Evaluation of a Simple Device for Bacteriological Sampling of Respirator-Generated Aerosols

KENNETH J. RYAN* AND STEPHANIE F. MIHALYI

Clinical Microbiology Laboratories, Department of Pathology, Arizona Medical Center, University of Arizona, Tucson, Arizona 85724

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The Aerotest sampler (Olympic Medical Corp., Seattle, Wash.) is designed to detect bacterial contamination of respirator-generated aerosols in a simple one-step process. The sampler was evaluated by controlled contamination of Bennett AP-5, PR-2, and MA-1 respirators with *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter*. Bacteria present as viable 1.4 to 3.5- μ m particles in each of 77 aerosols were quantitated by using the Andersen viable-particle sampler, and the results were compared with those of colonies obtained from 10-s or 15-cycle Aerotest samples. All aerosols containing counts greater than expected in hospital air were detected by the Aerotest method. Thirteen of 14 aerosols containing <20 bacteria/0.028 m³ showed fewer than 5 colonies, whereas all aerosols containing >1,000 bacteria/0.028 m³ showed at least 40 colonies. Aerosols with intermediate counts fell in between. Under the conditions described here, the Aerotest sampler was able to discriminate between the low levels of bacteria commonly found in hospital air and the bacterial contamination associated with nosocomial pneumonia. The Aerotest sampler provides a practical method for surveillance sampling of respirator nebulizers.

The potential hazard of contaminated inhalation therapy equipment has now been clearly documented both by systematic studies (13) and by reports of nosocomial pulmonary infection related to bacterial contamination of nebulizers and other apparatus used for respiratory care in the hospital (7, 10, 14). A number of methods for periodic disinfection of this equipment have been described (1, 5, 6, 9, 12, 15, 16), and there is evidence that programs which reduce the contamination of this equipment decrease the frequency of a type of pneumonia associated with gram-negative bacteria (12). Bacteriological sampling plays an important role in establishing the effectiveness of programs designed to decrease the infection hazard associated with inhalation therapy. Although periodic disinfection of respiratory equipment is widely practiced, the effect of variations in frequency of equipment changes, patient and hospital microbial flora, and the possibility of introduction of bacteria from other sources such as medications (7) make it necessary to demonstrate rather than assume the effectiveness of such programs. One approach is to monitor equipment that has been in use for bacterial contamination prior to changing or disinfecting the respirator circuit. Bacteriological monitoring then serves as a problem recognition device that can

detect failures at multiple points but, of course, does not pinpoint the contamination source.

Since equipment that has been in use is not expected to be sterile, nonquantitative methods such as surface swabbing are difficult to interpret, and the usual quantitative aerosol-sampling techniques are too cumbersome or expensive for routine hospital use. Edmondson and Sanford described a tube-funnel method for sampling nebulization equipment that correlates with results obtained by quantitative air sampling (3) and has been used for in-use evaluation of the frequency of respirator contamination (9, 12). The purpose of this study was to evaluate a commercially available respirator aerosol sampler, which has some advantages of convenience over the tube-funnel technique.

MATERIALS AND METHODS

Sampler. The Aerotest sampler (Olympic Medical Corp., Seattle, Wash.) is currently marketed as a complete unit and consists of a 60-mm petri dish designed so that the plate and lid fit on either end of a sampling cylinder (Fig. 1). The premolded sampling cylinder contains a truncated funnel, through which an aerosol can be directed toward the plate with escape vents around the top to prevent pressure build-up during operation. Once the aerosol stream has been directed through the cylinder onto the surface of the plate, the plate can be detached, cov-

FIG. 1. The Aerotest sampler. Shown as component parts (left) including sampling cylinder, lid, and plate (with colonies); and assembled (right), ready for use by removing lid and placing respirator tubing flush with central cone.

ered with the lid, and incubated like an ordinary petri dish without further processing. For this study, component parts of the sampler were supplied by the manufacturer.

