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Burkholderia cepacia respiratory tract acquisition: epidemiology and molecular characterization of a large nosocomial outbreak

C. F. PEGUES[1], D. A. PEGUES[2], D. S. FORD[1], P. L. HIBBERD[1,2], L. A. CARSON[3], C. M. RAINE[1] AND D. C. HOOPER[1,2]*

[1] *Infection Control Unit, Massachusetts General Hospital, Boston, MA*
[2] *Infectious Disease Unit, Massachusetts General Hospital, Boston, MA*
[3] *Hospital Infections Program, Centers for Disease Control and Prevention, Atlanta, GA*

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SUMMARY

In 1994 we investigated a large outbreak of *Burkholderia* (formerly *Pseudomonas*) *cepacia* respiratory tract acquisition. A case patient was defined as any patient with at least one sputum culture from which *B. cepacia* was isolated from 1 January to 31 December 1994. Seventy cases were identified. Most (40 [61%]) occurred between 1 February and 31 March 1994; of these, 35 (86%) were mechanically ventilated patients, 30 of whom were in an intensive-care unit (ICU) when *B. cepacia* was first isolated. Compared with control patients who were mechanically ventilated in an ICU, these 30 case-patients were significantly more likely to have been ventilated for 2 or more days (30/30 *v.* 15/30; $P < 0{\cdot}001$) or to have been intubated more than once (12/30 *v.* 2/30; OR = 9·3, 95% CI 1·6–68·8; $P = 0{\cdot}002$) before the first isolation of *B. cepacia*. By multivariate analysis, the 35 mechanically ventilated case-patients were significantly more likely to have received a nebulized medication (OR = 11·9, 95% CI = 1·6–553·1; $P < 0{\cdot}001$) and a cephalosporin antimicrobial (OR = 11·9, 95% CI = 1·6–553·1) in the 10 days before the first isolation of *B. cepacia*, compared with *B. cepacia*-negative control-patients matched by date and duration of most recent mechanical ventilation. Although *B. cepacia* was not cultured from medications or the hospital environment, all outbreak strains tested had an identical DNA restriction endonuclease digestion pattern by pulsed-field gel electrophoresis. Review of respiratory therapy procedures revealed opportunities for contamination of nebulizer reservoirs. This investigation suggests that careful adherence to standard procedures for administration of nebulized medications is essential to prevent nosocomial respiratory infections.

INTRODUCTION

Burkholderia (formerly *Pseudomonas*) *cepacia* is a multidrug-resistant Gram-negative bacillus that can cause respiratory infections among hospitalized patients, including those with cystic fibrosis [1–7]. The incidence of *B. cepacia* infections among patients in US hospitals increased significantly during the period 1980–5 and was 3·5 and 3·3 per 100000 admissions on medical and surgical services, respectively; 31% of all *B. cepacia* infections involved the lower respiratory tract [8]. Outbreaks of *B. cepacia* respiratory tract acquisition have been associated with inadequate or inappropriate disinfection or reuse of respiratory therapy equipment [9–11]. Intrinsic and extrinsic

* Address for correspondence: David C. Hooper, MD, Infectious Disease Unit, Gray 5, 32 Fruit St., Massachusetts General Hospital, Boston, MA 02114 USA.

contamination of nebulized medications or solutions have also been associated with outbreaks of nosocomial Gram-negative respiratory infections [12–20].

The Massachusetts General Hospital (MGH) is a 900-bed university teaching hospital and tertiary-care referral centre with 9 intensive-care units (ICUs) containing 113 beds. On 25 February 1994, the MGH Infection Control Unit was notified that two patients in the Neurosurgical ICU had sputum cultures that grew *B. cepacia*. Review of MGH microbiology records revealed that during February 1994, 20 patients had one or more sputum cultures that grew *B. cepacia*. In comparison, in the previous 13 months from 1 January 1993 to 31 January 1994, only 17 patients had one or more sputum cultures that grew this organism. In March 1994, an additional 20 patients were identified with *B. cepacia* isolated from sputum cultures. Preliminary review of records of these 40 patients revealed that 30 (75%) were resident in an ICU and that 35 (86%) were mechanically ventilated before the date of their first sputum culture that grew *B. cepacia*. We therefore conducted an analytic and molecular epidemiological investigation of *B. cepacia* respiratory acquisition among patients at the MGH to determine: (*a*) risk factors for respiratory acquisition of *B. cepacia*, particularly those procedures and medications associated with the care of mechanically ventilated patients; and (*b*) if *B. cepacia* sputum acquisition was associated with a unique strain of *B. cepacia*.

