

Outbreak of pulmonary *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* infections related to contaminated bronchoscope suction valves, Lyon, France, 2014

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In April 2014, pulmonary *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* co-infections potentially related to bronchoscopic procedures were identified in the intensive care units of a university hospital in Lyon, France. A retrospective cohort of 157 patients exposed to bronchoscopes from 1 December 2013 to 17 June 2014 was analysed. Environmental samples of suspected endoscopes were cultured. Bronchoscope disinfection was reviewed. Ten cases of pulmonary *P. aeruginosa*/*S. maltophilia* co-infections were identified, including two patients with secondary pneumonia. Eight cases were linked to bronchoscope A1 and two to bronchoscope A2. Cultures deriving from suction valves were positive for *P. aeruginosa*/*S. maltophilia*. Exposure to bronchoscopes A1 and A2 was independently coupled with increased risk of co-infection (adjusted odds ratio (aOR) = 84.6; 95% confidence interval (CI): 9.3–771.6 and aOR = 11.8, 95% CI: 1.2–121.3). Isolates from suction valves and clinical samples presented identical pulsotypes. The audit detected deficiencies in endoscope disinfection. No further cases occurred after discontinuation of the implicated bronchoscopes and change in cleaning procedures. This outbreak of pulmonary *P. aeruginosa*/*S. maltophilia* co-infections was caused by suction valve contamination of two bronchoscopes of the same manufacturer. Our findings underscore the need to test suction valves, in addition to bronchoscope channels, for routine detection of bacteria.

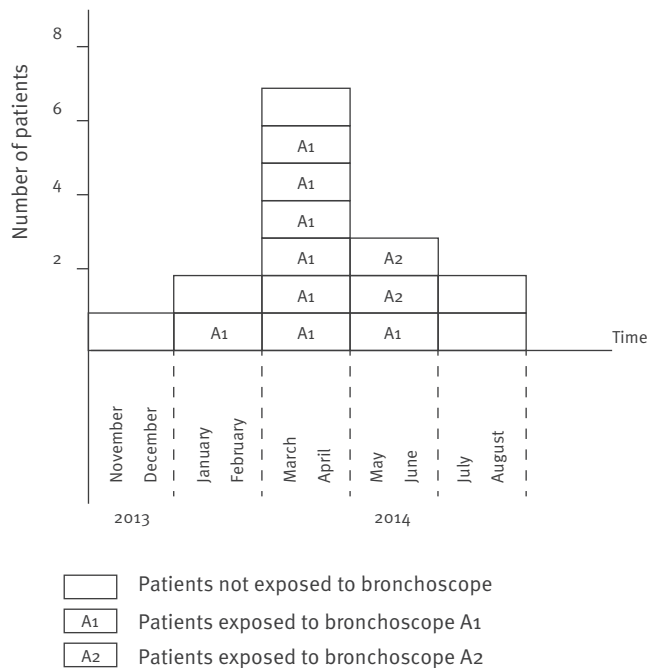
Introduction

Outbreaks and pseudo-outbreaks associated with bronchoscopic procedures have been reported in the literature [1-3]. The microorganisms most commonly implicated in these outbreaks are *Pseudomonas aeruginosa* [4-8], *Mycobacterium tuberculosis* [9,10], and *M. chelonae* [11,12]. In most cases, only a single microorganism is identified, infection by several microorganisms is less frequent [13,14]. Contamination in past outbreaks had various causes, including water from automated endoscope reprocessors [11,15], damaged [7] or defect bronchoscopes [6,13,16], misuse of connectors, deficiencies in the cleaning process and, much less frequently, contamination of suction valves [17,18]. To reduce the risk of nosocomial infections from bronchoscopic procedures, national bronchoscopy guidelines have been established in several countries, including France [19-24]. Despite the increasing experience of bronchoscopic teams, up-to-date guidelines and outbreak reports, patients might still be exposed to contaminated bronchoscopes.

In April 2014, we were alerted to two cases of early-onset pneumonia with *P. aeruginosa* and *Stenotrophomonas maltophilia* in young and immunocompetent trauma patients, after exposure to the same bronchoscope in Edouard Herriot Hospital (Hospices Civils de Lyon, Lyon, France). Here, we report the results of this outbreak investigation and the impact of control measures.

