EFFICACY OF ENVIRONMENTAL MEASURES IN REDUCING POTENTIALLY INFECTIOUS BIOAEROSOLS DURING SPUTUM INDUCTION

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ABSTRACT

OBJECTIVE: To evaluate the airborne viable bacterial concentrations generated during sputum induction and their reduction with exhaust ventilation, ultraviolet germicidal irradiation (UVGI), or both.

METHODS: Exhaust ventilation, upper air UVGI lights, and a portable UVGI unit were operated independently or in combination while and after sputum induction was performed for 58 patients suspected of having active tuberculosis. Viable airborne bacteria were sampled with volumetric air samplers, grown on blood agar, and identified with standard techniques.

RESULTS: During and immediately after sputum induction, concentrations of airborne bacteria, particularly respiratory tract or oropharyngeal organisms, increased rapidly, regardless of environmental conditions. The subsequent rate of reduction of airborne bacteria was most rapid with the portable UVGI unit, followed by upper air UVGI with air mixing. Exhaust ventilation achieved high air changes per hour, but efficacy in reducing airborne bacterial concentrations was low. However, the continuous entrainment of bacteria-laden air from the hallway outside may have resulted in underestimation. The efficacy of a wall-mounted upper air UVGI fixture was significantly less if there was no air mixing. The irradiation from this fixture was of adequate germicidal intensity only in a narrow horizontal plane 2.5 m above the floor.

CONCLUSION: Sputum induction was associated with a rapid and substantial increase in airborne bacteria despite the use of exhaust ventilation providing more than 30 air changes per hour, and the adjunct use of UVGI. This emphasizes that healthcare workers involved in similar cough-inducing procedures performed for patients with suspected tuberculosis must wear appropriate personal respirators (*Infect Control Hosp Epidemiol* 2003;24:483-489).

Interest in the nosocomial transmission of tuberculosis was reawakened in the late 1980s and early 1990s when more than a dozen institutions in the United States experienced major outbreaks. Subsequent recommendations to strengthen environmental controls emphasized proper ventilation. Ultraviolet germicidal irradiation (UVGI), although relatively inexpensive, was considered an adjunct measure.[1,2] Simultaneous implementation of administrative, personal, and environmental control measures resulted in rapid reduction of nosocomial transmission in several institutions.[3-6] However, the contribution of the relatively expensive environmental controls[7] could not be distinguished from that of other measures implemented at the same time.

Experimental studies have demonstrated that UVGI can achieve the equivalent of almost 20 air changes per hour (ACH) in killing airborne bacille Calmette–Guérin,[8] can kill airborne *Mycobacterium tuberculosis,*[9] and can prevent transmission of *M. tuberculosis* to experimental animals.[10] Although field studies have documented reduced transmission of other microorganisms,[11-13] no such studies have evaluated the efficacy of UVGI to prevent nosocomial transmission of tuberculosis. Therefore, the use of UVGI remains controversial, particularly because there are safety concerns.

The importance of cough-inducing procedures in the dissemination of airborne infection such as tuberculosis has been demonstrated experimentally,[14] in reports of outbreaks,[15] and in epidemiologic studies of respiratory technicians[16,17] and pulmonary trainees.[18] In 1996, the sputum induction room in our hospital was equipped with a wall-mounted upper air UVGI fixture, and exhaust ventilation providing at least 15 ACH.

We conducted this study to evaluate the efficacy of these environmental controls in eliminating potentially infectious aerosols generated by patients undergoing sputum induction.

