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Molecular Epidemiology of *Pseudomonas aeruginosa* in Intensive Care Units (ICUs) over a 10-Year Period (1998-2007)

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Abstract

Pseudomonas aeruginosa is one of the leading nosocomial pathogens in ICUs. The source of this microorganism can be either endogenous or exogenous. The proportion of cases due to transmission is still debated, and its elucidation is important for implementing appropriate control measures. In order to understand the relative importance of exogenous *versus* endogenous sources of *P. aeruginosa*, molecular typing was performed on all available *P. aeruginosa* isolated from ICU clinical and environmental specimens in 1998, 2000, 2003, 2004 and 2007. Patient samples were classified according to their *P. aeruginosa* genotypes in 3 categories: A) identical to faucet, B) identical to at least one other patient sample and not found in faucet, and C) unique genotype. Cases in categories A and B were considered as possibly exogenous, and cases in category C as possibly endogenous.

A mean number of 34 cases /1000 admissions per year were found to be colonized or infected by *P. aeruginosa*. Higher levels of faucet contamination were correlated with a higher number of cases in category A. The number of cases in category B varied from 1.9 to 20 cases/1000 admissions. This number exceeded 10/1000 admissions on 3 occasions and was correlated with an outbreak in one occasion. Number of cases considered as endogenous (category C) was stable and independent from the number of cases in categories A and B.

Repeated molecular typing over time allowed to document variations in the epidemiology of *P. aeruginosa* in ICU patients susceptible to require continuous adaptation of infection control measures.

Introduction

Pseudomonas aeruginosa is a ubiquitous environmental bacterium with minimal requirements for survival and a remarkable ability to adapt to a variety of environments. Although humans are frequently in contact with *P. aeruginosa*, the species only colonizes the normal human host intermittently. In contrast, it was found to be part of the intestinal flora of 2,6 to 24,0 % of hospitalized patients [1]. The importance of *P. aeruginosa* as an opportunistic pathogen stems from its ability to activate well-adapted phenotypes under environmental stress and to persist in adverse conditions such as in the presence of antibiotic or antiseptic substances. *P. aeruginosa* infections occur mostly in immunocompromised hosts and intensive care units (ICUs) patients. In one study, *P. aeruginosa* was responsible for 15.6 % of all nosocomial pneumonia cases in medical-surgical ICUs in the USA [2]. It was also found to be the most frequent Gram-negative bacterium recovered from patients with nosocomial pneumonia during ICU surveillance over the last two decades [3]. Furthermore, the overall rate of *P. aeruginosa* infections increased rapidly in some patients populations, even if the resistance profiles of *P. aeruginosa* from nosocomial infections remained stable [4].

As *P. aeruginosa* is ubiquitous in the environment and is also part of the endogenous flora of hospitalized patients, only studies using powerful molecular typing methods can explore the routes of colonization and/or infection. The identification of the source is only possible through comprehensive screening of the environment and the characterisation of the endogenous flora of patients, which requires significant resources. Molecular typing makes it possible to track the dissemination of specific strains and may facilitate the breaking down of endemic transmission to the level of micro-epidemics [5].

The question we wished to address is whether *P. aeruginosa* infections in ICUs patients are mainly due to endogenous or exogenous sources. We previously found that 42 % of ICU patients with *P. aeruginosa* harboured isolates identical to those found in the faucets [6].

Following control measures, the number of these patients decreased significantly [7]. These results showed that the epidemiology of *P. aeruginosa* is likely to vary over time. On the one hand, several recent studies using molecular typing in a non-epidemic ICU setting suggested that the major reservoir of *P. aeruginosa* was the endogenous flora of the patients [8,9,10,11]. At the same time, other investigators emphasized the role of the environment as a source of colonization and infection in ICUs [12,13]. It is still unclear whether the environment is mainly a passive site where strains are deposited, or whether it plays an active role in patient colonization [14]. In addition, the importance of other patients as exogenous sources should also be taken into consideration, even in periods free of recognized outbreak.