FIGURE 1

Epidemic curve of *Pseudomonas aeruginosa*- and *Stenotrophomonas maltophilia*-positive cultures isolated from respiratory samples of patients exposed or not exposed to bronchoscopes, France, November 2013–August 2014 (n=15)



Methods

Setting

Edouard Herriot Hospital is a 900-bed university-affiliated hospital from Hospices Civils de Lyon in Lyon, France, with four intensive care units (ICUs) accounting for 62 beds overall (ICUs #A, #B, #C and #D). Each year, more than 350 bronchoscopic and 2,000 cleaning procedures are performed in the hospital. In 2014, eight bronchoscopes were used in the endoscopy suite: three of the same model from manufacturer A (bronchoscopes A2, A2, A3) and five from manufacturer B (bronchoscopes B1, B2, B3, B4, B5). These bronchoscopes were deployed in ICUs, operating rooms or other care units.

Bronchoscope cleaning procedures

Bronchoscope cleaning and storage are centralised in ICU #C. Immediately after use, external bronchoscope surfaces are wiped with compresses and channels flushed with water. The bronchoscopes are taken to ICU #C for cleaning, as soon as possible, by authorised personnel, in accordance with a standardised local protocol adapted from French national recommendations [22]. A tightness test is performed before the bronchoscopes are soaked in detergent-disinfectant (Phagoclean NH₄, Laboratoire Phagogène, Christeys, France) and cleaned manually by wiping the outer surface, brushing and flushing internal channels. Each removable component is removed and cleaned. After rinsing, the bronchoscopes are processed in an

automated endoscope reprocessor (Soluscope Series 3 PA, Soluscope, Aubagne, France) with disinfectant (Soluscope P), additive (Soluscope A) and detergent (Soluscope C+). Finally, after drying, the bronchoscopes are kept in an aseptic storage cabinet (Medi 72, Medinorme, La Seyne-sur-Mer, France). Standardised forms are completed for each procedure to maintain traceability.

Outbreak investigations

In April 2014, two cases of early-onset pneumonia with *P. aeruginosa*/*S. maltophilia* in young, not immunocompromised trauma patients in ICU #C were reported to the Infection Control Unit. These patients were exposed to the same bronchoscope (A1). An investigation was launched. In June 2014, two further pulmonary *P. aeruginosa*/*S. maltophilia* co-infections in patients exposed to bronchoscope A2 were encountered in ICU #B. An additional investigation was conducted with a retrospective cohort of patients exposed to bronchoscopes from 1 December 2013 to 17 June 2014 in Edouard Herriot Hospital and a nested case-control study.

Cases were defined as patients exposed to bronchoscopes between 1 December 2013 and 17 June 2014, with *P. aeruginosa*/*S. maltophilia*-positive cultures isolated from clinical respiratory samples. We included only positive cultures from broncho-alveolar lavage, tracheobronchial aspiration or plugged telescoping catheter (Combicath), obtained during or after the bronchoscopic procedure. Sputum samples were not considered. Controls were defined as patients exposed to bronchoscopes in the same period but without positive respiratory sample cultures of the microorganisms found on bronchoscopes, namely *P. aeruginosa*, *S. maltophilia*, *Klebsiella pneumoniae*, *Enterobacter cloacae* or *Achromobacter xylosoxidans*. For the epidemic curve, the period of interest began on 1 November 2013.