METHODS

Setting and Patients

The Montreal Chest Institute is a tertiary-care university hospital specializing in respiratory diseases that

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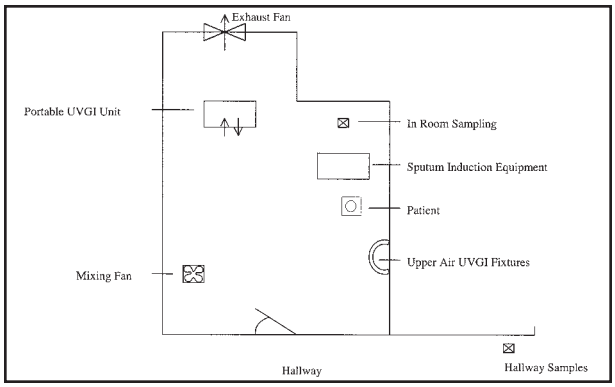

FIGURE 1. Schematic of the locations of environmental controls and sampling sites for the sputum induction room. UVGI = ultraviolet germicidal irradiation.

acts as a screening center for new immigrants to Canada and for tuberculin-positive contacts. Sputum induction is the primary method for bacteriologic investigation of patients with suspected active tuberculosis, but is not performed for other indications. As described elsewhere,[19] patients undergoing sputum induction are seated and inhale hypertonic saline (3%) delivered at 5 to 6 mL/min by an ultrasonic nebulizer (de Vilbiss Ultraneb 99, Sunrise Medical, Somerset, PA). Patients are instructed to breathe deeply and cough intermittently during the procedure. Sputum induction is continued for up to 15 minutes or until the patient is able to produce an adequate sample of sputum (defined as more than 5 mL). The interval between patients is at least 45 minutes.

This project was conducted as part of the quality assurance program of the hospital infection control program. Patients were informed of the environmental evaluation, but consent was not sought, as no patient data were gathered nor were there any patient-related interventions.

Environmental Conditions and Room Layout

As shown in Figure 1, exhaust ventilation was provided by a window-mounted variable speed fan unit that exhausted air directly outside through a high-efficiency particulate air filter (Hepaport, Modern Medical Systems, Farmingdale, NY). This unit provided 31.7 ACH, measured using a previously described tracer gas technique.[20] The window to this room was sealed and could not be opened. Upper air UVGI was supplied from a wall-mounted unit at a height of 2.5 m above the floor. A portable unit was also tested, which had an 85-W UVGI lamp and a variable speed fan with a maximum rate of 200 cu ft/min (Sanuvox Inc., Montreal, Quebec, Canada). The upper air UVGI, portable UVGI unit, and exhaust ventilation were operated so as to evaluate the efficacy of each separately and in combination. Each environmental condition was initiated before the pre-induction samples were taken, and continued at least until the last post-induction sample was obtained.

During the trials with the exhaust ventilation off, a 16-in oscillating pedestal fan was situated in one corner of the room and operated to evaluate the effect of air mixing on the efficacy of the upper air UVGI. This mixing fan was operated (1) not at all; (2) only during sputum induction to evenly mix airborne microorganisms, which enhances the accuracy and reproducibility of the measurement of their rate of removal[20]; or (3) constantly (ie, before, during, and after induction).

Environmental Measures

Before the environmental evaluations began, the dimensions of the sputum induction room, direction of air flow at the door and window, and ACH with the window exhaust ventilation were measured using previously described protocols and instruments.[20] In the morning and afternoon during the days of bacteriologic measurements, temperature, relative humidity, and carbon dioxide were measured using a hand-held direct reading instrument (Veloci-calc, TSI Inc., St. Paul, MN) outdoors, within the room with no patient present, and in the hallway.

The intensity of ultraviolet light was measured with a hand-held photometer with a sensor calibrated to measure ultraviolet C at a wavelength of 254 nm (International Light model IL1400A with model SEL240 sensor, International Light, Newburyport, MA). The intensity of UVGI from the wall-mounted fixture was measured every 5 cm from the floor in four directions and at different distances.

