Outbreak of Extensively Drug-Resistant *Acinetobacter baumannii* Indigo-Pigmented Strains

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**Acinetobacter baumannii** pigmented strains are not common in clinical settings. Here, we report an outbreak caused by indigo-pigmented *A. baumannii* strains isolated in an acute care hospital in Argentina from March to September 2012. Pan-PCR assays exposed a unique pattern belonging to the recently described regional CC113b/CC79p clonal complex that confirms the relevant relationships among the indigo-pigmented *A. baumannii* strains. All of them were extensively drug resistant and harbored different genetic elements associated with horizontal genetic transfer, such as the transposon Tn2006, 2 integrons, AbA-type islands, IS125, IS26, strA, strB, florR, and the small recombinase ISCR2 associated with the sul2 gene preceded by ISAbA1.

*Acinetobacter baumannii* is a well-known significant nosocomial pathogen that causes a variety of diseases (1–3). The ability of this bacterium to survive for long periods on inanimate surfaces and its extensive drug resistance make *A. baumannii* a successful microorganism that is able to cause outbreaks (4, 5). Many outbreaks due to *A. baumannii* have been documented in the literature (6–10). However, to date, no outbreaks due to indigo-pigmented *A. baumannii* strains have been documented.

The production of indigo pigment in the genus *Acinetobacter* was previously reported only in the environmental *Acinetobacter* sp. strain ST-550 and in the *A. baumannii* ATCC 19606 strain in the presence of indole as a carbon source (11–13). This production may be attributed to the activity of a monooxygenase or dioxygenase enzyme (11, 12). Here, we report the molecular characterization of an outbreak of indigo-pigmented *A. baumannii* strains that began in the traumatology service of an acute hospital in Argentina.

**MATERIALS AND METHODS**

**Bacterial strains.** A total of 13 pigmented *A. baumannii* strains were isolated in the traumatology service (n = 7), coronary care unit (n = 2), plastic surgery unit (n = 2), and intensive care unit (n = 2) of an acute care hospital in Argentina from March to September 2012 (Table 1). The strains were identified at the species level by using several criteria: (i) analysis on a Vitek 2 Compact (bioMérieux), (ii) amplified ribosomal DNA restriction analysis (ARDRA) using the primers 5'-TGGCCGCAATTGAGCCTGCCG and 5'-TACCTTGTAGACCTT CACCCCA with cycling conditions of initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min (14), (iii) amplification and sequencing of the 16S rRNA with the primers fD2 (5'-ACGGCTACCTTGTTACGACTT) (described by Weisburg et al. [15]) using 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min as the cycling conditions, and (iv) amplification and sequencing of the ropB gene using the primers Vic4 (5'-GGCGGAATTTGGG(AGT)GA(GA)AACCA) and Vic6 [GA(GA) T(GT)TGGAAGTTGTAACCC] and the same cycling conditions as described in iii above (see http://www.pasteur.fr/recherche/genopole/PP8 /mlst/Abaumannii.html). Antibiotic susceptibility tests were performed with the Vitek 2 system that uses the panel AST-082 (Gram-negative susceptibility [GNS] card). The MIC results were interpreted according to CLSI categories (16).

**DNA techniques.** Total DNA was extracted with a MasterPure DNA purification kit (Epipicent, Madison, WI, USA) according to the manufacturer’s instructions. To determine the presence of the most prevalent OXA carbapenemase genes in our region (17), such as *blaOXA-23-like* and *blaOXA-58-like* PCRAs were carried out using the primers and cycling conditions described in the literature (17). For the *blaOXA* amplification reactions, the strains AB3 (*blaOXA-23-like*), and AB1(*blaOXA-58-like*) were used as positive controls (17). The presence of insertion sequences (ISs) (ISAbA1, ISAbA3, IS125, IS26, IS825, ISCR1, and ISCR2), class 1 and 2 integrons, and the corresponding variable regions of integrons were determined using specific primers as previously described (17–20). To further characterize the strains, the occurrence of AbA-type islands using specific previously described primers (4F, 4R, 2F, and 2R) was determined (21, 22). We also searched for the presence of tetracycline-resistant genes using specific primers to amplify *tet(B), tet(A), tet(M), tet(39),* and *tet(H)* genes under the conditions described in the literature (21, 22). Amplification of the *iacA* gene (using the primers iacAF [5'-ATGAAATAGTGTGCTAAATG GAG] and iacAR [5'-GCAAACCAACCGGCCTAATG]) involved in indigo production, was carried out using the *A. baumannii* ATCC 19606 and ATCC 19778 strains as positive controls.

