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The role of water fittings in intensive care rooms as reservoirs for the colonization of patients with *Pseudomonas aeruginosa*

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Abstract *Objective:* To assess the role of the water environment in the *Pseudomonas aeruginosa* colonization of patients in intensive care units in the absence of a recognized outbreak. *Design and setting:* Prospective, single-centre study over an 8-week period in two adult ICUs at a university hospital. Environmental samples were taken from the water fittings of rooms once per week, during a 8-week period. Patients were screened weekly for *P. aeruginosa* carriage. Environmental and humans isolates were genotyped by using pulsed-field gel electrophoresis. *Results:* *P. aeruginosa* was detected in 193 (86.2%) of the 224 U-bend samples and 10 of the 224 samples taken

from the tap (4.5%). Seventeen of the 123 patients admitted were colonized with *P. aeruginosa*. Only one of the 14 patients we were able to evaluate was colonized by a clone present in the water environment of his room before the patient's first positive sample was obtained. *Conclusion:* The role of the water environment in the acquisition of *P. aeruginosa* by intensive care patients remains unclear, but water fittings seem to play a smaller role in non-epidemic situations than expected by many operational hospital hygiene teams.

Keywords Water fittings · *Pseudomonas aeruginosa* · Environment · Acquired infections · Intensive care

Introduction

In the past few decades, *Pseudomonas aeruginosa* has become a major hospital pathogen, due to both the number and severity of the infections it causes. A national prevalence survey indicated that *P. aeruginosa* was responsible for 10% of all nosocomial infections in France, only slightly fewer than *Escherichia coli* and *Staphylococcus aureus* [1]. In the hospital setting, intensive care units (ICUs) have a high endemic potential for this bacterium, which causes 18% of nosocomial infections in such units, versus only 6% in surgical and non-surgical units [2]. In many departments, *P. aeruginosa* is frequently involved in broncho-pulmonary infections and, to a lesser extent, urinary infections, infections of surgical sites, and bacteremia. The severity and excess mortality observed in cases of pulmonary infection and bacteremia are due to a combination of the intrinsic properties of the bacte-

ria – virulence factors and the natural resistance to antibiotics – and the immunocompromized status of the infected patients [3].

P. aeruginosa is an environmental bacterium. It exists as a saprophyte on damp soil, plant material, and in freshwater, wastewater, and seawater [4]. It is independent of humans but may be found as a commensal organism in the digestive tract. *P. aeruginosa* is rarely carried by subjects in good health, being found in only 2–10% of individuals, whereas it may be found in 50–60% of hospitalized patients, particularly on burns and scabs. The survival of *P. aeruginosa* seems to be particularly favored by damp environments, such as sinks, shower heads, and other water fittings [5, 6]. This enables this bacterium to contaminate medical and surgical equipment, hospital fittings, and other material [7]. Numerous hospital outbreaks have been blamed on the colonization of diverse items of equipment and/or damp materials [8, 9]. As a re-

sult, many hospital hygiene teams place great emphasis on the role of water in all infections with *P. aeruginosa*, particularly those occurring in ICUs in the absence of an outbreak.

The aim of this study was to evaluate the role of the water environment on the colonization of patients hospitalized in ICUs in the absence of a recognized epidemic.

Materials and methods

Background

This study was carried out in the two adult ICUs (one surgical and one medical) at Besançon University Hospital. Each of these two units has 15 beds, all of which are in individual rooms for the surgical ICU, with two double rooms for the medical ICU. This study was carried out (collection of data and strains) over 8 weeks, from 20 February to 10 April 2006. It was approved by the hospital's review board.

Design of the study

Environmental samples were taken weekly from the water fittings of the rooms, regardless of the *P. aeruginosa* infection status of the patients in the rooms. *P. aeruginosa* colonization status was monitored by testing samples taken for diagnostic purposes and samples taken weekly for screening purposes. We tested for isogenicity between clinical and environmental samples by determining macrorestriction profiles of total DNA, by pulsed-field gel electrophoresis (PFGE).

