

## High Genetic Diversity among *Stenotrophomonas maltophilia* Strains Despite Their Originating at a Single Hospital

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**The levels of genetic relatedness of 139 *Stenotrophomonas maltophilia* strains recovered from 105 hospitalized non-cystic fibrosis patients (51% from medical wards, 35% from intensive care units, and 14% from surgical wards) and 7 environmental sources in the same hospital setting during a 4-year period were typed by the pulsed-field gel electrophoresis (PFGE) technique. A total of 99 well-defined distinct *Xba*I PFGE patterns were identified (Simpson's discrimination index, 0.996). The dendrogram showed a Dice similarity coefficient ranging from 28 to 80%. Two major clusters (I and II), three minor clusters (III, IV, and V), and two independent branches were observed when using a 36% Dice coefficient, indicating a high diversity of genetic relatedness. It is of note that 84% of strains were grouped within two major clonal lineages. No special cluster gathering was found among strains belonging to the same sample type specimen, patients' infection or colonization status, and ward of precedence. Despite this fact, three different clones (A, B, and C) recovered from respiratory samples from six, three, and two patients, respectively, and two clones, D and E, in two bacteremic patients each, were identified. Isolation of an *S. maltophilia* strain belonging to the clone A profile in a bronchoscope demonstrated a common source from this clone. This study revealed a high genetic diversity of *S. maltophilia* isolates despite their origin from a single hospital, which may be related to the wide environmental distribution of this pathogen. However, few clones could be transmitted among different patients, yielding outbreak situations.**

*Stenotrophomonas maltophilia* is found in a great variety of environmental sources, including plant tissues, water, soil, sediments, and organic residues. Moreover, it has been identified in the hospital environment and has been recognized in human specimens (8). As a result of this adaptation to different habitats, great metabolic heterogeneity has been found (3, 15). During the last few years, *S. maltophilia* has risen to prominence, causing a wide spectrum of hospital-acquired infections, particularly pneumonia and bacteremia in severely debilitated or immunosuppressed patients with chronic underlying diseases admitted to intensive care units (ICUs) (8, 9, 11, 17, 19, 20). This situation may have been facilitated by its resistance to most of the currently available broad-spectrum antimicrobials (4, 30) and its ability to rapidly increase their multiresistance phenotype (1, 13).

On the other hand, information about the genomic relationship within this species and the degree of relationship among nosocomial strains remains scarce (3, 15). Nowadays, pulsed-field gel electrophoresis (PFGE) is considered to be the reference genotyping method for *S. maltophilia* and has been used not only to identify outbreaks and possible reservoirs and modes of transmission involving this species but also to understand population structure from isolates from cystic fibrosis patients (26). In addition, this technique provides a broad look at the whole chromosome of the microorganisms and is useful

for determining the genetic relatedness among isolates of a given species by comparison of their macrorestriction profiles (2). In a previous work, Berg et al. (3) determined the diversity of 40 clinical and environmental *S. maltophilia* isolates and analyzed phenotypic profiles and molecular types by several molecular methods. In their study, the most discriminatory method was PFGE under *Dra*I digestion. In our study, we have determined the genetic relatedness and epidemiological links among 139 isolates recovered in the same hospital over a long period by using profiles generated by PFGE under *Xba*I restriction. Moreover, detection of cross-transmissions among different patients is also presented.

### MATERIALS AND METHODS

**Bacterial strains.** One hundred thirty-nine *S. maltophilia* isolates (132 from 105 non-cystic fibrosis hospitalized patients and 7 from different hospital environments) were collected from 1995 to 1998 at Ramón y Cajal Hospital, a 1,200-bed university teaching hospital in northeast Madrid, Spain. Clinical isolates included all isolates from bacteremic episodes and 30% of isolates from nonblood samples recovered in our institution during the period studied. Moreover, all *S. maltophilia* isolates within a specific period and unit were included when an outbreak situation was suspected. The isolation sites of clinical isolates were the respiratory tract ( $n = 79$ ), blood ( $n = 19$ ), wounds ( $n = 15$ ), urine ( $n = 5$ ), rectal swabs ( $n = 2$ ), peritoneal fluids ( $n = 2$ ), ocular prosthesis ( $n = 2$ ), and other sites ( $n = 8$ ). All of these strains were previously analyzed for antimicrobial susceptibility profile (30). A control group including 12 *S. maltophilia* isolates was selected for molecular typing. This group included 10 clinical isolates (4 respiratory tract isolates, 3 blood isolates, and 1 isolate each from urine, ascitic fluid, and drainage) recovered in different Spanish hospitals in different geographic areas, one isolate from a tuna sample, and the *S. maltophilia* ATCC 13637 strain. Biochemical identification was performed with both the API-20NE (bioMérieux, La Balme Les Grottes, France) and WIDER (Fco. Soria Melguizo, Madrid, Spain) systems.