Airborne bacterial sampling was performed outdoors once each day, on the property of the hospital. Within the sputum induction room, all samples were taken at the height of the respiratory therapist's breathing zone (ie, 1.5 m above the floor) while standing and at 1 m from the patient in the direction of the exhaust fan (Fig. 1). Samples were taken in the room, with the door closed, before the patient entered for induction (baseline unoccupied), during induction, and on four occasions after induction. To enhance the functioning of the exhaust ventilation, the lower portion of the door to the sputum induction room had louvers, allowing air to enter. This meant that there was substantial entrainment of air from the hallway. Therefore, samples were also taken outside the room before each induction at a distance of 1 m from the door while it was closed.

The first tests of the portable UVGI unit were performed with the intake placed within a large sealed box and the outflow from the unit in a second large sealed box. Each box had single ports to allow air in or out. One Burkhard sampler (Burkhard Manufacturing, Hertfordshire, United Kingdom) was placed in each box to obtain airborne bacterial samples simultaneously from intake and outflow of the unit. Three pairs of samples were taken at each of three fan speeds: low, medium, and high. A hot-wire anemometer was used to measure air flow from which the volume of air was calculated. To test this portable UVGI unit during sputum inductions, it was

TABLE 1
AVERAGE AIRBORNE BACTERIAL COUNTS* (CFU/M^3) WITHIN THE SPUTUM INDUCTION ROOM UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Condition	Hall-to-Room Gradient† (Hall, Pre-Induction)	Increase‡ (Peak, Pre-Induction)	Decrease§ (Peak, Minimum)	Estimated ACH‖
Exhaust on (n = 29)				
All UVGI off (n = 16)	50	110	322	2.0
Upper air UVGI on (n = 9)	83	342	323	3.5
Portable UVGI on (n = 4)	107	88	156	6.9
Exhaust off (n = 29)				
Upper air UVGI on (n = 25)				
Mixing fan on throughout (n = 9)	247	238	339	4.5
Mixing fan on during cough only (n = 7)	112	308	247	1.8
Mixing fan off (n = 9)	193	271	390	2.5
Portable UVGI on (n = 4)	244	307	345	8.4

CFU = colony-forming units; ACH = air changes per hour; UVGI = ultraviolet germicidal irradiation.
*All samples of bacteria were taken with the door and window of the sputum induction room closed.
†Differences in airborne bacterial concentrations between the hallway and within the unoccupied sputum induction room. Samples were taken simultaneously with the door closed prior to the start of induction.
‡Change in the airborne bacterial concentration within the sputum induction room from pre-induction until peak concentration.
§Change in the bacterial concentration within the sputum induction room from peak level to minimum post-induction levels.
‖Equivalent ACH calculated from the rate of decrease in bacterial concentrations.

placed on the floor, facing the door at a distance 1 m between the patient and the exhaust fan (Fig. 1).

For 47 trials, airborne bacteria were collected by direct impaction onto Petri dishes containing 5% sheep blood agar, using Burkhard volumetric air samplers operated at 15 L/min for 5 to 7 minutes. During 11 trials, the bottom two stages of an Andersen 6-stage sampler (Graseby Andersen, Atlanta, GA) were used to estimate total and respirable airborne viable bacteria. Blank samples were taken by removing the lid of a blood agar plate within the procedure room for 1 minute. After 48 hours of incubation at 37°C, the colony-forming units (CFU) of bacteria were counted manually. Colonies of fungi, identified by their visible morphologic appearance, were not included. The total number of bacterial CFU was converted to CFU/m^3 using the following formula: CFU $\times$ 1,000/sampling time (in minutes) $\times$ flow rate (= 15 L/min).

For species identification, 18 samples were collected on blood agar from 8 patients under 4 environmental conditions. Each colony was identified, first using its morphologic appearance and Gram stain, then using catalase and coagulase reactions for gram-positive organisms and reaction on triple sugar iron medium, nitrate reduction, and oxidase reaction for gram-negative organisms, and finally by spore test for all bacteria suspected to be *Bacillus* species. The VITEK 1 system (bioMérieux, Hazelwood, MO) was used to identify all remaining organisms not otherwise identified.[21] Organisms were classified as respiratory if commonly found in the respiratory tract (including the nose) or oropharynx, such as *Gemella*, *Micrococcus*, or *Streptococcus* species; environmental if generally environmental organisms, such as *Bacillus cereus*, *Flavimonas oryzihabitans*, or *Pseudomonas stutzeri*; or mixed if the organisms could be from the skin, nose, or environment, such as *Corynebacterium*.

