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

**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-primer 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 broncho-pulmonary 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.  

**Keywords**  
*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° panels (Microscan, Dade Behring, Marly, France). The O-serotyping of isolates was performed by slide agglutination using commercial antisera (Sanofi/Diagnostica Pasteur, Marne-la-Coquette, 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 MgCl₂. Two primers were chosen for their ability to provide clear and discriminative patterns: 5'-AAGCGGCAAC-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...