J Infect Chemother (2008) 14:418–423
DOI 10.1007/s10156-008-0645-9

ORIGINAL ARTICLE

**Nobuyuki Shimono · Takahiro Takuma
Noriko Tsuchimochi · Akira Shiose · Masayuki Murata
Yoko Kanamoto · Yujiro Uchida · Shigeki Morita
Hiroko Matsumoto · Jun Hayashi**

An outbreak of *Pseudomonas aeruginosa* infections following thoracic surgeries occurring via the contamination of bronchoscopes and an automatic endoscope reprocessor

Received: June 5, 2008 / Accepted: September 25, 2008

Abstract An outbreak of *Pseudomonas aeruginosa* infections occurred after thoracic surgeries performed between May and June 2003. Clinical data of seven patients were reviewed and the fact was revealed that bronchoscopes were used during endotracheal intubation for one-lung ventilation in most patients. *P. aeruginosa* was recovered from the sputum of these patients at a very early stage postoperation. Environmental samples from bronchoscopes and an automated endoscope reprocessor (AER) were cultured and *P. aeruginosa* strains were recovered from all of them. All of these strains were confirmed to be identical by pulsed-field gel electrophoresis (PFGE). Inspection of the sterilization cycles of bronchoscopes revealed inappropriate management of bronchoscopes and a flaw in the AER; once its detergent tank was contaminated, it was not possible to disinfect it. After all the bronchoscopes had been disinfected, and the washing machine had been remodeled, with the washing process confirmed to be appropriate, the outbreak finally ended. This outbreak had two causes, a flaw in the AER and inappropriate disinfection procedures. Outbreaks associated with bronchoscopic examinations have been reported elsewhere. Bronchoscopes are widely used to facilitate endotracheal intubation, especially for one-lung anesthesia. Although they are used for only a short time during anesthetic procedures, we should handle them more carefully.

Key words *Pseudomonas aeruginosa* · Bronchoscope · Hospital infection

N. Shimono (✉) · M. Murata · Y. Kanamoto · Y. Uchida · H. Matsumoto · J. Hayashi
Department of Infection Control and Prevention/Infection Control Team, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
Tel. +81-92-642-5228; Fax +81-92-642-5247
e-mail: shimono@intmed1.med.kyushu-u.ac.jp

N. Shimono · T. Takuma · N. Tsuchimochi
First Department of Internal Medicine, Kyushu University Hospital, Fukuoka, Japan

N. Shimono · Y. Uchida
Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, Fukuoka, Japan

A. Shiose · S. Morita
Department of Cardiovascular Surgery, Kyushu University Hospital, Fukuoka, Japan

M. Murata · Y. Kanamoto · J. Hayashi
Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan

Introduction

Nosocomial infections are of great concern for all clinicians. Vigorous hand-washing is the most important procedure for preventing cross-infection. However, nosocomial infection via medical devices and surgical instruments is common. Bronchoscopes are semicritical instruments that contact mucous membranes and require high-level disinfection. Automatic endoscope reprocessors (AERs) are commonly used for the disinfection of endoscopes, including bronchoscopes. Outbreaks of bronchoscope contamination have been reported as being due to incomplete or improper manual processing prior to the placement of bronchoscopes in AERs or the inadvertent use of contaminated reservoirs.[1–3] In most cases, the causal microorganisms were environmental bacteria, particularly *Pseudomonas aeruginosa* and mycobacteria.[4]

For nosocomial pneumonia, it is important to reduce the following risk factors: prolonged mechanical ventilation, repeated intubations, supine positioning, and long-term antibiotic use.[5,6] The microorganisms causative of nosocomial pneumonia are related to the timing of onset. In early-onset nosocomial pneumonia, the responsible microorganisms are generally endogenous. In late-onset nosocomial pneumonia, the responsible microorganisms include potentially multidrug-resistant nosocomial organisms. We usually isolate *P. aeruginosa* from sputum as a late-onset nosocomial pneumonia pathogen.

Between May and June 2003, we experienced frequent recovery of *P. aeruginosa* from the sputum of patients with early-onset pneumonia that occurred within several days

after thoracic surgery. As a result of surveillance, we focused on anesthetic procedures using bronchoscopes for the one-lung ventilation technique.