Aerosol generation. The Aerotest sampler was evaluated by comparison with a viable particle sampler in a manner similar to that used by Edmondson and Sanford (3). To simulate the sampling conditions of a clinically contaminated machine, disposable volume ventilator circuits (no. 2234 Inspiron Co., Upland, Calif.) were set up as for clinical use on Bennett AP-5, MA-1, and PR-2 respirators, and the circuit nebulizer was contaminated with clinical laboratory isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* var. *anitratus*. Overnight broth cultures were adjusted to a MacFarland 0.5 turbidity standard, diluted in the 10^{-1} to 10^{-5} range in saline, and added to the nebulizer in succession, beginning with the most dilute solution. For pressure-cycled respirators, aerosols were produced with the machine set at maximum flow and pressure settings that gave a continuous aerosol at considerable velocity. For the AP-5, this was accomplished by turning the pressure and nebulizer settings to maximum. The PR-2 operated in this manner with the terminal flow, sensitivity, rate, and peak flow at maximum, the nebulizer expiration timer off, and the nebulizer inspiration one-half open. Aerosol generation was less efficient with the volume-cycled Bennett MA-1, and no combination of settings would produce a flow comparable to that of the pressure-cycled machines for more than 1.5 s. We elected to operate these respirators at a combination of settings (sensitivity, minimum; peak flow, 80; maximum pressure limit, 80; volume, 2,000; rate, 25) that would give maximal flow for approximately 1.5 s with 0.5-s pauses between cycles. Res-

pirators were run for 10 to 30 s with the nebulizer on before any samples were taken.

Aerosol sampling. Aerosols generated in all experiments were quantitated with an Andersen model 11-000 viable-particle sampler (Andersen-2000, Inc., Atlanta, Ga.) and operated as directed by the manufacturer, using Trypticase soy agar (Difco Laboratories, Detroit, Mich.) in the petri dishes supplied with the sampler. AP-5 and PR-2 respirator effluent tubing was held 1 to 2 cm from the sampler intake for 30 or 60 s, with the vacuum pump adjusted to draw $0.028 \text{ m}^3/\text{min}$. The MA-1 was sampled in the same manner for 45 cycles by using the cumulative positive-pressure time of 60 s as the sampling time, although the tubing was held at the sampler intake for a longer time due to the pauses between cycles. Bacterial colonies appearing after 24 h if incubation at 35°C in stage 5 of the sampler were counted, and the results were expressed as bacteria per cubic meter of air.

Aerotest samples were taken by holding the end of the respirator tubing (without mouthpiece) flush with the funnel portion of the sampling cylinder, forcing the entire flow through the funnel opening onto the plate containing Trypticase soy agar. Sampling time was 10 s for the AP-5 and PR-2 and 15 cycles for the MA-1. Although preliminary studies showed that sampling times of 20 to 30 s and 30 to 45 cycles increased the number of bacteria detected, the shorter sampling times were selected for this study because of convenience. Following sampling, the plate was separated from the sample cylinder, covered with the lid, and incubated at 35°C for 24 h. Colonies developing on the plates were counted, and the results were expressed as colonies per sample.

RESULTS

A total of 77 aerosols were tested by both the Aerotest and Andersen samplers in sets of three to eight per experiment. When early studies showed no difference between the three organisms, the remainder of the experiments were done only with *E. coli*, including all the studies with the MA-1. Nineteen of the aerosols were generated by the MA-1, 15 by the PR-2, and 43 by the AP-5 with no differences in performance, except that it was found to be particularly important to keep the tubing flush with the sampling cylinder cone throughout collection with the volume-cycled MA-1. In general, sampling was easier with the pressure-cycled respirators (AP-5 and PR-2), because the 10-s collection time was shorter than the approximately 30 s required for 15 MA-1 cycles, and the pressure-cycled machines had fewer settings to be adjusted.

