

## ORIGINAL ARTICLE

Emergence of Glutaraldehyde-Resistant *Pseudomonas aeruginosa*

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**OBJECTIVE.** In November 2009, routine sampling of endoscopes performed to monitor the effectiveness of the endoscope-cleaning procedure at our hospital detected *Pseudomonas aeruginosa*. Herein we report the results of the subsequent investigation.

**DESIGN AND METHODS.** The investigation included environmental cultures for source investigation, molecular analysis by pulsed-field gel electrophoresis (PFGE) to reveal the identity of the strains, and determination of the bactericidal activity of the glutaraldehyde-based disinfectant used for automated endoscope reprocessing. In addition, patient outcome was analyzed by medical chart review, and incidence rates of clinical samples with *P. aeruginosa* were compared.

**SETTING.** The University Hospital of Basel is an 855-bed tertiary care center in Basel, Switzerland. Approximately 1,700 flexible bronchoscopic, 2,500 gastroscopic, 1,400 colonoscopic, 140 endoscopic retrograde cholangiopancreatographic, and 140 endosonographic procedures are performed annually.

**RESULTS.** *P. aeruginosa* was detected in samples obtained from endoscopes in November 2009 for the first time since the initiation of surveillance in 2006. It was found in the rinsing water and in the drain of 1 of the 2 automated endoscope reprocessors. PFGE revealed 2 distinct *P. aeruginosa* strains, one in each reprocessor. The glutaraldehyde-based disinfectant showed no activity against the 2 pseudo-outbreak strains when used in the recommended concentration under standard conditions. After medical chart review, 6 patients with lower respiratory tract and bloodstream infections were identified as having a possible epidemiological link to the pseudo-outbreak strain.

**CONCLUSIONS.** This is the first description of a pseudo-outbreak caused by *P. aeruginosa* with reduced susceptibility to an aldehyde-based disinfectant routinely used in the automated processing of endoscopes.

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Since its introduction in the 1960s, flexible endoscopy has become standard of care for many indications in which open surgery is mandatory. Approximately 500,000 bronchoscopic examinations and 10 million gastrointestinal endoscopic examinations are performed in the United States annually.<sup>1</sup> Despite the large number of endoscopic examinations performed, hospital-acquired infections attributable to flexible endoscopy are rare. The American Society of Gastrointestinal Endoscopy estimated the risk as approximately 1 in 1.8 million gastrointestinal procedures.<sup>2</sup> The actual transmission of infectious organisms during endoscopic examination, however, may go unrecognized because of inadequate surveillance and the lack of formal, prospective surveillance data, which can lead to unrecognized or unreported outbreaks.<sup>3</sup> Although such outbreaks are relatively uncommon, endoscopes are the most frequent cause of outbreaks of device-related hospital-acquired infection.<sup>4</sup> Most of the outbreaks related to endoscopes involved waterborne organisms, such as gram-negative

rods and *Mycobacterium* species. *Pseudomonas aeruginosa* is the organism that is most commonly detected in gastrointestinal endoscopy-associated and bronchoscopy-associated outbreaks.<sup>5</sup>

Failure to follow recommended cleaning and disinfection procedures probably constitutes the single most important factor leading to endoscopic transmission of microorganisms and is the most common cause of outbreaks.<sup>6</sup> There have been a few notable exceptions in which problems related to the endoscopes themselves have led to contamination despite the use of an adequate reprocessing technique.<sup>7,8</sup>

In November 2009, the results of routine sampling of endoscopes showed growth of *P. aeruginosa*. Such microbiological surveillance is performed twice yearly and was initiated 1999 to monitor the performance of the endoscope reprocessor. Herein we report the results of the subsequent pseudo-outbreak investigation.

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## METHODS

### Setting

The University Hospital of Basel is an 855-bed tertiary care center in Basel, Switzerland. Approximately 1,700 flexible bronchoscopic, 2,500 gastroscopic, 1,400 colonoscopic, 140 endoscopic retrograde cholangiopancreatographic, and 140 endosonographic procedures are performed annually. A large majority of bronchoscopic examinations include bronchoalveolar lavage, in which sterile saline is instilled into the lower airways and then aspirated to obtain specimens. Biopsies are performed in most of the other endoscopic procedures.

