ORIGINAL ARTICLE

A Bundle of Measures to Control an Outbreak of *Pseudomonas aeruginosa* Associated With P-Trap Contamination

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OBJECTIVE. To describe an outbreak of multidrug-resistant *Pseudomonas aeruginosa* in which the hospital waste-pipe system was the likely source of contamination and to report the bundle of measures that facilitated the long-term control of the outbreak.

DESIGN. Outbreak investigation.

SETTING. The hematology unit of a tertiary-care referral center.

PATIENTS. Patients who were colonized or infected with *P. aeruginosa* belonging to the clonal outbreak.

METHODS. Patients admitted to our 15-bed stem-cell transplantation hematology unit were screened for *P. aeruginosa* carriage. *Pseudomonas aeruginosa* isolates were also obtained from diagnostic samples. We assessed the microbiological contamination of P-traps, water and toilets for 42 months. Extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) were screened and identified by polymerase chain reaction (PCR) and sequencing. Molecular typing of ESBL- or MBL-producing isolates was carried out using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

RESULTS. From 2009 to 2013, a biclonal outbreak of IMP-19–producing ST235 (11 cases) and IMP-29–producing ST111 (10 cases) of *P. aeruginosa* occurred. The environmental investigation strongly suggested that P-traps were the reservoirs for the outbreak strains. A bundle of infection control measures, including engineering interventions on water outlets and disinfection of P-traps, controlled the outbreak.

CONCLUSIONS. We report a prolonged outbreak of IMP-producing high-risk clones of *P. aeruginosa*, for which P-traps seems to play a major role in cross-transmission. It appears essential to implement proactive measures to limit the bacterial load in water fittings of high-risk units.

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Pseudomonas aeruginosa is an important opportunistic human pathogen causing infection in patients with impaired immune systems.[1] It is also ubiquitous in hospital water networks and thrives best in the distal parts of the water distribution system, such as taps, sinks, P-traps, or toilets.[2,3] Although a consensus has not been reached regarding the role of hospital water supplies in *P. aeruginosa* acquisition by inpatients, its presence in the water supply has been identified as a risk factor for *P. aeruginosa* acquisition,[3] and investigations of hospital outbreaks have frequently retrieved epidemic clones in the sinks.[4] Although *P. aeruginosa* has a nonclonal epidemic population structure, recent studies have provided evidence of the existence of multidrug-resistant global clones, denominated high-risk clones, and globally disseminated in hospitals.[5] We report a hospital outbreak involving 2 clones of multidrug-resistant *P. aeruginosa* in which the waste-pipe system was the likely reservoir for and source of contamination. The outbreak occurred from 2009 to 2013 in a hematology unit at the 1,200-bed Besançon University Hospital (eastern France). We also report the bundle of measures that facilitated the long-term control of the outbreak.

MATERIAL AND METHODS

Study Design

We performed an outbreak investigation and prospective environmental investigation. From January 2008 to November 2016, all patients admitted for more than 48 hours (n = 1,966) in our 15-bed stem-cell transplantation hematology unit were screened for *P. aeruginosa* carriage (with stools or rectal swab) on admission and once per week thereafter, throughout their stay.

Microbiological Methods

Swabs were streaked on *Pseudomonas* selective agar plates containing cetrimide, which were incubated for 48 hours at 35°

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C. *Pseudomonas aeruginosa* colonies were first detected using standard microbiology methods (ie, colony morphology, positive oxidase reaction, pigment production) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) with a log value ≥2 according to the manufacturer's recommendations (Bruker Daltonik GmbH, Bremen, Germany). *Pseudomonas aeruginosa* isolates were also obtained from routine diagnostic samples and identified accordingly. Susceptibility testing on all isolates was performed using disc diffusion methodology.[6] Extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) were screened in isolates resistant to third-generation cephalosporins using a phenotypic method described elsewhere[7] and identified by polymerase chain reaction (PCR) and sequencing with primers targeting ESBL- and MBL-encoding genes.[8] Molecular typing of ESBL- or MBL-producing isolates was carried out using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) as previously described.[9]

