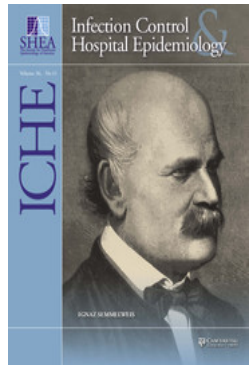

Post-Outbreak Investigation of *Pseudomonas aeruginosa* Faucet Contamination by Quantitative Polymerase Chain Reaction and Environmental Factors Affecting Positivity

Emilie Bédard, Céline Laferrière, Dominique Charron, Cindy Lalancette, Christian Renaud, Nadia Desmarais, Eric Déziel and Michèle Prévost

Infection Control & Hospital Epidemiology / Volume 36 / Issue 11 / November 2015, pp 1337 - 1343
DOI: 10.1017/ice.2015.168, Published online: 20 July 2015

ORIGINAL ARTICLE

Post-Outbreak Investigation of *Pseudomonas aeruginosa* Faucet Contamination by Quantitative Polymerase Chain Reaction and Environmental Factors Affecting Positivity

Emilie Bédard, PhD;[1,2] Céline Laferrière, MD;[3] Dominique Charron, MScA;[1] Cindy Lalancette, PhD;[2] Christian Renaud, MD;[3] Nadia Desmarais, ICS-PCI;[4] Eric Déziel, PhD;[2] Michèle Prévost, PhD[1]

OBJECTIVE. To perform a post-outbreak prospective study of the *Pseudomonas aeruginosa* contamination at the faucets (water, aerator and drain) by culture and quantitative polymerase chain reaction (qPCR) and to assess environmental factors influencing occurrence

SETTING. A 450-bed pediatric university hospital in Montreal, Canada

METHODS. Water, aerator swab, and drain swab samples were collected from faucets and analyzed by culture and qPCR for the post-outbreak investigation. Water microbial and physicochemical parameters were measured, and a detailed characterization of the sink environmental and design parameters was performed.

RESULTS. The outbreak genotyping investigation identified drains and aerators as the source of infection. The implementation of corrective measures was effective, but post-outbreak sampling using qPCR revealed 50% positivity for *P. aeruginosa* remaining in the water compared with 7% by culture. *P. aeruginosa* was recovered in the water, the aerator, and the drain in 21% of sinks. Drain alignment vs the faucet and water microbial quality were significant factors associated with water positivity, whereas *P. aeruginosa* load in the water was an average of 2 log higher for faucets with a positive aerator.

CONCLUSIONS. *P. aeruginosa* contamination in various components of sink environments was still detected several years after the resolution of an outbreak in a pediatric university hospital. Although contamination is often not detectable in water samples by culture, *P. aeruginosa* is present and can recover its culturability under favorable conditions. The importance of having clear maintenance protocols for water systems, including the drainage components, is highlighted.

Infect. Control Hosp. Epidemiol. 2015;36(11):1337–1343

Pseudomonas aeruginosa is a source of infection outbreaks, especially in intensive care units (ICUs).[1] Several of these outbreaks have been directly or indirectly linked to water distribution systems.[2–14] In ICUs, 30%–50% of *P. aeruginosa* infections have been associated with water.[15] A multicentric prospective study recently established tap contamination in patient rooms as an important environmental risk factor for *P. aeruginosa* acquisition.[16]

Several factors promote water contamination, including the type of faucet,[9,17,18] the presence and type of aerator on the faucet,[19] the volume of mixed hot and cold water,[17] the alignment of the sink drain,[20] construction or renovation settings, and ICU vs non-ICU settings.[3,20] Once contaminated, eradication of *P. aeruginosa* in the water system is challenging and often results in replacing related devices[5,7–12,14] or installing point-of-use 0.2-μm filters.[4,13,14]

Although cultivation is the reference method, it may not reveal background contamination that may flare up when the environment becomes favorable for growth and culturability. Environmental stressors present in water such as chlorine and copper decrease culturability without necessarily decreasing viability.[21,22] Most studies describing environmental contamination have been conducted using the culture detection method. Often, *P. aeruginosa* could not be isolated from water but was recovered from biofilm swabs. The use of quantitative polymerase chain reaction (qPCR) for the detection and measurement of bacteria in drinking water is not yet routinely used. However, this method could offer a valuable alternative for assessing the underlying contamination of systems as well as risk areas.