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**PFGE.** Chromosomal DNA was prepared in agarose plugs as described previously (31). After digestion with endonuclease *XbaI* (30 U; Roche Diagnostics, Barcelona, Spain), restriction fragments were resolved by PFGE with the CHEF-DRII system (Bio-Rad, Hemel Hempstead, United Kingdom). A second enzyme, *SpeI* (20 U; Roche), was used to confirm identical restriction *XbaI* profiles. The electrophoresis conditions were as follows: (i) for *XbaI*, pulse times were ramped from 10 to 60 s over 24 h at 5.4 V/cm with a second ramp from 5 to 20 s over 5 h at 5.4 V/cm at 12°C (30); and (ii) for *SpeI*, pulse times ranged from 25 to 45 s over 20 h at 6.0 V/cm at 12°C (22). Standard lambda ladders of 48.5-kbp concatemers (Roche) were run as molecular weight markers. Restriction fragments were visually compared and interpreted according to previous established criteria (27). Isolates with identical profiles were considered to represent a single strain.

**Computer-monitored fingerprinting and discriminatory power analysis.** Computer analysis of the PFGE banding patterns was performed with Bio-Rad Molecular Analyst software. The images analyzed included two reference lanes representing concatemer phage lambda ladders. Bands were automatically assigned by the computer and were manually corrected after observation in the computer screen. Only fragments exceeding 97.0 kbp were included in the analysis. Fingerprinting profiles were scored for the presence and absence of bands at given molecular weights, and strains that differed by one band were assigned different PFGE profiles, codified by a number. Only one profile was represented in the dendrogram for the isolates with identical *XbaI* profiles. The Dice correlation coefficient was used to analyze the similarities of the banding patterns (21). Strains with an identical PFGE banding pattern (100% similarity coefficient) were considered isogenic strains. Moreover, as previously stated (5, 31), closely related strains within the same clone were those with a similarity coefficient ranging from 80 to 95%. Clustering was based on the unweighted pair group method with arithmetic averages (UPGMA). The tolerance position was 1%.

The discriminatory power of PFGE between isolates from Ramón y Cajal hospital and isolates from the control group was evaluated by using Simpson's index of diversity (16), which expresses the probability that two unrelated strains will be placed in two different typing groups.

**Patient clinical data.** For all patients, demographic information and the presence of repetitive *S. maltophilia*-positive cultures and any other organism in the same positive culture were recorded. Upon identification of an outbreak situation, defined by indistinguishable PFGE profiles of isolates, the medical charts of patients colonized or infected with the outbreak strains were retrospectively reviewed. Hospital-acquired infections were classified according to the Centers for Disease Control and Prevention definitions (12). Clinical infection or colonization status by *S. maltophilia* was considered according to clinical judgment, high or moderate bacterial counts, tissue invasion, repetitive *S. maltophilia* isolation, monomicrobial culture, and antibiotic treatment response. Respiratory infection was considered when evidence of pulmonary infiltrates with X rays, fever (>38°C), cough, and respiratory function deterioration was observed (18, 20, 29). Nosocomial acquisition was considered as being 72 h after hospital admissions or within 30 days of a surgical patient's discharge (12).

## RESULTS

***S. maltophilia* macrorestriction profile analysis.** The PFGE conditions under *XbaI* restriction resolved DNA fragment sizes ranging from <48 kbp to >1,000 kb. Approximately 10 bands (>97.0 to about <1,000.0 kbp) were identified, and these were used for the scoring and computer analysis of *S. maltophilia* strains. The control typing group, including 12 strains from outside our clinical setting, displayed a Simpson's index of 1 (12 different profiles) with a genetic similarity coefficient ranging from 34 to 75%. Well-defined different profiles were obtained for 99 of the 139 isolates recovered in our hospital, and the Simpson's discrimination index value was 0.996.