Data Analysis

Peak CFU was the highest of the samples taken during induction or the first sample taken immediately after induction, whereas minimum was the lowest of all samples taken after induction. Equivalent ACH for removal of bacterial CFU was calculated as (log peak CFU - log minimum CFU)/(t/60), where t represents the interval in minutes from the midpoint of sampling for peak and the midpoint of sampling for minimum. Differences between average estimated ACH under different environmental conditions were tested for significance with the Student's *t* test.[22]

RESULTS

A total of 362 airborne samples were taken during 58 sputum inductions between October 1997 and April 1998. The sputum induction room had an area of 4.3 m^2, a volume of 13.5 m^3, an average ventilation of 31.7 ACH, an inward direction of air flow, an average temperature of 22°C, and a relative humidity of 30% (pre-induction). All outdoor bacterial concentrations were much lower than those indoors, at least in part because sampling was performed during colder seasons.

As seen in Table 1, there was a substantial increase in airborne bacterial concentrations during or immediately following induction. As shown in Figure 2, a rapid increase in concentrations occurred with most inductions, although there was substantial, and unexplained, variability between patients. This patient-to-patient variability accounted for the differences in average increases between different conditions in Table 1. There was a substantial decrease in bacterial concentrations under all conditions, but this was more rapid under certain conditions, resulting in large differences in estimated equivalent ACH.

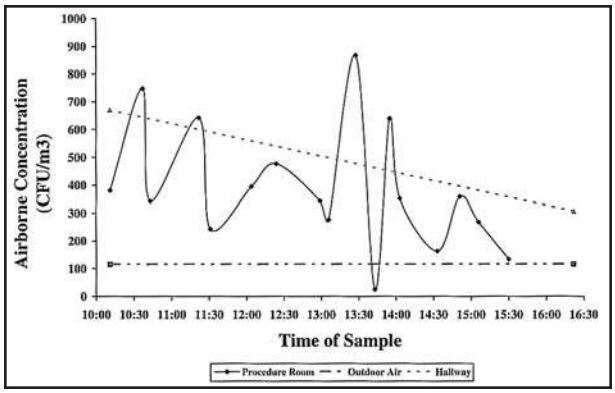

FIGURE 2. Airborne bacterial concentrations before, during, and after multiple sputum inductions in a single day. Upper air ultraviolet germicidal irradiation and the exhaust fan were both on constantly throughout the day. CFU = colony-forming units.

Bacterial concentrations within the induction room were closer to hallway concentrations when the exhaust ventilation was on, compared with when it was off (Table 2). The exhaust fan created strong directional air flow from the hall, into the induction room, and out the exhaust, which would have entrained airborne organisms from the hallway. These would more likely have been environmental, as the hall was generally unoccupied, although some patients and staff may have walked by. Removal of airborne bacteria was more rapid and estimated ACH were significantly higher with UVGI on compared with UVGI off, whereas the portable UVGI unit was significantly better than upper air UVGI and the upper air UVGI lamp with the mixing fan was better than upper air UVGI alone. There was no apparent effect of carbon dioxide concentrations, nor relative humidity, measured within the room before induction on the estimated germicidal efficacy of the interventions. Outdoor air bacterial concentrations were much lower than those indoors (data not shown), most likely because this study was conducted in late fall to early spring.

All organisms were identified for 18 samples taken in the room before, during, and after sputum induction under 4 different environmental conditions. As seen in Table 3, the increase in airborne bacterial concentrations during induction was entirely due to organisms from the oropharynx or respiratory tract. Environmental and mixed type organisms actually diminished, possibly because these organisms entered the room with the patient and the technician while the door was open but then declined steadily, even from the start of induction. Viable airborne fungal organisms were also identified in these 18 samples, but accounted for only 4% of the environmental organisms and thus less than 1% of the total airborne organisms identified.