METHODS

Case definition and ascertainment

A case-patient was defined as any patient who received care at the MGH from 1 January to 31 December 1994 and who had at least one sputum culture from which *B. cepacia* was isolated (*B. cepacia*-positive). Six patients with cystic fibrosis who were known to have chronic respiratory colonization with *B. cepacia* before 1994 were excluded. To identify cases, we reviewed microbiology records for sputum cultures positive for *B. cepacia* between 1 January and 31 December 1994.

Epidemiological studies

Case-control study 1

To identify risk factors for *B. cepacia* respiratory acquisition, we conducted two case-control studies. In the first study, because of the apparent association between *B. cepacia* respiratory acquisition and mechanical ventilation received in an ICU, we sought first to determine if duration of mechanical ventilation and ICU location were risk factors for acquisition of *B. cepacia*. We investigated the 30 case-patients who were mechanically ventilated in an ICU before the date of their first *B. cepacia*-positive sputum culture during the period 1 February to 31 March 1994 (Fig. 1). These patients were compared with 30 *B. cepacia*-negative control-patients who were identified by randomly selecting patients from the Respiratory Care Department's daily log of mechanically ventilated patients. Control-patients had to be mechanically ventilated in an ICU on the same day that case-patients had their first *B. cepacia*-positive sputum culture (i.e., match date) and never have had a *B. cepacia*-positive sputum culture. We then determined the frequency of intubation and the duration and location of mechanical ventilation for case- and control-patients, using the match date as the last date examined.

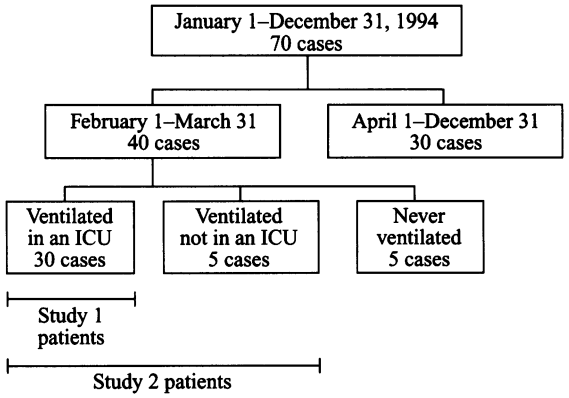

Fig. 1. Criteria for inclusion of cases in studies 1 and 2.

Case-control study 2

In the second case-control study, to determine risk factors for *B. cepacia* acquisition among mechanically ventilated patients, we compared the 35 mechanically ventilated case-patients identified from 1 February to 31 March with 35 mechanically ventilated control-patients (Fig. 1). To control for the potential confounding factor of duration of ventilation identified in Study 1, we selected control-patients by matching on the date and duration of the most recent intubation ± 2 days). Potential patient- and treatment-related risk factors were examined for each case-control pair from the date of admission to the match date including: date(s) and frequency of

intubation and total days of mechanical ventilation; surgical exposures, including performance of tracheostomy and bronchoscopy; nasogastric and gastric tube insertion; and receipt of chest physiotherapy. In addition, for the 10-day period before the match date, we determined exposure to the following medications: antimicrobials, gastric ulcer prophylaxis, metered dose inhalers, and nebulized medications.

Procedure review and environmental culturing

Patient-care and environmental cleaning procedures were reviewed, including endotracheal and nasotracheal intubation; administration of respiratory medications; and maintenance, cleaning, and disinfection of mechanical ventilator equipment.