The aim of our study was to investigate over a 10-year period the respective roles of exogenous (faucets or other patients) versus endogenous flora as sources of colonization or infection by *P. aeruginosa* in ICU's patients, and to document epidemiological variations over time.

Materials and Methods

Setting. The University Hospital of Lausanne is a 850-bed tertiary care hospital with a 32-bed adult (medical and surgical) ICUs and a 9-bed pediatric ICU. The ICUs have two distinct water distribution networks: i) medical ICUs (network I) and ii) surgical and pediatric ICUs (network II). Between 2006 and 2007, the medical ICUs were rebuilt and, consequently, all faucets and the water network were changed.

Study population. Patients were identified through the laboratory database. They were included in the study if they were hospitalized in one of the ICUs and had at least one culture of a clinical specimen positive for *P. aeruginosa*. No routine screening of *P. aeruginosa* carriage was performed. No clinical information was collected in order to distinguish between colonization and infection by *P. aeruginosa*. Epidemiological data (unit and room of

hospitalization, dates of admission and discharge) were retrieved from the hospital information system.

Study period. The study covered a period of ten years, from 1998 through 2007. Molecular and epidemiological analyses were conducted in 1998, 2000, 2003, 2004 and 2007.

Environment sampling. From 1998 to 2007, 18-56 ICU's faucets were swabbed once to three times a year (most usually twice). To identify the presence of *P. aeruginosa*, swabs were plated on cetrimide agar. All isolates from faucets were saved for molecular typing.

Molecular typing was performed in 1998, 2000, 2003 and 2004 on clinical and environmental isolates using pulsed-field-gel electrophoresis (PFGE), as described previously [6,7,15]. For the year 2007 samples, we used the recently developed double digest selective label (DDSL) typing technique, which is similar to PFGE (DNA restriction with the same rare cutting enzyme *Spe*I), but based on a much faster and easier simultaneous double digestion/labeling reaction procedure [16]. Indistinguishable isolates (identical bands) and closely related isolates (1-6 band differences) were considered to be of the same genotype.

Epidemiological definitions and classification of cases. A case was defined as the isolation of a given genotype of *P. aeruginosa* from a clinical specimen obtained during a patient's stay in an ICU. Thus, recovery of isolates having two different genotypes in a single patient yielded two distinct cases. Cases were classified into four categories (A-D) according to molecular typing data. Category A included cases with a *P. aeruginosa* genotype identical to that of an isolate recovered from a faucet during the same year or previously. Category B comprised cases where the genotype was identical to that of an isolate recovered from at least one other patient, but absent in isolates from the faucets. Cases from categories A and B were considered of possible exogenous origin. Category C comprised cases with a unique genotype; these cases were considered of possible endogenous origin. Finally, category D comprised cases for which isolates were not available for typing.

Cases which shared the same PFGE/DDSL genotype as isolates from faucets or other patients were defined as belonging to the same "genetic" cluster. Within a genetic cluster, if epidemiological links (see definition thereafter) were found between cases, they were considered as members of an "epidemiological" cluster. Epidemiological links were considered if i) a patient was hospitalized in the same ICU where a faucet was found to be contaminated with the same genotype during the same year, or ii) patients with identical genotypes were hospitalized during overlapping periods in the same ICU.

Results

Study population. During the 10-year period of the study, the number of admissions in ICUs remained stable (c.a. 2500 admissions per year, Table 1). A mean number of 86 ICU patients per year (34 per 1000 admissions) were observed to be colonized or infected by *P. aeruginosa*. The highest incidences were observed in 1998 and 1999 (57.1 and 42.2/1000 admissions, respectively), and then decreased below 40/1000 admissions. These incidences were not correlated with the number of clinical specimens received by the laboratory of bacteriology (Table 1; Pearson's test of correlation, p=0.1995), excluding a sampling bias.