Patients and methods

Clinical setting

Kyushu University Hospital is a 1300-bed tertiary care hospital in Fukuoka, Japan. Cardiac surgery is performed in 200 or more patients per year, with all operations done under general anesthesia. Bronchial tubes are sometimes placed with the aid of fiberoptic bronchoscopes. One-lung ventilation is used to promote better visualization during thoracic surgical procedures. Anesthetists use a fiberoptic bronchoscope to visually confirm the position of the tube and to make minor adjustments during operation.

Case definition

Cases were defined as patients who had undergone thoracic surgeries between May and June 2003, and from whose sputum *P. aeruginosa* strains were isolated within 1 month of the surgery.

Clinical investigation

Clinical investigation consisted of medical chart review, patient examination, and discussion with nurses and physicians. Nosocomial infection was determined by the isolation of the outbreak strains of *P. aeruginosa* and by clinical evidence of infection. Pneumonia or bronchitis was defined on the basis of respiratory signs of infection with fever and purulent sputum, or on radiographic appearance of a new pulmonary infiltrate. From the medical records of these patients, we reviewed the underlying diseases, the surgical procedures, the operation date, the date when *P. aeruginosa* was isolated, whether or not one-lung anesthesia had been done, the results of sputum cultures, antimicrobial administration records, chest X-ray or other radiological data, respiratory status, and blood examination.

Microbiology

Filtered sterile water specimens were collected from faucets, after first letting the tap run for 30 s. These specimens were collected in sterile 200-ml containers, and 50 ml of the specimen was centrifuged. The resultant pellet was inoculated onto 5% sheep blood agar and Bromothymol blue (BTB) agar plates. Solutions of 10 ml were taken from xylocaine bottles and 50-ml solutions were taken from unopened saline bottles, and these were also processed as described above.

Specimens were obtained from bronchoscopes and transesophageal echocardiographic scopes. The bronchoscopes were flushed with 20 ml of sterile water through the biopsy channel and the water was collected in sterile containers. Ultrasonographic scopes were wiped with a piece of premoistened gauze, which was wrung out to provide the specimen. Each specimen was centrifuged and the resultant pellet was inoculated onto 5% sheep blood agar and BTB agar plates.

Specimens of rinse-cycle water were drained aseptically from the AER. Detergent and disinfectant specimens were collected through valves in sterile 200-ml containers. Specimens from valves and the washer basin were taken for culturing by using premoistened sterile cotton-tipped applicators. The specimens were inoculated onto 5% sheep blood and BTB agar plates. Detergent and disinfectant specimens were also taken directly from the detergent tank and unopened detergent bottles for culturing. They were also processed as described above.

The results for the number of colonies recovered from the plates were expressed as (3+), (2+), and (1+), representing, more than 1000 colonies, 100 to 1000 colonies, and fewer than 100 colonies, respectively.

Molecular analysis

Pulsed-field gel electrophoresis (PFGE; Bio-Rad Laboratories, Richmond, CA, USA) was performed on available isolates obtained from patients and environmental specimens. Genomic DNAs were prepared by a method described in the instruction manual and application guide provided by Bio-Rad Laboratories. DNAs were restricted by the enzyme *Spe*I, electrophoresed on the Chef-DRII System (Bio-Rad Laboratories), and photographed, and the patterns were compared by the naked eye.

Results

Clinical investigation

Our infection control team (ICT) had not routinely surveyed for such nosocomial infections, but the cardiac surgeons noticed sequential pneumonia and reported this event to the ICT. We defined cases as patients who had received an operation and from whose sputum *P. aeruginosa* was isolated, and who suffered from pneumonia (three patients) or bronchitis (four patients). *P. aeruginosa* strains were sequentially isolated within 1 month, from May 14 to June 17. We surveyed all the clinical data and summarized them (Table 1). The operations performed were three thoracic aorta replacements two aortic valve replacements, one coronary artery bypass graft (CABG), and one radical operation for esophageal cancer. *P. aeruginosa* strains were isolated early, at 1 to 7 postoperative days. One lung anesthesia procedures were performed in five of the seven patients; for one lung ventilation during anesthesia, intubation was performed with aid of bronchoscopes. In the other two patients, we could not confirm whether or not intubation was done with the aid of bronchoscopes. All patients stayed in the intensive care unit (ICU) postoperatively.