Clinical samples

The clinical samples tested were taken for diagnostic and epidemiological (screening) purposes. Diagnostic samples were not taken systematically: they were taken only if there were clinical reasons to suspect infection, and the sampling site was chosen according to the site of suspected infection. Screening samples were taken systematically on admission of the patient to the ICU and once per week thereafter, throughout the patient's stay. They were taken from the nose, the rectum, and from tracheal aspirates. Colonization was defined as a positive result for at least one sample.

Environmental samples

Two types of environmental samples were taken once per week from the water fittings in each intensive care room: 10 ml of water from the U-bend under the sink and 150 ml

of cold water taken directly from the tap immediately after activating. The various volumes of water sampled were collected into sterile flasks containing sodium thiosulphate to inhibit the effects of water chlorination.

Culture

Diagnostic samples were plated on agar Columbia broth supplemented with 5% horse blood. Screening samples were plated on agar Mueller–Hinton broth. Environmental samples were plated on cetrimide agar after filtration and 24 h enrichment in trypticase-soy (TS) broth. Cetrimide agar plates were incubated for 72 h at 41°C.

Identification

Cetrimide agar is selective for *P. aeruginosa*, but other bacterial species may nevertheless develop. Identification of *P. aeruginosa* was based on positive oxidase activity, resistance to kanamycin, growth at 41°C and not at 4°C, and confirmed by biochemical tests (ID 32 GN, Biomérieux, Marcy l'Étoile, France).

Genotyping

Isolates were typed by determination of their total DNA macrorestriction profile (pulsotype) following digestion with *DraI* (Boehringer, Mannheim, Germany) as assessed by PFGE (CHEF DRIII, Bio-Rad Ivry sur Seine, France), according to a technique previously developed by our laboratory [10]. We used Gel-Compar (Applied Math, Kortrijk, Belgium) to establish a similarity matrix for the DNA based on calculation of the Dice coefficient (pairwise comparison of strains). A dendrogram was generated with the UPGMA (unweighted pair group using arithmetic means) hierarchical algorithm. We compared gels using *S. aureus* NCTC 8325 as a reference strain. Typing results were interpreted according to international recommendations [11].

Definitions

Clones including clinical isolates were defined as sporadic if they were isolated from only a single patient, microepidemic if they were found in two or three patients, and epidemic if from more than three patients. Clones including environmental isolates were defined as unique if the isolates were found in a single room and multiple if the isolates were present in several rooms.

Statistical analysis

The data were analyzed with EpiInfo version 6.04 (Epi-Info, CDC, Atlanta, GA, USA). Confidence intervals were calculated by the quadratic method of Fleiss.

Results

Incidence of colonization/infection

In total, 123 patients were admitted to the two ICUs (69 to the medical unit and 54 to the surgical unit) for a total of 1416 days of hospitalization (720 in the medical unit and 696 in the surgical unit). Seventeen patients (8 in the medical unit and 9 in the surgical unit) presented at least one sample positive for *P. aeruginosa*. The overall incidence of colonization was 13.8 (range 8.5–21.5) per 100 patients admitted: 11.6 (range 5.5–22.1) for the medical unit and 16.66 (range 8.4–29.8) for the surgical unit. The overall incidence density was 12.0 (range 7.2–19.6) per 1000 days of hospitalization: 11.1 (range 5.9–22.7) for the medical unit and 12.93 (range 8.3–25.3) for the surgical unit. In total, 63 samples tested positive for *P. aeruginosa*: 46 screening samples from 16 patients and 17 diagnostic samples from 7 patients.

Positivity of the water environment

In total, 448 samples were taken from both ICUs. *P. aeruginosa* was detected in 193 (86.2%) of the 224 U-bend samples and 10 of the 224 samples taken from the tap (4.5%; Table 1). More than half the samples taken from U-bends contained more than one strain of *P. aeruginosa*, with some samples containing up to four strains. Permanent colonization of the U-bend was observed in five of the 28 rooms. Two of these rooms were in the surgical ICU and the other three were in the medical ICU.