### Endoscope Cleaning Procedures

Endoscopes used for gastrointestinal and bronchoscopic procedures are cleaned by trained personnel in accordance with national guidelines<sup>9</sup> and the manufacturer's recommendations (Olympus and Pentax). After use, the instrument's outer surface is wiped to avoid surface drying. Endoscopes are then immediately soaked in cleaner and disinfectant solution (Sekusept aktiv) based on peracetic acid. Thereafter, they are cleaned manually by wiping the outer surface and brushing the inner channels. Endoscopes then undergo high-level disinfection in an automated endoscope washer disinfectant (Hamo Endoclean 2000; Steris) in which glutaraldehyde and glyoxal (3.5 g and 6.0 g per 100 g, respectively; neodisher Septo V459) are used as liquid disinfectant. According to the manufacturer's recommendation, the disinfectant was used at a working concentration of 1% at a temperature of 50°C–60°C for 5–10 min. Finally, the channels are dried with pressurized air. Logbooks are used to record when water filters and solutions are changed in the reprocessors.

### Surveillance

As part of the quality assurance program to monitor the effectiveness of the endoscope cleaning procedure, endoscopes were routinely sampled twice yearly.

### Reinforced Cleaning Procedures

From January through February 2010, after detection of the pseudo-outbreak of *P. aeruginosa* infection, the disinfectant used for high-level disinfection in the automated endoscope reprocessor was changed to Neodisher Septo DN 2 (20 g of glutaraldehyde per 100 g of disinfectant). According to the manufacturer's recommendation, the disinfectant was used at a working concentration of 1% at a temperature of 50–55°C for 5 min. In addition, a rinse aid (Neodisher mediklar) was added to the automated cleaning procedure, and thermal disinfection of the automated endoscope reprocessor was performed 3 times weekly by running the processor while empty at 90°C for 10 min.

From March 2010, the concentration of the disinfectant Neodisher Septo DN 2 was increased to 2% and the application time was increased to 10 min. In addition, the automated endoscope reprocessors were run while empty once a

week with the detergent Neodisher SeptoClean (consisting of <5% nonionic surfactants, amphoteric surfactants, and 15%–30% phosphates), and thermal disinfection was reduced to once weekly.

### Reinforced Surveillance

After introduction of the reinforced cleaning procedures, surveillance was intensified by sampling the rinsing water from the automated reprocessors twice weekly and sampling endoscopes twice monthly.

### Microbiological Methods

Routine sampling of endoscopes was performed by injecting 20 mL of sterile saline solution (NaCl 0.9%) into each air/water and suction/biopsy channel according to recommendations outlined by the Robert Koch Institute.<sup>10</sup> The volumes of solution injected were recovered at the distal end of the endoscope. The number of viable bacteria in the samples collected was determined by conventional culture after inoculation on blood agar and filtration on 0.45 µm membrane filters. After 72 hours of incubation at 35°C, the number of colony-forming units (CFUs) was counted, and the results were expressed as the number of viable microorganisms. Subcultures were performed for identification.

During the pseudo-outbreak investigation, swab samples from the interior of the automated endoscope reprocessor (Hamo Endoclean 2000; Steris), along with samples of cleaning solutions and of solutions and water used in the automated endoscope reprocessors, were sent for bacterial culture. Furthermore, swab samples were taken from all supplying water pipes. The water filters of the automated endoscope reprocessors were exchanged and were also cultured.

Surface samples were obtained with the use of sterile moistened cotton swabs. Bacterial cultures of the used rinsing water, obtained directly from the automated endoscope reprocessors, were performed once to twice weekly. Furthermore, routine sampling of endoscopes was performed at least weekly.

The bactericidal activity was determined using the standard method for testing the bactericidal activity of chemical disinfectants according to the German Society for Hygiene and Microbiology.<sup>11</sup> The disinfectant Neodisher Septo DN 2 (glutaraldehyde 20 g per 100 g) was tested for activity against the pseudo-outbreak isolate of *P. aeruginosa* and against the reference strain *P. aeruginosa* ATCC 15442. From 1.5 to  $5 \times 10^9$  CFU of the isolates were diluted to  $10^{-5}$  CFU/mL, then 8 mL of the disinfectant were added and a quantitative suspension test with and without an organic load (0.2% albumin) was performed. After exposure times of 5 and 10 min, respectively, at 50°C, the disinfectant was inactivated and the suspension was cultured for 48 hours at 36°C. If a reduction factor of 5 in the number of CFU was achieved, the disinfectant was regarded as effective according to EN 1040 (available at <http://www.cen.eu>).

Molecular typing to determine the clonality and the trans-

mission pattern of *P. aeruginosa* was performed by pulsed-field gel electrophoresis (PFGE) as described elsewhere.<sup>12</sup>

### Interpretation of Endoscope Sampling Results

Results were interpreted according to the guidelines of the Robert Koch Institute.<sup>10</sup> The threshold value for the concentration of CFU was less than or equal to 1 CFU/mL of recovery solution, and detection of any of the following bacteria was regarded as an indicator for insufficient disinfection, independent of the number of CFU: Enterobacteriaceae, Enterococci, *Pseudomonas aeruginosa*, other nonfermenting gram-negative bacteria, *Staphylococcus aureus*, and viridans streptococci.