Herein, we describe a follow-up investigation of the water system contamination by *P. aeruginosa* a decade after an

Affiliations: 1. Department of Civil Engineering, Polytechnique Montréal, Montréal, QC, Canada; 2. INRS-Institut Armand-Frappier, Laval, QC, Canada; 3. Department of Microbiology and Immunology (Infection Control), CHU Ste-Justine, Université de Montréal, Montréal, QC, Canada; 4. Nursing Care Division, CHU Ste-Justine, Montréal, QC, Canada.

Received March 8, 2015; accepted June 20, 2015; electronically published July 20, 2015

 0899-823X/2015/3611-0014. DOI: 10.1017/ice.2015.168

outbreak in a neonatal ICU (NICU). The objectives of this study were (1) to conduct a follow-up investigation on the water system using qPCR and cultivation methods to evaluate the level of contamination and (2) to identify the factors that contribute to the persistence of *P. aeruginosa* in the water system despite corrective measures. To our knowledge, this is the first study to report the use of qPCR to investigate the presence of *P. aeruginosa* in a hospital water system.

METHODS

The investigation was performed at Centre Hospitalier Universitaire (CHU) Sainte-Justine, a 450-bed pediatric university hospital in Montreal, Canada. A *P. aeruginosa* outbreak took place between January 2004 and November 2005 in neonatal intensive and intermediate care units.[23,24] To summarize, 27 *P. aeruginosa* infections were reported during the outbreak (2004–2005), and 6 patients died as a consequence of infection. The following types of infections were reported: sepsis, lower respiratory infection, urinary tract infection, catheter-related infection, cellulitis, and conjunctivitis. All infection episodes reported among neonates from the NICUs were healthcare-associated. Distribution and draining water systems including faucets, aerators, and sinks were approximately 50 years old, and all taps were equipped with aerators. Corrosion deposits were visible on faucets, aerators, sink traps, and mixing valves. Clogging of the drains, resulting in water stagnation into the sinks, was frequently reported by the hospital staff at the time of the outbreak. The clogging was caused by the accumulation of carbonate scale and microbial biofilm in the drain pipes. In the 15 years prior to the outbreak, construction and renovation had required several prolonged interruptions in the water supply.

The outbreak investigation led to environmental sampling within and outside the NICU conducted between March and April 2005.[23,24] *P. aeruginosa* was recovered by cultivation from 32 of 57 sink drains (56%), 3 of 56 faucet swabs (5%), and 5 of 16 aerators (31%) during the outbreak investigation. All swabs from other environmental surfaces, solutions, and water samples collected from taps (n = 69) or water distribution systems (n = 40) were negative. A survey of healthcare worker hands was unrevealing. A total of 66 *P. aeruginosa* isolates, all obtained during the outbreak period, were subjected to PFGE testing, resulting in the identification of 51 different genotypes and variants: 7 were identified exclusively from clinical isolates and 32 were identified exclusively from environmental isolates. Common strains were identified between the clinical and water system samples, notably between 12 patients and the sink aerator where the milk bottles were prepared. The genotyping gel of the clinical and environmental *P. aeruginosa* strains isolated during the outbreak is presented online as supplementary material. The environmental survey and the genotyping resulting from the outbreak investigation confirmed the water system as the source of the neonatal outbreak. The implementation of the following corrective measures was successful in drastically reducing the number of cases: use of sterile water for patient care, alcohol-based gel treatment following hand washing, wearing gloves for direct contact with body secretions, installation of point-of-use filters (0.2 μm), good practices in milk preparation, replacement of drain pipes, and prohibition of storage of medical material under or within 30 cm of a sink. These corrective measures have been maintained in place since their implementation; filters were removed when drains were identified as the potential source.