The results of a typical experiment are shown in Fig. 2, with both Aerotest and corresponding stage 5 Andersen sampler plates from four aerosols that were generated by sequentially adding 10^{-1} to 10^{-4} dilutions of an overnight broth

FIG. 2. Comparison of Aerotest and Andersen samplers with Bennett AP-5 respirator nebulizer contaminated with serial 10-fold dilutions of *E. coli*. Aerotest plates are shown above corresponding plates from stage 5 of Andersen model 11-000 viable particle sampler. Aerosol bacterial concentrations calculated from the Andersen plates are (left to right) 2, 146, 638, and >4,854 bacteria/0.028 m³ of air.

culture of *E. coli* to the nebulizer. Colonies on the Aerotest plates were counted directly, and those on the Andersen plates were converted to viable particles per cubic meter of air by relating sampling time to corrected particle counts derived from a probability table provided by the company. For each of the four aerosols, the Aerotest plate is shown above the stage 5 Andersen plate derived from the same aerosol. The aerosols contained 2, 146, 638 and >4,854 viable 1.4- to 3.5- μ m particles, as calculated from the Andersen plates. It can be seen that the Aerotest sampler detects bacteria over the same range as the Andersen sampler, and as one approaches its maximum, so does the other.

Correlations of Aerotest and stage 5 Andersen sampler microbial counts for all of the 77 aerosols are shown in Table 1. Forty-two of 43 Aerotest counts greater than 40 colonies correlated with contamination in excess of 100 bacteria/0.028 m³, with 30 of the 43 greater than 1,000. All of the 30 heavily contaminated aerosols (>1,000/0.028 m³) had Aerotest counts of greater than 40, and 22 of the 30 exceeded 200 colonies per plate. Aerosols with less than 20 bacteria/0.028 m³ showed four colonies or less, with the exception of one aerosol that contained two bacteria/0.028 m³ and had an Aerotest count of 5. Of the 25 aerosols between 100 and 999 bacteria/0.028 m³, 24 had 5 colonies or greater, and 12 had more than 40 colonies on Aerotest plates. Seven of eight aerosols in the

TABLE 1. Correlation of Aerotest counts with the level of bacterial contamination of 77 respirator-generated aerosols^a

Aerotest counts (colonies/plate) ^b	Level of bacterial contamination (bacteria/0.028 m ³) ^c			
	<20	20-99	100-1,000	>1,000
0	4 ^d	0	0	0
1-4	9	3	1	0
5-40	1	4	12	0
41-200	0	1	8	8
>200	0	0	4	22

^a Nebulizers of Bennett MA-1, AP-5, and PR-2 respirators seeded with *E. coli*, *Pseudomonas*, or *Acinetobacter*.

^b Mean of duplicate tests using 15-cycle sample with MA-1 and 10-s sample with AP-5 and PR-2.

^c Viable 1.4- to 3.5- μ m particles as determined by counts in stage 5 of Andersen model 11-000 sampler.

^d Number of aerosols.

20 to 99 bacteria/0.028 m³ range had Aerotest counts between 1 and 40; the exception was an aerosol with 98 bacteria/0.028 m³ with 118 colonies on the Aerotest sample.

DISCUSSION

The primary purpose of programs to monitor bacteriological contamination of respirators is prevention of nosocomial pneumonia. Unfortunately, studies in this area, including this one, do not directly correlate sampling methods or

bacteriological counts with risk or incidence of pulmonary infection. Because such studies are difficult to do, both from a clinical and ethical standpoint, we must currently make our best judgment based on available data. We have chosen to follow the in-use sampling approach developed by workers in Dallas (3, 12, 13), because they included estimates of disease incidence as well as bacteriological data. These studies correlated a decrease in autopsy findings of necrotizing pneumonia with a decreased frequency of contaminated respirators, as determined by in-use aerosol monitoring with the tube-funnel method (12). Since the tube-funnel method had been evaluated by comparison with 1.4- to 3.5- μm viable-particle counts (Andersen stage 5), the same measure was used in this study. This evaluation of the Aerotest sampler can be directly applied only to the in-use sampling approach, and conclusions regarding the relationship of Aerotest counts to risk of disease should be limited to those possible from the Dallas studies.

