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ORIGINAL

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Prospective study of nosocomial colonization and infection due to *Pseudomonas aeruginosa* in mechanically ventilated patients

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Abstract *Objective*: To investigate the respective contribution of endogenous and exogenous transmission of *Pseudomonas aeruginosa* in the colonization of lungs in the mechanically ventilated patient, to estimate the role of *P. aeruginosa* colonization in the occurrence of severe infections, and to extrapolate appropriate control measures for the prevention of *P. aeruginosa* ventilator-associated pneumonia.
Design: Prospective study of the presence of *P. aeruginosa* (in stomach fluid, throat specimens, stool, and sputum) on admission, twice a week throughout the patient's stay, and in their environment. O-serotyping, pulsed-field gel electrophoresis, and arbitrarily-primed polymerase chain reaction were used to characterize the strains.
Setting: The two intensive care units (ICUs 1 and 2) of a university hospital.
Patients: During a 6-month period, 59 patients were included (21 in ICU 1 and 38 in ICU 2).
Results: P. aeruginosa was isolated in 26 patients, including ten pneumonia cases and seven colonizations on admission. The incidence of acquired colonization was statistically different between the two ICUs: 5.5 and 20.5 per 1000 days of mechanical ventilation, in ICUs 1 and 2, respectively. Endogenous acquisition was the main origin of *P. aeruginosa* colonization (21 of 26 patients) and the upper respiratory tract was the main bacterial reservoir in bronchopulmonary colonization and infection. However, during the 6-month period of the study, a multidrug-resistant strain of *P. aeruginosa* O:11, isolated in the sink of the room of 12 patients, was found responsible for two colonizations (1 digestive, 1 throat/lungs) and one pneumonia. As a whole, from 26 cases of colonization/infection with *P. aeruginosa*, 5 were related to an exogenous contamination (environmental reservoir in 4 patients and cross-contamination in one patient).
Conclusions: These results emphasize the need for applying various infection control measures to prevent colonization of patients with *P. aeruginosa*, including strategies to limit the potential of sinks from acting as a source or reservoir for this bacterium.

Key words *Pseudomonas aeruginosa* · Colonization · Infection · Nosocomial pneumonia · Intensive care unit · Environment · Ventilator-associated pneumonia

Introduction

Pseudomonas aeruginosa is a gram-negative non-fermentative rod which can cause nosocomial infection and, more rarely, community-acquired infection. It was found to be responsible for up to 28 % of nosocomial infections in intensive care units (ICUs) during the European Prevalence of Infection in Intensive Care Study [1]. Indeed, such infections affect patients with severe underlying diseases (cystic fibrosis, severe burns) and/or immunosuppression. In the ICU, the case fatality rate of *P. aeruginosa* pneumonia can be as high as 80 % [2]. In mechanically ventilated patients (MVP), *Pseudomonadaceae* are the most common isolates in late onset pneumonia (developed beyond the 4th day of ventilation according to Van Saene et al. [3]). The main risk factors for the development of *P. aeruginosa* pneumonia are previous use of antibiotics and presence of chronic obstructive pulmonary disease [4, 5, 6].

P. aeruginosa is often recovered from watery environments (sinks, faucets, bedpans). It is not commonly isolated from healthy people. Under conditions of antibiotic treatment and/or hospital stay, the carriage of this bacterium, mainly in stool and throat, can increase [7]. The pathophysiology of a patient's pulmonary colonization with *P. aeruginosa* is still unclear: its main origin seems to be endogenous but in some outbreaks contaminated devices or environment have been found to be responsible for its transmission [8, 9, 10, 11, 12]. Moreover, in the ICU, the route of lung colonization with *P. aeruginosa* does not seem to be the same as with *Enterobacteriaceae* [13]; in the latter case, initial digestive colonization is thought to precede throat then lung colonization as a result of micro-inhalation [14]. Concerning *P. aeruginosa*, only a few studies have investigated the routes of pulmonary colonization and infection in MVP [5, 15, 16, 17, 18].

In the University Hospital investigated in this study, previous outbreaks due to *Pseudomonadaceae* had occurred in MVP [19, 20]. In 1995–96, a prospective study was conducted in the two ICUs of this hospital with the following aims: (1) to investigate the respective contribution of endogenous and exogenous transmission of *P. aeruginosa* in the colonization of lungs in MVP; (2) to estimate the role of *P. aeruginosa* colonization in the occurrence of severe infections; and (3) to extrapolate appropriate infection control measures for the prevention of *P. aeruginosa* colonization and pulmonary infection in the ICU.

Materials and methods

Study design and patients

The study was conducted prospectively in two ICUs (10 and 15 beds) of a French University Hospital during a 6-month period (November 1995–May 1996). ICU 1 mainly receives patients coming from other units of the same hospital (some of them being already infected on admission to the unit) while ICU 2 deals mainly with outpatients (medical, surgical, and trauma). The two units, which are 15 km apart, work independently. Consecutive patients were entered in the study if they were at least 18 years old and it was thought likely that they were to be ventilated for four or more days. Exclusion criteria were as follows: nosocomial pneumonia on admission, respiratory distress with contraindication to broncho-alveolar lavage (BAL), major coagulation abnormalities, tracheotomy on admission or selective digestive decontamination. The study was approved by the ethical committee of the hospital ("Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale Rhône-Alpes Loire"). Each included patient was screened for presence of *P. aeruginosa* on admission, twice a week throughout the patient's stay, and at extubation or death. Samples were obtained from stool, stomach, throat, and lungs (tracheal aspirates and BAL). The patient's environment was systematically screened for the presence of *P. aeruginosa* at the same time, including sinks (siphon, overflow), ventilator trap, bronchoscopes, and, in colonized patients, surfaces such as beds and tables. All bacteriological sampling was performed by the health care workers of each unit. A BAL was performed if a nosocomial pneumonia was suspected [21]. Data recorded prospectively from patients were as follows: demographic characteristics, history of prior hospitalizations and antibiotic treatment, diagnosis, clinical features, severity score, duration of mechanical ventilation, location of the patient in the unit, respiratory procedures during ICU stay, and treatment modalities. Admission and discharge diagnoses were divided into three groups according to the classification of Fernandez-Crehuet et al. [22]. The simplified acute physiology score SAPS 2 and the OMEGA daily workload score, widely used in France, were chosen to assess, respectively, the disease severity and the therapeutic activity [23, 24]. The OMEGA score combines three kinds of data: therapeutic procedures (51 items), diagnostic investigations, and nursing activities.