Molecular typing

The clinical isolates belonged to 17 clones. Sixteen of these 17 clones were sporadic (isolated from a single patient), and one clone was microepidemic including isolates from two patients. We typed 203 environmental isolates in all. These 203 environmental isolates belonged to 82 pulsotypes – 37 present in the medical ICU, 33 in the surgical ICU and 12 in both units; 54 of these 82 pulsotypes were isolated only once – 29 in the medical unit and 25 in the surgical unit – and the other 28 pulsotypes were multiple. Eight of these strains were present in the medical unit, 8 in the surgical unit, and

Table 1 Positive results (and numbers of clones) for samples taken from U-bends

Room		Date	21 Feb	27 Feb	6 Mar	13 Mar	20 Mar	27 Mar	3 Apr	10 Apr
Medical intensive care unit	A1	1 ^a	2	2 ^a	3	2 ^a	0	1	3	
	A2	1	3	2	2	1	2	2	0	
	A3	1	1	1	2	2	2	2	2	
	A4	1	1	3	3	3	1	0	2	
	A5	1	1	2	3	3	1	1	2	
	B1	1	3	2	1	2	2	1	2	
	B2	1 ^a	2 ^a	2 ^a	2 ^a	2 ^a	1	3	0	
	B3	1	3	1	3	2	2	2	2	
	B4	1	1	3	0	4	2	2	0	
	B5	1	1	2	2	1	1	2	0	
	C1	1	1	4	2	2	0	3	2	
	C2-3	1	3	3	3	3	3	2	0	
	C4-5	1	2	4	3	2	0	1	2	
	Surgical intensive care unit	A1	1	2	1	3	2	0	1	0
A2		1	2	3	2	2	4	0	2	
A3		1	3	1	2	1	0	1	2	
A4		1	2	2	1	2	3	2	2	
A5		1	2	1	3	2	2	1	3	
A6		1	2	3	2	2	0	2	0	
A7		1	2	2	3	2	0	1	0	
B1		1	2	2	2	3	1	2	0	
B2		1	1	2	2	2	2	0	0	
B3		1	1 ^a	0	2	1	1	2	0	
B4		1	3	1	2	3	2	1	2	
C1		1	0	4	2	2	1	0	1	
C2		0	0	2	2	2	1	3	2	
C3		1	3	1	2	3	1	2	0	
C4	1	0	3	2	1 ^a	0	1	0		

^a One positive sample from taps

Table 2 Timing of the colonization/infection of patients with respect to the timing of positive results for environmental samples from the water fittings

Patient no.	Clone no. First positive environmental sample	First clinical sample	Second environmental sample
1	Abs ^a	43	61
2	9, 48	23	5
3	43	43	43
4	43	34	31, 43
5	66	42	– ^b
6	42	40	– ^b
7	Abs ^a	7	41, 104
8	71	37	42, 64
9	Abs ^a	2	40, 42, 76
10	29	18	20, 38
11	42, 90	36	42
12	2	13	2, 10, 38
13	10, 27	31	10, 27
14	43	22	– ^b
15	42, 101	65	42
16	2, 66	12, 103	67
17	31, 32, 74	35	– ^b

^a Absence of environmental sample

^b Negative environmental sample

12 were present in both units (Table 1). Only two multiple clones included both clinical and environmental isolates: one was isolated from 7 environmental samples and 1 patient, and the other was isolated from 8 environmental samples and 2 patients. The time course of colonization was investigated by considering the timing of the clinical and environmental samples taken for the 17 patients colonized with *P. aeruginosa* (Table 2). Only 1 patient was colonized with a clone present in the water environment of his room before he gave his first positive sample. In the 3 cases without environmental sample E1, the environment perhaps acted as a reservoir, too. The environment was therefore a possible reservoir for the colonization of patients in 1 of 14 cases. In half the cases, the water environment (U-bends and taps) of the room contained several clones of *P. aeruginosa*. For a given water fitting, in 9 cases of 10, the tap and the U-bend were colonized with identical clones.

