Acinetobacter soli as a Cause of Bloodstream Infection in a Neonatal Intensive Care Unit

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Acinetobacter soli is a new bacterial species described from forest soil. Five cases of bloodstream infection caused by A. soli clonal isolates are reported here for the first time. The patients were neonates admitted to an intensive care unit. This is a new neonatal pathogen with the potential to cause outbreaks.

Bloodstream infections (BSIs) remain one of the most important complications in neonates admitted to hospitals (15). Although Acinetobacter sp. is a frequent pathogen in health care-associated infections, to date, only a few outbreaks of BSI caused by this agent in neonatal intensive care units (NICUs) have been described. Typically, these outbreaks are due to cross-transmission of strains, and a strong effort with combined interventions is essential to their control (9). Acinetobacter baumannii is the most pathogenic species of this genus (3); however, outbreaks caused by other species, such as Acinetobacter junii (2) and Acinetobacter ursingii (8), have been occasionally reported. In the present study, Acinetobacter soli, a species previously isolated only from forest soil (6), is described as the cause of an outbreak of BSI in neonates.

From 31 March to 8 April 2005, five neonates admitted to a 10-bed intensive care ward presented with symptoms of sepsis and isolation of a Gram-negative nonfermentative oxidase-negative rod from blood samples. The five isolates collected, one from each neonate, had identical colony morphology and biochemical reactions, but results were inconclusive. The infected neonates had been admitted to the unit on the days of their birth. The unit was a regular hospital ward adapted for intensive care, because the original neonatal intensive care unit in the hospital was closed a year before due to structural problems. On 8 April, after the fifth infant developed BSI with the unidentified rod, an outbreak became evident, and the neonates were transferred to another hospital; the ward was then closed. One neonate, who was severely compromised, progressed to death. The other four children were cured. All had negative blood cultures after 7 to 10 days of treatment. The clinical characteristics of patients with infection by the outbreak strain are shown in Table 1.

Biochemical properties of the study isolates were determined with API 20NE (bioMérieux, Hazelwood, MO) and conventional tests. Antimicrobial susceptibility to amikacin, ampicillin-sulbactam, cefepime, ceftazidime, gentamicin, imipenem, meropenem, piperacillin-tazobactam, sulfamethoxazole-trimethoprim, and tobramycin was tested by disk diffusion (1). The colistin MIC was determined by broth microdilution (1).

DNA fingerprints were determined by pulsed-field gel electrophoresis (PFGE) with SpeI (Promega, Madison, WI) (10) and randomly amplified polymorphic DNA (RAPD)-PCR (4). The species identification was performed by 16S rRNA (13), the rpoB gene, and flanking spacers (7) and gyrB gene (14) sequence analysis. To obtain the gyrB gene complete coding sequence, primers Rubgyr-sF (5'-CGC GTC GTT TAA GAG AGT T-3') and Rubgyr-sR (5'-CGC CAT AAT CAA CGC ACT T-3') were designed for PCR amplification and sequencing, with the following conditions: initial denaturation at 95°C for 2 min, 33 cycles at 95°C for 1 min, 51°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 5 min. The sequences obtained in our study were compared to those of the single fully characterized, first described A. soli strain B1T (6) and four other sequences of unpublished isolates deposited in GenBank, one rpoB sequence (GenBank accession number HM570037) and three 16S rRNA gene sequences (GenBank accession numbers FJ976560, FJ976568, and GQ258635).

All five study isolates shared very similar phenotypic characteristics. Cells grew well on Trypticase soy agar (TSA) and MacConkey agar media. On TSA, bacterial colonies were circular, convex, and smooth, had margins, and were approximately 2 mm in diameter after 24 h of incubation at 30°C. The isolates were Gram-negative rods, catalase positive, oxidase negative, nonmotile, and nonhemolytic on sheep blood agar. Biochemical test reactions were all negative, except for urease production. On API 20NE, the isolates had positive reactions for D-glucose assimilation, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, urease activity, and esculin hydrolysis. This biochemical profile gave an inconclu-

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TABLE 1. Clinical characteristics of patients with the outbreak strain