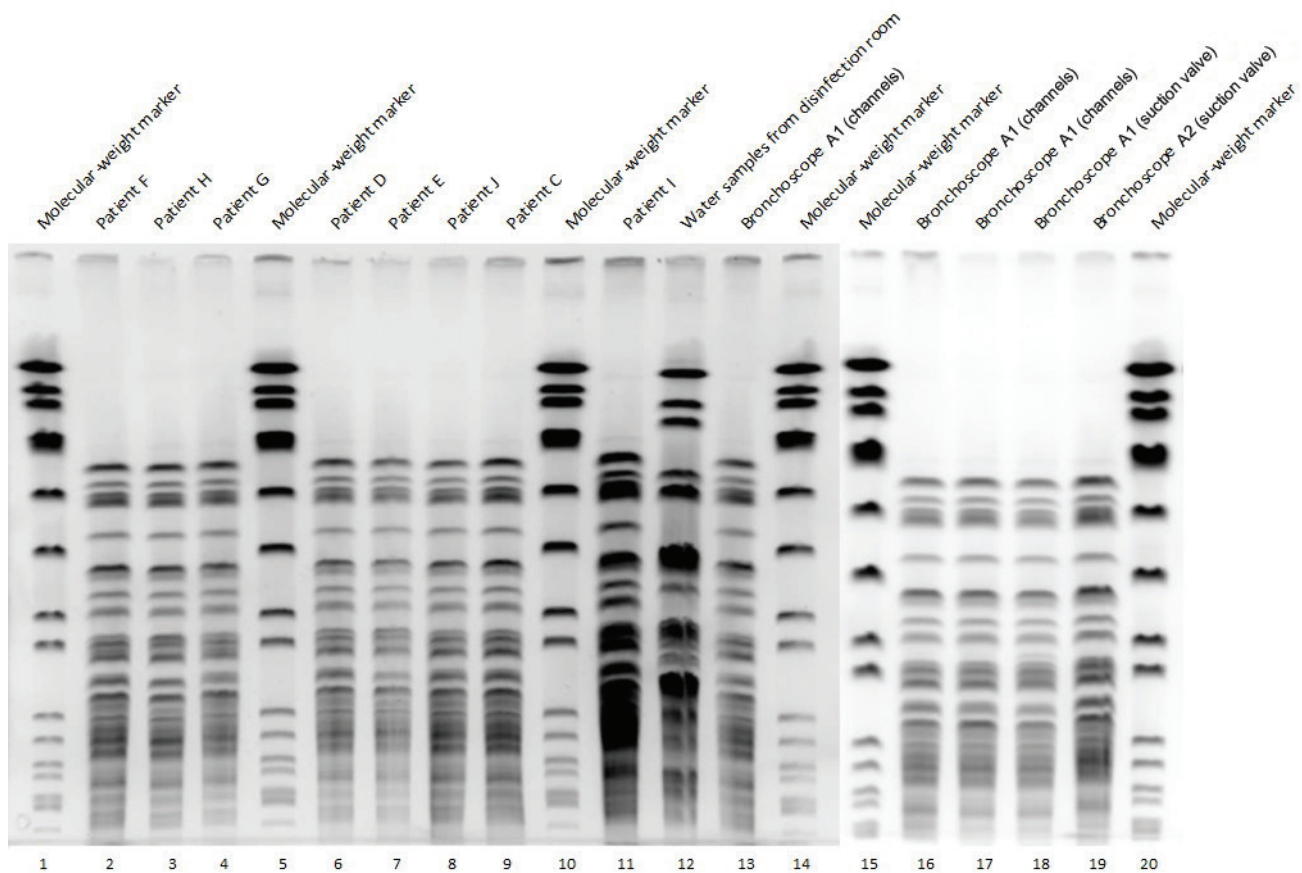
Patients exposed to bronchoscopes were identified from standardised, prospectively collected forms detailing bronchoscope use. Clinical sample results were obtained from the microbiological laboratory (according to European guidelines [25]) for patients exposed to bronchoscope for whom a microbiological sample was available, and medical case records were reviewed. Bronchoscope cleaning processes were audited by the Infection Control Unit. Prospective surveillance was implemented starting from the first investigation, as soon as the infection control team was informed. Every day, a member of the infection control unit was looking for new cases, checking results of cultures isolated from respiratory samples from patients in Edouard Herriot Hospital.