In addition, the relatedness of the strains was determined using two different molecular typing techniques, a PCR assay using degenerate oligonucleotide primers (DO-PCR) and the recently described pan-PCR assay, which consists of a multiplex PCR of 6 genes and allows for the identification of relevant relationships among strains (23, 24). To carry out the DO-PCR, the primer 19 (5'-GGTGAACGCTTNGTNGGRT)C was used employing a low-stringency amplification protocol (5 min of denaturation at 95°C, 40 cycles for 1 min at 93°C, and 1.5 min at 36°C, 2 min at 72°C, and 10 min at 72°C) (23, 24). The pan-PCR assay was performed as described by Yang et al. using the 6 designed pairs of primers that allow amplification of a group of genes whose variable presence enables identification of the strains of interest (23, 24). The cycling conditions employed for the reaction were initial denaturation at 95°C for 5 min, followed by 20
cycles of denaturation (95°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 1 min 30 s), and a single final extension at 72°C for 10 min.

**Sequence analysis.** Sequencing was performed on the two DNA strands using an ABI Prism 3100 BioAnalyzer and Taq FS terminator chemistry (Taq FS; Perkin-Elmer). The sequences were examined and assembled with Sequencher 4.7 software (Gene Codes Corp.) and BLAST (version 2.0) software (http://www.ncbi.nlm.nih.gov/BLAST/).

**Nucleotide sequence accession numbers.** The sequences determined in this study were submitted to GenBank under accession numbers KF410895 and KF410896.

### RESULTS

All strains were identified as *A. baumannii* using several methods: (i) the bionumber obtained by the Vitek 2 Compact was 02010303500352, giving identification of an *A. baumannii* complex with a 99% probability, (ii) the ARDRA profile obtained was 11123, which is characteristic of *A. baumannii*, (iii) the sequence analysis of the 16S rRNA gene (GenBank accession number KF410895) revealed 99% identity with the sequences corresponding to the 16S rRNA gene of *A. baumannii* (GenBank accession number CP003846), and (iv) the sequence of the *rpoB* gene (GenBank accession number KF410896) was 100% identical to that of the *rpoB* gene of *A. baumannii* (GenBank accession number DQ207471).

Although two antibiotic resistance profiles among the 13 indigo-pigmented *A. baumannii* strains were identified (Table 1), all strains were categorized as extensively drug resistant (XDR) according to the recent definitions suggested by Magiorakos et al. (25).

The clinical outcome of patients involved in the outbreak included five deaths. However, the association between *A. baumannii* colonization or infection and mortality could not be established because patients were medically compromised or had underlying diseases.

The indigo-pigmented strains showed a unique pattern by DO-PCR that clustered all of them in a single clone (Fig. 1a). We also decided to perform the new, recently described pan-PCR assay, which has been demonstrated to distinguish among strains with identical multilocus sequence types (MLSTs) (24). This technique showed unique amplification patterns, with the exception of one strain (M33405) in which one band is missing, confirming the relevant relationships among the indigo-pigmented *A. baumannii* strains (Fig. 1b). As this technique is defined as a highly discriminatory PCR assay, we consider that the pan-PCR assay showed a distinct variation in the gene content of this particular strain (33405).

Also, the use of pan-PCR allowed us to determine that the indigo-pigmented *A. baumannii* strains possessed the same amplification pattern as that obtained in the control strain for the CC113/CC79 clonal complex, which was shown to be prevalent in clinical *A. baumannii* isolates from Argentina (26) (Fig. 1b). This particular clonal complex, which differs from the international clones I, II, and III, was also described for *A. baumannii* isolates from Brazil and Spain (26–28).

