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Molecular Epidemiology of *Enterobacter aerogenes* Acquisition: One-Year Prospective Study in Two Intensive Care Units

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To evaluate the respective contributions of patient-to-patient transmission and endogenous acquisition of *Enterobacter aerogenes* isolates, we conducted a prospective epidemiologic study in two intensive care units (ICUs) between May 1994 and April 1995. We collected a total of 185 *E. aerogenes* isolates: 130 from 51 patients in a surgical ICU (SICU), 45 from 26 patients in a medical ICU (MICU), and 10 from the environments in these two ICUs. All isolates were typed by random amplification of polymorphic DNA and enterobacterial repetitive intergenic consensus PCR. Among the 175 clinical isolates, we observed 40 different profiles by random amplification of polymorphic DNA and 36 different profiles by enterobacterial repetitive intergenic consensus PCR. We identified a ubiquitous and prevalent clone, corresponding to 58% of SICU and 41% of MICU clinical isolates. Three epidemiologically related strains were specific to each ICU and represented 17% of SICU and 24% of MICU clinical isolates; unique type strains represented 17 and 29% of SICU and MICU clinical isolates, respectively, and *E. aerogenes* strains which were spread to a limited degree and which were isolated less than five times during the 1-year study period represented 8 and 6% of SICU and MICU clinical isolates, respectively. Our results show that *E. aerogenes* is acquired in the ICU in three different ways: patient-to-patient spread of a prevalent or an epidemiologically related strain, acquisition de novo of a strain from patients' own flora, and acquisition of a nonendemic strain followed by occasional patient-to-patient transmission. The findings point out the importance of patient-to-patient transmission in *E. aerogenes* acquisition and suggest that changes in *E. aerogenes* ecology in the hospital have taken place during the past decade.

Enterobacter aerogenes infections were not frequent before 1980 (8). The bacterium, however, has now replaced *Klebsiella pneumoniae* as the third leading cause of gram-negative nosocomial infections, mainly nosocomial pneumonias, after *Escherichia coli* and *Pseudomonas aeruginosa* (19). In our hospital, *E. aerogenes* was recognized as an important nosocomial problem in 1992. Since then it has been responsible for a wide range of infections, especially in intensive care unit (ICU) patients (15). The epidemiology of *E. aerogenes* infections is poorly documented. Although information exists on the possible reservoirs and portals of entry, opinions are divergent and information is available only for *Enterobacter* sp. isolates (2–4, 23). It is admitted that *E. aerogenes* is part of the normal flora of the human gastrointestinal tract and is, in most cases, endogenously acquired (8, 9). *E. aerogenes* has emerged as a pathogen because of the widespread prescription of broad-spectrum antibiotics, especially extended-spectrum cephalosporins (20). It is associated with the presence of medical devices such as endotracheal tubes or central venous catheters, which facilitate colonization (3, 8, 24). Other investigators believe that hand carriage plays an important role in patient-to-patient transmission of nosocomial *Enterobacter* sp. isolates (1, 6). In 1987, Flynn et al. (9) demonstrated by biotyping, serotyping, and phage typing that only 10% of *Enterobacter* sp. acquisitions were due to patient-to-patient transmission. These classical typing systems, however, are now considered not sufficiently discriminatory. Consequently, the relative importance of endogenous acquisition and patient-to-patient transmission in *E. aerogenes* acquisition is still unclear.

New molecular typing techniques such as plasmid profile analysis, restriction endonuclease analysis, and PCR-based techniques provide a better understanding of strain delineation in epidemiologic studies and allow differentiation between single epidemic strains and concomitant unrelated strains. Of the PCR-based techniques, random amplification of polymorphic DNA (RAPD) is based on the amplification of random DNA segments with a single primer with an arbitrary nucleotide sequence (25). It has been widely used for epidemiologic investigations (21). Because of the low level of stringency inherent in this procedure, the patterns generated by RAPD may be affected by experimental parameters. Personal investigations on the reproducibility of random PCR typing methods permitted us to standardize those parameters for routine use (5). Oligonucleotide primers based on families of short and repetitive sequences, such as the enterobacterial repetitive intergenic consensus (ERIC) sequence, have been used by Versalovic et al. (22) in a genomic fingerprinting method based on PCR called ERIC-PCR (22). ERIC sequences represent extragenic, highly conserved, and highly dispersed DNA sequences that have been observed in many eubacterial species. Consensus primers complementary to each end of the repeated sequence are oriented such that PCR amplification of DNA sequences proceeds between adjacent repeated ERIC elements. The PCR products have lengths reflecting distance polymorphisms between the 126-bp ERIC elements (22). Pulsed-field gel electrophoresis is considered to be the most reliable and reproducible typing procedure, allowing the detection of a high degree of DNA polymorphism. RAPD and ERIC-PCR have been compared with pulsed-field gel electro-