Environmental contamination. To measure the colonization rate with *P.aeruginosa* over the 10 years, only faucets that were regularly swabbed during the 10 years of the study (12 in the medical ICUs, and 17 in the surgical and pediatric ICUs) were included (Table 1). Results showed a difference of contamination over the years between the two water networks. Whereas <10% of the faucets were contaminated in the surgical and pediatric ICUs, the level of contamination was high in the medical network from 1998 to 2002, but dropped below 10% between 2003 and 2005 (no sampling was performed during renovation in 2006 and 2007; and an investigation done in 2008 revealed no contamination of the new faucets).

Faucets as a major environmental exogenous source of contamination. During the first year of investigation (1998), a high number of patients were found to be colonized with a *P. aeruginosa* strain identical to a strain found in the faucets (category A, Table 1, Figure). The higher contamination rates of faucets in the medical ICU (25%), compared to the surgical-pediatric ICUs (6.4%), was correlated with a higher number of cases of category A in this ICU (32.6 cases/1000 admissions), compared to the other ICUs (16.8 cases/1000 admissions) (Table 1, Figure). This correlation was also observed in 2000. The number of cases in category A in the medical unit only became similar to that of the surgical and pediatric ICUs in 2003, when the percentage of contaminated faucet dropped below 10%.

Other patients as exogenous source of contamination. Transmission was suspected when a patient shared a *P. aeruginosa* genotype with at least one other patient. The number of cases of this category varied over the 10 years of surveillance from 1,9 to 20 cases per 1000 admissions (category B, Table 1). This number exceeded 10 per 1000 admissions on 3 occasions: i) in both units in 1998, ii) in the surgical unit in 2003-4, and iii) in the same unit in 2007. In 1998, two large clusters of patients carrying the same *P aeruginosa* genotype (11 and 6 patients, respectively; Table 2) were identified, and one outbreak was recognized (epidemiological links between 6 patients in one cluster). Introduction of alcohol-based hand rub seemed to have played a role for the reduction of cases in this category [7]. In 2003, two large clusters of patients carrying the same *P aeruginosa* genotype (13 and 8 patients, respectively) were observed in the surgical unit, reflecting one outbreak due to the emergence and spread of two multi-resistant *P. aeruginosa* strains (susceptible to $\leq$1 of the 4 antibiotic classes used for therapy). This outbreak lasted until 2004. In addition, the present investigation revealed that several patients harbored non multi-resistant strains that were genetically identical to the epidemic multi-resistant strains. During the outbreak of 2003,

environmental sources of contamination (transoesophageal probe, siphons) were identified that may explain the high level of cases.

Endogenous source of contamination. Over the 10 years of the study, the number of cases with a unique *P. aeruginosa* genotype (category C) remained stable and independent from the variations in the number of cases in categories A and B (Table 1 and figure).

Concordance between molecular typing and epidemiological data. Within category A, 22 genetic clusters including 1 to 30 cases were observed. An epidemiological link could not be found for 2 cases in 2 genetic clusters (one patient in each of the 2 genetic clusters was hospitalized in another ICU than the one in the unit with a positive faucet) (Table 2). In 2007, we found 4 patients sharing the same genotype as one found in faucets during previous years, but not recovered from incriminated faucets in 2007. Within category B, 40 genetic clusters comprised of 2 to 13 cases were observed. Epidemiological links were found between all cases in only 8 of these clusters, between some of the cases for 6 other clusters, but not in the remaining 26 clusters (Table 2). During the years 2004 and 2007, when no outbreak was recognized, we observed many small genetic clusters, however without any concordance with epidemiological data (Table 2).

Discussion

Over the 10-year period under study, we observed several variations in the epidemiology of *P. aeruginosa* in our ICU setting. If endogenous sources appeared to remains stable over time, exogenous sources varied depending on environmental contamination and outbreak occurrence.

The relative importance of faucets as an environmental source of *P. aeruginosa* was high in 1998 but then decreased as a result of additional infection control measures. Taps contaminated with *P. aeruginosa* had already been shown to serve as a continuous source for

transmission [12,17]. However, our study is the first to investigate this contamination over a long period of time. A possible explanation for the observed decrease of contamination is a late effect of the increase in temperature of the water network from 50 to 65°C, a change that occurred in March 2000 [18]. Some experts recommend the use of microfilters in each faucet of the ICU (end-line filtration) [17,19]. Based on our surveillance data showing that cases linked to contaminated faucets have been very rare since 2003, we did not introduce such filters.