Environmental culturing was conducted on several dates during the investigation, including: ventilator equipment, suction irrigation solutions, and other solutions that could potentially be instilled into the airway, from each of 11 mechanically ventilated patients, including 7 case-patients and 4 patients known to be *B. cepacia* sputum culture negative; lidocaine (4% solution and 2% viscous gel) and benzocaine spray used in performing intubations in the MGH operating room; and 250 ml samples of hot and cold water and swabs from a total of 5 faucets in 3 anaesthesia preparation rooms in the MGH operating room. Ten μl of solutions and swabs of sink screens were inoculated directly onto Brucella, MacConkey, and *B. cepacia*-selective agars (Becton Dickinson, Cockeysville, MD). Water samples were filtered through a 0·45 μ filter; the filters were then plated onto Brucella and *B. cepacia*-selective agars. All cultures were incubated at 37 °C for > 72 h. All Gram-negative organisms were further identified by Vitek GNI (bioMerieux Vitek, Hazelwood, MO).

On a total of 3 days (one each in March, April and September 1994), we cultured opened and unopened vials of albuterol solution 0·5% from manufacturer A, including vials used by case-patients. One ml samples of albuterol solution 0·5% were inoculated directly onto Brucella agar. To determine if albuterol solution 0·5% would support the growth of *B. cepacia*, 1 ml of a diluted broth culture of *B. cepacia* (10^4 c.f.u./ml of dextrose phosphate broth) was inoculated into a previously unopened 20 ml vial of albuterol solution 0·5% and held at room temperature. One ml samples were cultured before inoculation and on days 2 and 7 after inoculation as described above.

Microbiological studies

The antimicrobial susceptibility of *B. cepacia* isolates from 1994 (outbreak period) and 1993 (pre-outbreak period) were compared. Antimicrobial susceptibility was determined by disk diffusion [21].

Eight *B. cepacia* isolates, including 6 from case-patients randomly selected from a total of 6 different ICU and non-ICU locations in February and March 1994, and 2 from cystic fibrosis patients known to have *B. cepacia* respiratory colonization, were typed at the Centers for Disease Control and Prevention (CDC) by one of us (LAC) by pulsed-field gel electrophoresis. Overnight broth cultures were adjusted to an absorbance of 0·3 at 610 nm, and agarose plugs were prepared from cell pellets according to the procedure of Smith and Cantor [22]. Agarose slices were digested with *Spe* I and *Xba* I restriction endonucleases according to the manufacturer's directions. Pulsed-field gel electrophoresis was carried out with a CHEF-DR II apparatus at 14 °C for 24 h at 200 V; pulse times were linearly ramped from 5–70 s for *Spe* I and 1–20 s for *Xba* I.

Statistical methods

The data were collected and analysed using EpiInfo software (Version 6·01) [23]. Odds ratios and exact 95% confidence intervals were calculated. The statistical significance of odds ratios were determined using the Mantel–Haensel corrected χ^2-test for unmatched analysis and the McNemar corrected χ^2-test for matched-pair analysis. Continuous variables were compared using the matched pairs *t*-test. Conditional logistic regression from the LOGXACT package (Cytel Software Corporation, Cambridge, MA) was used to estimate the odds ratio for risk factors for acquiring *B. cepacia*, while simultaneously adjusting for potential confounders [24].

RESULTS

From 1 January to 31 December 1994, we identified 70 cases of *B. cepacia* respiratory acquisition. The rate of *B. cepacia* respiratory acquisition was significantly greater in 1994 than in 1993 (17·2 [70/40, 772] *v.* 3·8 [14/36, 942] per 10000 discharges; χ^2 $P < 0{\cdot}001$). Cases increased abruptly beginning in the second week of February, peaked during the last week of February and the first week of March, and decreased

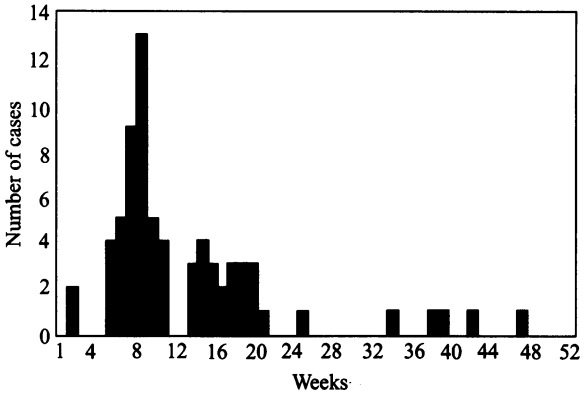

Fig. 2. Cases of *Burkholderia cepacia* respiratory acquisition, by week, 1 January–31 December 1994.