Our practice has been to collect an aerosol sample by the tube-funnel or Aerotest method just prior to the daily change of respirator circuits. The samples were collected by inhalation therapists and processed by the clinical microbiology laboratory, including quantitation and bacterial identification. Reports were sent to the inhalation therapy department and reviewed by the hospital epidemiologist, so they may be related to patient surveillance and acted on if a break in technique was suspected. If these samples contained no more viable 1.4- to 3.5- μm particles than are generally present in hospital air, we felt it reasonable to assume that the patient had not been exposed to a significant infectious hazard via his respirator. In practice, most samples are negative, with positive cultures appearing only sporadically, most likely representing patient or environmental contamination of the respirator. An increase in the contamination rate or the appearance of a pattern of positive cultures should lead to an investigation to determine the source, usually by methods other than aerosol testing. For example, we were alerted to an irrigating solution in an intensive care unit containing large numbers of *Serratia* by the appearance of the organism in multiple aerosol samples. The solution and the person responsible for the break in procedures were detected by a prompt epidemiological investigation before any disease occurred.

Whatever the frequency, timing, and nature of bacteriological monitoring, it should be recognized that its purpose is to detect breaks in

technique that could lead to pulmonary infection from a contaminated respirator. Disinfection procedures are one, but by no means the only, source of failure, since multiple factors on the ward, including the patient, could lead to contamination. Knowing that the respirator is sterile at the start of therapy does not remove the risk of respirator-associated pneumonia. In this setting, in-use sampling is useful, since it reflects what is being delivered to patients via their respirator and is likely to detect failures at both the disinfection and ward care level. For this purpose, surveillance methods that quantitatively or semiquantitatively sample the aerosol directly are preferred over less direct methods, such as surface swabbing (2, 11), primarily because (i) the environment in which respirators operate is not sterile, and (ii) high-level contamination of the respirator aerosol is the factor associated with pneumonia (12, 13). Aerosol sampling may not be appropriate for contamination of disinfected equipment prior to use, since bacteria in the tubing that are not aerosolized will not be detected. Swabbing or broth-rinse procedures would be more sensitive for these purposes. There is a need for sampling methods that are not too expensive or cumbersome to be readily used by inhalation therapists or hospital surveillance officers.

The Aerotest sampler described in this study utilizes the same principle as the funnel-tube method (3) but directs the aerosol stream onto an agar plate rather than a broth. This has the advantage of a single-step procedure, since it is not necessary for the laboratory to add agar prior to incubation as in the tube-funnel method. In addition, this sampler is a single compact unit, which facilitates sample collection by the therapist. A similar approach without the use of a built-in funnel has been described by Nazemi et al. (8). On theoretical grounds, both the Aerotest and tube-funnel methods would be expected to be inefficient for detecting bacteria suspended in small ($<3 \mu\text{m}$) particles, since there is no mechanism to entrap them other than impingement on the agar or broth surface. They also cannot be quantitative, in the same sense as the Andersen viable-particle counter, because neither the exact volume of gas passing through the sampler nor the portion of it contacting the broth or agar surface is known. Results must therefore be expressed as colonies per sample, which is less exact but satisfactory if the device detects contaminated respirators and approximate comparisons with more quantitative samplers can be made. This study was designed to evaluate the Aerotest sampler as a detection device by

using it to test respirator-generated aerosols of known bacterial concentration. It should be recognized that in this evaluation, and in the Dallas studies cited, only the 1.4- to 3.5- μm portion of the aerosol was counted. Although this has been judged an adequate sampling of the particles most likely to penetrate the bronchial tree distal to the terminal bronchioles (13), smaller or larger viable particles may also be important in respirator-associated pneumonia. The data discussed below pertain only to the aerosolized bacteria suspended in 1.4- to 3.5- μm particles, not to the total population capable of deposition in the tracheobronchial tree.