<table>
<thead>
<tr>
<th>Caucasian (wk)</th>
<th>Mothers health</th>
<th>Birth wt (g)</th>
<th>Respiratory distress</th>
<th>Date of isolation of BGN</th>
<th>Antibiotic therapy before BGN isolation</th>
<th>Days from admission to BGN isolation</th>
<th>Days from admission to bilateral pneumonia</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>1350</td>
<td>No</td>
<td>31 March 2006</td>
<td>Amoxicillin plus gentamicin</td>
<td>6</td>
<td>11</td>
<td>Death</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>2202</td>
<td>Yes</td>
<td>30 March 2006</td>
<td>Cefepime, vancomycin, (10)/pneumonia</td>
<td>4</td>
<td>12</td>
<td>Discharge</td>
</tr>
<tr>
<td></td>
<td>HIV positive</td>
<td>1360</td>
<td>Yes</td>
<td>31 March 2006</td>
<td>Clarithromycin, and ciprofloxacin (7)</td>
<td>4</td>
<td>15</td>
<td>Discharge</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>3450</td>
<td>Yes</td>
<td>31 March 2006</td>
<td>Meropenem (12) and ciprofloxacin (10)</td>
<td>7</td>
<td>20</td>
<td>Discharge</td>
</tr>
</tbody>
</table>

Note: The outbreak strain was isolated from a clinical specimen while this work was taking place, a genetic identification in more detail was undertaken.

The gyrB and the partial rpoB gene and spacer sequences of two of the five isolates (788BRRJ and 789BRRJ) were studied. All results for both isolates were identical, and only the sequences of strain 788BRRJ were deposited in GenBank. In the analysis by BLASTn, the highest similarities were with two A. soli isolates, as follows: 98% for gyrB of strain B1T (GenBank accession number EU622818) and 99% for rpoB zone 1 of strain K50-54 (GenBank accession HM570037.1). Thus, the 16S rRNA gene, gyrB, and rpoB sequences were concordant and defined the strain identification as A. soli.

In the present report, we describe an outbreak of BSI caused by A. soli, a species described in 2008 from an environmental specimen (6). The outbreak occurred in neonates admitted to an intensive care unit, a population prone to developing health care-associated infections. On the other hand, four of the five neonates were receiving antibiotics to which the bacterial isolate was susceptible, which is consistent with the hypothesis that a contaminated IV solution was the source of the outbreak. In fact, the five neonates had infections with a single genotype defined by two well-established fingerprinting methods, indicating that a point source and cross-transmission of the strain occurred in the unit. Alternatively, the laboratory reagents could have been contaminated by A. soli (a pseudo-outbreak). However, this is unlikely because all neonates had sepsis symptoms when blood cultures were drawn. Unfortunately, the unit was closed before any investigation could be carried out. Overcrowding and understaffing, conditions that facilitate the dissemination of pathogens, are common in neonatal units in many parts of the world and have been documented in a previous outbreak study in another NICU in our municipal health system (11). Hygienic conditions in the unit, a place originally used as a regular ward, were suboptimal and contributed to the decision to close the unit. No other isolates with similar characteristics were obtained from infants in the hospital to which they were transferred.

In distinction from the prototype A. soli strain B1T, which had resistance to amikacin and gentamicin, the A. soli isolates of this study were susceptible to all agents tested, including these two aminoglycosides. This full susceptibility profile may have contributed to the success of the antimicrobial therapy observed in almost all cases. In fact, the single infant who died had a congenital metabolic disease that was probably the major determinant of the outcome.

Because this was the first time A. soli was detected from a clinical specimen, three sequence signatures, those for 16S rRNA, gyrB, and rpoB, were determined. The three methods...
gave consistent results; however, sequencing of the 351-bp rpoB zone 1 fragment seemed most convenient, as it alone provided an adequate discriminatory power.

Recently, with the use of sequence-based methods to identify Acinetobacter isolates to the species level, it became clear that several non-A. baumannii species cause severe clinical infections (12). Nevertheless, the epidemiological features and clinical impact of non-A. baumannii infections are still poorly understood. Multidrug resistance, for example, has been an important issue in A. baumannii infections, leading to the extensive use of polymyxins as a last resort, even in neonates, as recently reviewed (5). However, as Turton and colleagues have observed (12), the prevalence of antimicrobial resistance may vary widely across non-A. baumannii species. In the present outbreak, isolates of A. soli were susceptible to several antimicrobial agents.

In conclusion, we show for the first time that A. soli may cause invasive disease and may spread among patients. Antimicrobial resistance does not seem to be a problem at the moment. Identification by sequencing a small rpoB fragment (zone 1) is feasible.

**Nucleotide sequence accession numbers.** All five isolates described here were deposited in GenBank under accession numbers FJ872517 to FJ872521. The sequences of strain 788BRRJ were deposited in GenBank under accession numbers FJ872512 to FJ872521. The sequences of strain 788BRRJ were deposited in GenBank under accession numbers FJ872517 to FJ872521.

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