Discussion

In this study, the incidence of colonization by *P. aeruginosa* was high, but molecular typing showed that there was no clonal outbreak. This high incidence was revealed by identification of simple colonization through testing screening samples. If diagnostic samples only had been used, the measured incidence would have been about half that reported. The water environment played only a minor role because only one of the 14 cases that could be evaluated was consistent with a colonization from the water sources tested (7.2% of cases).

Testing samples taken from water fittings in surgical and medical intensive care rooms in this study showed, nevertheless, that U-bends were frequently contaminated with *P. aeruginosa*, consistent with published findings [5, 6, 12, 13]. In half the cases, the water environment (U-bends and taps) of the room contained several clones of *P. aeruginosa*. This, together with the clonal diversity of the strains isolated from U-bends (differences between and within U-bends), provides evidence for colonization of the U-bend from the exterior rather than originating from the water supply. In addition, the presence of identical strains in the U-bends of sinks in both these physically independent units not shared by the same patients is suggestive of retro-colonization of the U-bend by the microflora present in wastewater pipes, via the biofilm, as previously proposed [14].

The role of the water environment in the *P. aeruginosa* colonization of patients was the key issue in this study. Many studies have attributed a major role to water fittings in the incidence of patient colonization with *P. aeruginosa* in ICUs [5, 12, 14]. Other studies have reported only a weak epidemiological link between environmental colonization and the occurrence of infections in patients [4, 15, 16]. All these previous studies were carried out during outbreaks. Some prospective studies have been published and they report frequencies of patient colonization by the environment of 14.2–50% [3, 6, 12–14, 17, 18]. In our study, in the absence of clonal epidemic, the frequency of colonization of patients via the water environment of their rooms seemed to be lower. Regardless of the epidemiological situation, which may differ between departments, measurement of the frequency with which

this event arises requires three methodological conditions which have never before been fulfilled:

1. The identification of all patients colonized by epidemiological sampling (screening) of patients. We found that the use of diagnostic samples alone would have identified only about half the colonized/infected patients.
2. The collection of samples from water fittings should be generalized and carried out regularly to make it possible to determine when the patients became infected or colonized. This is rarely the case in surveys motivated by the suspicion of an epidemic.
3. Strain comparisons should be based on a highly reproducible and discriminant typing method. The determination of the total DNA macrorestriction profile by PFGE can be considered to be the gold standard method for typing [10, 19].

The frequency of strains widely present in the environment (multiple clones) but never isolated from patients was high. Observations of this type led Valles et al. to suggest that there may be two different genetic groups in

this species: one group of strains that are mostly environmental and not very pathogenic in humans; and one group of strains better adapted to humans with a much higher pathogenic potential [13]. This view is supported by virulence surveys, analyses of bacterial populations by multi-locus sequence typing, and the application of several typing methods to strains of two different origins (clinical and environmental) [4, 20, 21].

Conclusion

In conclusion, although water fittings clearly play a role in the acquisition of *P. aeruginosa* by patients hospitalized in ICUs, the contribution of this phenomenon in non-epidemic situations appears to be much smaller than generally believed by many operational hospital infection control teams.