Bacteriological methods and typing

Standard identification

Specimens were cultured on selective *Pseudomonas* agar supplemented with 200 mg/l of cetrimide and 15 mg/l of nalidixic acid (Oxoid, Dardilly, France) and incubated up to 48 h at 41 °C. The identification of *P. aeruginosa* isolates and their sensitivity to antibiotics were determined by using Neg Combo 1^E panels (Microscan, Dade Behring, Maply, France). The O-serotyping of isolates was performed by slide agglutination using commercial antisera (Sanofi/Diagnotics Pasteur, Marnes-la-Cocquette, France).

Arbitrarily primed-polymerase chain reaction (AP-PCR) typing

DNA was extracted from bacterial cells and purified by using a lysis mixture containing guanidium thiocyanate and phenol-chloroform (Tri Reagent, Sigma Diagnostics, St Quentin Fallavier, France), following the recommendations of the manufacturer. AP-PCR analysis was done on 100 ng of template DNA in a mixture containing 6 μM of a 10-mer primer, 200 μM of each dNTP, 1.25 IU of *Taq* DNA polymerase (ATGC Biotechnology, Noisy le Grand, France), 10 mM Tris HCL, 50 mM KCl, and 1.5 mM $MgCl_2$. Two primers were chosen for their ability to provide clear and discriminative patterns: 5′-AACGCGCAAC-3′ (n°1) and 5′-GGTGGTGGCT-3′ (n°2). Each sample was submitted to a first cycle of denaturation, annealing, and hybridization for 5 min each at

Table 1 Demographic and clinical data of included patients in ICU 1 and 2

Variable	ICU 1 38 patients	ICU 2 21 patients
Gender (No. Males/%)	28/73.7	16/76.1
Mean age in years/SE	64.5/15.0	60.0/16.5
Mean stay in hospital before ICU admission (days)/SE	8.3/12.4	3.8/10.1
Category on admission (No. Patients/%)		
Medical	18/47.4	14/66.7
Emergency surgery	11/29.0	5/27.1
Scheduled surgery	8/21.1	1/1.1
Others	1/2.5	1/1.1
Prior antibiotic treatment on admission (No. Patients/%)	6/15.8	1/4.8
With broad spectrum antibiotics	5/13.2	1/4.8
Category of diagnosis at discharge[a] (No. Patients/%)		
1	10/26.9	1/4.8
2	9/23.6	5/23.8
3	19/50.5	15/71.4
Mean SAPS 2/SE	41.9/11.6	51.8/17.6
Mean duration of ICU stay (days)/SE	28.9/31.6	34.0/23.3
Mean duration of mechanical ventilation (days)/SE	22.8/23.5	31.4/23.4
Deaths (No./%)	12/31.6	10/47.6

[a] According to Fernandez-Crehuet et al. [20]. Group 1: cardiovascular disorders, genitourinary tract, skin and subcutaneous tissues, poisoning, complications of pregnancy, birth, and puerperium; group 2: infectious and parasitic disorders, digestive system, musculoskeletal system and connective tissue, symptoms and signs of undetermined conditions; group 3: nervous system and sensory organs; respiratory system; injuries, neoplasias; endocrine system; nutrition; metabolism, and immunological disorders

94 °C, 35 °,C and 72 °C, respectively, followed by 28 cycles of consecutive denaturation, annealing, and hybridization (94 °C, 1 min; 35 °C, 2 min; 72 °C, 2 min) and a final extension step of 10 min at 72 °C. Amplimers were separated in a 1 % agarose gel. A negative control without DNA was included in each reaction. The reproducibility of profiles was tested in at least two independent experiments. Strains were considered to be linked if they showed identical profiles or if minor differences in the intensity of one or two bands were not confirmed in repetitive experiments or with the use of another primer.

Pulsed-field gel electrophoresis (PFGE)

P. aeruginosa cells were embedded in 2 % agarose (Seaplaque, FMC BioProducts, Rockland, Me., USA) blocks and treated with lysis buffer containing 0.5 M EDTA, 1 % sodium dodecyl sulfate and 1 mg/ml of proteinase K, at 55 °C overnight. The agarose plugs were washed twice at 37 °C in TE-PMSF pH8 containing 10 mM Tris, 0.1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF) and then twice in TE buffer before storage. The genomic DNA inserts were digested at 37 °C for 3 h with 40 U of either *Dra*I or *Spe*I (Boehringer Mannheim, Meylan, France) enzymes and the fragments were separated by PFGE with a CHEF-DrII apparatus (Biorad, Ivry sur Seine, France) using 1 % agarose gel (Seakem GTG, FMC BioProducts) and Tris-borate-EDTA buffer, at 14 °C. Technical parameters were as follows: a field strength of 6 V/cm; two successive linear ramps of 4–8 s and 10–20 s for 11 h each for *Dra*I profiles; one linear ramp of 0.5–29 s for 24 h for *Spe*I profiles. After staining with ethidium bromide, the fragments were photographed. The PFGE profiles were visually analyzed. *P. aeruginosa* strains were classified as epidemiologically distinct if a difference of more than three bands was ascertained [25].