The dendrogram generated by computer-aided genotype analysis based on the unique PFGE patterns of all 139 *S. maltophilia* strains ranged from 28 to 80% similarity (Fig. 1). Two major clusters (designated clusters I and II, three minor clusters (III, IV, and V), and two independent branches (represented by profiles 6 and 62) were observed with a 36%

similarity coefficient. The major clusters, I and II, included 37.7 and 38.5%, respectively, of the strains studied, whereas the minor clusters III, IV, and V only contained 3.6, 2.2, and 5.8%, respectively, of the strains studied. Profiles 6 and 62 did not fit into any of the five clusters even at similarity values of 28 and 26%, respectively. The origin of *S. maltophilia* clinical isolates for each defined *XbaI* profile is included in the legend to Fig. 1.

Twenty-nine percent of isolates (40 of 139) showed an identical banding pattern to at least 1 other isolate. Repetitive isolates during the period studied were recovered in 18 patients: 14 patients presented two isolates each with identical profiles (100% similarity coefficient), and 4 patients presented three consecutive isolates each showing identical profiles (100% similarity coefficient). Persistence of *S. maltophilia* in these patients ranged from 2 to 25 days ( $8.5 \pm 6.5$  days).

It is of note that five isolates with different profiles (profiles 1, 4, 38, 52, and 61) were responsible for cross-transmission in 15 patients (see below). Profiles 1 (clone A), 4 (clone B), and 38 (clone C) were recovered from respiratory sources and were detected in six, three, and two patients, respectively. Moreover, profile 52 (clone D) was recovered from blood cultures in two patients, and profile 62 (clone E) was recovered from blood cultures in two patients. Strains belonging to these profiles represented different cross-infection situations among different patients and were associated with clusters II, II, V, and II and a nonclustered profile, respectively. These results were confirmed by *SpeI* restriction.

The degree of heterogeneity among control stains was also high. No new cluster or independent branches were observed when control strains were integrated in the dendrogram (data not shown).

**Demographic data and patient characteristics.** One hundred thirty-two isolates were recovered from 105 patients. We had access to demographic data for 97 patients (61 male and 36 female). Thirty-five percent of patients (34 of 97) were hospitalized in different ICUs: Medical ICU ( $n = 15$ ), Neurosurgical ICU ( $n = 6$ ), Digestive Tract Surgical ICU ( $n = 7$ ), Cardiovascular Surgery ICU ( $n = 3$ ), and Pediatric ICU ( $n = 3$ ). The other patients were from the Pulmonary Diseases ( $n = 12$ ), Digestive Tract Surgery ( $n = 13$ ), Infectious Diseases ( $n = 5$ ), and Hematology ( $n = 4$ ) Wards, while the rest of the patients were located in different units. *S. maltophilia* appeared as a monomicrobial culture in 13 of 19 (68.4%) blood cultures, 21 of 79 (26.6%) respiratory samples, 1 of 14 (7.1%) samples from wounds and cutaneous tissues, and 6 of 12 (50.0%) samples from other locations. In combination with other organisms, *S. maltophilia* appeared mainly associated with normal flora in respiratory samples (22%) and with gram-positive organisms (mainly coagulase-negative staphylococci) in blood (26%) and wound (61%) cultures.

**Detection of cross-transmission.** A total of five episodes of cross-transmission were detected during the period studied: (i) three episodes involved three different *S. maltophilia* strains (clones A, B, and C) recovered from respiratory samples from 11 patients grouped as 6, 3, and 2 patients, and (ii) two episodes involved two different *S. maltophilia* strains (clones D and E) implicated in episodes of bacteremia in 4 patients. Risk factors, previously defined by others (7, 9, 11, 19) for *S. maltophilia* acquisition in all of these patients are shown in Table 1.