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phoresis in some epidemiologic studies; they demonstrated that PCR-based typing methods represent a rapid and simple means of molecular typing, with a level of discrimination equivalent to that of pulsed-field gel electrophoresis (14, 17). Most of the DNA-based typing techniques have been widely used to examine isolates in *Enterobacter cloacae* outbreaks, demonstrating frequent patient-to-patient transmission (1, 12, 13). Recent reports showed that RAPD and ERIC-PCR techniques are useful tools for the epidemiologic typing of *E. aerogenes* isolates (6, 11).

We conducted a 1-year prospective epidemiologic study to compare by two PCR-based typing techniques, RAPD and ERIC-PCR, the genetic diversities of two populations of *E. aerogenes* strains isolated during the same period from ICU patients from two different units. The aims of the study were (i) to estimate the different means of contamination of patients by *E. aerogenes* (contamination by a nosocomial strain present in the hospital environment or in other hospitalized patients or endogenous acquisition from patients' own flora), (ii) to study the genetic diversity of the species, allowing the estimation of the notable spread of a particular epidemiologic type, and (iii) to evaluate the influence of the underlying diseases, environmental factors, and microbial ecologies of the two ICUs on the modes of acquisition of *E. aerogenes.*

MATERIALS AND METHODS

Clinical isolates and patient data. From 1 May 1994 to 30 April 1995 we collected all clinical *E. aerogenes* isolates from infected or colonized patients in two ICUs, a 14-bed surgical ICU (SICU) and an 8-bed medical ICU (MICU), located in two different hospitals in the city of Marseille, France. We obtained a total of 175 clinical *E. aerogenes* isolates: 130 from 51 patients in the SICU and 45 from 26 patients in the MICU. The strains were isolated on various media from routine clinical specimens submitted to our clinical laboratory. These specimens included bronchial secretions, urine specimens, closed-cavity drainage specimens, catheters, blood cultures, wound swabs, and nasal swabs. The number of strains studied per patient varied from 1 to 13 for patients in the SICU and from 1 to 4 for patients in the MICU, depending on the duration of hospitalization. Each strain studied was representative of a specific specimen selected in different weeks or from different anatomical sites. Isolates causing infection versus those thought to be colonizers were defined according to clinical criteria (fever and bacteremia) and biological criteria ($>10^4$ CFU in bronchial secretions, urine specimens, and closed-cavity drainage specimens). Isolates were considered nosocomially acquired when cultures of clinical specimens were positive at least 48 h after hospitalization. For cultures of clinical specimens positive before this delay, strains were considered community acquired.

In addition to bacteriology results, we collected clinical data for each infected or colonized patient. These data included age, underlying disease, presence and type of surgical procedure, usage of medical devices, antibiotic use, date of ICU admission, and duration of ICU hospitalization before and after isolation of *E. aerogenes*.

Environmental isolates. Bacteriological samples from the environments of the two ICUs were taken at six different times during the 1-year study period. Objects which were used in patient care or frequently handled by personnel were sampled with moistened Culturette swabs (Becton Dickinson and Co., Paramus, N.J.), which were subsequently plated on bromocresol purple agar plates (bioMérieux, Marcy-l'Etoile, France). Fingerprints of the patients and health care workers in the ICUs were taken on 5% sheep blood Mueller-Hinton agar (bioMérieux). A total of 10 *E. aerogenes* isolates (7 from the SICU and 3 from the MICU) from mechanical respiratory devices (3 strains), objects handled by personnel (2 strains), and fingerprints (5 strains) were collected for further investigation.

Controls. Twenty-four clinical *E. aerogenes* isolates from 20 patients hospitalized in several medical units of a third hospital in the city of Marseille during the study period were used as controls.

Identification of strains. All strains were identified by the API 20E system (bioMérieux) according to the manufacturer's instructions.