Table 1. Clinical data

Patient no.	Age (years)	Sex	Underlying disease	Operative procedure	Operation date	Date isolated	One-lung anesthesia
1	20	M	Infective endocarditis	Aortic valve replacement	5/10, 5/28	5/14 (4)	No
2	70	M	Myocardial infarction	CABG	5/28	5/29 (1)	Yes
3	75	F	Aortic valve failure	Aortic valve replacement	5/31	6/7 (7)	No
4	55	M	Aortic aneurysm	Thoracic aorta replacement	6/3	6/4 (1)	Yes
5	55	M	Aortic aneurysm	Thoracic aorta replacement	6/6	6/10 (4)	Yes
6	70	M	Aortic aneurysm	Thoracic aorta replacement	6/11	6/17 (6)	Yes
7	65	M	Esophageal cancer	Radical operation	6/11	6/13 (2)	Yes

Numbers in parentheses are postoperative days
CABG, coronary artery bypass grafting

Table 2. Results of cultures of specimens from environmental sources and bronchoscopes

Source and type of specimen	Organism isolated on culture
Operation rooms and preparation rooms	
Sterile tap water	None
Xylocaine	None
Saline	None
Bronchoscopes	
Bronchoscope (after use; no. 1039)	*P. aeruginosa* (3+)
Bronchoscope (before use; no. 1088)	*P. aeruginosa* (3+)
Bronchoscope (before use; no. 1114)	*P. aeruginosa* (3+)
Transesophageal echocardiographic probe	None
Transesophageal echocardiographic scope	GPC (+)
Washers (automated endoscope reprocessors)	
Rinse water	None
Liquid retained in the washer	*P. aeruginosa* (+)
Water from wall-intake filters	None
Disinfectant (Cidex OPA, Johnson & Johnson)	GNB (+)
Detergent tank	*P. aeruginosa* (3+)
Swab culture of the valve in the washer	GPC (+), GNB (+)
Detergent	None

(+), (2+), and (3+) represent the number of colonies recovered from the plates, at more than 1000 colonies, 100 to 1000 colonies, and fewer than 100 colonies, respectively
GPC, gram positive cocci; GNB, gram negative bacilli

Environmental cultures

All seven patients had been intubated. We first suspected that the nosocomial pneumonia or bronchitis could be attributed to the contamination of items in the ICU. But, unexpectedly, cultures from numerous items and liquid specimens from the ICU did not show any organisms (data not shown). The AER, bronchoscopes, transesophageal echocardiographic scopes, and numerous items and liquids used for operation were in the preparation room of the operation unit. The results of cultures from these sources are summarized in Table 2. Only cultures from the bronchoscopes and detergent from the washer tank showed contamination with *P. aeruginosa*.

Microbiology

Figure 1 depicts the PFGE patterns of genomic DNA after *Spe*I endonuclease digestion of all *P. aeruginosa* isolates from the machine, endoscopes, and from the sputum of the infected patients. It is apparent that each pair is of an identical pattern to the isolates from the machine, endoscopes, and the clinical isolates from the infected patients.

Bronchoscope cleaning procedures

According to our manual, bronchoscopes are usually cleaned just after use. They are manually cleaned by wiping the outer surface and brushing the inner channel and suction ports. The suction button is removed and cleaned. The bronchoscopes are then disinfected in an AER in which ortho-phthalaldehyde (Cidex OPA; Johnson & Johnson, Tokyo, Japan) is used as the liquid germicide. We always ensure that the bronchoscopes are immersed in the germicide and that all channel connectors are attached to the reprocessor according to the manufacturer's instructions. After high-level disinfection, the bronchoscopes are rinsed and the channels are flushed with sterile water to remove the disinfectant. The channels are then flushed with 70%

