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Outbreak of severe *Pseudomonas aeruginosa* respiratory infections due to contaminated nebulizers

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Summary: During the six months from January–June 1994, 10 cases of severe and 11 of less severe pulmonary infection caused by *Pseudomonas aeruginosa* were diagnosed in patients with chronic obstructive airways disease. Possible sources were evaluated. *P. aeruginosa* was isolated from four of the 22 nebulizers tested. The relationship of isolates from the patients and nebulizers was confirmed by sero- and phage-typing, and by arbitrarily-primed polymerase chain reaction (AP-PCR). Three types were identified and the distribution of types in patients with severe infection was as follows (one patient had a multiple infection). Type I was isolated from two nebulizers and from sputa, and/or blood and/or bronchial protected specimen brush samples or bronchial lavage fluid from four patients. Type II came from the sputa of three patients and a third nebulizer; and type III from sputa and/or blood of four further patients and another nebulizer. The data provided evidence for the relation between *P. aeruginosa* as a cause of infection and the contamination of the nebulizers. When nebulizer mouthpieces were changed every 24 h and sterilized between patients, no more contamination occurred, and the outbreak ceased.

Keywords: *Pseudomonas aeruginosa*; contamination of nebulizers; hospital-acquired pneumonia.

Introduction

Hospital-acquired infection represents a major source of morbidity and mortality for hospital patients.[1–3] Since the early 1960s hospital-acquired Gram-negative necrotizing pneumonia has been observed with increasing frequency.[1,2] Among the Gram-negative bacilli that cause nosocomial infection, *Psuedomonas aeruginosa* has the unique ability to infect a wide variety of animal and plant hosts, and is associated with infections in

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patients with burns, cystic fibrosis, neutropenia and traumatic wounds,[3] together with the respiratory-tract infections, which present an increasing problem in patients with chronic pulmonary disorders.[4]

Early in 1994, we observed an increased number of patients with respiratory infection caused by *P. aeruginosa* on the pulmonary ward. Despite adherence to a hand-washing protocol and isolation of all infected patients, new cases of severe infection continued to occur. Although hands are the usual mode of transmission of nosocomial infections,[5,6] the association between these infections and the introduction of new equipment for inhalation therapy suggested a possible source for the outbreak. This paper describes the outbreak and the measures taken to identify and eliminate the source of infection.

Methods

From January–June 1994, all patients admitted to the pulmonary ward with worsening chronic obstructive airways disease (COAD), who developed positive sputum cultures for *P. aeruginosa* were analysed. All had been treated with high doses of systemic steroids (75–100 mg/24 h, i.v.) together with inhalations of salbutamol and ipratropiumbromide using a Pari Boy inhaler. Severe hospital-acquired respiratory-tract infection was defined by development of a new infiltration on chest X-ray, together with positive cultures from sputum, and/or blood, bronchial protected specimen brush samples or bronchial lavage fluid. The characteristics of the 10 patients with severe infection are shown in Table I. Each patient who became infected was isolated immediately.

Inhalation therapy equipment

The inhalation device concerned is a Pari Inhaler (Par Werk, Starnberg, Germany). This is an electronically controlled device which uses compressed air to nebulize the drug. An aerosol is generated by high-speed air flow through a small hole in a plastic chamber designed to hold the medication. The chamber is connected by a rubber tube to a dedicated compressor and connected to a mouthpiece. The airflow is 15 L/min. A switch beside the compressor is used to control the apparatus, and to save unused medication. The reservoir is filled before each use with 0·5 mL (2·5 mg) salbutamol and 2 mL (500 μg) of ipratropiumbromide—giving 2·5 mL total fluid volume; although on occasion this is increased to 3–4·5 mL by addition of 0·9% sodium chloride—all from a sterile single-use vial. During their hospital stay, each patient had a designated nebulizer. The medication chamber was refilled when necessary. When the patient left hospital the mouthpiece was discarded, and the reservoir was disinfected by immersion in glutaraldehyde, then stored mounted at room temperature before use by the next patient according to hospital standard protocol.[7]

