Endemicity, molecular diversity and
colonisation routes of Pseudomonas
aeruginosa in intensive care units

Abstract  Objective: We carried out a prospective study to evaluate the endemicity of Pseudomonas aeruginosa in intensive care units (ICUs). Pulsed-field gel electrophoresis (PFGE) was used to determine the genotypes of P. aeruginosa isolates. This allowed us to determine the importance of cross-colonisation and the colonisation routes of P. aeruginosa.

Design: We screened epidemiological specimens (rectal swab, nose swab and tracheal aspiration) and routine clinical cultures from patients admitted to ICUs during a 2-year period, from 1st January, 1998, to 31st December, 1999.

Setting: The study was carried out in four separate adult ICUs located in the Franche-Comté region of France. These four units admitted a total of 1,500 patients per year.

Results: A total of 1,686 specimens were collected from 473 patients; 122 of these patients were positive on admission, 351 became positive during hospitalisation. The overall incidence of P. aeruginosa was 15.7 cases per 100 patients and 15.1 cases per 1,000 days of hospitalisation. Of 184 patients with at least one ICU-acquired positive clinical culture, 104 had been previously identified as carriers by a similar genotype. Typing of 208 non-replicate isolates revealed 101 major DNA patterns. Approximately 50% of P. aeruginosa carriage or colonisation/infection was acquired via cross-transmission; the other cases probably originated from endogenous sources.

Conclusion: Cross-colonisation seems to play an important role in the general spread of P. aeruginosa in ICUs.

Keywords Pseudomonas aeruginosa · Molecular epidemiology · Intensive care · Colonisation routes

Introduction

Pseudomonas aeruginosa is a common hospital-acquired pathogen of surgical wounds, the respiratory tract and urinary tract, in all departments of the hospital but especially in intensive care units (ICUs) [1, 2, 3]. The incidence of nosocomial P. aeruginosa infections has increased in recent decades [4]. This is partly due to the increase in the number of patients prone to such infection, particularly in ICUs. High mortality and morbidity rates
have been observed for *P. aeruginosa* infections, especially in cases of respiratory tract infection [5]. However, the general epidemiology of *P. aeruginosa* in ICUs suggests that infection represents merely the tip of an iceberg, whereas colonisation reflects the submerged part. Colonisation rates represent the true bacterial load within ICUs. Understanding the mechanisms establishing and maintaining endemicity of *P. aeruginosa* colonisation is therefore important. The environment used to be a major source of nosocomial *P. aeruginosa* infections, and outbreaks were reported in several ICUs [6, 7]. Effective infection control measures have reduced this phenomenon [7]. Nowadays, endemic nosocomial infections are thought to originate mainly from patients’ endogenous flora [8, 9]. In addition, colonised patients are a continuous exogenous source of micro-organisms that may go on to colonise other patients. To design targeted strategies to prevent infection, it is essential to understand the relative importance of exogenous and endogenous colonisations. The prevention strategies developed differ according to the dominant colonisation route.

We carried out a multicentre prospective study to examine endemicity, clonal diversity and colonisation routes of *P. aeruginosa* in ICUs.

**Materials and methods**

**Setting**

We studied four separate adult units: the medical and surgical ICUs at the University Hospital, Besançon, and the medical ICUs of Montbéliard and Vesoul General Hospitals. These three towns are located in Franche-Comté, a region of eastern France, with approximately 1,000,000 inhabitants. The surgical intensive care unit (SICU) and the medical intensive care unit (MICU) at Besançon have 15 beds each, the other MICUs each have 10 beds. These four units admit a total of 1,500 patients per year, giving a mean of 15,500 patient-days per year.

**Study design**

Patients were tested for *P. aeruginosa*. Routine clinical specimens and screening specimens (rectal swabs, nasal swabs and tracheal aspiration) were collected and analysed in order to identify *P. aeruginosa*. Screening cultures were collected from each patient on the day of admission and then once a week for the duration of hospitalisation in the ICU. *P. aeruginosa* strains were characterised using pulsed-field gel electrophoresis (PFGE), the genomic fingerprinting method now regarded as the most accurate method for the typing of *P. aeruginosa* for epidemiological purposes [10].

**Bacteriological culture**

Columbia agar, containing 5% horse blood, was used for the primary isolation of clinical specimens, and Mueller-Hinton agar for screening specimens. Suspect colonies were identified based on characteristic morphology, by Gram staining and by the oxidase test. Further biochemical tests were carried out to confirm their identity (API-20NE, Biomérieux, Marcy l’etoile, France).

**Data collection**

All patients admitted to these four ICUs between 1st January, 1998, and 31st December, 1999, were included in a prospective study. We recorded the patient’s age and sex, admission date and length of stay. Patients admitted to the same ICU several times, or to another participating ICU, with an interval of more than 3 months, were entered once for each admission.

**Definitions**

Patients with screening cultures testing positive in the absence of, or before isolation of, positive clinical specimens were considered to be carriers. Due to the lack of clinical data confirming infection, patients with positive clinical specimens were considered to be colonised/infected. When both clinical and screening cultures tested positive on the same day, the patient was considered as colonised/infected.

*Pseudomonas aeruginosa* carriage and colonisation/infected were considered to be ICU-acquired if *P. aeruginosa* was not detected in any specimen during the first 48 h after admission to the ICU. Carriage and colonisation/infection were considered to be endogenous if the strain of *P. aeruginosa* had not previously been isolated from another patient. Cross-acquisition was defined as carriage of, or colonisation/infected by, a strain of *P. aeruginosa* with a PFGE pattern identical or closely related to that of isolates from another patient in one of the ICUs.

**Statistical analysis**

Data are expressed as absolute numbers with or without percentages. Frequencies were compared using the $\chi^2$ test, a $p$ value of less than 0.05 being considered statistically significant. To determine whether screening cultures could predict further positive clinical cultures, we calculated the sensitivity, specificity, positive and negative predictive values of samples screening.

**Genotyping**

The genetic similarity of strains was investigated by pulsed-field gel electrophoresis (PFGE; CHEF DR-II, Bio-Rad, Ivry sur Seine, France) using Dral (Boehringer Mannheim, Germany) as previously described [11]. Five hundred ninety isolates from 242 patients were genotyped. We used PFGE data in two ways. Firstly, to study the route of colonisation by identified clones of *P. aeruginosa*, we compared the PFGE pattern of strains isolated from clinical cultures to that of strains previously isolated from screening cultures (we typed one strain per week if the antibiotic susceptibility pattern was identical or all the strains had changed). With this aim in view, we compared photographs of PFGE patterns by eye to identify identical-sized bands. Indistinguishable isolates (no band differences) and closely related isolates (2–3 band differences) were considered to be of the same genotype.

Secondly, to investigate the general epidemiological aspects of *P. aeruginosa*, we investigated *P. aeruginosa* isolates from patients testing positive (clinical or screening cultures, one strain per week if the antibiotic susceptibility pattern was identical or all the strains