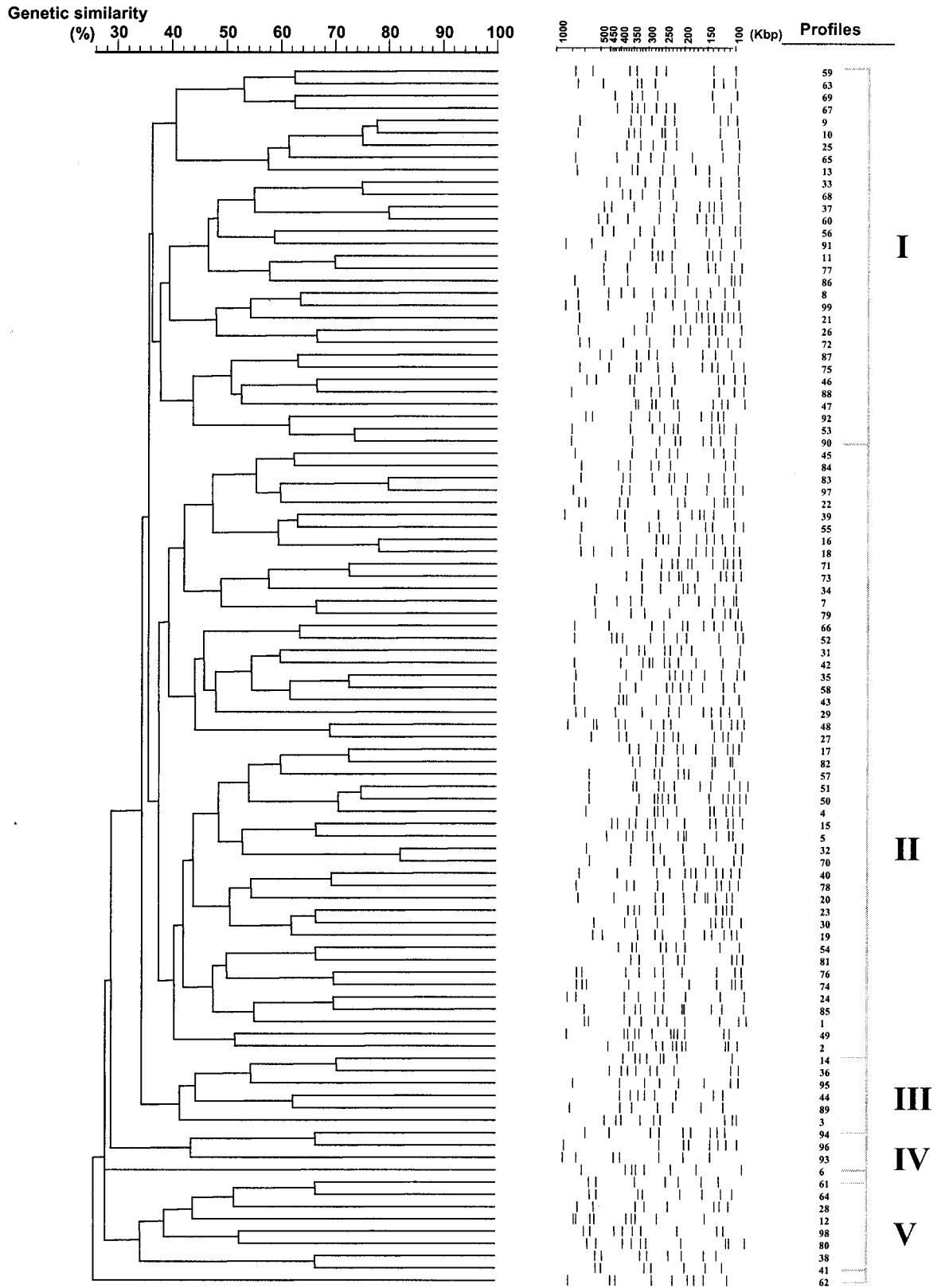


FIG. 1. Phylogenetic analysis of digitized 99 PFGE *Xba*I profiles of 132 clinical and 7 environmental *S. maltophilia* isolates recovered in our hospital during a 4-year period. The dendrogram was constructed with PFGE data by similarity and clustering analysis by using the Dice coefficient and UPGMA with the Molecular Analysts software. A percent genetic similarity scale is shown above the dendrogram. Profile types are marked on the left in arabic numerals, and the clusters (cutoff value of 36% similarity) are marked on the right in roman numerals. The clinical origins of the isolates are as follows: respiratory specimens, profiles 1 to 51; blood, profiles 52 to 67; wounds, profiles 70 to 77; organic fluid, profiles 69 and 79; ocular specimens, profiles 68 and 78; urine, profiles 80 to 83; stool, profiles 84 and 85; environmental isolates, profiles 1, 86 to 91; and others, profiles 92 to 99.

