

## Home-Use Nebulizers: a Potential Primary Source of *Burkholderia cepacia* and Other Colistin-Resistant, Gram-Negative Bacteria in Patients with Cystic Fibrosis

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**Inhalation of aerosols contaminated with gram-negative bacteria generated from home-use nebulizers used by cystic fibrosis (CF) patients may be a primary route for bacterial colonization of the lung. *Burkholderia cepacia* was isolated from 3 to 35 home-use nebulizers, and *Stenotrophomonas maltophilia* was isolated from 4 of 35 home-use nebulizers. Sputum cultures for two patients whose nebulizers were contaminated with *B. cepacia* did not yield the organism. However, DNA macrorestriction analysis by pulsed-field gel electrophoresis confirmed that one of two strains of *B. cepacia* recovered from the nebulizer of a third patient was also present in the sputum of that patient. Although *Pseudomonas aeruginosa* was isolated from 34 patients, none of the nebulizers were positive for the organism. Sixty-nine percent of nebulizers were contaminated, and up to 16 different environmental colistin-resistant, gram-negative species were identified. The heaviest contamination was found beneath the chamber atomizer. A questionnaire survey showed that the majority of patients (28 of 34) were receiving nebulized colistin and/or gentamicin. Patients who followed recommended instructions for good nebulizer hygienic practice and paid particular attention to drying had minimal or no contamination of their nebulizers.**

The problem of acquisition of *Burkholderia cepacia* continues to be of great concern to cystic fibrosis (CF) patients and their caregivers (17). Transmission of the organism by close contact and aerosol transfer between patients both in and out of the hospital has been repeatedly documented (5, 11, 13, 16), but the lack of reported primary environmental reservoirs is puzzling. A large, recent survey (12) of the domestic environment of CF patients found no significant difference in the recovery of *B. cepacia* from CF patient versus control homes (21 versus 15%), and the species was also infrequent in salads and fresh foods.

Nebulizers are frequently used to deliver aerosols of antibiotics and other drugs to patients with chronic conditions. A case-controlled study of five adult CF patients in hospitals showed a significant association between outpatient nebulizer use and colonization with *B. cepacia*, and the organism was cultured from nebulizers used by colonized patients (2). This has been widely recognized in the treatment of patients with asthma or chronic obstructive disease, in which reservoirs of aqueous inhaled drugs may be contaminated with bacteria, including *B. cepacia* (1, 3, 7).

Over half of approximately 800 CF patients at the Royal Brompton Hospital, London, United Kingdom, participate in a program for the administration of aerosolized antibiotics and bronchodilators in their homes. The patients are encouraged to exchange old plastic tubing and atomizing chambers for new ones at 6-month intervals, but some units are used longer before renewal. An examination of the state of some of the

returned tubing and chambers prompted a formal study of the colistin-resistant, gram-negative bacterium (CRGNB) flora and a questionnaire survey of nebulizer hygiene practices of the patients.

### MATERIALS AND METHODS

**Patients.** Thirty-five adult CF patients were recruited for the study. At Physiotherapy Out Patients they were given replacement circuits (administration and exhaust plastic tubing, nebulizer chamber, and mouthpiece [Fig. 1]) in exchange for the used counterparts. The patients were also asked to complete a questionnaire on length and type of usage and method of cleaning circuits. They were instructed to clean their nebulizers thoroughly in running warm water after each treatment and to dry the circuit as much as possible with a clean paper towel before leaving it to air dry. It was recommended that disinfection of circuits be carried out weekly with shop-purchased hypochlorite (bleach) solution at a use concentration of 125 ppm of available chlorine.

Sputum culture results were provided by the diagnostic laboratory of the Royal Brompton Hospital.

**Sampling and culturing.** Nebulizer circuits with visible moisture were sampled with a bacteriological loop or micropipette. The tubing (3-cm lengths) and chambers were wiped out with a sterile swab moistened in Trypticase soy broth (Unipath, Ltd., Basingstoke, United Kingdom). Following culture of the internal parts, the chambers were then submerged in Trypticase soy broth in a sterile jar and subcultured after 48 h at 30°C. All primary and enrichment samples were plated on Trypticase soy agar (Unipath) and *Pseudomonas cepacia* selective medium (Mast Laboratories, Ltd., Merseyside, United Kingdom). The agar plates were incubated at 30°C for 48 h. Colonies were purified, and gram-negative, glucose-nonfermenting species were identified with the aid of the API 20NE system (BioMérieux, Basingstoke, United Kingdom).

