Journal of Hospital Infection 96 (2017) 238–243

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Association between *Pseudomonas aeruginosa* positive water samples and healthcare-associated cases: nine-year study at one university hospital

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ARTICLE INFO

Article history:
Received 21 September 2016
Accepted 8 December 2016
Available online 18 December 2016

Keywords:
Pseudomonas aeruginosa
Water
Healthcare-associated infections

SUMMARY

Objective: To study the association between the results of water samples and *Pseudomonas aeruginosa* healthcare-associated cases in a French university hospital.
Methods: Generalized Estimating Equations were used on complete case and imputed datasets. The spatial unit was the building and the time unit was the quarter.
Results: For the period 2004–2013, 2932 water samples were studied; 17% were positive for *P. aeruginosa*. A higher incidence of *P. aeruginosa* cases was associated with a higher proportion of positive water samples ($P=0.056$ in complete case analysis and $P=0.031$ with the imputed dataset). The association was no longer observed when haematology and intensive care units were excluded, but was significant in analyses of data concerning intensive care units alone ($P<0.001$).
Conclusion: This study suggests that water outlet contamination in hospitals can lead to an increase in healthcare-associated *P. aeruginosa* cases in wards dealing with susceptible patients, but does not play a significant role in other wards.

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http://dx.doi.org/10.1016/j.jhin.2016.12.007
0195-6701/

Introduction

Pseudomonas aeruginosa infections may cause severe morbidity and mortality.[1] The possible presence of *P. aeruginosa* in water systems is well established.[2] Some mechanisms of exposure are common and can occur even when guidelines for the control of *P. aeruginosa* are adhered to, such as when showering or bathing, via drinking water or through contact with contaminated surfaces. Outbreaks of infection with *P. aeruginosa* have also been reported in relation to failure to comply with guidance, such as use of contaminated water to rinse endoscopes or surgical equipment, or use of non-sterile water for antiseptic dilution.[3]

The literature review of Anaissie *et al*. provided a summary of water-borne healthcare-associated infections other than *Legionella* spp.[3] Most of the reports related to *P. aeruginosa*, and all were concerned with outbreaks of infection; no large-scale long-term studies were found. Many of the studies went no further than to report on the relationship between micro-organisms found in water systems and those identified in patients using genotyping methods.

Two recent literature reviews reported a link between water system contamination and *P. aeruginosa* infections.[4,5] However, although a link between water network contamination and healthcare-associated infection is suspected, the strength of the association is still not clear. As a result, water sampling remains controversial and guidelines differ between countries.

This study aimed to examine the association between the results of water samples and cases of healthcare-associated infection with *P. aeruginosa*.

Methods

Study setting and population

The University Hospital of Dijon is located in France and has 1800 beds, with medical and surgical wards and intensive care units (ICUs). There are 10 buildings across five sites, one of which was constructed recently (wards occupied since 2010).

All *P. aeruginosa* positive samples from 1st January 2005 to 31st March 2013 were extracted from the bacteriology laboratory database. Duplicates (isolates with the same antibiogram according to the Antimicrobial Committee of the French Society for Microbiology,[6] and identified within six months of an earlier isolate from the same patient) were excluded. Patients were also excluded if they had been hospitalized for less than 48 h when their samples were collected.

Water samples

In the study facility, water samples had to be taken from taps, showers and drinking fountains in each ward for testing once every quarter. Water points that were sampled on each occasion were selected at random; however, rooms that housed patients and those 'disfavoured' outlets furthest away from the water supply entry point into the hospital had to be tested at least annually. Samples from water outlets on the ICUs with filters fitted were taken after removing the filter. Filters in the study hospital are ready to use and are changed every 31 days. Samples were taken according to French standards: after disinfection of the water point and a short flush (1 or 2 min), 100 mL of water was sampled in sterile bottles containing 20 mg/L of sodium thiosulphate and filtered through a sterile membrane (pore size 0.45 μm).[7] The membranes were placed face upwards on a selective agar medium with cetrimide, and were incubated for 44±4h at 36°C. The culture results for the water samples were obtained from the bacteriology laboratory database and were available from 1st July 2004 to 15th February 2013. A water outlet was considered positive if either the cold or hot water contained *P. aeruginosa*.