Antimicrobial susceptibility testing. Susceptibilities to 43 antimicrobial agents and 6 combinations of agents were determined by the standard disk diffusion method on Mueller-Hinton agar (bioMérieux). These agents and associations were penicillin, ampicillin, ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, mezlocillin, piperacillin, piperacillin-tazobactam, methicillin, cloxacillin, cephalothin, cefamandole, cefoxitin, cefotetan, cefotiam, cefsulodin, moxalactam, cefotaxime, ceftazidime, ceftazidime-sulbactam, ceftriaxone, cefoperazone, cefoperazone-sulbactam, cefmenoxime, cefepime, cefpirome, imipenem, meropenem, aztreonam, gentamicin, tobramycin, kanamycin, streptomycin, netilmicin, amikacin, neomycin, erythromycin, spiramycin, clindamycin, doxycycline, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, fosfomycin, colistin, pefloxacin, ofloxacin, and ciprofloxacin.

The presence of an extended-spectrum β-lactamase (ESBL) was determined by the double-disk synergy test, i.e., by placing three disks containing either cefotaxime, ceftazidime, or ceftriaxone near a disk containing 20 μg of amoxicillin plus 10 μg of sodium clavulanate. Extension of the zone of inhibition toward the disk containing amoxicillin-clavulanate indicated the presence of an ESBL (16).

Epidemiologic typing. The isolates were investigated by using two molecular PCR-based typing methods: RAPD and ERIC-PCR. For RAPD, we used primer AP12H (5′-CGGCCCCTGT-3′), first described by Williams et al. (25). For ERIC-PCR, we used a primer based on the enterobacterial repetitive intergenic sequence named ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) (22).

(i) DNA preparation. Isolates were grown overnight at 37°C on Mueller-Hinton agar (bioMérieux). Total cellular DNA was extracted by the Chelex technique (7), and DNA concentrations were estimated on agarose gels.

(ii) Amplification conditions. Amplification reactions were performed in a total volume of 47 μl containing 100 μM dATP, 100 μM dCTP, 100 μM dGTP, and 100 μM dTTP plus 0.2 μM primer, 25 ng of template DNA, and 1.25 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) in 1× PCR buffer (20 mM Tris-HCl [pH 8.3], 50 mM KCl, 3 mM $MgCl_2$, 0.001% [wt/vol] gelatin). A negative control without template DNA was included in each experiment. The reaction mixtures were overlaid with mineral oil and were subjected to amplification in a DNA thermal cycler (TR2; Cera-Labo, Aubervilliers, France) programmed for 45 cycles of 1 min at 94°C, 1 min at 45°C, and 1 min at 74°C. Amplification products (10-μl samples) were electrophoresed in 1.2% agarose gels in Tris-acetate buffer (0.04 M Tris-acetate, 0.001 M EDTA [pH 8.2]) and were then stained with ethidium bromide and photographed on a UV light transilluminator. A molecular weight standard (Marker VI; Boehringer GmbH, Mannheim, Germany) was included on each gel. The photographed gels were scanned and the profiles were analyzed with a Bio Image whole-band system (Scanner 3+; Millipore, Ann Arbor, Mich.), which allows automatic band size determination with 1% precision. Fingerprint patterns were interpreted and compared without knowledge of epidemiologic data. Heterogeneity with respect to the intensities and the shapes of bands was not considered to be a difference. Strains were considered different if their profiles differed by two or more bands according to preceding studies (18, 26).

(iii) Reproducibility. For each of the PCR-based techniques used, reproducibility was determined by testing independent DNA preparations extracted from cultures of single colonies at different times and amplified separately.

RESULTS

Patient data. Clinical and bacteriological data for SICU and MICU patients infected with or colonized by *E. aerogenes* are given in Table 1. The types of patients in the two ICUs differed; they were essentially postoperative and polytraumatic patients in the SICU and medical patients with respiratory failure in the MICU. The sites of isolation also differed; intra-abdominal infection was more frequent in SICU patients, who were also more frequently colonized at multiple anatomical sites. Isolation of *E. aerogenes* from a respiratory tract sample was more frequent in MICU patients, who developed more infections as a result of their colonization. Nasal carriage of *E. aerogenes* was notable in SICU patients but could not be evaluated in MICU patients since routine nasal swabs were not taken from patients in this ICU. Despite all of these differences, the mean age, duration of hospitalization, and delay before isolation of *E. aerogenes* were similar for the patient populations in these two ICUs.

