Survival of Micro-Organisms in Aerosols Produced in Cleaning and Disinfecting

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RESPIRATORY diseases arising in the alveoli of the lungs; that is, psittacosis, Q fever, pulmonary mycosis, tuberculosis, and inhalation anthrax, are exclusively airborne infections. Numerous other diseases also may be airborne (*1*), and the organisms causing them must become aerosolized in sizes small enough to inhale.

Aerosols less than 5μ in size may pass the barriers in the nose and trachea and move into the terminal bronchioles and alveoli. Aerosols are created when liquid or dry material is subdivided into particles small enough to remain suspended in the air. Most often the aerosol is produced from a liquid substrate, and subdivisions of a liquid may occur by splashing, bursting of bubbles, forcing liquid through small orifices, and by vibrating reeds. The droplets ejected into the air evaporate as they fall, while the residue (droplet nuclei) remain suspended indefinitely, being wafted along in the air until a force exerted on them causes separation (*2*).

This study was made to determine the size of the aerosolized particles produced by cleaning methods and to compare the number of viable organisms surviving in the aerosol produced when either different detergents or disinfectant solutions, or both, were used to clean soiled wall surfaces inoculated with selected micro-organisms. Tenacious soil is frequently removed from the surfaces of equipment, floors, and walls by scrubbing with a brush, and by use of high-pressure spray pumps developed to reduce the time and labor needed for repetitive cleaning in large or inaccessible areas.

The procedures studied are those commonly used in terminal cleaning of quarters housing diseased animals and roosting areas of birds where etiologic agents of histoplasmosis and other respiratory diseases may be concentrated. The study's results, however, may be significant in cleaning any facility contaminated by infective micro-organisms where high-pressure sprays or brushes are used.

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Materials and Methods

The rooms (see photo) studied were located at the National Animal Disease Laboratory, Ames, Iowa. The walls were of concrete plaster, finished with an impervious coating, and room ventilation was adjusted to one change of air each 8 to 10 minutes.

A soiling material, developed by Peabody and co-workers (*3*), containing flour, wheat paste, dried egg yolk, evaporated milk, peanut butter, water, and India ink, for evaluation of steam cleaning procedures, was modified by adding 10 milliliters of bovine serum and one paper towel to each 200-gram portion. The suspension of test organisms was added to this mixture just before application.

A commercial high-presure spray cleaner, with a 6-liter per minute capacity at 500 pounds per square-inch nozzle pressure, was used to apply the solutions to the wall surface. Solutions in the concentrations normally recommended for use on heavily soiled areas were prepared with 51°C. tapwater. The following commercially available disinfectants and detergents were used in the evaluation.

Sodium hypochlorite, liquid bleach type, 0.03 percent available chlorine

Quaternary ammonium (benzalkonium chloride), 0.08 percent (*A*)

Peracetic acid, 2.0 percent solution of 40 percent acid

Phenolic detergent-disinfectant A, 2.0 percent (*B*)

Phenolic detergent-disinfectant B, premeasured dry form (*C*)

Phenolic disinfectant C, 1.0 percent (*D*)

Neutral nontoxic liquid detergent, 0.5 percent solution added to water, sodium hypochlorite, and peracetic acid solutions for detergent effect (*E*)

