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

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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 1686 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 1000 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 targetted 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'étoile, 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/infection 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/infection 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 χ_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 DRIII, Bio-Rad, Ivry sur Seine, France) using *Dra*I (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 if this pattern 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

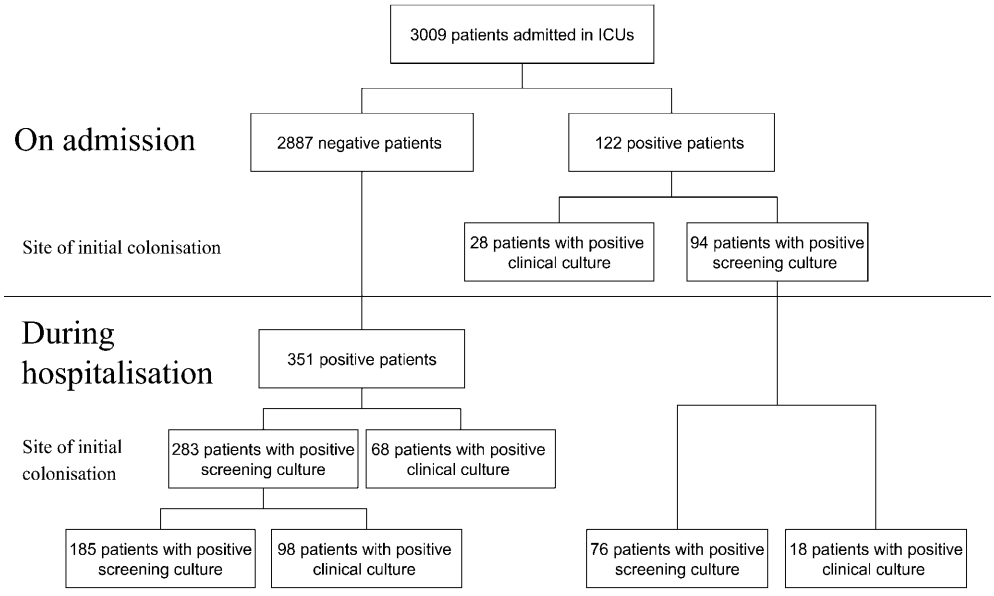

Fig. 1 Carriage and colonisation/infection with *Pseudomonas aeruginosa* on admission and during hospitalisation of the study population

if this pattern had changed) for at least 1 week. Samples of *SmaI*-restricted DNA of *Staphylococcus aureus* NCTC 8325 were included in each run as an internal reference. The banding patterns were analysed by scanning photographic negatives. GelCompar software was used for cluster analysis (Applied Maths, Kortrijk, Belgium). Each strain was first compared with all other strains and the Dice correlation coefficient was used to calculate similarity. The strains were then grouped and the UPGMA clustering algorithm was used to depict the groups as a dendrogram. Major restriction patterns were defined as those differing by more than three fragments, with a similarity index below 85 %, as described by Tenover et al. [12].

Results

Endemicity of *P. aeruginosa* in intensive care units

During the study period, 3009 patients were admitted to the four ICUs for a total of 31,219 days. In total, 1686 specimens (clinical and screening) testing positive for *P. aeruginosa* were taken from 473 patients (Fig. 1). Of these patients, 212 were colonised/infected and 261 were only carriers. The overall incidence of having at least one positive specimen was 15.7 cases per 100 patients and 15.1 cases per 1000 days of hospitalisation. The incidence of colonisation/infection was significantly higher [$p < 10^{-5}$, RR = 5.12 (3.43–7.64)] in the university hospital than in the other hospitals, whereas the incidence of carriage was not . The prevalence rate of patients tested positive upon admission and the incidence rate of patients with clinical samples tested positive are reported in Table 1. Figure 1 shows the distribution of carriage or colonisation/infection with *P. aeruginosa* of the study population. The distribution of initial positive sites is reported in Table 2.

The overall mean length of stay in an ICU before *P. aeruginosa* carriage or colonisation/infection was acquired was 16.6 days. The frequency of ICU-acquired *P. aeruginosa* carriage or colonisation/infection 7 and 14 days after hospitalisation was 23.4 % and 57.8 %, re-

Table 1 Prevalence rate of patients tested positive upon admission and incidence of colonisation/infection during the stay according to the size and type of ICU

	Type of ICU (number of admissions)	Prevalence rate of patient tested positive upon admission per 100 patients *(n)*	Incidence rate of patients with clinical samples tested positive *(n)*
Besançon University hospital	SICU (841) MICU (805)	2.73 (23) 6.71 (54)	9.6 (73^a+8^b) 14.0 (94^a+19^b)
Vesoul community hospital	MICU (656)	4.57 (30)	3.4 (21^a+1^b)
Montbéliard community hospital	MICU (707)	2.12 (15)	0.3 (6^a+0^b)
Total		4.05 (122)	7.05 (194^a+28^b)