To assess the possibility that the apparently low efficacy of upper air UVGI was artifactual, due to poor penetration of ultraviolet irradiation into larger particles, an Andersen sampler was used during 7 inductions with this

TABLE 2
COMPARISON OF THE EFFECT OF DIFFERENT ENVIRONMENTAL CONTROLS ON AIRBORNE BACTERIA CONCENTRATIONS (CFU/M^3)

Control	No. of Inductions	Hall-to-Room Gradient* (Prior to Induction)	Increase† (Peak, Pre-Induction)	Decrease‡ (Peak, Minimum)	Estimated ACH§
All measures	58				
Exhaust off	29	247	274	334	3.8
Exhaust on	29	69	179	300	3.2
P‖		.07	NS	NS	NS
UVGI off	16	50	110	322	2.0
UVGI on (all)	42	192	271	315	4.0
P‖		NS	.06	NS	**< .05**
UVGI upper (all)	34	201	289	330	3.1
UVGI portable	8	166	187	251	7.7
P‖		NS	NS	NS	**< .05**
Exhaust off only	29				
UVGI upper (all)	25	248	270	332	3.0
UVGI portable	4	243	307	345	8.4
P‖		NS	NS	NS	**< .05**
UVGI upper, mixed	9	247	238	339	4.5
UVGI upper, unmixed	16	249	287	328	2.2
P‖		NS	NS	NS	**< .01**

CFU = colony-forming units; ACH = air changes per hour; UVGI = ultraviolet germicidal irradiation; NS = difference not statistically significant ($P > .1$).
*Difference between measures in the hallway and measures in the unoccupied sputum induction room prior to sputum induction.
†Change in the airborne bacterial concentration from pre-induction until peak concentration.
‡Change in the bacterial concentration from peak level to minimum post-induction levels.
§Equivalent ACH calculated from the decrease in bacterial concentrations from peak to minimum.
‖The values from testing the significance of differences of means using the Student's *t* test (bold indicates significant).

TABLE 3
AVERAGE AIRBORNE VIABLE BACTERIAL COUNTS* (CFU/M³) BY TYPE, IDENTIFIED BEFORE, DURING, AND AFTER SPUTUM INDUCTION

Type	Before	During	(% Change)	After	(% Change)
Environmental	73	57	(-22)	29	(-49)
Mixed	90	50	(-45)	50	(0)
Respiratory	223	351	(57)	106	(-70)
Total	386	458	(19)	184	(-60)
% of total, respiratory	58	77	(33)	57	(-26)

CFU = colony-forming units.
*Based on 18 samples taken within the sputum induction room with the door and window closed under 4 different environmental conditions: (1) exhaust on, but no ultraviolet germicidal irradiation (UVGI); (2) exhaust on with upper air UVGI; (3) exhaust off with upper air UVGI and mixing; and (4) exhaust off with portable UVGI.

TABLE 4
EFFICACY OF THE PORTABLE ULTRAVIOLET LIGHT UNIT AT DIFFERENT FAN SPEEDS*

Air Volume Setting (cu ft/min)	Trial	Intake (Pre-UVGI) (CFU/m³)	Outflow (Post-UVGI) (CFU/m³)	Mean Efficacy By Trial	Mean Efficacy By Setting
155 (high)	1	116	0	100%	
	2	107	19	82%	92%
	3	420	29	93%	
70 (medium)	4	89	19	79%	
	5	63	10	85%	87%
	6	348	10	97%	
23 (low)	7	170	38	78%	
	8	205	10	95%	88%
	9	116	9	92%	
Overall efficacy, 89%					

UVGI = ultraviolet germicidal irradiation; CFU = colony-forming units.
*These tests were performed with the intake and the outflow of the portable unit flowing within separate large sealed boxes, each with single ports to allow air in or out. One sampler was placed in each box to obtain simultaneous intake and outflow samples.

