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Direct Dilution Sampling, Quantitation, and Microbial Assessment of Open-System Ventilation Circuits in Intensive Care Units

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In a systematic approach, 37 duplicate samples of open system circuits (Bennett MA-1 ventilators) of patients in medical and surgical intensive care units were processed by direct and serial (APHA guidelines) dilutions. The paired difference test on 15 of the in-use circuitry solution samples indicated no difference between the direct and serial dilution methods ($P < 0.001$). Seventy-seven additional respiratory therapy circuitry samples from similar intensive care patients were analyzed via a direct dilution method alone and processed microbiologically. The direct dilution procedure was a rapid and accurate means of evaluation of microbial contamination in the range of ≥ 10 to $\leq 10^6$ CFU/ml. High densities of organisms frequently were found. Sites of contamination included the proximal or patient end of the circuitry (heaviest), the nebulizer trap, and the distal or humidifier portions of the circuitry. The contaminants found were predominantly gram-negative nonfermenters: *Acinetobacter calcoaceticus* var. *antitratus, Pseudomonas aeruginosa, Pseudomonas maltophilia,* and *Flavobacterium meningosepticum.* Fermenters were *Klebsiella pneumoniae, Proteus* sp., *Enterobacter cloacae, Citrobacter diversus,* and *Enterobacter agglomerans.* Infrequently, gram-positive *Streptococcus* spp. and *Staphylococcus* spp. were noted.

Investigators during the 1960s and early 1970s demonstrated that contaminated respiratory therapy (RT) equipment was a potential and actual infectious disease hazard in hospitals (7, 8, 16–18). In 1978, we investigated RT equipment in several hospitals and noted that approximately 35 to 45% of the nebulizers and large reservoir wall humidifiers were contaminated with microorganisms (19). Currently, respiratory infections are the third most common type of nosocomial disease (10, 25). Because of the persistence and regularity of reservoir contamination in several hospitals, we expanded our direct dilution method of sampling (19) and evaluated other surveillance methods for their potential value in assessing microbial burdens of in-use RT equipment and solutions (20, 21). An additional and hitherto unemphasized potential problem is the breathing circuitry of in-use RT equipment and its management by personnel. These circuits, if contaminated, would be reservoirs of microbial pathogens and might cause infectious disease or colonization of the patients and personnel, and they could contaminate the environment. This study was undertaken to compare two methods of sampling and to evaluate the extent and degree of microbial contamination of RT circuits at the termination of the usual 24-h use with high-risk patients in intensive care units (ICUs). The RT circuits were open system circuits, i.e., those periodically exposed to the environment by disconnection and subsequent reconnection. The data obtained from this study might serve to alert personnel to the potential biohazards associated with contaminated wet in-use circuitry and help to establish appropriate management and surveillance guidelines for operating the equipment.

MATERIALS AND METHODS

Direct and serial dilution comparisons. The direct dilution procedure was compared with the saline wash procedure (4) in 37 duplicate samples obtained from labeled and bagged Bennett MA-1 ventilatory circuits used for 24 h. Humidifier (Cascade) solutions were pipetted (1 ml) into sterile, screw-capped test tubes (18 by 150 mm). Both solutions and bagged circuits (disconnected at the wye connector to prevent spillage from expiratory to inspiratory side) were delivered for processing to our microbiology laboratory within 1 to 2 h after patient use. The corrugated tubing of each breathing circuit was stretched to release trapped condensate (usually 1 to 5 ml) from the tubing into separate, sterile, 150-ml breakers. The condensate was swirled several times to mix the effluent. Aliquots of

the samples were obtained with 0.01- and 0.001-ml iridium–rhodium-calibrated loops (Fisher Scientific Co.) and were streaked on sectored blood agar (BA) plates containing 5% sheep blood in Trypticase soy agar (BBL Microbiology Systems), or plain Trypticase soy agar plates. Fluid from the humidifiers (collected in the sterile screw-capped tubes) and fluid from nebulizer traps were plated on sectored plates with calibrated loops as described above. A 0.1-ml sample of each fluid was serially diluted and plated on BA and Trypticase soy agar plates.