Table 1. *Characteristics of case patients, 1 January–31 December 1994*

Characteristic	Number (%) ($n = 70$)
Sex	
Male	46 (66)
Female	24 (34)
Age (years)	
Mean ± SD*	63·0 ± 17·4
Median (range)	65 (1 month–92 years)
Patient location†	
Neurosurgical ICU	16 (23)
Coronary-care Unit	10 (14)
Cardiac surgical ICU	7 (10)
Respiratory ICU	7 (10)
General surgical ICU	6 (9)
Medical ICU	5 (7)
Burn ICU	1 (1)
Neonatal ICU	1 (1)
Non-ICU location	15 (20)
Outpatient	2 (3)
In-hospital mortality	23/68‡ (34)

* SD, standard deviation.
† Hospital location on the date of the first *B. cepacia*-positive sputum culture.
‡ Among hospitalized case-patients.

sharply after the fourth week of May (Fig. 2). No additional cases were reported after 26 November 1994. Sixty-eight (97%) of the 70 case-patients were inpatients, and 52 (74%) were resident in one of seven adult ICUs on the date of their first *B. cepacia*-positive sputum culture (Table 1). Twenty-six cases were identified among patients in the neurosurgical ICU (16 [23%]) or the coronary-care unit (10 [14%]). The median interval between the date of admission and the first *B. cepacia*-positive sputum culture for the 68 hospitalized case-patients was 11 days (range 1–111 days). The in-hospital mortality rate among these case-patients was 34% (23/68); no deaths were directly attributed to *B. cepacia* respiratory acquisition.

B. cepacia was cultured only from sputum in 68 (97%) of the 70 case patients. One case-patient had *B. cepacia* isolated from blood collected through an arterial line 8 days after his first *B. cepacia*-positive sputum culture; another patient had *B. cepacia* cultured from a central venous catheter tip 10 days after his initial *B. cepacia*-positive sputum culture. Of the 40 cases identified from 1 February to 31 March, 9 (22%) met the CDC definition of ventilator-related pneumonia associated with *B. cepacia* respiratory acquisition [25]. Of the 9 patients, 4 (44%) had sputum cultures that grew moderate to abundant *B. cepacia*, including one patient whose culture grew abundant *B. cepacia* as the only respiratory pathogen. Eight patients had other organisms isolated from the sputum cultures that first grew *B. cepacia*; five of these patients had other nosocomial pathogens isolated (*Proteus mirabilis* [$n = 3$], *Pseudomonas aeruginosa* [$n = 1$], *Acinetobacter* sp. [$n = 1$]).

Case-control study 1

Of the 40 cases that occurred from 1 February to 31 March, 30 of the patients were resident in an ICU and were receiving mechanical ventilation immediately before their first *B. cepacia*-positive culture (Fig. 1). Compared with 30 randomly selected control-patients who were mechanically ventilated in an ICU, the 30 case-patients were significantly more likely to have been ventilated for 2 or more days (30/30 [100%] *v.* 15/30 [50%]; $P < 0{\cdot}001$) and to have been intubated more than once (12/30 [40%] *v.* 2/30 [7%]; OR = 9·3, 95% CI = 1·6–68·8; $P = 0{\cdot}002$) before the match date. There was no significant difference in the ICU location of case- and control-patients on the match date (data not shown). Of the remaining 10 case-patients identified in February and March 1994, 5 were mechanically ventilated in a non-ICU location (operating room [$n = 4$] and emergency department [$n = 1$]), and 5 were not ventilated before the first *B. cepacia*-positive culture (Fig. 1).