Environmental investigation

According to French guidelines [24], samples from suspected bronchoscope channels were taken by two authorised personnel, after cleaning and at least six

FIGURE 2

Pulsed-field gel electrophoresis of *Pseudomonas aeruginosa* isolates from clinical (n = 8) and environmental (n = 6) samples, France, November 2013–August 2014



Macrorestriction profiles of total DNA from clinical and environmental isolates were acquired by pulsed-field gel electrophoresis (PFGE) on a CHEF-DR III unit (Bio-Rad, Hercules, US). Isolates of *P. aeruginosa* from clinical samples (patients C, D, E, F, G, H, I and J) were identical to isolates from channels and suction valve of bronchoscope A1 and to isolates from the suction valve of bronchoscope A2, but differed from tap water isolates from the disinfection room. *Staphylococcus aureus* NCTC 8325 (with *Sma*I as restriction enzyme) was used as a reference (molecular weight marker), and PFGE patterns were analysed visually.

hours of storage. Sixty mL of Pharmacopeia dilution solution with antimicrobial inactivators (DNP buffer, AES Chemunex, bioMérieux, Marcy l’Etoile, France) were flushed into proximal ports and collected in sterile cups at the distal end of the operating channel. As the first set of cultures from bronchoscope channel samples were negative, bronchoscope suction valves and biopsy valves from suspected bronchoscopes were sampled.

In addition, surface samples from the aseptic storage cabinet for bronchoscopes, water samples from automated endoscope reprocessors and tap water samples from ICU #C were cultured.

Molecular typing

Macrorestriction profiles of total DNA from clinical and environmental isolates were acquired by pulsed-field gel electrophoresis (PFGE) on a CHEF-DR III unit

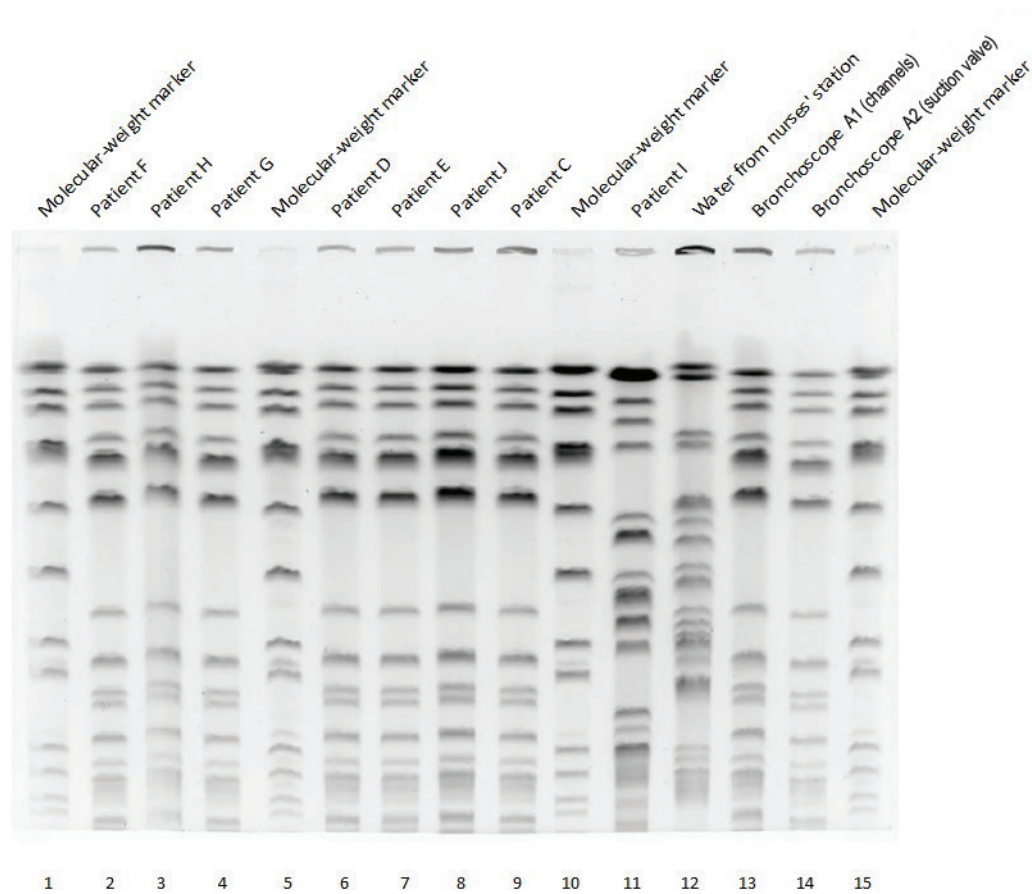
(Bio-Rad, Hercules, United States (US)) [26]. *Dra*I and *Xba*I served as restriction enzymes for *P. aeruginosa* and *S. maltophilia*, respectively. We ensured that the gels were comparable by including *Staphylococcus aureus* NCTC 8325 (with *Sma*I as restriction enzyme) as a reference, and PFGE patterns were analysed visually.

