Molecular Epidemiology of Sequential Outbreaks of *Acinetobacter baumannii* in an Intensive Care Unit Shows the Emergence of Carbapenem Resistance

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The molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* was investigated in the medical-surgical intensive care unit (ICU) of a university hospital in Italy during two window periods in which two sequential *A. baumannii* epidemics occurred. Genotype analysis by pulsed-field gel electrophoresis (PFGE) of *A. baumannii* isolates from 131 patients identified nine distinct PFGE patterns. Of these, PFGE clones B and I predominated and occurred sequentially during the two epidemics. *A. baumannii* epidemic clones showed a multidrug-resistant antibiotype, being clone B resistant to all antimicrobials tested except the carbapenems and clone I resistant to all antimicrobials except ampicillin-sulbactam and gentamicin. Type 1 integrons of 2.5 and 2.2 kb were amplified from the chromosomal DNA of epidemic PFGE clones B and I, respectively, but not from the chromosomal DNA of the nonepidemic clones. Nucleotide analysis of clone B integron identified four gene cassettes: *aacC1*, which confers resistance to gentamicin; two open reading frames (ORFs) coding for unknown products; and *aadA1a*, which confers resistance to spectinomycin and streptomycin. The integron of clone I contained three gene cassettes: *aacA4*, which confers resistance to amikacin, netilmicin, and tobramycin; an unknown ORF; and *blaOXA-29p*, which codes for a class D β-lactamase that confers resistance to amoxicillin, ticarcillin, oxacillin, and cloxacillin. Also, the *blaIMP* allele was amplified from chromosomal DNA of *A. baumannii* strains of PFGE type I. Class 1 integrons carrying antimicrobial resistance genes and *blaIMP* allele in *A. baumannii* epidemic strains correlated with the high use rates of broad-spectrum cephalosporins, carbapenems, and aminoglycosides in the ICU during the study period.

*Acinetobacter baumannii* is a glucose-nonfermentative gram-negative coccobacillus that is widely distributed in the hospital environment and is an important opportunistic pathogen responsible for a variety of nosocomial infections, comprising bacteremia, urinary tract infection, secondary meningitis, surgical-site infection, and nosocomial and ventilator-associated pneumonia, especially in intensive-care-unit (ICU) patients (4, 6, 7, 10, 15, 16, 24, 29). Extensive use of antimicrobial chemotherapy within hospitals has contributed to the emergence and increase in the number of *A. baumannii* strains resistant to a wide range of antibiotics, including broad-spectrum beta-lactams, aminoglycosides, and fluoroquinolones (4, 7, 10, 15, 24, 28). In recent years, several outbreaks of nosocomial infections caused by carbapenem-resistant *A. baumannii* have been documented (3, 6, 10, 16, 25). Because of the multiple antibiotic resistance exhibited by *A. baumannii*, nosocomial infections caused by this organism are difficult to treat. These therapeutic difficulties are coupled with the fact that these bacteria have a significant capacity for long-term survival in the hospital environment, thus favoring the transmission between patients, either via human reservoirs or via inanimate materials (3, 4, 6, 10). Studies of antibiotic resistance mechanisms in *Acinetobacter* spp. have demonstrated the presence of specific genes located on integrons (8, 11, 12, 21). These are genetic elements consisting of a gene encoding an integrase (*intI*) flanked by a recombination site, *attI*, where mobile gene cassettes, often comprising antibiotic resistance genes, can be inserted or excised by a site-specific recombination mechanism (22). Several classes of integrons have been described on the basis of the sequence of the integrase gene, with class 1 integrons being the most common and widely distributed among gram-negative bacteria (13, 14, 22). The presence of type 1 and type 2 integrons has already been described in *A. baumannii* strains of both clinical and environmental origin (8, 11, 12, 21), with epidemic strains of *A. baumannii* containing significantly more integrons than nonepidemic strains (12).