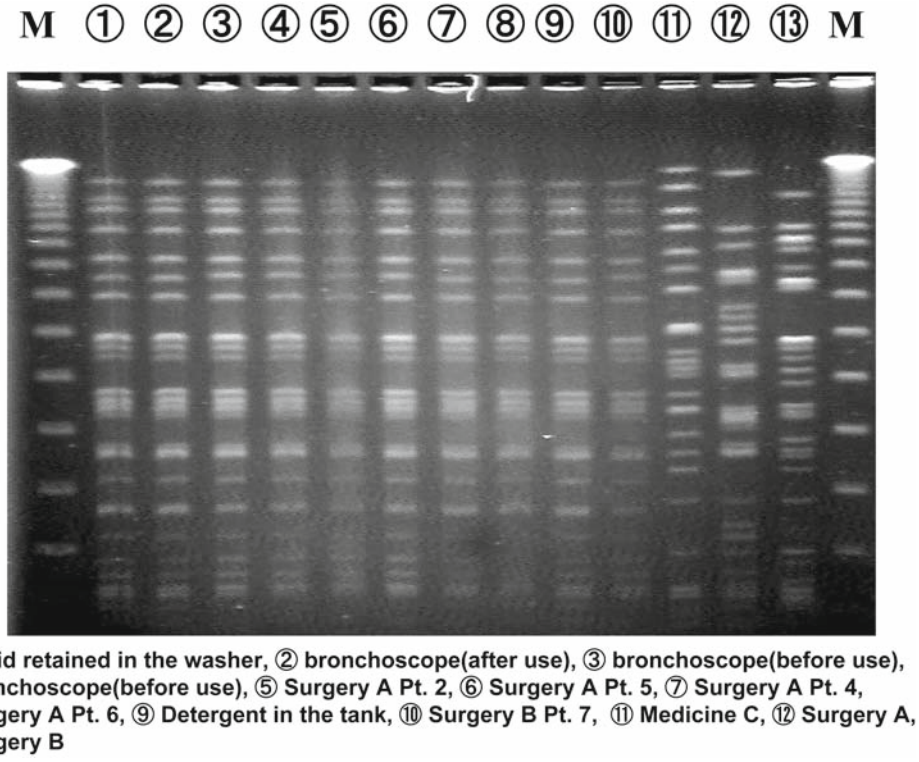

Fig. 1. Results of pulsed-field gel electrophoresis are shown. Strains from environmental samples (*1–4, 9*) and strains from patients (*5–8, 10*) were shown to be identical. In contrast to these samples, strains from other patients were different. *Pt*, Patient; *Surgery A*, surgical ward A; *Medicine C*, medical ward C; *M*, Marker (lambda ladder)

alcohol. Finally, the bronchoscopes are air-dried and stored in a vertical position.

All the steps for cleaning and disinfection of the endoscope equipment were checked. We have a center for the disinfection of endoscopes and most of the endoscopes in the hospital are disinfected or sterilized there according to our manual. However, the bronchoscopes used in the operation unit are disinfected separately from other scopes. Unfortunately, there was poor compliance with the guidelines and recommendations for the cleaning of bronchoscopes. The following steps were sometimes skipped: brushing the internal channels as the first step and rinsing the inner channels with alcohol as the last step. Cleaning should be done promptly after each use of an endoscope to prevent drying of secretions. What was worse, bronchoscopes used in the operation rooms had been left close beside an anesthetist and reused several times during all the operations without disinfection.

Remodeling the AER

Figure 2 depicts the AER. The disinfectant solution did not reach the water inlet lines or the detergent holding tank. Once the tank or lines were contaminated, it was impossible to remove the holding tank and difficult to disinfect or sterilize it. We asked the manufacturer to install a detergent bottle outside the machine, and we are careful to change the detergent bottle before it becomes empty.