Statistical analysis

In the nested case–control study, all exposures to bronchoscopes were considered to be potential risks. Other potential risk factors were unit, patient age, sex and number of bronchoscopic procedures per patient. To identify characteristics linked with the risk of being a case, categorical variables were compared by chi-square test, and continuous variables by the Mann-Whitney U test. All tests were two-tailed. A p value of <0.05 was considered significant. Univariate and multivariate logistic regression was undertaken with Stata 11.0 software (StataCorp, College Station, US).

FIGURE 3

Pulsed-field gel electrophoresis of *Stenotrophomonas maltophilia* isolates from (n = 8) and environmental (n = 3) samples, France, November 2013–August 2014



Macrorestriction profiles of total DNA from clinical and environmental isolates were acquired by pulsed-field gel electrophoresis (PFGE) on a CHEF-DR III unit (Bio-Rad, Hercules, US). Isolates of *S. maltophilia* isolates from clinical samples (except for patient I) were identical to isolates from the suction valve of bronchoscope A2 and to isolates from the channels of bronchoscope A1, but differed from tap water isolates found in the nurses' station. *Staphylococcus aureus* NCTC 8325 (with *Sma*I as restriction enzyme) was used as a reference (molecular weight marker), and PFGE patterns were analysed visually.

Results

Between 1 December 2013 and 17 June 2014, 157 patients were exposed to at least one bronchoscope, and 216 bronchoscopic procedures were undertaken. Median age was 62 years (interquartile range (IQR): 49–73 years), and 111 patients (71%) were male. Overall, 10 patients had *P. aeruginosa*/*S. maltophilia*-positive cultures isolated from respiratory sampling; 35 patients had at least one respiratory sample with *P. aeruginosa*, *S. maltophilia*, *K. pneumonia*, *E. cloacae* or *A. xylosoxidans*, but did not fulfil the criteria of the case definition, and the respiratory samples of 112 patients were negative for all of these pathogens. The 10 cases identified were all men, with a median age of 52 years (IQR: 23–67 years) (Table 1), three were previously hospitalised and nine were intubated during their ICU stay. Among them, two patients had secondary pneumonia, nine and 11 days after bronchoscopy.

Three cases died during ICU stay and their deaths were not related to bronchoscope contamination. Eight cases were associated with bronchoscope A1 and two cases with bronchoscope A2. During the outbreak, the attack rate among cases exposed to bronchoscopes was 9.4% between February and June 2014 compared with 0% between December 2013 and January 2014 ($p < 0.05$); five patients had *P. aeruginosa*/*S. maltophilia* positive respiratory samples but had not been exposed to a bronchoscope (Figure 1).

We compared exposed patients co-infected with *P. aeruginosa* and *S. maltophilia* (n = 10) to non-infected patients (n = 112) during the outbreak period (Table 2). Univariate analysis disclosed that exposure to bronchoscope A1 or A2, hospitalisation unit and number of bronchoscopic procedures per patient were associated with increased risk of being a case. After multivariate

TABLE 1

Demographic and clinical characteristics of patients with pulmonary *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* co-infection exposed to bronchoscopes, France, November 2013–August 2014 (n=10)