**DNA typing.** Isolates of *B. cepacia* were compared by DNA macrorestriction analysis as previously described (8).

### RESULTS

We examined all plastic parts and tubing of home-use nebulizers of 35 CF patients for bacterial contamination. Twenty-four (68.8%) nebulizer circuits were contaminated with CRGNB (Table 1), as evidenced by their growth on *P. cepacia* selective

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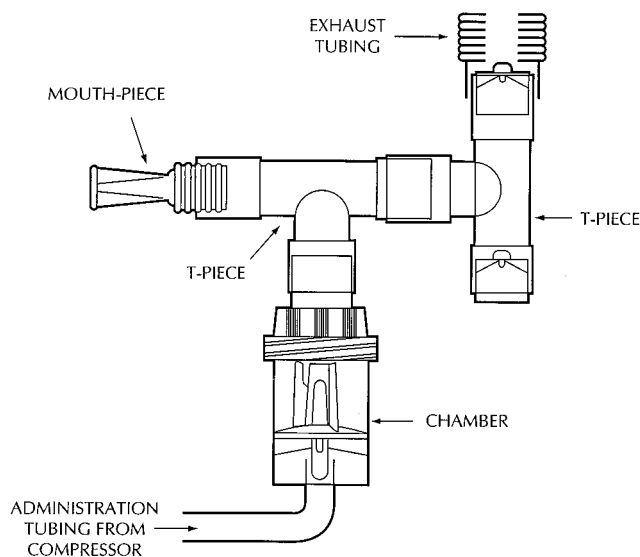


FIG. 1. Diagram of home-use nebulizer parts and tubing.

medium. The antibiotic chamber was the most frequently contaminated part of the circuitry (21 of 24 chambers), followed equally by the T-piece and mouthpiece. The administration and exhaust tubing were the least contaminated parts.

*B. cepacia* was isolated from three nebulizers (of patients 22, 24, and 31), from the antibiotic chamber only in two of these and throughout the circuitry in the third (from patient 24). The sputum from one of these patients (patient 22) also yielded *B. cepacia*, the DNA of which was indistinguishable from that of the nebulizer isolate by pulsed-field gel electrophoresis. The nebulizer also yielded a genotypically distinct *B. cepacia* strain which had not been recovered from the patient's sputum (Fig. 2). Sputum cultures for the remaining two patients whose nebulizers were contaminated with *B. cepacia* did not yield the organism.

All but one of the 35 patients were colonized with *Pseudomonas aeruginosa*, but this organism was not recovered from any of the nebulizers. In contrast, *Stenotrophomonas maltophilia* was isolated from four nebulizers (from patients 5, 9, 11, and 12), but none of the patients was colonized by this organism. A syringe and needle used by one patient (patient 5) to add antibiotics to the nebulizer chamber were available for sampling, and *S. maltophilia* and *Flavobacterium indologenes* were isolated from them.

At least 15 different species of gram-negative bacteria, in addition to *B. cepacia*, grew on the *P. cepacia* selective medium and were therefore termed colistin resistant (CRGNB in Table 2). Fourteen species of CRGNB each were recovered from the nebulizers of patients 5, 6, and 12; the majority of the remainder of the nebulizers yielded one to three species of CRGNB each. The number of bacteria present in droplets of moisture recovered from nebulizers ranged from  $10^2$  to  $10^9$  CFU/ml, and the heaviest contamination was found beneath the antibiotic chamber atomizer and between the T-joints. Five of seven nebulizers with visible moisture throughout yielded the heaviest growths of bacteria, including three with *S. maltophilia* and two with *B. cepacia*. Of the 11 nebulizers not contaminated with CRGNB, 9 were dry on receipt.

Thirty-four patients completed the questionnaire survey, and the results are shown in Table 3. The mean period of use of the nebulizers before being submitted for sampling was 2.4

months (range, 3 weeks to 2 years). Six patients had used the same circuitry for 6 months, and another six had used the same circuitry for 1 year without change. The duration of use did not correlate with the degree of contamination. Twenty-one patients were receiving aerosolized colistin, 11 in combination with gentamicin. Three patients did not use their nebulizers for antibiotics, but two of these circuits yielded CRGNB. Seven of eight circuits from patients who apparently did not use disinfectants were contaminated with CRGNB, but even 10 circuits from 18 patients who disinfected their apparatus at least once per week were still contaminated.

## DISCUSSION

We have demonstrated the potential for the acquisition of apparently harmful bacterial species by the use of nebulizers for medications at home. Aerosols generated by the nebulizer and contaminated by gram-negative species may travel deep into the lung airways of CF patients in sufficient numbers to compete with any microflora already present and establish themselves as residents.