A large environmental microbiological investigation was carried out for 42 months from May 2013 to October 2016. P-traps from all sinks of the unit (n = 19) were sampled monthly from May 2013 to February 2015 and biannually until the end of the survey. For each sink, 50 mL of P-trap content was collected using a suction catheter and a syringe and centrifuged for 5 minutes at 5,000 × *g*. All toilets (n = 18) were sampled with a swab monthly from May 2013 to April 2014. Water samples were collected monthly from taps from May 2013 to February 2015. Filters, if present, were removed prior to the sampling of the first 250 mL flushed into a sterile flask containing 5 mg sodium thiosulphate. Then, 100 mL of this solution was filtered through a 0.45-mm pore-size membrane filter. All environmental samples were cultured on *Pseudomonas* selective agar plates as described above. Identification, susceptibility testing, detection of resistance determinants and typing were carried out using methods described for clinical samples.

Infection Control Intervention

In May 2013, we implemented a bundle of infection control measures that included (1) a global clinical audit carried out by infection control nurses and practitioners and a reminder on recommendations of hand disinfection opportunities, (2) excreta management, (3) use of gloves, (4) recall of cleaning practices, (5) discontinuation of feces discharge in the toilets, and (6) removal of hand showers for rinsing the toilets. Considering the spatiotemporal distribution of acquisition of *P. aeruginosa* outbreak strains (Figure 2), rooms 2208 and 2210 were closed for 1 month, and a large microbiological surveillance program was implemented. After the first results of environmental sampling, we replaced all taps and all drains of sinks and toilets starting with rooms 2208 and 2210. These replacements took 1 year due to the maintenance of medical activity. New water outlets were equipped with lockable P-traps (Geberit, Avon, France) and disposable point-of-use water filters (Anios, Lille-Hellemmes, France) that were changed monthly. We recommended pouring a bleach solution (water with 2.6% active chlorine) twice weekly into the blocked P-traps to allow a contact time of 15 minutes before rinsing with water. An additional measure was implemented in April 2014: P-traps were changed at patient discharge whenever a patient stay exceeded 1 week.

RESULTS

Outbreak Description

From November 2009 to September 2013, 21 patients were colonized or infected with an IMP-producing *P. aeruginosa*. Figure 1 shows the epidemic curve. We observed a biclonal

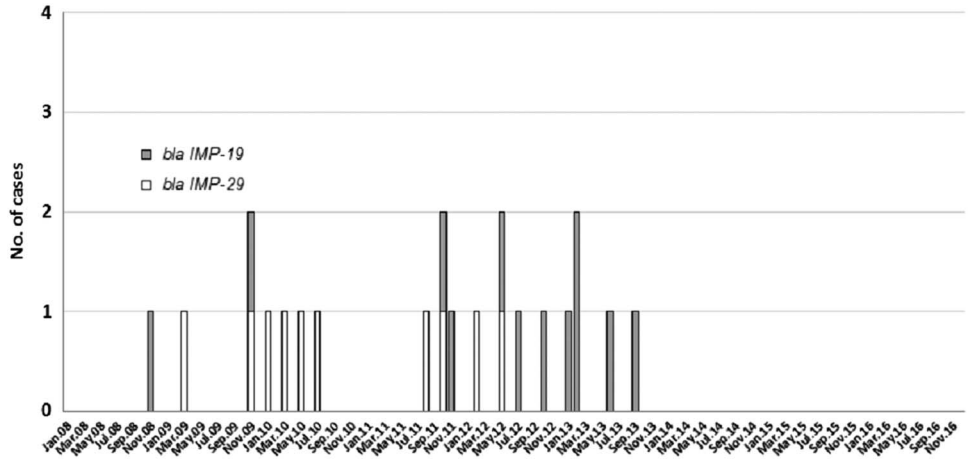

FIGURE 1. Epidemic curve of the outbreak of IMP-producing *P. aeruginosa* in the hematology unit of the University Hospital of Besançon (2008–2016). The grey bars and white bars represent the number of patients positive with IMP-19– and IMP-29–producing *P. aeruginosa*, respectively.

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outbreak with contemporary spread of IMP-19–producing ST235 (belonging to a unique PFGE pattern called A) and IMP-29–producing ST111 (belonging to a unique PFGE pattern called B). Overall, 11 patients were colonized or infected with ST235 and 10 patients were colonized or infected with ST111. Table 1 summarizes the main characteristics of these patients. The first infection occurred in May 2012, and 5 more patients were diagnosed between August 2012 and May 2013. Of these 6 patients, 5 died, and the deaths were considered attributable to *P. aeruginosa* infection in 4 cases.