Temperature and relative humidity

Data on mean outdoor temperatures and relative humidity by month were obtained from the French meteorological database (Météo France).

Building

Several wards moved during the study period, either within the same building or to a new building. This led to potential changes in the risk profiles of patients for acquiring *P. aeruginosa* in any building over time. To overcome this, data for the quarter that patients moved between buildings were erased from the database for the affected buildings, and for analysis purposes, the building was regarded as two different buildings, pre- and post-ward movement. This led to 12 buildings being considered in the analysis, instead of 10 actual buildings within the hospital complex.

Population at risk

The number of person-days (population at risk) was obtained from administrative databases, which record all hospitalizations for each ward.

Analyses

Data about water sample results were aggregated by building and by quarter in order to study the association between water results and cases. Generalized Estimating Equations (GEE) were used in order to take autocorrelation in time within each building into account, with binomial negative regression of the number of *P. aeruginosa* cases, with patient-days as the exposure variable. Exchangeable, first-order autoregressive and independent autocorrelation structures were tested. The autocorrelation structure was chosen with regard to the Quasi-likelihood Information Criterion (QIC),[8] the correlation coefficient between the different periods for the same building, and the graphical evolution of the incidence of *P. aeruginosa* cases in the different buildings. Robust variance estimators were used and allowed standard errors to overcome poor specification of the correlation structure.[9] The proportions of positive water samples among the number of samples in the building during the current, previous and next quarters were tested. A time trend was examined. The effect of quarter and mean outdoor temperature during the quarter and the previous quarter were tested in separate models to avoid collinearity. The QIC was used to choose the model among the models with the same correlation structure. Analyses were repeated without ICUs and haematology units, in which patients are more susceptible to *P. aeruginosa* infections. Finally, due to the small number of

buildings concerned, data about ICUs were grouped and a classical negative binomial regression with robust standard errors was performed to assess the association between positive water samples and *P. aeruginosa* cases in these units. The incidence of *P. aeruginosa* cases in the previous quarter was introduced in these models (lag 1). Data on haematology units could not be analysed separately because of the few water samples in these units.

No samples were taken during several quarters for several buildings (84/289). These missing data could be a source of bias and lead to inefficient analyses. Multiple imputations are recommended in this case. Imputations of the proportion of positive water samples were therefore performed using Imputation with Chained Equations (ICE).[10] The arc-sine of the square root of the proportion of positive water samples was used to approach normal distribution, and was back-transformed after multiple imputations. Stata Version 11 (StataCorp, College Station, TX, USA) was used (ice module) to obtain 50 imputed datasets.[11,12] In other terms, 50 datasets were created by replacing missing values with values predicted according to observed data. Analyses were repeated on each of the 50 databases and combined, taking the variability in results obtained between datasets into account.[10] Positive water samples in the current, previous and next quarters were imputed. Other variables were not missing. Bootstrapping was used to relax the assumption of multi-variate normality on the distribution of regression coefficients in multiple imputations.[13]

A threshold of $P=0.2$ was used in univariate analyses to select variables to include in the multi-variate analysis. $P<0.05$ was considered to be significant.