An increase in the number of cases of *A. baumannii* has been observed over the past few years in the medical-surgical ICU of our university hospital in Italy. The objectives of the present study were (i) to investigate the molecular epidemiology of *A. baumannii* colonization and infection in the ICU of our university hospital, (ii) to determine whether the increase in *A. baumannii* acquisition from ICU patients was due to the spread of epidemic clones, (iii) to study the molecular epidemiology of *A. baumannii* antimicrobial resistance, and (iv) to identify clinical and therapeutic factors contributing to the selection of multidrug-resistant *A. baumannii* in the hospital environment.

(This study was presented in part at the 6th International Conference on Antimicrobial Resistance, Florence, Italy, on October 1, 2007.)

MATERIALS AND METHODS

Setting and study period. The medical-surgical ICU of the 1.470-bed teaching hospital of the University “Federico II” of Naples, Naples, Italy, consists of six rooms, five two-bed rooms and one room with a maximal capacity of six patients for room. Washing sinks are available in each room, and gloves are used routinely. The bacterial isolates selected for the present study included 131 A. baumannii isolates from 131 patients from the medical-surgical ICU of University “Federico II” of Naples during two window periods from August 1999 to February 2001 and from January 2002 to December 2002.

Microbiological methods. A. baumannii strains were collected from clinical specimens by using standard methods, in pure cultures on MacConkey agar plates, and stored at –80°C with glycerol for subsequent typing. Organisms were identified by using the Vitek 2 automatic system for the identification and susceptibility testing (bioMerieux, Marcy l’Etoile, France). Environmental cultures (room surfaces, including walls, floor, beds and drug trolley, washing sinks, disinfectants, equipment) were obtained by swabbing all surfaces with a brain heart broth infusion moistened cotton swab (3). Culture specimens were enriched overnight at 37°C in brain heart infusion broth and then isolated in pure cultures on MacConkey agar plates. Staff hands were sampled with the direct contact method on MacConkey agar plates (3). The isolates were identified as A. baumannii spp, by using the Vitek 2 automatic system with ID-GNB card for identification of gram-negative bacilli, according to the manufacturer’s instructions (bioMerieux).

Antimicrobial susceptibilities. Antimicrobial resistance was determined by the disk diffusion method according to National Committee for Clinical Laboratory Standards document M7-A4 (18). Isolates showing an intermediate level of susceptibility were classified as resistant. Susceptibility tests were also performed by using the Vitek 2 system with AST-GN09 card according to the manufacturer’s instructions (bioMerieux). The following antimicrobial agents at the indicated concentrations were tested: amikacin at 8, 16, and 64 µg/ml; ampicillin-sulbactam at 4 and 2, 16, and 32 µg/ml; aztreonam at 2, and 32 µg/ml; cefazolin at 4, 16, and 64 µg/ml; cephalosporin at 2, 8, 16, and 32 µg/ml; ceftolozine at 2, 8, and 32 µg/ml; ceftazidime at 1, 2, 8, 16, and 32 µg/ml; ciprofloxacin at 0, 5, 2, and 4 µg/ml; gentamicin at 4, 16, and 32 µg/ml; imipenem at 2, 4, and 16 µg/ml; levofloxacin at 0, 5, 4, and 8 µg/ml; meropenem at 0, 5, 4, and 16 µg/ml; pipercillin at 4, 16, and 64 µg/ml; and tobramycin at 8, 16, and 64 µg/ml. Throughout the present study, results were interpreted according to the National Committee for Clinical Laboratory Standards criteria for broth microdilution and disk diffusion methods (18).