Discussion

The main route of nosocomial infection is contact transmission, directly from body surface to body surface, or indirectly via contaminated inanimate objects.[7,8] Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections and the leading cause of death related to transtracheal tubes. Among mechanically ventilated patients, the incidence of nosocomial pneumonia ranges from 10% to 68%, and the mortality rate is twofold higher than that in non-mechanically ventilated patients.[5,9,10] As to the causative microorganisms, the National Nosocomial Infection Surveillance (NNIS) System has reported *P. aeruginosa* and *Staphylococcus aureus* to be the most frequent isolates (each 17.4%).[11,12] Generally late-onset VAP is related to the duration of mechanical ventilation and the length of hospital stay.[13] The previous use of broadspectrum antimicrobial agents is also one of the risk factors for VAP. In our cases, *P. aeruginosa* strains were isolated in succession from the sputum of patients who underwent cardiac surgeries. Indeed, the cardiac surgery patients were immunocompromised because of their extracorporeal circulation and tremendous surgical stress, but the sequential occurrence of VAP seemed unusual and our patients had not received broadspectrum antimicrobial agents. Moreover, we isolated *P. aeruginosa* and noticed the appearance of pneumonia soon after the operation. These facts suggested the possibility of direct inoculation of the microorganism into the bronchus.

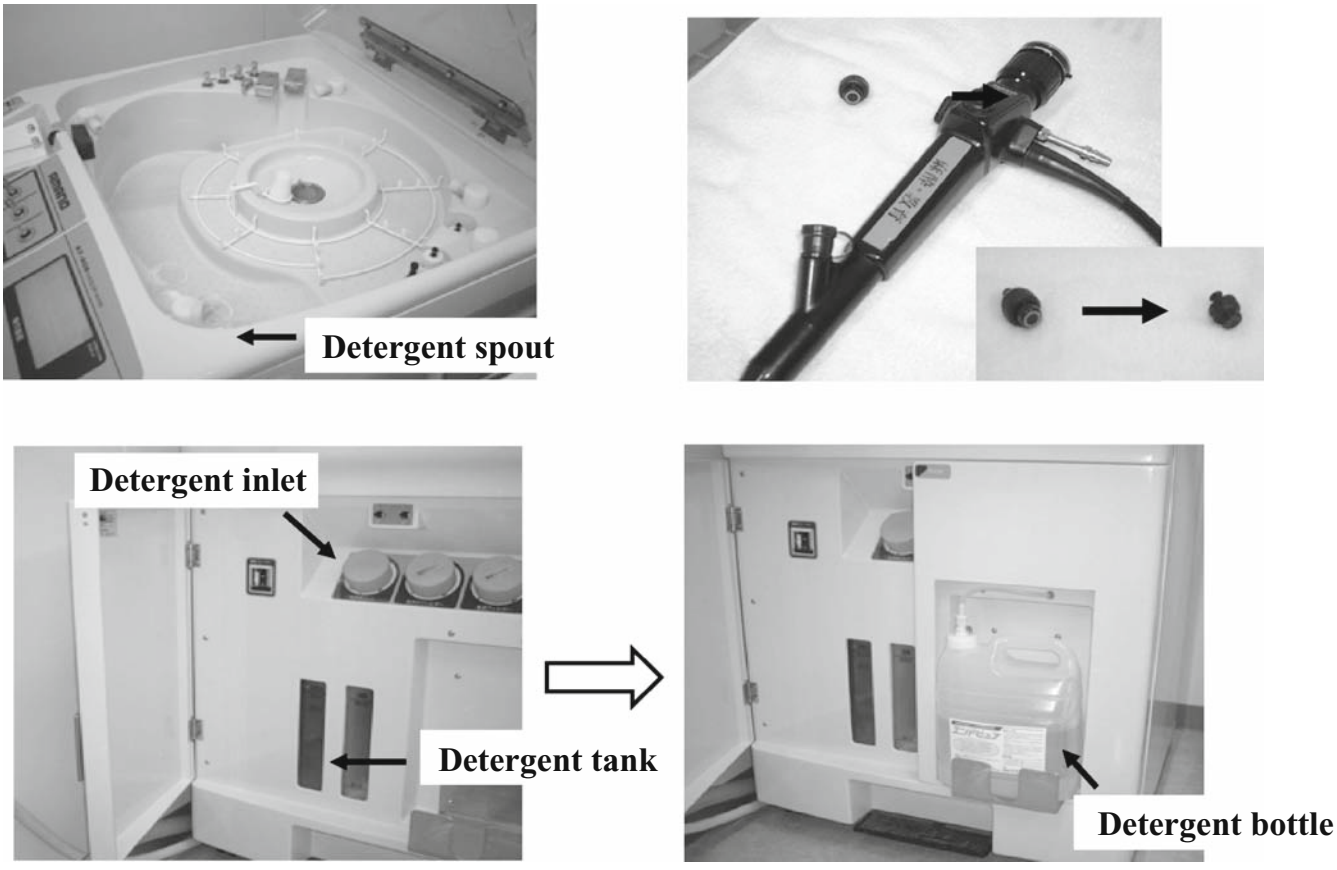

Fig. 2. The automatic endoscope reprocessor has a washing tub in which a bronchoscope is placed. The detergent tank and antiseptic tank are built in the reprocessor and detergent is automatically poured into the tub. In this reprocessor, detergent had been poured through the inlet into the tub when the volume of detergent got less. The tank was difficult to clean. So we attached the detergent bottle outside and changed the bottle when it was empty. At the same time, we changed to the use of disposable suction-port caps

Many reports can be found on nosocomial infections associated with endoscopes. Proper disinfection and sterilization is essential if we are not to transmit infectious pathogens to patients via endoscopes, including bronchoscopes. *P. aeruginosa* and mycobacteria in particular are the most important organisms related to bronchoscope-associated nosocomial infections. Failure to comply with hygiene guidelines has led to numerous outbreaks of infection.[14–18] Flaws related to bronchoscopes; for example, a loose biopsy-port cap, have also caused large nosocomial outbreaks.