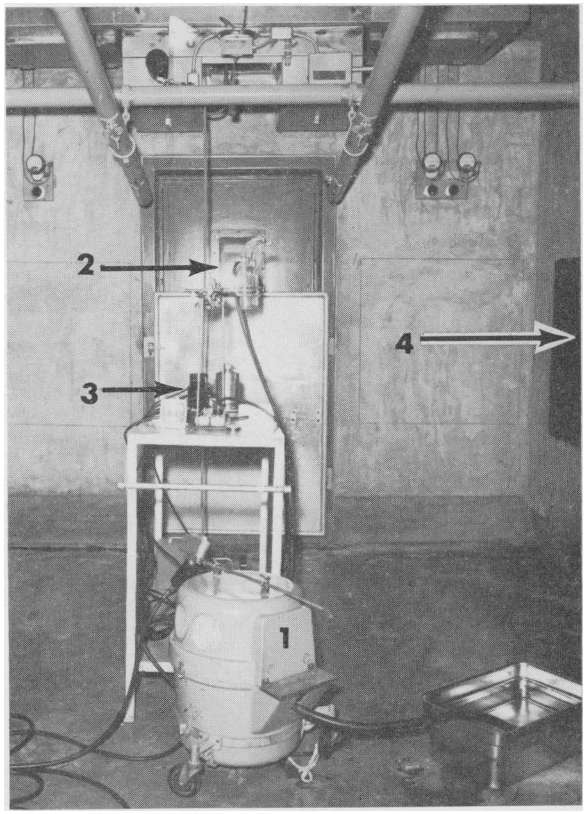
Test organisms selected represented a cross section of micro-organisms generally used in disinfection studies. Selection was based on availability, ease of assay, and relative nonpathogenicity for man. The organisms used were as follows:

Serratia marcescens (ATCC274) was grown for 4 days on standard plate count agar (*F*). Colonies were scraped off the media, placed in nutrient broth, and 10 milliliters of this suspension was added to each 200 grams of the soiling material.

Bacillus subtilis spores were obtained from the U.S. Army Chemical Corps, Fort Detrick, Md. One gram of spores, suspended in sterile water, was added to 200 grams of the soiling material.

Staphylococcus aureus, a coagulase positive strain, meeting the requirements of phenol coefficient testing of disinfectants, was prepared as described for *S. marcescens*.

Mycobacterium smegmatis was grown on Middlebrook 7H10 medium (*G*) and prepared as described for *S. marcescens*.



Testing room showing 1. high pressure cleaner, 2. all glass impingers, 3. Andersen sampler, 4. soil on wall surface

T–3 coliphage, titering 10^9 organisms per milliliter, was prepared as described by Songer and associates (*4*). Ten milliliters of virus suspension were added to each 200 grams of soiling material.

Air samplers. An Andersen sampler (*H*) was used to determine the size range of particles produced during cleaning. Improved all-glass impingers (*I*) were used for collection and subsequent quantitation of the microbial aerosols. Ten milliliters of tryptose broth with Dow Corning nonfoaming agent and appropriate disinfectant neutralizers was used as the impinging fluid.

Neutralizers for impinging fluid. One-tenth milliliter of 10 percent $Na_2S_2O_3$ solution was added to the fluid in each impinger when aerosols containing sodium hypochlorite and peracetic acid were sampled. One milliliter of 10 percent solution of Tween 80 (*J*) was added to each impinger when phenolic disinfectants were sampled, and one-half milliliter of 10 percent Tamol N (*K*) was added to neutralize the quaternary ammonium disinfectant.

Assay procedures. One milliliter portions of undiluted impinger fluid and 1 milliliter portions of diluted 10^{-1}, 10^{-2}, and 10^{-3} of impinger fluid were plated on standard plate count agar. For *M. smegmatis*, Middlebrook 7H10 agar was used. Dilutions of impinging fluid containing T–3 coliphage were assayed by a plaque technique using *Escherichia coli B* as host cells (*4*).

Test procedure. In the afternoon preceding the day of sampling, the organisms were thoroughly mixed with the soiling material. This material was applied to several 1-square meter areas of wall in the test room. The soil was air dried until the following morning when composites of the dried soiling material were collected for assay to assure that approximately equal numbers of organisms were present in the material before the cleaning-disinfection procedure started.

The cleaning and disinfection solution was applied with the sprayer for one-half minute before taking samples in two all-glass impingers. The cleaning and sampling continued for 3 additional minutes as the nozzle of the sprayer was moved back and forth across the test area 15 to 20 centimeters from the surface. After a 2-minute intermission, aerosal sampling with two additional impingers was continued for 3 minutes. At the start of the second minute, the Andersen sampler was operated for the desired time, usually one-fourth or one-half minute. At the end of the sampling period, all samples were refrigerated at 5° C. until assayed. This procedure was repeated three times and duplicate samples were collected each time, providing six determinations for each organism with each disinfectant.