The dates when the *A. baumannii* strains were recovered clearly show that the outbreak began in the traumatology service and then spread to the other services (Table 1). Attempts to recover *A.

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**TABLE 1 Indigo-pigmented *A. baumannii* strain characteristics and associated clinical data of the patients**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation date</th>
<th>Patient age (yr)/sex</th>
<th>Underlying disease(s)</th>
<th>Diagnosis at admission(s)</th>
<th>Nosocomial diagnosis(s)</th>
<th>Service</th>
<th>Source of infection</th>
<th>Antibiotic resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>3/28/2012</td>
<td>47/F</td>
<td>ND</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>Surgical wound</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
<td></td>
</tr>
<tr>
<td>M30393</td>
<td>6/25/2012</td>
<td>55/M</td>
<td>DBT</td>
<td>Diabetic foot infection</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M30121</td>
<td>6/19/2012</td>
<td>31/F</td>
<td>NUD</td>
<td>Pyomyositis</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>167</td>
<td>6/25/2012</td>
<td>65/M</td>
<td>DBT</td>
<td>Diabetic foot infection</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>192</td>
<td>7/17/2012</td>
<td>64/M</td>
<td>DBT, CRF</td>
<td>Prosthesis infection</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>Mini-BAL</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
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<tr>
<td>186</td>
<td>7/12/2012</td>
<td>56/M</td>
<td>DBT</td>
<td>Diabetic foot infection</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M32757</td>
<td>8/15/2012</td>
<td>64/M</td>
<td>DBT, CRF</td>
<td>Prosthesis infection</td>
<td>Surgical site infection</td>
<td>TS</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M32467</td>
<td>8/8/2012</td>
<td>Unknown/F</td>
<td>NUD</td>
<td>Burn infection</td>
<td>Surgical site infection</td>
<td>PSU</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M33405</td>
<td>8/8/2012</td>
<td>Unknown/F</td>
<td>NUD</td>
<td>Burn infection</td>
<td>Surgical site infection</td>
<td>PSU</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
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<tr>
<td>M33045</td>
<td>8/28/2012</td>
<td>65/M</td>
<td>Scheduled CABG</td>
<td>Burn infection</td>
<td>Surgical site infection</td>
<td>PSU</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M33614</td>
<td>9/3/2012</td>
<td>45/M</td>
<td>Hemorrhagic stroke</td>
<td>VAP</td>
<td>ICU</td>
<td>Mini-BAL</td>
<td>ICU</td>
<td>TMP, AMK, CAZ, FEP, CIP, PIP-TZ, IMP, MEM, GEN</td>
</tr>
<tr>
<td>M34050</td>
<td>9/10/2012</td>
<td>60/M</td>
<td>Hemorrhagic stroke</td>
<td>VAP</td>
<td>ICU</td>
<td>Tracheal aspirate</td>
<td>TS</td>
<td>ICU</td>
</tr>
<tr>
<td>F33943</td>
<td>9/7/2012</td>
<td>82/F</td>
<td>Respiratory failure, CAP</td>
<td>VAP</td>
<td>CCU</td>
<td>Tracheal aspirate</td>
<td>Mini-BAL</td>
<td>ICU</td>
</tr>
</tbody>
</table>

**a** F, female; M, male.

**b** DBT, diabetes; NUD, nonunderlying disease; CRF, chronic renal failure.

**c** ND, not determined; CABG, coronary artery bypass graft; CAP, community-acquired pneumonia.

**d** VAP, ventilator-associated pneumonia.

**e** SSTI, skin and soft tissue infection; BAL, bronchoalveolar lavage.

**f** TS, traumatology service; PSU, plastic surgery unit; CCU, coronary care unit; ICU, intensive care unit.

MEM, GEN, meropenem; GEN, gentamicin.

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This particular clonal complex, which differs from the international clones I, II, and III, was also described for *A. baumannii* isolates from Brazil and Spain (26–28).