Bacterial contamination of nebulizer apparatus has been recognized for many years. In 1958, Macpherson (10) showed that the majority of samples of water from humidifiers used in oxygen therapy were contaminated with bacteria. This was subsequently confirmed by a number of studies of humidifier and nebulizer apparatus in hospitals. Gelbart et al. (4) reported the isolation of *B. cepacia* from water reservoirs of unheated nebulizer units and suggested that this equipment might serve as a source of respiratory tract exposure. Two recent hospital outbreaks of *B. cepacia* infection involving 37

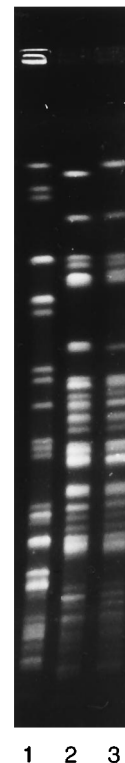


FIG. 2. Macrorestriction profiles of *Xba*I digests of chromosomal DNAs of three isolates of *B. cepacia*. Lanes 1 and 2, DNAs of isolates from nebulizer chamber used by patient 22; lane 3, sputum isolate from the same patient.

TABLE 1. Parts of nebulizer apparatus contaminated by CRGNB<sup>a</sup>

Patient	Compressor tubing	Chamber	T-piece	Mouth-piece	Exhaust tubing
1		b			
2					
4					
5		Sm <sup>c</sup>			
6					
7					
8					
9		Sm			
11		Sm			
12		Sm	Sm	Sm	
14					
15					
16					
17					
21					
22		Bc <sup>d</sup>			
23					
24	Bc	Bc	Bc	Bc	
27					
29					
31		Bc			
32					
33					
35					

<sup>a</sup> Patients 3, 10, 13, 18, 19, 20, 25, 26, 28, 30, and 34 had no CRGNB in their nebulizer apparatus.

<sup>b</sup> Shaded blocks in field, CRGNB isolated.

<sup>c</sup> Sm, *S. maltophilia*.

<sup>d</sup> Bc, *B. cepacia*.

TABLE 2. Gram-negative bacterial species recovered from enrichment cultures plated on *B. cepacia* selective medium and number of nebulizer circuits contaminated

Species isolated	No. of circuits
<i>Acinetobacter johnsonii</i> .....	1
<i>Acinetobacter junii</i> .....	1
<i>Agrobacterium radiobacter</i> .....	1
<i>Alcaligenes xylosoxidans</i> .....	1
<i>Burkholderia cepacia</i> .....	3
<i>Comamonas acidovorans</i> .....	13
<i>Comamonas testosteroni</i> .....	1
<i>Flavobacterium indologenes</i> .....	5
<i>Flavobacterium meningosepticum</i> .....	1
<i>Ochrobactrum anthropi</i> .....	2
<i>Oligella urethralis</i> .....	1
<i>Pseudomonas aureofaciens</i> .....	1
<i>Pseudomonas fluorescens</i> .....	7
<i>Pseudomonas vesicularis</i> .....	2
<i>Sphingomonas paucimobilis</i> .....	7
<i>Stenotrophomonas maltophilia</i> .....	4

and 42 patients were linked to nebulized polymyxin B and amphotericin B and to albuterol therapy, respectively (6, 19).

The practice of nebulization therapy at home was widely introduced in the 1980s, particularly for the delivery of drugs for the treatment of chronic asthma. A number of papers describing investigations of the microbiological safety of the equipment subsequently appeared. Popa et al. (15) reported contamination of the majority of large-volume saline reservoirs with gram-negative species, and a similar investigation (7) found 32 of 52 nebulizer solutions for asthmatic patients to be contaminated with pseudomonads. A study of nebulized drugs used by CF patients has shown *Bacillus cereus* contamination of solutions dispensed for use in nebulizers at home only 5 days after dispensation (9). Transmission of gram-negative bacilli from nebulizers to patients was first demonstrated by Wexler and colleagues (18), who isolated these organisms from 5 of 20 patients who were not colonized with them prior to nebulization.