Molecular typing by pulsed-field gel electrophoresis (PFGE) and dendrogram analysis. The preparation of genomic DNA of A. baumannii isolates was performed as previously described (29). DNA restriction was done with Apal enzyme (New England Biolabs, Beverly, Mass.) at 25°C for 1 h. The gels were run on a CHEF-DR2 system (Bio-Rad Laboratories, Hercules, Calif.) over 20 h at 14°C with 5 to 13 s of linear ramping at 200 V. Images of ethidium bromide-stained gels were digitized by using a Howtek Scammaster-3 system (Pharmacia Biotech Inc., Cologno Monzese, Italy) and analyzed by using the computer software RFLPrint (PDI, Huntington Station, N.Y.). Clusters of possibly related isolates were identified by using the Dice coefficient of similarity and unweighted group method with arithmetic averages at 80%, which indicates four-to-six fragment differences in gels with an average of 20 bands (26).

DNA purification and PCR methods. Plasmid DNA preparation was performed by using the Wizard Plus SV Miniprep DNA purification system (Promega Corp., Madison, Wis.) according to manufacturer’s procedure. Genomic DNA preparation was performed by using the Wizard Genomic DNA purification kit (Promega Corp.) according to manufacturer’s procedure. PCR amplification of class 1 integron and mapping of resistance genes was performed on 0.5 µg of genomic DNA as described previously (13). Primers for the detection of class 1 integron were located in the 5′ conserved segment (5′-CS) and in the 3′-CS regions (12). Detection of class 1 and class 2 integrons by integrase gene PCR was performed according to the method of Koeleman et al. (6). Amplification of the blaIMP-1 gene was performed with the primers 5′-ATGGCAAGATTGCATTGTGATCT-3′ (sense, positions 1 to 22, as numbered from the start of the IMP-1 gene) and 5′-TTAGTGGCGTGTTGTTGATGG-3′ (antisense, positions 721 to 741) specific for Acinetobacter IMP-1 gene (accession number AY055216). PCR conditions for IMP comprised a thermal ramp to 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by 10 min at 72°C.

DNA sequencing and computer analysis of sequence data. PCR products were purified by low-melting-point agarose gel electrophoresis, phenol-chloroform extraction, and ethanol precipitation. Cycle sequencing of the purified PCR products was performed by using the ABI Prism BigDye Terminator v3.0 ready reaction cycle sequencing kit as recommended by the manufacturer (Applied Biosytems, Foster City, Calif.). DNA products were analyzed with an Applied Biosystems 3100 Genetic Analyzer (Applied Biosystems). Similarity searches of the DNA sequences obtained were performed against nucleic acid sequence databases with an updated version of the BLAST program (1).

Nucleotide sequence accession numbers. The nucleotide sequence data of the class integrons from A. baumannii isolate AB-11/99 of PFGE type B and A. baumannii isolate AB-2105/02 of PFGE type I have been deposited in the GenBank nucleotide database under accession numbers AY307113, and AY307114, respectively.

Surveillance procedures. Nosocomial infection surveillance in the medical-surgical ICU was performed by a trained physician, who reviewed the following sources for evidence of infection: physician and nurse personnel in the unit, patient charts, and diagnostic microbiological laboratory culture reports. The data collected on nosocomial infections included sites of infection, pathogens, time of acquisition from admission, and major risk factors (i.e., urinary catheterization, intravenous catheterization, and mechanical ventilation). Information about infections in the unit was recorded on a standardized form by the surveillance physician and was reviewed regularly with the attending clinician and the hospital epidemiologist. Nosocomial infections were defined by standard Centers for Diseases Control and Prevention definitions (9). Antimicrobial use rates in the ICU were calculated according to National Nosocomial Infections Surveillance (NNIS) system report (19).