For 12 patients hospitalized in the SICU, *E. aerogenes* was isolated in the first 48 h after admission; however, 9 of these patients were hospitalized in surgical wards of the same hospital before SICU admission. For three patients hospitalized in the MICU, *E. aerogenes* was isolated in the first 48 h; two of these patients had been transferred from ICUs in another city. We therefore concluded that the *E. aerogenes* isolates were probably not hospital acquired for only four patients (three patients in the SICU and one patient in the MICU).

Antibiotyping. Among the 175 clinical *E. aerogenes* isolates, 164 presented an ESBL associated with an inducible chromosomal cephalosporinase. They were resistant to a majority of the antibiotics and combinations of antibiotics tested except

TABLE 1. Clinical data, treatment, and details about the isolates from patients in the two ICUs

Parameter	SICU (n = 51)	MICU (n = 26)	P
Age (yr)	57.4 (19–86)	61.5 (30–84)	NS[a]
No. (range) of days in ICU	28.18 (1–126)	33 (2–73)	NS
Disease or status (no. [%] of patients)			
Postsurgery	30 (60)	1 (4)	<0.001
Polytrauma	5 (9.7)		<0.005
Respiratory failure	16 (31.3)	21 (81)	<0.0001
Other		4 (15)	NS
No. (range) of days of hospitalization before first isolation of *E. aerogenes*	12.5 (0–51)	15 (0–55)	NS
Invasive procedure (no. [%] of patients)			
Surgery	30 (60)	1 (4)	<0.001
Mechanical ventilation	45 (88)	21 (81)	NS
Potential portal of entry (first isolation) (no. [%] of patients)			
Abdomen	3 (6)		
Urinary tract	7 (13)	4 (15)	
Respiratory tract	22 (43)	19 (73)	
Nasal cavity	10 (20)		
Wound	6 (12)	2 (8)	
Catheter	3 (6)	1 (4)	
No. of sites infected			
Intra-abdominal focus	9		
Urinary tract	16	7	
Respiratory tract	29	21	
Nasal cavity	16		
Wound	11	3	
Catheter	4		
Blood culture	2	3	
No. (%) of patients with indicated no. of infected sites			
1	29 (57)	20 (77)	
2	18 (35)	3 (11.5)	
3	2 (4)	3 (11.5)	
4	2 (4)		
Previous antibiotic therapy (before isolation of *E. aerogenes*) (no. [%] of patients)			
β-Lactams	15 (29)	7 (27)	NS
β-Lactams − quinolones	21 (41)	16 (62)	<0.005
β-Lactams + aminoglycosides	6 (12)		NS
Other	2 (4)	2 (7.7)	NS
None	7 (14)	1 (3.3)	NS
No. (%) of patients infected	25 (49)	20 (77)	<0.01
No. (%) of patients colonized	26 (51)	6 (23)	<0.01
No. (%) of patients with other infections	27 (53)	16 (61)	NS
Mortality (no. [%] of patients)	17 (33)	6 (23)	NS

[a] NS, not significant.

imipenem, colistin, and cefepime, to which all strains were susceptible. These strains were also generally susceptible to moxalactam and gentamicin (only 4 of 164 strains tested were resistant to each of these two antibiotics).

According to their antimicrobial susceptibility patterns, the remaining 11 clinical *E. aerogenes* isolates (8 from SICU patients and 3 from MICU patients) were considered to be wild-type strains. They were isolated from nasal swabs (five strains), wound swabs (four strains), and bronchial secretions (two strains). In each case, they corresponded to the first strain isolated from these patients. Four of these strains were similar to the four strains previously considered non-hospital acquired, thus confirming that they were nonnosocomial in origin. Five other strains were isolated from surgical wounds of five patients 2 to 3 days after abdominal surgery. For these five patients, the patients' digestive flora could be considered the source of the pathogen.

The 10 *E. aerogenes* isolates from the environment of the two ICUs presented an ESBL associated with an inducible chromosomal cephalosporinase and were resistant to a majority of the antibiotics or antibiotic combinations tested.