Of the 77 aerosols tested, only those containing fewer than 20 bacteria/0.028 m³ gave negative Aerotest results (no colonies), and, with one exception, all aerosols over 100 bacteria/0.028 m³ showed at least 5 colonies on the Aerotest plate (Table 1). No respirator generating an aerosol with over 1,000 bacteria/0.028 m³ showed fewer than 40 colonies, and most were over 200. These data indicate that the Aerotest sampler is surprisingly sensitive, detecting all contaminated aerosols containing more than 20 bacteria/0.028 m³, but it only gives an approximate quantitation with positive aerosols. For example, in order to include all aerosols containing over 1,000 bacteria/0.028 m³, it is necessary to use an Aerotest count of 40 or greater, which then includes 12 aerosols in the range of 100 to 1,000 bacteria/0.028 m³. However, even if this or any other simple sampler were able to precisely quantitate aerosols in this range, it would not improve our current ability to interpret the results. Until we know more about the infectious risk of various concentrations of aerosolized bacteria, we are left with the evidence from outbreaks and indirect studies that have only associated heavily contaminated respirators with pneumonia.

Thus, under the conditions of these tests, the Aerotest sampler was able to discriminate between low-level contamination, consistent with that found in hospital air (4, 13), and the high-level contamination that has been associated with pulmonary infection (12, 13). Those wishing to use this sampler for monitoring purposes might reasonably conclude that a negative result indicated that the respirator aerosol contained fewer than 20 bacteria/0.028 m³ (1.4- to 3.5- μm range), or no more than the hospital air, whereas a colony count of more than 40 colonies would indicate heavy contamination and a significant risk of nosocomial infection. Aerotest counts between 1 and 40 correlate with aerosol counts in the range of 20 to 1,000 bacteria/0.028 m³, which exceeds the numbers generally found in hospital air, but the clinical sig-

nificance of these intermediate counts is unknown. Until more experience is gained, we should probably regard these low counts as suspicious.

The results described here with the Aerotest sampler are comparable to those reported by Edmondson and Sanford with the tube-funnel method (3). In controlled nebulizer contamination experiments with pressure-cycled respirators, they found negative cultures when aerosols contained fewer than 20 bacteria/7.5 liters of air, whereas aerosols with over 1,000/7.5 liters, showed more than 20 colonies, with most showing more than 100 colonies. Published reports of hospital surveillance performed with the tube-funnel method indicate that in the presence of an effective disinfection program over 90% of respirator aerosols will be negative after up to 24 h of use, with fewer than 5% showing large numbers of colonies (3, 9). Based on this study and our initial experience in hospital surveillance, similar results may be expected with the Aerotest sampler.

A wide variety of respirators, nebulizers, humidifiers, and aerosol generators are currently implicated in nosocomial infection, but relatively few have been studied to determine optimal surveillance sampling methods. The problem for the clinical microbiologist and hospital epidemiologist is magnified by the frequent changes in design and use of this equipment. Until some measure of standardization is achieved, direct quantitative or semiquantitative sampling of the aerosol is more readily interpreted than less direct methods, particularly when the results can be compared with studies that assessed disease prevalence as well as simple contamination (12, 13). In this paper, methods have been described for the use of a simple semiquantitative device for in-use sampling of aerosols generated by both volume and pressure-cycled respirators. Using an Aerotest count of 40 colonies to indicate heavy contamination would include all the aerosols with over 1,000 bacteria/0.028 m³ plus some false positives (100 to 1,000 bacteria/0.028 m³), if that term can be used at our present state of knowledge of infectious risk. In this study, there were no false negatives, i.e., aerosols with over 20 bacteria/0.028 m³ that did not show at least one colony. The Aerotest sampler appears to provide a practical method for detecting significant bacterial contamination of aerosols generated by machines that can be operated under the conditions described.

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