The following formula was used to determine the number of viable organisms per liter of air:

$$\frac{\text{Number colonies or plaques} \times \text{dilution of impinging fluid}}{\text{Number liters of air sampled} \times \text{time in minutes}} = \text{Organisms per liter of air}$$

Results of all six determinations were averaged.

Results

Size of aerosol particles. The Andersen sampler was used to determine the size of the aerosol particles generated by cleaning. The combined results of 46 samples of air taken after spray cleaning and four samples taken after brush cleaning are shown in table 1.

This data in table 1 indicate that about 50 percent of the colonies collected after spraying were 5μ or less and, therefore, small enough to pass the barriers in the upper respiratory tract and penetrate the lungs. The information in table 1 indicates that brush cleaning as well as the high-pressure spray method produced aerosol particles in the size range known to be a respiratory hazard, although with the brush method, a larger proportion of the particles were in the nonhazardous range.

Table 2 shows the number of viable microorganisms recovered per liter of room air during and after each cleaning solution was used.

The numbers of each type of organism recovered varied considerably even when the water-detergent solution used as a base of reference was the cleaning agent. This reference solution exerted little adverse effect on the micro-organisms, thereby producing an aerosol with the maximum number of viable organisms per unit of air. The numbers of organisms in the dried soiling material were relatively consistent at 10^8 to 10^9 per gram.

Differences in the susceptibility of the microorganisms to the various disinfectants occurred with the most resistant being the *B. subtilis* spores. Sodium hypochlorite had the least effect

Table 1. Distribution of organisms (composite results of six determinations) collected on each stage of Andersen sampler after cleaning

Stage	Aerosol size range(μ)	Spray method		Brush method	
		Total colonies	Percent	Total colonies	Percent
1	8.2 and above	6,746	27.5	800	49.2
2	5.0–10.4	6,618	26.9	378	23.2
3	3.0–6.0	5,305	21.6	220	13.5
4	2.0–3.5	3,129	12.7	106	6.5
5	1.0–2.0	2,271	9.2	81	5.0
6	Less than 1.0	496	2.0	43	2.6

on this organism, followed by the phenolic A, quaternary ammonium compound, phenolic B, and peracetic acid, in that order. The action of the hypochlorite on *B. subtilis* is in direct contrast to its effect on the other test organisms.

M. smegmatis is similar to *Mycobacterium tuberculosis* in its resistance to many chemical disinfectants; hypochlorites and quarternary ammonium compounds have almost no effect on this acid-fast organism. Use of the hypochlorite resulted in fewer viable airborne mycobacteria than the phenolics A and B. Use of phenolic disinfectant C resulted in a low concentration of airborne *M. smegmatis*.

Similar results were obtained with *S. aureus* and *S. marcescens*. All disinfectant solutions markedly reduced numbers of aerosolized viable organisms in comparison with water. The greatest reduction occurred with peracetic acid followed by the hypochlorite, and the phenolics were the least effective. In comparison with water, sodium hypochlorite and peracetic acid were the only disinfectants to lower substantially the concentration of T–3 coliphage during and after the cleaning period.

When brushes were used for cleaning with water and with phenolic disinfectant A, fewer *B. subtilis* spores were aerosolized than by the spray cleaning method (table 2). There were more spores in the air, however, during and after the use of the disinfectant than when water was used. Furthermore, the concentration of airborne spores remained high after the cleaning period. The vibrating reed effect of the stiff brush probably was a major addition to the other aerosol producing phenomena present during the spray cleaning.

Discussion

Several factors influence the number of viable micro-organisms aerosolized during the cleaning of a surface with detergent-disinfectant solution—the number of organisms present, their natural resistance to the disinfectant, the protective capacity of the material in which they are embedded, and the ability of the cleaning procedure to produce an aerosol. Ventilation, resistance to irradiation, temperature, and humidity also affect the numbers of organisms surviving in the area after aerosolization. These environmental and conditional factors were not delineated in this study.