RAPD and ERIC-PCR typing of clinical isolates. Both RAPD and ERIC-PCR allowed typing of all of the isolates, and a good correlation was observed between the two techniques. Representative RAPD profiles of the *E. aerogenes* clinical isolates are shown in Fig. 1. We identified 25 distinct

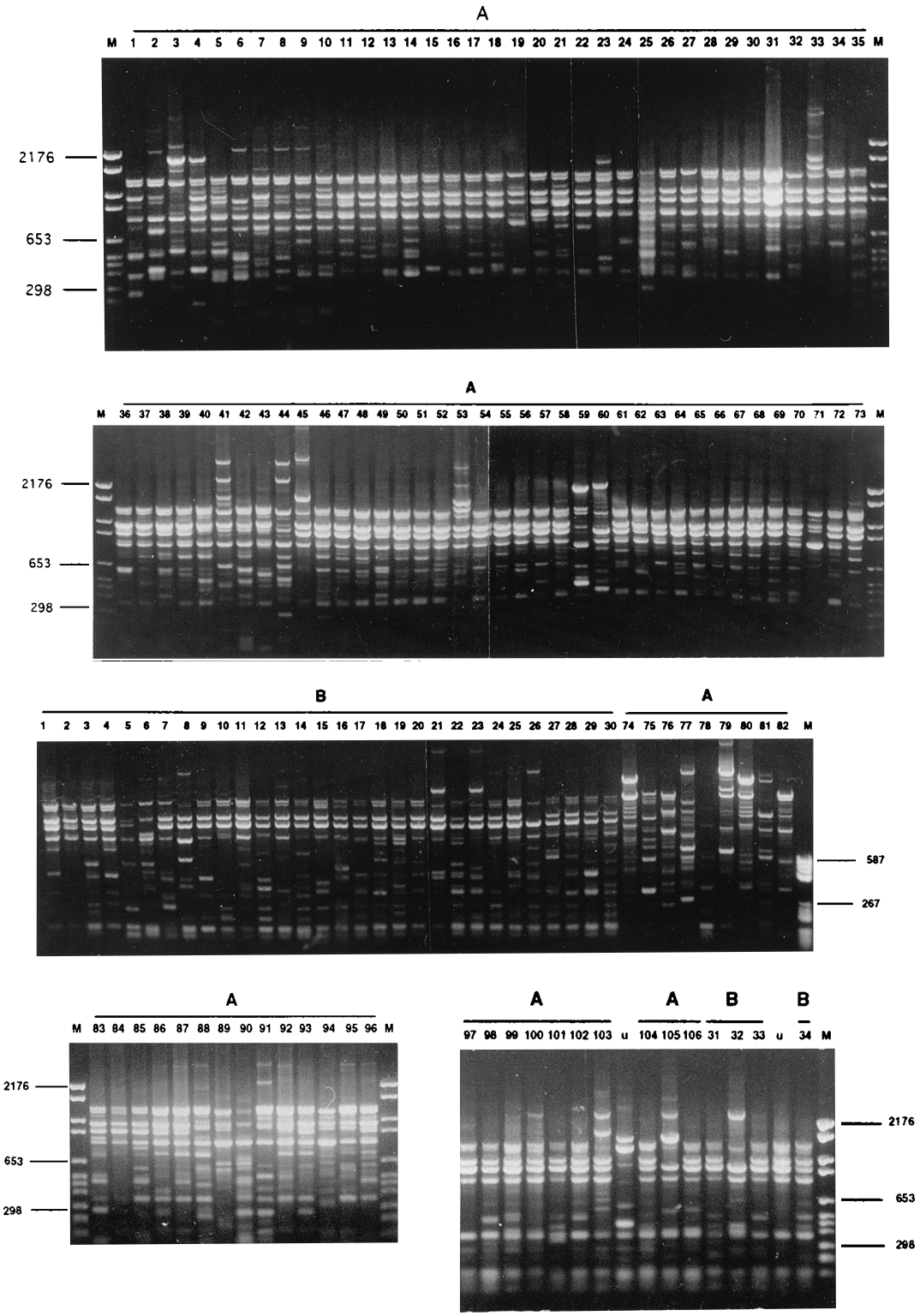

FIG. 1. Representative RAPD fingerprints of 106 clinical *E. aerogenes* isolates from the SICU (A) and 34 clinical isolates from the MICU (B) with primer AP12H. Lanes u, unrelated isolates; lanes M, size markers V or VI. The numbers to the left and right are base pairs.

RAPD or ERIC-PCR types among the 130 strains from SICU patients. The 45 strains from MICU patients gave 15 different RAPD types and 11 different ERIC-PCR types (Fig. 2).