Although a contaminated faucet can be the source of a cluster of *P. aeruginosa* colonization or infection, alternative hypotheses should be taken into consideration to explain the cases in category A that could not be distinguished in our study. Firstly, the patient him- or herself may be the source of the contamination of a faucet. We have previously shown that such events are probably infrequent [6]. Secondly, a mixed scenario can also occur when a patient contaminated from a faucet becomes the source of patient-to-patient transmission.

In our study, patient-to-patient transmission was mainly measured as the number of patients sharing identical *P. aeruginosa* genotypes. To understand this epidemiology, we should not only consider the number of cases, but also the size and the number of genetic clusters. Small clusters probably reflect sporadic patient-to-patient transmissions, whereas larger clusters suggest the contribution of a persisting source, most probably from the environment. A large outbreak due to the emergence and spread of multi-resistant *P. aeruginosa* was observed in 2003 and 2004. Molecular investigation enabled us to understand this outbreak, which involved two strains and multiple exogenous sources of contamination (transoesophageal probe, siphons, and other patients). The duration of the outbreak was probably due to the persistence of the epidemic strains in the environment, and to the lack of additional contact measures of ICU patients harboring the epidemic strains that did not show a multi-resistant phenotype. This episode suggests that environmental sources other than faucets should also be

suspected when a *P. aeruginosa* outbreak involves several patients. This is in accordance with reports of *P. aeruginosa* outbreaks in the literature always showing the implication of the environment [20,21,22,23,24].

Molecular typing results of patients' isolates should disclose unsuspected clusters. Indeed, the axiom of molecular epidemiology is that when two isolates are genetically identical, they should originate from the same chain of transmission [25]. However, even if the population diversity of *P. aeruginosa* is very high, presence of predominant genotypes in patients without epidemiological links should also be taken into consideration. Therefore, to investigate transmission, it is important to analyze the concordance of molecular results with epidemiological data. A low concordance suggests that either the epidemiological link was missing, or that a predominant *P. aeruginosa* genotype was found in epidemiologically unrelated patients. Our definition of epidemiological link relies mainly on the existence of overlapping periods of hospitalization in the same ICU or hospitalizations in the same ICU where a faucet was contaminated with the same genotype. In 2003, the outbreak involving two multi-resistant strains was not restricted to the ICUs (operating theatre, other wards), which strongly suggests exogenous origin of contamination for all patients harboring these genotypes. Similarly, we might have been able to find epidemiological links during the other years, if deeper investigations had been conducted.

In conclusion, the epidemiology of *P. aeruginosa* in the ICU setting shows that the contribution of endogenous versus exogenous reservoirs to the colonization and infection of patients varies over time. The major factors contributing to such variations are probably the contamination of the environment, the compliance of health care workers with infection control measures, and the genetics of the pathogen itself. As *P. aeruginosa* is ubiquitous in humid environments and colonizes up to 15% of hospitalized patients, eradication of the reservoir may prove quite difficult. We have demonstrated that, in our setting, this eradication

was possible in faucets by increasing the temperature of hot water. In addition, when a multi-resistant strain is repeatedly recovered from patients and from the environment, efforts should be undertaken to achieve eradication of this strain from the environment. However, patient-to-patient transmission was also found to be an important and recurrent cause of exogenous origin. Therefore, additional efforts should be deployed to reinforce standard precautions and specific infection control measures and thus limit the transmission of *P. aeruginosa*.

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Transparency declaration

Potential conflict of interest. All authors : none

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Figure legend

Figure : Evolution of the annual no. of cases per 1,000 admissions and per category, and of the percentage of faucets contaminated with *P. aeruginosa* in the medical ICU (A), and in the paediatric-surgical ICUs (B)(category A : identical to faucet ; category B : identical to at least one other patient ; category C : unique genotype ; category D : not typed).