Potent disinfectants may kill most micro-organisms before they have a chance to become airborne. This discovery and the fact that peracetic acid also exerts a strong oxidizing action on the aerosolized micro-organisms accounted for the low survival rate of most micro-orga-

Table 2. Number of micro-organisms (average of six determinations) recovered from 1 liter or more of air using various cleaning or disinfecting solutions, or both

Organism and collecting period	Water	Hypo-chlorite	Quaternary ammonium	Phenolics			Peracetic acid
				A	B	C	
Bacillus subtilis:							
Cleaning	560	250	140	160	75	------	4
Post cleaning	400	180	130	140	57	------	(1)
Mycobacterium smegmatis:							
Cleaning	530	16	93	54	53	(2)	(3)
Post cleaning	340	1	51	14	11	(3)	(3)
Staphylococcus aureus:							
Cleaning	1, 900	8	187	334	323	------	(3)
Post cleaning	1, 900	1	85	127	147	------	(3)
Serratia marcescens:							
Cleaning	390	1	4	34	36	------	(3)
Post cleaning	190	(3)	2	5	1	------	(3)
T-3 coliphage:							
Cleaning	27	(3)	28	21	28	------	(3)
Post cleaning	19	(3)	7	16	17	------	(4)
Bacillus subtilis:[5]							
Cleaning	35	------	--------	88	------	------	--------
Post cleaning	22	------	--------	96	------	------	--------

[1] None in 6.0 liters of air. [2] 1 in 3.6 liters of air. [3] None in 3.6 liters of air. [4] None in 7.2 liters of air.
[5] Brush cleaning.

nisms when this disinfectant was used. Many chemical solutions suitable for the disinfection of surfaces are not effective on airborne micro-organisms. The mechanism of action of bactericides in the air is quite different from that in solution (*5*).

The most effective chemicals known for aerial disinfection are the ethylene and propylene glycols and certain α-hydroxycarboxylic acids. The lethal effect of these chemicals appears to be related to a dehydrating action on the organism. The phenolic C disinfectant has propylene glycol incorporated into its formula. However, it was not determined if the glycol was the ingredient responsible for the greater effectiveness of this solution in reducing airborne *M. smegmatis*.

Data from this study indicate that viable micro-organisms are aerosolized in sufficient numbers of suitable size to be a hazard to susceptible hosts or sterile products and processes. Often 10 organisms or less can be considered an infective dose when the particle size is small enough to enter the alveoli of the lung (*2*).

The danger of inhaling 10 liters of contaminated air per minute is easily recognized. Certainly, respiratory protective equipment should be worn by a person cleaning in areas contaminated with human pathogens. This equipment would also permit the use of stronger and more effective disinfectants such as peracetic acid.

The addition of a disinfectant to a cleaning solution cannot be relied on to protect the operator and adjacent areas from the micro-organisms suspended in the air by the cleaning process; however, addition of an effective disinfectant will lower the number of viable organisms. The choice of disinfectant is very important and cannot be made without experimental evidence of its effectiveness against a specific aerosolized organism.

Summary

This study was made to determine the size of aerosolized particles produced by cleaning walls with a sprayer or brush, and to determine the number of viable organisms in the aerosol when different detergents or disinfectants, or both, were used in the cleaning solution.

Suspensions of *Staphylococcus aureus*, *Serratia marcescens*, *Mycobacterium smegmatis*, T-3 coliphage, and *Bacillus subtilis* spores were incorporated separately into artificial soiling material which was then painted on a wall surface and dried. Either disinfectant or detergent solutions, or both, were applied with a high-pressure sprayer or a stiff-bristled brush to clean the wall. The concentration in the room air of particles 10μ or less in diameter that contained viable micro-organisms was determined.

Substantial numbers of each of the micro-organisms were recovered from the room air when water, a quaternary ammonium compound, and three phenolic disinfectants were employed as cleaning agents. No T-3 coliphage and smaller numbers of the other micro-organisms, except *B. subtilis*, were recovered from the air when sodium hypochlorite solution was the disinfectant.

Peracetic acid solution was the most effective in reducing the concentration of aerosolized micro-organisms including the bacterial spores. One phenolic disinfectant was effective in reducing the concentration of viable aerosolized *M. smegmatis*. Assay of the aerosols disclosed that approximately 50 percent of the particles were of a suitable size (5μ or less) to remain airborne indefinitely and could pose a potential disease hazard when inhaled by a susceptible host.