### Patient Outcomes

All patients who underwent endoscopic examination from April through November 2009 were identified from the endoscopy database and were retrospectively assessed for the presence of colonization or infection due to *P. aeruginosa*. If *P. aeruginosa* was detected in any sample after endoscopic examination, an epidemiological link was assessed by full chart review of 2 board-certified infectious diseases specialists. Transmission was regarded as possible if the strain detected in the clinical specimen revealed the same phenotypic features (ie, the same susceptibility test result) as the strain detected in the endoscopes and if prior colonization or infection of the patient with *P. aeruginosa* was not documented in either the medical records or in the computerized database of the microbiology laboratory or if prior colonization or infection with *P. aeruginosa* was present (as identified by medical chart review or documented in the database of the microbiology laboratory) but susceptibility test results differed for at least 2 of the following antibiotic classes: quinolones, aminoglycosides, carbapenems, cefepime, and piperacillin-tazobactam. Upper and lower respiratory tract and bloodstream infections were defined according to the criteria of the Centers for Disease Control and Prevention.<sup>13</sup> From December 2009 through January 2010, all consecutive samples (specimens recovered from the respiratory tract or from tissue and blood cultures) with detection of *P. aeruginosa* from patients who had undergone endoscopic examination within the previous 6 months were prospectively collected and molecularly typed.

Furthermore, incidence rates of the number of clinical samples with detection of *P. aeruginosa* were compared for the years 2009 and 2008. The number of patients who underwent endoscopic examination within 30 days before detection of *P. aeruginosa* in a respiratory tract or tissue sample or in blood cultures was compared for the years 2009 and 2008.

## RESULTS

Routine surveillance to monitor the effectiveness of the endoscope cleaning procedure detected *P. aeruginosa* in 23 (32%) of 73 samples from a total of 40 endoscopes in November 2009. This pathogen had not been detected since the

initiation of routine surveillance in 1999. In the following samples, obtained from endoscopes during the period from November 4 through December 7, 2009, *P. aeruginosa* was detected in 29 (29%) of 99 investigational samples.

### Endoscope Cleaning Procedures

Endoscopic and reprocessing procedures were observed on-site by an infection control practitioner and hospital epidemiologist. No significant breaches in technique were noted, but several changes were noted during the last year before the pseudo-outbreak. Examination of the maintenance records for the automated endoscope reprocessors demonstrated that the drying program had been reduced from 10 min to 5 min to expedite turnaround time. In addition, the rinse aid was discontinued in July 2009 to diminish foaming. The microprocessor running the process was double-checked by a representative of the manufacturer, and the written protocol did not reveal any deviation from the normal procedure. However, a manual check identified failure of the reprocessor to reach the required target temperature, although only in very few of the monitored cycles.

### Sampling because of Pseudo-Outbreak Investigation

A total of 103 environmental samples were taken from November through December 2009. *P. aeruginosa* could be detected in the rinsing water as well as in the drain of 1 of the 2 automated endoscope reprocessors (Table 1). However, the pathogen could not be detected in the supplying water pipes, filters, or ventilation shafts of the reprocessors, nor could it be detected in the hospital water supply tanks or in any of the cleaning solutions or disinfectants used for reprocessing the endoscopes.

### Molecular Typing

PFGE revealed 2 distinct *P. aeruginosa* strains (A and B) recovered from the rinsing water of the 2 different automated endoscope reprocessors as well as from the drain of 1 of the reprocessors. One automated reprocessor was contaminated with strain A, whereas the other was contaminated with strain B.

During the pseudo-outbreak, all strains from patients with *P. aeruginosa* infection who had undergone endoscopic examination within the previous 6 months were stored at  $-70^{\circ}\text{C}$ . These strains were detected from clinical samples submitted to the laboratory or were obtained by the infectious disease consultation service. Molecular typing of all consecutive patient samples ( $n = 18$ ) in which *P. aeruginosa* was detected did not reveal a clone that matched one of the strains isolated from the automated reprocessors.