Analysis of the study

For each colonized or infected patient, a chronological analysis of the isolation and mode of acquisition of *P. aeruginosa* strain(s) was performed. All *P. aeruginosa* strains isolated from patients or their environment were analyzed by molecular typing, with the exception of successive strains isolated from the same site within the same week. AP-PCR was only used for comparing strains of *P. aeruginosa* isolated successively from the same site in the same patient. All strains isolated from different sites in the same patient and from their environment were definitively classified by PFGE using two different enzymes [26]. Data collected from patients were used to identify risk factors for colonization and/or infection with *P. aeruginosa*. Acquired colonization in ICU was stated in a patient who did not harbor *P. aeruginosa* in samples systematically recorded on admission and who acquired this bacterium in at least one clinical sample collected during the ICU stay. The colonization was considered as endogenous if *P. aeruginosa* was isolated in at least one patient's sample taken on admission or later without previous isolation of the same strain in environment samples or in other patients of the ICU. Conversely, the colonization was considered as exogenous if *P. aeruginosa* was not present in any sample taken at the entry of the patient in the ICU and if a strain of *P. aeruginosa* was further isolated in his clinical samples with previous isolation of the same strain in environmental sample(s) or in other patients of the ICU. *P. aeruginosa* pneumonia was defined by the occurrence of a new and persistent chest radiographic infiltrate, not otherwise explained, with suggestive clinical symptoms and of a BAL with more than 10^4 CFU/ml of *P. aeruginosa*.

Two multivariate analysis were carried out to evaluate: (1) risk factors for *P. aeruginosa* colonization on admission in ICU patients (comparing patients colonized or not colonized with *P. aeruginosa* in at least one sample on admission); and (2) risk factors for *P. aeruginosa* acquisition during ICU stay in patients not colonized on admission (comparing patients who acquired or did not acquire

Table 2 Clinical and bacteriological characteristics of mechanically ventilated patients ($n = 26$ patients) found colonized or infected by *P. aeruginosa*

Patient[a]	No. ICU	Year/Month of admission	Duration of stay (days)	Colonization		Pneumonia[b]	
				Pulmonary tract	Digestive Tract	Y/N	Day of diagnosis
A	1	95/11	39	–	–	Yes	3
B	1	95/11	13	–	+	No	
F	1	95/12	26	–	+	No	
G	1	96/01	12	+	–	No	
H	1	96/01	38	–	+	No	
I	1	96/01	20	–	–	Yes	8
L	1	96/02	5	–	+	No	
R	1	96/02	126	–	–	Yes	38
Z	1	96/04	9	+	–	No	
AC	1	96/04	28	–	+	No	
AD	1	96/05	153	–	–	Yes	51
AL	1	96/06	45	+	–	No[c]	
A'	2	95/11	81	–	–	Yes	21
B'	2	95/12	42	–	–	Yes	14
E'	2	95/12	26	–	+	No	
G'	2	95/12	10	–	+	No	
I'	2	95/12	33	–	+	No	
J'	2	95/12	26	–	+	No	
K'	2	95/12	14	–	+	No	
L'	2	95/12	45	–	+	No	
M'	2	96/01	44	–	–	Yes	19
N'	2	96/01	50	–	–	Yes	25
O'	2	96/01	33	–	–	Yes	8
Q'	2	96/02	16	+	–	No	
R'	2	96/02	7	+	–	No	
U'	2	96/03	86	–	–	Yes	5

[a] The patient's identification letters were given chronologically and independently between the two ICUs
[b] Pneumonia: see text for the criteria of pneumonia
[c] Pulmonary colonization treated by antibiotics

P. aeruginosa in at least one clinical sample during ICU stay). Imipenem, third-generation cephalosporins, and fluoroquinolones were considered as broad-spectrum antibiotics [6]. Antibiotic treatments received prior to colonization and/or infection with *P. aeruginosa* were analyzed as described previously [5].

Statistical methods

Data were analyzed using Access 7.0 (Microsoft), Epi-info 6.04b (CDC-OMS), and SPSS 7.0 software. For the univariate and bivariate analysis, Fisher's exact test and *t*-tests were used (P value below the 0.05 level was considered as significant). To adjust for confounding factors, variables with a P value below the 0.2 significance level in univariate analysis were entered into a multiple logistic regression model. For the multivariate analysis of risk factors for colonization with *P. aeruginosa* on admission, a logistic regression was used. For the multivariate analysis of risk factors for *P. aeruginosa* acquisition during ICU stay, the Kaplan-Meier method (log-rank test) and the Cox model were used.

Results

Incidence of *P. aeruginosa* colonization and infection in the ICU

During the 6-month period of the study, from 332 patients admitted to the ICU (133 to ICU 1 and 199 to ICU 2), 59 (18%) patients were definitively included in the study [38 (29%) from ICU 1 and 21 (11%) from ICU 2]. The reason for the non inclusion of most of the patients admitted the unit was a foreseeable duration of stay of less than 4 days. Demographic and clinical data of the included patients are given in Table 1. The mean number of diagnoses at discharge was 3.4 (SE = 1.1). Globally, included patients had severe diseases with a mean SAPS 2 score equal to 41.9 and 51.8 in ICU 1 and 2, respectively, explaining high mortality rates of 31.6% in ICU 1 and 47.6% in ICU 2.