A post-outbreak investigation was conducted in July 2013 in various areas of the hospital, including the NICU. A total of 28 faucets were sampled as follows: (1) a swab of the drain, (2) 1 L of first-flush cold water in sterile polypropylene bottle with 1.1 mg/L sodium thiosulfate, and (3) a swab of the aerator. Water samples from 3 additional faucets were tested. Cultivation, heterotrophic plate counts (HPCs), qPCR, and viable and total cell counts were performed on the water samples. Cultivation and qPCR were performed on swabs. HPC was determined on R2A agar after 7 days of incubation at 22°C.[25] Viable and total cell counts were performed using LIVE/DEAD BacLight Kit (Thermo Fisher Scientific, Waltham, MA). Cultivation was performed according to ISO16266:2006,[26] as previously described.[17] Briefly, the water samples were filtered (0.45 μm pore-size filter), and the filters were incubated on cetrimide agar with 15 mg/L nalidixic acid at 37.5°C and colonies were counted after 24 hours and 48 hours. Using qPCR, the presence of *P. aeruginosa* was assessed by targeting the *gyrB* gene (Corbett Rotor-Gene 6000) for 50 cycles: 10 minutes initial denaturation (95°C), denaturation (95°C, 30s), and annealing and elongation (60°C, 90 s).[27] DNA was extracted after filtration of 450 mL on a 0.45-μm mixed-cellulose esther filter using a bead-beating method followed by ammonium acetate precipitation and ethanol washes, as before.[28]

Each faucet and its environment were characterized in detail, including the type of activating device, the connecting pipe material, the faucet internal diameter, the faucet alignment to the drain, and the drainage efficiency. The copper water distribution line was fed by municipal surface water with an average residual chlorine level of 0.4 mg Cl_2/L. Chlorine levels in the distribution system were maintained at similar levels, and no corrosion control initiatives or water quality changes occurred during the period between the outbreak and the follow-up study.

Statistical analyses (*t* test, *z* test, and multivariate adaptative regression spline [MARSpline]) were performed with Statistica10 (StatSoft, Dell, Aliso Viejo, CA). MARSpline regression is a nonparametric analysis in which continuous, categorical, and nominal variables can be added to the model and from which a better fit from a few or all variables is proposed. The significance level was set at $P = .05$.

RESULTS

Water characteristics measured at the time of the outbreak (2005) and during the post-outbreak investigation (2013) are

TABLE 1. Mean Tap Water Microbiological and Physicochemical Characterization

	During Outbreak, 2005 (n = 4)	Post Outbreak, 2013 (n = 28)
HPC (CFU/mL)	$5.2 \pm 3.7 \times 10^1$	$3.7 \pm 10 \times 10^2$
Viable (Bact/mL)	$1.1 \pm 0.3 \times 10^5$	$0.9 \pm 1.5 \times 10^5$
Total (Bact/mL)	$1.8 \pm 0.3 \times 10^5$	$1.9 \pm 2.6 \times 10^5$
Residual chlorine (mg Cl_2/L)	n/a	0.05 ± 0.05
Total copper (μg/L)	n/a	570 ± 140
Hot water temperature (°C)	n/a	53 ± 10

NOTE. HPC, heterotrophic plate counts; Bact, bacteria; n/a, not applicable.

TABLE 2. Proportion of *P. aeruginosa* Culture and qPCR Positive Post-Outbreak Samples for Each Type of Sampling Site and for ≥2 Corresponding Sampling Sites

				Proportion of Sinks with Corresponding Positive Samples for ≥2 Sampling Locations			
	Water	Aerator	Drain	Water and Aerator	Water and Drain	Aerator and Drain	Water, Aerator, and Drain
Culture	2/28 (7%)	1/28 (3.5%)	16/28 (57%)	1/2 (50%)	0/2 (0%)	0/1 (0%)	0/2 (0%)
qPCR	14/28 (50%)	18/28 (64%)	25/28 (89%)	9/14 (64%)	10/14 (71%)	14/18 (78%)	6/14 (43%)

NOTE. qPCR, quantitative polymerase chain reaction.

presented in Table 1. Total and viable cell counts were comparable between the 2 sampling campaigns, but HPCs were 1 log higher during post-outbreak sampling measurements. However, results were not significantly different between the 2 periods according to *t* test statistics ($P > .05$).

The post-outbreak investigation results are presented in Table 2. Results obtained by qPCR detection revealed dramatically higher positivity for all sampling sites. *P. aeruginosa* was detected in the water and the biofilm from the corresponding aerator and drain for 6 faucets, and 12 faucets had 2 positive sites (water/aerator, water/drain, or aerator/drain) (Table 2).

Figure 1 shows the mean concentrations of copper and residual chlorine and the hot water temperature at sampled taps for *P. aeruginosa*–positive and –negative water from faucets as evaluated by qPCR (Table 2). The impacts of various environmental parameters on *P. aeruginosa* detection are presented in Table 3. For each parameter, the positivity in the water and corresponding aerator and drain swabs are presented. Three additional faucets were sampled for water only and were included in the water sample analysis. A statistical comparison of positivity obtained for each configuration within an environmental design parameter was performed. A *P* value below .05 suggested that the results obtained for the different configurations within a group were significantly different and therefore affected positivity results.

