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

THESE

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par

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Rapport de synthèse

Pseudomonas aeruginosa, une bactérie environnementale ubiquitaire, est un des pathogènes nosocomiaux les plus fréquents aux soins intensifs. La source de ce microorganisme peut être soit endogène, 2,6 à 24 % des patients hospitalisés étant colonisés au niveau digestif, soit exogène. La proportion des cas d'infections à *P. aeruginosa* d'origine exogène, donc secondaires à une transmission par manuportage ou par l'eau du réseau utilisée pour la toilette ou d'autres soins, reste débattue. Or une meilleure évaluation du taux d'infections exogènes est importante pour la mise en place de mesures de contrôle appropriées.

Le but de cette étude était de déterminer sur une période de 10 ans les rôles respectifs des sources exogènes (robinets, autres patients) et endogène dans la colonisation et/ou l'infection par *P.aeruginosa* chez les patients des Soins Intensifs, ainsi que de documenter les variations épidémiologiques au cours du temps.

L'étude a été menée dans les unités de Soins Intensifs du Centre Hospitalier Universitaire Vaudois (CHUV). Les patients colonisés et/ou infectés par *P. aeruginosa* entre 1998 et 2007ont été identifiés via la base de données du laboratoire de microbiologie. Ils ont été inclus dans l'étude s'ils étaient hospitalisés dans une des unités de Soins Intensifs, Durant cette période, des prélèvements pour recherche de *P. aeruginosa* ont été effectués sur des robinets des soins intensifs. Un typage moléculaire a été effectué sur toutes les souches cliniques et environnementales isolées en 1998, 2000, 2003, 2004 et 2007.

Les patients inclus dans l'étude ont été répartis en quatre catégories (A-D) selon le résultat du typage moléculaire leur souche de *P. aeruginosa*. La catégorie A inclut les cas pour lesquels le

génotype de *P. aeruginosa* est identique à un des génotypes retrouvé dans l'environnement. La catégorie B comprend les cas pour lesquels le génotype est identique à celui d'au moins un autre patient. La catégorie C comprend les cas avec un génotype unique et la catégorie D comprend les cas pour lesquels la souche était non disponible pour le typage. Les cas des catégories A et B sont considérés comme ayant une origine exogène.

Au cours des années de l'étude, le nombre d'admissions aux soins intensifs est resté stable. En moyenne, 86 patients par année ont été identifiés colonisés ou infectés par *P. aeruginosa* aux Soins Intensifs. Durant la première année d'investigation, un grand nombre de patients colonisés par une souche de *P. aeruginosa* identique à une de celles retrouvées dans l'environnement a été mis en évidence. Par la suite, possiblement suite à l'augmentation de la température du réseau d'eau chaude, le nombre de cas dans la catégorie A a diminué. Dans la catégorie B, le nombre de cas varie de 1,9 à 20 cas/1000 admissions selon les années. Ce nombre est supérieur à 10 cas/1000 admissions en 1998, 2003 et 2007 et correspond à des situations épidémiques transitoires. Tout au long des 10 ans de l'étude, le nombre de cas dans la catégorie C (source endogène) est demeuré stable et indépendant des variations du nombre de cas dans les catégories A et B.

En conclusion, la contribution relative des réservoirs endogène et exogène dans la colonisation et/ou l'infection des patients de soins Intensifs varie au cours du temps. Les facteurs principaux qui contribuent à de telles variations sont probablement le degré de contamination de l'environnement, la compliance des soignants aux mesures de contrôle des infections et la génétique du pathogène lui-même. Etant donné que ce germe est ubiquitaire dans l'environnement aqueux et colonise jusqu'à 15% des patients hospitalisés, la disparition de son réservoir endogène semble difficile. Cependant, cette étude démontre que son contrôle est possible dans l'environnement, notamment dans les robinets en augmentant la température de l'eau. De plus, si une souche multi-résistante est retrouvée de manière répétée dans l'environnement, des efforts doivent être mis en place pour éliminer cette souche. Des efforts doivent être également entrepris afin de limiter la transmission entre les patients, qui est une cause importante et récurrente de contamination exogène.