P. aeruginosa was isolated in 26 of the 59 patients, including 16 colonizations (eight in each unit), five of them in the pulmonary tract, and ten pneumonia (four in ICU 1 and six in ICU 2) (Table 2). On admission, seven patients were colonized with *P. aeruginosa* in ICU 1 (six in digestive tract, two in throat specimens and tracheal aspirates) and none in ICU 2. Only one of the seven patients colonized on admission developed a pneumonia. The rate of ICU-acquired colonization (digestive and pulmonary) was 5.5 and 20.5 per 1000 days of mechanical ventilation in ICU 1 and 2, respectively.

Typing results

During the study period, 1383 samples were taken: 830 in ICU 1 and 553 samples in ICU 2. *P. aeruginosa* was re-

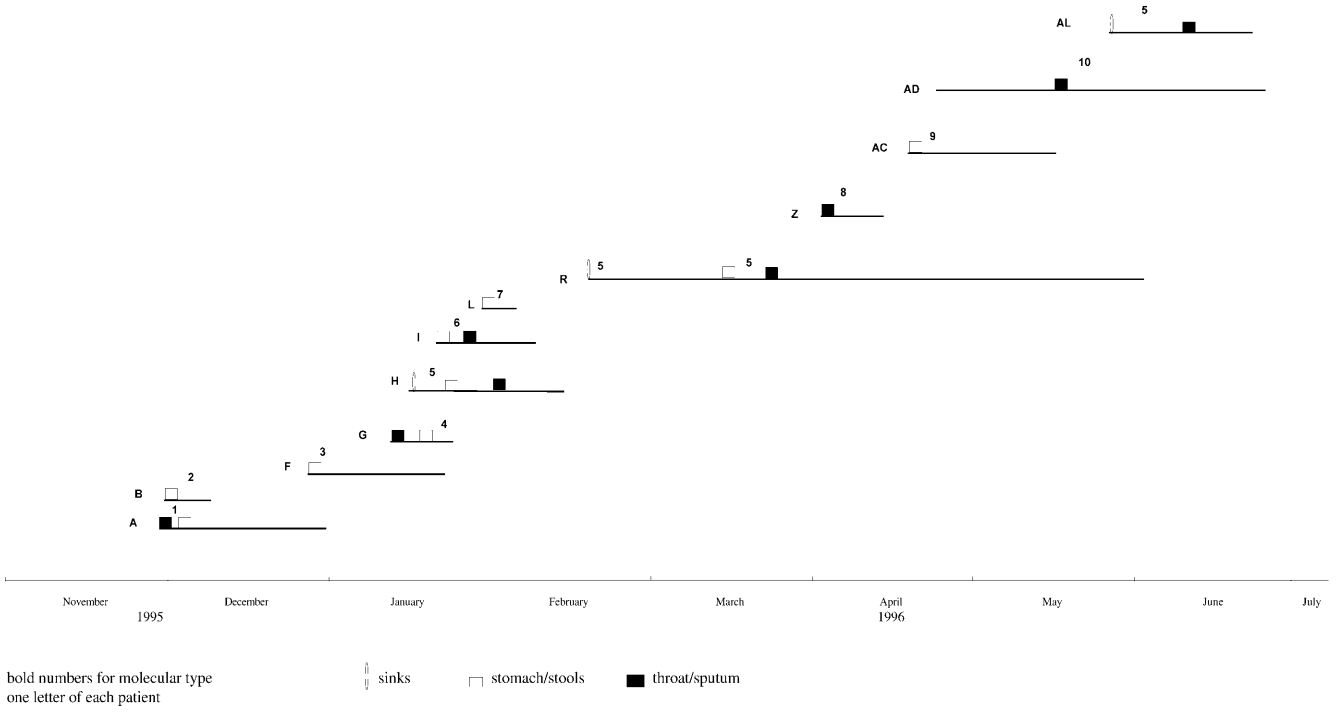

Fig. 1 Chronology of *Pseudomonas aeruginosa* colonization and infection in patients of ICU 1

covered from 323 samples: 170 (including 46 environmental samples) in ICU 1 and 153 (including 45 environmental samples) in ICU 2. From the 323 *P. aeruginosa* strains, 207 (64%) were typed by molecular techniques including 68 clinical (18 tracheal aspirates, four BAL, 19 throat, 27 digestive tract) and 34 environmental strains (14 siphons, 7 overflows, 13 traps) in ICU 1, and 100 clinical (30 tracheal aspirates, 6 BAL, 27 throat, 37 digestive tract) and 34 environmental strains (14 siphons, 7 overflows, 13 traps) in ICU 2. As specified above, the samples that were excluded from typing corresponded to strains isolated from specimens of the same patient or his/her environment collected in the same site within the same week.

The chronology and sites of isolation of *P. aeruginosa* according to the results of the typing experiments are reported in Figs. 1 and 2. The acquisition of *P. aeruginosa* was endogenous in 21 out of 26 patients (80.8%). Conversely, in five patients (three in ICU 1 and two in ICU 2), the bacterium was isolated from the patients' environment prior to isolation from clinical specimens.

In the 15 patients with colonization and/or infection of the respiratory tract, the first site of isolation of *P. aeruginosa* was the upper respiratory tract in seven patients (two colonizations and five infections), the digestive tract in five patients (two colonizations and three infections), the throat in six patients (two colonizations and four infections) and the patients' environment in four patients (two colonizations and two infections); in five of these 15 patients (two colonizations and three infections), the bacterium was first isolated in at least two compartments simultaneously (Figs. 1 and 2). In summary, from these 15 patients, three experienced a probable exogenous contamination whereas 12 were colonized endogenously, nine of them having harbored the strain of *P. aeruginosa* in the upper respiratory tract (tracheal aspirates and/or throat) since the first isolation.