The effects of environmental factors and microbiological quality of the water on the *P. aeruginosa* load detected in water, aerator, and drain swabs by qPCR were analyzed using a multivariate regression (MARSpline). In addition, the impact of environmental factors on the water microbiological quality (HPC and viable cell counts) was assessed using the same regression method. Significant variables contributing to model response variables are presented in Table 4.

DISCUSSION

Environmental monitoring and prevention concentration thresholds of *P. aeruginosa* in water are proposed in infection control guidelines from the United Kingdom.[29] Previous *P. aeruginosa* outbreak investigations have also confirmed the waterborne source through the detection of *P. aeruginosa* in swabs from drains and faucets.[5,6,11,13,14,20] Notably, in this case and despite the established link between aerators and drains swabs and clinical cases, *P. aeruginosa* was not isolated from water by culture during the outbreak. The high level of aerator positivity (ie, 4 of the 5 positive aerators harbored strains shared with clinical isolates during the outbreak) led to the hypothesis of retrograde contamination from the drain.

Recent evidence has shown that the first-flush volume (<1 L) at the faucet contains strikingly higher levels of HPCs (30–100× higher) and culturable *P. aeruginosa*.[17,30–32] In light of these findings, it is not surprising that all 2005 environmental outbreak investigation samples were negative for *P. aeruginosa* because these samples were collected after a 30-second flush. Furthermore, bacteria present in the water distribution system are exposed to stressors such as chlorine, copper, and high temperatures.[21,22] In drinking water, stressed cells that lose their culturability can be detected by qPCR; these cells can quickly recover their culturability under favorable conditions.[21]

These findings suggest the need to re-evaluate the prevalence of *P. aeruginosa* in the water system using a standardized sample volume for culture and the need to apply qPCR methods recently validated in drinking water.[27] To investigate the importance of environmental parameters, the post-outbreak investigation included detailed monitoring of water quality, including the main chemical stressors and bacterial indicators and the prevalence of *P. aeruginosa* in drain and aerator.

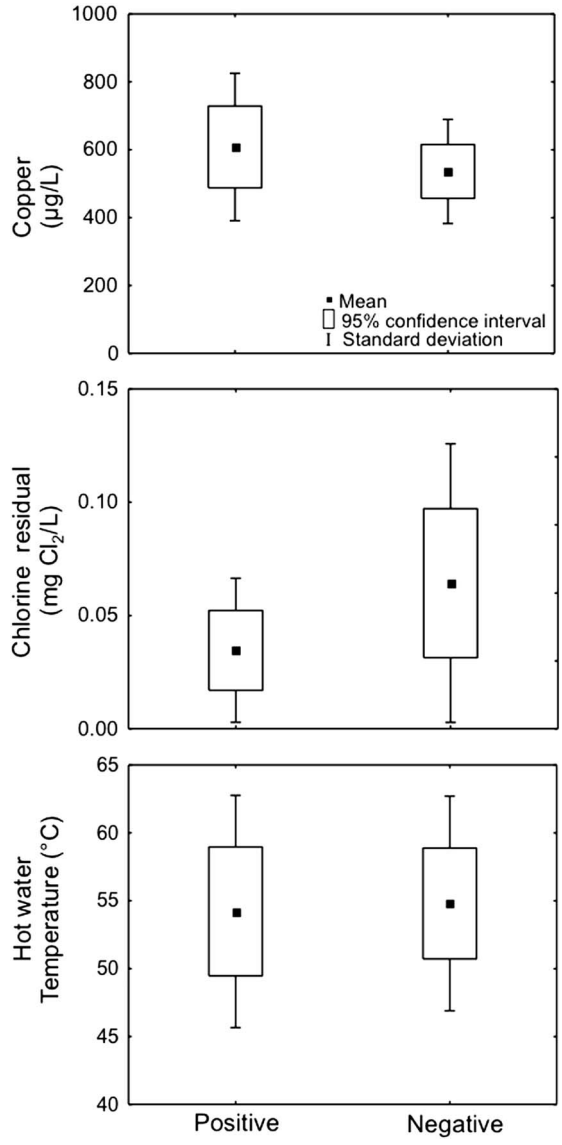

FIGURE 1. Mean values for copper concentration, chlorine residual and hot water temperature for positive and negative *P. aeruginosa* water samples as measured by qPCR (n = 28).