Patient	A	B	C	D	E	F	G	H	I	J
Age group (years)	40-49 ^a	70-79	50-59	30-39	18-29	18-29	50-59	18-29	60-69	70-79
ICU	ICU #A	ICU #B	ICU #A	ICU #C	ICU #C	ICU #C	ICU #A	ICU #C	ICU #B	ICU #B
Cause of hospital admission	Pneumonia	Respiratory distress syndrome, coma	Fever and aplasia after autologous transplantation	Abdominal wound	Polytrauma	Polytrauma, coma	Septic shock, pneumonia, waiting for lung transplant	Polytrauma	Cardiac arrest	Multiple organ failure (post-operative)
Immunosuppression	No	No	Yes	No	No	No	Yes	No	No	Yes
Death (cause of death)	No	Yes ^b	Yes ^b	No	No	No	Yes ^b	No	No	No
Time between hospitalisation and bronchoscopic procedure (days)	6	0	3	3	3	1	0	9	10	7
Bronchoscope exposure	A1	A1	A1	A1	A1	A1	A1	A1	A2	A2
Microbiology results	<i>P. aeruginosa</i> , <i>S. maltophilia</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>E. coli</i> , <i>C. tropicalis</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>influenza A virus</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>S. aureus</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>S. aureus</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>E. cloacae</i> , <i>K. pneumoniae</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>K. pneumoniae</i> , <i>C. albicans</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i>	<i>P. aeruginosa</i> , <i>S. maltophilia</i> , <i>A. xylosoxidans</i>
Secondary pneumonia	No	Yes	No	No	No	Yes	No	No	No	No

ICU: intensive care unit; M: male; A1: bronchoscope A1; A2: bronchoscope A2.

^a To anonymise the description of cases, the age is reported by stratum.

^b Unrelated to bronchoscopic procedure.

TABLE 2

Factors associated with the risk of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* co-infection, France, November 2013–August 2014 (n=122)

Characteristics	<i>Pa/Sm</i> co-infection n (%)	Non-infected ^a n (%)	p	Crude odds ratio (95% CI)
Total	10	112		
Age (years)	52 (23-67) ^b	62 (49-72) ^b	0.07	0.96 (0.92–1.0) ^{b,c}
Sex (female)	0 (0)	39 (35)	0.02	NE
Bronchoscope exposure ^d				
Bronchoscope A1	8 (80)	9 (8)	<0.001	45.8 (8.4–248.7) ^e
Bronchoscope A2	4 (40)	16 (14)	0.03	4.0 (1.02–15.8) ^e
Bronchoscope A3	2 (20)	17 (15)	0.69	1.4 (0.3–7.2) ^e
Bronchoscope B1	0 (0)	0 (0)	NA	NE
Bronchoscope B2	1 (10)	24 (21)	0.39	0.4 (0.05–3.4) ^e
Bronchoscope B3	2 (20)	25 (22)	0.86	0.9 (0.2–4.4) ^e
Bronchoscope B4	0 (0)	27 (24)	0.08	NE
Bronchoscope B5	0 (0)	13 (12)	0.25	NE
Unit				
Intensive care unit	10 (100)	70 (62)	0.02	NE
Operating rooms	0 (0)	30 (27)	0.06	NE
Other units	0 (0)	12 (11)	0.28	NE
Number of bronchoscopic procedures				
1	5 (50)	95 (85)	0.006	1.0 (reference)
≥ 2	5 (50)	17 (15)		5.6 (1.5–21.4)

NA: not applicable; NE: could not be estimated; *Pa/Sm*: *Pseudomonas aeruginosa*/*Stenotrophomonas maltophilia*.

^a Patients for whom *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Klebsiella pneumonia*, *Enterobacter cloacae* or *Achromobacter xylosoxidans* could not be isolated from respiratory samples.

^b Median interquartile range.

^c For one year older.

^d More than one exposure was possible.

^e Compared with the absence of exposure to this particular endoscope.

analysis, exposure to bronchoscope A1 and bronchoscope A2 was independently associated with heightened risk of *P. aeruginosa*/*S. maltophilia* co-infection (adjusted odds ratio (aOR) = 84.6, 95% confidence interval (CI): 9.3–771.6 and aOR = 11.8, 95% CI: 1.2–121.3, respectively). No further cases occurred after sequestration of the two implicated bronchoscopes.