### Bactericidal Tests

The disinfectant Neodisher Septo DN 2 (glutardialdehyde 20 g per 100 g) was tested for activity against the pseudo-outbreak isolate of *P. aeruginosa* and against the reference strain *P. aeruginosa* ATCC 15442. For the reference strain, a

TABLE 1. Results of Cultures of Specimens from Environmental Sources

Source and type of specimen	<i>Pseudomonas aeruginosa</i> isolated on culture
Washers (automated endoscope reprocessors)	
Interior of washer (surface samples)	No
Supplying waterpipes (surface samples)	No
Filters	No
Rinsing water	Yes
Ventilation shaft	No
Drains	Yes
Water-supplying tanks of the hospital	
Water	No
Disinfectant	No
Cleaning solutions	No

reduction factor greater than 5 log<sub>10</sub> CFU was achieved when using concentrations of 0.5% to 2% for 5 min and 10 min at 20°C and 55°C. For the 2 pseudo-outbreak strains, a reduction factor greater than 5 could only be achieved when using 2% of the disinfectant for 10 min at 55°C (Table 2). At 20°C, bacterial growth could still be detected with a reduction factor of less than 5, which confirmed dramatically reduced bactericidal activity (Table 3).

#### Reinforced Cleaning Procedures

After introduction of the reinforced cleaning procedures, *P. aeruginosa* could no longer be detected in the samples obtained from endoscopes. However, in the cultures of the rinsing water, which was checked twice weekly, *P. aeruginosa* reemerged at the beginning of March 2010. PFGE revealed identity to 1 of the 2 pseudo-outbreak strains (strain A). Cleaning procedures were changed as described above, on the basis of the bactericidal test results. After March 2010, *P. aeruginosa* was no longer detected in either the samples obtained from the endoscopes or the rinsing water of the automated reprocessors.

#### Patient Outcomes

A total of 63 patients who underwent endoscopic examination from April through November 2009 had samples (obtained either from the respiratory tract or from tissue and blood cultures after endoscopy was performed) that revealed *P. aeruginosa*. The epidemiologic investigations failed to identify a link in 20 of 63 patients. After medical chart review by 2 infectious disease specialists, lower respiratory tract and bloodstream infections possibly caused by the pseudo-outbreak strain were detected in 6 patients.

The comparison of the number of patients who underwent endoscopic examination 30 days before detection of *P. aeruginosa* in a respiratory tract or tissue sample or in blood cultures showed no increase, with 30 patients fitting this definition in 2008 and 27 patients fitting this definition in 2009. The number of procedures performed was 5,544 in 2008 and 5,630 in 2009, which accounted for 0.54% (30 of 5,544) and 0.48% (27 of 5,630) of the patients with detection of *P. aeru-*

*ginosa* in a clinical specimen within 30 days after endoscopic examination.

#### DISCUSSION

This is, to our knowledge, the first report of a pseudo-outbreak caused by contamination of automated endoscope reprocessors with 2 different strains of *P. aeruginosa* resistant to aldehydes and heat. No increase in the incidence of infection caused by this pathogen could be detected during the outbreak period. However, 6 patients could have potentially been infected by contaminated endoscopes.

Glutaraldehyde has been used as a high-level disinfectant for approximately 30 years and displays potent bactericidal, fungicidal, mycobactericidal, sporicidal, and virucidal activity, which is based on its interaction with amino groups in proteins and enzymes.<sup>14</sup> Glutaraldehyde-resistant mycobacteria have been revealed as a source of recontamination of endoscopes and have been detected in endoscope washers, which has led to misdiagnosis of infections.<sup>15,16</sup> Furthermore, these pathogens were found to be cross-resistant to peracetic acid.<sup>16</sup> Nevertheless, an increase in the activity of glutaraldehyde could be obtained by increasing the temperature of the washer to 50°C.<sup>16</sup> A strain of *P. aeruginosa* resistant to disinfection with glutaraldehyde was isolated from endoscopes and from tap water by Kovacs et al<sup>17</sup> in 1998. However, glutaraldehyde was used at room temperature, and resistance testing was performed at 37°C. In contrast, tests revealed our pseudo-outbreak strains to be resistant even when using the recommended glutaraldehyde concentration of 1% at 55°C, and they survived thrice-weekly thermal disinfection at 90°C.

Resistance to high-level disinfection with glutaraldehyde of *P. aeruginosa* has been reported in dental and medical devices<sup>18</sup> but was associated with the pathogen being entrapped in lubricants used in dental handpieces and endoscopes and with the disinfectant not being able to penetrate the lubricant.