Results

Water samples

In total, 2932 water samples were studied for the period from July 2004 to February 2013. More samples were taken at the end of the study period because of the move of wards and because more water outlets were available in the new building. Samples were mainly taken from patients' rooms (1445 samples), drinking fountains (602 samples), and in nursing stations or disinfection rooms (388 samples) (Table A, see online supplementary material). Among these, 493 (16.8%, 95% confidence interval 15.5–18.2%) were positive for *P. aeruginosa*, 183 (6.2%) were sampled in disfavoured water outlets, and 128 (4.5%) were showers. Among the positive samples, 282 were contaminated with more than 100 colony-forming units (cfu) per 100 mL of *P. aeruginosa*, and the quantity *of P. aeruginosa* was not known in 11 cases. In the other positive samples, the median quantity of *P. aeruginosa* was 10 (interquartile range 3–30) cfu/100 mL. After the removal of quarters in which wards were moved, 2742 water samples remained.

The absence of water sampling in a building in a quarter was negatively associated with time ($P<0.001$), with the incidence of *P. aeruginosa* cases ($P=0.002$), and with the quarter globally ($P=0.046$, no significant difference for the second, third and fourth quarter compared with the first), but was not associated with other variables. In multi-variate analyses, missing water sampling data were still negatively associated with time ($P<0.001$), incidence of *P. aeruginosa* cases ($P=0.020$) and quarter ($P<0.001$).

The proportion of positive water samples was positively associated with the fourth quarter, the temperature in the previous quarter, the incidence of *P. aeruginosa* cases, and the proportion of positive samples in the previous and next quarters (Table B, see online supplementary material). These variables were thus used in the imputation model. Time and building were also used because of the structure of the data (longitudinal data). Temperature in the previous quarter was not used in the imputation model because of collinearity with quarter.

P. aeruginosa colonizations or infections

During the study period, 3405 isolates of *P. aeruginosa* were recovered from 2605 patients, equating to 10,532 patient-

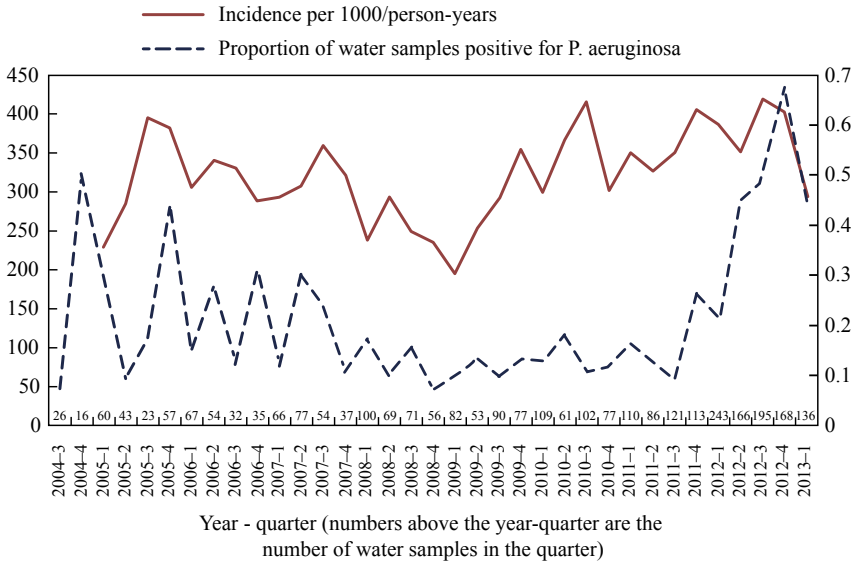

Figure 1. Evolution of the number of water samples, the proportion of water samples positive for *Pseudomonas aeruginosa* (black dashed line), and the incidence of *P. aeruginosa* healthcare-associated cases per 1000 patient-years (red line) between July 2004 and February 2013, by quarter.

years. The incidence rate was 323.3 per 1000 patient-years (95% confidence interval 312.4–334.2). The mean age of patients was 67.3 years (standard error 0.6 years) at the time of the first positive sample, and 22% were women. The median time to acquisition of the isolate was 15 days (interquartile range 7–34). The main sampling site was the lung [1246 isolates (37%)], followed by urine [916 (27%)] and superficial samples [437 (13%)]. Incidence by quarter is shown in Figure 1.