Case-control study 2

Compared with matched control patients, the 35 mechanically ventilated case-patients were significantly more likely to have received a nebulized

medication, nebulized albuterol, a cephalosporin, or the combination of a cephalosporin and aminoglycoside antimicrobial in the 10-day period before the match date (Fig. 1, Table 2). In addition, among patients who underwent surgery, case-patients were significantly less likely than control-patients to have had a nasogastric tube placed in the operating room (Table 2). Case- and control-patients did not differ significantly in any of the other potential risk factors examined including: the frequency and location of intubations; receipt of metered dose respiratory medications; hospital unit on the match date; other surgical procedures performed; or receipt of chest physiotherapy, bronchoscopy, or tracheostomy (Table 2). By multivariate analysis, receipt of a nebulized medication and a cephalosporin antimicrobial in the 10-day period before the match date were the only risk factors independently associated with *B. cepacia* respiratory acquisition (Table 2).

Administration of nebulized medications

Thirteen (41%) of 35 case-patients received nebulized medications in the 10 days before the match date. Of these, 9 case-patients received albuterol only, 3 received albuterol and at least one other nebulized medication (glycopyrolate and/or acetylcysteine), and 1 received acetylcysteine only. Because of the high degree of correlation between receipt of any nebulized medication and receipt of albuterol, we next determined the number of doses of albuterol that were administered to case- and control-patients during selected intervals and reviewed MGH pharmacy records for purchases of albuterol solution. Compared with matched control-patients, mechanically ventilated case-patients were significantly more likely to have received nebulized albuterol at any time from admission to match date (15/35 [43%] *v.* 6/35 [17%], matched OR = 3·6, 95% CI = 1·1–13·2). In addition, among patients who received albuterol, case-patients received significantly more doses than did control-patients when examined from the date of admission to the match date (median, 18 *v.* 1·5 doses; $P = 0{\cdot}04$) or during the 10-day period before the match date (median, 8 *v.* 1 dose; $P = 0{\cdot}004$). The findings were similar when the number of doses of any nebulized medication was determined for case- and control-patients during the 10-day period before the match date (median, 12 *v.* 1 dose; $P = 0{\cdot}001$).

Since 1993, the MGH pharmacy obtained albuterol solution 0·5% exclusively from manufacturer A. Original quality assurance testing and retesting of retained samples of each of the lots of albuterol solution 0·5% potentially in use at the time of the outbreak revealed the absence of microbial growth, including *B. cepacia.*

Review of patient-care procedures

At MGH, nebulized medications are administered almost exclusively to non-mechanically ventilated patients by either respiratory therapists or nurses trained in their administration. Nebulized medications are stored at the patient's bedside and are used exclusively by that patient. Review of procedures with personnel indicated that during the outbreak nebulizer reservoirs were not always rinsed after each use nor discarded after 24 h.

Microbiological studies

All environmental cultures were negative for *B. cepacia*, including solutions, medications, and water samples. Opened and unopened vials of albuterol solution 0·5% from a single lot purchased on 23 January 1994 were all negative for microbial growth, including three opened vials from three case-patients who first became *B. cepacia*-positive 3, 11 and 14 days, respectively, before the albuterol culture was performed. Following inoculation of two unopened 20 ml vials of albuterol solution 0·5% with 10^4 c.f.u. of *B. cepacia* (mean, 480 c.f.u./ml), *B. cepacia* remained viable and grew slowly, reaching mean concentrations of 1600 c.f.u./ml and 4600 c.f.u./ml at 2 and 7 days, respectively.

The *B. cepacia* isolates from 6 case-patients had an identical restriction fragment length polymorphism (RFLP) pattern by pulsed-field gel electrophoresis. This RFLP pattern differed substantially compared with that of two *B. cepacia* isolates from cystic fibrosis patients not associated with the outbreak (Fig. 3).