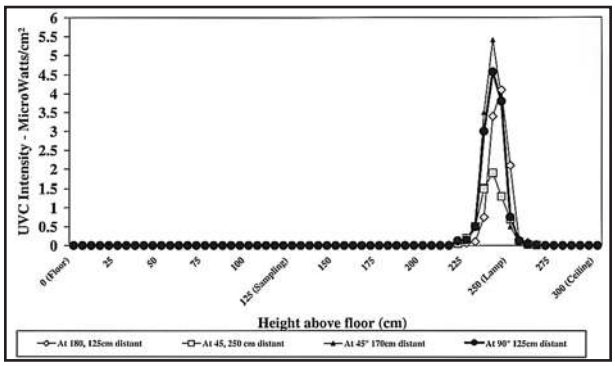

FIGURE 3. Intensity of the upper air ultraviolet germicidal irradiation (UVC) from a wall-mounted fixture by height from the floor, as well as the angle and distance from the fixture.

modality. This permitted estimation of germicidal efficacy for airborne viable bacteria in respirable-size particles. The Andersen sampler was used in 4 additional trials with exhaust ventilation, as clearance of airborne bacteria by this method should not be affected by particle size. In all 11 trials, clearance of bacteria with upper air UVGI was not significantly nor substantively different for bacteria in respirable-size particles compared with all sizes of particles (data not shown in tabular form).

As seen in Table 4, the germicidal efficacy of the portable unit was consistently close to 90% at all fan speeds assessed. At the highest fan speed, the equivalent of the entire volume of air of the sputum induction room should have passed through this unit in 3 minutes. This meant that the unit should have provided the equivalent of 15 ACH in clearing airborne organisms.

As shown in Figure 3, the ultraviolet light from the wall-mounted upper air unit provided irradiation of effective germicidal intensity in a plane only 20 cm wide, centered 2.5 m (8 ft) above the floor or 125 cm (almost 4 ft) above the breathing zone of the respiratory technicians. Even within this narrow horizontal plane, the intensity of the ultraviolet light was less than half in the farthest corner of the room or along the walls where the light was mounted. (For comparison, on a sunny day in late March at 12 noon, the outdoor ultraviolet intensity was 40 $\mu W/cm^2$, measured with the same instrument.)

DISCUSSION

The key finding of this study was the dramatic increase in airborne bacterial concentrations during and immediately after sputum induction, despite the presence of exhaust ventilation providing more than 30 ACH, UVGI, or both. A second important observation was the reduced effectiveness of upper air UVGI fixtures with louvers without mechanical air mixing, likely related to the narrow horizontal plane of ultraviolet light emitted. The performance of cough-inducing procedures for patients with active pulmonary tuberculosis has been associated with a high risk of nosocomial transmission in outbreaks and population-based studies.[16-18] Modeling studies suggesting that environmental controls will be insufficient[23] are corroborated by the rapid and substantial increases in bacterial concentrations with sputum induction measured in this study. These findings demonstrate that cough-inducing procedures can result in bursts of potentially infectious aerosols,

causing short-lived but intense occupational exposure, despite the operation of environmental controls exceeding current recommendations.

Modern, commercially available upper air UVGI fixtures, such as those installed in our sputum induction room, have louvers that release the light in a narrow horizontal plane. These are designed to prevent the occurrence of keratoconjunctivitis and sunburn among occupants from accidental exposure.[24] However, our data suggest that the enhanced safety features of modern UVGI fixtures, releasing light in a narrow plane (Fig. 3), may reduce their germicidal effectiveness relative to earlier designs.[11-13,25] The finding that equivalent ACH was substantially enhanced by adding a mixing fan has been predicted in a modeling study.[26] In settings such as waiting rooms or homeless shelters where UVGI lights may be used,[24] additional fans should also be added to enhance the air mixing.