[1,19] In these reports of nosocomial outbreaks, the bronchoscopes had been used for bronchoscopic examination, for collecting bronchoalveolar lavage fluid samples, for therapy for medical disorders, and as an aid in medical procedures. But we should not forget that bronchoscopes are also used during anesthesia procedures. We focused on the anesthesia procedures of the one-lung ventilation technique. The one-lung technique is widely used to facilitate thoracic surgical visualization.[20–22] For both-lungs ventilation, the aid of bronchoscopes is not always necessary during intubation. For one-lung ventilation, however, we always use a flexible bronchoscope during endotracheal intubation to confirm the positioning.

At our institution, bacteriological investigations of the bronchoscopes yielded a pure culture of *P. aeruginosa* after saline flushing. We also identified contamination by *P. aeruginosa* in the detergent tank of the AER. The disinfection process requires the following five steps. (1) Cleaning the internal and external surfaces, and brushing and flushing internal channels with water and an enzymatic cleaner; (2) immersing the endoscope in high-level disinfectant, exposing and ensuring that all accessible channels are perfused; (3) following disinfection, the endoscope and channels are rinsed with water; (4) rinsing the insertion tube and inner channels with alcohol and drying them with forced air; and (5) storing the endoscope in a way that prevents recontamination and promotes drying.[23–25] These recommendations must be strictly followed, and the cleaning step cannot be overemphasized. Cleaning itself dramatically reduces the bioburden on endoscopes. From several reports, it was shown that cleaning alone reduced the microbial contaminants, with a reduction rate of 99.99%.[3] Without the removal of protein, disinfectant becomes useless for killing bacteria.[26] Unfortunately, we sometimes skipped this cleaning step before the outbreaks. What was worse, bronchoscopes used to be left, without cleaning, beside the bed during

surgery. Cleaning should be done promptly after each use of an endoscope to prevent the drying of secretions. Once secretions have dried, they may form thick biofilms, which result in reduced effectiveness of the detergent or disinfectant.

We concluded that the bronchoscopes were contaminated with *P. aeruginosa*, and from them it spread to the AER. Because of a defect in the AER, the contaminant microorganisms persisted for a long time and caused the sequential nosocomial pneumonia. We disinfected all of the bronchoscopes and cleaned and remodeled the AER. After the washing process was properly practiced, the outbreaks ended. Bronchoscopes are widely used as an aid for intubation, especially for one-lung anesthesia. Although they are used for only a short time during anesthetic procedures, we should handle them more carefully.

Acknowledgments We thank Ms. Kazuko Hosokawa and Ms. Mikiko Ushijima for their excellent technical assistance.

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