Table I. *Characteristics of the patients who developed a* Pseudomonas aeruginosa *respiratory infection with infiltration on chest X-ray*

Patient No.	Sex	Age (years)	Number of days before infiltrate	Underlying diseases	Pack-years*	Antimicrobial treatment before isolation of *P. aeruginosa*	Temperature (°C)	X-ray localization of infiltrate	Leucocytes‡ $\times 10^9$/L
1	M	66	20	COAD; aspergilloma LUL	50	—	39·1	LLL	26·2
2	F	68	6	COAD	—	—	38·4	LUL	24·8
3†	M	61	6	COAD; silicosis pulmonum	50	—	38·8	LUL	29·0
4	M	73	7	COAD	60	Amoxycillin 1 day Erythromycin 6 days	36·7	LLL	30·4
5	M	79	20	COAD	40	Cotrimoxazole 10 days	38·8	LUL	35·3
6	M	65	5	COAD; atherosclerosis	40	Co-amoxyclav acid 2 days Cotrimoxazole 5 days	39·2	LUL/RUL	27·6
7†	M	81	16	COAD; silicosis pulmonum; pulmonary embolism	20	Amoxycillin 1 day Cotrimoxazole 11 days	37·8	RUL	16·8
8†	M	67	7	COAD; radiotherapy because of bronchus carcinoma LUL	50	—	38·0	LUL/RLL	21·6
9†	M	78	25	COAD; reflux esophagitis	60	—	36·8	LUL	44·0
10	F	64	9	COAD; alcohol abuse	50	Cotrimoxazole 2 days	39·4	LUL	15·8

* A pack-year is a year when the patient smoked 20 cigarettes/day. (40/day in one year = 2 pack years.)
† Patients who died. COAD: chronic obstructive airway disease; LUL: left upper lobe; LLL: left lower lobe; RUL: right upper lobe; RLL: right lower lobe.
‡ Leucocyte count in peripheral blood.

Investigation of the outbreak

As soon as a common source outbreak was suspected the following investigations were performed. Samples were taken from all disinfected nebulizer chambers, tap water and showers in the patient rooms. The nebulizers were sampled using cotton swabs. Before collecting water samples 1–2 L of water were discarded then 20 mL were collected. The swabs and water samples (100 μL) were streaked semi-quantitatively using the four-quadrant method onto the following culture media: blood agar; cysteine-lysine-electrolyte deficient (CLED) agar; pseudomonas-specific agar (Oxoid CM559+supplement SR 103E and glycerol) for total bacterial count, facultative aerobic Gram-negative rods and *Pseudomonas* sp. respectively. *P. aeruginosa* was identified using standard methods, i.e., positive oxidase test, growth at 42°C, failure to ferment glucose and lactose or to produce H_2S. All strains were kept at −70°C.

Typing of pseudomonas strains

The antibiotic susceptibilities were determined by measurement of minimal inhibitory concentrations using the microdilution method (NCCLS, guidelines) with *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218 as reference strains. Phage typing was performed using the following phages: 7, 16, 21, 24, 31, 44, 68, 73, F7, F8, F10, 109, 119, 352, 1214, M4, M6, Co121, Co111, Co118. Serotyping was performed using antisera to the IATS types (Difco). Each different reaction was considered in analysis of phage patterns. Strains with similar serotypes but at least one major difference in phage type were considered different. Further identification was done by arbitrarily-primed polymerase chain reaction (AP-PCR) finger printing.[8] The antibiotic susceptibility, sero- and bacteriophage type was determined for all strains, but AP-PCR was done on selected strains (Table II).

Results

During the six months from January–June 1994, 10 patients developed a severe respiratory-tract infection with new infiltrates on X-Ray (NIX), and 11 patients developed milder infection all with positive sputum cultures for *P. aeruginosa* as a single pathogen. Six of the patients developed infiltrates within 10 days of admission. Because initial sputum cultures grew other bacteria (*Haemophilus influenzae* or *Streptococcus pneumoniae*), six patients were treated with antibiotics before developing *P. aeruginosa* infection. The infection was fatal in four cases, despite appropriate antibiotic therapy (determined by antibiotic susceptibility tests). In one the blood culture was the only sample to reveal *P. aeruginosa*, whereas in the fourth, although sputum was negative, the protected specimen brush and broncho-alveolar lavage fluid samples were also positive.