RESULTS

Molecular epidemiology of A. baumannii colonization and infection in the ICU. The molecular epidemiology of A. baumannii was studied in the medical-surgical ICU of University “Federico II” of Naples during two window periods, from August 1999 to February 2001 and from January 2002 to December 2002 in which an increase in the number of A. baumannii isolates was observed in the ward. Between August 1999 and February 2001, A. baumannii was isolated from 87 patients in the medical-surgical ICU, 29 of which were classified as infected and 58 of which were classified as colonized on the basis of the evaluation of the clinical chart (Fig. 1). In this study period, the four most common isolated pathogens were Pseudomonas aeruginosa, Staphylococcus aureus, A. baumannii, and Candida albicans, which were responsible for 24.8, 18.6, 17.9, and 13.2% of the infections, respectively. During the second window period, A. baumannii was isolated from 44 patients in the medical-surgical ICU, 34 of which were classified as infected and 10 of which were classified as colonized (Fig. 1). Between January 2002 and December 2002, A. baumannii was responsible for 34 of the 160 infections, which occurred in the unit (21.5%). Other less frequently isolated pathogens were P. aeruginosa (20.2% of all infections), S. aureus (16.9%), and C. albicans (10%).

To determine whether the increase of A. baumannii isolation in ICU patients during the two window periods was due to the spread of epidemic strains, all A. baumannii isolates were genotyped by ApaI digestion, PFGE, and dendrogram analysis. Genotypic analysis of A. baumannii isolates from ICU patients identified nine major PFGE patterns, which we named from A to I, that differed in migration of at least four DNA fragments and showed a similarity of < 80% at dendrogram analysis. Of

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these, PFGE type B could be further classified into five subtypes, B1 to B5, that showed one-fragment to three-fragment variations in the macrorestriction pattern and a similarity of \( >80\% \) upon dendrogram analysis (Fig. 2). Although seven PFGE patterns were single isolates, PFGE patterns B and I predominated, being isolated from 81 and 43 different patients, respectively. Interestingly, these two PFGE patterns occurred in two very well defined temporal clusters, with PFGE pattern B being isolated between August 1999 and January 2001 and PFGE pattern I being isolated between March and November 2002. Sporadic PFGE clones A, C, D, E, F, and G were isolated in the first window period, whereas sporadic PFGE clone H was isolated in the second window period. Multiple isolates from the same patients always showed identical PFGE patterns.

Features of clinical isolates from patients in the ICU colonized or infected with different \textit{A. baumannii} PFGE clones are shown in Table 1. PFGE clone B was responsible for 52 colonizations and 29 infections, whereas PFGE clone I was responsible for 9 colonizations and 34 infections. The lower respiratory tract was the most frequent site of isolation (70 of 81 and 36 of 43, respectively) and was associated with clinical infection in 18 of 70 and 27 of 36 patients for PFGE clones B and I, respectively. PFGE clones B and I were also isolated from the urinary tract of four and two patients or from the blood of six and five patients, respectively, and were always associated with clinical infection. PFGE clone B was also isolated once from an infected surgical wound. Sporadic PFGE clones A, C, D, E, F, G, and H all colonized the upper respiratory tract.

Extensive environmental investigations were performed during the second study period to identify sources and reservoirs of infection. Samples were obtained from various sites of the ICU, including room surfaces (6), bed frames (6), sinks (4), monitors (6), humidifiers (6), and staff hands (4). \textit{A. baumannii} was isolated from three monitors and three humidifiers of three different beds located in two different rooms and from the hands of two nurses. All \textit{A. baumannii} environmental isolates showed the identical PFGE pattern I.