Endoscopic and environmental cultures

As soon as the first two cases with *P. aeruginosa*/*S. maltophilia* in patients exposed to bronchoscope A1 were reported, the device was investigated and taken out of service. However, as bronchoscope channel samples were negative in bacteriological testing, we allowed it to be used again. Meanwhile, ICU #C tap water was sampled. *P. aeruginosa* grew from one sample in the disinfection room, and *S. maltophilia* grew from one sample in the nurses' station. Contaminated washbasins were disinfected, and control samples were negative. Thus, contaminated tap water was deemed to be the potential source of infection. At that time, the source of contamination was considered to be controlled, and active surveillance was implemented.

However, in May 2014, another case of *P. aeruginosa*/*S. maltophilia* pneumonia attributed to bronchoscope A1 was detected. Bronchoscope A1 was withdrawn from circulation and sent to the manufacturer for technical expertise. Bronchoscope channels and valves were sampled by the infection control team. Channel samples grew both *P. aeruginosa* and *S. maltophilia*, and the suction valve grew *Burkholderia cepacia*, *E. cloacae*, *K. pneumonia* and *P. aeruginosa*. The biopsy valve culture remained negative. The expert report noted that the suction valve had a porous seal. The environmental investigation was extended to the aseptic storage cabinet and water from automated endoscope reprocessors, but these sample cultures were negative.

In June 2014, two additional *P. aeruginosa*/*S. maltophilia* pneumonia cases were reported in patients exposed to bronchoscope A2 in ICU #B; it was removed from use. Channel and biopsy valve samples were negative, but the suction valve grew *P. aeruginosa* and *S. maltophilia*. Sampling was extended to bronchoscope A3, but channel and valve cultures were negative. Routine samples from the endoscopes of other

brands were all negative for *P. aeruginosa* or *S. maltophilia* during the outbreak period.

Molecular typing

PFGE revealed that isolates of *P. aeruginosa* (Figures 2 and 3) from clinical samples (patients C, D, E, F, G, H, I and J) were identical to isolates from channels and suction valve of bronchoscope A1 and to isolates from the suction valve of bronchoscope A2, but differed from isolates obtained from tap water in the disinfection room. Similarly, *S. maltophilia* isolates from clinical samples (except for patient I) were identical to isolates from the suction valve of bronchoscope A2 and to isolates from the channels of bronchoscope A1, but differed from tap water isolates found in the nurses' station. Clinical isolates from patients A and B could not be recovered for typing.

Bronchoscope cleaning processes

Bronchoscope cleaning processes were audited by the Infection Control Unit with a standardised form. Some deficiencies were detected such as delays between endoscopy and cleaning. Moreover, the tightness test was not always performed before manual cleaning. However, these deficiencies were not specific to bronchoscopes from manufacturer A. Corrective actions were taken. Protocols were updated, traceability was improved, and single-use bronchoscopes were provided during the night and on-call duties in order to avoid latency between bronchoscopy and cleaning. As of 24 June 2016, no contamination of bronchoscope with *P. aeruginosa/S. maltophilia* has been identified, no new case related to bronchoscope exposure has occurred since bronchoscope disinfection was improved.

Discussion

From December 2013 to June 2014, an outbreak of *P. aeruginosa/S. maltophilia* co-infections was investigated in 10 patients undergoing bronchoscopy. These cases were related to two bronchoscopes of the same model from which *P. aeruginosa/S. maltophilia* were isolated from the suction valves. Clinical and contaminated bronchoscope isolates showed similar PFGE patterns. Two secondary pneumonia infections were identified among the cases. The respiratory samples may have been contaminated in the eight other cases, but antibiotic therapy was initiated for all patients and may have prevented the development of nosocomial pneumonia.

One of the key issues is to know how bronchoscope A1 was contaminated. As environmental sources of contamination were excluded, it may have been tainted during a bronchoscopic procedure on a patient colonised or infected by *P. aeruginosa/S. maltophilia*. Persistent contamination was probably partially due to defective bronchoscope cleaning as some deficiencies were highlighted by the audit. Furthermore, the complexity of suction valve cleaning and disinfection

compared to other bronchoscopes might have contributed to the event.