The follow-up water system investigation showed a low level of *P. aeruginosa* contamination in the system; 2 of 28 water samples were positive by culture. Water positivity increased drastically when measured by qPCR, which detected the presence of all cells in the sample, including viable but unculturable cells. When the aerator was positive, mean concentrations of *P. aeruginosa* in water were 2 log higher than in faucets in which the aerator was not positive. These results reveal a link between the aerator and water contamination.

Mean copper concentration in water was not significantly different between qPCR-positive and -negative samples (Fig. 1a). In this study, mean copper concentration in water was higher (570 µg/L) than the concentration reported to inhibit *P. aeruginosa* culturability (250 µg/L).[21,22] However, this threshold concentration was obtained using strains not previously acclimated to elevated copper concentrations. Viable but nonculturable *P. aeruginosa* cells can persist in the presence of copper concentrations up to 2 mM, which is orders of magnitude higher than copper concentrations found in drinking water.[33] Copper concentrations observed in drinking water would therefore affect culturability without affecting viability, with the capacity to recover culturability once copper stress is reduced.[22]

Mean residual chlorine (Fig. 1b) was slightly more elevated in negative samples and was a significant variable for aerator positivity according to qPCR and water *P. aeruginosa* load (Table 4). Hot water temperature mean values were also comparable between samples positive and negative for *P. aeruginosa* (Fig. 1c). Temperature in the hot water system can affect water and aerator positivity if it is high enough to perform thermal disinfection. In the present study, hot water temperatures were below 65°C, a temperature lower than that required for thermal disinfection of water and biofilm.[34,35]

Further analyses performed on qPCR-positive water samples, aerator swabs, and drain swabs revealed the impact of environmental parameters (Table 3). The type of faucet activating device, the faucet internal diameter, and the speed of drainage did not lead to statistically different *P. aeruginosa* positivity of either the water or the aerator and drain biofilms. However, the drainage efficiency was a significant variable to predict the HPC in water as well as drain positivity (Table 4). Water and aerator positivity were not significantly impacted by the connection pipe material, although it was reported as significant in previous studies.[17,19] As stated previously, copper affects culturability of *P. aeruginosa*, and previous studies evaluated positivity based on culture detection method. The present study suggests that viable and total water contamination is not significantly influenced by the connecting material. However, faucet alignment to drain configuration led to significantly different positivities of the water. Results suggest that water from a faucet aligned behind the drain has a higher rate of contamination by *P. aeruginosa* than other configurations. In previous studies direct water flow into the drain was determined to be a source of retrograde contamination of the faucet, and sink designs were changed to avoid alignment between the faucet and the drain.[5,14,36] However, to our knowledge, no other study has reported the impact of the actual positioning of the faucet with regard to the drain on positivity. Drains have high rates of contamination by *P. aeruginosa*,[5,14,37] and the risk of retrograde contamination can be managed by ensuring appropriate design of the sink and maintenance of efficient drainage. Further investigation with a larger number of each drain configuration will be needed to determine the optimal drain positioning with relation to the faucet. The relation between the operating and design parameters of a sink and the drain contamination is highlighted by the correlation obtained in the modeling of drain positivity as measured by qPCR (Table 4).

TABLE 3. Summary of *P. aeruginosa* Occurrence and Percentage Measured by qPCR in Water, Aerator Swab, and Drain Swab Samples Grouped by Sink Environmental Design Parameters[a]