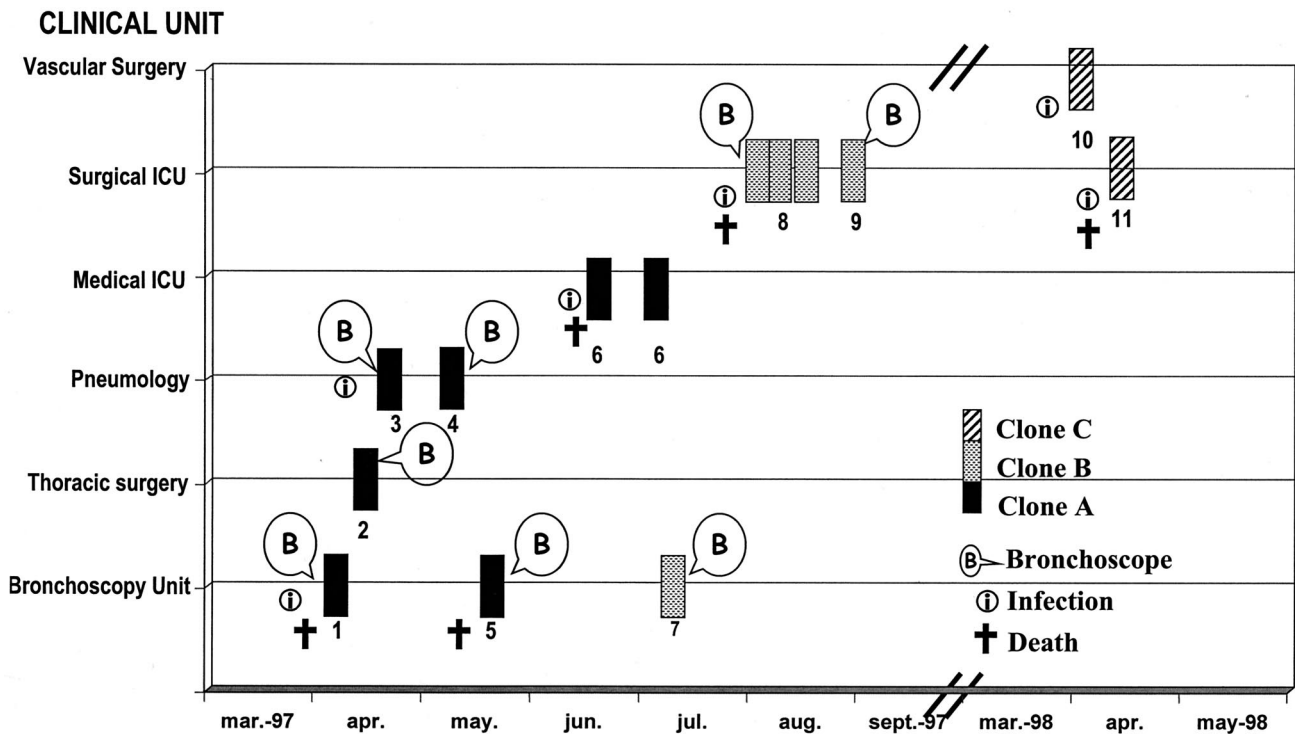


FIG. 2. Progression of clones A, B, and C of *S. maltophilia*. Boxes represent one isolate each; the number below corresponds to the number of the patient. The time period, ward of precedence, bronchoscopy procedure, infection, and patient deaths are also indicated.

Figure 2 shows progression of the respiratory outbreaks, the number of patients involved, the patients who underwent a bronchoscope procedure, and the patients' origin.

Seven isolates with an indistinguishable profile (clone A, profile 1) were recovered from respiratory sources from one outpatient (patient 5) and from five patients hospitalized in different wards (patients 1, 2, 3, 4, and 6) during a period of 84 days (Fig. 2). With the exception of patient 6, all patients had undergone a bronchoscope procedure. In patient 6, *S. maltophilia* was isolated twice during a period of 13 days. It is of note that as a result of an epidemiological investigation performed by our Hospital Infection Control Committee, an *S. maltophilia* strain with the same PFGE profile (profile 1) was cultured in a fiberbronchoscope in our Bronchoscopy Unit (Fig. 3). Cross-transmission of clone B was also demonstrated. Five strains with an indistinguishable pulse type (profile 4) were collected over a period of 47 days from an outpatient followed by the Pulmonary Diseases Unit (patient 7) and from two patients (patients 8 and 9) from the Digestive Tract Surgical ICU. *S. maltophilia* was isolated three times from a liver transplant recipient (patient 8) over an 11-day period as a monomicrobial culture. It is of note that a bronchoscope procedure was carried out in all patients, but epidemiological investigation failed to identify *S. maltophilia* in any of the fiberbronchoscopes used. Moreover, the same clone (clone C, profile 38) was observed in respiratory samples from two patients (patients 10 and 11) with a difference of 12 days in isolation. Neither of them had undergone a bronchoscope procedure, but both had undergone surgery. After the last patients positive for clones A, B and C, no further cases were detected.