Figures 3 and 4 show the distributions of the isolates during the 1-year period according to their RAPD type. Interestingly, the type distribution differed for strains from the two ICUs. Numerous strains (74 from the SICU and 21 from the MICU) with an identical type (type I) were recovered during the whole

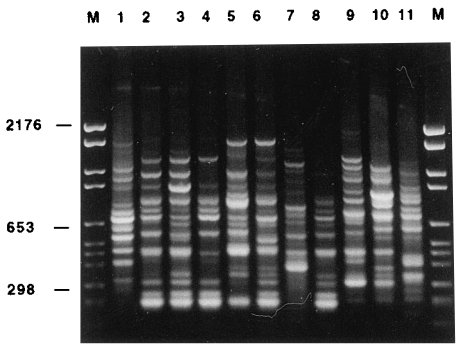

FIG. 2. Representative ERIC patterns with primer ERIC2 obtained with clinical *E. aerogenes* isolates from the MICU. Lanes M, size marker VI. The numbers to the left are base pairs.

1-year period. Over shorter periods in the SICU, two other types were common to 19 strains (types II) and 9 strains (type III). In the MICU a less prevalent type (type IV) was found to be common to nine strains. Lastly, we distinguished a few types isolated two to four times and those which appeared once during the study period.

Between one and four different types were observed among isolates from each patient. We also observed same-day colonization or infection by strains of different types in nine patients in the SICU and two patients in the MICU. Finally, the 24 control clinical isolates from a third hospital gave seven different RAPD types and five ERIC-PCR types.

Clustering of isolates. Figure 5 shows the percentage of isolates from the two ICUs in each category. Type I was the most prevalent type; 58% of clinical *E. aerogenes* isolates from the SICU and 41% from the MICU were type I. Epidemiologically related strains, types II and III from the SICU and type IV from the MICU, represented 17 and 24% of the clinical isolates, respectively. Finally, four major types corresponded to epidemiologically related strains, representing 75 and 65% of clinical *E. aerogenes* isolates from the SICU and the MICU, respectively. Fewer than five clinical *E. aerogenes* strains isolated during the 1-year study period were other types. They corresponded to isolates with unique types (17 and 29% of clinical isolates from the SICU and the MICU, respectively) and to small clusters of isolates responsible for occasional spread to fewer than five patients (8 and 6% of clinical isolates from the SICU and the MICU, respectively).

The prevalent type I strains and all other epidemiologically related strains presented an ESBL associated with an inducible chromosomal cephalosporinase. On the other hand, they presented the particular characteristic of being resistant to all quinolones.

RAPD and ERIC-PCR typing of environmental isolates. Among the 10 strains recovered from the environments, 9 were type I strains and 1 corresponded to an epidemiologically unrelated strain.

DISCUSSION

Since 1992, multiply resistant *E. aerogenes* isolates have been responsible for numerous colonizations and infections in patients hospitalized in the ICUs of hospitals in Marseille. The present study was designed to clarify the mechanisms of acquisition of this nosocomial pathogen in the ICU setting.

Both RAPD and ERIC-PCR have previously been used to type *E. aerogenes* isolates (6, 11). In those studies, they showed comparable discriminatory powers and good correlations with other genotypic typing methods such as restriction endonuclease analysis, ribotyping, and plasmid profile analysis. In our study, RAPD and ERIC-PCR provided stable profiles and allowed differentiation between epidemiologically related and unrelated strains.

Using these two techniques, we identified a ubiquitous and prevalent clone responsible for about two-thirds of epidemiologically related transmissions in the SICU and the MICU. This clone was also recovered from the environments of the two ICUs. As expected, this prevalent clone was also present in other hospitals in the city of Marseille; some of the strains selected as controls from a third hospital presented the same RAPD and ERIC-PCR profiles. During the study period, our laboratory was asked to investigate by the same techniques two *E. aerogenes* outbreaks: one in the military hospital in Marseille and the other in a hospital in the city of Avignon, 100 km away. Surprisingly, we found that our prevalent clone was also responsible for those two outbreaks (data not shown). We also identified two other epidemiologically related strains in the SICU (types II and III) and one in the MICU (type IV). Those strains may be considered responsible for widespread infec-