Molecular epidemiology of *Pseudomonas aeruginosa* in intensive care units over a 10-year period (1998–2007)

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Abstract

Pseudomonas aeruginosa is one of the leading nosocomial pathogens in intensive care units (ICUs). The source of this microorganism can be either endogenous or exogenous. The proportion of cases as a result of transmission is still debated, and its elucidation is important for implementing appropriate control measures. To understand the relative importance of exogenous vs. 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 into three categories: (A) identical to isolate from 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 of 34 cases per 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 per 1000 admissions. This number exceeded 10/1000 admissions on three occasions and was correlated with an outbreak on one occasion. The number of cases considered as endogenous (category C) was stable and independent of the number of cases in categories A and B. The present study shows that repeated molecular typing can help identify variations in the epidemiology of *P. aeruginosa* in ICU patients and guide infection control measures.

Keywords: Environment, epidemiological tracking, faucets, intensive care, molecular typing, *Pseudomonas aeruginosa*, water
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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. By contrast, it has been found to be part of the intestinal flora of 2.6–24.0% 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 the presence of antibiotic or antiseptic substances. *P. aeruginosa* infections occur mostly in immunocompromised hosts and in intensive care unit (ICU) patients. In one US study, *P. aeruginosa* was responsible for 15.6% of all nosocomial pneumonia cases in medical-surgical ICUs [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 nosocomial *P. aeruginosa* infections has been found to increase rapidly in some patient populations, even when the resistance profiles of *P. aeruginosa* isolates remained stable [4].

Because *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, which is only possible through comprehensive screening of the environment and characterization of the endogenous flora of patients, requires significant resources. Molecular typing makes it possible to track the dissemination of specific strains and may facilitate the analysis of endemic transmission to the level of micro-epidemics [5].

The present study aimed to investigate whether *P. aeruginosa* infections in ICU patients are mainly the result of endogenous or exogenous sources. We previously found that 42% of ICU patients with *P. aeruginosa* harboured isolates identical to those found in faucets [6]. The number of these patients decreased significantly subsequent to the introduction of control measures [7]. These results showed that the epidemiology of *P. aeruginosa* is likely to vary over time. Several recent studies using molecular typing in a non-epidemic ICU setting have suggested that the major reservoir of *P. aeruginosa* was the endogenous flora of the patients [8–11], whereas other studies have emphasized the role of the environment as a source [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 patients as exogenous sources outside of outbreaks also needs to be elucidated.

The present study investigates, over a 10-year period, the respective roles of exogenous (faucets or other patients) vs. endogenous flora as sources of colonization or infection by *P. aeruginosa* in ICU patients, and documents epidemiological variations over time.

Materials and Methods

Setting

The University Hospital of Lausanne is an 850-bed tertiary care hospital with a 32-bed adult (medical and surgical) ICU and a nine-bed paediatric ICU. The ICUs have two distinct water distribution networks: (i) medical ICUs (network I) and (ii) surgical and paediatric ICUs (network II). Between 2006 and 2007, the medical ICUs were rebuilt and 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 clinical specimen positive for *P. aeruginosa*. No routine screening of *P. aeruginosa* carriage was performed. No agttempt was made 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 10 years, from 1998 to 2007. Molecular and epidemiological analyses were conducted in 1998, 2000, 2003, 2004 and 2007.

Environment sampling

From 1998 to 2007, 18–56 ICU faucets were swabbed once to three times a year (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 by using pulsed-field-gel electrophoresis (PFGE), as described previously [6,7,15]. For samples from 2007, 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 uses a faster and easier simultaneous double digestion/labelling reaction procedure [16]. Indistinguishable isolates (identical bands) and closely-related isolates (one to six band differences) were considered to belong to 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, the 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 comprised cases with a *P. aeruginosa* genotype identical to that of an isolate recovered from a faucet during the same year or during previous years.

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 to be of possible exogenous origin. Category C comprised cases with a unique genotype; these cases were considered to be of possible endogenous origin. Finally, category D comprised cases for which isolates were not available for typing.

Cases that 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 were found between cases, they were considered as members of an 'epidemiological' cluster. Epidemiological links were identified if: (i) a patient was hospital-

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

Environmental contamination

The *P. aeruginosa* environmental colonization rate was measured by regularly swabbing the faucelts during the 10 years of the study (12 faucets in the medical ICUs, and 17 in the surgical and paediatric ICUs) (Table 1). The results obtained revealed a difference of contamination over the years between the two water networks. Less than 10% of the faucets in the surgical and paediatric ICUs were contaminated. Higher contamination rates were seen in the medical network from 1998 to 2002, although rates dropped below 10% between 2003 and 2005 (no sampling was performed during renovation in 2006 and 2007; and an investigation carried out in 2008 revealed no contamination of the new faucets).