The same *S. maltophilia* clone (clone D, profile 52) was identified in two bacteremic patients (patients 12 and 13) (Table 1) hospitalized in the Pediatric ICU and in the Rheumatology Ward, respectively. Finally, the same clone (clone E, profile 61) was identified in blood cultures in two bacteremic

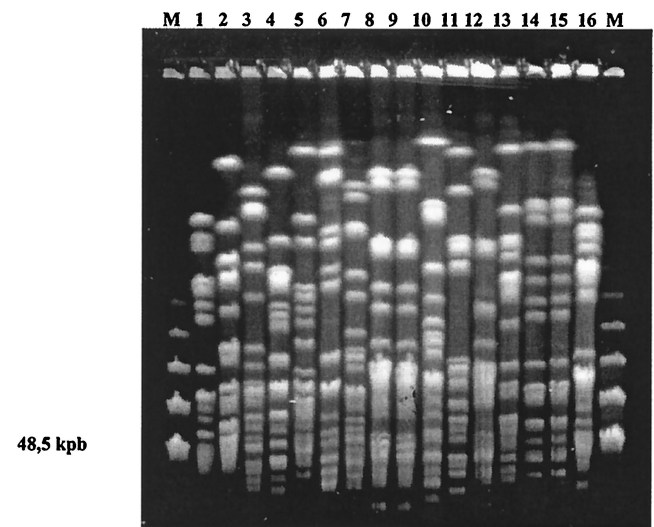


FIG. 3. *Xba*I profiles obtained in *S. maltophilia* isolates from blood from 16 patients. Two episodes of cross-transmission were suspected (clone E, lanes 8 and 9, and clone D, lanes 14 and 15). Lane M, molecular size marker.



patients (patients 14 and 15) (Table 1) hospitalized in the Neurosurgery ICU and the Hematology Ward.

DISCUSSION

Several multiresistant pathogens, including both gram-negative and gram-positive organisms, have been recognized during the last decades as nosocomial pathogens. Most of these organisms, such as *Acinetobacter baumannii*, *Enterobacter* spp., *Klebsiella pneumoniae*, or methicillin-resistant *Staphylococcus aureus*, are generally found in epidemic or outbreak situations (10). In some institutions, these organisms have represented a clinical concern as a result of endemic isolation in hospitalized patients. Moreover, isolation of *S. maltophilia* in the nosocomial setting is not an infrequent situation, and different outbreaks have been identified (28). Until the present, few studies have focused on the genetic relatedness of *S. maltophilia* isolates, and most of them only included a small number of both environmental and nosocomial isolates (3, 7, 8, 20) or were focused on cystic fibrosis isolates (5, 31). In the present work, we studied a total of 139 *S. maltophilia* isolates from non-cystic fibrosis patients and recovered in the same hospital during a 4-year period and investigated their genetic relatedness.

The low interstrain variability of phenotypic typing methods in *S. maltophilia* (23, 34) makes molecular techniques, particularly PFGE, widely accepted methods for epidemiological typing of this species (3, 20, 26). As previously demonstrated in *S. maltophilia*, PFGE under *Xba*I restriction is appropriate for tracing isolates and generating stable profiles in long-term-colonized patients. In the present study, the discriminatory ability of the *Xba*I PFGE technique reached a Simpson coefficient value of 1.0, allowing us to establish the molecular relationships among clinical *S. maltophilia* isolates recovered in a single hospital. Despite being from the same institution, the majority of strains tested (71.7%) displayed different PFGE profiles, and only identical genotypic patterns were observed in isolates recovered from 15 patients (14.3%), suggesting a common source of these strains. Moreover, the PFGE fingerprinting analysis for the strains tested revealed a great discriminatory ability, resulting in a Simpson coefficient value of 0.996. Berg et al. (3), using PFGE under *Dra*I restriction, also demonstrated a high intraspecific diversity in *S. maltophilia* clinical and environmental isolates from different locations and niches. A similar conclusion was obtained with arbitrarily primed PCR (32), ERIC-PCR (6), randomly amplified polymorphic DNA (35), and amplified-fragment length polymorphism (15) techniques in *S. maltophilia* isolates from different environments. However, the high intraspecific diversity decreased, because closely related strains could be detected in chronically colonized cystic fibrosis patients with this pathogen (5).