The *B. cepacia* isolates from all 70 case-patients were resistant to gentamicin, tobramycin, amikacin, chloramphenicol, and ticarcillin and were susceptible to trimethoprim-sulphamethoxazole, ciprofloxacin, mezlocillin, piperacillin, and imipenem. In contrast, the *B. cepacia* respiratory isolates from 17 patients in 1993 (pre-outbreak period) were variably resistant to tobramycin (67%), gentamicin (80%), chloramphenicol (56%), and ticarcillin (50%) and were not uniformly susceptible to trimethoprim-sulphamethoxazole (50%), ciprofloxacin (30%), and imipenem (67%).

Table 2. *Risk factors associated with* B. cepacia *respiratory acquisition, matched univariate and multivariate analyses*

Risk factor	Cases: number (%) exposed	Controls: number (%) exposed	Matched OR (95% CI)	Multivariate OR (95% CI)
Demographic characteristics	(*n* = 35)	(*n* = 35)	—	—
Sex				
Male	25 (71)	21 (60)	1·7 (0·6–5·6)	—
Female	10 (29)	14 (40)	—	—
Age (years)				
Mean ± S.D.	62 ± 15·4	65 ± 16·4	—	—
Median (range)	63 (28–85)	71 (19–82)	—	—
Underlying disease				
Cardiovascular	12 (34)	9 (26)	1·5 (0·5–5·1)†	—
Neurological	9 (26)	8 (23)	—	—
Respiratory	1 (3)	8 (23)	—	—
Gastrointestinal	5 (14)	2 (6)	—	—
Carcinoma	4 (11)	1 (3)	—	—
Trauma	2 (6)	5 (14)	—	—
Other	2 (6)	2 (6)	—	—
In-hospital mortality	11 (31)	15 (43)	0·6 (0·2–1·8)	—
Exposures from admission	(*n* = 35)	(*n* = 35)	—	—
Oral or intravenous steroids	13 (37)	8 (23)	2·7 (0·7–10·0)	—
Residence in:				
Neurosurgical ICU	7 (20)	2 (6)	3·5 (0·7–16·8)‡	—
Coronary-care Unit	6 (17)	2 (6)	—	—
Cardiac surgical ICU	5 (14)	5 (14)	—	—
Respiratory ICU	3 (9)	6 (17)	—	—
Other ICU	9 (26)	13 (37)	—	—
Non-ICU location	5 (14)	7 (20)	—	—
Number of intubations:				
3–5	6 (17)	2 (6)	2·2 (0·7–8·1)§	—
2	7 (20)	5 (14)	—	—
1	22 (63)	28 (80)	—	—
Intubation in coronary-care unit	6 (17)	1 (3)	6·0 (0·7–49·8)	—
Gastrostomy tube	5 (14)	12 (34)	0·4 (0·1–1·2)	0·2 (0·02–1·1)
Chest physiotherapy	31 (88)	30 (85)	1·5 (0·2–9·0)	—
Bronchoscopy	5 (14)	11 (31)	0·3 (0·09–1·2)	—
Tracheostomy	10 (28)	8 (22)	1·5 (0·4–5·3)	—
Any surgery	27 (77)	24 (68)	1·8 (0·4–8·2)	—
Surgical exposures	(*n* = 27)	(*n* = 24)	—	—
Neurosurgery	8 (23)	1 (3)	5·3 (0·66–UD)‖	—
Anaesthesia resident A	5 (14)	0	3·8 (0·41–UD)	—
Nasogastric tube	4 (11)	10 (28)	0·1 (0·0–0·9)	—
Operating room A	4 (11)	0	2·4 (0·18–UD)	—
Anaesthesia machine A	5 (14)	1 (3)	5·0 (0·6–236·8)	—
Exposures in 10-day period before match date	(*n* = 35)	(*n* = 35)	—	—
Any cephalosporin	32 (91)	22 (63)	6·0 (1·3–55·2)	11·9 (1·6–553·1)
Any aminoglycoside	22 (63)	17 (49)	1·8 (0·6–6·0)	—
Any cephalosporin and aminoglycoside	21 (60)	10 (29)	4·7 (1·3–25·3)	—
Any penicillin	19 (54)	12 (34)	2·4 (0·8–8·7)	—
Vancomycin	26 (74)	19 (54)	3·3 (0·9–18·8)	—
Any nebulized medication	13 (37)	3 (9)	6·0 (1·3–55·3)	11·9 (1·6–553·1)
Nebulized albuterol	12 (34)	3 (9)	5·5 (1·2–51·0)	—
Any metered dose inhaler	25 (71)	23 (66)	1·3 (0·5–3·8)	—