Environmental Screening

A total of 1,067 environmental samples were analyzed: 437 samples of P-traps, 414 water samples, and 216 toilets samples. All the water samples were negative for IMP-producing *P. aeruginosa*. However, 3 toilets sampled in May and June 2013 were positive for IMP-19–producing *P. aeruginosa* ST235 (rooms 2207, 2208, and 2212), and all toilets tested negative thereafter. The results of the search for *P. aeruginosa* in P-traps are summarized in Table 2. Broadly, *P. aeruginosa* was detected in 149 of 437 P-trap samples (34.1%) and IMP-like–producing *P. aeruginosa* were recovered in 19 samples (12 containing IMP-19–producing *P. aeruginosa* and 7 containing and IMP-29–producing *P. aeruginosa*). Additionally, 4 isolates producing the class A PER-1 ESBL were identified without being involved in the current outbreak. IMP-19–producing *P. aeruginosa* ST235 was identified in 7 rooms for a prolonged period (from May 2013 to June 2015). IMP-29–producing *P. aeruginosa* ST111 was recovered in only 4 rooms from May 2013 to August 2014.

DISCUSSION

In the present study, we describe a 4-year-long outbreak of IMP-producing carbapenem-resistant *P. aeruginosa* where P-traps were the likely reservoir. Numerous studies have reported that water systems can act as a source of *P. aeruginosa* acquisition by patients in high-risk settings, but the route of transmission is often unclear and becomes a cause of chicken-and-egg debate.[4] In this outbreak, the role of the environment was clear because the acquisition of the 2 outbreak strains was mainly associated with 2 specific rooms where the environment was contaminated. A recent in situ study using a handwashing sink laboratory gallery showed that transfer of bacteria from P-traps to patients is a multistage process: (1) the development of the biofilm up from the P-trap, (2) the contamination of the strainer, and (3) the subsequent droplet dispersion when the

TABLE 1. Patients Infected or Colonized With IMP-Producing *P. aeruginosa* in the Hematology Unit

Patient	Age, y	Sex	Underlying Diseases/Causes of Hospitalization	Date of First Isolation (mm/dd/yyyy)	Site of First Isolation	Duration From Admission to First Isolation, d	Room No.	Clinical Outcome at Discharge	MBL Gene	PFGE Pattern	ST
PA1	52	M	AML, alloSCT	11/03/2009	Stools	27	2208	Alive	bla_{IMP-19}	A	235
PB1	48	M	AML	03/16/2010	Stools	107	2210	Alive	bla_{IMP-29}	B	111
PA2	66	F	AML	11/29/2010	Rectal swab	26	2208	Alive	bla_{IMP-19}	A	235
PB2	43	M	MS, alloSCT	01/18/2011	Stools	22	2209	Alive	bla_{IMP-29}	B	111
PB3	51	M	AML, alloSCT	03/23/2011	Stools	17	2210	Alive	bla_{IMP-29}	B	111
PB4	55	F	MM, alloSCT	05/26/2011	Stools	20	2207	Alive	bla_{IMP-29}	B	111
PB5	54	M	CML	07/12/2011	Stools	19	2203	Alive	bla_{IMP-29}	B	111
PA3	42	F	MM, alloSCT	10/31/2011	Rectal swab	34	2208	Alive	bla_{IMP-19}	A	235
PB6	45	F	AML, alloSCT	11/07/2011	Stools	34	2210	Alive	bla_{IMP-29}	B	111
PA4	70	M	AML	05/14/2012	Blood	14	2208	Attributable death	bla_{IMP-19}	A	235
PA5	56	F	AML, alloSCT	07/05/2012	Stools	21	2208	Alive	bla_{IMP-19}	A	235
PB7	62	M	AML, alloSCT	08/06/2012	Blood	26	2210	Not attributable death	bla_{IMP-29}	B	111
PB8	27	M	ALL	10/26/2012	Blood	16	2210	Attributable death	bla_{IMP-29}	B	111
PA6	30	F	AML	10/29/2012	Stools	24	2214	Alive	bla_{IMP-19}	A	235
PA7	66	M	AML	01/28/2013	Rectal swab	32	2208	Alive	bla_{IMP-19}	A	235
PA8	44	F	ALL, alloSCT	02/11/2013	Rectal swab	71	2214	Alive	bla_{IMP-19}	A	235
PB9	71	M	AML	02/24/2013	Blood	23	2210	Attributable death	bla_{IMP-29}	B	111
PA9	73	M	MS / AML	02/27/2013	Blood	19	2208	Alive	bla_{IMP-19}	A	235
PB10	44	F	AML	05/10/2013	Blood	20	2210	Attributable death	bla_{IMP-29}	B	111
PA10	53	F	AML	06/03/2013	Stools	21	2214	Alive	bla_{IMP-19}	A	235
PA11	66	M	AML	09/09/2013	Stools	18	2208	Alive	bla_{IMP-19}	A	235