Detection of this outbreak may have been further delayed because there was no specific surveillance of patients exposed to bronchoscopes. Moreover, the source of contamination was found by extended bronchoscope sampling. Bronchoscope disinfection is routinely assessed by channel sampling, as recommended in French guidelines [24]. The first results of bronchoscope contamination detection were probably false negatives. This outbreak highlights the benefits of routinely testing suction valves to look for bacterial contamination of bronchoscopes. In case of suspected contamination, suction valves should be systematically tested. If contaminated, they should be removed and replaced or sterilised. This outbreak raises questions about the cleaning process for suction valves. Indeed, there is no consensus on whether single-use suction valves, high-level suction valve disinfection or sterilisation after manual cleaning should be preferred. The manufacturer confirmed the lack of recommendations for suction valve management. The expert report stated that the submitted suction valve had porous seals which increased the risk of contamination. Preventive replacement of suction valves should be considered.

Faced with the contamination of two bronchoscopes of the same model, within the same part (suction valves), we wonder about increased risks posed by these devices. We therefore reported the event to the French National Agency for Medicines and Health Products Safety (Agence nationale de sécurité du médicament et des produits de santé (ANSM)), where no other notifications concerning these bronchoscopes were filed. Disparities in the hospital's stock of bronchoscopes regarding brands or preventive maintenance and lack of preventive maintenance were probable contributing factors. The two bronchoscopes under investigation were bought in 2007 and 2008 and did not have preventive maintenance contracts with the manufacturer.

Other outbreaks or pseudo-outbreaks tied to suction valve contamination have been described, mostly before the 2000s, but they involved mycobacteria [17,18]. Bronchoscope contamination by *P. aeruginosa/S. maltophilia* was reported in the investigation of a pseudo-outbreak in Baltimore, US in 2008 [27] and more recently, contamination by *S. maltophilia* was reported in the Netherlands [28].

Our investigations had some limitations. We did not find the index case, and the route of pathogen transmission from bronchoscopes A1 and A2 was not clearly identified. Transmission may have occurred through one secondary case exposed to both bronchoscopes, or perhaps through the connectors. Moreover, *B. cepacia*, *E. cloacae* and *K. pneumonia* were identified on one bronchoscope suction valve. Our case definition did not include patients with respiratory samples positive

for these microorganisms. We may have underestimated the magnitude of the outbreak.

Conclusion

We investigated an outbreak of *P. aeruginosa*/*S. maltophilia* pulmonary infections caused by suction valve contamination of two bronchoscopes from the same manufacturer. While bronchoscope contamination might be attributed to deficiencies in bronchoscope cleaning processes, suction valves of these bronchoscopes have a particular design which may increase the risk of contamination; the manufacturer was informed in the process and they were cooperative. No further confirmed cases exposed to bronchoscope have been detected as at 24 June 2016. Our findings underscore the need to test not only bronchoscope channels but also suction valves regularly for routine detection of bacteria. The large number of patients worldwide who are exposed daily to bronchoscope examinations highlights the necessity for regular updates of guidelines, appropriate hygiene procedures and reporting new risks to improve patient safety.

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Conflict of interest

None declared.

Authors' contributions

Marine Guy: performed epidemiological investigations, analysis, manuscript writing. Philippe Vanhems: project leader, performed epidemiological investigations, reviewed the manuscript. Cédric Dananché: performed epidemiological investigations, reviewed the manuscript. Pierre Cassier: performed environmental microbiologic analysis, reviewed the manuscript. Anne Regard: performed epidemiological investigations, reviewed the manuscript. Monique Hulin: performed epidemiological investigations, reviewed the manuscript.

Olivier Dauwalder: performed microbiologic analysis, reviewed the manuscript. Xavier Bertrand: performed pulsed-field gel electrophoresis analysis, reviewed the manuscript. Jullien Crozon-Clauzel: participated in epidemiological investigation, reviewed the manuscript. Bernard Floccard: participated in epidemiological investigation, reviewed the manuscript. Laurent Argaud: participated in epidemiological investigation, reviewed the manuscript. Michel Perraud: performed epidemiological investigations, reviewed the manuscript. Thomas Bénet: project leader, performed epidemiological investigations, analysis and interpretation of results, manuscript writing.

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