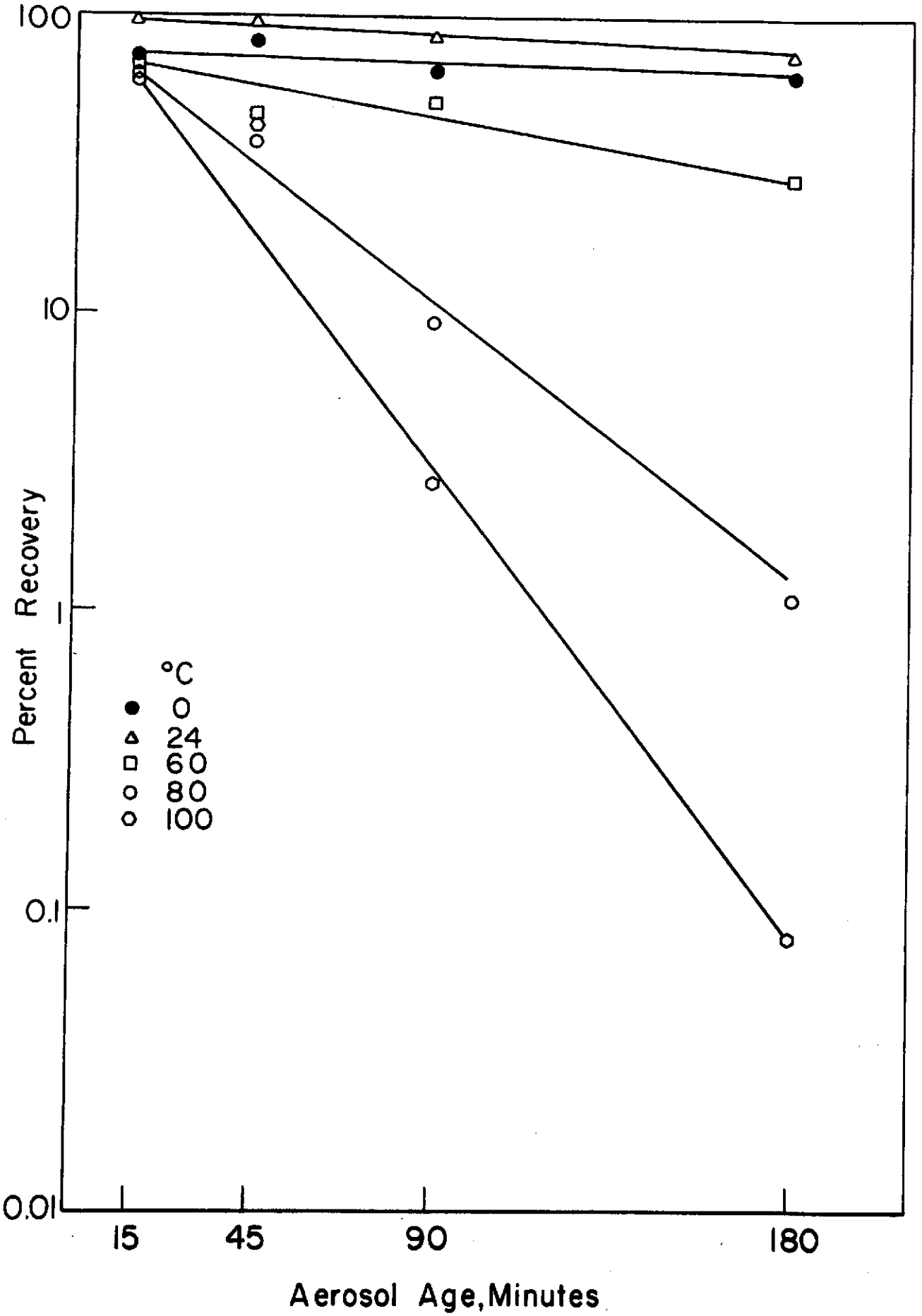


Fig. 3 AEROSOL SURVIVAL OF S. aureus 52A/79 AT VARIOUS ENVIRONMENTAL TEMPERATURES

## REFERENCES


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