After the removal of quarters in which wards were moved, 3255 cases remained including 1185 cases in ICUs and haematology units (908 in ICUs and 277 in haematology units).

Association between the proportion of water samples positive for P. aeruginosa and P. aeruginosa cases in patients

The lowest QICs were obtained with the exchangeable autocorrelation structure in an empty model of the incidence of *P. aeruginosa* cases and in the final model (Table C, see online supplementary material). Moreover, correlation coefficients and graphical analysis of evolution of the incidence of *P. aeruginosa* cases by building showed that incidence rates of *P. aeruginosa* cases were correlated within buildings [Table D (see online supplementary material) and Figure 1]. Autoregressive or exchangeable working correlation structures seemed to be more appropriate. The exchangeable structure was thus used in the analyses.

Univariate complete case analysis showed a positive association between the incidence of *P. aeruginosa* cases and the proportion of positive water samples in the quarter in the building (Table E, see online supplementary material). Higher incidences of *P. aeruginosa* cases were associated with higher mean temperatures in the current and previous quarters, and with the second, third and fourth quarters with the first as a reference. The incidence of *P. aeruginosa* healthcare-associated cases was not significantly associated with other variables.

In multi-variate analysis, the incidence of *P. aeruginosa* cases was still positively associated with the proportion of positive water samples ($P=0.056$ in complete case analysis and $P=0.031$ with the imputed dataset) (Table I). Higher incidences of *P. aeruginosa* cases were still associated with higher mean temperatures in the current and the quarters, and with the second, third and fourth quarters with the first as a reference. The association was no longer observed when ICUs and hematology units were excluded, but was significant in analyses of data concerning ICUs alone ($P<0.001$) (Table F, see online supplementary material). In multi-variate models using mean temperature instead of quarter, the incidence of *P. aeruginosa* cases was still significantly associated with mean temperature in the current quarter, but not with temperature in the previous quarter (not shown). The model including the quarter had the best QIC and was thus kept.

Discussion

The incidence of healthcare-associated *P. aeruginosa* cases was positively associated with the proportion of positive water samples in the current quarter in both the total population and in subgroup analysis of ICUs. In the ICUs, filters were fitted principally on outlets used for bathing patients, but another water outlet could have been used for handwashing as recommended by the infection control unit. Moreover, the non-filtered outlet was sometimes used for washing patients for practical reasons. No association was observed in subgroup analysis excluding ICUs and haematology units. Lower power could be suspected to explain that the association was no longer observed, but this subgroup included nearly two-thirds of *P. aeruginosa* cases. This suggests that outside particular

Table I
Multi-variate analysis of the incidence of *Pseudomonas aeruginosa* healthcare-associated colonizations or infections

	Total population		ICUs and haematology units excluded	
	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
Complete case analysis				
Proportion of positive water samples				
- During the current quarter	0.338 (−0.009–0.686)	0.056	0.108 (−0.303–0.519)	0.606
- During the previous quarter	0.078 (−0.209–0.365)	0.595	0.088 (−0.192–0.367)	0.538
- During the next quarter	−0.193 (−0.404–0.017)	0.072	−0.097 (−0.317–0.123)	0.386
Quarter (reference: first quarter)				
- Second quarter	0.151 (0.031–0.272)	0.014	0.049 (−0.07–0.168)	0.423
- Third quarter	0.328 (0.209–0.448)	<0.001	0.338 (0.225–0.452)	<0.001
- Fourth quarter	0.251 (0.117–0.385)	<0.001	0.219 (0.016–0.423)	0.035
Constant	−15.251 (−15.733 to −14.768)	<0.001	−15.466 (−15.953 to −14.978)	<0.001
Imputed data set				
Proportion of positive water samples				
- During the current quarter	0.347 (0.032–0.662)	0.031	0.181 (−0.175–0.537)	0.318
- During the previous quarter	0.006 (−0.3–0.311)	0.970	−0.072 (−0.35–0.206)	0.611
- During the next quarter	−0.004 (−0.176–0.168)	0.967	0.074 (−0.18–0.329)	0.567
Quarter (reference: first quarter)				
- Second quarter	0.130 (0.044–0.215)	0.003	0.088 (−0.036–0.211)	0.163
- Third quarter	0.240 (0.12–0.359)	<0.001	0.254 (0.123–0.385)	<0.001
- Fourth quarter	0.201 (0.099–0.303)	<0.001	0.194 (0.064–0.324)	0.003
Constant	−15.362 (−15.822 to −14.902)	<0.001	−15.643 (−16.04 to −15.247)	<0.001