**Antimicrobial susceptibility patterns of \textit{A. baumannii} isolates.** It has been previously shown that \textit{A. baumannii} infections can be selected because of the broad antibiotic resistance exhibited by this organism (3–7, 10, 15, 16, 24, 25, 28). We therefore evaluated whether the spread of the two \textit{A. baumannii} epidemic PFGE clones B and I in the ICU would have been sustained by a particular multidrug-resistant phenotype. To address this issue, we analyzed the antibiotype of different \textit{A. baumannii} strains isolated in the ward. As shown in Table 2, \textit{A. baumannii} strains with different PFGE profiles all exhibited a multiply resistant antibiotype characterized by resistance to monobactams and ceftriaxone and resistance or intermediate susceptibility to ampicillin-sulbactam, piperacillin-tazobactam, broad-spectrum cephems, fluoroquinolones, and aminoglycosides. On the contrary, eight of nine \textit{A. baumannii} PFGE types were susceptible to carbapenems. \textit{A. baumannii} epidemic strains of PFGE type B were resistant to the majority of antimicrobials, including ampicillin-sulbactam, but sensitive to cefepime, carbapenems, netilmicin, and tobramycin. \textit{A. baumannii} strains of PFGE type I showed a particular multidrug-resistant antibiotype characterized by resistance to the majority of antimicrobials tested, including carbapenems, and...
susceptibility to ampicillin-sulbactam and gentamicin. All A. baumannii strains of identical PFGE profile showed the same antibioticotype (data not shown). Antibiotic susceptibility pattern of A. baumannii strains of PFGE type I were also analyzed by using a broth microdilution method. These experiments showed that A. baumannii strains of PFGE type I were susceptible to ampicillin-sulbactam (MIC, 8 mg/liter) and gentamicin (MIC, 4 mg/liter) and of intermediate resistance to tobramycin (MIC, 8 mg/liter), imipenem (MIC, 8 mg/liter), and meropenem (MIC, 8 mg/liter) but resistant to all other antimicrobials (data not shown).

Characterization of class 1 integrons in epidemic A. baumannii strains. To further investigate the mechanisms of antibiotic resistance in A. baumannii strains, we sought to determine whether antibiotic resistance genes might be located in mobile gene cassettes. To address this issue, A. baumannii isolates were analyzed for integron content and sequences of the amplification product. The variable regions of type 1 integrons were amplified with the primers 5'/H11032 CS and 3'/H11032 CS, which annealed with the DNA regions flanking the recombination site attI (13). Single amplification products of approximately 2.5 and 2.2 kb were obtained from chromosomal DNA of all A. baumannii strains of identical PFGE types B and I, respectively (Fig. 3 and data not shown). Also, an amplicon of 2.5 kb was detected in all A. baumannii strains of different PFGE B subtypes. No amplification products were obtained from sporadic PFGE clones A, C, D, E, F, G, and H (data not shown).

Nucleotide analysis of integron from A. baumannii strains of PFGE type B showed an amplicon of 2,542 bp containing four gene cassettes: aacC1, encoding an AAC (3)-Ia aminoglycoside acetyltransferase, which confers resistance to gentamicin; two open reading frames coding for unknown products; and aadA1a gene, which encodes an AAD(3′)-Ia aminoglycoside acetyltransferase, which confers resistance to gentamicin; two open reading frames coding for unknown products; and aadA1a gene, which encodes an AAD(3′)-Ia aminoglycoside acetyltransferase.

TABLE 1. Features of clinical isolates from patients in the ICU colonized or infected with different A. baumannii PFGE clones

<table>
<thead>
<tr>
<th>PFGE clone</th>
<th>No. of isolates from:</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>URT</td>
<td>LRT</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>52</td>
<td>18</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9</td>
<td>27</td>
</tr>
</tbody>
</table>

Notes: Abbreviations: URT, upper respiratory tract; LRT, lower respiratory tract; UT, urinary tract; C, colonized patients; I, infected patients.

TABLE 2. Antimicrobial susceptibility patterns of A. baumannii PFGE clones in the ICU

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Antimicrobial susceptibility pattern of PFGE clone:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>r</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>r</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>s</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>s</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>r</td>
</tr>
<tr>
<td>Aztreomycin</td>
<td>r</td>
</tr>
<tr>
<td>Imipenem</td>
<td>s</td>
</tr>
<tr>
<td>Meropenem</td>
<td>s</td>
</tr>
<tr>
<td>Amikacin</td>
<td>s</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>s</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>s</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>s</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>s</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>s</td>
</tr>
</tbody>
</table>