* S.D., standard deviation. † Cardiovascular *v.* other underlying disease. ‡ Neurosurgical ICU *v.* other ICU.
§ More than one intubation *v.* one intubation. ‖ UD = undefined.

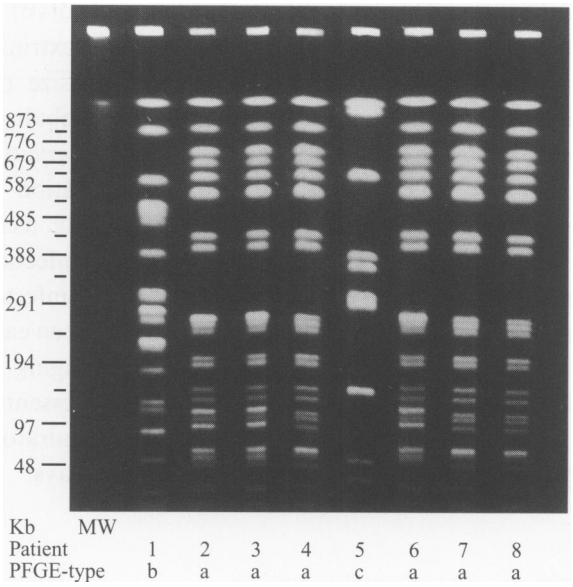

Fig. 3. Analysis of *Spe* I restriction endonuclease-digested genomic DNA of *B. cepacia* by pulsed-field gel electrophoresis. Strains isolated from the sputum of case-patients (patients 2–4, 6–8) had an identical RFLP pattern (designated 'a' that differed substantially from that of strains from cystic fibrosis patients with *B. cepacia* respiratory colonization (patients 1 and 5; RFLP patterns 'b' and 'c'). Bacteriophage λ ladder (lane MW) was used as molecular size standard. The results were confirmed using *Xba* I restriction endonuclease digestion.

DISCUSSION

Our investigation of *B. cepacia* respiratory acquisition at MGH demonstrated that the outbreak first affected mechanically ventilated ICU patients. Although many of the *B. cepacia*-positive sputum cultures probably represented upper airway colonization, at least 9 (23%) of 40 patients identified during February–March 1994 had evidence of lower respiratory tract infection associated with *B. cepacia* acquisition. Additional cases of *B. cepacia* respiratory colonization may have occurred, especially among patients in non-ICU locations, and may not have been detected if a sputum culture was not obtained. However, because control-patients had to have had one or more sputum cultures obtained, each of which was negative for *B. cepacia*, failure to ascertain cases fully would not have altered the results of the risk factor analysis.

Although we were unable to identify a source of *B. cepacia*, several findings suggested the outbreak may have spread from a single source. First, the dramatic increase in the incidence of *B. cepacia* in February 1994, which declined rapidly thereafter without specific intervention, suggested a possible point-source outbreak. In addition, the outbreak strains of *B. cepacia* had identical antimicrobial susceptibilities and RFLP patterns by pulsed-field gel electrophoresis. The occurrence of scattered cases after the peak in February and March 1994 suggested that following its introduction into the hospital environment, *B. cepacia* acquisition may have been facilitated by multiple modes of transmission, including person-to-person transmission via the hands of health-care workers [7].

To limit potential confounding by later cases, we restricted the study of risk factors for *B. cepacia* acquisition to patients who were ventilated during the first 2 months of the outbreak. Because we found that *B. cepacia* acquisition was associated with a longer duration of mechanical ventilation, we also controlled for this potential confounder by matching by date and duration of the most recent mechanical ventilation. We did not control for severity of illness using clinical scoring systems such as APACHE because such systems have not been validated for non-ICU patients and are subject to misclassification bias when determined retrospectively [26].