The results of the study indicate that criteria for evaluating the effectiveness of disinfectant solutions for surface application against a specific micro-organism cannot be applied to an aerosol containing the same micro-organism.

REFERENCES

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(*2*) Wells, W. F.: Airborne contagion and air hygiene. Harvard University Press, Cambridge, Mass., 1955.

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(*4*) Songer, J. R., Sullivan, J. F., and Hurd, J. W.: Testing air-filter systems. I. Procedure for testing high-efficiency air filters on exhaust systems. Appl Microbiol 11: 394–397 (1963).

(*5*) Sykes, G.: Disinfection—how, when, where? J Appl Bact 30: 1–5 (1967).

SUPPLY AND EQUIPMENT REFERENCES

(*A*) Quaternary ammonium, Roccal. Winthrop Laboratories, New York, N.Y.
(*B*) Micro-Bac. Economics Lab, Inc., St. Paul, Minn.
(*C*) Premeasured Tergisyl. Lehn and Fink Products Corp., Bloomfield, N.J.
(*D*) Amphyl. Lehn and Fink Products Corp., Bloomfield, N.J.
(*E*) 7X concentrate. Linbro Chemical Co., Inc., New Haven, Conn.
(*F*) Standard plate count agar. Difco Laboratories, Inc., Detroit, Mich.
(*G*) Middlebrook 7-H-10 agar. Difco Laboratories, Inc., Detroit, Mich.
(*H*) Andersen sampler. Andersen Samplers and Consulting Service, Provo, Utah.
(*I*) All-glass impingers. Ace Glass Co., Vineland, N.J.
(*J*) Tween 80. Atlas Chemical Industries, Inc., Wilmington, Del.
(*K*) Tamol. Rohm and Haas Corp., Philadelphia, Pa.

20 Years of Public Health on TV

The Baltimore City Health Department and the Medical and Chirurgical Faculty of Maryland in cooperation with WMAR–TV recently celebrated the 20th anniversary of a television series, "Your Family Doctor," initiated by Dr. Huntington Williams, former commissioner of health of Baltimore.

Through a variety of presentations—films, demonstrations, drama, question and answer sessions, and combinations of these—the production staff has developed an informative, accurate, as well as entertaining program.

The program is centered around a fictitious character, Dr. John Worthington, a general practitioner whose namesake was Baltimore's first health officer appointed by the Governor of Maryland in 1792 to fight yellow fever. The program's basic aims are (*a*) to promote a public understanding of the basic practices for keeping well, (*b*) to encourage consultation with the family physician when there is a question of illness, (*c*) to present public health problems and their local application to the community, (*d*) to inform and familiarize the public with the activities of the city health department, and (*e*) to demonstrate the close working relations between the health department and the physicans in the community.

This series has received an honorable mention award, "For outstanding educational value and distinguished television production," at the Ohio State University Institute for Education by Radio-Television.

Programing of "Your Family Doctor" is guided by a television committee consisting of members of the medical society and the health department. Working with production supervisers are specialist advisers—physicians, health workers, and educators—many who represent the medical faculty and governmental, voluntary, and private agencies. A sample of titles over the years shows the emphasis given to local health programs and problems and education in relation to heart disease, cancer, accident prevention, nutrition, maternal and child health, and environmental health. Also included are programs that have dealt with the World Health Organization, the research functions of large medical centers, the work of voluntary health agencies, careers in health, the historical aspects of medicine and public health, and the relation of medical subjects to the arts.

In recent years this program has been an important means of developing community support for preventive medicine and safety projects. For example the Sabin poliomyelitis campaign, the measles vaccine drive and the mass anti-rabies program were furthered through this TV series, not only in Baltimore but throughout the State. In safety, the series has provided community education in such areas as poison prevention, home accident prevention, automobile safety, eye safety, swimming and boating safety, hunting and fishing safety, and fire prevention.—JOSEPH GORDON, *director, bureau of health information, Baltimore City Health Department.*