	Water		Aerator swab		Drain swab	
	n	%	n	%	n	%
Faucet activating device						
Manual with 2 levers	5/13	38	6/13	46	10/13	74
Manual with 1 lever	5/8	62	5/6	83	5/6	83
Foot operated	6/10	60	5/9	56	9/9	100
P value	*.10*		*.06*		*.20*	
Connecting pipes						
Flexible hoses	4/6	67	4/6	67	3/6	50
Copper pipes	11/21	52	10/18	56	17/18	94
Flexible hoses and copper pipes	1/4	25	2/4	50	4/4	100
P value	*.20*		*.30*		*<.05*	
Faucet internal diameter						
≥1 cm	10/21	48	10/20	50	17/20	85
<1 cm	6/10	60	6/8	75	7/8	88
P value	*.30*		*.10*		*.40*	
Faucet alignment to drain						
Behind	8/10	80	6/9	67	9/9	100
Direct	2/5	40	4/5	80	4/5	80
Forward	6/12	50	6/10	60	8/10	80
Side	0/3	0	0/3	0	2/3	67
P value	*<.05*		*.20*		*.10*	
Heterotrophic plate counts						
<10 CFU/mL	3/11	27	4/11	37	10/11	91
≥10 CFU/mL	10/17	59	12/17	71	14/17	82
P value	*.05*		*<.05*		*.30*	
Speed of water drainage in sink						
Good	6/14	43	6/13	46	11/13	85
Average	8/13	62	8/11	73	9/11	82
Poor	2/4	50	2/4	50	4/4	100
P value	*.20*		*.10*		*.40*	
Room usage						
Patient care	13/17	76	9/14	64	13/14	93
Other	3/14	21	7/14	50	11/14	79
P value	*<.05*		*.20*		*.20*	

NOTE. CFU, colony-forming units.
[a]Statistically significant if $P \leq .05$.

Samples from taps with HPC higher than 10 CFU/mL had twice the positivity for *P. aeruginosa* in water and aerator biofilm samples. Heterotrophic plate counts are generally used as indicators of the general microbial quality of the water in main distribution systems.[38] Water positivity was significantly higher if the faucet sampled was in a patient room. A detailed utilization survey of both faucets and drains in sampled rooms would help provide an explanation for this finding. Ehrhardt et al[20] reported higher positivity for NICU faucets (71%) vs faucets sampled outside the NICU (12.5%), but there was no mention of either the faucet or the drain usage inside the NICU compared to outside.

Our study has a number of limitations. First, because of the large number of parameters investigated, some categories had a limited number of samples. Second, these results apply to 1 hospital system and may vary in different settings. Third, the post-outbreak sampling was conducted in the absence of active clinical cases.

The results from this study reveal that although bacteria may not be detectable in the water by traditional culture methods, *P. aeruginosa* is present and can recover its culturability under favorable conditions. In a hospital environment, this finding suggests that failure to maintain good practices, or presence of disrupting events such as renovation, may act as promoting factors leading to increased concentrations and risk of patient exposure. These results demonstrate the importance of defining a clear and detailed protocol to determine the design of and the precise maintenance required for water systems, including drains. Furthermore, detection by qPCR is a valuable tool for rapidly identifying positive

TABLE 4. Multivariate Adaptive Regression (MARSpline) Results Presenting Model Input, Response Variable and Significant Variables for Environmental and Microbiological Parameters at the Sink

Model Input	Response Variable	Significant Variables	R^2
HPC, viable and total cell counts, drain and aerator positivity by culture	*P. aeruginosa* in water (GU/mL)	HPC, viable cell counts, drain and aerator positivity by culture	.59
HPC & environmental factors (connection pipe material and diameter, copper and chlorine concentration, type of faucet, mitigated volume, faucet activating device, HPC, drain alignment, drainage efficiency, room usage)	*P. aeruginosa* in water (GU/mL)	HPC, chlorine residual	.47
	P. aeruginosa drain positivity (qPCR)	Connection material, copper concentration, mitigated volume, drain alignment and drainage efficiency	.998
	P. aeruginosa aerator positivity (qPCR)	HPC, copper and chlorine concentration	.35
Environmental factors (same as above)	HPC	Connection material, drainage efficiency	.52
Environmental factors (same as above), HPC, *P. aeruginosa* positivity by culture (water, aerator, drain)	Viable cell counts	Copper concentration, drain alignment	.50

NOTE. HPC, heterotrophic plate counts; GU, genomic units; qPCR, quantitative polymerase chain reaction.

sites in an outbreak investigation and in direct sampling and typing efforts. Finally, qPCR provides complementary information to culture-based results, especially for samples subjected to inhibitors such as elevated copper concentrations or disinfectant residual.

ACKNOWLEDGEMENTS

The authors would like to thank Chair staff, the participating healthcare facility personnel, Johanne Ismaïl (LSPQ) for the genotyping analysis, and Dr. Maude St-Jean and Dr. Mireille Lemay for their scientific contribution.

Financial support: This research was supported by NSERC through the Industrial Chair on Drinking Water and EB Vanier Canada Graduate Scholarship. ED holds a Canada Research Chair.

Potential conflicts of interest: All authors report no conflicts of interest relevant to this article.