Notes: Abbreviations: s, Susceptible; r, resistant. Antimicrobial resistance was determined by the disk diffusion method. Isolates showing an intermediate level of susceptibility were classified as resistant.
FIG. 3. (A) PCR amplification, with the 5'-CS and 3'-CS primers, of variable regions of integrons from *A. baumannii* epidemic PFGE clones B and I. The PCR products were separated by electrophoresis in 1.0% agarose. Lane 1, genomic DNA from *A. baumannii* epidemic PFGE clones B; lane 2, genomic DNA from *A. baumannii* epidemic PFGE clones I; lane 3, negative control (no DNA); lane M, 1-kb DNA ladder. Sizes in base pairs (bp) of 1-kb DNA ladder molecular mass markers are indicated on the right of the panel. (B) Structure of the variable region of type 1 integron amplified in *A. baumannii* epidemic PFGE clones B. (C) Structure of the variable region of type 1 integron amplified in *A. baumannii* epidemic PFGE clones I. Coding sequences are indicated by arrows with the corresponding names; the *attC* sites are indicated by black filled rectangles.
adenyltransferase that confers resistance to spectinomycin and streptomycin. Sequence analysis of integron from *A. baumannii* strains of PFGE type I showed an amplicon of 2,230 bp containing three gene cassettes: an *aacA4* allele encoding an AAC(6’)-Ib aminoglycoside acetyltransferase that confers resistance to amikacin, netilmicin, and tobramycin; an open reading frame coding for an as-yet-undetermined product; and *blaOXA-20*, a gene coding for a class D β-lactamase that confers resistance to amoxicillin, ticarcillin, oxacillin, and cloxacillin (17). The presence of integrons in *A. baumannii* strains was also evaluated by integrase gene PCR to detect *intI1* and *intI2* genes (12). Class 1 integrons were detected in all *A. baumannii* strains of PFGE type B and I, but in none of PFGE clones A, C, D, E, F, G, and H. On the other hand, class 2 integrons were not found in any of *A. baumannii* strains (data not shown).

Molecular analysis of carbapenem resistance of *A. baumannii* strains of PFGE type I. To characterize the resistance to carbapenems of *A. baumannii* strains of PFGE type I, PCR analysis was performed on chromosomal DNA with primers specific for either IMP- or VIM type class B carbapenemase genes. As shown in Fig. 4, the *blaIMP* allele compatible with the expected size of 741 bp was amplified from chromosomal DNA of all *A. baumannii* strains of identical PFGE type I but not from those of PFGE type B (Fig. 4 and data not shown). On the other hand, no VIM-type class B carbapenemase genes were amplified by PCR analysis of chromosomal DNA from *A. baumannii* strains of PFGE type I (data not shown).

**Antimicrobial use rates in the ICU.** To identify clinical and therapeutic factors contributing to the selection of multidrug-resistant *A. baumannii* epidemic clones in the hospital environment, we analyzed the use of selected antimicrobial agents in the ICU from January 1999 to December 2002. As shown in Table 3, use rates of antimicrobials of the ampicillin group were close to the 50th percentile of use rates reported by the NNIS for medical-surgical ICU (19) and increased to the 75th percentile during year 2002. Use rates of antipseudomonal penicillins during the entire study period were close to the 50th percentile of use rates reported by the NNIS (19). Use rates of carbapenems, although decreasing in 2001 and 2002, were always higher than the 90th percentile of the NNIS (19). Use rates of fluoroquinolones increased from the 50th percentile in 1999 and 2000 to the 75th percentile in 2001 and 2002. On the other hand, use rates of aminoglycosides, although elevated, decreased from 182 to 73.2 defined daily doses/1,000 patient-days during years 1999 and 2002, respectively.

**DISCUSSION**

Multidrug-resistant *A. baumannii* has increasingly been recognized as being responsible for large and sustained hospital outbreaks, particularly in ICU wards (4, 6, 7, 10, 15, 16, 24, 29). Invasive diagnostic and therapeutic procedures used in hospital ICUs have been demonstrated to predispose subjects to severe infections with *A. baumannii* (4, 6, 15, 24, 29).
months. The impact of *A. baumannii* on ICU-acquired infections was substantial and differed in the two study periods, with *A. baumannii* being the third most prevalent cause of infection from August 1999 to February 2001 and the first most prevalent cause of infection from January 2002 to December 2002. Thus, at least during the two window periods, *A. baumannii* epidemic infections became endemic in the ICU.