The dates when the *A. baumannii* strains were recovered clearly show that the outbreak began in the traumatology service and then spread to the other services (Table 1). Attempts to recover *A.
A. baumannii strains from environmental sources other than the hospitalized patients yielded no indigo-pigmented A. baumannii strains. A nonpigmented XDR A. baumannii strain (A179) was recovered from the wound-healing chamber used in the traumatology service.

The molecular characterization of the outbreak was carried out by PCRs, and sequence analyses were performed to identify the presence of the antibiotic-resistant genes and the genetic elements associated with the antibiotic resistance. We also searched for the iacA gene and found positive results in all the indigo-pigmented A. baumannii strains and also in the XDR nonpigmented A. baumannii strain A179.

While all the strains harbored the Tn2006 transposon, which carries bla\textsubscript{OXA-23}, amplification of the bla\textsubscript{OXA-58} and bla\textsubscript{OXA-143}
carbapenemase genes gave negative results. All indigo-pigmented strains possessed IS215 and IS26, and the strA, strB, and floR genes. Class 2 integrons were also found in all the indigo-pigmented A. baumannii strains, whereas no class 1 integrons were found. These results are in accordance with our previous studies, which showed that class 2 integrons are more abundant than class 1 integrons in A. baumannii strains from Argentina (20, 29, 30).

In addition, all indigo-pigmented strains were positive for the presence of not only AbaR-type genomic islands but also a cluster containing ISAba1, sul2, and ISCR2. The A. baumannii A297 strain harboring a similar cluster, which contains ISAba1, sul2, ISCR2, strB, and strA, has been described (19). However, no positive result to link the strB and strA genes with ISCR2 was obtained in our A. baumannii indigo-pigmented strains.

The PCRs we used to amplify tetracycline-resistant determinants, such as tet(A) and tet(B), gave negative results. In addition, no evidence of the aadA2, aac(6’)-Ib, and aphA1 genes was found.

Our first thoughts were that the A179 strain, which was found in a wound-healing chamber in the traumatology service, may have been the source of the described outbreak and/or the source of the antimicrobial-resistant mechanisms found in the indigo-pigmented strains. To confirm our hypothesis, we characterized the A179 strain.

The nonpigmented A. baumannii A179 strain was susceptible only to minocycline, tigecycline, amikacin, and colistin. The same resistance profile was also observed in the A179 strain harboring the A297 integron array. This integron was previously described not only in the wide-antibiotic-spectrum A297 strain but also in the indigo-pigmented A179 strain. Instead of class 2 integrons, we found that the A179 strain belongs to a different clone (Fig. 1a and b). Also, the clonal relationship found by DO-PCR and pan-PCR showed that the A179 strain harbors Tn2 at the insertion site (Fig. 1a and b). However, no evidence of the A. baumannii CR2 cluster, which contains IS215 and IS26, was detected in the A179 strain harboring Tn2 and the AbaR-type genomic island. However, no evidence of the ISAba125 or ISCR2 or the strA, strB, or floR gene was detected.

DISCUSSION

To our knowledge, this is the first report of an outbreak of XDR indigo-pigmented A. baumannii strains. The molecular characterizations of the strains clearly exposed the large number of genetic elements present in these strains and thus support the general idea that A. baumannii has a particular ability to acquire different genetic elements to evolve rapidly to XDR and pandrug-resistant (PDR) strains.

In all the strains, we observed the presence of not only ISAba1 and ISAba125, which are the most prevalent ISs in this microorganism, but also IS26 and the ISCR2 elements. Our findings are in agreement with the concept that insertion sequences have a predominant role in the acquisition and dissemination of antibiotic resistance within A. baumannii. The virulence associated with indigo-pigmented isolates remains to be established.

Our study also highlights the importance of rigorous infection prevention and control measures for managing an outbreak of A. baumannii. Once the organism is identified, universal hygiene measures should be observed to further spread and outbreaks.

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We declare no conflicts of interest.

REFERENCES