Address correspondence to: Emilie Bédard, NSERC Industrial Chair in Drinking Water, Polytechnique Montréal, P.O. Box 6079 Station Centreville, Montréal, QC, Canada, H3C 3A7 (emilie.bedard@polymtl.ca).

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/ice.2015.168.

REFERENCES

1. Jefferies JMC, Cooper T, Yam T, Clarke SC. *Pseudomonas aeruginosa* outbreaks in the neonatal intensive care unit—a systematic review of risk factors and environmental sources. *J Med Mic* 2012;61:1052–1061.
2. Trautmann M, Michalsky T, Wiedeck H, et al. Tap water colonization with *Pseudomonas aeruginosa* in a surgical intensive care unit (ICU) and relation to *Pseudomonas* infections of ICU patients. *Infect Control Hosp Epidemiol* 2001;22:49–52.
3. Reuter S, Sigge A, Wiedeck H, Trautmann M. Analysis of transmission pathways of *Pseudomonas aeruginosa* between patients and tap water outlets. *Crit Care Med* 2002;30:2222–2228.
4. Aumeran C, Paillard C, Robin F, et al. *Pseudomonas aeruginosa* and *Pseudomonas putida* outbreak associated with contaminated water outlets in an oncohaematology paediatric unit. *J Hosp Infect* 2007;65:47–53.
5. Hota S, Hirji Z, Stockton K, et al. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* colonization and infection secondary to imperfect intensive care unit room design. *Infect Control Hosp Epidemiol* 2009;30:25–33.
6. Ferroni A, Nguyen L, Pron B, et al. Outbreak of nosocomial urinary tract infections due to *Pseudomonas aeruginosa* in a paediatric surgical unit associated with tap-water contamination. *J Hosp Infect* 1998;39:301–307.
7. Bert F, Maubec E, Bruneau B, et al. Multi-resistant *Pseudomonas aeruginosa* outbreak associated with contaminated tap water in a neurosurgery intensive care unit. *J Hosp Infect* 1998;39:53–62.
8. Durojaiye OC, Carbarns N, Murray S, Majumdar S. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* in an intensive care unit. *J Hosp Infect* 2011;78:154–155.
9. Halabi M, Wiesholzer-Pittl M, Schöberl J, Mittermayer H. Non-touch fittings in hospitals: a possible source of *Pseudomonas aeruginosa* and *Legionella* spp. *J Hosp Infect* 2001;49:117–121.
10. Merrer J, Girou E, Ducellier D, et al. Should electronic faucets be used in intensive care and hematology units? *Int Care Med* 2005;31:1715–1718.
11. Yapicioglu H, Gokmen TG, Yildizdas D, et al. *Pseudomonas aeruginosa* infections due to electronic faucets in a neonatal intensive care unit. *J Paediatr Child Health* 2011;48:430–434.
12. Romano S, Bourdier A, Parer S, et al. Peripheral venous catheter and bloodstream infection caused by *Pseudomonas aeruginosa* after contaminated preoperative shower. *Infect Control Hosp Epidemiol* 2013;34:544–546.
13. Vianelli N, Giannini MB, Quarti C, et al. Resolution of a *Pseudomonas aeruginosa* outbreak in a hematology unit with the use of disposable sterile water filters. *Haematologica* 2006;91:983–985.
14. Schneider H, Geginat G, Hogardt M, et al. *Pseudomonas aeruginosa* outbreak in a pediatric oncology care unit caused by an errant water jet into contaminated siphons. *Pediatr Infect Dis J* 2012;31:648–650.