Molecular typing of *A. baumannii* isolates showed that the two sequential outbreaks were caused by the spread of two different epidemic clones, which coexisted with unrelated sporadic different strains. The first epidemic clone showed an unstable PFGE pattern with the presence of several subtypes during the 18 months of isolation. On the other hand, the second outbreak episode was caused by the spread of a single different epidemic clone. This is in agreement with our previous data showing that sequential *A. baumannii* epidemics in the same ICU were caused by different clones, one replacing the other in a well-defined temporal order (29). Contaminated environmental sources, including humidifiers and monitors, and hand carriage by patient-care personnel were identified during the second *A. baumannii* outbreak, suggesting the horizontal transmission of the epidemic strains from one patient to another through the hospital staff. Because multivariate analysis has previously identified mechanical ventilation as a major risk factor for *A. baumannii* acquisition in the same ICU ward (29), we postulate that any maneuver associated with mechanical ventilation might have been the mode of *A. baumannii* patient-to-patient transmission during the two sequential outbreaks described in the present study. In partial support of this hypothesis, the respiratory tract was the most frequent site of isolation for both sporadic and epidemic clones, with the latter being isolated only from the lower respiratory tract. Our data also demonstrated that the two epidemic *A. baumannii* clones resulted in a worse clinical outcome compared to sporadic clones; the epidemic clone of the B genotype was responsible for 29 infections, and the epidemic clone of the I genotype was responsible for 34 infections, whereas the sporadic clones resulted only in colonizations.

Several studies indicate that *A. baumannii* strains responsible for nosocomial infections have been selected because of their highly resistant phenotype (3–7, 10, 15, 16, 24, 25, 28). In particular, the emergence and spread of resistance to amikacin (8, 28) or carbapenams (3, 6, 10, 16, 25) have been reported during hospital outbreaks of multidrug-resistant *A. baumannii*. Accordingly, the analysis of the antibiotype of *A. baumannii* clones isolated in the present study showed that the two sequential epidemic clones and three of the seven sporadic strains were highly resistant. In particular, the *A. baumannii* epidemic clone of the B genotype was resistant to ampicillin-sulbactam, broad-spectrum cephalosporins, gentamicin, and amikacin but sensitive to carbapenams. On the other hand, *A. baumannii* epidemic genotype I clone was resistant to the majority of antimicrobials tested, including carbapenams and most aminoglycosides, but sensitive to ampicillin-sulbactam and gentamicin. The simultaneous occurrence of resistance to amikacin and carbapenams in *A. baumannii* epidemic genotype I clone might have been responsible for the high rate of infection during the second *A. baumannii* outbreak in our ICU. It has been recently demonstrated that the major selection pressure driving changes in the frequency of antibiotic resistance is the volume of drug use (2). The use of antibiotics can contribute to the persistence and spread of the outbreaks caused by multidrug-resistant *A. baumannii* (3, 5–8, 10, 15, 16, 25, 28).

Our data show elevated use rates of broad-spectrum cephalosporins, carbapenams, and aminoglycosides in the ICU from 1999 to 2002. This may have selected the two sequential epidemics caused by multidrug-resistant *A. baumannii*, particularly strains resistant to antibiotics highly used in the ICU. In accordance with this, it has been shown that the risk of *A. baumannii* acquisition increases in case of use of broad-spectrum cephalosporins and aminoglycosides (29). Also, prior aminoglycoside therapy has been identified as risk factor for multidrug-resistant *A. baumannii* bloodstream infections (24).