*P. aeruginosa* is a pathogen that is well known for its ability to adapt to changing environmental conditions. In chronic infection, this pathogen can survive attacks by the host immune system and the action of antimicrobial substances by undergoing phenotypic and genetic adaptation, resulting in

TABLE 2. Bactericidal Tests with Glutaraldehyde (20 g/100 g) Applied for 10 Min at 55°C

Strain, glutaraldehyde concentration, %	Bacterial growth	Reduction factor of no. of CFU
<i>Pseudomonas aeruginosa</i> (strain A)		
0.5	Yes	<4.12
1.0	Yes	>5.50
2.0	No	>5.50
<i>P. aeruginosa</i> (strain B)		
0.5	Yes	<4.00
1.0	Yes	4.42
2.0	No	>5.39
<i>P. aeruginosa</i> (ATCC 15442)		
0.5	No	>5.55
1.0	No	>5.55
2.0	No	>5.55

the progressive loss of virulence and the development of increased persistence. However, the underlying strategies and mechanisms employed by *P. aeruginosa* are still relatively poorly understood.<sup>19</sup>

The question arises whether resistance to disinfectants is attributable to primary resistance or to the development of resistance, similar to the development of resistance to antibiotics. In 1976, 5 fully susceptible laboratory strains were described that were exposed for longer periods of time to sublethal doses of 4 basic constituents of disinfectants (phenol, isopropanol, formaldehyde, and chloramine 80). After multiple passages, an increase in resistance against formaldehyde and chloramine 80 could be noted.<sup>20</sup> The organisms tested were *Staphylococcus aureus*, *Escherichia coli*, *Aerobacter cloacae*, *P. aeruginosa*, and *Klebsiella pneumoniae*. Spicher et al<sup>21</sup> reported that strains of *P. aeruginosa* vary widely in their susceptibility to formaldehyde, with the need to use concentrations almost 3 times as high for the most resistant strain than for the most susceptible strain to obtain equal microbicidal effects.

Possible mechanisms of action could be enzymatic degradation and extracellular and intracellular penetration barriers. Formaldehyde degradation could be detected photometrically in *P. aeruginosa* strains. The highest formalde-

hyde resistance correlated with a formaldehyde dehydrogenase activity that was 100 times higher than that for a relatively susceptible ATCC strain.<sup>22</sup> Interestingly, the tested strains exhibited markedly differing resistance to formaldehyde, with some environmental isolates growing even at concentrations in the range of commonly used disinfectant solutions.

Our strains may have become resistant because of the use of glutaraldehyde at suboptimal application durations and possibly because of inadequate temperatures, because our automated reprocessors may not have consistently achieved a temperature of 55°C as a result of inconsistent failure of the microprocessors caused by wear. In addition, the rinse aid, which was discontinued, facilitates and accelerates the drying process, and the drying program had been reduced from 10 min to 5 min; these changes may have contributed to the contamination of the endoscopes, especially because gram-negative bacteria do not survive as long as gram-positive bacteria on dry surfaces.<sup>23</sup>

The susceptibility of *P. aeruginosa* to different chemical germicides was also tested by Sagripanti et al,<sup>24</sup> which revealed that this pathogen, together with *S. aureus*, showed the most resistance.<sup>24</sup> Less than 4 logs inactivation were observed after treatment with glutaraldehyde or hypochlorite of an ATCC

TABLE 3. Bactericidal Tests with Glutaraldehyde (20 g/100 g) Applied for 10 Min at 20°C

Strain, glutaraldehyde concentration, %	Bacterial growth	Reduction factor of no. of CFU
<i>Pseudomonas aeruginosa</i> (strain A)		
0.5	Yes	<4.11
1.0	Yes	<4.11
2.0	Yes	<4.67
<i>P. aeruginosa</i> (strain B)		
0.5	Yes	<4.16
1.0	Yes	<4.16
2.0	Yes	<4.16
<i>P. aeruginosa</i> (ATCC 15442)		
0.5	No	>5.54
1.0	No	>5.54
2.0	No	>5.54

strain (ATCC 27853) of *P. aeruginosa*. Sagripanti et al<sup>24</sup> suggested that resistance to disinfectants may be more important than pathogenicity in determining the relative prominence of an organism as an agent responsible for hospital-acquired infection. Species that more frequently survived exposure to germicidal agents were also the most commonly reported in association with hospital-acquired infection.<sup>24</sup>

Although glutaraldehyde is the most commonly used chemical for reprocessing endoscopic equipment, our results suggest that it may not be the most effective product. *P. aeruginosa*, which is one of the most important hospital-acquired pathogens, can become resistant, especially when there is not compliance with the recommended temperature and application time of disinfection. This new data may expedite the current trend to replace aldehydes with peracetic acid. Resistance to disinfectants and resistance to heat have emerged and can cause pseudo-outbreaks in healthcare settings.

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