Faucets as a major environmental exogenous source of *P. aeruginosa*

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 and Fig. 1). The higher contamination rates of faucets in the medical ICU (25%) compared to the surgical–paediatric ICUs (6.4%) were correlated with a higher number of category A cases in this ICU (32.6 cases per 1000 admissions) compared to the other ICUs (16.8 cases per 1000 admissions) (Table 1 and Fig. 1). This correlation was also observed in 2000. The number of category A cases in the medical unit only became similar to those in the surgical and paediatric ICUs in 2003, when the percentage of contaminated faucet dropped below 10%.

Other patients as exogenous sources of *P. aeruginosa*

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 surveil-

TABLE 1. **Epidemiological data of patients with *Pseudomonas aeruginosa* in the intensive care units (ICUs) over a 10-year period**

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Number of admissions in ICUs	2434	2538	2449	2586	2427	2400	2554	2530	2567	2675
Number of patients with *P. aeruginosa*	139	107	75	52	87	92	82	64	75	83
Number of patients with *P. aeruginosa* per 1000 admissions	57.1	42.2	30.6	20.1	35.8	38.3	32.1	24.9	29.2	31.0
Number of clinical specimens[a] per 1000 patient-days of hospitalization	NA	NA	668	792	786	817	815	727	757	815
Percent of faucets[b] 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 paediatric ICUs (water network II)	6.4	0	0	4.3	3.1	4.3	2.9	0	0	0
Categories of cases[c] (number of cases per 1000 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 paediatric 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).
[a]Clinical specimens received by the laboratory of bacteriology.
[b]The percentage of contaminated faucets was based on data from faucets that were regularly swabbed over the 10 years.
[c]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.

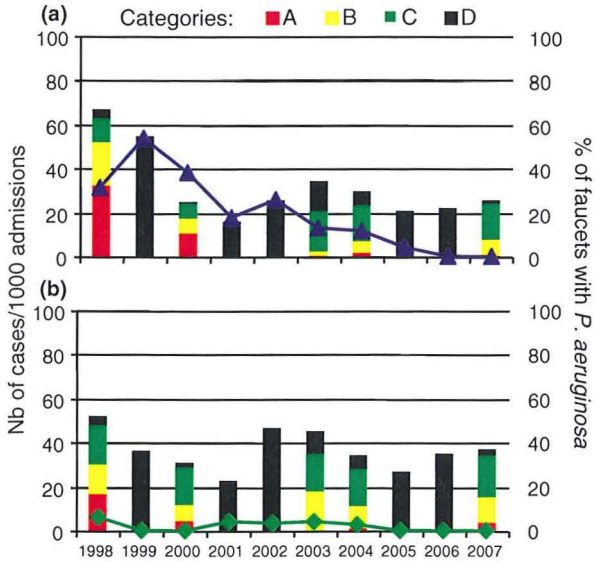

FIG. 1. Evolution of the annual number of cases per 1000 admissions and per category, and of the percentage of faucets contaminated with *Pseudomonas aeruginosa* in the medical intensive care unit (ICU) (a), and in the surgical–paediatric ICUs (b). Category A, identical to faucet; category B, identical to at least one other patient; category C, unique genotype; category D, not typed.

lance from 1.9 to 20 cases per 1000 admissions (category B; Table 1). This number exceeded ten cases per 1000 admissions on three 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 six patients in one cluster). The introduction of alcohol-based hand rub appaered to have played a role in the reduction of cases in this category [7]. In 2003, two large clusters of patients carrying the same *P. aeruginosa* genotype (13 and eight patients, respectively) were observed in the surgical unit, reflecting an outbreak resulting from the emergence and spread of two multiresistant *P. aeruginosa* strains (susceptible to ≤1 of the four antibiotic classes used for therapy). This outbreak lasted until 2004. In addition, the present study revealed that several patients harboured non multiresistant strains that were genetically identical to the epidemic multiresistant strains. During this 2003 outbreak, several contaminated environmental sites (transoesophageal probe, siphons) were identified that may explain the large number of cases.