These findings must be interpreted with caution because of several limitations. We did not measure actual ACH, but rather estimated this parameter based on clearance of airborne viable bacteria. This method would have underestimated the effectiveness of exhaust ventilation, because the high volume of air exhausted outdoors caused rapid entrainment of air from the hallway. This hallway air had significant levels of airborne viable bacteria because of patient and personnel activity there. Therefore, the patient-generated aerosol was likely rapidly exhausted and replaced with other bacteria that would not have the same potential infectious risk. However, the estimates of the relative efficacy of exhaust only or exhaust with various UVGI delivery strategies should have been valid because they were derived from increases and decreases from bacterial levels measured within the induction room prior to each induction. Because the hallways were largely unoccupied, there should not have been major changes in the hallway levels of airborne bacteria from these baseline measures until the end of sampling for each induction. Also, the comparisons between upper air UVGI and the portable UVGI unit and the assessment of the effect of mixing on upper air UVGI with the exhaust fan off should have been valid. During these inductions, the hallway levels should have had minimal effect on the relative efficacy of different UVGI strategies.

We did not measure tuberculosis infection among healthcare workers. This would have required repeated tuberculin testing of many workers. Nor did we measure airborne tuberculosis bacteria, as few patients undergoing induction proved to have active tuberculosis and such sampling is technically difficult because of the low concentration of airborne tuberculosis bacteria relative to other bacteria and fungi.[27] More than 80% of all microorganisms identified during and after induction were potential respiratory pathogens or colonizers of the mouths and noses of humans. Respiratory organisms also accounted for the increase in organisms seen with sputum induction. Therefore, the measured airborne bacteria appear to have resulted from patients' coughing, and so represented a reasonable proxy of exposure.

Each measure of airborne bacteria represented a 7-minute average because of the sampling time required. Given the rapidly changing airborne bacterial concentrations, this may have underestimated the difference between peak and minimum concentrations, resulting in an underestimation of apparent efficacy. However, this problem should not have affected the comparison of the relative effectiveness of different environmental control strategies. Finally, relative humidity may have transiently increased during generation of the saline aerosols to more than 60% to 70%, which would have reduced the efficacy of UVGI.[28] However, this would have been a true limitation of UVGI for sputum induction, albeit not for other situations in which humidity would not be affected by the procedure itself.

Experimental studies with controlled release of bacille Calmette–Guérin or similar bacteria have provided more precise estimates of equivalent ACH,[8] because the numbers of bacteria released and the resultant concentrations were known. In this study, the environmental controls were assessed under real-world conditions with actual patients. Although this had limitations, there were important advantages. First, it allowed detection of the unexpected rapid and substantial increase in airborne bacterial concentrations despite adequate environmental controls, and substantial inter-subject variability in these aerosol bursts. Second, the inter-patient variability in generating airborne aerosols meant that the relative efficacy of environmental controls was determined over a broad range of airborne bacterial concentrations generated by a large number of patients. As a result, the findings should be more robust and generalizable to other settings with similar patient populations.

Overall, the efficacy of upper air UVGI was modest and appeared to be dependent on adequate air mixing. The greater efficacy of the portable UVGI unit might simply have been due to better movement of airborne bacteria to subject them to UVGI lights within the unit. However, the efficacy of portable air filter units can be reduced by handling them improperly, positioning them incorrectly within the room, or turning them off. In contrast, upper air UVGI should function continuously and without problem if installed correctly initially.

Sputum induction can result in brief but significant increases in airborne bacterial concentrations despite high air exchange rates with or without UVGI. The efficacy of upper air UVGI was reduced by inadequate air mixing within the room. This study reinforces recommendations[1,2] that healthcare workers performing cough-inducing procedures for patients with possible active tuberculosis must wear effective personal respiratory protection, even in the presence of adequate environmental controls.

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