Samples from tap water and showers in the patients' rooms did not grow

Table II. *Characteristics of* Pseudomonas aeruginosa *isolated from nebulizers and patients with infiltrate on Chest-X-ray*

Source		Sero	Type phage	AP-PCR*
Nebulizer I		5	119x,1214	a
Patient 1	Sputum	5	119x,1214	a
	Blood	5	119x,1214	a
Patient 2	Sputum	5	119x,1214	a
	Sputum	5	119x,1214	a
	Sputum	5	119x,1214	a
Patient 3	Protected specimen brush	5	119x,1214	a
	Bronchoalveolar lavage fluid	5	119x,1214	a
Patient 9	Blood	5	119x,1214	a
Nebulizer II		5	7,24,F8,119x	b
Patient 4	Sputum	6	7,24,F8,119x	b
Patient 5	Sputum	6	7,24,F8,119x	b
Patient 10	Sputum	6	7,24,F8,119x	b
Nebulizer III		11	21,68,1214,M6,Co118	a
Patient 3	Blood	11	21,68,1214,M6,Co118	a
Patient 6	Sputum	11	21,68,1214,M6,Co118	a
Patient 7	Sputum	10	not typable	not tested
Patient 8	Sputum	10	not typable	not tested
	Sputum	10	not typable	not tested
	Sputum	10	not typable	not tested

* AP-PCR, arbitrarily primed polymerase chain reaction.

Table III. *Characteristics AP-PCR of* Pseudomonas aeruginosa *isolated from nebulizers and sputum samples*

Source	Number of *Pseudomonas aeruginosa* positive patients		Sero/phagetype	AP-PCR*
	With infiltrate	Without infiltrate		
Nebulizer I	4	1	5 119x,1214	a
Nebulizer II	3	9	6 7,24,F8,119x	b
Nebulizer III	1	1	11 21,68,1214,M6,Co118	a

* AP-PCR, arbitrarily primed polymerase chain reaction.

P. aeruginosa. Twenty-two disinfected and ready for use nebulizers were sampled. *P. aeruginosa* was isolated from four. The isolates from two nebulizers were identical by sero/phage and AP-PCR patterns, and are designated as type I. The types of *P. aeruginosa* isolated are listed in Tables II and III. The antibiotic susceptibility results are excluded as these were identical for all strains, namely: susceptible to piperacillin, ceftazidime, gentamicin and tobramycin.

Serotyping and bacteriophage typing results were in agreement and identified three types. AP-PCR revealed only two, together with clustered isolates of sero/phage types I and III. The three types corresponded well

with the distribution of isolates from nebulizers and patients. Table II shows the comparison of strains from nebulizers with those from the 10 patients showing NIX. The type I strain resembled those isolated from four patients. The isolate from nebulizer 2 (type II) corresponded with isolates from another three patients. Finally, the strain from nebulizer 3 (type III) resembled those from another four patients. Table III shows a comparison of all the nebulizer and patient isolates which had been typed by AP-PCR (i.e., omitting patients 7 and 8). Five patients (four with and one without NIX) had a type I infection, 12 (three with and nine without NIX) had a type II infection, and two patients (one with, one without NIX) had a type III infection.

Discussion

Our study confirms earlier reports that respiratory therapy equipment (i.e., humidifiers/nebulizers) can become contaminated by Gram-negative bacilli while in use.[9–15] Like previous authors we think that the equipment can become contaminated by the patient receiving therapy, and become a source of cross infection. Failure of staff to wash hands is generally considered as the main route of spread of Gram-negative bacilli but our data suggest that storage of disinfected but inadequately dried nebulizers was the likely cause of this 'epidemic', although staff hands were not cultured.