For the wash analysis (4), 50 ml of sterile 0.85% saline was added to the tubing and, while the tubing was held at each end, it was alternately raised and lowered 50 times to wash organic debris into the saline. The wash sample was poured into the beaker that contained the condensate first expressed from the tubing (minus the 0.1-, 0.01-, and 0.001-ml initial test samples). After thorough mixing of the pooled samples, 0.01- and 0.001-ml portions were streaked onto BA or Trypticase soy agar plates as described above. Then 0.1 ml of each pooled sample was serially diluted and plated in the same manner. The plates were incubated for 18 to 24 h at 37°C and, if negative, for up to 1 week at room temperature to detect slow-growing bacteria. Gram stains and oxidase tests were done on microorganisms obtained from representative quantitated colonies. The Oxiferm and Enterotube (Roche Diagnostics, Nutley, N.J.) and the API 20E (Analytab Products, Plainview, N.Y.) systems with respective code books (1, 22, 23) were used for the identification of the gram-negative bacteria isolated from the tubing and reservoirs. Tests for motility, and, when indicated, growth at 42°C were included. The *Manual of Clinical Microbiology* (15) was used to verify identifications.

Direct dilution sampling. Seventy-seven samples of breathing circuits of Bennett MA-1 ventilators were collected after the machines were in use for 24 h. The entire circuit from each patient was labeled (disconnected at the wye connector as explained above), carefully packaged in sterile plastic bags, and immediately transported by cart to a hospital laboratory. Each circuit was carefully disassembled, and selected sites were sampled directly with a 0.001-ml calibrated loop: condensate two to three inches within the ports of (i) inspiratory, (ii) expiratory, (iii) humidifier tubing, and fluid remaining in the (iv) nebulizer traps and (v) humidifiers (Cascade). The 0.001-ml inoculum was streaked onto BA plates. The 0.1-ml aliquots from the humidifiers and nebulizer traps (obtained with 1-ml pipettes) were placed on BA plates and spread over the surface of the agar with a glass rod that was dipped in 70% alcohol, flamed, and cooled. If the humidifier or nebulizer was almost empty, a flamed 0.001-ml loop was used to obtain the remaining small quantity of fluid for sampling.

Disinfection and sterilization of RT circuits. All reusable RT circuit equipment in this hospital was routinely washed with soap and water and thoroughly rinsed. If circuits were received "contaminated" from isolation units, they were decontaminated with cold glutaraldehyde for 20 to 30 min, washed with soap and water, rinsed and thoroughly dried, packaged, sterilized with ethylene oxide, chamber aerated, dated, and shelved. Selected routine samples of this equipment were sterile in quality control checks.

RESULTS

Direct and serial dilutions. Comparison of direct and serial dilution procedures revealed a close correlation. The paired difference test on 15 solution samples obtained at various sites indicated no significant difference between the two procedures ($P < 0.001$). No comparisons of both procedures could be made when the number of CFU per milliliter exceeded 10^6, because thousands of colonies resulted from these platings, even when diluted with the 0.001-ml loops. Nevertheless, the direct dilution procedure provided a rapid, accurate means of evaluating the degree of microbial contamination at the various circuitry sites in the range of ≥ 10 to $\leq 10^6$ CFU/ml.

A total of 37 duplicate samples of in-use circuits was processed via the direct and serial dilution methods. The data in Table 1 show the results obtained during consecutive sampling periods of the circuitry of patients who exhibited varied respiratory diseases and who were compromised by one or more additional conditions. These samplings reinforced the paired difference test results previously obtained. By the use of serial dilution, microbial burdens were detected at 10^8 to 10^9 CFU/ml in fluids of several circuitry sites after 24 h of use. Similar species (Table 1) of microorganisms were concurrently found in the RT equipment and sputum cultures or tracheal washings. The predominant organisms were gram-negative non- or weak fermenters. These bacteria and the incidence of their occurrence in the circuitry of 19 patients were as follows: *Acinetobacter calcoaceticus* var. *anitratus* (79%, 15 of 19), *Pseudomonas aeruginosa* (32%), *Pseudomonas maltophilia* (16%), and *Flavobacterium meningosepticum* (5%). Also isolated in high densities were members of the *Enterobacteriaceae: Klebsiella pneumoniae* (21%), *Proteus* sp. (21%), *Enterobacter cloacae* (11%), *Citrobacter diversus* (11%), and *Enterobacter agglomerans* (5%). Occasionally, gram-positive *Streptococcus* spp., *Staphylococcus* spp., and diphtheroids (5% each) were isolated.