NOTE. MBL, metallo-beta-lactamase; PFGE, pulsed-field gel electrophoresis; ST, sequence type; AML, acute myeloid leukemia; alloSCT, allogeneic stem cell transplantation; MS, myelodysplastic syndromes; MM, multiple myeloma; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia.

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TABLE 2. Results of P-Trap Sampling for Presence of *Pseudomonas aeruginosa* in the Environment of Hematology Unit of the University Hospital of Besançon (2013–2016)

Date (MMM-YY)	Room No.																		
	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	Sluice 13-14	2215	2216	Sluice 15-16	Nurse	Shower
May 2013																			
Jun 2013																			
Jul 2013																			
Aug 2013																			
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Mar 2016																			
Oct 2016																			

NOTE. Red, presence of IMP-19–producing *P. aeruginosa*; blue, presence of IMP-29–producing *P. aeruginosa*; yellow, presence of PER-1–producing *P. aeruginosa*; green, presence of *P. aeruginosa* not producing ESBLs or MBLs; white, absence of *P. aeruginosa*.

water flow hits the strainer. This process leads to the contamination of the sink and the surrounding environment.[10] Therefore, patients can be contaminated when grooming, brushing their teeth, or after contact with the environment. Healthcare workers are also at risk for hand contamination and further patient contamination during care.[11,12]

The comprehensive review by Oliver et al[5] gathered the evidence of the existence of multidrug-resistant global clones, denominated high-risk clones and disseminated worldwide. Our outbreak involved the 2 most successful high-risk clones, ST235 and ST111. Unexpectedly, both epidemic strains produced an IMP-like enzyme, which was not the most common MBL hosted by *P. aeruginosa* worldwide.[13] The PFGE pattern A-ST235 produced an IMP-19 (an IMP-2 variant) that was described in 2007 at Dijon University Hospital. This pattern was first hosted by *Aeromonas caviae*[14] and subsequently by 8 other species of gram-negative bacilli, including *P. aeruginosa*.[15] Stem-cell transplantation of patients from the Dijon hospital was performed in our Besançon hospital. Considering these facts, IMP-19–producing *P. aeruginosa* was very likely introduced in our hospital via colonized patients from the Dijon hospital. The MBL IMP-29 was described for the first time in our hospital during this outbreak.[16] Since then, IMP-29–producing *P. aeruginosa* has been recovered in the wastewater network of our town but has not been described in another region or country.[9] $bla_{\text{IMP-19}}$ and $bla_{\text{IMP-29}}$ nucleotide sequences share only 88.8% identity, ruling out the possibility of a direct filiation between the 2 genes.

A bundle of measures was implemented progressively starting in May 2013, and it appears to be efficient to prevent transmission of epidemic strains to newly admitted patients. Indeed, 1 new patient become colonized in September 2013, but no patients were positive for *P. aeruginosa* producing IMP-like enzymes from this date to November 2016, and all patients were screened weekly for *P. aeruginosa* carriage during that period. Importantly, the outbreak was controlled while the epidemic strains were still present in the environment and were sporadically detected in P-traps in 2013, 2014, and 2015 (Figure 2). In other words, we witnessed a recolonization of the new P-traps in rooms hosting patients who were not colonized by the epidemic strains. This finding suggests, as shown by Kotay et al,[10] that *P. aeruginosa* producing IMP-like enzymes stayed in the main pipe and recontaminated the P-traps.[10] This explains how the pathogen contaminated new P-traps and drains of rooms hosting patients negative for IMP-like–producing *P. aeruginosa*.