In ICU 1, 12 different profiles were characterized by PFGE. An epidemic *P. aeruginosa* strain (molecular profile V in Fig. 1; and lanes 1–18 in panel A and 1–12 in panel B, Fig. 3) belonging to serotype O:11 with a particular pattern of antibiotic susceptibility (resistant to ticarcillin, ceftazidime, and imipenem, and sensitive to ciprofloxacine) was recovered from the sinks of 12 patients hospitalized in four different rooms throughout the investigation period (Fig. 4). Later on, during the hospitalization of three of these patients (patients H, AL, and R in Fig. 1), it was found responsible for digestive colonization, pulmonary colonization treated by antibiotics, and pneumonia, respectively, as demonstrated by the similarity of the PFGE profiles both with *Dra*I (Fig. 3, panel A) and *Spe*I (Fig. 3, panel B) between environmental and clinical strains. A cross contamination between the patients H and R can be excluded since no overlapping hospitalization period occurred between these patients. Conversely, patients R and AL spent 17 days in two consecutive rooms simultaneously. In addition, neither patients from the ICU who were not in-

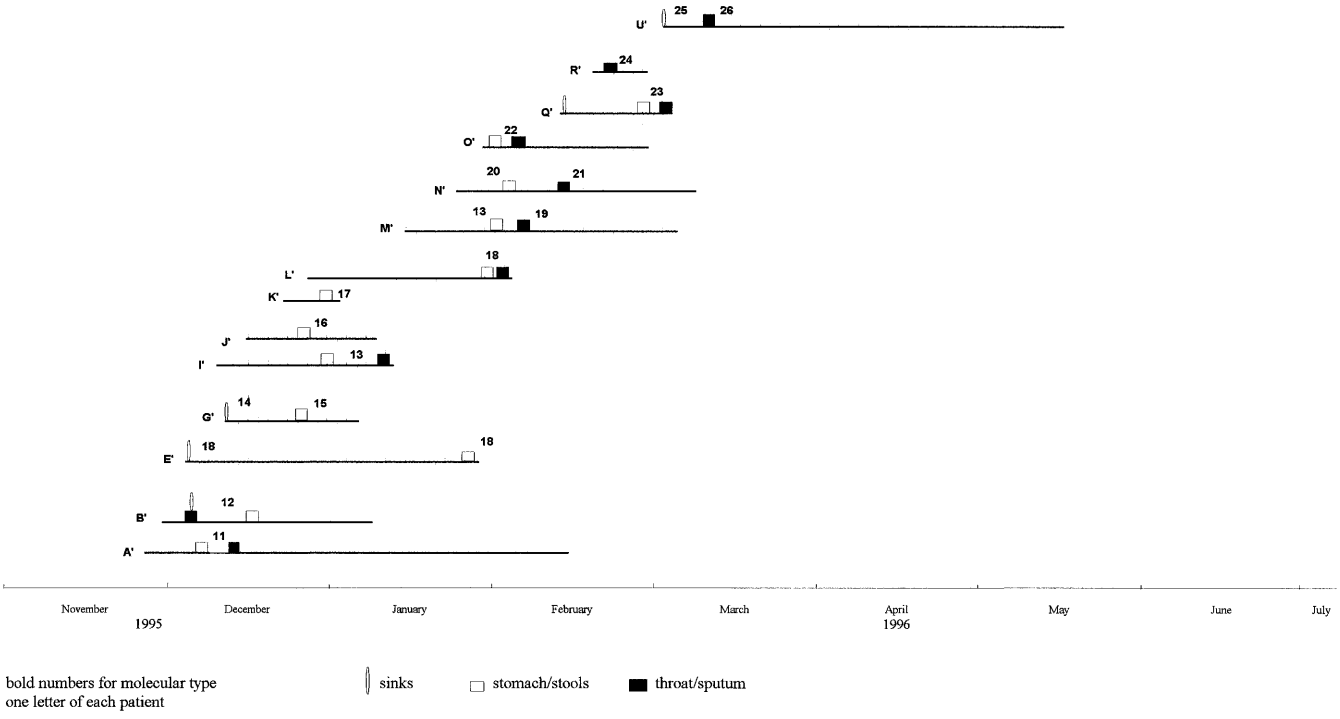

Fig. 2 Chronology of *Pseudomonas aeruginosa* colonization and infection in patients of ICU 2

cluded in the study, nor patients hospitalized in the ICU during the last 2 months preceding the study exhibited a *P. aeruginosa* strain of serotype O:11 with the same antibiotype in their clinical samples (data not shown). Moreover, during the 6-month period of the study, three bacteriological controls performed systematically from the hospital circuit delivering tap water to ICU 1 showed no *P. aeruginosa* contamination.

In ICU 2, more than 25 different molecular profiles were characterized by PFGE; two patients harbored two distinct strains of *P. aeruginosa* in different sites. Two cross transmissions occurred; they were confirmed by the chronology of *P. aeruginosa* isolation (overlap in hospitalization periods) and the similarity of molecular profiles (patients I' and M' in Fig. 2). In addition, in two cases of colonization (respiratory in patient Q' and digestive in patient E' in Fig. 2), a strain first isolated in the sink of the room was genetically similar to the strain responsible for the secondary colonization of the patient occupying this room.