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References

1. Enquête nationale de prévalence des infections nosocomiales 2006. (2007) Résultats préliminaires au 12/01/2007. Institut de veille sanitaire (InVS). available at <http://www.invs.sante.fr>
2. Bertrand X, Thouverez M, Patry C, Balvay P, Talon D (2001) *Pseudomonas aeruginosa*: antibiotic susceptibility and genotypic characterization of strains isolated in the intensive care unit. Clin Microbiol Infect 7:706–708
3. Berthelot P, Grattard F, Mahul P, Pain P, Jospe R, Venet C, Carricajo A, Aubert G, Ros A, Dumont A, Lucht F, Zeni F, Auboyer C, Bertrand JC, Pozzetto B (2001) Prospective study of nosocomial colonization and infection due to *Pseudomonas aeruginosa* in mechanically ventilated patients. Intensive Care Med 27:503–512
4. Ruiz L, Dominguez MA, Ruiz N, Vinas M (2004) Relationship between clinical and environmental isolates of *Pseudomonas aeruginosa* in a hospital setting. Arch Med Res 35:251–257
5. Lasheras A, Guisset O, Boulestreau H, Rogues AM, Fiore M, Szajner S, Bezian MC, Gabinski C, Gachie JP (2006) Reservoirs and transmission of *Pseudomonas aeruginosa* in intensive care unit. Med Mal Infect 36:99–104
6. Blanc DS, Nahimana I, Petignat C, Wenger A, Bille J, Francioli P (2004) Faucets as a reservoir of endemic *Pseudomonas aeruginosa* colonization/infections in intensive care units. Intensive Care Med 30:1964–1968
7. Schelenz S, French G (2000) An outbreak of multidrug-resistant *Pseudomonas aeruginosa* infection associated with contamination of bronchoscopes and an endoscope washer-disinfector. J Hosp Infect 46:23–30
8. Cobben NA, Drent M, Jonkers M, Wouters EF, Vaneechoutte M, Stobberingh EE (1996) Outbreak of severe *Pseudomonas aeruginosa* respiratory infections due to contaminated nebulizers. J Hosp Infect 33:63–70
9. Bert F, Maubec E, Bruneau B, Berry P, Lambert-Zechovsky N (1998) Multi-resistant *Pseudomonas aeruginosa* outbreak associated with contaminated tap water in a neurosurgery intensive care unit. J Hosp Infect 39:53–62
10. Talon D, Cailleaux V, Thouverez M, Michel-Briand Y (1996) Discriminatory power and usefulness of pulsed-field gel electrophoresis in epidemiological studies of *Pseudomonas aeruginosa*. J Hosp Infect 32:135–145
11. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239
12. Trautmann M, Michalsky T, Wiedeck H, Radosavljevic V, Ruhnke M (2001) 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 22:49–52
13. Valles J, Mariscal D, Cortes P, Coll P, Villagra A, Diaz E, Artigas A, Rello J (2004) Patterns of colonization by *Pseudomonas aeruginosa* in intubated patients: a 3-year prospective study of 1,607 isolates using pulsed-field gel electrophoresis with implications for prevention of ventilator-associated pneumonia. Intensive Care Med 30:1768–1775
14. Trautmann M, Lepper PM, Haller M (2005) Ecology of *Pseudomonas aeruginosa* in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. Am J Infect Control 33:S41–S49

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15. Talon D, Mulin B, Rouget C, Bailly P, Thouverez M, Viel JF (1998) Risks and routes for ventilator-associated pneumonia with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 157:978–984
 16. Bonten MJ, Bergmans DC, Speijer H, Stobberingh EE (1999) Characteristics of polyclonal endemicity of *Pseudomonas aeruginosa* colonization in intensive care units. Implications for infection control. *Am J Respir Crit Care Med* 160:1212–1219
 17. Reuter S, Sigge A, Wiedeck H, Trautmann M (2002) Analysis of transmission pathways of *Pseudomonas aeruginosa* between patients and tap water outlets. *Crit Care Med* 30:2222–2228
 18. Ferroni A, Nguyen L, Pron B, Quesne G, Brusset MC, Berche P (1998) Outbreak of nosocomial urinary tract infections due to *Pseudomonas aeruginosa* in a paediatric surgical unit associated with tap-water contamination. *J Hosp Infect* 39:301–307
 19. Speijer H, Savelkoul PH, Bonten MJ, Stobberingh EE, Tjhih JH (1999) Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit. *J Clin Microbiol* 37:3654–3661
 20. Fenner L, Richet H, Raoult D, Pappazian L, Martin C, La Scola B (2006) Are clinical isolates of *Pseudomonas aeruginosa* more virulent than hospital environmental isolates in amebal co-culture test? *Crit Care Med* 34:823–828
 21. Vernez I, Hauser P, Bernasconi MV, Blanc DS (2005) Population genetic analysis of *Pseudomonas aeruginosa* using multilocus sequence typing. *FEMS Immunol Med Microbiol* 43:29–35