ICUs, intensive care units; CI, confidence interval.

wards (immunocompromised patients, patients with cystic fibrosis or in ICUs), the exposure of patients to *P. aeruginosa* in water is not a major risk factor of *P. aeruginosa* acquisition. It could be proposed to sample units dealing with susceptible patients, as advised in guidelines from the Department of Health and Irish guidelines.[14,15]

As the water outlet may have been contaminated for a long time before the positive result, the proportion of positive water samples in the next quarter was tested as a factor associated with *P. aeruginosa* cases. In the study facility, after a positive sample, the water outlet had filters fitted. Thus, exposure after a positive sample at a *P. aeruginosa* affected water outlet was assumed to be nil. However, the proportion of positive water samples in the previous quarter was still tested in the model to take long incubation periods into account. Despite the filtration strategy, in ICUs, positive water samples were associated with *P. aeruginosa* cases. This could be due to the exposure of patients to contaminated water points before installation of filters. Moreover, the random sampling procedure meant that water outlets near a positive water outlet were rarely sampled at the same time. These outlets may have been colonized but not had filters fitted due to the absence of water samples. The appropriateness of the study strategy for use of filters could therefore be questioned.

The incidence of *P. aeruginosa* cases was associated with a higher mean temperature in the current quarter, but not with mean relative humidity. The association between the incidence of Gram-negative bacteria and higher temperatures or summer has been observed in other studies.[16,17] Ramos *et al.* found an association between relative humidity and the incidence of *P. aeruginosa* cases.[18]

In this study, the water samples taken in an entire hospital for more than eight years were studied. There has been little previous analysis of such data, probably because few countries require water sampling in all areas of the hospital. However, this study had several limitations. Firstly, strong assumptions were made, including that the sampling strategy reflected the contamination of the water system accurately. Secondly, isolates were not typed to determine the similarity between water outlet and clinical isolates. Thirdly, *P. aeruginosa* can also be transmitted by cross-transmission,[19–21] which could have led to overestimation of the effect of the proportion of cases that were directly water-borne; for example, a water-borne transmission could have occurred and then been amplified by cross-transmission between patients. Nevertheless, any such secondary cases would still be an indirect consequence of water contamination. Endogenous infections with *P. aeruginosa* can also occur; these, and any secondary cases arising from such infections, could have caused background noise in this analysis. Finally, this study is subject to classical ecological fallacy.[22]

In conclusion, the results of this long-term large-scale study suggest that water outlet contamination in hospitals can lead to an increase in healthcare-associated *P. aeruginosa* cases in wards dealing with susceptible patients, even when point-of-use filters are applied to some outlets, but does not play a significant role in other wards. Moreover, no evidence was found to support the strategy of applying filters to individual outlets found to be positive for *P. aeruginosa*. The data suggest that regular monitoring of water outlets in high-risk wards may be justified, but that quarterly monitoring of water outlets in all clinical areas of hospitals, as is required by French recommendations, appears to be excessive.

Acknowledgements

The authors wish to thank Philip Bastable for his editorial assistance.

Conflict of interest statement
None declared.

Funding sources
None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jhin.2016.12.007.

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