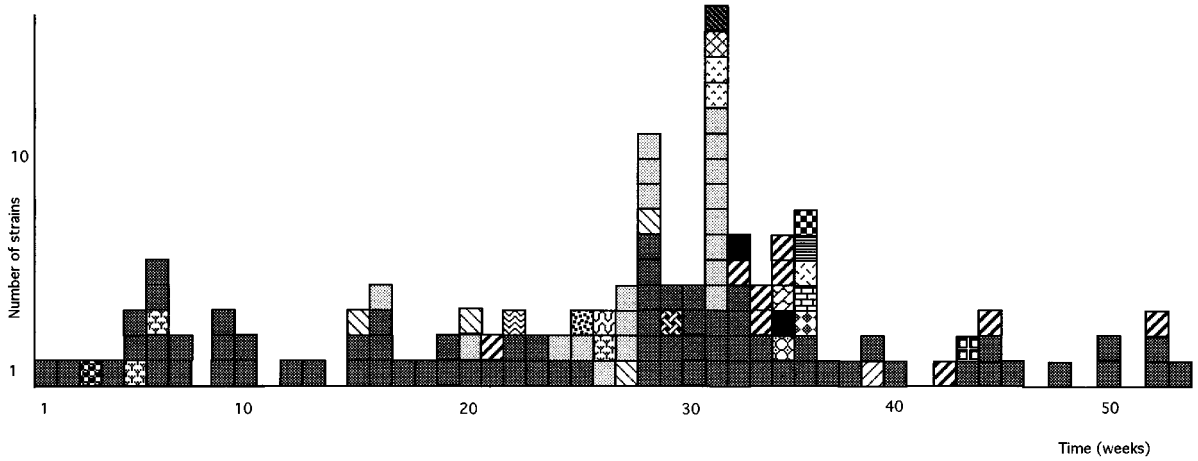

FIG. 3. Diagrammatic representation of the distribution of the different clinical *E. aerogenes* isolates from the SICU during the 1-year period. A characteristic RAPD type corresponded to a specific graphical representation: ▨, type I; ▤, type II; ▨, type III.

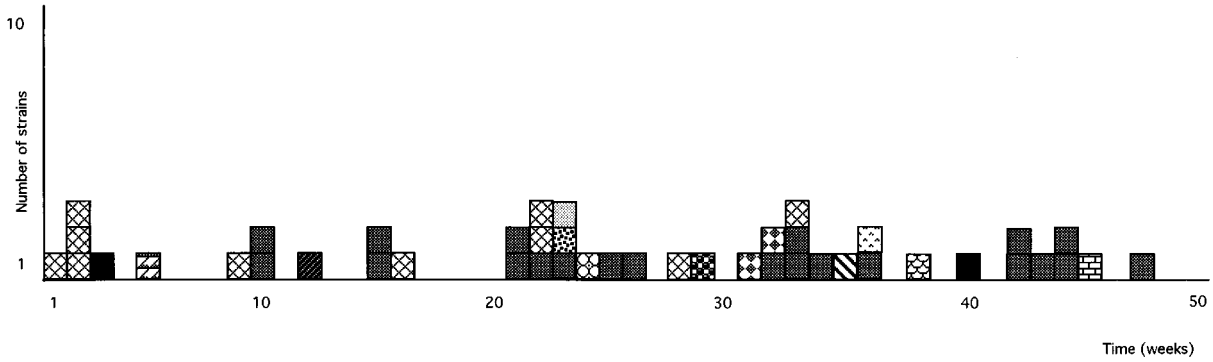

FIG. 4. Diagrammatic representation of the distribution of the different clinical *E. aerogenes* isolates from the MICU during the 1-year period. A characteristic RAPD type corresponded to a specific graphical representation: ▨, type I; ⊠, type IV.

tions and explained the remaining one-third of the epidemiologically related transmissions in our two ICUs.

Flynn et al. (9) and Gaston (10) showed that cross-infections caused by *Enterobacter* spp. were usually limited to a few patients. A recent study by Georghiou et al. (11), however, showed that 79% of the isolates from patients in several units of their hospital corresponded to two epidemic clusters. All of those isolates came from patients with previous hospital exposure. We confirm these results since, in our study, 65 to 75% of the *E. aerogenes* colonizations or infections corresponded to the acquisition of a prevalent or epidemiologically related multiresistant strain.

Georghiou et al. (11) showed that 21% of their strains were not epidemiologically related and were recovered from patients without hospital exposure. In our study, 17 to 29% of patients acquired a non-epidemiologically related *E. aerogenes* strain. Some of these strains were wild-type strains isolated from localized peripheral sites such as the nasal cavity, an external wound from patients without known previous hospital exposure, and deep wounds after an abdominal surgical procedure. The finding of such isolates in patients demonstrated the notion of endogenous acquisition described by Flynn et al. (9).