[a] ICU-acquired, [b] Upon admission

Table 2 Distribution of initial sites of carriage and/or colonisation/infection

	Site	Number of patients positive on admission *(n)*	Number of patients positive during hospitalisation *(n)*
Carriage	Rectum	60	158
	Respiratory tract[a]	34	125
Colonisation/infection	Broncho-alveolar lavage (with $\geq 10^4$ CFU/ml)	13	15
	Urine (with $\geq 10^5$ CFU/ml)	6	16
	Catheter	1	9
	Pus/draining wound	2	8
	Blood	2	4
	Cerebrospinal fluid	0	2
	Superficial swab	4	12
	Gynaecological swab	0	2
Total		122	351

[a] Nasal swab and tracheal aspiration

Table 3 Site of initial colonisation with clones of *P. aeruginosa* identified by PFGE

Number of carriage or colonisation/infection belonging to	Site of initial carriage or colonisation/infection			
	Carriage			Colonisation/infection
	Intestinal tract	Respiratory tract[a]	Both	
Unique PFGE pattern ($n = 69$)	30	38	0	1
Micro-epidemic[b] PFGE pattern ($n = 32$)	15	9	1	7
Epidemic[c] PFGE pattern ($n = 66$)	26	26	5	9
Major epidemic PFGE pattern ($n = 41$)	16	11	4	10
Total	87	84	10	27

[a] Including broncho-alveolar lavage, tracheal aspiration and nose
[b] PFGE pattern including two non-replicate isolates
[c] PFGE pattern including more than two non-replicate isolates

spectively. Carriers or colonised/infected patients had a longer median stay in ICUs than other patients (26.3 versus 7.4 days).

Of the 3009 patients admitted to the ICUs during the survey, 377 were identified as carriers, 116 of them had positive clinical cultures later and 104 with identical PFGE patterns. The sensitivity, specificity, positive and negative predictive values of epidemiological screening for the detection of further positive clinical cultures with identical PFGE pattern were 53.6 %, 90.3 %, 27.6 % and 96.6 %, respectively.

Routes of colonisation/infection

Of 184 patients with at least one ICU-acquired colonisation/infection, 104 had previously been identified as carriers [63 out of 221 rectal carriage(28.5 %) and 41 out of 156 respiratory tract carriage (26.3 %)] with similar genotypes and 80 patients were not identified as carriers. Patients colonised by one clone of *P. aeruginosa* and with a positive clinical culture ($n = 12$) of a different clone were considered as not having been previously colonised.

Seventy-six patients had positive broncho-alveolar lavage for *P. aeruginosa*. Of these, 48 had previously been colonised (24 had respiratory tract colonisation, 20 had intestinal colonisation and 4 had both). In 52 cases (68.4 %) these episodes of broncho-alveolar colonisation/infection were classified as ICU-acquired. Sixty-four patients had positive urine samples for *P. aeruginosa*. Of these, 44 had previously been colonised (33 had intestinal colonisation, 14 had respiratory tract colonisation and 3 had both). In 50 cases (78.1 %) these episodes of urinary tract colonisation/infection were classified as ICU-acquired.

Incidence of cross-colonisation

We identified 208 cases of carriage and/or colonisations/infections with a single clone, which lasted at least 7 days. These episodes occurred in 193 individual patients (15 patients were colonised/infected for at least 1 week by two different clones).

The 208 non-replicate isolates typed yielded 101 major DNA patterns: 69 unique patterns, 16 patterns including two isolates, 8 patterns including 3 isolates, 3

patterns including 4 isolates, 1 pattern including 6 isolates, 2 patterns including 7 isolates, 1 pattern including 10 isolates and 1 pattern including 41 isolates. The dates of stay, the location of hospitalisation and transfers of patients colonised/infected by a strain of *P. aeruginosa* with a PFGE pattern including 2 or more isolates (32 patterns) are consistent with there being cross-transmission. Indeed, cross-transmission would have been possible, in both space and time, for all these patterns except three. The outbreak involving the epidemic clone, including 41 isolates, was reported in a previous paper [13].

Finally, of the 208 identified cases of colonisation, 107 (51.4 %) were acquired via cross-transmission and the 101 others probably originated from endogenous sources. The initial positive sites of the 208 clones characterised by PFGE are summarised in Table 3. They are equally distributed between intestinal and respiratory tract sources. Cross-transmission was significantly higher [$p = 0.0002$, RR = 1.49 (1.17–1.90)] in the SICU [68.4 % (52/76)] than in the MICU [47.8 % (44/92) at Besançon, 36.3 % (8/22) at Vesoul and 37.5 % (3/8) at Montbéliard].