In the United Kingdom, home nebulization of antibiotics has only recently become widespread as a treatment for CF patients, and no bacteriological surveys, to our knowledge,

TABLE 3. Survey of nebulizer usage by CF patients and bacterial contamination

Patient no.	Length of use	Antibiotic used <sup>a</sup>	Disinfection <sup>b</sup>	Agent <sup>c</sup>	Contamination
1	6 mo	Co	Once/wk	HPC	CRGNB
2	3 mo	Co	None	—	CRGNB
3	15 mo	Gn	Once/wk	Salt water	None
4	1 yr	None	Twice/wk	HPC	CRGNB
5	6 mo	Gn + Ca	Once/6 mo	HPC	<i>S. maltophilia</i>
6	5 mo	Co	Once/mo	HPC	CRGNB
7	14 mo	Gn	Once/wk	HPC	CRGNB
8	3 wk	Co	None	—	CRGNB
9	8 mo	Co	Twice/wk	HPC	<i>S. maltophilia</i>
10	1 yr	None	Once/wk	HPC	None
11	4 mo	Gn + Co	Once/2 wk	Metabisulfite	<i>S. maltophilia</i>
12	5 mo	Co	Once/2 wk	HPC	<i>S. maltophilia</i>
13	12 mo	Gn + Co	None	—	None
14	12 mo	Gn + Co	Once/wk	HPC	CRGNB
15 <sup>d</sup>					CRGNB
16	18 mo	Gn + Co	Once/wk	HPC	CRGNB
17	2 mo	Gn	Once/wk	HPC	CRGNB
18	4 mo	Gn + Tob	Once/wk	HPC	None
19	2 yr	Gn + Co	Once/day	HPC	None
20	3 mo	Tn + Ca	Once/mo	HPC	None
21	7 mo	Gn + Ca	Once/wk	HPC	CRGNB
22	12 mo	Cz	None	—	<i>B. cepacia</i>
23	1 mo	Gn + Co	Once/wk	HPC	CRGNB
24	2 yr	Gn + Co	None	—	<i>B. cepacia</i>
25	14 mo	Gn + Co	Once/day	HPC	None
26	4 mo	Gn + Co	Once/wk	HPC	None
27	6 mo	Gn	None	—	CRGNB
28	6 mo	Co	Once/wk	HPC	None
29	3 mo	Gn + Co	Once/2 mo	HPC	CRGNB
30	14 mo	Gn + Co	Once/2 wk	HPC	None
31	6 mo	Co	Once/mo	HPC	<i>B. cepacia</i>
32	3 mo	Tn	None	—	CRGNB
33	1 mo	None	None	HPC	CRGNB
34	12 mo	Co	Once/wk	HPC	None
35	6 mo	Co	Once/wk	HPC	CRGNB

<sup>a</sup> Ca, carbenicillin; Cz, ceftazidime; Co, colistin; Gn, gentamicin; Tn, trimethoprim; Tob, tobramycin.

<sup>b</sup> Frequency with which nebulizer unit was disinfected or cleaned.

<sup>c</sup> Agent used for disinfection. HPC, hypochlorite (125 ppm of available chlorine); —, disinfecting agent not stated.

<sup>d</sup> Questionnaire not completed.

have been reported. We found contamination of the majority of home-use nebulizers and, most significantly, isolated either *B. cepacia* or *S. maltophilia* from 7 of 35 nebulizers. We were also able to show by DNA fingerprinting that one of two isolates of *B. cepacia* from a nebulizer chamber was indistinguishable from the patient's sputum isolate. Moreover, none of the other six patients whose nebulizers yielded *B. cepacia* or *S. maltophilia* showed these organisms in cultures of sputum specimens taken when the nebulizer was sampled. Thus, the nebulizers were likely to be primary sources rather than sites of secondary contamination derived from the patients' secretions. The sources of contamination of the nebulizers in the home have not been established, but a recent survey suggests that the occurrence of these bacteria in the home environment is not unusual (12). A recent genotyping survey of *B. cepacia* isolates from CF patients in the United Kingdom showed that at least two-thirds of patients with the organism are colonized with unique strains (14), despite the high frequency of the British epidemic strain in this population (5). It follows, therefore, that although transmission of *B. cepacia* is significant in the colonization of some patients, other primary sources of infection must account for many of the cases.

Our results suggest that contamination of home-use nebulizers is common and that it is compounded by the variety of maintenance practices observed. Thirty of 35 nebulizers had been in use for at least 3 months, and most patients (33 of 35) used them two or more times each day. Although 26 patients rinsed their nebulizer after every use, only 5 stated that they dried the unit. Patients received standard instructions, yet most allowed their nebulizers to air dry, which, although recommended by some manufacturers, is contrary to Royal Brompton Hospital's standard recommendation of drying with clean paper towels. The necessity of dismantling, cleaning, careful drying, and correct storage of the nebulizer parts and associated tubing should be emphasized to patients, and there should be regular follow-up to ensure that their home equipment is correctly maintained. The education and training of home caregivers should include the principles of disinfection of circuitry so that the caregivers are more able to identify inadequacies in practices and are able to advise patients of the correct procedures.

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