Risk factors for *P. aeruginosa* colonization

In univariate analysis, the only significant risk factor for *P. aeruginosa* acquisition in the ICU was the duration of stay (Table 3). If the analysis was restricted to ICU 1, the presence of a sink in the room colonized with *P. aeruginosa* was a significant risk factor for a later acquisition of this strain ($P = 0.04$ by Fisher's exact test).

As all patients harboring *P. aeruginosa* on admission originated from ICU 1, the analysis of the risk factors for *P. aeruginosa* colonization at entry was restricted to this ICU. The variables significantly associated with colonization with *P. aeruginosa* on admission after univariate analysis are the previous isolation of a *P. aeruginosa* strain in clinical samples ($P < 0.001$), the number of antibiotics received before ICU ($P = 0.01$), the patient's age ($P = 0.03$), the origin of the patient (home, hospital unit, other ICU, $P = 0.03$), a previous use of broad-spectrum antibiotics ($P = 0.04$) and a history of hospital antibiotics 12 months before admission ($P = 0.05$). After multivariate analysis, only the variables previous isolation of a *P. aeruginosa* strain in clinical samples and the previous use of broad-spectrum antibiotics (third-generation cephalosporins, fluoroquinolones, imipenem) remained in the model but the number of patients studied did not allow us to draw conclusions about this result. All seven patients had stayed in an hospital unit (two in another ICU) before their transfer to the ICU participating to the study. The review of their medical file showed that, in five of them, a *P. aeruginosa* strain had been isolated previously.

The rate of acquisition of *P. aeruginosa* by a patient during the ICU stay was significantly higher in ICU 2 than in ICU 1 ($P < 0.001$ by log-rank test). In univariate analysis, the ICU location ($P < 0.001$), the duration of hospital stay ($P < 0.001$), and the OMEGA daily work-