Table 1 . Epidemiological data of patients with *Pseudomonas aeruginosa* in the Intensive Care Units over a 10-year period

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
No. of admissions in ICUs	2434	2538	2449	2586	2427	2400	2554	2530	2567	2675
No. of patients with *P. aeruginosa*	139	107	75	52	87	92	82	64	75	83
No. of patients with *P. aeruginosa*/1,000 admissions	57,1	42,2	30,6	20,1	35,8	38,3	32,1	24,9	29,2	31,0
No. of clinical specimens[#]/1000 patient-days of hospitalization	NA	NA	668	792	786	817	815	727	757	815
Percent of faucets[$] contaminated with *P. aeruginosa*										
in the medical ICUs (water network I)	25	53,1	38,1	13,3	22,7	8,3	8,7	4,2	ND	ND
in surgical and pediatric ICUs (water network II)	6,4	0	0	4,3	3,1	4,3	2,9	0	0	0
Categories of cases* (no. of cases/1,000 admissions)										
in the medical ICU:										
Category A	32,6	ND	8.5	ND	ND	1,0	1,9	ND	ND	0
Category B	20	ND	7,4	ND	ND	1,9	5,6	ND	ND	8,3
Category C	10,5	ND	8.5	ND	ND	18,4	16,7	ND	ND	16,5
Category D	4,2	ND	1,1	ND	ND	13,6	5,6	ND	ND	0,9
Total	67,3	55,4	25,5	16,2	26,1	34,9	29,8	21,1	22,3	25,7
in the surgical and pediatric ICUs:										
Category A	16,8	ND	4,0	ND	ND	0	1,4	ND	ND	0
Category B	13,5	ND	6,7	ND	ND	18,3	10,2	ND	ND	12,6
Category C	18,2	ND	18.0	ND	ND	16,8	16,9	ND	ND	20,2
Category D	4,0	ND	2,0	ND	ND	10,2	6,1	ND	ND	2,5
Total	52,5	36,5	30,7	23,1	46,7	45,3	34,6	27,5	35,5	35,3

NA, not available

ND, not done (see text)

[#], Clinical specimens received by the laboratory of bacteriology.

[*], Category A : cases with a *P.aeruginosa* genotype identical to one found in faucets during the same year or previously; category B : identical to at least one other patient; category C : unique genotype ; category D : not typed.

[$], the percentage of contaminated faucets was based on data from faucets that were regularly swabbed over the 10 years.

Table 2. Analysis of genetic and epidemiological clusters in cases of category A (patients with a *P. aeruginosa* genotype identical to that found in faucets) and category B (patients with a *P.aeruginosa* genotype identical to at least one other patient and not found in faucets).

	1998	2000	2003	2004	2007
No. of cases in category A	**57**	**17**	**1**	**4**	**0**
No. of genetic clusters	10	7	1	4	0
No. of patients in genetic clusters	30, 6*, 5, 4, 4, 3, 2, 1, 1, 1	8, 4, 1, 1, 1,1,1	1*	1, 1, 1, 1	0
No. of cases in category B	**39**	**17**	**27**	**21**	**29**
No. of genetic clusters	11	4	5	8	12
No. of patients in genetic clusters (no. of patients in epidemiologic clusters)	11 (6, 2)	8 (3)	13 (9,2)	5 (3)	3 (3)
	6 (0)	4 (0)	8 (3,3,2)	3 (0)	3 (0)
	3 (3)	3 (2)	2 (0)	3 (0)	2 (2)
	3 (2)	2 (0)	2 (0)	2 (2)	2 (2)
	3 (0)		2 (0)	2 (0)	2 (0)
	3 (0)			2 (0)	2 (0)
	2 (2)			2 (0)	2 (0)
	2 (2)			2 (0)	2 (0)
	2 (0)				2 (0)
	2 (0)				2 (0)
	2 (0)				2 (0)
					2(0)

* one patient harbored a genotype identical to that found in faucet, but was hospitalized in a different ICU, no probable transmission was thus considered.