Because nebulized medications were administered almost exclusively to patients who were not mechanically ventilated, matching cases and controls by duration of mechanical ventilation could have resulted in detection bias if controls did not have a similar opportunity to be exposed to nebulized medications before intubation or following extubation. However, a strong dose-response relationship was observed among patients receiving nebulized medications and its correlate nebulized albuterol. In addition, among the 13 case-patients exposed to nebulized medications during the 10-day period before the match date, ten (77%) either received these medications before the intubation date ($n = 9$) or while receiving mechanical ventilation ($n = 1$); these cases and their matched controls thus had a similar opportunity to be exposed to nebulized medications. When the three cases that were exposed to nebulized medications only after extubation and their matched controls were excluded from analysis, receipt of nebulized medications was still associated with *B. cepacia* respiratory acquisition (10/32 *v.* 3/32; matched OR = 4·5, 95% CI = 1·1–30·6). Case- and control-patients also had similar demographic and clinical characteristics, including in-hospital mortality rates, suggesting that differences in severity of underlying lung disease did not confound the association with receipt of nebulized medications.

In this investigation, cultures of all patient-care equipment, solutions, and medication associated with the outbreak were negative for *B. cepacia*; thus we were unable to identify a definitive source. Because

nebulizer devices were used almost exclusively on non-ventilated patients, they were not available when we cultured the respiratory therapy equipment of seven mechanically ventilated case-patients. However, nebulizer reservoirs were not always rinsed with sterile water and dried after each use nor discarded after 24 h use in compliance with the MGH policy, thus increasing the risk for extrinsic contamination. Previous studies have demonstrated that nebulized medication devices can become contaminated by using non-sterile water to rinse nebulizers between use [17], by extrinsic contamination of nebulized medications [18, 20] and presumably by the hands of health-care workers manipulating the equipment. Although we did not examine exposure to health-care workers nor perform handwiping cultures of personnel, exposure to an individual health-care worker appears to be an unlikely source of the outbreak, because each individual worked in only one or two patient care units while cases were widely distributed throughout the hospital.

Receipt of selected antimicrobials may increase the risk of upper airway colonization of ventilated patients with resistant Gram-negative organisms [27–30]. In this investigation, receipt of a cephalosporin and the combination of a cephalosporin and an aminoglycoside were associated with *B. cepacia* acquisition by univariate analysis; these were antimicrobials to which the *B. cepacia* isolates were resistant.

Although receipt of a nebulized medication and a cephalosporin were significant independent predictors of becoming a case, together they accounted for only 36·5% of the variance in the logistic regression model. This suggests that other risk factors not studied contributed to the outbreak. Once *B. cepacia* was introduced into multiple ICUs, its transmission among mechanically ventilated patients could be facilitated by contamination of ventilator circuitry or nebulizer reservoirs via frequent manipulation or by direct inoculation into the patients' airways (e.g., via suctioning). Consistent with this, longer duration of ventilation and multiple intubations both were associated with *B. cepacia* acquisition.

This investigation emphasizes the potential for multidrug-resistant Gram-negative organisms to disseminate rapidly in the hospital and cause both respiratory colonization and infection, especially among mechanically ventilated patients. Our investigation indicated that *B. cepacia* respiratory acquisition was associated with receipt of nebulized medications. This outbreak and the recent report of *B. cepacia* respiratory acquisition linked to extrinsic contamination of albuterol solution emphasize the potential for microbial contamination of nebulized medications despite the presence of the preservative benzalkonium chloride [20]. The administration of nebulized medications should be done carefully, using standard administration techniques and disinfection practices. Nebulizer reservoirs should be disinfected or rinsed with sterile water and air dried between each use [31]. Strict handwashing and glove-changing (if gloves are used) between patient contacts are essential to prevent microbial contamination of respiratory therapy equipment and/or the patients' airways.

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