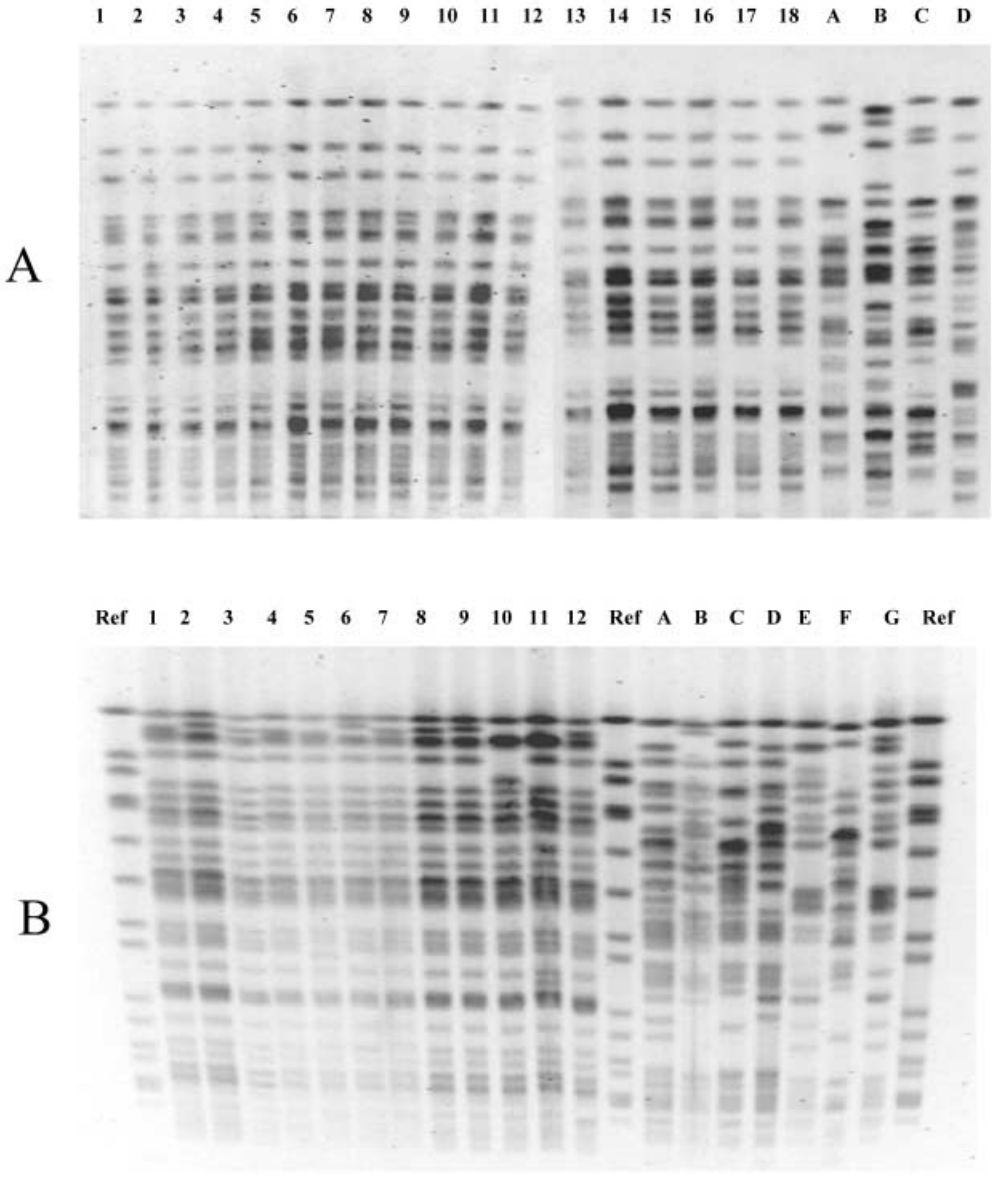

Fig. 3 Pulsed-field gel electrophoresis patterns of strains of *P. aeruginosa* O11 isolated in ICU 1 in the course of the study *(numbers)* and in other units of the same hospital *(letters)*. *Ref* corresponds to reference strain of *S. aureus* NCTC 8325 and was used as a size marker. *Panels A* and *B* illustrate profiles obtained with *Dra*I and *Spe*I, respectively. In *panel A*, lanes 1–11 show successive strains isolated from patient R (Table 2) and his environment over a 4-month period (lanes 1 and 2: different isolates from sinks, 02/23/96; lane 3: stool, 03/21/96; lane 4: wound, 03/26/96; lane 5: tracheal aspirate, 04/01/96; lane 6: stool, 04/01/96; lane 7: bronchoalveolar lavage, 04/12/96; lane 8: tracheal aspirate, 04/23/96; lane 9: stool, 05/23/96; lanes 10 and 11, ventilator trap, 04/01/96 and 04/15/96); lanes 12–16 show strains isolated from sinks of four other patients; lanes 17 and 18 show clinical strains isolated from patients H and AL (Table 2). In *panel B*, lanes 1–8 show strains isolated over a 6-month period from sinks of the rooms of seven patients includingthree colonized or infected ones (patients H, R, and AL in Table 2); lanes 9–12 show clinical isolates of patients AL (lanes 9 and 12), H (lane 10), and R (lane 11)

load score ($P < 0.01$) are significantly associated with *P. aeruginosa* colonization during the ICU stay. In multivariate analysis, the ICU location [OR = 3.47, CI 95 % = (1.01–11.90)] and OMEGA score [OR = 1.002 (per unit), CI 95 % = (1.001–1.004)] are associated with increase risk of colonization while the duration of antibiotic treatment is associated with a decreased risk [OR = 0.78 (per day), CI 95 % = (0.69–0.87)].

Discussion

Because of the severity of *P. aeruginosa* pneumonia in MVP, taking into account factors that promote the colonization of patients with *P. aeruginosa* is crucial to improve the prevention of these infections. As *P. aeruginosa* is ubiquitous in the environment and that different mechanisms of patients' colonization have been described (endogenous infection, cross contamination, contamination from watery hospital environments) [8, 9, 10, 11, 12], only prospective studies using powerful molecular typing methods [26, 27, 28] can allow us to explore the route of colonization/infection of MVP with this bacterium [5, 13, 15].

A goal of this study was to analyze the routes of respiratory colonization with *P. aeruginosa*. The upper respiratory tract (throat and/or trachea) was the main reservoir of *P. aeruginosa* in 3/4 of the patients experienc-

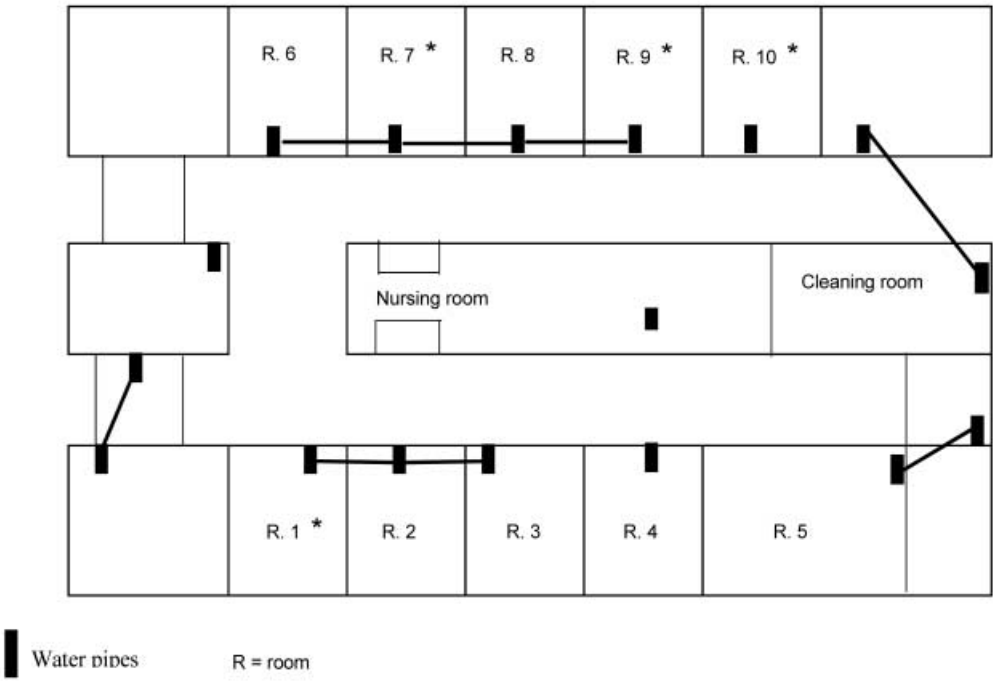

Fig. 4 Map of ICU 1 showing the layout of patients' rooms, sinks *(black rectangles)*, and water pipes *(black bars)*; rooms with an asterisk (*) correspond to those with sinks found contaminated with *P. aeruginosa*

Table 3 Univariate and multivariate analysis of risk factors for colonization with *P. aeruginosa*