Direct dilution sampling. A total of 77 samples was collected from selected sites and analyzed by direct dilution. In many instances, high densities of gram-negative organisms at various sites were detected at the 24-h sampling (Table 2). The circuitry of each of four patients (F, K, L, and P) was tested two or more times on different days. Circuits of the remaining patients were sampled once. Microbial burdens ranged from no growth on admission or intubation to densities of $\geq 10^5$ CFU/ml. Subject K temporarily had small numbers of *Candida* sp. in both the circuitry and sputum (approximately 1×10^3 to 2×10^3 CFU/ml per sample). There were as many as four or five different species of organisms in

TABLE 1. Quantitative comparison of serial (S) and direct (D) dilutions of condensate and wash samples from MA-1 ventilators used for 24 h

Patient no.	Date sampled	Organism(s) isolated	No. of bacteria (CFU/ml) isolated from:											
			Expiratory tubing				Inspiratory tubing				Nebulizer		Humidifier	
			Condensate		50-ml wash		Condensate		50-ml wash					
			S	D	S	D	S	D	S	D	S	D	S	D
1[a]	12/27	*A. calcoaceticus* var. *anitratus* *E. coli*	ND[b]	ND	3.4×10^6	$>10^6$	ND	ND	1.1×10^6	$>10^5$	7.4×10^5	6.3×10^5	ND	ND
	12/28	*A. calcoaceticus* var. *anitratus*	1.0×10^9	$>10^6$	2.3×10^8	$>10^6$	5.8×10^8	$>10^6$	1.2×10^8	$>10^6$	ND	ND	6×10^2	1.1×10^3
	12/29	*A. acinetobacter* var. *anitratus*	1.4×10^7	$>10^6$	5.1×10^6	$>10^6$	7.3×10^6	$>10^6$	ND	ND	ND	ND	ND	ND
	12/30	*A. calcoaceticus* var. *anitratus* *Proteus* sp. (expiratory only)	1.5×10^7	$>10^6$	1.2×10^7	$>10^5$	$>10^5$	$>10^5$	2.7×10^5	1.7×10^5	ND	ND	1.6×10^3	1.5×10^3
2[a]	12/28	*A. calcoaceticus* var. *anitratus*	5.8×10^5	8.3×10^5	3.0×10^4	4.3×10^4	6.5×10^5	5.9×10^5	1.7×10^5	2.3×10^5	1.4×10^6	1.0×10^6	2.1×10^3	3.0×10^3
	12/29	*A. acinetobacter* var. *anitratus*	1.4×10^5	1.1×10^5	5.3×10^4	3.4×10^4	1.5×10^6	8.2×10^6	4.8×10^5	3.1×10^5	ND	ND	ND	ND
3	12/27	*P. aeruginosa*[c] *P. maltophilia* *A. calcoaceticus* var. *anitratus*	ND	ND	1.8×10^8	$>10^6$	ND	ND	0	0	ND	ND	ND	ND
4	12/27	*P. aeruginosa*[c] *E. cloacae* *F. meningosepticum* *A. calcoaceticus* var. *anitratus*	$>10^5$	$>10^5$	6.5×10^6	2.2×10^5	ND	ND	ND	ND	ND	ND	ND	ND
5	12/29	*P. aeruginosa*[c] *K. pneumoniae*	2.7×10^4	7.3×10^4	7.0×10^3	5.7×10^3	3.5×10^2	2.5×10^2	1.0×10^2	2.0×10^2	0	0	ND	ND
6	12/27	*P. aeruginosa*[c] *A. calcoaceticus*[c] var. *anitratus*	3.0×10^8	$>10^5$	2.1×10^7	$>10^5$	$>10^5$	$>10^5$	8.0×10^7	$>10^5$	ND	ND	ND	
7	12/29	*Streptococcus*[d] sp. *Staphylococcus* sp. Diphtheroids	1.0×10^7	$>10^6$	1.0×10^7	$>10^6$	0	0	0	0	ND	ND	0	0

[a] Microbiological laboratory reports not available.
[b] ND, Not done.
[c] Organisms found in sputum cultures or tracheal washings concurrent with sampling; sputum may have contained added organisms.
[d] *Streptococcus* not identified to species level; *S. pneumoniae* found in sputum culture.