Reinarz *et al.*[9] reported contamination of nebulizers in 1965; 84% of those sampled grew Gram-negative bacteria. Pierce *et al.*[12] documented the potential role of contaminated nebulizers in the pathogenesis of nosocomial pneumonia; while Christopher *et al.*[13] showed that aerosols of Gram-negative bacteria could spread up to 15 ft from a heated humidifier in use on a patient with a pseudomonas pneumonia. Studies on the infectivity of aerosols have shown that the minimal infective dose is less if the particle size is small enough to allow deposition in the airways beyond the level of the ciliated epithelium.[11] At least 50% of particles of 1–2 μm diameter will enter the airways distal to the terminal bronchioles.[10] Aerobiological studies have been difficult to interpret because the quantity of air sampled, the sampling method, and particle sizes collected have all varied; and the entire issue is complicated by the fact that the minimum infective dose to cause pulmonary infection is for the most part unknown.[13]

P. aeruginosa is becoming an important pathogen in patients with COAD, contributing considerably to morbidity and mortality.[16] The factors accounting for its virulence are complex and include extracellular enzymes, toxins and cellular components such as lipopolysaccharide. Motility also seems to be a virulence factor.[16] Because of its unique growth requirements (wide temperature range and use of atmospheric CO_2 as a carbon source) *P. aeruginosa* can survive or grow in moist environments.[3] Infection has been associated with infrequent or inadequate cleaning of hot-water pipes containing carbon-rich debris.[3] Distilled water and water in flower vases

have been found to contain *P. aeruginosa*.[17,18] Water in flower vases can support the growth of many different Gram-negative bacteria so even a small inoculum derived from the hospital environment could reach large numbers within a few days.[18] Contaminated tap water has also been reported as a source of pseudomonas infection in a burns unit.[16]

The combination of AP-PCR typing with the phenotyping techniques enabled us to conclude that an outbreak did occur. AP-PCR is a recently described technique, which can differentiate prokaryotes. In general the typability is greater than that obtained with bacteriophage- or sero-typing, while the three methods have similar discrimination.[8] In this study however the phenotyping methods provided higher discrimination than the AP-PCR.

Having established that an outbreak existed, the next question was one of prevention. Pierce *et al.* in 1970 demonstrated that a reduction in the incidence of Gram-negative pneumonia from 7·9–2·2% correlated with the introduction of effective equipment sterilisation. Use of 0·25% acetic acid provided satisfactory anti-pseudomonas activity despite corrosion of the metal alloy in the equipment.[12]

The small sized nebulizers used for medication in our patients are unlikely to generate bacterial aerosols. The main risk occurs when multi-dose medication vials are used. In our ward the nebulizer reservoir is filled from sterile 'single-use' vials and disinfection of the reservoir plus disposal of the mouthpieces was instituted. However although a presumptively adequate disinfection method was used for the nebulizers, they were stored in a damp condition. We concluded that a disinfection protocol must include instructions for storage, and be monitored regularly. Brändli[7] described the following protocol for use of a nebulizer: wash hands, refill before use from sterile container or single-use vial. After use (or at least daily) wash in hot water, dry with a paper towel, immerse in glutaldehyde for 30 min, and never use tap water. Before the outbreak we also used glutaraldehyde, and stored the nebulizers mounted ready for use. Following the problem, we changed to changing the mouthpieces daily and sterilizing the other components with ethylene oxide in between patients. This proved highly effective in prevention of cross infection despite admission of further cases of severe respiratory infections with *P. aeruginosa*. Flower vases are banned in both the pulmonary and intensive-care wards. Hospital tap water is monitored regularly for both *Legionella* and *Pseudomonas*, although all cultures to date have been negative.

Our observations stress the importance of continuous surveillance of the measures used to prevent and control hospital-acquired infection. Although we paid special attention to handwashing and isolation procedures, the disinfection and especially storage of the nebulizers was not adequately controlled. When tracing the cause of an outbreak of hospital-acquired infection all possible critical steps must be carefully analysed.

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