Endogenous sources of *P. aeruginosa*

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

Concordance between molecular typing and epidemiological data

Within category A, 22 genetic clusters involving one to 30 cases were observed. An epidemiological link could not be found for two cases in two genetic clusters (one patient in each of the two genetic clusters was hospitalized in another ICU than the unit with a positive faucet) (Table 2). In 2004, we found four patients sharing the same genotype found in faucets during previous years (1998–2003), but not recovered from faucets in 2007.

Within category B, 40 genetic clusters involving two to 13 cases were observed. Epidemiological links were found between all cases in only seven of these clusters, between

TABLE 2. Analysis of genetic and epidemiological clusters in cases of category A (patients with a *Pseudomonas 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
Number of cases in category A	**57**	**17**	**1**	**4**	**0**
Number of genetic clusters	10	7	1	4	0
Number of patients in genetic clusters	30, 6[a], 5, 4, 4, 3, 2, 1, 1, 1	8, 4, 1, 1, 1, 1, 1	1[a]	1, 1, 1, 1	0
Number of cases in category B	**39**	**17**	**27**	**21**	**29**
Number of genetic clusters	11	4	5	8	12
Number of patients in genetic clusters	11 (6, 2)	8 (3)	13 (9, 2)	5 (3)	3 (3)
(number of patients in epidemiologic clusters)	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)

[a]One patient harboured a genotype identical to that found in faucet but was hospitalized in a different intensive care unit; no probable transmission was thus considered.

some of the cases in seven other clusters, but with no links in the remaining 26 clusters (Table 2). During 2004 and 2007, when no outbreaks were seen, we observed many small genetic clusters without any concordance with the epidemiological data (Table 2).

Discussion

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

Faucets were an important environmental source of *P. aeruginosa* in 1998 but became less common as a result of additional infection control measures. Taps contaminated with *P. aeruginosa* have previously been shown to be a continuous source for transmission [12,17] but the present 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] but, because our data showed that cases linked to contaminated faucets were rare from 2003 onwards, we did not introduce such filters, except in rooms with burned or solid transplant organ patients.

Although a contaminated faucet can be the source of a cluster of *P. aeruginosa* colonizations or infections, there are other hypotheses that could explain category A cases in the present study. First, patients may be the source of faucet contamination; however, we have previously shown that such events are probably infrequent [6]. Second, a mixed scenario can occur when a patient contaminated from a faucet becomes the source of patient-to-patient transmission.

In the present 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 transmission, whereas larger clusters suggest the contribution of a persisting source, most probably from the environment. A large outbreak as a result of the emergence and spread of multiresistant *P. aeruginosa* was observed in 2003 and 2004; molecular investigation showed that this outbreak involved two strains and multiple exogenous sources of contamination (transoesophageal probe, siphons and other patients). The duration of this outbreak was probably a result of the persistence of the epidemic strains in the environment and transmission between patients harbouring the epidemic strains that did not show a multiresistant phenotype (and consequently not placed on contact precautions). 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 other studies reporting on *P. aeruginosa* outbreaks involving the environment [20–24].

Molecular typing results of patient isolates may 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, the presence of predominant genotypes in patients without epidemiological links should also be taken into consideration. Therefore, during the investigation of transmission, it is important to analyze the concordance of molecular results with epidemiological data. A low concordance suggests that either an epidemiological link was missing or that a predominant *P. aeruginosa* genotype was found in epidemiologically unrelated patients. Our definition of epidemiological link relies 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 multiresistant strains was not restricted to the ICUs (operating theatre, other wards), which strongly suggests an exogenous source for all patients harbouring these genotypes. Similarly, we might have found epidemiological links in other years if more extensive investigations had been conducted.

In conclusion, the relative contribution of endogenous vs. exogenous reservoirs to the colonization and infection of ICU patients with *P. aeruginosa* varies over time. The major factors contributing to such variations are most likely the contamination of the environment, the compliance of health care workers with infection control measures and the genetics of the pathogen itself. Because *P. aeruginosa* is ubiquitous in humid environments and colonizes up to 15% of hospitalized patients, elimination of the reservoir may be difficult. We have demonstrated that, in our setting, this eradication from faucets was possible by increasing the temperature of hot water. In addition, when a multiresistant strain is repeatedly recovered from patients and from the environment, efforts should be made to eradicate this strain from the environment. However, patient-to-patient transmission was also found to be an important and recurrent cause of exogenous origin; the strict maintenance of infection control measures is also essential for limiting the transmission of *P. aeruginosa*.

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Transparency Declaration

All authors declare that they have no potential conflict of interest.

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