some samples on day 5 or 6 after instrumentation. Contamination tended to be higher at the proximal (patient) end of the circuitry, and frequently, species of organisms common to the circuitry were found concurrently in sputum cultures or tracheal washings. On eight occasions, the same organisms were found in the inspiratory/expiratory tubing, the nebulizer trap, and the humidifier (Tables 1 and 2). Interestingly, humidifier fluid and humidifier tubing condensates (in tubing leading from humidifier to the patient) were both positive in 8 of 20 (40%) samples; both were negative in 10 of 20 (50%) samples. In two samples (10%), the humidifier fluid was negative but the humidifier tubing condensate was positive. In summary, humidifier tubing condensate was positive for gram-negative rods in 50% of the samplings.

In ICU no. 1, the circuitry of five different patients became colonized with *A. calcoaceticus* var. *anitratus* during a 10-day period. Sputum cultures or tracheal washings of three of these patients concurrently contained *A. calcoaceticus* var. *anitratus*; one patient had "unidentified gram-negative rods" in the sputum; the remaining patient was colonized with other organisms reported in the sputum. In ICU no. 2, four of five patients, with *A. calcoaceticus* var. *anitratus* in the circuitry, likewise had the organism in their sputum cultures or tracheal washings during the same 10-day period. Two sputum cultures of the third patient had been discarded due to saliva or other contamination; therefore, no comparison could be made.

DISCUSSION

To the best of our knowledge, this work represents the first systematic quantitative and qualitative microbiological study of RT breathing circuits via direct dilution samplings. The results clearly showed significant microbial contamination. The results of the paired difference test of the data in Table 1 reflected comparable values with serial and direct dilutions. Although serial dilutions gave valuable definitive quantitation in densities $>10^6$ CFU/ml, direct dilution proved a useful and relatively easy way to quantitate microbial burdens at selected sites in wet equipment. This method allowed for the substitution of the laborious washings of tubing for a more rapid, equally sensitive ($\leq 10^6$ CFU/ml), direct process.

The data in Tables 1 and 2 showed colonization with large numbers of gram-negative aerobic bacilli in the circuitry of 19 ventilated patients, sampled in medical and surgical ICUs during two different periods. The group included males and females ranging in age from 17 to 78 years, with a mean of 52. The length of hospitalization of the patients in the ICUs ranged from 1 to 39 days. All patients had multisystem maladies; all were on antibiotics; all were instrumented via intubation or tracheostomies; 95% of the ventilator circuits were colonized with aerobic gram-negative bacilli (Tables 1 and 2). Twelve patients concurrently had one or two similar species in the sputum or tracheal washings, and two of these had as many as five organisms in the circuitry after being on the ventilator for 5 to 6 days; 74% were on ventilators for 4 or more days. According to chart diagnoses, 79% had pneumonia on or after admission. Within this high-risk population, 42% expired, 42% were discharged home, and 16% were transferred to other institutions. Many characteristics of these patients were consistent with patients of other studies (11, 14, 24–27).

Thirteen patients had been admitted before the study and were sampled once or on an intermittent basis. Four of six remaining patients, admitted during the study and sampled on a consecutive basis, showed rapid gram-negative bacterial colonization. In 1969, Johanson et al. (12) found that healthy individuals had a 2% colonization rate with transient gram-negative rods, but the rate rose rapidly to 35% in moderately ill hospital patients and was up to 60 to 70% in very ill hospitalized patients. They showed that the incidence of colonization increased as the severity of illness increased. Thus, the presence of greater numbers of organisms among these high-risk patients could result in their used equipment becoming greater microbial biohazards. In another study by Johanson and his associates (13), microbial colonization of patients rose rapidly during the first 4 days in a medical ICU and then leveled off at 45%. Although pneumonia did not develop in all of those colonized patients, 23% succumbed to pneumonia. Only 3.3% of noncolonized patients developed pneumonia.

The progressive pattern of colonization of circuitry from the proximal to the distal end of a circuit (in relation to the patient) focused attention on the sampled sites as potential environmental biohazards among this compromised population. Various kinds of organisms seeded into the circuitry may grow and multiply in the warm, moist environment, using such nutrients as dissolved gases (2), absorbed compounds (9), patient secretions, and by-products of other cells.