It is of note that unlike isolates from cystic fibrosis patients (5, 31), isolates serially recovered from the same patient showed identical PFGE profiles (100% similarity). In a previous work from our group (31), which included only isolates from cystic fibrosis patients, the presence of strains with a similarity coefficient ranging from 80 to 95% was observed within the same patient. This fact represents the dynamic situation of *S. maltophilia* isolates in the pulmonary environment in cystic fibrosis patients, which may persist for more than 6 years (31). On the contrary, nosocomial patients present a

TABLE 1. Comorbidities and potential risk factors for *S. maltophilia* infection and colonization in patients sharing similar clone types<sup>a</sup>

Patient (clone)	Age <sup>b</sup> (gender)	Infection or colonization type <sup>c</sup>	Ward	Central venous catheter	Mechanical ventilation	Bronchoscope procedure	Underlying illness	Previous hospitalization (< 6 mo)	Previous surgery (< 3 mo)	Previous antimicrobial therapy (< 1 mo)	Antimicrobial therapy	Outcome
1 (A)	58 (M)	Respiratory (I)	ICU	Yes	Yes	Yes	Malignancy	Yes	Yes	AMX	CAZ + TOB	Death
2 (A)	82 (M)	Respiratory (I)	Thoracic Surgery	No	No	Yes	COPD	Yes	No	AMX	ERY	Recovered
3 (A)	24 (F)	Respiratory (C)	Pneumology	No	No	Yes	Tuberculosis	Yes	No	CRO	no	Recovered
4 (A)	76 (M)	Respiratory (C)	Outpatient	No	No	Yes	COPD	No	No	CRO	CIP	Recovered
5 (A)	76 (M)	Respiratory (C)	Outpatient	No	No	Yes	COPD, anaemia	No	No	No	No	Death
6 (A)	60 (F)	Respiratory (I)	ICU	Yes	Yes	No	COPD, anaemia, neutropenia, hypertension	Yes	No	AMX + TOB	MER + VAN	Death
7 (B)	70 (M)	Respiratory (C)	Outpatient	No	No	Yes	Malignancy	No	No	No	No	Recovered
8 (B)	61 (F)	Respiratory (I)	ICU	Yes	Yes	Yes	Liver transplant	Yes	No	VAN + TOB + MER	CIP	Death
9 (B)	73 (M)	Respiratory (C)	ICU	No	No	Yes	COPD, traumaism	No	No	No	AMX	Recovered
10 (C)	75 (M)	Respiratory (I)	Vascular Surgery	No	Yes	No	Diabetes	Yes	Yes	CTX + TOB + MER	PIP-TAZ	Recovered
11 (C)	77 (M)	Respiratory (I)	ICU	Yes	No	No	COPD, heart failure	No	No	VAN + FLZ	AMP + TOB	Death
12 (D)	18 (M)	Bacteremia (I)	ICU	Yes	No	No	Malignancy, neutropenia	No	No	No	No	Recovered
13 (D)	56 (F)	Bacteremia (I)	Rheumatology	Yes	No	No	Pleurocarditis, anaemia	No	No	No	No	Recovered
14 (E)	60 (F)	Bacteremia (I)	ICU	Yes	Yes	No	Craniocebral traumaism	Yes	No	No	No	Recovered
15 (E)	64 (F)	Bacteremia (I)	Hematology	Yes	Yes	No	Malignancy, neutropenia, diabetes	Yes	No	No	CAZ + VAN	Recovered

<sup>a</sup> M, male; F, female; AMX, amoxicillin-clavulanate; AMP, ampicillin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; FLZ, fluconazol; MER, meropenem; PIP-TAZ, piperacillin-tazobactam; TOB, tobramycin; VAN, vancomycin; COPD, chronic obstructive pulmonary disease.  
<sup>b</sup> Ages are given in years.  
<sup>c</sup> Infection (I) or colonization (C) is indicated parenthetically.

lower length of persistence of *S. maltophilia* isolates ( $8.5 \pm 6.5$  days in the present study).