15. Exner M. Wasser als Infektionsquelle: Leitungswasser: Klar und sauber? *Heilberufe* 2012;64:24–27.
16. Venier AG, Leroyer C, Slekovec C, et al. Risk factors for *Pseudomonas aeruginosa* acquisition in intensive care units: a prospective multicentre study. *J Hosp Infect* 2014;88:103–108.
17. Charron D, Bédard E, Lalancette C, et al. Impact of electronic faucets and water quality on the occurrence of *Pseudomonas aeruginosa* in water: a multi-hospital study. *Infect Control and Hosp Epidemiol* 2015;36:311–319.
18. Blanc DS, Nahimana I, Petignat C, et al. Faucets as a reservoir of endemic *Pseudomonas aeruginosa* colonization/infections in intensive care units. *Intensive Care Med* 2004;30:1964–1968.
19. Walker JT, Jhutty A, Parks S, et al. Investigation of healthcare-acquired infections associated with *Pseudomonas aeruginosa* biofilms in taps in neonatal units in Northern Ireland. *J Hosp Infect* 2014;86:16–23.
20. Ehrhardt D, Terashita D, English T. An outbreak of *Pseudomonas aeruginosa* in neonatal intensive care unit, Los Angeles County, 2006: acute communicable disease control program; 2006 December 6. http://publichealth.lacounty.gov/wwwfiles/ph/dcp/acd/2006SpecialStudies.pdf.
21. Bédard E, Charron D, Lalancette C, et al. Recovery of *Pseudomonas aeruginosa* culturability following copper- and chlorine-induced stress. *FEMS Microbiol Lett* 2014;356:1–9.
22. Dwidjosiswojo Z, Richard J, Moritz MM, et al. Influence of copper ions on the viability and cytotoxicity of *Pseudomonas aeruginosa* under conditions relevant to drinking water environments. *Int J Hyg Environ Health* 2011;214:485–492.
23. Renaud C, Vallée J, Perpête C, et al. Clinical aspects of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* infections related to contaminated tap water in a neonatal intensive care unit. Society for Healthcare Epidemiology of America (SHEA), 16th Annual Scientific Meeting, March 18–21, 2006, Chicago, IL.
24. Laferrière C, Perpête C, Scrivo C, et al. Nosocomial *Pseudomonas*, *Stenotrophomonas*, *Burkholderia* infections in a neonatal intensive care unit linked to contaminated tap water. Society for Healthcare Epidemiology of America (SHEA), 16th Annual Scientific Meeting, March 18–21, 2006, Chicago, IL.
25. American Public Health Association (APHA). American Water Works Association (AWWA), Water Environment Federation (WEF). Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association, 2012.
26. International Organization for Standardization (ISO). Water quality—detection and enumeration of *Pseudomonas aeruginosa*—method by membrane filtration; 2006.
27. Lee CS, Wetzel K, Buckley T, et al. Rapid and sensitive detection of *Pseudomonas aeruginosa* in chlorinated water and aerosols targeting *gyrB* gene using real-time PCR. *J Appl Microbiol* 2011;111:893–903.
28. Bédard E, Fey S, Charron D, et al. Temperature diagnostic to identify high risk areas and optimize *Legionella pneumophila* surveillance in hot water distribution. *Water Res* 2015;71:244–256.
29. Department of Health (DH). Government of Great Britain (2013) Water systems: HTM 04-01: Addendum. *Pseudomonas aeruginosa*—advice for augmented care units.
30. Cristina ML, Spagnolo AM, Casini B, et al. The impact of aerators on water contamination by emerging Gram-negative opportunists in at-risk hospital departments. *Infect Control Hosp Epidemiol* 2014;35:122–129.
31. Lipphaus P, Hammes F, Kotzsch S, et al. Microbiological tap water profile of a medium-sized building and effect of water stagnation. *Environ Technol* 2014;35:620–628.
32. Lautenschlager K, Boon N, Wang Y, et al. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. *Water Res* 2010;44:4868–4877.
33. Teitzel GM, Parsek MR. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 2003;69:2313–2320.
34. Van der Mee-Marquet N, Bloc D, Briand L, et al. Non-touch fittings in hospitals: A procedure to eradicate *Pseudomonas aeruginosa* contamination. *J Hosp Infect* 2005;60:235–239.
35. Park H, Park HJ, Kim JA, et al. Inactivation of *Pseudomonas aeruginosa* PA01 biofilms by hyperthermia using superparamagnetic nanoparticles. *J Microbiol Methods* 2011;84:41–45.
36. Breathnach AS, Cubbon MD, Karunaharan RN, et al. Multidrug-resistant *Pseudomonas aeruginosa* outbreaks in two hospitals: association with contaminated hospital waste-water systems. *J Hosp Infect* 2012;82:19–24.
37. Döring G, Ulrich M, Müller W, et al. Generation of *Pseudomonas aeruginosa* aerosols during handwashing from contaminated sink drains, transmission to hands of hospital personnel, and its prevention by use of a new heating device. *Int J Hygiene Env Med* 1991;191:494–505.
38. Bartram J, Cotruvo J, Exner M, et al. Heterotrophic plate counts and drinking-water safety. London, United Kingdom: World Health Organization (WHO) and International Water Association (IWA) Publishing, 2003.