Discussion

Pseudomonas aeruginosa has a remarkable ability for colonising certain subpopulations of patients. Intensive care units have been clearly established as endemic settings for this pathogen. Several risk factors are significantly associated with the acquisition of this bacterium in ICUs: length of stay [14], extensive use of antibiotics [15, 16, 17], use of indwelling urinary catheters [17], mechanical ventilation [14] and alcoholism [14, 17]. Although colonisation by *P. aeruginosa* frequently precedes overt infection [17], the original source of the organism and the precise mode of transmission are often unclear. Some authors have suggested that endogenous colonisation occurs rather than exogenous nosocomial acquisition [8, 9, 18]. However, the environment has been clearly shown to be a source and to be involved in horizontal transmission [19]. The relative importance of different colonisation pathways has rarely been determined by genotyping.

Comparison of incidence rates with those in other ICUs is difficult, mainly because of differences in patient populations. However, the incidences of *P. aeruginosa* carriage and colonisation/infection upon admission and during hospitalisation are consistent with other French [20] and European [21] studies. The rate of colonisation upon admission was below 5 %, and 74.2 % of carriage or colonisations/infections were ICU-acquired. The prevalence of carriers may reflect the pressure of colonisation, which did not differ significantly between the four ICUs studied here. Thus, the difference in the incidence of colonisation/infection between the university hospital and the other hospitals may be associated with differences in the two populations (underlying disease, severity of illness), or with differences in the intensity of care, antibiotic pressure or sampling policy.

The two principal sites of colonisation/infection were the bronchopulmonary (35.8 %) and urinary tracts (30.2 %). These two types of specimen gave highly significant levels of infection. Screening samples may enable us to predict further *P. aeruginosa* infection. The specificity and negative predictive value of screening make this technique useful for screening cultures. Weekly sampling would appear to be a good compromise between an additional workload and sufficient sensitivity of the technique. If these samples are negative, probabilistic antibiotic treatment could minimise the risk of *P. aeruginosa* infection and limit the use of anti-pseudomonas antibiotics. In the absence of a screening programme, 55.2 % (261/473) of all patients tested positive for *P. aeruginosa* would have remained unknown. Thus, *P. aeruginosa* clinical cultures do not present the full epidemiological picture. Surveillance cultures are indispensable in epidemiological studies of *P. aeruginosa* in ICUs.

Our study provides a detailed analysis of colonisation routes in ICUs. More than half of the ICU-acquired colonisations/infections (56.5 %) were preceded by colonisation with a genotypically identical strain. The intestinal tract is regarded as the most important reservoir of this bacterium [9, 21]. Our observations suggest that the respiratory tract is also important, and confirm the lower respiratory tract carriage of *P. aeruginosa* in patients admitted to ICUs [14]. However, our detection methods for *P. aeruginosa* may not be sensitive enough for rectal swabbing and thus cases of rectal colonisation may have been missed. Patients may, therefore, have unidentified intestinal reservoirs responsible for further respiratory tract colonisations. Thus, the pattern and the route of tracheobronchial colonisation appear to differ from those of enteric Gram-negative bacteria. A screening programme for *P. aeruginosa* carriage in ICUs must therefore include both rectal and respiratory tract specimens if it is to identify a large proportion of the positive patients.

Our findings suggest that cross-colonisation may be an important means of *P. aeruginosa* colonisation and infection, although the overall incidence rates do not suggest the occurrence of an outbreak. Indeed, we identified an epidemic clone of *P. aeruginosa* that had been propagated for at least 30 months in a SICU without significant changes in the incidence of colonisation/infection [13]. The possibility of cross-colonisation in non-epidemic situations has rarely been studied. Bergmans et al. [21] studied 100 patients admitted to an ICU ward; cross-colonisation accounted for 50 % of all cases of acquired *P. aeruginosa* colonisation, the other 50 % of patients were probably colonised from endogenous

sources. Bonten et al. [9] did not find cross-transmission to be important, with only 8% (3/44) of patients colonised in this way. The relative importance of endogenous and exogenous sources of *P. aeruginosa* is unclear. Our analysis of cross-transmission is based on the carriage or colonisation/infection of patients who stayed in ICUs for a long period (at least 7 days). Length of stay in ICU is an identified risk factor for *P. aeruginosa* acquisition. Subsequent analysis of the cases depending on the duration of colonisation may therefore correspond to skewed recruitment. Differences in cross-transmission rates between the wards studied may be due to differences in compliance with infection-control measures, which were not monitored. The epidemic clone previously described [13] was responsible for 73% of the cases of cross-transmission in the SICU, which led to an overestimate of cross-colonisation. In its absence, rates of cross-transmission decreased from 51.4% to 40.1%.

In conclusion, the epidemiological pattern of *P. aeruginosa* infection and colonisation may be described as a silent epidemic, similar to those of other nosocomial pathogens, such as vancomycin-resistant enterococci. Our results also show that molecular typing is a potentially powerful screening method for continuous quality improvement. Epidemiological surveillance combined with PFGE should help to improve the targetting of preventive strategies.