On the basis of *Xba*I PFGE profiles, 99 distinct profiles were identified among 139 isolates studied. Isolates were grouped into five phylogenetic clusters when a cutoff of 36.0% in the genetic similarity scale was considered. The resolved profiles showed a great genetic distance, and the genetic diversity extended from low (28.0%) to high (80.0%) similarity. These results clearly demonstrated the low homogeneity level of *S. maltophilia* strains, irrespective of the time frame of collection in the same clinical setting. Interestingly, the major clonal lineages (I and II) were nearly grouped 84.0% of the strains analyzed.

As previously noted, prolonged hospitalization and broad-spectrum antimicrobial therapy may facilitate the selection of *S. maltophilia* from respiratory or gastrointestinal locations (32, 33). In our study, the great number of distinct *S. maltophilia* profiles may reflect a wide environmental distribution of this species, allowing acquisition from different environmental sources. Moreover, *S. maltophilia* strains from different patients may have been acquired independently, discarding the presence of specific nosocomial clones. In *S. maltophilia*, the epidemiological relationship among different isolates needs to be analyzed, because unexpected results can be obtained. This was the case in a Croatian hospital in which, over a 4-month period (28), nine different profiles were observed in *S. maltophilia* isolates recovered from 20 patients. Six of these profiles were observed in different groups, including up to four hospitalized patients in the same or different units. Moreover, in an Italian university hospital, an epidemiological investigation of ICU patients revealed that although most patients were infected or colonized by different *S. maltophilia* clones, strains with identical genotypes were isolated, and two separate outbreaks were identified (7). Similarly, in our investigation, three consecutive respiratory outbreaks were detected in an approximately 1-year period. As previously noted, the acquisition of *S. maltophilia* isolates could be due to defective sterilization of the bronchoscope rather than dissemination of the organisms from other environmental sources or between patients. This hypothesis was reinforced when an *S. maltophilia* isolate with an indistinguishable profile from clone A was isolated in a fiberbronchoscope. However, in other clones, the sources and vehicles of infection could not be detected, as has occurred in most *S. maltophilia* outbreaks (8). Recently, an outbreak of *Pseudomonas aeruginosa* infections after bronchoscopic procedures was demonstrated (25), but to our knowledge, the involvement of these procedures as a potential source for *S. maltophilia* transmission has not been published. In contrast, faucet aerators and contaminated faucet water, electronic ventilator temperature sensors, and disinfectants have been recognized as contributing to disseminate epidemic *S. maltophilia* isolates (8).

In general, *S. maltophilia* is recovered from mixed cultures, particularly from respiratory tract and skin and soft tissue infections, which makes it difficult to establish an unequivocal role of this organism as a pathogen (8). In our study, *S. maltophilia* appeared as a single etiological agent in 26.6% of respiratory samples and 7.1% of wounds. This value reached 68.4% in blood cultures, similar to that found by Jang et al. (17). The pathogenicity of *S. maltophilia* is still controversial;

however, in a systematic retrospective case control study excluding polymicrobial bacteremia, the mortality rate for *S. maltophilia* (26.7%) was similar to that observed for other nosocomial pathogens causing bloodstream infections (24). As in other clinical studies (14), most of our *S. maltophilia* isolates (79 of 139) were recovered from respiratory specimens. However, clinical evidence of true infection was difficult to determine. Previous risk factors for *S. maltophilia* pneumonia have been reported (29, 32, 33), including neutropenia, immunosuppression, use of H2 antagonists, previous antibiotic exposure, hospitalization and surgery, catheterization, mechanical ventilation support, prolonged hospitalization, and ICU stay (7–9, 11, 19). Most of these risk factors were observed in our 11 patients involved in respiratory cross-transmission (Table 1).

In conclusion, our study revealed a high genetic diversity among *S. maltophilia* isolates despite their origin in a single hospital. This result may be related to the high potential environmental distribution of this pathogen. However, a few clones could have been transmitted among different patients, producing outbreaks and epidemic situations. Transmission of *S. maltophilia* isolates was in fact demonstrated in six patients after the use of a bronchoscope device. Molecular typing investigations of *S. maltophilia* isolates are useful for control strategies to decrease infections due to this emerging pathogen.

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