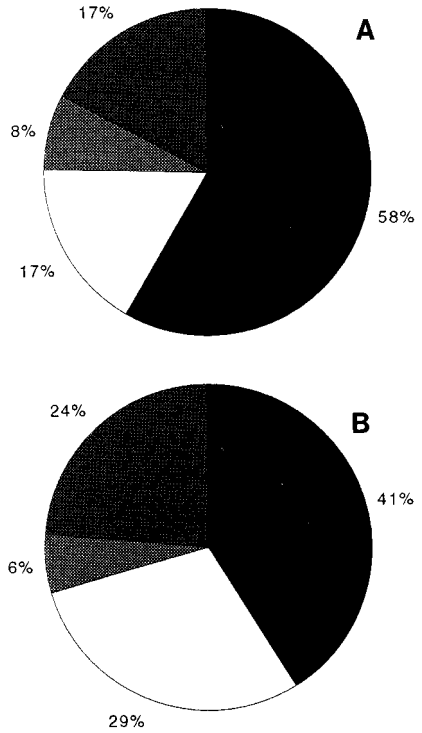

FIG. 5. Percentage of clinical isolates from the SICU (A) and the MICU (B) in each category. Black portion, prevalent type I; dark grey portion, epidemiologically related strains (type II, III and IV); light grey portion, occasionally isolated strains; white portion, unique type strains.

We also identified an intermediate situation, corresponding to 6 to 8% of the *E. aerogenes* acquisitions, in which a strain from a patient's endogenous flora was probably responsible for occasional cases of patient-to-patient transmission.

Although differences in underlying diseases and sites of isolations were observed between SICU and MICU patients, it is interesting that the distribution of the modes of acquisition was similar in the two ICUs and did not seem to be influenced by the differences in patient characteristics and environmental factors between the two types of ICUs. In both ICUs, the most common portal of entry for *E. aerogenes* was the respiratory tract, as observed previously (2, 23). However, more than 80% of the patients from both ICUs had mechanical ventilation, which is known to represent a major risk factor for nosocomial pneumonia in the ICU. In the SICU, where nasal swabs were obtained, nasal carriage preceded colonization of the respiratory tract. An intra-abdominal or wound focus of infection was particularly notable in surgical patients, as reported elsewhere (3, 24). Finally, colonization by distinct *E. aerogenes* strains at different anatomical sites was observed in some patients, as described in previous reports (6, 9).

The first colonization or infection by *E. aerogenes* was observed 12 to 15 days after admission, which is consistent with that reported for other studies (11). During this period, 70 to 90% of the patients had received β-lactam antibiotics, alone or in association with fluoroquinolones. This explains the high percentage of *E. aerogenes* isolates that were resistant to broad-spectrum β-lactams and fluoroquinolones. The high frequency of antibiotic therapy is explained by broad-spectrum antibiotic prophylaxis in surgical patients and the relatively high frequency of primary infections caused by gram-positive or gram-negative bacterial species in MICU and SICU patients.

Our data suggest that ICU patients are frequently first colonized by their endogenous *E. aerogenes* strain. When patients are colonized before ICU admission, the strain is generally of the wild type. In previously colonized patients, substitution of the original strain with a multiresistant strain generally occurs

during hospitalization. This can be explained by the selection of resistant strains after broad-spectrum antibiotic therapy or by cross-transmission from critically ill, heavily colonized patients who require prolonged and intense nursing and medical care. In patients not previously colonized, significant exposure in the ICU may lead to colonization or infection by a prevalent or epidemiologically related multiresistant *E. aerogenes* strain. The presence of *E. aerogenes* isolates on the hands or on surfaces is a good opportunity for contamination by strains present in the environment, and such strains can be transmitted by personnel during nursing. The prevalent *E. aerogenes* strains were recovered from the environment and can thus represent possible sources of cross-infections. Cross-contaminations are not due to direct contact between patients, but contact with nursing personnel is required either by direct patient-personnel contact or after the staff member has touched a contaminated surface.

In conclusion, our results show that *E. aerogenes* can be acquired in the ICU in three ways: patient-to-patient transmission of a prevalent or epidemiologically related strain, de novo emergence of a nonendemic strain from the patient's own flora, and acquisition of a nonendemic strain followed by occasional patient-to-patient transmission. Our results also point out the importance of patient-to-patient transmission. It corresponds to about two-thirds of the acquisitions of this bacterium in the ICU and suggests that changes in *E. aerogenes* ecology in the hospital have taken place during the past decade.

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