References

1. Jarvis WR, Martone WJ (1992) Predominant pathogens in hospital infections. J Antimicrob Chemother 29 (Suppl A):19–24
2. Spencer RC (1996) Predominant pathogens found in the European prevalence of infection in intensive care study. Eur J Clin Microbiol Infect Dis 15: 281–285
3. Richards MJ, Edwards JR, Culver DH, Gaynes RP (1999) Nosocomial infections in medical care units in the United States. National Nosocomial Infections Surveillance System. Crit Care Med 27: 887–892
4. Cross AS (1985) Evolving epidemiology of *Pseudomonas aeruginosa* infections. Eur J Clin Microbiol 4: 156–159
5. Fagon JY, Chastre J, Vuagnat A, Trouillet JL, Novara A, Gibert C (1996) Nosocomial pneumonia and mortality among patients in intensive care units. JAMA 275: 866–869
6. Bert F, Maubec E, Bruneau B, Berry P, Lambert-Zechovsky N (1998) Multi-resistant *Pseudomonas aeruginosa* lung outbreak associated with contaminated tap water in a neurosurgery intensive care unit. J Hosp Infect 39: 53–62
7. Cobben NAM, Drent M, Jonkers M, Wouters EFM, Vaneechoutte M, Stobberingh EE (1996) Outbreak of severe *Pseudomonas aeruginosa* respiratory infections due to contaminated nebulizers. J Hosp Infect 33: 63–70
8. Grüner E, Kropec A, Huebner J, Altwegg M, Dashner F (1994) Ribotyping of *Pseudomonas aeruginosa* strains isolated from surgical intensive care patients. J Infect Dis 167: 1216–1220
9. Bonten MJ, Bergmans DC, Speijer H, Stobberingh EE (1999) Characteristics of polyclonal endemicity of colonization in intensive care units. Implications for infection control. Am J Respir Crit Care Med 160: 1212–1219
10. Speijer H, Savelkoul PHM, Bonten MJ, Stobberingh EE, Tjhie JHT (1999) Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit. J Clin Microbiol 37: 3654–3661
11. Talon D, Cailleaux V, Thouverez M, Michel-Briand Y (1996) Discriminatory power and usefulness of pulsed-field gel electrophoresis in epidemiological studies of *Pseudomonas aeruginosa*. J Hosp Infect 32: 135–145
12. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33: 2233–2239
13. Bertrand X, Bailly P, Blasco G, Balvay P, Boillot A, Talon D (2000) Large outbreak in a surgical intensive care unit of colonization or infection with *Pseudomonas aeruginosa* that overexpressed an active efflux pump. Clin Infect Dis 31: E9–E14
14. Talon D, Mulin B, Rouget C, Bailly P, Thouverez M, Viel JF (1998) Risks and routes for ventilator-associated pneumonia with *Pseudomonas aeruginosa.* Am J Respir Crit Care Med 157: 978–984
15. Baddour LM, Hicks DV, Tayidi MM, Roberts SK, Walker E, Smith RJ, Sweitzer DS, Herrington JA, Painter BG (1995) Risk factor assessment for the acquisition of fluoroquinolone-resistant isolates of *Pseudomonas aeruginosa* in a community-based hospital. Microb Drug Resist 1: 219–222
16. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH (1999) Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. Antimicrob Agents Chemother 43: 1379–1382
17. Blanc DS, Petignat C, Janin B, Bille J, Francioli P (1998) Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: a prospective epidemiologic study. Clin Microbiol Infect 4: 242–247
18. Talon D, Capellier G, Boillot A, Michel-Briand Y (1995) Use of pulsed-field gel electrophoresis as an epidemiological tool during an outbreak of *Pseudomonas aeruginosa* lung infections in an intensive care unit. Intensive Care Med 21: 996–1002
19. Panzig B, Schroder G, Pitten FA, Grundling M (1999) A large outbreak of multiresistant *Pseudomonas aeruginosa* strains in north-eastern Germany. J Antimicrob Chemother 43: 415–418
20. Cattoen C, Levent T, Grandbastien B, Descamps D, Bouillet L, Coignard B, Beaucaire G, réseau de microbiologistes de l'ARECLIN (1999) Observatoire régional *Pseudomonas aeruginosa* du Nord-Pas-de-Calais : données épidémiologiques et microbiologiques. Med Mal Inf 29: 160–166
21. Bergmans DC, Bonten MJ, Van Tiel FH, Gaillard CA, Van der Geest S, Wilting RM, De Leeuw PW, Stobberingh EE (1998) Cross-colonization with *Pseudomonas aeruginosa* of patients in an intensive care unit. Thorax 53: 1053–1058