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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.

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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 AAT ATC 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 B1^T (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

Characteristic	Information for patient:				
	1	2	3	4	5
Sex	Female	Female	Male	Female	Male
Gestational age (wk)	35	39	34	37	38
Mother's health	HIV positive	Healthy	Healthy	Healthy	Healthy
Childbirth	Cesarean section	Vaginal	Cesarean section	Cesarean section	Vaginal
Birth wt (g)	1,350	3,360	2,285	2,670	2,655
Twin pregnancy	Yes	No	No	No	No
Baseline condition	First twin stillborn, hypoactive	Hypoxia	Congenital metabolic disease	Hypoxia	Meconium aspiration syndrome
Respiratory distress	No	Yes	Yes	Yes	Yes
Date of isolation of BGN[a]	31 March 2005	2 April 2005	5 April 2005	6 April 2005	8 April 2005
Days from admission to BGN isolation	6	3	5	4	17
Medical procedure(s) before BGN isolation	Peripheral vein	Oxygen hood, peripheral vein	Intermittent mechanical ventilation, peripheral vein	Intermittent mechanical ventilation, peripheral vein	Central venous catheter, total parenteral nutrition
Antibiotic therapy before BGN isolation (no. of days)/reason	Ampicillin plus gentamicin (6)/clinical sepsis of maternal origin	None	Ampicillin plus gentamicin (2)/clinical sepsis of hospital origin	Ampicillin plus gentamicin (4)/clinical sepsis of maternal origin	Ampicillin plus gentamicin (10)/clinical sepsis of maternal origin; cefepime (7)/pneumonia
Antibiotic therapy to treat BGN (no. of days)	Cefepime (7)	Ampicillin plus gentamicin (14)	Cefepime (1)	Cefepime (2) followed by meropenem (12) and ciprofloxacin (10)	Cefepime, vancomycin, clarithromycin, and ciprofloxacin (7)
Outcome	Discharge	Discharge	Death	Discharge	Discharge

[a] BGN, Gram-negative bacillus in the present study.

sive identification. Isolates were susceptible to all antimicrobial agents tested, including colistin (MIC, 0.25 μg/ml). All had indistinguishable PFGE and RAPD-PCR fingerprint patterns. PFGE fingerprints had 15 bands of about 194 kb to less than 48.5 kb.

All isolates shared identical 1,108-bp 16S rRNA gene sequences and were deposited in GenBank. Those sequences had 99 to 100% similarity with those of strain B1^T (6) and the three other *A. soli* sequences in GenBank (accession numbers FJ976560, FJ976568, and GQ25863). This high level of similarity with *A. soli* strongly indicated the identification of the strain. However, since this species had not been previously 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 B1^T (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 B1^T, 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

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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 HQ418340 to HQ418343.

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