Additional epidemiological information was provided by the molecular typing of *A. baumannii* antimicrobial resistance. Type I integrons were amplified from the genomic DNA of the two *A. baumannii* epidemic clones but not from the genomic DNA of sporadic clones. This is in agreement with previous data showing that integron-located antimicrobial resistance genes are frequently found in epidemic strains of *A. baumannii* (12). PCR and sequence analysis of antimicrobial resistance genes located in mobile DNA elements demonstrated the presence of integron-located *aacC1* and *aadA4* resistance genes in *A. baumannii* strains of PFGE type B, a finding consistent with their phenotypic resistance to gentamicin. On the other hand, integron isolated in *A. baumannii* strains of PFGE type I contained an *aacA4* allele that confers resistance to amikacin, netilmicin, and tobramycin and the *bla* gene coding for a class D β-lactamase, which confers resistance to amoxicillin, ticarcillin, oxacillin, and cloxacillin (17). The presence of *aacA4* resistance gene correlates well with the resistance of *A. baumannii* strains of PFGE type I to all aminoglycosides, with the exception of gentamicin. *A. baumannii* strains of PFGE type I were also characterized by intermediate resistance to carbapenams, with imipenem and meropenem having MICs of 8 mg/liter. Our data showed that *bla* allele was amplified from chromosomal DNA of *A. baumannii* strains of PFGE type I. This is consistent with several reports showing that allelic variants of IMP-type β-lactamase are responsible for carbapenem resistance in *Acinetobacter* spp. (5, 23, 27). Also, in keeping with our data, *Acinetobacter* clinical isolates carrying *bla* alleles exhibit different levels of carbapenem resistance, with MICs varying from 4 to 32 mg/liter (5, 27).

Antimicrobial resistance in *A. baumannii* strains might have been acquired either through horizontal gene transfer or selection of novel resistant clones. The data reported here show that the same cassette arrays are found in all integrons isolated in *A. baumannii* strains of PFGE types B and I, suggesting that antimicrobial resistances have been acquired through selection of two independent resistant clones. This finding is in agreement with previous data showing that the spread of both amikacin (8, 28) and carbapenem (6) resistances in *A. baumannii* strains isolated from different hospitals in Spain was due to the acquisition of two new epidemic strains. However, we cannot rule out the possibility that antimicrobial resistances might have been acquired through horizontal gene transfer. In partial support of this hypothesis, the same cassette array of *A. baumannii* strains of PFGE type B was found also in type I integrons of several clinical isolates from Italian hospitals belong-
ing to different ribotype groups (11). Also, the same cassette array of integron of A. baumannii strains of PFGE type I was found in type 1 integrons of A. baumannii isolates from Italy (11), France (21), and Spain (20). In the latter case, however, the gene, designated blaOXA-2, differs from the OXA-20 gene in 2 bp, with one of the mutations being silent and the other generating a substitution of glutamic acid for aspartic acid (20). Moreover, the presence of integrons containing the same organization of cassettes in A. baumannii strains with different genotypes further suggests a horizontal gene transfer of integrons (11, 21).

In conclusion, we show here that A. baumannii strains cause large and sustained hospital outbreaks and identify factors involved in the emergence and spread of their antimicrobial resistances. The two epidemics were due to the dissemination of two distinct clones that were selected because of the presence of aminoglycoside and beta-lactam resistance gene cassettes within class 1 integrons, as well as a chromosomal integron of A. baumannii, and a chromosomal cassette array. This program would require monitoring ICU-acquired bacteria and antimicrobial resistance selection and dissemination. In conclusion, we show here that A. baumannii strains cause large and sustained hospital outbreaks and identify factors involved in the emergence and spread of their antimicrobial resistances. The two epidemics were due to the dissemination of two distinct clones that were selected because of the presence of aminoglycoside and beta-lactam resistance gene cassettes within class 1 integrons, as well as a chromosomal integron of A. baumannii, and a chromosomal cassette array. This program would require monitoring ICU-acquired bacteria and antimicrobial resistance selection and dissemination.

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