The use of bleach solution and changing the of P-traps (ie, at patient discharge whenever a patient stayed >7 days) may have reduced the microbial load in this environment. However, we did not assess the bacterial load of epidemic strains before and after implementation of the measures. Finally, it is quite difficult to identify measures that are most important in the bundle implemented in a crisis.

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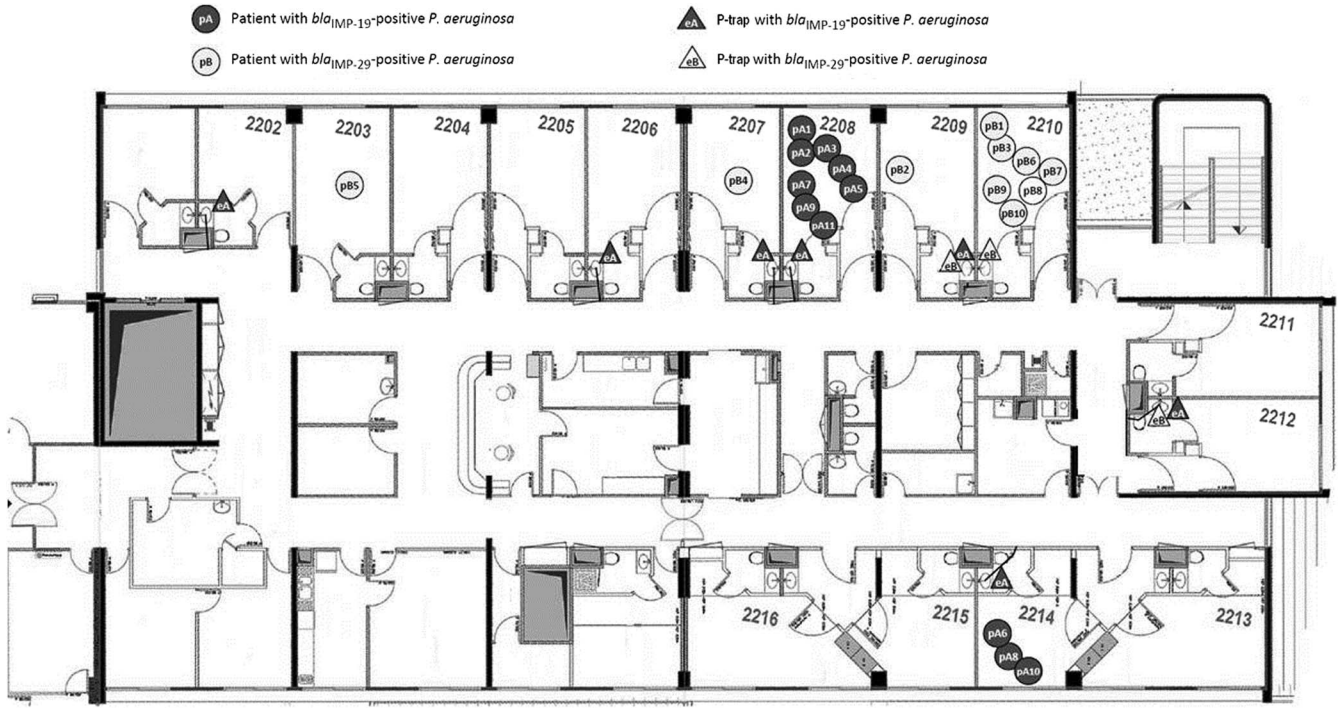

FIGURE 2. Location of patients and P-traps positive for *P. aeruginosa* isolates carrying bla_{IMP-19} or bla_{IMP-29} genes in the hematology unit of the University Hospital of Besançon.

In summary, we report a prolonged outbreak in a hematology unit due to 2 IMP-producing high-risk clones of *P. aeruginosa*, for which the environment, particularly P-traps, played a major role in cross-transmission. Our infection control measures efficiently controlled the outbreak. However, our extensive microbiological investigation indicated that the eradication of *P. aeruginosa* from the wastewater network is unrealistic. Hence, a reservoir can persist even after full replacement of the equipment at the water point of use. In that context, it appears critical to implement proactive measures reducing the bacterial load in water fittings of high-risk units to limit the transmission of nosocomial pathogens to fragile patients.

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