Risk factor	Univariate analysis	Multivariate analysis	
For colonization with *P. aeruginosa* on admission (n = 38 patients):	*P*		
Age	0.03		
Origin of patients (home, hospital unit, other ICU)	0.03		
History of hospital antibiotics (12 months before admission)	0.05		
Previous use of broad spectrum antibiotics (third-generation cephalosporins, fluoroquinolones, imipenem)	0.04	Variable remaining in the model but not statistically significant	
Previous isolation of PA in clinical samples	< 0.001	Variable remaining in the model but not statistically significant	
Number of antibiotics received before ICU	0.01		
For *P. aeruginosa* acquisition during ICU stay (n = 52 patients)	*P*	*P*	Exp (B) CI 95 %
ICU	< 0.001	0.05	3.47 [1.01–11.90]
Duration of ICU stay (per day)	< 0.001	0.74	
OMEGA score (per unit)	< 0.01	0.001	1.002 [1.001–1.004]
Number of antibiotics received during ICU stay	0.07	0.49	
Duration of antibiotic treatment in ICU (per day)	0.08	< 0.0001	0.78 [0.69–0.87]
Albumin < 30 g/l^{-1}	0.11	0.56	

ing a colonization/infection of the respiratory tract with this bacterium. In most cases (9/10), ventilator-acquired pneumonia followed endogenous colonization with strains showing similar genomic profiles by two different typing methods. These observations confirm those of previous studies [13, 14, 15, 16], including two recent studies using molecular typing [5, 17]. By proving the genetic identity between strains colonizing initially the upper respiratory tract (throat and trachea) without previous digestive colonization and strains causing pneumonia, our study emphasizes the predominant role of this reservoir in *P. aeruginosa* ventilator-associated pneumonia.

Besides the main endogenous origin of respiratory colonization with *P. aeruginosa* in MVP, this study illustrates the complexity of the epidemiology of these colonizations by demonstrating, in some cases, the role of the patient's environment as a reservoir of *P. aeruginosa* strains. Actually, in five patients (three in ICU 1, two in ICU 2), three of them involving the respiratory tract, a

strain of *P. aeruginosa* with a similar molecular profile was primarily isolated from the sink of the patients' room. The epidemiological situation of ICU 1 is particularly interesting since an epidemic multidrug-resistant strain of *P. aeruginosa* serotype O:11 (profile V by PFGE in Fig. 1) was isolated during all the course of the study in the sink of four rooms. Serotype O:11 is one of the most common serotypes of *P. aeruginosa* causing human infections [10, 12, 29] and is found more frequently in clinical isolates from ICU patients than in those from other patients [29]. The arguments which demonstrate that sinks acted as a reservoir in some colonizations/infections of our patients are as follows: (1) colonized patients were hospitalized in rooms whose sinks were contaminated with strains of *P. aeruginosa* sharing the same molecular profile; (2) there was no time-overlapping between two of three patients; (3) this multidrug-resistant strain failed to be isolated from 192 other environmental samples or from any of the clinical samples taken from ICU patients not included in the study, despite systematic testing on admission, then once a week, and review of their microbiological files; and (4) the occurrence of patients' colonization with this strain stopped when chlorination of sinks (siphons and overflow) was reinforced (data not shown).

As shown in Fig. 3, there was no link between the contaminated sinks and the design of water pipe supply, suggesting that tap water was not the source of contamination. Moreover, no *P. aeruginosa* strain was isolated from tap water distributed in ICU 1. In the investigated units, patients' broncho-pulmonary tracts are humidified with cascade humidifiers. Because of water condensation in inspiratory and expiratory gas tubing, traps are emptied several times per day into the sinks of the patient's room. We hypothesize that sinks were contaminated, probably at the siphon level, with the epidemic strain during this practice. A similar mechanism was suggested in a recent outbreak of *P. aeruginosa* O:11 [12] with presumable contamination of sinks during the washing of hands or soiled utensils. *P. aeruginosa* can survive and multiply in sinks [11]; if it is present at densities of more than 10^5 CFU/ml in sink drains, hands can be contaminated during washing with this bacterium via aerosol generation [30]. The role of cross contamination of patients by *P. aeruginosa* via hand carriage in intensive care unit outbreaks has already been suggested [31, 32]. Nurses and nurse assistants also used the sink to wash patients' facecloth flannels and the bowls used for the washing. In some outbreaks, the bath has been presumed to be the source of patients' colonization with *P. aeruginosa* [10, 11]. We thus hypothesize that sinks may have been a reservoir of *P. aeruginosa* and that staff hands or washing equipment could have transmitted the bacterium to patients.

Additional evidence for the role of the environment in the spread of some cases of *P. aeruginosa* colonization was brought up by the results of the multivariate analysis. Actually, the pressure of selection of antibiotics did not influence the rate of bacterial colonization and the risk of acquiring *P. aeruginosa* colonization was reduced in patients with long-term antibiotic therapy using drugs active against this bacterium. Similarly, Cook et al. reported recently that exposure to antibiotics conferred protection against the occurrence of ventilator-associated pneumonia [33].

From a practical point of view, these results emphasize the need to reinforce the measures of infection control to limit the transmission of *P. aeruginosa* from watery hospital environments, including regular cleaning and disinfecting of sinks and exclusive use of sinks of the patient's room for hand-washing of health-care workers. Use of disposable gloves or alcoholic handrub, as recently proposed [34], could help to reduce this transmission.

Conclusions

This study confirms the mainly endogenous origin of *P. aeruginosa* pneumonia and emphasizes the role of the upper respiratory tract as an important reservoir of *P. aeruginosa*. It also demonstrates that contamination of sinks with *P. aeruginosa* via hand-carriage can contribute to the dissemination of the bacterium to patients. Thus, in ICUs, it is important to develop strategies which prevent watery hospital environments from acting as a source of *P. aeruginosa* colonization/infection in MVP.

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