Logically, effluent gases and mucous secretions from the patient would cause greater contamination in the expiratory than in the inspiratory tubing. However, our data suggest that in 23 of 26 (88%) instances, inspiratory tubing was also contaminated, sometimes with high densities of microbes. An important observation, in trying to account for this high microbial density,

TABLE 2. Type and density of microorganisms isolated from selected sites of in-use MA-1 ventilators used for 24 h

Patient	Condition	Days in hospital	Days on machine	Sample no.	Organism(s) and density (CFU/ml) isolated from the following sample site[a]: Expiratory tubing	Inspiratory tubing	Nebulizer trap	Humidifier
F	Aspiration pneumonia	4	3	1	*C. diversus* (10,000)	*C. diversus* (72,000)	0	0
		7	6	2	*K. pneumoniae, P. mirabilis*[b] (>200,000[c])	*K. pneumoniae, P. mirabilis, C. diversus, A. calcoaceticus*[b] (>200,000[c])	*K. pneumoniae, C. diversus, A. calcoaceticus*, (>200,000[c])	*K. pneumoniae, C. diversus* (>100,000[c])
K	Chest infiltration[d]	3	2	1	ND[e]	*C. albicans* (1,000)	0	0
		4	3	2	*A. calcoaceticus* (3,000)	0	0	0
		5	4	3	*A. calcoaceticus* (>50,000)	*A. calcoaceticus* (>50,000)	*A. calcoaceticus* (90)	*A. calcoaceticus* (50)
L	Aspiration pneumonia	2	2	1	ND	ND	0	ND
		3	3	2	*Pseudomonas* sp., *K. pneumoniae, E. cloacae* (>100,000[c])	*E. cloacae* (>100,000)	*P. maltophilia*[b] (10), *K. pneumoniae* (260)	0
		5	5	3	*P. aeruginosa* (>50,000), *P. maltophilia*[b] (25,000)	*P. aeruginosa* (>25,000), *P. maltophilia* (30,000), *A. calcoaceticus*[b] (9,000)	*P. aeruginosa* (200), *P. maltophilia* (450), *A. calcoaceticus* (420)	*P. aeruginosa, P. maltophilia* (500[c])
P	Respiratory failure	2	2	1	0	0	0	0
		5	5	2	*A. calcoaceticus, C. diversus* (55,000[c])	*A. calcoaceticus, C. diversus* (45,000[c])	0	0
A	Pneumonia	15	15	1	ND	ND	*A. calcoaceticus*[b] (300)	ND
B	*Pseudomonas* pneumonia	11	10	1	*Proteus*[b] sp. (>200,000) plus another gram-negative species (>200,000)		ND	*A. calcoaceticus*[b] (25,000)
C	Aspiration pneumonia	39	12	1	*P. aeruginosa*[b] (>100,000)	*P. aeruginosa* (>100,000)	*P. aeruginosa* (>10,000)	*P. aeruginosa* (1,500), *A. calcoaceticus*[b] (200)
D	Status asthmaticus	16	2	1	ND	*K. pneumoniae, A. calcoaceticus*[b] (>85,000[c])	*A. calcoaceticus* (>100,000)	*A. calcoaceticus* (80)

E	Recurrent pneumonia	13	2	1	*K. pneumoniae, A. calcoaceticus*[b] (>100,000[c])	*A. calcoaceticus* (>100,000)	*A. calcoaceticus* (>100,000)	*A. calcoaceticus* (>100,000)
J	Lung abscess; tuberculosis	13	2	1	*P. maltophilia* (2,000)	*P. maltophilia* (1,000)	0	0
O	Aspiration[d] pneumonia	3	4	1	ND	*A. calcoaceticus*[b] (>200,000)	*A. calcoaceticus* (>200,000)	0
R	Pneumonia	10	7	1	*P. mirabilis*[b] (>50,000)	*P. mirabilis* (>50,000)	*P. mirabilis* (90)	*P. mirabilis* (1,500)

[a] 0, Negative sample; no microorganisms found.
[b] Organisms found in sputum cultures concurrent with sampling; sputum may have contained additional organisms.
[c] Density for two or three species combined.
[d] Two sputums discarded due to saliva or contamination; unidentified gram-negative rods in sputum.
[e] ND, Not done.

was the unstable positioning of the wye connector as the patient changed position. No valve or guard is found at the bifurcation of the wye connector to prevent spillage. This situation, therefore, may permit condensate to flow from the expiratory to the inspiratory side of the tubing as the body position of the patient is changed, or as the tubing is maneuvered by personnel.

Entrance into the breathing circuit while one is manually administering a medication or aerosolizing with an in-line medication nebulizer opens the circuitry to potential contamination (24). Even though the devices and the sterilization and disinfection processes for these devices have improved since the early 1970s (24), great care must be exercised in their use. In this study, the hospital used in-line nebulizers as traps in the circuits. There was contamination in 12 of 21 (57%) nebulizer traps, particularly with *A. calcoaceticus* var. *anitratus*, which was highly endemic during the period of this study (Tables 1 and 2). This contamination could result from one or more of the following: backflow of microbes, apparently from the patient, into the tubing condensate and into the nebulizer trap; exposure to air; or manipulation at the connector sites.

At the distal end of the circuits, Cascade humidifiers were contaminated in 11 of 20 (55%) samplings. This most distal device could pose a hazard for cross-contamination. In conjunction with the possibilities listed for contamination of nebulizer traps, unused portions of opened sterile water or saline added to residual portions in the humidifier could account for some of the contamination of these humidifiers.

An observed and potentially hazardous practice was the emptying of microbe-laden condensate into an open trash can or sink rather than into a closed container (10, 25). The condensate in tubing leading from the humidifier to the patient was positive in 50% of 20 samplings. Addition of an effective concentration of disinfectant such as iodophor (10) to a closed discard container possibly would prove safer. A study is planned to determine the effectiveness of this suggested disinfection-discard measure. Also observed was the discarding of dismantled, unbagged, used circuitry into open trash cans and the collection of used circuits into brown paper bags. This contaminated equipment should be bagged into biohazard or impervious plastic (3) bags, tightly secured, and transported from an ICU for disposal or microbial control processing.

The results of this study strongly indicate that even after use, RT equipment may pose a distinct biohazard if not judiciously handled. Further studies are needed on used equipment,

especially among this high-risk population, to determine whether similar equipment could be a focal point for cross-contamination. The high frequency of the occurrence of *A. calcoaceticus* var. *anitratus* in the medical and surgical ICUs (Tables 1 and 2) may also indicate cross-contamination. Study of the microbial interactions between animate and inanimate sources is warranted in these ICUs.

The inspiratory effluent or aerosol entrapment method has been traditionally used to assess RT equipment; however, the direct sampling technique is apparently more sensitive (detecting ≥10 CFU/ml) than at least one aerosol entrapment method we tested previously (20). Direct sampling, thus, can assist in the detection of low numbers of microbes that have the potential to serve as infective agents in the compromised host. In a study of 914 patients on respiratory assistance devices at Walter Reed Hospital, the effluent (inspiratory) gas method of sampling was typically able to detect contamination of approximately 10^3 CFU/ml (6). Inspiratory gases from ventilators of 32 patients with hospital-acquired pneumonia were positive in 69% of these cases and in 40% of 33 ventilated patients with no hospital-associated pneumonia. There were 63 negative nebulizer samples collected by a Vacutainer (6), but no statement was made as to the use of the nebulizer if a trap or an aerosolizer. Possible explanations of this difference of nebulizer data from those in the present study are the different use of the nebulizer, the method of sampling, the patients and personnel, and the prevalence of *A. calcoaceticus* var. *anitratus* in the current study. In a more recent report, Craven (5) and his associates compared the results of 24- and 48-h changes of ventilator equipment in medical and surgical ICUs. They studied inspiratory-phase gas entrapment, using a tube broth method (Edmondson and Sanford) and air samplers (Aerotest and Anderson). Humidifier fluids were plated on BA plates. These workers found high levels of contamination in some inspiratory tubing and humidifiers but no statistically significant difference in the amount of bacterial contamination at 24 or 48 h.

In our studies, the direct dilution method proved more sensitive than the analyses of inspired gases. The higher contamination rates reported in this study are likely due to the greater sensitivity in detecting bacterial contamination via direct dilution sampling and an actually greater incidence of bacteria, particularly *A. calcoaceticus* var. *anitratus*, which seemed highly endemic in this situation. The direct dilution method should be very useful in assessing contaminated RT equipment and evaluating potential or actual environmental biohazards for patients, personnel, and the public.

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